Message

From: Grevatt, Peter [Grevatt.Peter@epa.gov]
Sent: 10/24/2016 11:54:23 AM
To: Beauvais, Joel [Beauvais.Joel@epa.gov]
Subject: FW: Perchlorate Peer Review Advance Materials

To keep you in the loop. I agree that the call was quite constructive. Jim L. spent a fair amount of the time bringing the discussion Ex. 5 Deliberative Process (DP) Suggestions from OMB and others were reasonable and helpful. I'll keep you posted as this progresses.

From: Burneson, Eric
Sent: Thursday, October 20, 2016 5:37 PM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>
Cc: Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Kevin:
I wanted to thank you for your assistance in setting expectations for the interagency discussion we just had. The call just concluded, and it was constructive and helpful. We agreed to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) We also will be receiving written comments from our federal colleagues next Thursday. OMB was very helpful in facilitating the discussion. Thanks again.

Eric G. Burneson, P.E.
Director of Standards and Risk Management
Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency

From: Burneson, Eric
Sent: Thursday, October 20, 2016 3:18 PM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>
Cc: Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Got it. We will endeavor to be clear in responding to Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Thanks

From: Rennert, Kevin
Sent: Thursday, October 20, 2016 3:10 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Cc: Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Short summary is that Jim understands that our position is that Ex. 5 Deliberative Process (DP)
Ex. 5 Deliberative Process (DP)
What they really want is to be able to have us Ex. 5 Deliberative Process (DP) To the extent

that we can use today's call to do so, the less friction we'll have on this point moving forward, so I'd encourage you to use the call for that as much as possible. Thanks.

On Oct 20, 2016, at 1:33 PM, Burneson, Eric <Burneson.Eric@epa.gov> wrote:

Kevin: The interagency call about the perchlorate peer review charge is today at 4:00. We have not responded to Jim Kim's request for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) I assume that our answer is that Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Let us know if we should take a different approach to responding.

Eric G. Burneson, P.E.
Director of Standards and Risk Management
Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency

From: Rennert, Kevin
Sent: Monday, October 17, 2016 2:00 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Eric – thanks for flagging. I thought we had actually put this to bed on our call with Jim on Friday, as I'd stated that we were planning Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) He thought that sounded fine, and in alignment with Jim Kim, which is what he cared about. It looks from the below email like that's not the case. I'll call Jim L. and discuss, and will follow up afterwards. Thanks. Kevin

---

From: Burneson, Eric
Sent: Monday, October 17, 2016 10:11 AM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: Fw: Perchlorate Peer Review Advance Materials

Kevin: Apologies if we have already worked through a path forward on Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) but OMB continues to ask for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) We think this sets up a dynamic in which Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) We would appreciate any guidance as to how to respond this request from Jim.

I am at the ASDWA meeting in Milwaukee but am happy to discuss if needed
Eric Burneson Ex. 6 Personal Privacy (PP)

From: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) >
Sent: Monday, October 17, 2016 2:02 PM
To: Christ, Lisa
Cc: Dorjets, Vlad EOP/OMB; Schwab, Margo EOP/OMB; Grevatt, Peter; Greene, Ashley; Burneson, Eric
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

I forgot to ask -- can we schedule a teleconference for interagency discussion of Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) I think it is important to understand Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)

Jim

From: Kim, Jim H. EOP/OMB
Sent: Monday, October 17, 2016 9:58 AM
To: 'Christ, Lisa' <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thank you so much!

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Monday, October 17, 2016 9:56 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP)
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Jim,
I wanted to provide the information for the October 20th call on the perchlorate BBDR model charge questions.
Conference Ex. 6 Personal Privacy (PP) access code Ex. 6 Personal Privacy (PP)

Interagency comments on the charge questions will be provided to EPA and comments on the BBDR model and report will be submitted to the docket. We respectfully request comments on the charge as soon as possible after the meeting -- October 27 would be ideal to allow ample time for peer reviewers consideration.

Sincerely,
Lisa

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 Personal Privacy (PP)
Sent: Thursday, October 06, 2016 2:56 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks. Also – I wanted to confirm that if Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)? That would give EPA a little more than 2 weeks to consider interagency comments. So our goal will be for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Also – I wanted to confirm that our comments on the peer review charge would be submitted to you, while comments on the BBDR draft report and model will be submitted to the docket.

I am thinking that a 3rd meeting prior to the end of November would be useful to Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Please let me know your thoughts.

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Thursday, October 6, 2016 7:35 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP)
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Hi Jim
Of course. I held the time on EPA participants calendars, so I'll send confirmation. I'll set up logistics and send you the information soon.
Lisa

Sent from my iPhone

On Oct 5, 2016, at 4:40 PM, Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) > wrote:

> Hi Lisa,
>
> Thanks for the info. Sorry for the late response. Can we go ahead and schedule a meeting for October 20? Thanks for your patience.
>
> Best regards,
> Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 11:04 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) Dorjets, Vlad EOP/OMB Ex. 6 Personal Privacy (PP) Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim
The closing date for public comments on the charge questions is October 21. The public comment period for the model and report ends November 14. We intend to have our contractor identify the peer reviewers and we'll finalize charge questions between October 21 and November 14. [potential call dates are Oct 20 & 27]

Public comments will be summarized and provided to the peer reviewers at the end of November around the same time we publish the next federal register notice announcing a meeting date sometime in early January (based on availability of peer reviewers and at least 30 days before the panel meeting). [potential call dates are Nov 7 & 8]

Lisa

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Sent: Friday, September 30, 2016 10:27 AM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks for the dates and times. I was hoping to get something on our calendars earlier to ensure that Ex. 5 Deliberative Process (DP) Can you tell me the timeline that is planned in regards to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)

Thanks,
Jim

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To: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) ; Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) ; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim,

I'd like to suggest we schedule two meetings and see if an additional meeting, as suggested, is needed. I looked at calendars for relevant EPA folks and can offer the following dates/times.
1st meeting: October 20 from 4-5pm OR October 27 from 405pm
2nd meeting: November 7 from 11-11am OR November 8 from 11a-noon

I am unable to find availability sooner than Oct 20. However, that provides time for review of materials before discussions begin.
Please let me know at your earliest convenience which times work and I will set up a conference call.
Lisa

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 Personal Privacy (PP)
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB
Ex. 6 Personal Privacy (PP)
< Ex. 6 Personal Privacy (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, September 28, 2016 10:36 AM
To: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) ; Kim, Jim H. EOP/OMB
< Ex. 6 Personal Privacy (PP)
Ex. 6 Personal Privacy (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

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Lisa Christ, Chief
Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M
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Message

From: Rennert, Kevin [Rennert.Kevin@epa.gov]
Sent: 10/3/2016 1:38:21 PM
To: Beauvais, Joel [Beauvais.Joel@epa.gov]
CC: Vaught, Laura [Vaught.Laura@epa.gov]; Grevatt, Peter [Grevatt.Peter@epa.gov]
Subject: RE: Perchlorate Charge Questions Interagency Comments

Thanks.

From: Beauvais, Joel
Sent: Monday, October 03, 2016 9:29 AM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>
Cc: Vaught, Laura <Vaught.Laura@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>
Subject: RE: Perchlorate Charge Questions Interagency Comments

Thanks, Kevin -- I think we Ex. 5 Deliberative Process (DP) I'll let Peter connect with his folks and with me and get back to you (and then Jim)

From: Rennert, Kevin
Sent: Sunday, October 02, 2016 10:48 PM
To: Beauvais, Joel <Beauvais.Joel@epa.gov>
Cc: Vaught, Laura <Vaught.Laura@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>
Subject: Fwd: Perchlorate Charge Questions Interagency Comments

Joel, Peter, see below from Jim on additional meetings on perchlorate charge questions. I'll follow your lead. Thanks. Kevin

Begin forwarded message:

From: "Laity, Jim A. EOP/OMB" <James_A. Ex. 6 EOP (PP)
Date: September 30, 2016 at 7:01:26 PM EDT
To: "Rennert, Kevin" <Rennert.Kevin@epa.gov>
Subject: Perchlorate Charge Questions Interagency Comments

Hi Kevin, I'm back in the office today. OMB folks were generally pleased with how the perchlorate briefing went and with the opportunity that will be provided to the interagency scientists to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) You will see below that EPA has tentatively offered Ex. 5 Deliberative Process (DP) The team here asks if we could possibly have Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP) Understanding the sensitivities here, I reiterate that we understand that Ex. 5 Deliberative Process (DP) We understand that EPA has Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)

I can ask this of the DW office directly but thought I would start with you. Feel free to call if you would like to discuss. Jim

From: Kim, Jim H. EOP/OMB
Sent: Friday, September 30, 2016 2:27 PM
To: Laity, Jim A. EOP/OMB <Ex. 6 EOP (PP)>
Subject: FW: Perchlorate Peer Review Advance Materials

Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
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Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
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Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric

<Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

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Lisa

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To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB Ex. 6 EOP (PP)
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Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

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Thank you,
Lisa

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Lisa Christ, Chief
Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M
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Message

From: Beauvais, Joel [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=088A99C4B0F04E2AA5784349BCCF7B95-BEAUVAIS, JOEL]
Sent: 10/24/2016 12:46:07 PM
To: Grevatt, Peter [Grevatt.Peter@epa.gov]
Subject: Re: Perchlorate Peer Review Advance Materials

Glad to hear it and thanks for keeping me updated

On Oct 24, 2016, at 7:54 AM, Grevatt, Peter <Grevatt.Peter@epa.gov> wrote:

To keep you in the loop. I agree that the call was quite constructive. Jim L. spent a fair amount of the time bringing the discussion Ex. 5 Deliberative Process (DP) Suggestions from OMB and others were reasonable and helpful. I'll keep you posted as this progresses.

From: Burneson, Eric
Sent: Thursday, October 20, 2016 5:37 PM
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Cc: Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
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Eric G. Burneson, P.E.
Director of Standards and Risk Management
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Ex. 5 Deliberative Process (DP)

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Subject: Re: Perchlorate Peer Review Advance Materials

Short summary is that Jim understands that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) What they really want is to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) To the extent that we can use today's call to do so, the less friction we'll have on this point moving forward, so I'd encourage you to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Thanks.

On Oct 20, 2016, at 1:33 PM, Burneson, Eric <Burneson.Eric@epa.gov> wrote:

Kevin: The interagency call about the perchlorate peer review charge is today at 4:00. We have not responded to Jim Kim's request for Ex. 5 Deliberative Process (DP) expect that he will raise this question in our discussion. I assume that our answer is that we will be

Ex. 5 Deliberative Process (DP)

Let us know if we should take a different approach to responding.

Eric G. Burneson, P.E.
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Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency

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Eric – thanks for flagging. I thought we had actually put this to bed on our call with Jim on Friday, as I'd stated that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) It looks from the below email like that's not the case. I'll call Jim L. and discuss, and will follow up afterwards. Thanks. Kevin

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Kevin: Apologies if we have already worked through a path forward on the interagency review of the perchlorate peer review charge but OMB continues to

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I am at the ASDWA meeting in Milwaukee but am happy to discuss if needed
Eric Burneson Ex. 6 Personal Privacy (PP) 7

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Sent: Monday, October 17, 2016 9:58 AM
To: 'Christ, Lisa' <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) ; Schwab, Margo EOP/OMB < Ex. 6 EOP (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thank you so much!

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Monday, October 17, 2016 9:56 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) >
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) ; Schwab, Margo EOP/OMB < Ex. 6 EOP (PP) ; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Jim,
I wanted to provide the information for the October 20th call on the perchlorate BBDR model charge questions.

Conference [redacted] access code [redacted]

Interagency comments on the charge questions will be provided to EPA and comments on the BBDR model and report will be submitted to the docket. We respectfully request comments on the charge as soon as possible after the meeting -- October 27 would be ideal to allow ample time for peer reviewers consideration.

Sincerely,
Lisa

From: Kim, Jim H. EOP/OMB [mailto:[redacted]]
Sent: Thursday, October 06, 2016 2:56 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB <[redacted]>; Schwab, Margo EOP/OMB <[redacted]>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks. Also -- I wanted to confirm that if [redacted]
[redacted]
[redacted]? That would give EPA a little more than 2 weeks to consider interagency comments. So our goal will be for [redacted]
[redacted]
[redacted] Also -- I wanted to confirm that our comments on the peer review charge would be submitted to you, while comments on the BBDR draft report and model will be submitted to the docket.

I am thinking that a 3rd meeting prior to the end of November would be useful to discuss
[redacted]

Please let me know your thoughts.

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Thursday, October 6, 2016 7:35 AM
To: Kim, Jim H. EOP/OMB <[redacted]>
Cc: Dorjets, Vlad EOP/OMB <[redacted]>; Schwab, Margo EOP/OMB <[redacted]>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Hi Jim
Of course. I held the time on EPA participants calendars, so I'll send confirmation. I'll set up logistics and send you the information soon.

Lisa

Sent from my iPhone

On Oct 5, 2016, at 4:40 PM, Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)> wrote:

> Hi Lisa,
>
> Thanks for the info. Sorry for the late response. Can we go ahead and schedule a meeting for October 20? Thanks for your patience.
>
> Best regards,
> Jim
>
> ---
>
> **From:** Christ, Lisa [mailto:Christ.Lisa@epa.gov]
> **Sent:** Friday, September 30, 2016 11:04 AM
> **To:** Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>
> **Cc:** Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
> **Subject:** RE: Perchlorate Peer Review Advance Materials
>
> Jim
> The closing date for public comments on the charge questions is October 21. The public comment period for the model and report ends November 14. We intend to have our contractor identify the peer reviewers and we'll finalize charge questions between October 21 and November 14. [potential call dates are Oct 20 & 27]
>
> Public comments will be summarized and provided to the peer reviewers at the end of November around the same time we publish the next federal register notice announcing a meeting date sometime in early January (based on availability of peer reviewers and at least 30 days before the panel meeting). [potential call dates are Nov 7 & 8]
>
> Lisa
>
> ---
>
> **From:** Kim, Jim H. EOP/OMB [mailto Ex. 6 EOP (PP)
> **Sent:** Friday, September 30, 2016 10:27 AM
> **To:** Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB
> **Cc:** Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
> **Subject:** RE: Perchlorate Peer Review Advance Materials
>
> Hi Lisa,
>
> Thanks for the dates and times. I was hoping to get something on our calendars earlier to ensure that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Can you tell me the timeline that is planned in

regards to the peer review (e.g. deadline date for peer review charge revisions, dates of peer review meeting)?

Thanks,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 9:42 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) Schwab, Margo EOP/OMB Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim,
I'd like to suggest we schedule two meetings and see if an additional meeting, as suggested, is needed. I looked at calendars for relevant EPA folks and can offer the following dates/times.
1st meeting: October 20 from 4-5pm OR October 27 from 405pm
2nd meeting: November 7 from 11-11am OR November 8 from 11a-noon

I am unable to find availability sooner than Oct 20. However, that provides time for review of materials before discussions begin.
Please let me know at your earliest convenience which times work and I will set up a conference call.
Lisa

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 EOP (PP)
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB Ex. 6 EOP (PP) Ex. 6 EOP (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) the second would be to discuss Ex. 5 Deliberative Process (DP), and the third would Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, September 28, 2016 10:36 AM
To: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>; Ex. 6 EOP (PP) <Ex. 6 EOP (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

~~~~~~

Lisa Christ, Chief
Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M
~~~~~~

Message

From: Beauvais, Joel [Beauvais.Joel@epa.gov]
Sent: 7/28/2016 7:17:37 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
CC: Grevatt, Peter [Grevatt.Peter@epa.gov]; Shapiro, Mike [Shapiro.Mike@epa.gov]; Christ, Lisa [Christ.Lisa@epa.gov]
Subject: Re: URGENT: Perchlorate actions

Thanks Eric.

On Jul 28, 2016, at 10:30 AM, Burneson, Eric <Burneson.Eric@epa.gov> wrote:

Just had a conversation with Linda W. and clarified the approach that we had described to OMB.

Ex. 5 Deliberative Process (DP)

Linda stated that she understood this process and that many of her concerns were relieved but that she would still have concerns about Ex. 5 Deliberative Process (DP) And she also stated that we need to be Ex. 5 Deliberative Process (DP)
Ex. 5 Deliberative Process (DP)

Eric

From: Grevatt, Peter
Sent: Thursday, July 28, 2016 1:44 PM
To: Beauvais, Joel <Beauvais.Joel@epa.gov>
Cc: Shapiro, Mike <Shapiro.Mike@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: FW: URGENT: Perchlorate actions
Importance: High

Joel, FYI. This misrepresents our process. I'll work with Mike and Eric to develop a response.

From: WENNERBERG, LINDA S. (HQ-LD020) [mailto:linda.s.wennerberg@nasa.gov]
Sent: Thursday, July 28, 2016 12:35 PM

To: Shapiro, Mike <Shapiro.Mike@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>
Cc: Laity, James A. <Ex. 6 EOP (PP)>; Dorjets, Vlad <Ex. 6 EOP (PP)>; Ex. 6 EOP (PP); Mcneill, Mike A (HQ-LD020) <mike.a.mcneill@nasa.gov>; james.leatherwood-1@nasa.gov; Ex. 6 EOP (PP)
Subject: URGENT: Perchlorate actions
Importance: High

Mike and Peter;

I just received a call from a senior colleague at DoD regarding Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) I am led to believe that Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP). I am writing to dispel any confusion or misrepresentation.

I, on behalf of NASA, am most concerned about Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) I request that you get back to me on EPA's plans and strongly request that you consider Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) The federal family has long worked together to ensure sound science is utilized for perchlorate.

NASA requested Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Please contact me as soon as possible to communicate EPA's plans.

Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89
300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

Message

From: Beauvais, Joel [Beauvais.Joel@epa.gov]
Sent: 7/28/2016 7:17:00 PM
To: Grevatt, Peter [Grevatt.Peter@epa.gov]
CC: Shapiro, Mike [Shapiro.Mike@epa.gov]; Burneson, Eric [Burneson.Eric@epa.gov]
Subject: Re: URGENT: Perchlorate actions

Ok thanks for the heads up - keep me posted

On Jul 28, 2016, at 9:44 AM, Grevatt, Peter <Grevatt.Peter@epa.gov> wrote:

Joel, FYI. This misrepresents our process. I'll work with Mike and Eric to develop a response.

From: WENNERBERG, LINDA S. (HQ-LD020) [mailto:linda.s.wennerberg@nasa.gov]
Sent: Thursday, July 28, 2016 12:35 PM
To: Shapiro, Mike <Shapiro.Mike@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>
Cc: Laity, James A. <James_A. Ex. 6 EOP (PP)>; Dorjets, Vlad <Ex. 6 EOP (PP)>; Ex. 6 EOP (PP) Mcneill, Mike A (HQ-LD020) <mike.a.mcneill@nasa.gov>; james.leatherwood-1@nasa.gov; Ex. 6 EOP (PP)
Subject: URGENT: Perchlorate actions
Importance: High

Mike and Peter;

I just received a call from a senior colleague at DoD regarding proposed Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) I am led to believe that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) I am writing to dispel any confusion or misrepresentation.

I, on behalf of NASA, am most concerned about Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) I request that you get back to me on EPA's plans and strongly request that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) The federal family has long worked together to ensure sound science is utilized for perchlorate.

NASA requested Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Please contact me as soon as possible to communicate EPA's plans.

Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89

300 E Street SW
Washington, DC 20546-0001

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Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

Predicted Effect of Perchlorate on Thyroid Hormone Levels in the Pregnant & Lactating Mother, Fetus, and Breast- and Bottle-Fed Infant: Model Dose-Response Application

Paul M Schlosser

U.S. Environmental Protection Agency, ORD, NCEA-Washington

September 27, 2016

DO NOT CITE OR QUOTE

ED_005043_00018642-00001

Background

- In 2012, as required by the Safe Drinking Water Act, EPA sought guidance from the Science Advisory Board on how best to consider and interpret life stage information, epidemiologic and biomonitoring data, physiologically-based pharmacokinetic (PBPK) analyses and the totality of perchlorate health information to derive an MCLG for perchlorate.
- In 2013, the SAB recommended that:

  "…EPA derive a perchlorate MCLG that addresses sensitive life stages through physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling based upon its mode of action rather than the default MCLG approach using the reference dose and specific chemical exposure parameters"

  "…the following MOA-based approach for using PBPK/PD modeling and additional clinical and toxicological data to inform the derivation of a health-protective MCLG recognizing that the sensitive populations for perchlorate exposure are the fetuses of hypothyroxinemic pregnant women, and infants exposed to perchlorate through either water-based formula preparations or the breast milk of lactating women"
- A team of scientists lead by Dr. Jeff Fisher, U.S. FDA, developed the Biologically Based Dose-Response (BBDR) model
  - Integrates PBPK models for perchlorate and iodide with BBDR models for thyroid hormones
  - Predicts effect of perchlorate on thyroid hormone levels in formula-fed and breast-fed infants, and lactating women, for postnatal days 7 to 90

DO NOT CITE OR QUOTE

ED_005043_00018642-00002

Background II

- June 2013: SAB Recommendations – EPA should:
  - Use hypothyroxinemia (hX) as immediate endpoint vs. Radio-Iodide Uptake (RAIU) Inhibition suggested by NAS in 2005
  - hX = moderate TH (T4 and T3) reduction, with thyroid stimulating hormone (TSH) in normal range
- Excluding TSH feedback makes the modeling less complex, but limits range for which it is valid
- Report includes statistical analyses to define range for hX
  - Range defined *relative to model-predicted fT4 levels for each life-stage*, given suggested iodine nutrition levels (i.e., euthyroid state)
  - This approach used to control for bias due to possible over- or under-prediction of the model vs. actual population mean TH levels
- Study populations providing data for model calibration, hence "controls" for perchlorate exposure, are assumed to have similar exposures to other goitrogens (SCN^-, NO_3^-) as populations of concern
  - Hence no specific correction for exposure to these other common goitrogens

DO NOT CITE OR QUOTE

ED_005043_00018642-00003

General Picture

- **Idealized view:**

Ex. 5 Deliberative Process (DP)

Pregnancy Dose-Response: 1st View

Ex. 5 Deliberative Process (DP)

Pregnancy Dose-Response: 2nd View

Ex. 5 Deliberative Process (DP)

Bottle/formula-fed Infant: Day 7

Ex. 5 Deliberative Process (DP)

Bottle/formula-fed Infant: Days 7-90
200 µg/d iodine during pregnancy

Ex. 5 Deliberative Process (DP)

Breast-feeding mother: maternal nutrition & perchlorate exposure = pregnancy levels

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Summary: Formula-/Breast-Fed Infant & Lactating Mom (Perchlorate ≤ 20 µg/kg/d)

Ex. 5 Deliberative Process (DP)

Message

From: Bertrand, Charlotte [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=F044D768E05842E1B75321FF6010E1B8-BERTRAND, CHARLOTTE]
Sent: 9/26/2016 6:50:26 PM
To: Monell, Carol [Monell.Carol@epa.gov]; Maryt Cooke (Cooke.Maryt@epa.gov) [Cooke.Maryt@epa.gov]
Subject: FW: Perchlorate Peer Review Briefing with EPA
Attachments: Perchlorate Peer Review Briefing with EPA.ics

Appointment

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 7/10/2018 7:05:23 PM
To: Kim, Jim H. EOP/OMB; Ex. 6 EOP (PP)

Subject: TWG - Perchlorate

Start: 7/11/2018 2:00:00 PM
End: 7/11/2018 2:30:00 PM
Show Time As: Tentative

Appointment

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 7/9/2018 8:56:05 PM
To: Ex. 6 EOP (PP)

Subject: Meeting Forward Notification: TWG - Perchlorate

Start: 7/9/2018 9:00:00 PM
End: 7/9/2018 9:30:00 PM
Show Time As: Busy

Appointment

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 7/9/2018 5:27:13 PM
To: Ex. 6 EOP (PP)

Subject: Accepted: TWG - Perchlorate
Location: Conference Call

Start: 7/11/2018 6:00:00 PM
End: 7/11/2018 7:00:00 PM
Show Time As: Busy

Appointment

From: Hafez, Ahmed [Hafez.Ahmed@epa.gov]
Sent: 10/19/2017 1:07:04 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]; Miller, Gregory [Miller.Gregory@epa.gov]; Flowers, Lynn [Flowers.Lynn@epa.gov]; Christ, Lisa [Christ.Lisa@epa.gov]
CC: Hernandez-Quinones, Samuel [Hernandez.Samuel@epa.gov]

Subject: Canceled: Finalizing Perchlorate Charge Questions
Attachments: Proposed Draft SBA Edits to Percholorate Charge Questions v2 October 5.docx; RE: Perchlorate Charge Question - question about Lisa's email discussion below
Location: Call-in or Eric's Office (WJC-E 2331A)

Start: 10/19/2017 3:30:00 PM
End: 10/19/2017 4:30:00 PM
Show Time As: Free

Importance: High

Message

From: Christ, Lisa [Christ.Lisa@epa.gov]
Sent: 10/5/2017 6:55:17 PM
To: kevin.bromberg@sba.gov; Burneson, Eric [Burneson.Eric@epa.gov]; Hafez, Ahmed [Hafez.Ahmed@epa.gov]
CC: Ex. 6 EOP (PP); Hernandez-Quinones, Samuel [Hernandez.Samuel@epa.gov]
Subject: RE: Perchlorate Charge Question - question about Lisa's email discussion below

Hello Kevin and Jim,
Here's my attempt to clarify a couple things and respond to your questions.

JK: Basically, the explanation is that Ex. 5 Deliberative Process (DP)
Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

KB: My reaction, as noted in my earlier email, is this result COULD also be attributed to Ex. 5 Deliberative Process (DP)
Ex. 5 Deliberative Process (DP)
I would suggest Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

From: Bromberg, Kevin L. [mailto:kevin.bromberg@sba.gov]
Sent: Wednesday, October 04, 2017 5:20 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>; Hafez, Ahmed <Hafez.Ahmed@epa.gov>
Cc: Ex. 6 EOP (PP) Hernandez-Quinones, Samuel <Hernandez.Samuel@epa.gov>
Subject: RE: Perchlorate Charge Question - question about Lisa's email discussion below

Lisa, I am confused by your statement in the last paragraph that Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

http://www.nejm.org/doi/full/10.1056/NEJMoa1606205#t=articleResults Casey Study

The results of our trials are consistent with those of the CATS study, which was a thyroid-screening trial involving 21,846 pregnant women, primarily from the United Kingdom. Women in that trial were either screened immediately and treated with levothyroxine if they were identified as having subclinical hypothyroidism or hypothyroxinemia or had their serum frozen to be analyzed on completion of the pregnancy. **The results of IQ testing of the children at 3 years of age did not differ significantly between children whose mothers had been immediately treated during the pregnancy and those whose mothers had treatment that was deferred.**

Thyroid hormone–replacement therapy in the CATS study was initiated at a median gestational age of 13 weeks 3 days, as compared with 16 weeks 4 days and 18 weeks in our two trials.[17] However, 24% of the children in the CATS study were lost to follow-up. In the current trials, the follow-up rate at 5 years of age was more than 92%.

Several studies have also suggested that either a high maternal thyrotropin level or hypothyroxinemia is associated with externalizing behavioral problems such as attention deficit–hyperactivity disorder (ADHD).[25,26] In the Generation R Study, 8-year-old children of women from an iodine-deficient geographic area who had been identified with hypothyroxinemia before 20 weeks of gestation were found to have higher ADHD index scores than children of women without hypothyroxinemia.[26] This association persisted after adjustment for IQ. **We did not identify any significant between-group differences in ADHD index scores in either of our trials, and the scores were well within the normal range. Moreover, the Child Behavior Checklist scores did not reveal any evidence of behavioral problems in children whose mothers with subclinical hypothyroidism or hypothyroxinemia were in the placebo group. The CATS study also did not detect any behavioral improvements in children who had been exposed to thyroid hormone replacement.**[17]

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, October 04, 2017 11:56 AM
To: Bromberg, Kevin L.; Burneson, Eric; Hafez, Ahmed
Cc: Ex. 6 EOP (PP) Hernandez-Quinones, Samuel
Subject: RE: Perchlorate Charge Question - My latest thoughts - feel free to modify

Kevin,
Thank you for the additional input on the charge. Also, I have summarized the discussion from yesterday.

You asked: Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Response: Ex. 5 Deliberative Process (DP)

To develop these tables, EPA used Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Best,

Lisa

From: Bromberg, Kevin L. [mailto:kevin.bromberg@sba.gov]
Sent: Wednesday, October 04, 2017 11:33 AM
To: Burneson, Eric <Burneson.Eric@epa.gov>; Hafez, Ahmed <Hafez.Ahmed@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Ex. 6 EOP (PP)
Subject: Perchlorate Charge Question - My latest thoughts - feel free to modify

Thanks for that thoughtful conversation yesterday.

Lisa and I just talked – and not I'm memorializing my latest thoughts.

The apparent discrepancy with regard to Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

A better home for these thoughts would be in Ex. 5 Deliberative Process (DP) See italicized additions.

Ex. 5 Deliberative Process (DP)

Kevin Bromberg
Assistant Chief Counsel for Environmental Policy
SBA // Office of Advocacy
409 3rd St. SW, Washington, D.C. 20416
kevin.bromberg@sba.gov 202.481.2963
202.205.6964

- Use Ballot image search to depopulate. The file may have been moved, renamed, or deleted. Verify that the file path in the search for and retrake.

- Use Ballot image above to duplicate. The file may have been moved, renamed, or deleted. Verify that the file path in the search for and retrake.
- Use Ballot image search for displayed. The file may have been moved, renamed, or deleted. Verify that the file path in the search for and retrake.
- Use Scan Image Search to displayed. The file may have been moved, renamed, or deleted. Verify that the file path in the search for and retrake.
- Use Ballot image search for displayed. The file may have been moved, renamed, or deleted. Verify that the file path in the search for and retrake.

- Use Ballot image search for displayed. The file may have been moved, renamed, or deleted. Verify that the file path in the search for and retrake.
- Use Ballot image search for displayed. The file may have been moved, renamed, or deleted. Verify that the file path in the search for and retrake.
- Use Ballot image search for displayed. The file may have been moved, renamed, or deleted. Verify that the file path in the search for and retrake.

Appointment

From: Neumayr, Mary B. EOP/CEQ Ex. 6 EOP (PP)
Sent: 9/28/2017 4:36:05 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]

Subject: Declined: EPA Perchlorate Peer Review Charge follow up
Location: Teleconference # Ex. 6 Personal Privacy (PP) code Ex. 6 Personal Privacy (PP) or EPA WJC East Room 2123

Start: 10/2/2017 7:00:00 PM
End: 10/2/2017 8:00:00 PM
Show Time As: Busy

Recurrence: (none)

Appointment

From: Wennerberg, Linda S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
Sent: 9/27/2017 7:31:55 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]

Subject: Accepted: EPA Perchlorate Peer Review Charge follow up
Location: Teleconference # Ex. 6 Personal Privacy (PP) code Ex. 6 Personal Privacy (PP) or EPA WJC East Room 2123

Start: 10/2/2017 7:00:00 PM
End: 10/2/2017 8:00:00 PM
Show Time As: Busy

Recurrence: (none)

Appointment

From: Jensen, Michael C. EOP/CEQ Ex. 6 EOP (PP)
Sent: 9/22/2017 2:02:44 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]

Subject: Accepted: EPA Perchlorate Peer Review Charge follow up
Location: Teleconference # Ex. 6 Personal Privacy (PP) code Ex. 6 Personal Privacy (PP) or EPA WJC East Room 2123

Start: 10/2/2017 7:00:00 PM
End: 10/2/2017 8:00:00 PM
Show Time As: Busy

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To: Burneson, Eric [Burneson.Eric@epa.gov]

Subject: Accepted: EPA Perchlorate Peer Review Charge follow up
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To: Burneson, Eric [Burneson.Eric@epa.gov]

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From: Laity, Jim A. EOP/OMB Ex. 6 EOP (PP)
Sent: 9/22/2017 11:38:04 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]

Subject: Tentative: EPA Perchlorate Peer Review Charge follow up
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Appointment

From: Seale, Viktoria Z. EOP/CEQ Ex. 6 EOP (PP)
Sent: 9/25/2017 3:31:01 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]

Subject: Tentative: EPA Perchlorate Peer Review Charge follow up
Location: Teleconference # Ex. 6 Personal Privacy (PP) code Ex. 6 Personal Privacy (PP) or EPA WJC East Room 2123

Start: 10/2/2017 7:00:00 PM
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Appointment

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 7/10/2018 7:05:20 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]; Kim, Jim H. EOP/OMB [Ex. 6 EOP (PP)]

Subject: TWG - Perchlorate

Start: 7/11/2018 2:00:00 PM
End: 7/11/2018 2:30:00 PM
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Message

From: Christ, Lisa [Christ.Lisa@epa.gov]
Sent: 9/28/2016 2:36:16 PM
To: Ex. 6 EOP (PP)
CC: Grevatt, Peter [Grevatt.Peter@epa.gov]; Greene, Ashley [Greene.Ashley@epa.gov]; Burneson, Eric [Burneson.Eric@epa.gov]
Subject: Perchlorate Peer Review Advance Materials
Attachments: 1. Draft Charge Questions for Perchlorate BBDR Model Peer Review 9-28-16.pdf; 2. Draft Perchlorate BBDR Model Report for Peer Review 9-28-16.pdf; 3. Draft Perchlorate BBDR Model Report for Peer Review - Appendices 9-28-16.pdf; 4. Link to Obtain Draft Perchlorate BBDR Model for Peer Review 9-28-16.pdf

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

~~~~~~

Lisa Christ, Chief
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Perchlorate BBDR Model External Peer Review
Draft Charge Questions

The U.S. EPA's Office of Water is in the process of developing National Primary Drinking Water Regulations for perchlorate under the Safe Drinking Water Act. In 2012, EPA submitted a white paper *Life Stage Considerations and Interpretation of Recent Epidemiological Evidence to Develop a Maximum Contaminant Level Goal for Perchlorate* (U.S. EPA, 2012) to the Science Advisory Board (SAB) for review. This white paper presented scientific information published since the National Research Council (NRC) released their 2005 Report "Health Implications of Perchlorate Ingestion" and described how EPA derived a range of Maximum Contaminant Level Goal (MCLG) values for life stages of concern. In their 2013 report, the SAB concluded that EPA should: 1) consider exposure to the following sensitive life stages: the fetuses of hypothyroxinemic pregnant women and infants exposed to perchlorate through either water-based formula or the breast milk of lactating women, and 2) derive the MCLG using a physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) model to account for exposure differences and biological differences across life stages, including sensitive life stages. Specifically, the SAB found that the 'default algebraic approach provides limited ability to address the various exposure and biological factors affecting sensitivity to perchlorate at different life stages. The SAB concluded that, from a scientific standpoint, it would be more appropriate to base the MCLG derivation on the perchlorate mode of action, using PBPK/PD modeling to relate perchlorate concentrations in drinking water to biological effects rather than the default approach.'

The SAB report referenced a report by Lumen et al. (2013) on the effect of perchlorate on thyroid hormone perturbations for pregnant mothers and fetuses as well as their work to extend the physiologically-based pharmacokinetic/pharmacodynamic iodine uptake inhibition (PBPK/PD-IUI) model with links to serum thyroid hormone levels. The extension of the PBPK/PD-IUI model to thyroid hormones is also referred as the BBDR model (Biologically Based Dose Response model).

The new model presented here integrates the previous EPA PBPK model (2009) for perchlorate with the model developed by Lumen et al. (2013) for pregnant mothers and fetuses and iodide models developed by Fisher et al. (2013) for lactating rats and Fisher et al. (2016) for lactating mothers and nursing infants. The BBDR model predicts thyroid hormone perturbations for lactating mothers, breast-fed infants and bottle-fed infants resulting from exposure to perchlorate under different dietary iodine intake levels. EPA is relying on the Lumen et al. (2013) model, peer reviewed and published previously, to predict effects for pregnant mothers and fetuses. It is important to note a modification to a key parameter was made (Michaelis-Menten constant for the sodium-iodide symporter for perchlorate, Km) and the thyroid hormone predictions were adjusted for pregnant mothers and fetuses in this model report.

EPA is seeking comments on the scientific rigor of the BBDR model for evaluating the effects of perchlorate on thyroid hormones in lactating mothers, breast-fed infants and bottle-fed infants under different iodine intake levels. EPA is also seeking comment on modifications made to the Lumen et al. (2013) model for evaluating the effects of perchlorate on thyroid hormones in pregnant mothers and fetuses.

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Note that the model is only calibrated for and expected to be valid for conditions where feedback regulation due to variations in thyroid-stimulating hormone (TSH) levels is minimal, so all questions regarding the model are for that range.

1. **Please comment on the following aspects of the BBDR model structure and integration of the submodels for iodide/thyroid hormones and perchlorate for each of the life stages of focus (i.e., lactating mother, breast-fed infant, and bottle-fed infant):**
    A. **Does the developed model structure adequately and accurately describe the physiology and kinetics of iodine, thyroid hormones, and perchlorate during different life stages specified above? Please specifically note the strengths and weaknesses of the model structure for each life stage.**
    B. **Are the physiological and kinetic parameters for iodine, thyroid hormones, and perchlorate for these life stages supported by the published sources for those parameters or data used to identify parameters via calibration? Please identify any critical data gaps or additional data on these model parameters.**

2. **EPA has modified one parameter in the 2013 Lumen et al. model (and other previous models): KM_TPNG, the Michaelis-Menten constant for the sodium-iodide symporter for perchlorate based on Schlosser (2016), and a second key parameter in the 2009 USEPA/2007 Clewell et al. PBPK models: VMAXC_MKP, the maximal velocity for perchlorate transfer to breast milk from those components in the USEPA 2009 models. These two parameters affect perchlorate transport from the mammary plasma to breast milk and from the thyroid plasma to thyroid tissue. Were the changes made to the 2013 Lumen et al. model for pregnant mothers and fetuses (Km only) and lactating mothers and infants (Km and Vmax) scientifically supported and reasonably estimated?**

3. **Please address the strengths or limitations of the data used for model calibration and evaluation and identify any additional literature that EPA should consider.**
    A. **Specifically, is the model calibration and evaluation adequately compared with what is reported in the literature for iodine and perchlorate concentrations in urine, and breast milk and thyroid hormone plasma concentrations under different iodine intake and perchlorate exposure scenarios?**
    B. **Are the influential parameters identified in the local sensitivity analysis for total thyroxine (T4) and free thyroxine (fT4) plasma concentrations consistent with known biology? Are there critical data gaps for the influential parameters, which would impact the thyroid hormone predictions?**

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4. **The BBDR model is limited to control of thyroid hormone production based on iodine concentrations in the thyroid and does not include hypothalamus-pituitary thyroid (HPT) feedback control based on TSH levels. Therefore, the model predictions are reliable for a limited range of dietary iodine intake levels and perchlorate exposure combinations, which is not inclusive of individuals with deficient iodide intake. Please comment on the adequacy of the range of iodine intake levels (adequate versus iodide deficient conditions) selected in predicting the thyroid hormone predictions for the pregnant mothers, fetuses, lactating mothers, breast-fed infants and bottle-fed infants. Please also suggest any options for accounting for individuals with deficient dietary iodine when evaluating the effects of perchlorate on thyroid hormones.**

5. **Given the lack of a specific quantitative clinical definition of hypothyroxinemia for the specific life stages evaluated (pregnant mothers, infants and lactating mothers) and that reference intervals of thyroid hormones reported in the literature are influenced by many factors (life stage differences, analytical method used, laboratory utilized, ethnicity, iodide nutrition, population size etc.), EPA developed a normalization procedure to derive hypothyroxinemia reference ranges.**

   A. **Please comment on the hypothyroxinemia reference ranges derived for pregnant mothers, fetuses, lactating mothers, breast-fed infants and bottle-fed infants.**

   B. **Based on the perchlorate dose level at which each life stage is predicted to become hypothyroxinemic (for a given iodine ingestion rate), it appears that the lactating mother is much more sensitive than the breast-fed infant for PND 30-90. In part this occurs because breast feeding reduces the iodide available for the maternal thyroid. Is this prediction biologically realistic and if so, should the subsequent analysis also consider the lactating mother as a life-stage of concern for MCLG derivation?**

6. **On PND 7 the breast-fed infant is predicted to be more sensitive than at later PNDs (though still somewhat less or equally sensitive compared to the breast-feeding mother). This sensitivity is effectively a residual effect of fetal exposure during pregnancy, so while it is relatively transient within the postnatal period, it reflects the dose-response during fetal development. However, the BBDR model is considered more uncertain at PND 7 vs. PND 30-90, because of the rapid changes in physiology just after birth and the lack of calibrating data. Given these factors, please comment on the potential focus of an MCLG analysis on the PND 7 infant.**

7. **The pregnancy model (but not the lactation/infant models) uses empirical functions to make urinary clearance of iodine and metabolic degradation of T3 dependent on**

the iodine ingestion level, in order to predict observed nonlinearity in the relationship between thyroid hormone levels and iodine intake. Does this use of empirical functions result in predicted outcomes or trends that are not plausible for serum thyroid hormones, particularly fT4?

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Biologically Based Dose Response Models for the Effect of Perchlorate on Thyroid Hormones in the Infant, Breast Feeding Mother, Pregnant Mother, and Fetus: Model Development, Revision, and Preliminary Dose-Response Analyses

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1 **LIST OF ABBREVIATIONS[1]**

Abbreviation	Definition
BBDR	Biologically based dose response
BW	Body weight
CHO	Chinese hamster ovary
ClO_4	Perchlorate
EPA	Environmental Protection Agency
FDA	Food And Drug Administration
fT4	Free thyroxine
GI	Gastrointestinal
HRCCA	Health Risk Reduction And Cost Analysis
Km	Michaelis Menten saturation constant
LMS	Lambda-mu-sigma transformed normal distribution
MCLG	Maximum contaminant level goal
MOA	Mode of action
NHANES	National Health And Nutrition Examination Survey
NIS	Sodium-iodide symporter
NPDWR	National Primary Drinking Water Regulation
PBPK	Physiologically based pharmacokinetic
PBPK-PD	Physiologically based pharmacokinetic-pharmacodynamic
PND	Postnatal day
POD	Point of departure
RAIU	Radio-iodide uptake
RfD	Reference dose
SAB	Science Advisory Board
SD	Standard deviation
SDWA	Safe Water Drinking Act
T3	Triiodothyronine
T4	Thyroxine
TH	Thyroid hormone
TSH	Thyroid stimulating hormone

2 [1]Table 3 lists model parameter abbreviations and their definitions

3

EXECUTIVE SUMMARY

The U.S. EPA is developing a National Primary Drinking Water Regulation (NPDWR) for perchlorate. The 1996 Safe Drinking Water Act Amendment (SDWA) regulatory efforts require assessment of effects of the contaminant on potentially sensitive subgroups of the general population. The SAB (2013) recommended that the EPA focus the application of physiologically based pharmacokinetic (PBPK) modeling on the condition of hypothyroxinemia in which free thyroxine (fT4) levels are decreased to the lower end of the normal range with TSH levels maintained in the normal range. Hypothyroxinemia is considered a more sensitive and therefore better "indicator of the potential adverse health effects than the more pronounced decreases in thyroid function associated with hypothyroidism" (SAB, 2013), where T4 is reduced to low levels and TSH is elevated. To meet the regulatory requirement and to follow the 2013 Science Advisory Board (SAB) perchlorate report recommendations, EPA and FDA worked collaboratively to develop and integrate PBPK models for perchlorate and iodide[1] with biologically based dose response (BBDR) models for thyroid hormones to predict the effect of perchlorate on the thyroid gland in formula-fed and breast-fed infants, as well as lactating women. (BBDR models describe biologically processes and how they change as a result of chemical interaction, while the PBPK model describes the delivery of a chemical to the biological site of action). The model developed is limited to the range of perchlorate doses and dietary iodine intakes for which thyroid homeostasis is expected to be auto-regulated. Under this condition, TSH is in its normal range and thyroid hormone excretion is only a function of organified iodide content in the thyroid gland.

Model development efforts focused on: 1) the lactating mother; and 2) the breast-fed and formula-fed infant for the postnatal period from postnatal day (PND) 7 to 90. With a few modifications, the existing PBPK models for perchlorate dosimetry in the infant (formula- or breast-fed) and lactating mother (U.S. EPA, 2009), which were used in previous work predicting inhibition of radiolabeled iodide uptake (RAIU), were integrated with PBPK models for iodide and BBDR models for thyroid hormones, which were based on the Lumen et al. (2013) model for pregnant women and fetuses and Fisher et al. (2013) for lactating rats and nursing pups. The new, integrated infant and maternal models predict the effects of perchlorate on serum thyroid hormone concentrations in the lactating mother exposed to perchlorate in the diet and in infants exposed via ingestion of perchlorate in formula or breast milk.

---

[1] Elemental iodine can be present in many forms, including ionic, non-bound iodide, and as incorporated into thyroid hormones (often referred to as "organified" or "organified iodine"). Since iodine nutrition is determined by the total amount of available forms in the diet, dietary levels (amount ingested) are referred to as "iodine" in this report. Since model-predicted levels of un-bound iodine in the blood, breast milk, and urine are assumed to be in the ionic form, these *concentrations* are usually referred to as "iodide". However, when discussing the nutritional levels and intake for the breast-fed infant, the term "iodine" will be used, to be consistent in that regard, even though it is assumed to be present in breast milk as iodide.

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Lifestage-specific model parameters were obtained from the literature, when available, or calibrated either by empirical estimates or model fits to literature data. While there was a paucity of data in the literature for the population targeted by these models, data were available to compare with model predictions for 1) infant urinary iodide and perchlorate concentrations for both breast-fed and formula-fed infants; 2) breast milk iodide and perchlorate concentrations; and 3) maternal and breast-fed infant free T4 (fT4) and total bound and free T4 (T4) serum concentrations. Overall, the model predictions when compared with literature data were considered adequate.

Dose-response analyses for perchlorate effects on thyroid hormones were performed with the newly developed models, as well as with the pregnant woman and fetus model developed previously (Lumen et al., 2013)[2]. For the analyses, maternal iodine intakes ranged from 75 to 250 µg/day and formula iodine concentrations ranged from 108 to 384 µg/L, with perchlorate doses up to 20 µg/kg/d. In initial analyses for formula-fed infants, perchlorate reduced fT4 levels at PND 7 but not PND 30 to 90 for all formula concentrations of iodine. For all postnatal days and formula iodine concentrations, the model predicted fT4 would be within the reference intervals (i.e., the range considered to be normal and healthy) for infants (Lem et al., 2012).

For the breast-fed infant, the model predicted that maternal perchlorate exposure would reduce serum fT4 concentrations from PND 7 to 90, with the magnitude of the decrease dependent upon the maternal iodine intake. The model predicted that breast-fed infants from lactating mothers with iodine intake $\geq$ 150 µg/d and perchlorate exposure up to 20 µg/kg/d would have serum fT4 levels within the reference intervals for infants (Lem et al., 2012) from PND 30 to 90; however, the lactating mothers were predicted to become hypothyroxinemic or hypothyroid at perchlorate exposures below 20 µg/kg/d, even with daily iodine intakes of 250 µg/d. For PND 7, serum fT4 levels in breast-fed infants would be below the reference intervals at lower perchlorate doses < 12 µg/kg/d, depending on maternal iodine intake.

The difference in the predicted effect of perchlorate on the breast-fed vs. formula-fed infant is due primarily to a larger perchlorate dose from breast milk versus formula, which is exacerbated by low iodide levels in breast milk when the mother's iodine ingestion is inadequate. The local sensitivity analysis performed with the model showed that, in addition to the kinetic parameters governing the formation and metabolism of thyroid hormones and urinary clearance of iodide, maternal parameters controlling iodide concentration in milk and the ingested amount of milk were influential on predictions of fT4 and T4 in the breast-fed infant.

Dose-response analyses were conducted for the pregnant mother and fetus, since the fetus in particular is considered a sensitive lifestage for developmental effects, and results were compared with those for the lactating mother and breast-fed infant. The pregnant mother was predicted to have somewhat lower base-line fT4 levels (in the absence of perchlorate) than the

[2] As detailed in this report, the affinity of perchlorate for the sodium-iodide symporter (NIS), a key parameter in the PBPK/BBDR models, was revised based on a re-evaluation of original data. The revised value was substituted into the model of Lumen et al. (2013) in the analyses conducted here.

lactating mother at PND 7 for the same iodine ingestion levels. However, the steepness of the perchlorate dose-response for the pregnant mother is about the same as for the PND 7 lactating mother. And from PND 7 to 90, fT4 levels in the breast-feeding mother were predicted to decrease as a result of postnatal physiological changes and the demands of breast-feeding, making the lactating mother potentially more vulnerable than during pregnancy. On the other hand, the estimated variance in serum fT4 levels for the pregnant mother, hence the width of the reference range used to define normal vs. hypothyroxinemic conditions, was somewhat smaller than that estimated for the lactating mother. The model predicts that a pregnant mother and a lactating mother at PND 60 or 90 who only ingest 75 µg/d of iodine would be hypothyroid with no perchlorate exposure. With an iodine intake of 100 µg/d (considered medically insufficient), both pregnant and lactating mother would be barely within or just below the reference range. The model does predict that at 150 µg/d iodine or higher, the mother stays within the reference range during both pregnancy and lactation as long as perchlorate exposures are below 4 µg/kg/d.

The late-gestation fetus is predicted to be potentially more sensitive than nursing infants, since compared with the breast-fed infant, base-line fT4 levels are lower and the decrease in fT4 levels with increasing perchlorate dose is greater even with moderate to high maternal iodine intakes of 150-250 µg/d. However, because there is not a well-established thyroid hormone reference range for fetuses, an absolute determination of the relative risk to the fetus vs. the breast-fed infant cannot be made.

INTRODUCTION

Regulatory Background & Needs for Perchlorate

On February 11, 2011, EPA published a determination to regulate perchlorate (ClO_4^-) under the Safe Drinking Water Act (SDWA) (U.S. EPA, 2011) based on the following:

- perchlorate may have an adverse effect on the health of persons;
- perchlorate is known to occur or there is a substantial likelihood that it will occur in public water systems with a frequency and at levels of public health concern; and,
- regulation of perchlorate, in the sole judgment of the Administrator, presents a meaningful opportunity for health risk reduction for persons served by public water systems.

1 EPA is now in the process of developing a National Primary Drinking Water Regulation
2 (NPDWR) for perchlorate. The Safe Drinking Water Act (SDWA) requires EPA to propose a
3 Maximum Contaminant Level Goal (MCLG) simultaneously with the NPDWR. The MCLG is a
4 non-enforceable goal defined under the SDWA (§1412.b.4.B) as *"the level at which no known or*
5 *anticipated adverse effects on the health of persons occur and which allows an adequate*
6 *margin of safety."* In addition to the MCLG, EPA must also prepare a Health Risk Reduction and
7 Cost Analysis (HRRCA) when proposing a regulation that includes an assessment of the
8 quantifiable and non-quantifiable health risk reduction benefits likely to occur as a result of
9 treatment to remove the contaminant. SDWA further requires that the HRRCA include an
10 assessment of the effects of the contaminant on the general population and on groups within
11 the general population such as infants, children, pregnant women, the elderly, individuals with a
12 history of serious illness, or other populations that are identified as likely to be at greater risk of
13 adverse health effects.

14 In support of SDWA regulatory efforts, on May 29, 2012, EPA evaluated the evidence relating
15 perchlorate exposures and health effects in humans since the NRC 2005 report (NRC, 2005)
16 including the EPA's 2009 PBPK model findings and derived a range of possible MCLGs for
17 different life-stages in a white paper, titled, "*Life Stage Considerations and Interpretation of*
18 *Recent Epidemiological Evidence to Develop a Maximum Contaminant Level Goal for*
19 *Perchlorate*" (U.S. EPA, 2012). In the report, EPA developed a range of potential MCLGs for
20 different life stages based on the reference dose of 0.7 µg/kg/day and relevant exposure
21 assumptions/parameters that are needed as part of the formula to derive the MCLG. The RfD of
22 0.7 µg/kg/day was developed by NRC and later adopted by EPA (U.S. EPA, 2012; NRC, 2005).
23 It is based on the No Observed Effect Level of 7 µg/kg/day and corresponds to a RAIU inhibition
24 of 1.8 percent reported in healthy human adults (Greer et al., 2002) with application of an
25 uncertainty factor of 10 to account for differences in sensitivity between the healthy adults in the

study and the most sensitive population, namely "fetuses of pregnant women who might have hypothyroidism or iodide deficiency." The exposure parameters and assumptions included body weights and drinking water intake specific to those life stages as well as relative source contribution which are needed as part of the formula to derive the MCLG.

The Science Advisory Board (SAB) evaluated the white paper in 2012 and provided recommendations to EPA (SAB, 2013). Specifically, the SAB recommended that the EPA derive a perchlorate MCLG that addresses sensitive life stages through use of a physiologically based pharmacokinetic/pharmacodynamic (PBPK-PD) model which incorporates perchlorate's mode of action, rather than the default MCLG equation that uses the RfD and a limited number of exposure parameters (e.g., body weight, drinking water intake). PBPK-PD modelling is referred to as Biologically Based Dose Response (BBDR) modeling in this document. This is the first time that EPA has considered a BBDR model to set an MCLG rather than the traditional algebraic equation using the RfD. The SAB further found "that hypothyroxinemia (i.e., [moderately] low levels of thyroid hormone) is a more appropriate indicator of the potential adverse health effects than the more pronounced decreases in thyroid hormones associated with hypothyroidism." SAB's recommendation is based on a biological endpoint, i.e., hypothyroxinemia, that is different from the endpoint used to derive the RfD for perchlorate in 2005, i.e., the inhibition of radioactive iodide uptake into the thyroid, used to derive the RfD for perchlorate. SAB also recommended that EPA should consider sensitive populations including fetuses of hypothyroxinemic pregnant women and infants exposed to perchlorate through either water-based formula preparations or the breast milk of lactating women.

The mode of action (MOA) of perchlorate is well understood and involves perchlorate's ability to disturb thyroid homeostasis by limiting iodide uptake by the thyroid, which in turn can lead to reduced production of thyroid hormones (THs) (SAB, 2013). Developing fetuses are considered particularly sensitive to the potential effects of *in utero* exposure. From the SAB (2013):

> "Children exposed gestationally to maternal hypothyroxinemia (without hypothyroidism) show reduced levels of global and specific cognitive abilities, as well as increased rates of behavior problems including greater dysregulation in early infancy and attentional disorders in childhood (Kooistra et al., 2006; Pop et al., 2003; Pop et al., 1999; Man et al., 1991). Notably these effects are correlated with both degree (Henrichs et al., 2010; Pop et al., 1999) and duration (Pop et al., 2003) of maternal hypothyroxinemia. The Henrichs et al. (2010) study, which stratified children into severe (<5th percentile) and mild (5-10th percentile) maternal hypothyroxinemia subgroups, showed that while effects were stronger and broader in the severe subgroup, the mild subgroup still showed delayed language development, thus suggesting that any factor that lowers maternal fT4, even slightly, can affect the offspring."

Li et al. (2010), Henrichs et al. (2010), Román et al. (2013), and Min et al. (2016) provide further evidence and discussion of the effects of hypothyroxinemia *in utero*.

While Costeira et al. (2011) and Kooistra et al. (2006) evaluated neurodevelopmental effects in infants associated with iodine deficiency and low maternal fT4 (hypothyroxinemia), the association was with iodide nutrition and fT4 levels during gestation. Hence these studies do not provide information on the sensitivity of the neonate to iodine/fT4 decrements occurring after birth. Other studies of neurodevelopmental endpoints either focused on premature infants or severe thyroid hormone changes such as hypothyroidism. Thus adverse effects of moderate postnatal thyroid hormone decrements (hypothyroxinemia) on neurodevelopment in full term (born healthy), nursing or bottle-fed infants have not been studied. However, as described in a detailed review by U.S. EPA (2012),

> "A reduction in the storage of thyroid hormone and iodine, as well as rapid thyroid hormone turnover (i.e., shorter half-life) in the fetus, neonates and children makes these life stages more sensitive to thyroid disrupting agents such as perchlorate compared to adults. Furthermore, the physiological demand for thyroid hormones is far greater in the developing fetus, neonate and child compared to the adult, again increasing the vulnerability to thyroid disrupting agents."

The conclusion that infants are also particularly sensitive to perchlorate exposure is strongly supported by the SAB (2013) report (section 3.1) and introduction to Fisher et al. (2016). On the other hand, because the previous analysis based on the initial iodide uptake effect of perchlorate, quantified as RAIU inhibition, indicated that older children would be less sensitive than infants (also because they ingest less fluid/kg BW than infants) (U.S. EPA, 2009), EPA decided that the current modeling effort and analysis could be limited to infants between birth and 3 months of age, and the analysis was not extended to older children.

Within this exposure/MOA framework, a BBDR model can provide a tool for integrating exposure (e.g., different drinking water consumption scenarios), perchlorate and iodide pharmacokinetics, thyroid function description predicting serum thyroid hormone levels, and dose-response relationships for perchlorate effects on thyroid function at the different life stages. The SAB concluded that a data-driven approach with a BBDR model represents a more rigorous way to address differences in biology and exposure between adults and sensitive life stages than is possible with the default approach for deriving an MCLG.

In 2009, EPA reviewed and revised the available perchlorate life stage PBPK models (U.S. EPA, 2009; Clewell et al., 2007; Merrill et al., 2005). In discussion with the original authors, EPA improved the PBPK model by addressing the minor errors in mathematical equations and model code and inconsistencies among model code files and reflecting the biology in the models. The resulting model (U.S. EPA, 2009) predicted radioactive iodide uptake inhibition for different life stages in response to perchlorate exposures. The model did not include dietary iodine or address the effects of perchlorate under iodine sufficient or deficient conditions. The SAB reviewed this model and recommended that the EPA PBPK model for different life stages (U.S. EPA, 2009) needed to be expanded to include THs in order to predict hypothyroxinemia as a critical endpoint. Such a model integrating perchlorate, dietary iodine, and THs could then be

used to predict what levels of perchlorate could result in hypothyroxinemia for various life stages.

Shortly before the SAB provided their recommendations to EPA, Lumen et al. (2013) reported development of a TH/perchlorate BBDR model for pregnant women and fetuses. Specifically, the model predicted perturbations in serum THs during human pregnancy due to dietary iodine and perchlorate exposures. In order to implement the SAB recommendations, EPA expanded the Lumen et al. model to address formula-fed infants and lactating mothers and breast-fed infants.

The purpose of this document is to describe the perchlorate BBDR model development, including calibration, to predict the TH perturbations (i.e., free T4 and total T4 in serum) for the lactating mother, breast-fed infant, and formula-fed infant upon exposure to various perchlorate dose levels under different iodine intake conditions. In addition, this document describes the model application and preliminary dose-response predictions for the pregnant mothers and fetuses, lactating mothers and breast-fed infants and formula-fed infants. This document provides support for verification of model coding, evaluation of model calibration, and evaluation of the accuracy of model predictions for different perchlorate doses and iodide intake levels to determine if the model is adequate for perchlorate MCLG derivation.

Technical Background for Model Development

Model Introduction

The new BBDR model is intended to predict and evaluate the potential combinatory effects of iodine nutrition and exposure to perchlorate on thyroid function in the infant and lactating mother. The infant, 7 to 90 days of age, and lactating mother were selected for BBDR model development to address the most sensitive period of thyroid function maturation (SAB, 2013).

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To make the modeling efforts more efficient in responding to SAB recommendations, EPA and FDA worked collaboratively and integrated the perchlorate PBPK models developed by EPA (U.S. EPA, 2009) for the infant (formula- or breast-fed) and lactating mothers with newer models for iodine nutrition and thyroid hormone (TH) production in those life stages, developed from Lumen et al. (2013) and the thyroidal iodide binding description from Fisher et al. (2013).

Figure 1 shows the overall workflow for creation and calibration of the models for the infant and lactating mother. The existing EPA peer-reviewed model (McLanahan et al., 2014; U.S. EPA, 2009) was incorporated as the perchlorate sub-model for the formula-fed and breast-fed infant and lactating mother with the modifications as noted below in Integration of Thyroid BBDR Model with Perchlorate PBPK Model. Physiological parameters were set to the life-stage-specific human values. Iodide and TH parameters for the lactating human mother were either: 1) adapted from the human pregnancy model (Lumen et al., 2013); 2) set based on literature references to the extent possible for this life-stage; or 3) fit to available iodide and thyroid hormone data. More published data on thyroid function during infant development was available for setting model parameters than for the lactating mother, allowing 5 of 19 infant iodide and TH parameters to be identified directly from those data. In the absence of literature references, 14 of 19 parameters were adapted from the fetal model (Lumen et al., 2013), the rat lactation model (Fisher et al., 2013), or visually fit to available data (blood and urine levels of thyroid hormones and iodide). The fitting was accomplished by first calibrating the BBDR model for dietary iodine, perchlorate, and TH kinetics for the formula-fed infant, and then expanding it to include the lactating mother with exposure to the infant through breast milk.

The model code is available separately on U.S. EPA's HERO website via the following citations for the acslX implementation of the pregnancy (U.S. EPA, 2016a) and lactation (U.S. EPA, 2016c) models and R/MCSim versions of the pregnancy (U.S. EPA, 2016b) and lactation (U.S. EPA, 2016d) models. The models were originally developed in acslX (AEgis Technologies,

Huntsville, Alabama) and model simulation plots shown in this report were produced with that software (packages cited/lined above). However AEGis has discontinued sales and support of the software, so the models have been converted to also run as MCSim models within the open-source R programming language. Instructions for downloading and installing R and MCSim are included in the HERO citations, (U.S. EPA, 2016c) and (U.S. EPA, 2016b). The R/MCSim versions reproduce the corresponding acslX versions to within 1% accuracy, although the formatting of the plots produced is not identical.

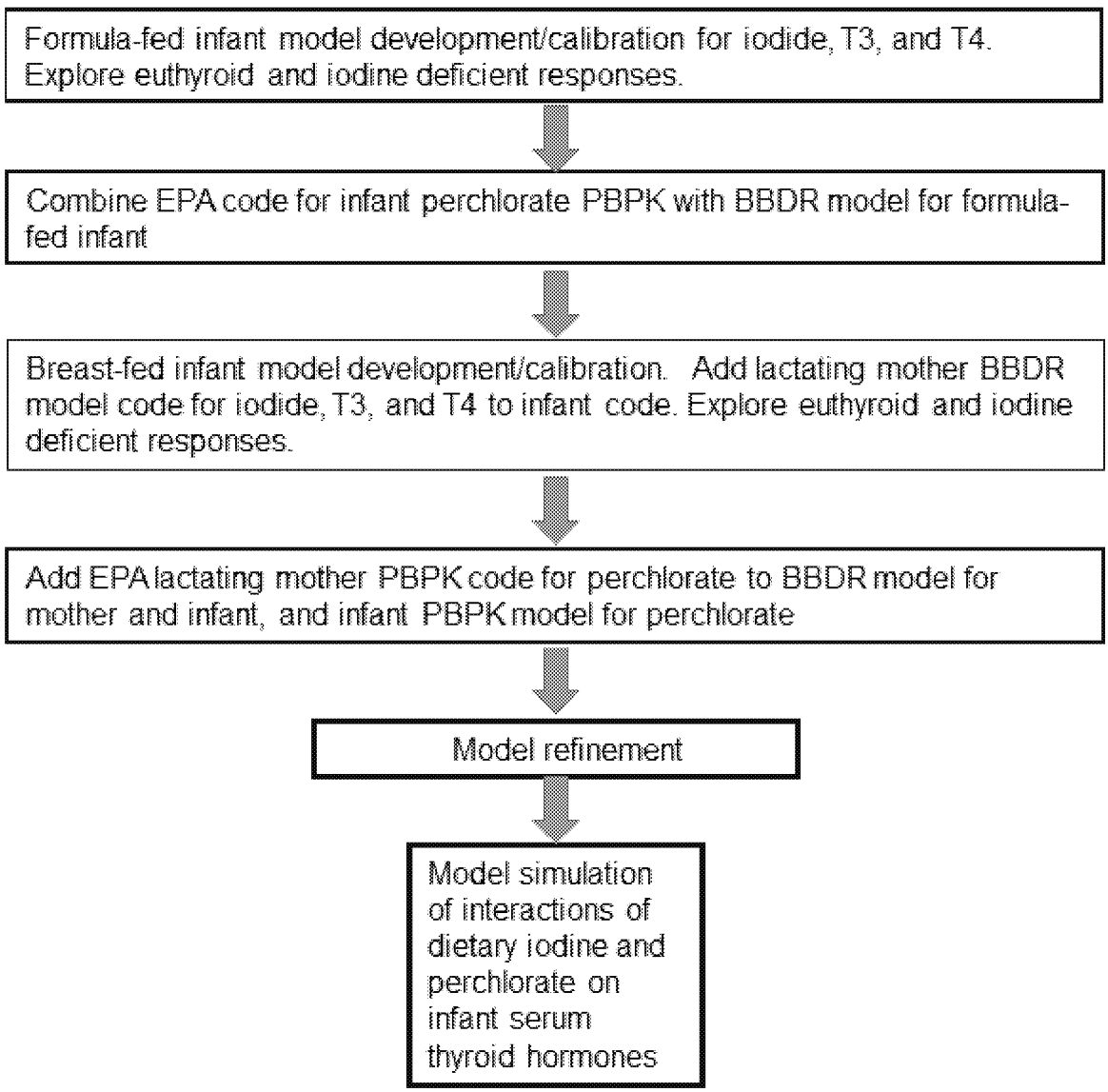

Figure 1. Work flow for model development and calibration.

Domain of Model Applicability

At the suggestion of the SAB (2013), the developed maternal lactation and infant models are limited to the range of iodine dietary concentrations within which the thyroid's function is effectively autonomous; i.e., both euthyroid conditions, where T4 is within the normal (reference[3]) range, and hypothyroxinemic conditions, where T4 is decreased below the reference range but with TSH within its reference range (Silva and Silva, 1981). Therefore, these newly developed models do not address conditions at which the hypothalamic-pituitary-thyroid axis[4] is involved with regulating thyroid hormone concentrations in the lactating mother or infant. In the range of evaluation, it is assumed that there is insufficient feedback control of the T4 concentrations on TSH levels to warrant model description. Hence, the dynamic ranges where the models are considered valid for predicting thyroid hormone disruption are, qualitatively, the reference intervals for serum T4 and fT4 concentrations in lactating women and in infants that are aged 7 to 90 days (Lem et al., 2012) (i.e., the range for these hormones that is normal for healthy individuals) and a range just below the reference range where there is a clinically significant reduction in T4 or fT4, but TSH is expected to remain within its normal range (i.e., the hypothyroxinemic range)[4]. Likewise predictions of the late-gestation pregnant mother and fetus models (Lumen et al., 2013) are only assumed valid for T4 and fT4 levels in

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[3] Here we use "reference range" to refer to the range of a hormone that is considered normal and healthy for a population. Hence some in the population will have hormone levels above or below that reference range and be identified as having an adverse clinical condition. The "population distribution" refers to the distribution of hormone levels in the entire population, including those outside of the reference range.

[4] The model does not describe TSH levels, but assumes they stay within the normal reference range. Clinically, significant elevations in TSH can be observed whether or not T4 of fT4 are in the reference range. Thus there are not specific levels of T4/fT4 that are definitively associated with TSH elevation. This may be due to inter-individual variability in hormonal control mechanisms. But mechanistically it is known that TSH production and release are stimulated by decreasing T4 levels. Hence for the purpose of this model, specific, life-stage-specific fT4 levels are identified below which TSH levels are expected to be sufficiently stimulated as to rise above their reference range and introduce significant feedback control on the thyroid, which would make model predictions invalid.

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the reference interval and expected hypothyroxinemic range for that life stage.

While the preceding paragraph discusses the qualitative distinction between euthyroid and hypothyroxinemic condition, and the corresponding range in which the model is considered valid, there are no unified quantitative definitions for reference ranges or hypothyroxinemia during pregnancy (Moleti et al., 2011), lactation (maternal), or infancy. For example, within the context of pregnancy, some authors use the 2.5th percentile of the fT4 population distribution while others use the 10th percentile and the reference ranges vary between populations and studies (Moleti et al., 2011). Elevated TSH levels, the marker of hypothyroidism, can occur in women with fT4 levels in the same range as other women with normal TSH levels (e.g., see Figure 1 in Moleti et al. (2008)), so there is also not a specific fT4 level below which this transition occurs.

Given the lack of a unified clinical definition, for the purpose of this report it is assumed that euthyroid conditions occur for fT4 levels between the 10th and 90th percentile of the entire population in a given life stage, and that hypothyroxinemia occurs for fT4 levels between the 2.5th and 10th percentile for the entire population. When fT4 falls below the 2.5th percentile for the entire population in a life stage, it is assumed that TSH levels would be sufficiently elevated to trigger hormonal feedback, invalidating the model predictions. While model simulation results are shown beyond this range for the purpose of illustration, simulation curves for these results are shown in grey (vs. black) to indicate that the confidence in those model predictions is low (e.g., **Figure 10.** Free T4 (fT4) levels as a function of perchlorate exposure and dietary iodine ingestion in the gestation-week 40 pregnant mother and fetus..

The actual range of model accuracy is uncertain, due to the fact that the data on urinary and breast milk iodide levels, and TH blood levels, are generally not correlated with specific iodine intake levels (doses). Hence model predictions of fT4 as a function of iodine dose cannot be

evaluated or calibrated against corresponding dose-response data. The pregnancy model (Lumen et al., 2013) was primarily developed and calibrated using an assumed iodine intake of 200 µg/d, the level recommended for pregnant women. Because fT4 levels are predicted to only decline slightly as iodine intake drops to 150 µg/d, and data for pregnant women (compared to urinary iodide, a measure of nutrition) show a similar pattern (Silva and Silva, 1981), pregnancy model dose-response predictions for ≥ 150 µg/d iodine intake are judged to be quite reliable. Model predictions are also shown for 100 and 75 µg/d iodine, but the results for 100 µg/d are near the bottom of the provisional reference range, and are much more uncertain. At 75 µg/d the pregnant mother is predicted to be below the provisional 2.5th population percentile, hence in the hypothyroid range, even without perchlorate exposure (Figure 10, lowest curve, shown in grey). Those results are included, even though the entire 75 µg/d curve is currently considered uncertain, in the event that it is later determined that model predictions are valid for lower levels of fT4.

The infant model was first developed using iodine levels reported for infant formulas, which have iodine concentrations in the range of 100-300 µg/L. These concentrations effectively saturate the infant thyroid's capacity to produce THs and model dose-response predictions are judged to have relatively low uncertainty for the formula-fed infant.

Iodine Ingestion & T4 Reference Ranges

The lactating mother model was primarily calibrated or tuned assuming iodine ingestion of 250 µg/d, the level recommended for breast-feeding women. However, given that the dependence on iodine nutrition is quite similar to that predicted for the pregnant mother and that only a 6-14% difference is predicted in fT4 levels at 150 vs. 250 µg/d iodine (depending on postnatal day (PND) and perchlorate exposure), the model predictions are likewise judged to be reliable for ≥ 150 µg/d iodine, but to have increasing uncertainty at 100 µg/d iodine (where maternal fT4 approaches the bottom of the reference range). At 75 µg/d iodine, maternal fT4 is predicted to

be at or below the provisional 2.5th percentile (the point where hypothyroidism is expected to begin), similar to the pregnant mother. Results for 75 µg/d iodide are included because they may be relevant if the provisional reference range is adjusted downwards, but are considered highly uncertain. Since maternal predictions provide the nutritional intake for the breast-fed infant, the same degree of uncertainty is assumed for that life stage.

The fT4 reference range reported in different studies varies depending on the size, ethnicity, and iodide nutrition of the population examined, the analytic method, and laboratory where the analyses are conducted. As a result, the mean for a given population might not correspond to the control level of fT4 predicted by the model given the recommended iodine intake (e.g., 200 µg/d during pregnancy). Therefore, a normalization procedure was used to avoid apparent discrepancies and resulting bias. If the model predicts control fT4 levels above the actual population mean, this normalization adjusts the reference range upward to the same extent, and vice-versa.

Specifically, fT4 reference ranges or population percentiles from a given study or analysis were normalized to the mean fT4 for that study/analysis (or median, if the mean is not available), resulting in a conversion to a fraction of the mean. The model-predicted results for an iodine intake of 200 µg/d and no perchlorate intake for both pregnancy and lactation was used to set the model-mean fT4 for the pregnant mother, lactating mother, and breast-fed infant. (At 200 µg/d, fT4 levels in the lactating mother were 1-6% lower than at 250 µg/d, and this choice was simpler than simulating a woman who changes dietary intake from 200 to 250 µg/d just after giving birth.) Since results for the lactating mother and breast-fed infant changed with PND, the assumed population mean for each life stage therefore varied with PND. The relative fT4 level (fraction of that mean) for the lactating mother estimated as the 10th and 2.5th population percentile did not depend on PND, due to limited data for lactation, but the infant percentiles did vary, based on a larger data set.

For the formula-fed infant, the age-specific mean was defined by model predictions for an infant ingesting formula with an average concentration of 159 µg/L iodine (average of 8 formulas reported by Pearce et al. (2004)), born to a mother who ingested 200 µg/d iodide during pregnancy; i.e., an infant with fully sufficient iodine levels and intake.

For the pregnant mother, results from 6 studies characterizing T4 reference intervals during pregnancy (Khalid et al. (2014); Wang et al. (2011); Yan et al. (2011); Gong and Hoffman (2008); Stricker et al. (2007); Panesar et al. (2001)) were combined by first normalizing the reported percentiles (2.5th, etc.) from each study to the respective mean or median. Because the resulting percentiles clearly showed a right-skewed normal distribution, the lambda-mu-sigma transformation of the normal distribution (LMS, Box and Cox (1964)) was then fit to the resulting collection of normalized percentiles (Appendix E). From the resulting distribution, the 10th and 2.5th population percentiles were then calculated to be 83.5% and 75.7% of the mean, respectively. Since the Lumen et al. (2013) model predicts an fT4 level of 14 pM for a 3rd-trimester pregnant mother consuming 200 µg/d iodide, the corresponding 10th and 2.5th fT4 percentiles were estimated to be 11.7 and 10.6 pM, respectively.

For the lactating mother, an analysis of fT4 levels in 48 lactating women from the NHANES (2007-2008 and 2009-2010) was used to directly estimate the 10th and 2.5th percentiles for fT4 as 81% and 74% of the group mean relative values close to those estimated for the pregnant mother (Appendix F). As mentioned above, mean fT4 levels are predicted to change with PND in the lactating mother, but *not* the relative variation around the mean, so these percentiles are applied to the PND-specific fT4 levels from the model (assumed to represent the mean, for a woman consuming 200 µg/d iodide) to obtain PND-specific percentiles.

For the infant, an age-dependent reference analysis was conducted by Lem et al. (2012), who fit their data using the LMS-transformed normal distribution with age-dependent parameters. While

Lem et al. (2012) did not provide specific parameters for their statistical fit, it was possible to identify a single value for the shape parameter (λ) and age-specific values for the coefficient of variation (σ), consistent with their summary statistics (**Table 1**). For example, the hypothyroxinemic fT4 range for the 1-month, breast-fed infant, for example, is assumed to be 61-66% of the predicted fT4 in a 1-month infant of a mother who ingests 200 µg/d iodide.

Table 1: Provisional Reference Intervals for fT4 in Normal-Term Infants[5]

Infant age	-2 SDs	-1 SD	Mean	+1 SD	+2 SDs	λ†	σ†	Ratio of 2.5th%** to mean	Ratio of 10th%** to mean
1 week*	12.32	15.91	21.61	31.65	52.54	-0.64	0.34	0.58	0.62
1 month*	12.81	16.12	21.12	29.28	44.33	-0.64	0.29	0.61	0.66
2 months	--	--	--	--	--	-0.64‡	0.266‡	0.64	0.68
3 months*	13.41	16.28	20.31	26.29	36.83	-0.64	0.24	0.66	0.71

† Selected to match values for +/- 1 and +/- 2 SD in the preceding columns, given the reported mean for 1 week, 1 month, and 3 months.
* Data from Lem et al. (2012); range of levels for normal, healthy infants.
‡ Average of 1- and 2-month values, used for this analysis
** Calculated using the LMS-transformed normal distribution (Box and Cox, 1964) with mean = 1, λ and σ as identified for each age.

The Fisher et al. (2013) BBDR model for iodide nutrition in the suckling rat pup and lactating dam served as the foundation for development of a model to predict serum TH concentrations in the lactating mother and breast- and formula-fed infant, with many parameters taken from the BBDR model for thyroid hormones in the 3rd trimester pregnant woman and fetus Lumen et al. (2013). The infant BBDR model was calibrated for PND 7 to 90 in the euthyroid, full-term infant (> 37 weeks of gestation). Changes in TH kinetics have been noted in the first few days after birth (Fisher and Klein, 1981; Jacobsen et al., 1977; Erenberg et al., 1974; Abuid et al., 1973). Apparent surges in TSH and T4 production result in high serum levels of TSH and T4 at birth.

[5] All of the ranges proposed here for reference intervals and hypothyroxinemia should be considered preliminary, pending review and comments from the proposed peer review.

Serum TSH and T4 concentrations decline in the newborn over the first week after birth. By PND 7 the rate of change in infant serum TSH is much slower (Lem et al., 2012), and thus model predictions beginning at PND 7 are more reliable. As stated above, TH production in these models was calibrated based on euthyroid conditions (normal iodine intake, serum TSH and TH levels).

BBDR MODEL DEVELOPMENT AND PARAMETERIZATION

Literature Data for Model Development

A PubMed search was conducted in December, 2013, using the following strategy, and the resulting papers were entered into EPA's HERO database. Other relevant papers known to the various project contributors (see list of contributors on title page), or later identified as potentially relevant where subsequently added, resulting in a set of 1471 references, listed in the available spreadsheet "ReviewHERO416.xlsx", from which data might be obtained.

Search	Query	Items found	Time
#13	**Search (#4) AND #12 Filters: Humans**	**1408**	8:47:19
#12	Search ((((#7) OR #8) OR #9) OR #10) OR #11 Filters: Humans	161800	8:40:58
#11	Search (iodine) OR iodide Filters: Humans	61544	8:40:08
#10	Search (triiodothyronine) OR triiodothyronine[MeSH Terms] Filters: Humans	14122	8:40:08
#9	Search (thyroid) OR thyroid[MeSH Terms] Filters: Humans	110777	8:40:08
#8	Search (thyroxine) OR thyroxine[MeSH Terms] Filters: Humans	26695	8:40:08
#7	Search (thyroid hormone) OR thyroid hormone[MeSH Terms] Filters: Humans	46330	8:40:08
#6	**Search (#3) AND #4 Filters: Humans**	**22**	8:22:19
#5	Search (#3) AND #4	48	8:13:57
#4	Search (#1) OR #2	92212	8:13:47
#3	Search perchlorate	4826	8:13:47
#2	Search postpartum	74486	8:13:22
#1	Search (lactat*) NOT lactate	51482	8:13:22

The literature search was performed with the objective of identifying sufficient data to calibrate the BBDR model. Additional citations were also identified from reference lists from reviewed articles, general internet searches, and the EPA HERO database. Of the papers identified in the searches, 566 were reviewed for inclusion. Criteria for inclusion included that the data be specifically identifiable for infants or breast feeding mothers from 7-90 days after birth. Also, data were only included from studies with full-term, healthy infants and healthy mothers that were not being treated for thyroid or other diseases while breast-feeding, and who were considered to have sufficient iodine in their diets. Of the papers reviewed, 403 articles were excluded because the titles indicated that these inclusion criteria were not met, or that they were unlikely to include useful information on thyroid hormone levels. Of the 163 articles that were reviewed more thoroughly, 21 articles had no useful information, 10 were review articles, and data from 70 articles was excluded for specific reasons, such as disease or not having subjects specifically identified as being in the range of 7 to 90 days postnatal. The remaining 62 articles had data that were used for calibration, validation or setting model parameters. Information for these 62 specific articles are provided in ReviewHERO416.xlsx. The review process was halted after it was judged that the objective had been met; i.e., sufficient data for model calibration had been identified.

In-vivo data were collected from studies for lactating mothers, breast-fed infants, formula-fed infants, and infants for which the nutrition source was not indicated. The in-vivo, life-stage specific studies identified with iodide, perchlorate, or thyroid hormone data for calibrating the lactation BBDR model, are summarized in **Table 2**.

Table 2: Publications providing life-stage-specific, in-vivo data for calibration and evaluation of the BBDR models

Reference	Infant diet*	Maternal			Infant	
		Plasma	Urine	Breastmilk	Plasma	Urine
Cao et al. (2010)	FF, BF				Iodide, fT4	Iodide, ClO4, fT4
Gordon et al. (2014)	FF, BF					Iodide
Kirk et al. (2013)	BF		ClO4	ClO4		
Lem et al. (2012)	NS				T3, T4, fT4	
Leung et al. (2012)	BF		Iodide, ClO4	Iodide, ClO4	fT4	Iodide, ClO4
NHANES†	BF	T3, T4, fT4	Iodide, ClO4			

*BF=Breast-fed, FF=Formula-fed, NS=Nutrition source not specified.
† http://wwwn.cdc.gov/nchs/nhanes/2007-2008/THYROD_E.htm, http://wwwn.cdc.gov/nchs/nhanes/2009-2010/THYROD_F.htm

Physiological parameters are life-stage specific, and were taken from appropriate sources (identified in the data tables) where possible. However many of the iodide/thyroid hormone and perchlorate parameters where not available for specific life-stages, and so were extrapolated from other life-stages or in-vitro data.

The parameters that were calibrated are listed with their respective definitions in **Table 3**. **Table 4** and **Table 5** list the data sources and description of the method of estimation (empirical or model fit to the data) for the infant and lactating mother, respectively. Parameter values (with units) are provided in **Table 6**-**Table 8**. The primary data sets used to calibrate the model to predict serum THs for the lactating mother and formula-fed or breast-fed infant were taken from Leung et al. (2012), NHANES (NHANES, 2007-2008, Thyroid Profile[6] and NHANES 2009-2010, Thyroid Profile[7]), Caldwell et al. (2011), Cao et al. (2010), Gordon et al. (2014), and Lem et al. (2012). Some of these authors also measured iodide in infant and mother's urine, and breast

[6] http://wwwn.cdc.gov/nchs/nhanes/2007-2008/THYROD_E.htm
[7] http://wwwn.cdc.gov/nchs/nhanes/2009-2010/THYROD_F.htm

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milk, which were used for model calibration or validation. In cases where limited or no data are available for aspects of iodine and TH homeostasis in the lactating mother, some model parameter values for the lactating mother were taken from the pregnant woman (Lumen et al., 2013)

Table 3: Symbols and definition for calibrated parameters*

PARAMETER		DEFINITION
Lactating Mother	Infant	
CTGIPOOL	CTGIPOOL_N	Euthyroid organified iodide stores
CLUX_I	CLUCX_NI	Urinary clearance of iodide; scaled table for infant
CLFECESFT3C	KFECEST3C_N	Scaled fecal clearance of T3
CLFECESFT4C	KFECEST4C_N	Scaled fecal clearance of free T4 (mother);T4 (infant)
CLMILKT3C	---	Scaled clearance of T3 to milk
CLMILKFT4C	---	Scaled clearance of free T4 to milk
CLUT3C	---	Scaled urinary clearance of T3
CLUFT4C	CLUFT4C_N	Scaled urinary clearance of free T4
FRCONVT4	FRCONVT4_N	Ratio of serum free T4 to total T4
KBINDC_I	KBINDC_NI	2nd order scaled rate constant for organification of free iodide in thyroid
KMETT3C	CLMETT3CX_N	1st order scaled T3 degradation rate constant (mother) or T3 metabolic clearance (infant)
KMETT4C	CLMETT4CX_N	1st order scaled T4 degradation rate constant (mother) or T4 metabolic clearance (infant)
KM_MKI	---	M-M constant for iodide uptake to milk
KMTHY_I	KMTHY_NI	M-M constant for iodide uptake in thyroid
KPRODT3C	VPRODT3F_N	1st order scaled rate constant for production of T3; ratio of T3:T4 production in infant
KPRODT4C	KPRODT4CX_N	1st order scaled rate constant for production of T4(mother); Scaled production rate table (infant)
PATHYC_I	PATHYC_NI	Scaled permeability- area (PA) product for iodide
PAMKC_I	--	PA product for iodide into milk
---	PBODY_NI	Rest of body tissue: plasma partition coefficient (PC) for iodide
PMK_I	---	Milk: mammary plasma PC for iodide
PRP_I	---	Richly perfused: plasma PC for iodide
PSP_I	---	Slowly perfused: plasma PC for iodide
PTHY_I	PTHY_NI	Thyroid tissue: plasma PC for iodide
T3FRAC	T3FRAC_N	Fraction of T4 metabolized to T3
VDT3C	VDT3C_N	Volume of distribution for T3
VDT4C	VDT4C_N	Volume of distribution for T4
VMAXCX_MKI	---	Scaled Vmax for NIS: iodide into milk
VMAXTHYX_I	VMAXTHYCX_NI	Vmax table for NIS: iodide into thyroid

* "X" in the parameter name indicates that it is a time-dependent table function.

1 **Table 4:** Calibration of Parameter Values for Infants

Parameter	Data Source and parameter calibration
Iodide	
CTGIPOOL_N	Fisher et al. (2016): set so that the total pool equals amount predicted in fetus at end of pregnancy (Lumen et al., 2013) for 250 µg/d maternal iodide.
CLUCX_NI	Ponchon et al. (1966): Set table values to 0.044 L/h/kg at birth, increasing to 0.055 L/h/kg at 3 weeks (n=3), and then to 0.6 L/h/kg at 6 weeks (n=4).
VMAXTHYCX_NI	Ponchon et al. (1966): Estimated based on reported clearance of 2.5 ml/min/kg (n=3, birth-21 days of age) and 1.1 ml/min/kg (n=4, 21-90 days of age), for healthy infants from Belgium, VmaxthyC_Ni = Clearance (L/h)×Km_NISi for NIS (3.15×10^4 nmol/L)
KMTHY_NI	Lumen et al. (2013):Values reported in Gluzman and Niepomniszcze (1983)
KBINDC_NI	Fitted in Fisher et al. (2013); scaled to (thyroid weight)$^{0.75}$ for growth.
PATHYC_NI	(Delange, 1998): Visually Fitted to maintain ATGIPOOLC_N.
PBODY_NI	Body/plasma value of 0.21 is intermediate between slowly perfused (0.18) and richly perfused (0.4) from Lumen et al. (2013) for pregnant mother
PTHY_NI	Lumen et al. (2013): Set to maternal values
Thyroxine (T4)	
VDT4C_N	Cottino et al. (1961): 0.31 L/kg
VPRODT4CX_N	Fisher (1996): Estimated from reported T4 utilization per day for infants in 3 age groups up to 12 months: 10 µg T4/kg/day from birth to 4 days of age, 7 µg T4/kg/day at 28 days, and 6 µg T4/kg/day from 1-12 months of age. (Data originally from Neison et al. (1993).)
CLMETT4CX_N	Model fit to visually predicted plasma T4 and fT4 from Lem et al. (2012).
T3FRAC_N	Qualitative pattern based on ratio of T3 to T4 serum concentration profiles over 30 days in 18 infants from the United States in Chopra et al. (1975), Lumen et al. (2013) estimates in the term fetus, and Richard et al. (1998) reported ontogeny of iodothyronine deiodinases. Fit to T3 data from Lem et al. (2012), Eimlinger et al. (2001), Franklin et al. (1985), and Williams et al. (2004).
FRCONVT4_N	Model fit to visually predict Lem et al. (2012) data with Gemelli et al. (1990) (n = 11 newborns) (ratio of fT4/T4 = 9.6×10^{-4}) as initial estimate.
CLUFT4C_N	Based on Cao et al. (2010) and Sillén (2001), infant urinary excretion estimated to be < 0.1% of total synthesis and ingestion; hence this rate was set to zero.
CLFECEST4C_N	Set value based on adult humans- 15% of administered ^{131}I-T4 was found in feces from Oddie et al. (1964), and targeted 7.8% of T4 formed per day excreted based on estimates for maturation from Saghir et al. (2012).
Triiodothyronine (T3)	
VDT3C_N	Lumen et al. (2013): Set to fetal value, which was fit to data of Hume et al. (2004)
VPRODT3F_N	Lumen et al. (2013): Used assumption from Lumen model that thyroidal production of T3 vs. T4 is similar to the adult value of 1/11 of T4 production rate, based on Thorpe-Beeston et al. (1992) and Hume et al. (2004)
CLMETT3CX_N	Model fit to describe visually T3 time course data from Lem et al. (2012).
CLFECEST3C_N	Set value based on 10.3% of administered ^{131}I-T3 was found in feces of adults from the United States (n=18) from Fisher and Oddie (1964b) and assumed that 4.5% of T3 formed per day is excreted in feces based on immature status reported by Saghir et al. (2012).

2

Table 5: Calibration of Parameter Values for Lactating Mother*

Parameter	Data Source and Calibration
Iodide	
CTGIPOOL	Lumen et al. (2013): Set to target 15 mg for thyroidal iodide stores
CLUX_I	Fisher et al. (2016): set to vary over time, model fit to central tendency for Leung et al. (2012) data; values initially from Aboul-Khair et al. (1964).
VMAXCX_MKI	Leung et al. (2012): Fit to iodide concentrations in breast milk
VMAXTHYX_I	Aboul-Khair et al. (1964) reported thyroidal clearance of systemic iodide equal to 2.25, 1.48, and 1.22 L/h at 2, 6 and 12 weeks. Converted to Vmax (clearance (L/h)×Km (nmol/L)). Last time-point value coded as occurring at 90 days rather than 84 day. Value at birth similar to pregnant woman (Lumen et al., 2013).
KBINDC_I	Dunn and Delange (2005): Fit value to predict thyroid stores near the maximal concentration for euthyroid adults (10-20 mg/thyroid)
KM_MKI	Lumen et al. (2013): Set equal to the KMNIS_I based on commonality of NIS molecule; binding affinity not expected to vary with site of expression.
KMTHY_I	Set equal to pregnancy values that were fit in Lumen et al. (2013) based on commonality of NIS molecule; binding affinity not expected to vary with site of expression.
PAMKC_I	Fitted parameter to Leung et al. (2012)
PATHYC_I	Lumen et al. (2013): Chosen to be consistent with expected thyroidal iodide concentration stores in adults; i.e., very low levels of free iodide. (No data are available for pregnant or lactating women.)
PMK_I, PRP_I, PSP_I, PTHY_I	Lumen et al. (2013); McLanahan et al. (2014): Set to values used previously in pregnancy model
Thyroxine (T4)	
CLMILKFT4C	van Wassenaer et al. (2002): Model fit to predict < 1 µg/day of T4 ingested in milk (0.6 µg/day)
CLFECESFT4C	Model fit to target 10% of T4 excreted in feces for euthyroid lactating woman. Oddie et al. (1964) reported that in adults 15% of administered ^{131}I-T4 was found in feces.
CLUFT4C	Habermann et al. (1976): Model fit to target of less than 2 µg/day of T4 in urine, 1.6 µg/day euthyroid lactating woman. Habermann et al. (1976) reported excretion of 1.4 µg/day of T4 in urine over 24 h in 20 subjects
FRCONVT4	Fisher et al. (2016): Slightly increased from pregnancy value (0.9×10^{-4}; Lumen et al. (2013)) to 1.0×10^{-4}
KPRODT4C	Decrease in model-fit value from Lumen et al. (2013) for pregnant women (2.45×10^{-6} $h^{-1}kg^{-0.75}$) to 1.1×10^{-6} for lactating woman
KMETT4C	Reduced to 1.4×10^{-4} $h^{-1}kg^{-0.75}$ to fit data for lactating woman vs. 1.9×10^{-4} used for pregnant women Lumen et al. (2013)
VDT4C	Slight decrease in model-fit value from Lumen et al. (2013) for pregnant women (8.4% BW for lactating woman vs. 12.1% for pregnant women)

* "X" in the parameter name indicates that it is a time-dependent table function.

Table 5 (cont.): Calibration of Parameter Values for Lactating Mother

Parameter	Data Source and Calibration
Triiodothyronine (T3)	
CLFECEST3C	Fisher and Oddie (1964b): Model fit to target < 8% of T3 excreted in feces, Fisher and Oddie (1964b) reported 10.3% of administered ^{131}I-T3 was found in feces of adults from the U.S. (n=18)
CLUT3C	Model fit to target < 3 µg/d excreted in urine, lactating woman was 2.3 µg/d; Habermann et al. (1976) reported excretion of 1.7 µg/day of T3 in urine over 24 h in 20 adults.
CLMILKT3C	Fit to predict < 1 µg/day of T4 ingested in milk (0.6 µg/day reported in van Wassenaer et al. (2002))
T3FRAC	Lumen et al. (2013): Set to pregnancy value
KMETT3C	Lumen et al. (2013): Set to upper limit of pregnancy value
KPRODT3C	Increased from 1/11 of T4 production to reflect thyroidal metabolic shift which occurs when iodine intake is low
VDT3C	Slight increase from pregnant woman fitted value (0.46 L/kg) from Lumen et al. (2013) to 0.47 L/kg for lactating woman

Infant temporal reference intervals for serum thyroid hormones reported over the course of 90 days of age (Lem et al., 2012; Elmlinger et al., 2001) were used to set the distribution of serum thyroid hormone concentrations for normal euthyroid thyroid function in infants, regardless of the method of feeding. Reference intervals (percentiles) for serum THs were not found in the peer-reviewed literature for lactating women; however, individual data at time-points from PND 1 to 90 were available from the NHANES studies and were used as guidance for model development. These NHANES data were used to calibrate the model for the lactating mother with lactation-day-specific information. While other sources report thyroid hormone data during the postpartum period, they were deemed less useful for a variety of reasons, including failure to specifically identify results for lactating vs. non-breast-feeding mothers, failure to specify the postnatal age (day) on which the data were collected, or only providing data for a portion of the 90-day period for which data was needed (e.g., Ardawi et al. (2002) only provides data for a single time-point). Hence these other data sets were not used.

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1 Model Structure

2 The iodide and perchlorate models assume that iodide does not distribute into red blood cells
3 (RBCs) and distribution of perchlorate into RBCs is diffusion-limited. Therefore transport of
4 iodide between tissues occurs only in the plasma, and while perchlorate is carried in both RBCs
5 and plasma, uptake of perchlorate into tissues is only assumed to occur from the plasma.

6 The infant iodide sub-model is a physiologically based pharmacokinetic model (**Figure 2**) with
7 the same compartments as the iodide sub-model for the fetus (Lumen et al., 2013): including
8 plasma, rest of body, thyroid plasma, and thyroid gland tissue. The model structure is both
9 simpler and distinct from the previous radiolabeled PBPK models (McLanahan et al., 2014; U.S.
10 EPA, 2009). Differences include the reduction of the thyroid gland to two compartments (plasma
11 and tissue instead of plasma, follicle, and lumen), input of ingested iodine directly into the
12 plasma (GI tract not specified for iodide), and lumping of adipose and skin with tissues in the
13 rest-of-body compartment.

14 The model compartments for the mother (**Figure 3**) consist of plasma, slowly perfused, richly
15 perfused, thyroid gland and thyroid plasma, and mammary gland plasma and milk. Active iodide
16 uptake is modeled between the thyroid plasma and thyroid tissue and between the mammary
17 plasma and milk. Two structural changes made for this model compared with the previous
18 kinetic models for radiolabeled iodide are: 1) a reduction in the thyroid subcompartments
19 (plasma and tissue instead of plasma, follicle, and lumen); and 2) direct transfer of iodide from
20 the mammary plasma to the milk (with mammary gland tissue not specifically included). Similar
21 to the infant, the dietary intake of iodine is treated as infusion directly into the plasma (GI tract
22 not specifically described), and the adipose and skin compartments are lumped into the slowly
23 perfused compartment.

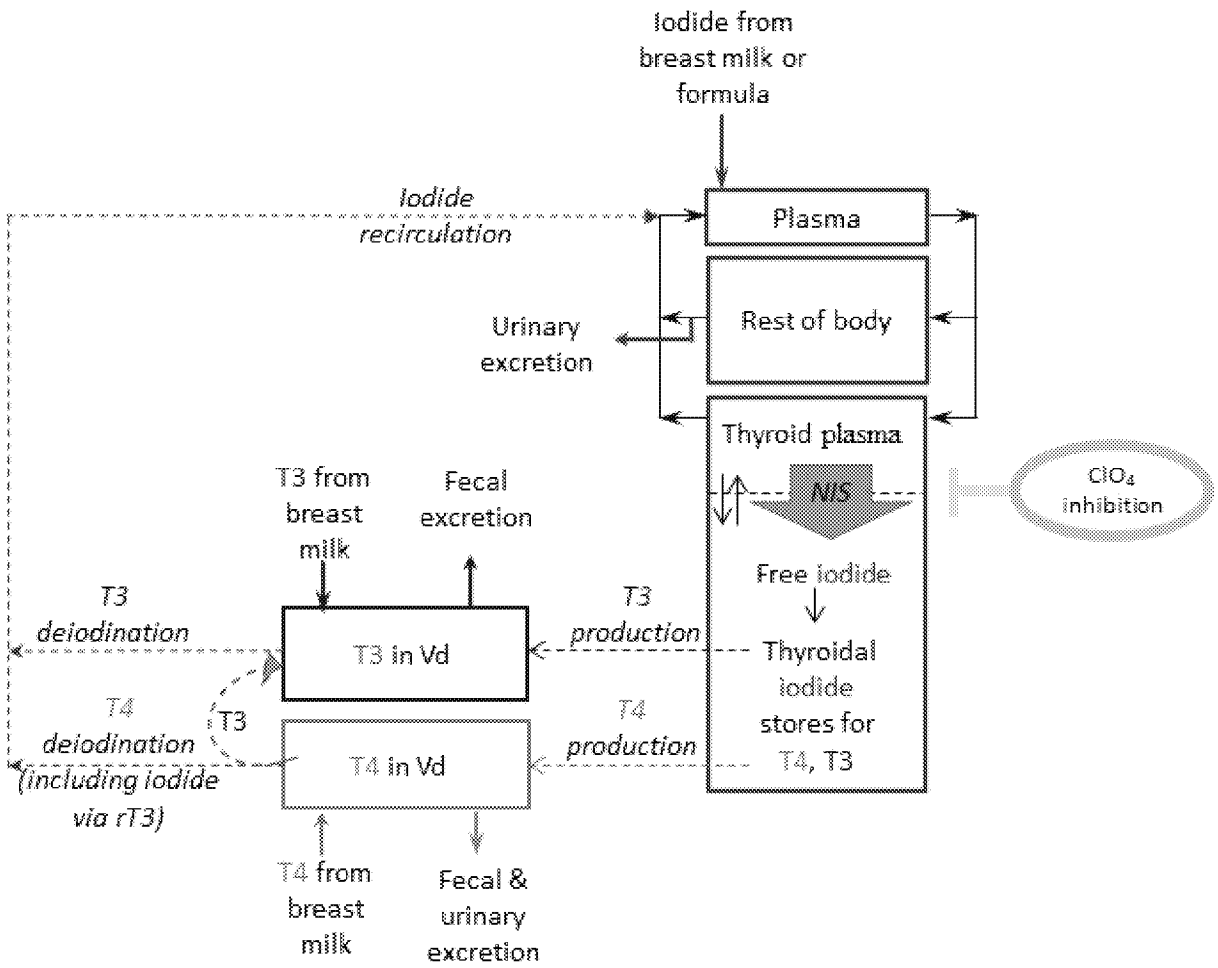

Figure 2. Infant iodide/thyroid hormone sub-model.

In mammary and thyroid, free diffusion between plasma and tissue is indicated by ↓↑ and active uptake by NIS is indicated by ⬇. Vd denotes volume of distribution

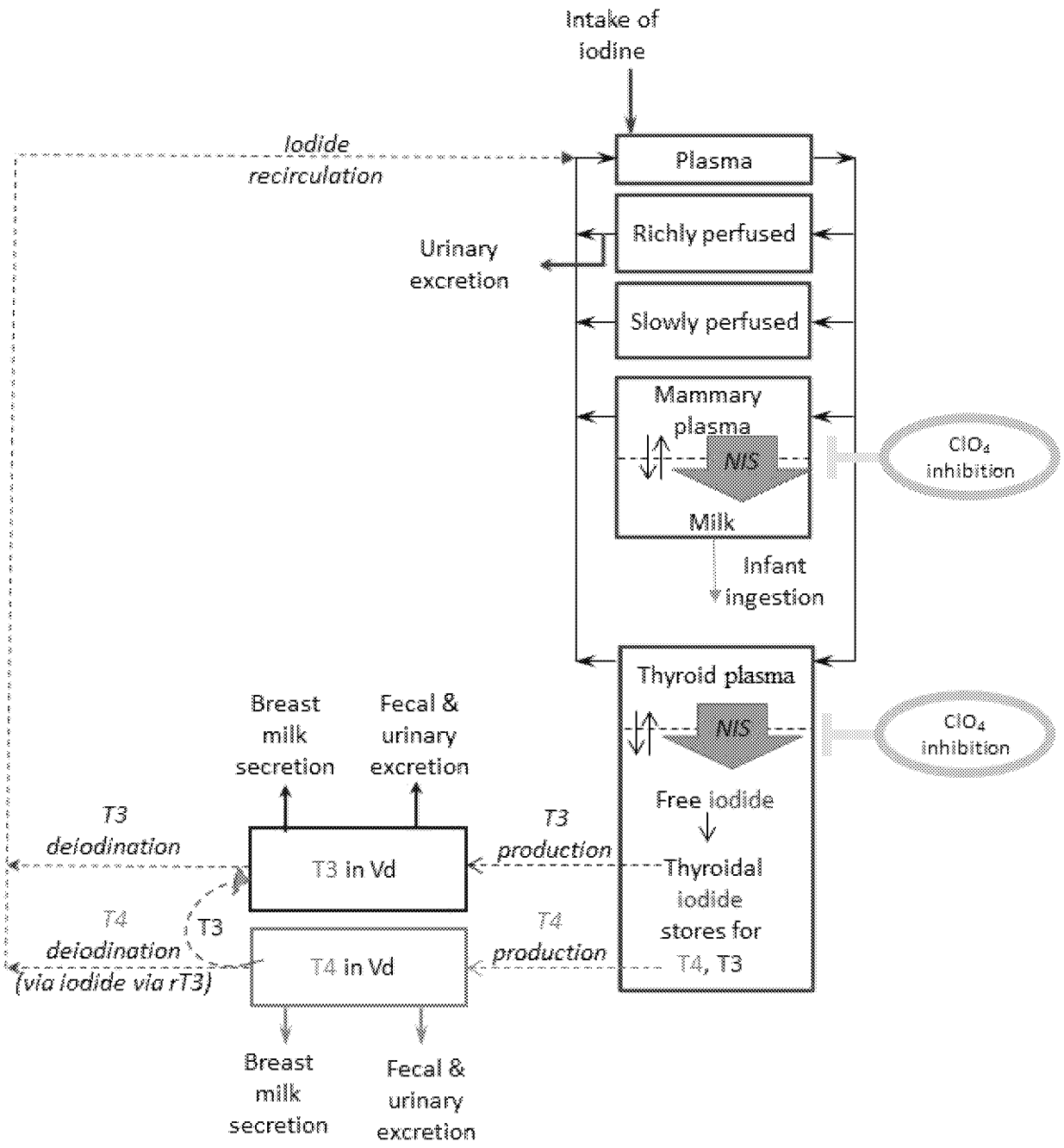

Figure 3. Maternal iodide/thyroid hormone sub-model.

In mammary and thyroid, free diffusion between plasma and tissue is indicated by ↓↑ and active uptake by NIS is indicated by ⬇. Vd denotes volume of distribution

Physiological Model Parameters

Values and units for physiological parameters for the infant and lactating mother in comparison with non-pregnant and pregnant women, when available, are listed in **Table 6**. A description of the literature sources for the values is given below.

Infant

Edginton et al. (2006) reported reference ranges from published literature for cardiac output (L/min) versus age in children, which was used to set up a table function to linearly increase the cardiac output over the 3 month simulation period. Whole blood cardiac output was converted to plasma flow based on the ratio of red blood cell volume to plasma volume (i.e., the hematocrit) in the infant (Brines et al., 1941). Plasma volume was calculated with information from Brines et al. (1941), who estimated plasma volume in 4 infants, age 7 to 21 days old, and the red blood cell volume was set assuming the adult hematocrit value from Altman and Dittmer (1971). The current model used set values for the hematocrit, while the U.S. EPA (2009) model used a time-dependent function. The plasma flow rate to the thyroid gland was set to the adult value, 1.6% of cardiac output, based on sources used for the Lumen et al. (2013) model (i.e., Brown et al. (1997), Kiserud et al. (2006)).

Infant body weight (BW_N) growth was expressed by an algebraic equation for infants aged less than 11 months (Clewell et al. (2007); originally from Clewell et al. (2004)). Thyroid gland weight changes in the infant over the course of 90 days were taken from Clewell et al. (2007) and verified with data from Ogiu et al. (1997), while thyroid plasma volume (fraction of total thyroid mass) was set using the thyroid blood fraction used for the pregnant mother and fetus in Lumen et al. (2013), which obtained the estimate from Brown et al. (1986), and circulating blood hematocrit. Sillén (2001) reported urine production of 5 ml/h/kg BW for the newborn based on 4-

hour experimental periods. To better predict urinary excretion of iodide, urine production was assumed to be constant at 5 ml/h/kg from birth to 90 days of age.

Lactating Mother

The compartment volumes and plasma flow rates were calculated with equations from McLanahan et al. (2014). The plasma flow rate to the mammary gland was set to a constant rate from birth to postpartum day 90, based on data reported by Geddes et al. (2012). The previous U.S. EPA (2009); McLanahan et al., 2014 model used a flow rate relative to the size of the mammary gland, which was modeled as increasing from birth to 2 weeks postpartum and then held constant up to 6 months postpartum. The excretion rate for breast milk was described with a non-linear function fitted to milk yield data (Arcus-Arth et al., 2005; Casey et al., 1986; Hofvander et al., 1982), and the residual volume of milk was set to mean value reported in Kent et al. (2006) and Daly et al. (1992).

Iodide and Thyroid Hormone Kinetics

Infant Iodide

Infant-specific, iodide model parameters (**Table 7**) included urinary excretion of iodide, active uptake of iodide into the thyroid gland, organification of iodide, storage in the thyroid gland, and tissue/plasma partition coefficient values for the thyroid gland and body. Values include those obtained from literature reference and calibrated values (**Table 4**).

Urinary excretion of iodide has been reported to increase over the first 90 days in the infant (Ponchon et al., 1966) with an excretion rate of 0.74 ml/h/kg from birth to 13 days (n=3), followed by an increase to 1.1 ml/h/kg over the next few months (n=4). The maximal velocity for thyroidal uptake of iodide (VMAXTHYCX_NI) was derived from thyroidal clearance calculations (Ponchon et al., 1966). Ponchon et al. (1966) reported that thyroidal clearance of iodide

decreased with age (2.5 ml/min-kg for the first 3 weeks of life, then 1.1 ml/min-kg for up to 6 months of age), therefore, VMAXTHYCX_NI decreased with postnatal age in the model. The Michaelis-Menten constant for the infant thyroid (KMTHY_NI) was set to the maternal value. Passive, bi-directional diffusion of iodide into and out of the infant thyroid gland was described with a fitted permeability coefficient value of 0.01 L/h/kg $BW^{0.75}$ based on euthyroid, organified iodide stores. Once iodide was translocated into the thyroid gland the organification of iodide was described with a second order constant, which increases with age, fitted to provide thyroidal iodide stores near the maximal value for euthyroid infants. The maximal organified iodide stored in the thyroid gland of the newborn (n=13, average 292 µg iodide) was expressed as a concentration, 1716.7 nmol iodide/g thyroid weight, based on 13 healthy newborns from Canada (Delange, 1998). The thyroid/plasma partition coefficient (0.15) was set equal to that used by Clewell et al. (2007) and Merrill et al. (2005), as was done by Lumen et al. (2013), and the rest-of-body: plasma partition coefficient (0.21) to an intermediary value between maternal slowly (0.18) and richly perfused (0.4) values.

Intake of iodide from formula was described as the concentration of iodide in the formula times the rate of formula ingested. Iodide concentrations in formula were taken from measurements reported in Pearce et al. (2004) for 8 commercial formulas: 17.9, 22.2, 17.9, 17.3, 56.8, 16.2, 24.2 and 15.9 µg per 5 oz. The average of these iodide concentrations is 159 µg/L of formula, with a range of 108-384 µg/L. The 50th percentile volume of formula ingested daily decreased with age from 0.199 L/day/kg for the first month of life (n=56, days 8-30), to 0.189 (n=62) and 0.174 (n=69) L/day/kg for the second and third months of life (Fomon et al., 1964).

Intake of iodide from breast milk or formula was described as the breast milk or formula concentration times the ingestion rate, which was described with a non-linear function fitted to milk ingestion data. (The model code also allows use of a table function going through the same data.) The data were calculated from mean daily milk ingestion rates reported by Casey et al.

(1986) over a 5-day period. Nursing rates for birth, 12 h, and days 1, 2, 3, 4, and 5 were 13, 13, 13, 40, 98, 140 and 155 g milk per kg BW of infant per day (g/kg/d). For lactation months 1 and 2, Hofvander et al. (1982) reported milk ingestion rates of 154 and 148 g/kg/d and, at lactation month 3, Dewey et al. (1991) reported 130 g/kg/d. Arcus-Arth et al. (2005) also summarized milk ingestion data, including the sources identified here.

Infant Thyroid Hormones

The model structures for T4 and triiodothyronine (T3) are one compartment volumes of distribution (**Figure 2**) similar to Lumen et al. (2013) and for the lactating rat and breast-fed pup (Fisher et al., 2013) with losses from metabolism by deiodination or conjugation, and excretion into breast milk, urine (T4 only), and feces. T4 is formed in the thyroid gland and secreted into systemic circulation, while T3 is primarily formed from deiodination of T4 in the body and to a much lesser degree from formation in the thyroid gland.

The thyroid endocrine system in the body of an infant is highly active compared to an adult, with high throughput or turnover in the infant thyroid gland (Fisher, 1996). Consequently, several model parameter values associated with T4 were age-dependent in the infant, where data were available. **Table 4** lists data sources that were used for fitting or setting the value of the parameters in the model. **Table 8** contains the thyroid hormone model parameters for infants compared with the lactating and pregnant mother.

Maximum T4 secretion (production) from the thyroid gland from birth to day 4 was set to 0.535 nmol/h/kg BW; it then decreased linearly from 0.535 nmol/h/kg at day 4 to 0.375 nmol/h/kg at day 7, linearly from there to 0.322 nmol/h/kg at day 28, and then remained constant at 0.322 nmol/h/kg for up to 6 months based on the data reviewed by Fisher (1996).

For the current model, whole body estimates of deiodination rates of T4 were estimated by visually fitting the model-predicted plasma T4 concentrations with literature data for T4 and fT4

plasma concentrations (Lem et al. (2012) resulting in age-dependent rates of 3.5 ml/h/kg at birth, declining to 2.3 ml/h/kg at 10 days, then to 2.0 ml/h/kg at 30 days, where it stayed constant to 90 days of age.

Conversion of T4 to reverse T3 (rT3), an inactive form of T3, was described as an age-dependent function. Deiodinase III is present at birth at relatively high levels and decreases with age (Richard et al., 1998; Chopra et al., 1975). Chopra et al. (1975) collected serum profiles in 18 infants for rT3. From birth to 5 days of age, 50% of the T4 was assumed to be converted to rT3 (1–T3FRAC_N), declining to 45% on day 6, 40% on day 7, 30% on day 30, and ultimately to 20% at 6 months. This time-dependence was coded into the model by defining the parameter T3FRAC_N (the fraction of T4 metabolized to T3), the remainder going to the inactive form of T3 (rT3) with a time-dependent table function (linear interpolation between defined time-points). rT3 was not tracked in the model but was assumed to be completely deiodinated, releasing three iodide atoms.

A small fraction of T4 was assumed to be secreted into feces (up to 8% in the infant) based on adult experimental findings (Oddie et al., 1966). These authors found 15% of administered ^{131}I-thyroxine excreted in feces of adults. For the infant, the maturation of uridine 5'-diphospho-glucuronosyltransferase (UGT) enzymes (Saghir et al., 2012) responsible for conjugation of T4 was assumed to be nearly 50% (or less) of adult values (e.g., for UGT1A1). No data were found for the infant to verify this assumption.

For urinary excretion of T4 in the infant, information was derived from adults. Habermann et al. (1978) collected 24-h urine samples from 20 adults and found trace amounts of T4 in urine (1-2 µg), which is nearly 1% of the T4 formed each day for the adult. For the infant, 0.3% or less of T4 was assumed to be excreted in urine. No data were found for the infant to verify this assumption. The free fraction of total T4 in serum (fT4) was set to an initial constant value of

9.6×10^{-4} (i.e., [fT4] = $9.6\times10^{-4}\times$ [T4], based on Gemelli et al. (1990), n=21), but was then fitted to measured and free T4 in serum (Lem et al., 2012); FRCONVT4_N = 1.7×10^{-4}.

The volume of distribution of T4 in the infant was set to 0.31 L/kg based on an analysis of 7 children up to 12 months of age, who were injected with radiolabeled thyroxine (Cottino et al., 1961). The volume of distribution for T3 in the infant was set equal to the fetus (Lumen et al., 2013) and the production rate was set equal to 1/11 of the infant T4 production rate, similar to the T3:T4 production ratio of 1/11.8 used for the fetus by Lumen et al. (2013).

As with T4, T3 metabolic rates were estimated visually and set to age-dependent parameter values of 0.12 L/h/kg from birth to 10 days, then 0.09 L/h/kg for 30-90 days of age, which allowed the model to predict the mean serum T3 values reported by Lem et al. (2012). Fisher and Oddie (1963) reported that fecal excretion of radiolabeled T3 was about 10% in adults. For the infant the model value targeted 4.5% of the adult T3 excretion rate because of the immature status for conjugation (Saghir et al., 2012). Urinary excretion of T3 was not included in the model.

Lactating Mother Iodide

Several iodide-specific model parameters were obtained from the literature for the lactating mother. **Table 5** lists data sources that were used for fitting or setting the value of the parameters for the iodide sub-model for the lactating mother. Values of the kinetic parameters for the iodide sub-model, compared with the pregnant female and infant, are listed in **Table 7**.

Urinary excretion of iodide was found to decrease over time in 7 lactating women (Aboul-Khair et al., 1964) for postpartum weeks 2, 6, and 12. These urinary iodide excretion clearance values (L/h) and the estimated urinary iodide excretion clearance value from the 3rd trimester mother at birth (Lumen et al., 2013) were used for the lactating mother model for iodide.

In a similar trend, the calculated thyroidal uptake of iodide declined over 12 weeks of lactation (Aboul-Khair et al., 1964). Thyroidal clearance of iodide was converted to a maximum velocity term (VMAXTHYX_I) for NIS mediated uptake of thyroidal iodide by multiplying the clearance values by the affinity constant, KMTHY_I; it was not scaled by body weight but was estimated to decrease with time. At birth, the lactating mother maximal velocity was set equal to the 3rd trimester pregnant mother. The maximal velocity for NIS mediated uptake of iodide into the breast milk was fit visually to measured iodide levels in breast milk (Leung et al., 2012); it was scaled to body weight and assumed to be constant during the 3 months postpartum.

Partition coefficients for the slowly and richly perfused tissue groups, and the thyroid gland were set to previously used values (McLanahan et al., 2014; Lumen et al., 2013).

While generally assumed to represent diffusion-limited transport, in reality the permeability-area (PA) coefficients are empirical terms which allow for the probable movement of the charged anions across membranes by transporters other than NIS, including ion gates, and through gaps in cell membranes. Because there are no measures of free iodide in the human thyroid, the value of the thyroidal PA coefficient is not validated in any strict sense, but is selected to allow the system of model equations to work without numerical errors. The diffusion-limited term is included in the model to make it more biologically realistic, but because model results are not sensitive to the value of the thyroidal PA, the exact choice of its value has a negligible impact on model predictions.

Iodide Transfer via Breastmilk

The mammary gland is capable of concentrating iodide in breast milk via the sodium iodide symporter (NIS), a membrane bound transport protein that is under the control of prolactin and other hormones (Micali et al., 2014; Semba and Delange, 2001). There are likely several mechanisms involved, including other transporters such as pendrin, but data by which their

contribution to iodide transport could be quantified (vs. that of NIS) is lacking. Therefore the model structure only includes NIS-mediated transport between blood plasma and breast milk.

Semba and Delange (2001) and Dorea (2002) reviewed reports for low breast milk concentrations of iodide in regions of the world with goiter (13-18 µg/L) compared to those with breast milk iodide concentrations exceeding 100 µg/L. Breast milk measurements of iodide in the United States are limited. Leung et al. (2012) compared three reports of mean or median iodide measurements in breast milk for Boston, MA (155 µg/L,178 µg/L and 45.5 µg/L) with Texas (34 to 52 µg/L). The range of iodide concentrations in spot breast milk samples can exceed two orders of magnitude, so factors other than variation in dietary intake must influence this range.

The volume of mammary plasma (VMB) was set to 27.6% of the volume of the whole mammary gland. Reported breast volumes range from 0.2 to over 1.0 L (Xu et al., 2014; Ramsay et al., 2005; Vandeweyer and Hertens, 2002). A mean value of 1.74% of pre-pregnancy body weight was used for the total mammary tissue (VMAMC) (ICRP, 1975), which yields a volume of 0.59 L. The volume of breast milk (VMK) was based on reports of milk yield and was considered to be a constant volume (0.369 L). This simplifying assumption is suitable because the synthesis rate of milk (11-58 ml/hr/breast, n=6, Daly et al. (1992)) rapidly replaces the volume of milk suckled by the infant (29 to 33 ml/hr during the first months of life). The residual amount of breast milk remaining in the breast immediately after nursing (mean value of 109 g with high variability; Dewey et al. (1991)) helps ensure an ample supply of milk each nursing. This residual breast milk (109 ml per breast) plus average feeding volume of 84 ml for productive breast and 67 ml for less productive breast (Kent et al., 2006) equals a total theoretical milk volume of 0.369 L or 0.1845 L per breast. The mammary glands will hold up to 193 ml of breast milk for a productive breast and 164 ml for a less productive breast (Kent et al., 2006).

Iodide was assumed to be transported unidirectionally by the NIS protein into breast milk and to a limited extent by passive diffusion. The NIS in the mammary gland on the basolateral membrane of alveolar cell mediates transfer of iodide from the plasma into milk. NIS expression is stimulated by the hormones oxytocin, prolactin, and estrogens (Micali et al., 2014). The maximal velocity for NIS mediated uptake of iodide into the breast milk (VMAXCX_MKI) was fit to predict both measured iodide levels in breastmilk (Pearce, 2007) and infant urinary iodide (Leung et al., 2012). No in vitro or in vivo data were found for NIS protein expression in the mammary gland as a function of postpartum days.

The mammary blood: milk PA coefficient values were adjusted in the course of describing breast milk iodide concentration and thyroid total iodine storage data for iodine intake from about 50-100 µg iodide/d to about 400 µg iodide/d, to maintain sufficient thyroidal iodine stores. The permeability-area coefficient for thyroid blood: tissue bidirectional transport (PATHYC_I) was set to 10^{-5} L/h/kg$^{0.75}$ compared to 10^{-4} L/h/kg$^{0.75}$ used by Clewell et al. (2007), Lumen et al. (2013) and McLanahan et al. (2014), because the current model was based on the lactating rat model (Fisher et al., 2013). The permeability-area coefficient value for the milk: mammary plasma bidirectional transport (PAMKC_I) was set to 0.001 L/h/kg$^{0.75}$ to better predict transfer to the infant, which determines the infant urinary iodide concentration, compared to 0.02 L/h/kg$^{0.75}$ used for both plasma: mammary tissue and mammary tissue: milk by Clewell et al. (2007) and McLanahan et al. (2014).

Lactating Mother Thyroid Hormones

A list of the kinetic parameters for the thyroid hormone sub-model and their values compared with the pregnant female and infant is found in **Table 8**. **Table 5** list data sources that were used for fitting or setting the value of the parameters for the thyroid hormone sub-model for the lactating mother.

As shown in Table 5, the primary data set used to calibrate the model to predict serum thyroid hormones for the lactating mother was taken from NHANES. Measurements of iodide in infant and mother's urine, and breast milk, were also used for model calibration or validation. For comparison, Soldin et al. (2007) reported postpartum fT4 levels for a number of other countries, all with values between 13 and 14 pM. In contrast, the mean levels for U.S. lactating women, with the combined NHANES 2007-2008 and 2009-2010 data sets, was 9.3 pM (SE = 0.3 pM, *n* = 39). It is not clear if these differences are due to differences in analytical methodology or represent true population differences. Iodine levels in the diet vary with geographic region and there may also be ethnic differences in hormone levels. Whether the differences are analytical or demographic, careful interpretation is needed when comparing predictions with the model described here, having been calibrated to the NHANES data, to reported thyroid hormone values from other countries.

At parturition, the lactating mother's Vmax for iodide uptake in the thyroid (VMAXTHY_I) was set equal to the value used for the 3rd trimester pregnant mother Lumen et al. (2013). Organification, the process of formation of precursor thyroid hormones in the thyroid gland, was a second order process with a rate constant (KBINDC_I) used in a similar manner as the adult rat (Mclanahan et al., 2008) and lactating rat (Fisher et al., 2013). KBINDC_I was fitted to predict data on near maximal concentrations of thyroidal iodide stores (CTGIPOOL), which were reported by Dunn Dunn and Delange (2005). Bidirectional diffusion of iodide between the thyroid gland and plasma (PATHYC_I) was assumed to be minimal to predict very low concentrations of free thyroidal iodide in the thyroid gland.

Several other model parameters for the thyroid hormones in the lactating mother were derived from Lumen et al. (2013) for the pregnant woman, which were calibrated from baseline values obtained from adult studies in men and non-pregnant women. For T4, the volume of distribution and secretion (production) rate from the thyroid gland were adjusted slightly from those reported

in Lumen et al. (2013) (**Table 6**); VDT4C = 0.084 L/kg in the lactating mother, VDFT4_MI = 0.121 L/kg in the pregnant mother (Lumen et al., 2013); KPRODT4C = 1.1×10^{-6} $h^{-1}kg^{-0.75}$ in lactating mother and KPRODT4F_MI = 2.45×10^{-6} $h^{-1}kg^{-0.75}$ in the pregnant mother (Table 8). Fifty percent of deiodinated T4 was assumed to be converted to rT3 and 50% to T3, similar to Lumen et al. (2013). In the model rT3 was not tracked, but was assumed to be completely deiodinated, releasing three iodide atoms. The iodide atoms were immediately available for systemic circulation. The clearance rate constant for metabolism or deiodination of T4 was set equal to the value for the pregnant woman. The concentration of fT4 was set to a small fraction of total T4 (Lumen et al., 2013). Fecal and urinary elimination clearance constants for T4 were fitted to predict less than 2 µg/d of T4 in urine (Habermann et al., 1976) and for feces, 10% excretion as hepatic conjugates (Oddie et al., 1964). Excretion of T4 in milk was described with a fitted clearance term to predict transport of less than 1 µg/d of T4 (van Wassenaer et al., 2002).

For T3 the volume of distribution for the lactating woman was increased from the pregnant woman (Lumen et al., 2013), while the production rate of T3 and its deiodination rate were set to values reported in Lumen et al. (2013). Fecal clearance of T3 was estimated to target elimination of less than 8% of the T3 formed (Fisher and Oddie, 1964a). Fisher and Oddie (1964a) reported 10% of radiolabeled T3 was secreted in feces of adults. Urinary clearance of T3 was estimated to target excretion of less than 3 µg/d (Habermann et al., 1976). For euthyroid mothers with healthy, full-term infants, very limited data was found on excretion of T3 into milk. Specifically, Sato and Suzuki (1979) measured 0.14 ± 0.11 and 0.07 ± 0.07 µg T3/L breast milk for postnatal ages of 0-1 and 1-2 months, respectively. (Other sources report T3 in breast milk, but for mothers with thyroid disease (Mizuta et al., 1983) or pre-term infants (van Wassenaer et al., 2002).) Based on these data, the rate constant for T3 clearance to milk was estimated to target less than 0.1 µg secreted per day.

Integration of Thyroid BBDR Model with Perchlorate PBPK Model

For perchlorate, the previously evaluated and modified models (McLanahan et al., 2014; U.S. EPA, 2009) were integrated into the new lactating mother and infant model code for dietary iodine and thyroid hormones. The details of the EPA perchlorate and radioiodide PBPK models for the lactating mother and breast-fed and formula-fed infant can be found in (McLanahan et al., 2014; U.S. EPA, 2009) and are not discussed in this document except for changes that were made to the model structure and parameters during the current process of model evaluation and refinement, as discussed below.

As described earlier under *Model Structure*, the structure in the iodide and thyroid hormone BBDR model differs slightly from the radioiodide and perchlorate PBPK models (McLanahan et al., 2014; U.S. EPA, 2009). Therefore, the perchlorate PBPK model was changed in parallel to match the iodide model structure for the thyroid and mammary gland. The thyroid was reduced to two subcompartments, the plasma and tissue, and the mammary gland was simplified to a one-step process with transport occurring directly from mammary plasma to the milk.

Since the perchlorate PBPK model structure was simplified to match the iodide model structure, it was necessary to determine if the revised perchlorate model still matched the human perchlorate exposure radioiodide data (Greer et al., 2002) or breast milk data, or whether some of the parameters needed to be revised. Also, preliminary results with the current lactating mother/breastfeeding infant model indicated that higher levels of perchlorate than previously considered (above 7 µg/kg/d) could have negligible effect on thyroid hormone levels, but performance of the model at these higher exposure levels had not been evaluated.

Key Perchlorate PBPK Model Parameter Changes

Evaluation of the simplified perchlorate PBPK model revealed the need to update some model parameters. Values of two key model parameters were changed from those used in previous

PBPK models (U.S. EPA, 2009). Details of the analyses conducted are provided in Appendix B, "Model Structure Changes and Additional Evaluation." The most important changes are summarized here. Based on re-evaluation of the in vitro data of Kosugi et al. (1996), the affinity constant (Km) for binding of perchlorate to the NIS (KM_TPNG) was reduced from 1.6×10^5 ng/L (McLanahan et al., 2014; Clewell et al., 2007; Merrill et al., 2005) to 6×10^4 ng/L (Schlosser, 2016). This lower value was shown to provide model predictions much more consistent with the radioiodide uptake inhibition data of Greer et al. (2002) for doses up to 100 µg/kg/d than those obtained with the former value for the Km.

Similar to the revision of the Km for perchlorate on the NIS in the thyroid, the Km for perchlorate transport in other tissues in which it is expressed and incorporated into the model, for the lactating mother and infant, and for the pregnant mother and fetus, were revised to match the new maternal value noted above. Other tissues with NIS-mediated transport described in the model include the skin, GI tissue, and mammary. The molecular form of NIS is not known to vary between the tissues in which it is expressed and the NIS-iodide Km is assumed to be the same in all tissues, hence a single value for NIS-perchlorate Km was used to be consistent with both the underlying biology and the iodide sub-model.

The second parameter to be revised, Vmax for perchlorate transport from the mammary plasma to the milk (VMAXC_MKP), also had a significant impact on model predictions. Analysis of perchlorate levels in human breast milk vs. urinary excretion from Kirk et al. (2013) showed a range in the milk:urine ratio of perchlorate among the subjects in that study. Based on the mean value of the ratio of milk concentration to 24-hour urinary excretion from Kirk et al. (2013), the VmaxC for perchlorate expression in breast milk, VMAXC_MKP, was increased by a factor of 2.24 from that used by Clewell et al. (2007) and McLanahan et al. (2014).

1 Perchlorate Clearance – Uncertainty

2 An uncertainty that was identified in the process of model evaluation, but appears to be of minor
3 consequence, is that the terminal clearance half-life for perchlorate in the pregnancy and
4 lactating mother PBPK models (McLanahan et al., 2014; Clewell et al., 2007) is much longer
5 than that for the "average adult" (Merrill et al., 2005): ~ 56 h vs. 7 h. As shown in Appendix D,
6 the difference in distribution parameters significantly affects the time course of perchlorate after
7 exposure begins or ends, but does *not* affect the predicted blood perchlorate concentration at
8 steady-state, which is the level used in evaluating the dose-response for perchlorate. Therefore,
9 while we are uncertain about the validity of this significant life-stage difference in humans, that
10 uncertainty has little impact on model predictions shown here. This difference could significantly
11 impact the results of meal-by-meal ingestion simulations, vs. the continuous ingestion assumed
12 here for simplicity.

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Table 6: Physiological parameters

Parameter	Symbol [a]	Units	Value or Range	Source/notes
Initial Body Weight				
Nonpregnant female		kg	67.8	Clewell et al. (1999), Table 1, mean BW
Pregnant	BW_M	kg	72.3	Lumen et al. (2013)
Lactating	BWPOST	kg	74.6	Clewell et al. (2007); Gentry et al. (2002)
Infant	BW_N0	kg	3.512	Clewell et al. (2007; 2004), males
Body weight over time				
Pregnant female	BW_M	kg	72.3	Gestation week 40; Lumen et al. (2013); Hytten and Leitch (1971)
Lactating female	BW [a]	kg	74.6 – 71.6	Clewell et al. (2007); BW = BWPRE + Vft (increased fat) + (Vwholem-Vmampre) (increased mammary tissue)+ VMK (residual milk)
Infant	BW_N [a]	kg	3.512 – 5.6	Clewell et al. (2007; 2004), growth equation for males
Cardiac output				
Nonpregnant		$L/h/kg^{0.75}$	13.2	Adapted from Brown et al. (1997); provided for comparison
Pre-pregnancy	QCPRE	L/h	Calculated	Pre-pregnancy plasma flow, 55.7% of total blood flow; QCPRE = $0.557*QCC*BWPRE^{0.75}$
Pregnant	QFC_M	$L/h/kg^{0.75}$	15.6	Lumen et al. (2013); Hytten and Leitch (1971)
Lactating	QCC	$L/h/kg^{0.75}$	15.35	QC = plasma flow then matches average results of Robson et al. (1987a) & Robson et al. (1987b), using plasma = 55.7% of total blood; QC varies in proportion to VF & VM, weighted by QFC and QMC, respectively
Infant	QCx_N [b]	L/h	Age (d) / Cardiac output (L/h): 0 / 30; 14 / 40.8; 60 / 55.8; 135 / 66; 180 / 66	Edginton et al. (2006); linear interpolation of QCC_N between points; infant plasma is 57.83% of total blood volume, so: QC_N = 0.5783*QCC_N(t);
Fractional blood flows				
Fat				
Nonpregnant		Unitless	0.085	Brown et al. (1997); Table 27, Females
Lactating	QFC [a]	Unitless	0.085	Brown et al. (1997);QF = QFC*QCPRE*VF/(VFC*BWPRE); QF/QC range = 0.094-0.104
Infant	Calculated [c]	Unitless	0.064-0.074	Clewell et al. (2007; 2004); QF_N = QFC*QC_N*VFC_N/VFC
Gastric tissue				
Nonpregnant		Unitless	0.21	Brown et al. (1997); Table 27, "Portal Vein" flow for Females
Lactating	QGC	Unitless	0.21	Brown et al. (1997); QG = QGC*BWPRE
Infant	Calculated [c]	Unitless	0.021-0.022	Clewell et al. (2007; 2004); QG_N = QGC*QC_N*VGC_N/VGC

Table 6: Physiological parameters, **Fractional Blood Flows** (cont'd)

Kidney				
Nonpregnant female		Unitless	0.17	Brown et al. (1997); Table 27, Females
Lactating	QKC	Unitless	0.17	Brown et al. (1997); QK = QKC*BWPRE
Infant	Calculated [c]	Unitless	0.27-0.31	Clewell et al. (2007; 2004); QK_N = QKC*QC_N*VKC_N/VKC
Liver				
Nonpregnant female		Unitless	0.06	Brown et al. (1997); Table 27, total liver flow minus portal vein flow for Females
Lactating	QLC	Unitless	0.06	Brown et al. (1997), Clewell et al. (2007); QL = QLC*BWPRE
Infant	Calculated [c]	Unitless	0.09-0.10	Clewell et al. (2007; 2004); QL_N = QLC*QC_N*VLC_N/VLC
Mammary gland (not present as separate compartment in infant or nonpregnant adult)				
Lactating	QM [a]	L/h	9.83	Geddes et al. (2012)
Rapidly perfused (RP) *(not present as separate compartment for iodide in the infant)*				
Nonpregnant female		Unitless	0.695	Brown et al. (1997); Table 27, Females, 100% minus % flow to slowly perfused tissue;
Pregnant	QFRP_M	Unitless	0.76	Lumen et al. (2013); QRP_M = (QFRP_M–QFTHY_M – QFPLC)*QCM; QRP_M/QCM = 0.644
Lactating-perchlorate	QRC	Unitless	0.695	Brown et al. (1997); Table 27, Females, 100% minus % flow to slowly perfused tissue. Flow to remaining RP tissue is: QR = (QRC–QMC)*QCPRE – QG – QT– QL– QK. Range of fraction flow (QR/QC) is 0.21-0.2125
Lactating-iodide	QRC	Unitless	0.695	Remaining RP tissue flow is: QR_I = (QRC–QMC)*QCPRE – QT, since thyroid is the only separate RP tissue for iodide. Range of fractional flow (QR_I/QC) is 0.64-0.65.
Infant (perchlorate only)	QRC_N	Unitless	0.657	Brown et al. (1997); Table 27, "PCMT", 100% minus % flow to slowly perfused tissue; Remaining RP flow is: QR_N = QRC_N*QC_N – QG_N – QTHY_N – QL_N – QK_N; Range of fractional flow (QR_N/QC_N) is: 0.20-0.26
Slowly perfused (SP) *(not present as a separate compartment for iodide in the infant)*				
Nonpregnant female			0.305	Brown et al. (1997); Table 27, Females, sum of adipose, bone, muscle, and skin
Pregnant-iodide	QFSP_M	Unitless	0.24	Lumen et al. (2013)
Pregnant-perchlorate	QFSP_M	Unitless	0.24	Lumen et al. (2013); Qsp_mp = QFSP_M – QFSK_MP since skin is separate
Lactating-perchlorate	QSC	Unitless	0.305	Brown et al. (1997); Clewell et al. (2007); Remaining SP flow (QS) is: (QSC – QFC – QSKC)*QCPre. Range of fractional flow (QS/QC) is 0.166-0.168
Lactating-iodide	QSC	Unitless	0.305	Brown et al. (1997); Clewell et al. (2007); fat & skin included for iodide, so QS_i = (QSC-QFc)*QCPre + QF. Range of fractional flow (QS_i/QC) is 0.311-0.319
Infant-perchlorate	QSC_N	Unitless	0.343	Brown et al. (1997); Table 27, "PCMT", Sum of flows for adipose, bone, muscle, & skin; QS_N = QSC_N*QCN – QSK_N – QF_N; Range of QS_N/QC_N = 0.19-0.21
Skin				
Nonpregnant female		Unitless	0.05	Brown et al. (1997); Table 27, Females
Pregnant-perchlorate	QFSK_MP	Unitless	0.058	Clewell et al. (2007)
Lactating	QSKC	Unitless	0.05	Brown et al. (1997); Table 27, Females; QSK=QSKC*QCPRE; QSK/QC ~ 0.049
Infant	--	Unitless	0.074-0.078	Clewell et al. (2007); QSK_N = QSKC*VSKC_N/VSKC

Table 6: Physiological parameters, **Fractional Blood Flows** (cont'd)

Thyroid				
Nonpregnant female		Unitless	0.015	Brown et al. (1997); Table 27, Females
Pregnant	QFTHY_M	Unitless	0.016	Lumen et al. (2013)
Lactating	QTC	Unitless	0.015	Brown et al. (1997); QT = QTC*QCPRE; Range of QT/QC = 0. 0146-0.0148
Infant	QTC_N	Unitless	0.016	Brown et al. (1997); Table 27, PCMT
Fractional Volumes				
Rest of body *Infant*	VbodyC_N	Unitless	0.92	Lumen et al. (2013); Used for iodide in infant to determine the rest of body volume: VBODY_N = VBODYC_N*BW_N – VTHY_N – VPLASMA_N
Fat				
Nonpregnant female	VFC	Unitless	0.327	Brown et al. (1997); Table 14, Female
Lactating (increase from nonpregnant) [a]	VFT	Unitless	T(h) / VFT 0 / 0.0836 4380 / 0	Gentry et al. (2002) for day 0; Gentry et al. (2003) for 6 months (4380 h); VF = (VFC + VFT(t))*BWPRE; Range of VF/BW = 0.35-0.37
Infant [a]	VFC_N	Unitless	0.244-0.286	Clewell et al. (2007), VF_N(t) from Haddad et al. (2001); VFC_N = VF_N(t)/BW_N
GI tract				
Nonpregnant female		Unitless	0.021	Brown et al. (1997); Tables 7 & 21; whole GI tract plus spleen & pancreas, for consistency with use of hepatic portal flow, which comes from all of those tissues
Lactating	VGC	Unitless	0.021	Brown et al. (1997); VwholeG = VGC*BWPre; range of VwholeG/BW = 0.019-0.02. For tissue only VG = VGC*(1-VGBC)*BWPRE; Range of VG/BW = 0.018-0.019
Infant (stomach only)	VGC_N	Unitless	0.0021	Clewell et al. (2007), Haddad et al. (2001); empirical function for VWHOLEG_N(t); VG_N(t) = VWHOLEG_N(t)*(1-VGBC_N); Range of VG_N/BW_N = 0.002-0.0021
GI tissue blood *(all)*	VGBC, VGBC_N	Unitless	0.041	Clewell et al. (2007), Merrill et al. (2005), extrapolated from Altman and Dittmer (1971); fraction of whole GI volume
Gastric juices *(all)*	VGJC	Unitless	0.00071	Clewell et al. (2007), Merrill et al. (2005), Licht and Deen (1988) (0.05 L/70 kg); VGJ = VGJC*BWPRE; VGJ_N = VGJC*BW_N
Kidney				
Nonpregnant female		Unitless	0.0044	Brown et al. (1997)
Lactating	VKC	Unitless	0.0044	Clewell et al. (2007);VK = VKC*BWPRE; Range of VK/BW = 0.0040-0.0042
Infant [a]	VKC_N	Unitless	0.007-0.008	Clewell et al. (2007), VK_N(t) from Haddad et al. (2001); VKC_N = VK_N/BW_N
Liver				
Nonpregnant female		Unitless	0.026	Brown et al. (1997)
Lactating	VLC	Unitless	0.026	Clewell et al. (2007);VL = VLC*BWPRE; Range of VL/BW = 0.024-0.025
Infant [a]	VLC_N	Unitless	0.039-0.045	Clewell et al. (2007), VL_N(t) from Haddad et al. (2001); VLC_N=VL_N/BW_N

Table 6: Physiological parameters, **Fractional Volumes** (cont'd)

Volume of milk	VMK	L	0.357	Kent et al. (2006); Daly et al. (1992)
Mammary tissue	VMAMC	Unitless	0.0174	Fraction of pre-pregnancy body weight (ICRP, 1975); lumped with other richly perfused for distribution, but used in tissue blood volume calculation
Mammary blood	VMBC	Unitless	0.276	Fraction of total mammary tissue volume; set equal to blood fraction in thyroid (VTBC), from (Brown et al., 1986)
Plasma				
Nonpregnant female		Unitless	0.044	Altman and Dittmer (1971)
Pregnant	VFPLS_M	Unitless	0.052	Lumen et al. (2013)
Lactating	VPLASC	Unitless	0.044	Clewell et al. (2007), Altman and Dittmer (1971); VPLAS = VPLASC*BWPRE; VPLAS/BW range = 0.040-0.042
Infant	VPLASC_N	Unitless	0.048	Clewell et al. (2007); Brines et al. (1941)
Red blood cells				
Nonpregnant female		Unitless	0.035	Merrill et al. (2005); Marieb (1992); Altman and Dittmer (1971)
Pregnant-perchlorate	VRBCC_MP	Unitless	0.035	Clewell et al. (2007)
Lactating	VRBCC	Unitless	0.035	Clewell et al. (2007); VRBC = VRBCC*BWPRE; VRBC/BW range = 0.032-0.033
Infant [a]	VRBCCC_N	Unitless	0.035	Clewell et al. (2007)
Richly perfused (RP)				
Nonpregnant female		Unitless	0.192	Brown et al. (1997), Tables 21: Sum of brain, GI, heart, kidneys, liver, lung, "rest of body" and blood
Pregnant	VFRP_M	Unitless	0.19	Brown et al. (1997), all RP
Lactating	VRC	Unitless	0.192	Brown et al. (1997), Clewell et al. (2007; 2004); VR = VRC*BWPRE –VTTOT–VL–VK –VWHOLEG–VMAMPRE–VBLOOD; VR/BW range=0.051-0.053
Infant-perchlorate (remaining RP only) [a]	VRC_N	Unitless	0.032-0.033	Clewell et al. (2007; 2004); Calculated, VRC_N = 2.596*VGIT_N/BW_N, where VGIT_N is the age-based volume of the total GI tract from Haddad et al. (2001)
Slowly perfused (SP)				
Nonpregnant female		Unitless	0.794	Brown et al. (1997); Table 21, sum of adipose, bone, muscle, and skin
Pregnant	VFSP_M	Unitless	0.793	Brown et al. (1997), all SP
Lactating	VSC	Unitless	0.794	Brown et al. (1997), Clewell et al. (2007);VS = VSC*BWPRE – VWHOLESK –VFPRE; VS/BW range = 0.39-0.41
Infant (remaining SP only) [a]	VSC_N	Unitless	0.40-0.46	Clewell et al. (2007); Calculated, VSC_N = 0.92 – (all other tissues, including remaining richly perfused, liver, kidney, blood, thyroid, stomach, stomach contents, skin, & fat)

Table 6: Physiological parameters, **Fractional Volumes** (cont'd)

Skin				
Nonpregnant female		Unitless	0.037	Brown et al. (1997)
Pregnant-perchlorate	VFSK_MP	Unitless	0.037	Brown et al. (1997)
Lactating	VSKC	Unitless	0.037	Ciewell et al. (2007); VwholeSk = VSkC*BWPre, VwholeSk/BW range = 0.034-0.035; skin tissue only VSK = VSKC*(1 – VSKBC)*BWPRE; VSK/BW range = 0.031-0.032
Infant [a]	VSKC_N	Unitless	*0.053-0.055*	VWHOLESK_N(t) from Haddad et al. (2001); VSKC_N = VWHOLESK_N(t) /BW_N; skin tissue only, VSK_N = VWHOLESK_N(t)*(1 – VSKBC_N), Range of VSK_N/BW_N range = 0.051-0.053
Blood fraction of skin				
Nonpregnant female		Unitless	0.08	Brown et al. (1997)
Pregnant-perchlorate	VFSK_MP	Unitless	0.037	Ciewell et al. (2007)
Lactating	VSKBC	Unitless	0.08	Brown et al. (1997); VSKB = VSKBC*VSKC* BWPRE
Infant	VSKBC_N	Unitless	0.08	Assumed same as adult
Thyroid (total volume)				
Nonpregnant female		Unitless	3×10^{-4}	Brown et al. (1997)
Pregnant	VFTHY_M	Unitless	2.2×10^{-4}	Lumen et al. (2013)
Lactating	VTTOTC	Unitless	2.5×10^{-4}	Yokoyama et al. (1986); VTTOT=VTTOTC*BWPRE, VTTOT/BW range = $2.27\text{-}2.36\times10^{-4}$; thyroid tissue only: VT=VTTOTC*(1 – VTBC)*BWPRE; VT/BW range = $1.64\text{-}1.71\times10^{-4}$
Infant [a]	VTTOTC_N	Unitless	$2.82\text{-}3.95\times10^{-4}$	Ciewell et al. (2007); Ogiu et al. (1997); calculated from empirical function, VTG_N = $1.3857\times10^{-3} + 6.3\times10^{-5}\times$months (grams); VTTOTC_N = VTG_N$\times10^{-3}$ /BW_N; for tissue only, VT_N= VTG_N*(1-VTHYBC_N); Range of VT_N/BW_N = $3.34\text{-}2.39\times10^{-4}$
Blood fraction of thyroid				
Rat		Unitless	0.18	Brown et al. (1997)
Pregnant (human)	VFTHYB	Unitless	0.276	Lumen et al. (2013)
Lactating (human)	VTBC	Unitless	0.276	Brown et al. (1986), Table 2; average for decades 3-6 (i.e., ages 20-59) of the sum of mean % volume for stroma & vessels; VTB = VTBC* VTTOTC*BWPRE
Infant	VTHYBC_N	Unitless	0.153	Ciewell et al. (2007)
Urine volume				
Pregnant	VURINE	L/d	1.5	Lumen et al. (2013); Thorp et al. (1999)
Lactating	VURINE	L/d	1.453	Fisher et al. (2016), Table S1 based on NHANES 2009-12[e]
Infant [a]	VURINEC_N	L/h/kg	0.005	Sillén (2001); Estimated based on 4-h infant void volume at 3 months of age

[a] Calculated by equation over time.

[b] Entered in model code as table function. Values between time points are linearly interpolated.

[c] Calculated based on neonate to adult volume of tissue

[d] Changes are based on changes in body weight over time relative to tissue

[e] Centers for Disease Control & Prevention (CDC), National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Data Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Diseases Control and Prevention; 2009-2012. Available from: http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm.

Table 7: Iodide kinetic parameters

Parameter	Model Symbol	Units	Value		Source
Ingestion					
Formula ingestion rate	VFORMULAC	L/h/kg	Time (d)	Value	Fomon et al. (1964)
			0	0.00829	
			31	0.00789	
			61	0.00725	
			91	0.00658	
Breast milk ingestion rate	KTRANS	L/d/kg	Time (d)	Value	Arcus-Arth et al. (2005); Casey et al. (1986); Hofvander et al. (1982)
			0	0.013	
			1	0.013	
			2	0.04	
			3	0.098	
			4	0.14	
			5	0.155	
			30	0.154	
			60	0.148	
			90	0.13	
Formula concentration	FORMULAUGL_I	µg/L	~ 100-400		Pearce et al. (2004)

Table 7: Iodide kinetic parameters (cont'd)

Urinary clearance

Pregnant mother	CLF_UIM	L/h/kg$^{0.75}$	0.17		Lumen et al. (2013) (model fit)
Lactating	CLUx_I [a]	L/h	Time (d)	Value	Fisher et al. (2016); model fit to predict central tendency for Leung et al. (2012) data; values initially from Aboul-Khair et al. (1964), 3.9±0.6 L/h (mean ± SE) at GW 39, then 3.1±0.4, 2.2±0.2, and 2.1±0.1 (mean ± SE) at postnatal weeks 2, 6, and 12, respectively.
			0	5	
			14	4	
			42	4	
			90	3	
Infant	CLUCx_NI [a]	L/h/kg	Time (d)	Value	Fisher et al. (2016): values at d=0 and d=180 calculated from Ponchon et al. (1966); functions predictions were smoothed out by adding transitions between the two urinary excretion estimates.
			0	0.044	
			12.5	0.05	
			20.8	0.055	
			89.5	0.06	
			180	0.066	

Thyroid Euthyroid Iodide Stores

Pregnant mother	(n/a)	--	--	Term not included in model
Lactating	CTGIPOOL [a]	nmol/kg thyroid	1.13×10^{7}	Fisher et al. (2016): set to yield > 15 mg thyroidal iodide stores
Infant	CTGIPOOL_N [b]	nmol/g thyroid	1717	Fisher et al. (2016): set so that the total pool equals amount predicted in fetus at end of pregnancy (Lumen et al., 2013) for 250 µg/d maternal iodide.

Binding for organified iodide in thyroid

Pregnant mother	(n/a)	--	--	Binding rate set = net uptake of iodide
Lactating	KBINDC_I [a]	$L^{1}nmol^{-1}\ h^{-1}$ kg-thyroid$^{-0.75}$	1×10^{-4}	Model fit to predict thyroid stores near the maximal concentration for euthyroid condition [a]
Infant	KBINDC_NI [b]	$L^{1}nmol^{-1}\ h^{-1}$ kg-thyroid$^{-0.75}$	0.1	Fisher et al. (2013); model fit, scaled to thyroid weight because of growth of the thyroid gland

Table 7: Iodide kinetic parameters (cont'd)

Maximal thyroidal uptake					
Pregnant mother	VmaxNISF_THY_mi	nmol/h/kg$^{0.75}$	3800		Lumen et al. (2013) (model fit)
Lactating	Vmaxthyx_i [a]	nmol/h	Time (d)	Value	Aboul-Khair et al. (1964) for PND 14, 42, and 90. Value at PND 0 calculated using the allometric coefficient from Lumen et al. (2013) and the post pregnancy body weight for the lactating mother: 3800x(BWpost^0.75).
			0	96500	
			14	70875	
			42	46620	
			90	38430	
Infant	VmaxthyCx_Ni [b]	nmol/h/kg	Time (d)	Value	Estimated from Ponchon et al. (1966) [b]
			0	4763	
			21	2060	
			90	2060	
Thyroid Michaelis-Menten constant					
Pregnant mother	KMNIS_I	nmol/L	3.15×10^4		Lumen et al. (2013) (literature data)
Lactating	KMTHY_I [a]	nmol/L	3.15×10^4		Set to pregnancy value [a]
Infant	KMTHY_NI [b]	nmol/L	3.15×10^4		Set to pregnancy value [b]
Maximal milk uptake (n/a for pregnant female and infant)					
Lactating	VMAXCx_MKI [a]	nmol/h/kg	1.05×10^4		Model fit [a]
Milk Michaelis-Menten constant (n/a for pregnant female and infant)					
Lactating	KMMk_I [a]	nmol/L	3.15×10^4		Set to thyroid value [a]

Table 7: Iodide kinetic parameters (cont'd)

Scaled permeability surface area				
Thyroid				
Pregnant mother	PAFTHY_MI	L/h/kg$^{0.75}$	1×10^{-4}	Lumen et al. (2013) (previous model fit)
Lactating	PATHYC_I [a]	L/h/kg$^{0.75}$	1×10^{-5}	Model fit [a]
Infant	PATHYC_NI [b]	L/h/kg$^{0.75}$	1×10^{-2}	Model fit [b]
Milk				
Lactating mother	PAMKC_I [a]	L/h/kg$^{0.75}$	1×10^{-3}	Model fit I [a]
Partition Coefficients				
Milk: plasma	PMK_I	unitless	1.0	Clewell et al. (2007); Merrill et al. (2005)
Thyroid: plasma				
Pregnant mother	PTHY_MI	unitless	0.15	Clewell et al. (2007); Merrill et al. (2005)
Lactating	PTHY_I	unitless	0.15	Clewell et al. (2007); Merrill et al. (2005)
Infant	PTHY_NI	unitless	0.15	Set to maternal value
Rest of body: plasma (n/a for pregnant and lactating *mother*)				
Infant	PBODY_I [b]	unitless	0.21	Estimated; between maternal richly and slowly perfused values
Richly perfused: plasma (n/a for infant)				
Pregnant mother	PRP_MI	unitless	0.4	Lumen et al. (2013) (model fit)
Lactating	PRP_I	unitless	0.4	Clewell et al. (2007); Merrill et al. (2005)
Slowly perfused: plasma (n/a for infant)				
Pregnant mother	(n/a)	unitless	0.18	Lumen et al. (2013) (model fit)
Lactating	PS_P	unitless	0.18	Set to pregnancy value

[a] Information on data source and calibration in table 5
[b] Information on data source and calibration in table 4

Table 8: Thyroid hormone kinetic parameters

Parameter	Model Symbol	Units	Value		Source
Thyroxine (T4)					
Volume of Distribution					
Pregnant mother	VDFT4_MI	L/kg	0.121		Lumen et al. (2013) (model fit)
Lactating	VDT4C [a]	L/kg	0.084		Estimated based on Lumen et al. (2013)
Infant	VDT4C_N	L/kg	0.31		Cottino et al. (1961)
T4 Production in thyroid					
Pregnant Female	KPRODT4F_MI	$h^{-1}kg^{-0.75}$	2.45×10^{-6}		Lumen et al. (2013) (model fit)
Lactating mother	KPRODT4C[a]	$h^{-1}kg^{-0.75}$	1.1×10^{-6}		Estimated based on Lumen et al. (2013)
			Time (d)	Value	
Infant	VPRODT4CX_N [b]	nmol/h/kg	0	0.535	Set equal to T4 utilization reported from Fisher (1996)
			4	0.535	
			7	0.375	
			28	0.3218	
			30	0.3218	
			180	0.3218	
Total T4 metabolism					
Pregnant mother	KDEGT4F_MI	$h^{-1}kg^{-0.75}$	1.9×10^{-4}		Lumen et al. (2013) (model fit)
Lactating	KMETT4C	$h^{-1}kg^{-0.75}$	1.4×10^{-4}		Model fit
			Time (d)	Value	
Infant	CLMETT4CX_N [b]	L/h/kg	0	0.0035	Estimated clearance
			10	0.0023	
			30	0.0020	
			90	0.0020	

Table 8: Thyroid hormone kinetic parameters (cont'd), Fractional conversion of T4 to T3

Pregnant Female	(set in equations)	unitless	0.5		Lumen et al. (2013) (model fit)
Lactating mother	T3FRAC	unitless	0.5		Set to pregnancy value
Infant	T3FRAC_N [b]	unitless	Time (d)	Value	Estimated, fraction of T4 metabolism going to T3 vs. rt3. Day 0 value from Lumen et al. (2013) for fetus; days 5-30 fit to infant T3 data (Lem et al., 2012; Williams et al., 2004; Elmlinger et al., 2001; Franklin et al., 1985) and to qualitatively match declining trend for rT3/T4 from Chopra et al. (1975); day 180 value set based on ontogeny of iodothyronine deiodinases (near adult activity at 6 months of age) from Richard et al. (1998).
			0	0.5	
			5	0.5	
			6	0.55	
			7	0.6	
			30	0.7	
			180	0.8	
Free fraction of total T4					
Pregnant Female	FRCONVT4_MI	unitless	9×10^{-5}		Lumen et al. (2013) (literature data estimate)
Lactating mother	FRCONVT4	unitless	1×10^{-4}		Fisher et al. (2016): Slightly increased from 0.9×10^{-4}; (Lumen et al., 2013) to 1.0×10^{-4}
Infant	FRCONVT4_N [b]	unitless	1.7×10^{-4}		Fit to data from Lem et al. (2012)
Urinary clearance fT4 (not specified in pregnant female; composite urinary/fecal clearance given below)					
Lactating mother	CLUFT4C [a]	$L/h/kg^{0.75}$	0.6		Fisher et al. (2016): Estimated for free T4
Infant	CLUFT4C_N [b]	$L/h/kg^{0.75}$	0		Amount not quantitatively significant
Fecal elimination (not specified in pregnant female; composite urinary/fecal clearance given below)					
Lactating mother	CLFECESFT4C[a]	$L/h/kg^{0.75}$	2		Fisher et al. (2016): Estimated for *free* T4
Infant	CLFECEST4C_N [b]	$L/h/kg^{0.75}$	2×10^{-4}		Fisher et al. (2016): Estimated for *total* T4
Milk clearance fT4					
Lactating mother	CLMILKFT4C[a]	$L/h/kg^{0.75}$	0.3		Fisher et al. (2016): Estimated for *free* T4
Composite urinary/fecal clearance for Total T4					
Pregnant Female	CLFT4_mi	$L/h/kg^{0.75}$	1.85×10^{-4}		Lumen et al. (2013) (model fit)
Lactating mother	(n/a)	$L/h/kg^{0.75}$	2.61×10^{-4}		Sum corrected for free fraction
Infant	(n/a)	$L/h/kg^{0.75}$	2×10^{-4}		Sum corrected for free fraction

Table 8: Thyroid hormone kinetic parameters (cont'd),
TRIIODOTHYRONINE (T3)

Volume of Distribution					
Pregnant mother	VDFT3_MI	L/kg	0.46		Lumen et al. (2013) (model fit)
Lactating	VDT3C [a]	L/kg	0.47		Fisher et al. (2016)
Infant	VDT3C_N	L/kg	0.304		Lumen et al. (2013) (model fit for fetus)
T3 Production in thyroid					
Pregnant mother	KPRODT3F_MI	$h^{-1}kg^{-0.75}$	2.2×10^{-7}		Lumen et al. (2013) (model fit)
Lactating	KPRODT3C [a]	$h^{-1}kg^{-0.75}$	4×10^{-7}		Increased from 1/11 T4 production [a]
Infant	$VPRODT3F_N * VPRODT4Cx\_N$ [b]	nmol/h/kg	Time (d)	Value	Assumed 1/11 production ratio T4:T3 from Lumen (2013)
			0	0.0487	
			4	0.0487	
			7	0.0341	
			28	0.0293	
			30	0.0293	
			180	0.0293	
Total T3 metabolism					
Pregnant mother	KDEGT3F_MIt	$h^{-1}kg^{-0.75}$	Pdoseµg_i (µg/d)	Value	Lumen et al. (2013) (model fit); *function of iodide dose-rate*
			50	9.9×10^{-4}	
			75	2.6×10^{-3}	
			100	1.425×10^{-3}	
			150	1.6×10^{-3}	
			200	1.67×10^{-3}	
			250	1.705×10^{-3}	
			300	1.72×10^{-3}	
			350	1.725×10^{-3}	
Lactating	KMETT3C	$h^{-1}kg^{-0.75}$	2.5×10^{-3}		Model fit
Infant	CLMETT3CX_N [b]	L/h/kg	Time (d)	Value	Fisher et al. (2016): Estimated [b]
			0	3.5×10^{-3}	
			10	2.3×10^{-3}	
			30	2.0×10^{-3}	
			90	2.0×10^{-3}	

Table 8: Thyroid hormone kinetic parameters (cont'd),

Urinary clearance T3 (not specified in pregnant female; composite urinary/fecal clearance given below; not included for infant)				
Lactating mother	CLUT3C_I [a]	L/h/kg$^{0.75}$	3×10^{-3}	Fisher et al. (2016): Estimated [a]
Fecal elimination (not specified in pregnant female; composite urinary/fecal clearance given below)				
Lactating mother	CLFECEST3C [a]	L/h/kg$^{0.75}$	5×10^{-3}	Fisher et al. (2016): Estimated [a]
Infant	CLFECEST3C_N [b]	L/h/kg$^{0.75}$	9×10^{-4}	Fisher et al. (2016): Estimated [b]
Milk clearance				
Lactating mother	CLMILKT3C [a]	L/h/kg$^{0.75}$	2×10^{-4}	(*Lactating mother only*) Estimated
Composite clearance for Total T3				
Pregnant mother	CLFT3_MI	L/h/kg$^{0.75}$	2.7×10^{-3}	Lumen et al. (2013) (model fit)
Lactating	(n/a)	L/h/kg$^{0.75}$	8.1×10^{-3}	Sum milk, urine, fecal
Infant	(KFECESCT3_N)	L/h/kg$^{0.75}$	9×10^{-4}	Fecal only

[a] Information on data source and calibration in table 5.
[b] Information on data source and calibration in table 4.

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MODEL CALIBRATION RESULTS

The figures below show the model comparison with the data that was used for calibrating the model parameters in both the formula-fed and breast-fed infant exposure scenarios. The parameters that were calibrated from model fits are listed in **Table 4** for the infant and **Table 5** for the lactating mother. The values for the parameters are given in **Table 7** and **Table 8** for iodide and the thyroid hormones, respectively. While comparisons of model predictions to data for infants less than 7 days old are shown, the model was not calibrated to those data because of the rapid changes in TSH levels which occur during this period (Lem et al., 2012). Overall, the model predictions were considered adequate.

First, model predictions of urinary iodide concentrations for the formula-fed infant were compared with data from Gordon et al. (2014) and Cao et al. (2010). The model simulations were performed based on formula concentrations of iodine reported by Pearce et al. (2004) and perchlorate by Schier et al. (2010). Urinary iodide predictions were not sensitive to perchlorate exposure levels, so for these simulations, the geometric mean perchlorate concentration (0.92 µg/L) of all formulas analyzed by Schier et al. (2010) was used for the iodide comparisons (**Figure 4**, top panel). The model-predicted urinary iodide concentration for formula-fed infants with iodine concentrations at the lowest reported value of 108 µg/L by Pearce et al. (2004) were within the range of literature-reported values (**Figure 4**) from Gordon et al. (2014) and from Cao et al. (2010) for both cow milk-based and soy-based formula. Model-predicted urinary concentrations of iodide for the average formula iodine concentration (159 µg/L) also were consistent with Gordon et al. (2014) and cow formula data from Cao et al. (2010) but were higher than the majority of values for soy formula. However, the reported iodine concentrations for soy formula were 108 and 117 µg/L from Pearce et al. (2004), and therefore, the average iodine concentration of 159 µg/L likely does not reflect the iodide intake of infants consuming

soy formula in the Cao et al. (2010) study. The model-predicted urinary iodide concentrations with formula iodide = 384 µg/L, the highest reported iodine formula concentration from Pearce et al. (2004), were only within the higher range of values for infants consuming cow formula from Cao et al. (2010). This higher formula concentration of iodine was greater than 2 standard deviations from the mean of the 8 formulas analyzed by Pearce et al. (2004).

For perchlorate, data for urinary concentrations were only available for formula-fed infants from Cao et al. (2010). To cover the range of literature-reported perchlorate concentrations, simulations are shown for 0.18 µg/L (minimum measured by Schier et al. (2010)), 0.92 µg/L (geometric mean of values for both soy and cow milk formulas from Schier et al. (2010)), and 4.1 µg/L (maximum value from Pearce et al. (2004), which is higher than the maximum measured by Schier et al. (2010)). Model-predicted concentrations of perchlorate were within the reported values for cow and soy formulas (**Figure 4**, bottom panel).

For the breast-fed infant, model predictions of urinary iodide were compared with literature data (**Figure 5**) from Gordon et al. (2014) and Cao et al. (2010). Because the actual breastmilk iodide concentrations were not reported in the studies, the data was compared with a range of iodine intakes. Model simulations only varied slightly for perchlorate exposures up to 350 ng/kg/d (1/2 the RfD; results not shown); very small differences could be seen in simulation plots but these were well within the variation among the data. Since average (mean) perchlorate exposures were previously estimated to be between 80 and 123 ng/kg/d for women of childbearing age in different subgroups (U.S. EPA (2008)[8], **Table 4**), an approximate overall average perchlorate intake of 100 ng/kg/d was used while evaluating fits to the iodine data. The

[8] https://www.gpo.gov/fdsys/pkg/FR-2008-10-10/html/E8-24042.htm

model-predicted urinary iodide concentrations for breast-fed infants were lower than the majority of the literature reported values at postnatal days < 45 but were within the range of reported values for later simulated postnatal days.

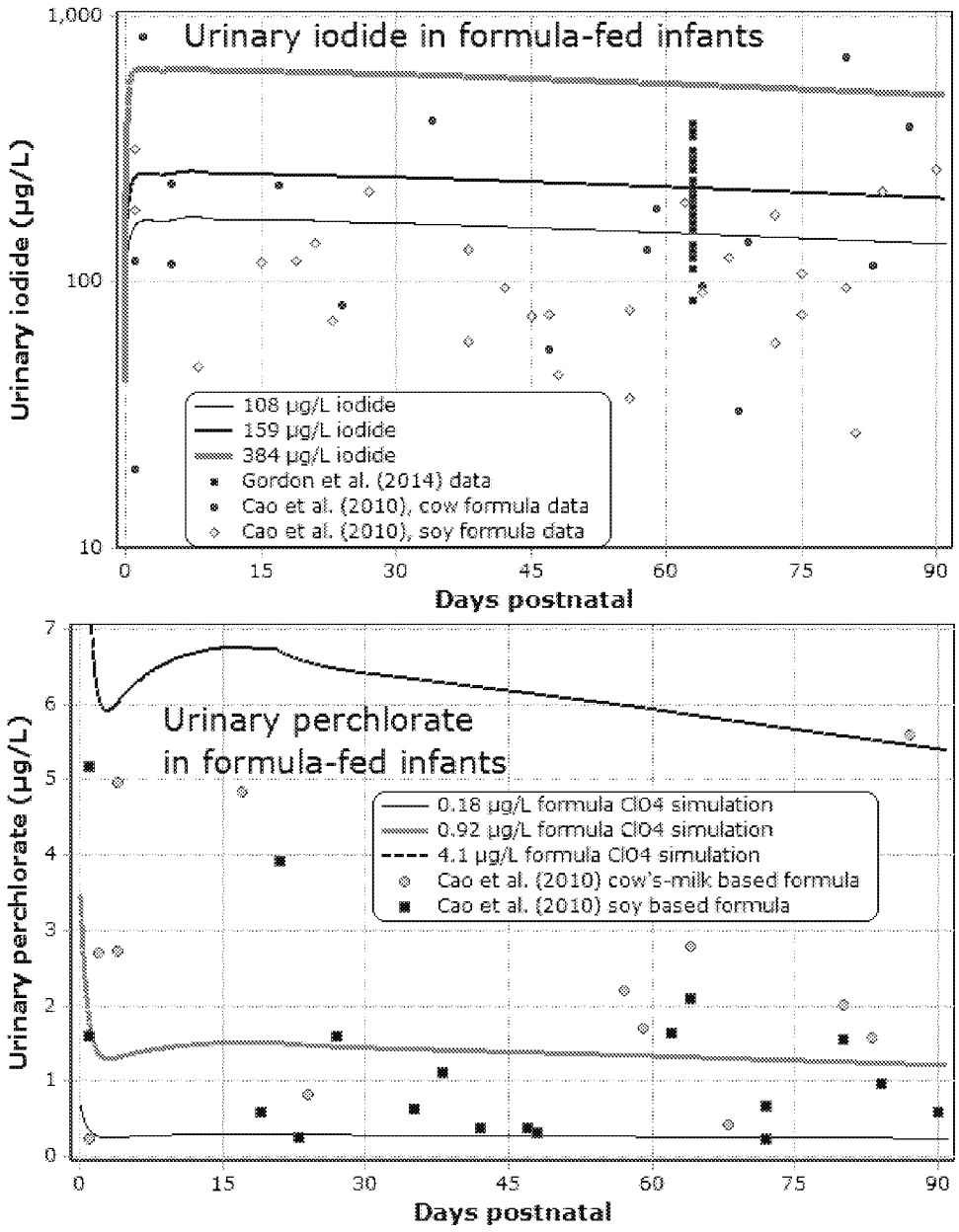

Figure 4. Comparison of formula-fed model simulations of urinary iodide and perchlorate with literature data.

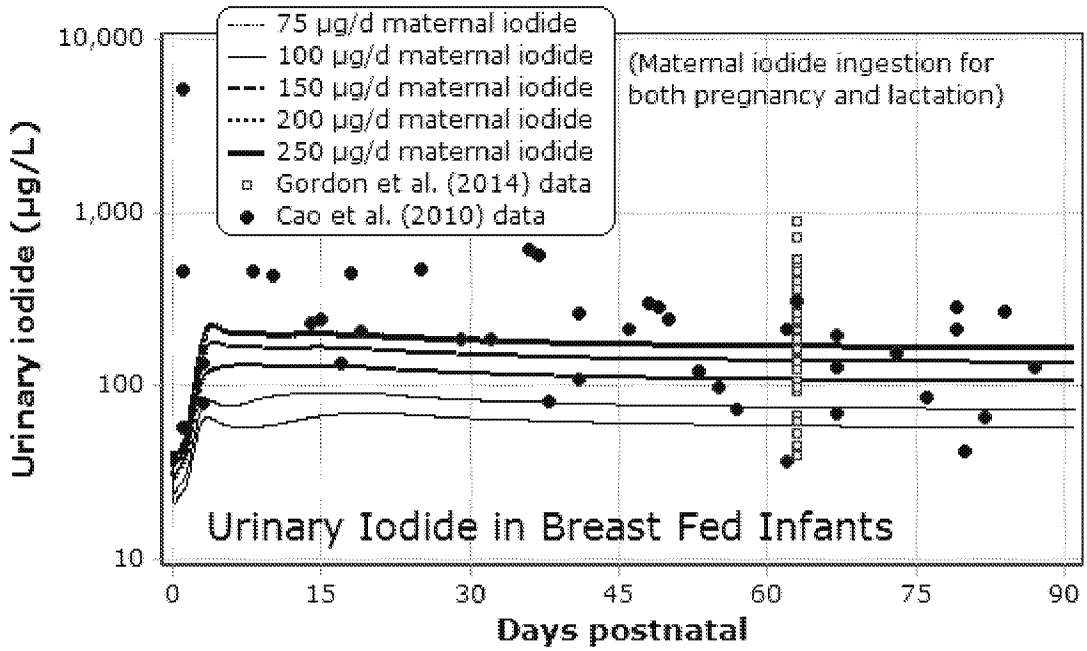

Figure 5. Model simulations for breast-fed infant from a range of maternal iodine intakes and 100 ng/kg/d perchlorate compared with literature data for urinary concentrations of iodide.

Maternal iodine and perchlorate exposures from Cao et al. (2010) and Gordon et al. (2014) are unknown. Data from Cao et al. (2010) are weekly averages obtained separately for boys (*n* = 1-5 per point) and girls (n = 2-6). Data from Gordon et al. (2014) are for 30 individual formula-fed children. Iodine and perchlorate exposures are unknown for both studies.

The model-predicted urinary perchlorate concentrations were within the range of reported values for birth to 90 days for both formula-fed and breast-fed infants, when perchlorate daily intake was assumed to vary between 0.001 and 1 µg/kg/d (**Figure 6**; data from Leung et al. (2012) and Cao et al. (2010)). Since iodine intake between 75 and 250 µg/day had no effect on predicted urinary perchlorate levels, the iodine intake by the lactating mother was assumed to be 150 µg/day for the perchlorate comparison: a low level within the plausible range for which the model is assumed valid. Since iodine intake has very little effect on predicted perchlorate kinetics, the exact choice of iodine level is not important for this comparison.

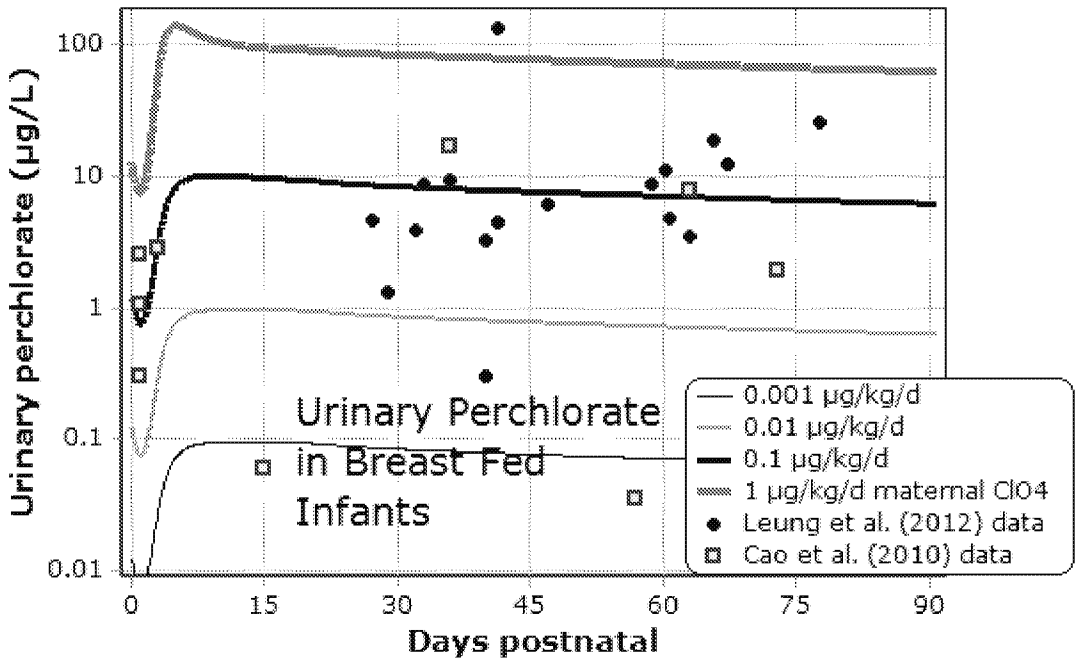

Figure 6. Model simulations for the breast-fed infant from a range of maternal perchlorate levels (with 150 µg/d dietary iodine) compared with literature data for urinary perchlorate.

Maternal iodine and perchlorate exposures from Cao et al. (2010) and Leung et al. (2012) are unknown.

Note: it has been suggested that iodide levels are not stable in breast-milk samples (Dr. Benjamin Blount, CDC, personal communication), which may explain in part why many of the data in **Figure 7** (also see **Figure 17**) are well below the model simulations for 150 and 250 µg/d maternal iodine intake. The predictions for the lowest iodine intake of 75 ug/d are closer to the middle of the range of the data.

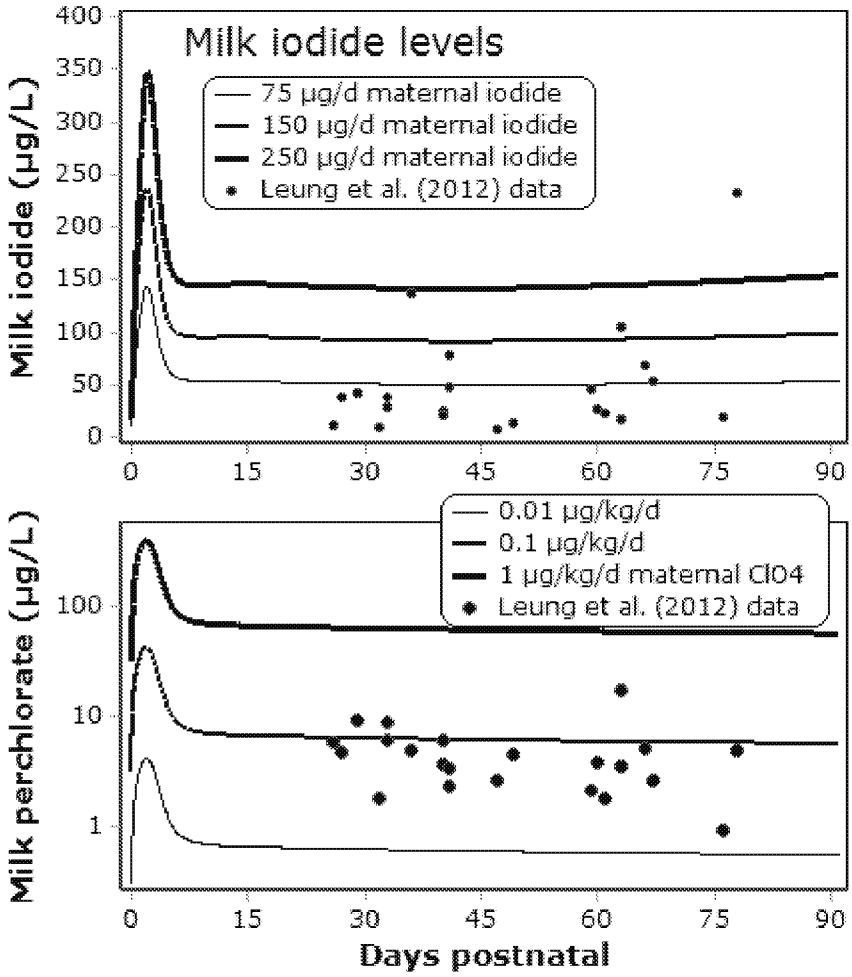

Figure 7. Model simulated iodide and perchlorate breast milk concentrations compared with data of Leung et al. (2012).

Data are from an observational study where the iodine and perchlorate exposures are unknown.

For comparison with literature data on thyroid hormones, the model predictions were performed only for lactating mothers (**Figure 8**) and breast-fed infants (**Figure 9**) because literature data was not specifically available for formula-fed infants. The model predictions of T4 and fT4 were

compared with data obtained from NHANES (NHANES, 2007-2008, Thyroid Profile[9] and NHANES 2009-2010, Thyroid Profile[10]) for the lactating mother. Model-predicted infant serum T4 concentrations were compared with data from Lem et al. (2012), and model-predicted serum fT4 were compared with data from Lem et al. (2012), Leung et al. (2012), and Cao et al. (2010). Because the maternal intakes of iodine from the literature sources are not known, the model simulations were performed at maternal iodine intakes ranging from 75 to 250 µg/d with perchlorate exposure of 100 ng/kg/d. Model-predicted concentrations of T4 and fT4 in both lactating mothers and breast-fed infants were within the range of literature reported values. Model-predicted concentrations of T4 and fT4 for maternal intake of 75 µg/d iodine differed significantly from the iodine intakes of 150-250 µg/d. As shown in the dose-response section, for maternal iodide intake of 100 µg/d, maternal fT4 was predicted to be near or slightly below the bottom of the reference range (when the reference range is calculated relative to model predictions for 200 µg/d iodide). And for maternal intake of 75 µg/d iodide, maternal fT4 is predicted to be in or below the hypothyroxinemic range; i.e., in the range of hypothyroidism. However, infant fT4 levels were predicted to be still within the reported reference intervals for normal infants, ages 7 to 90 days (Lem et al., 2012), for maternal intake as low as 75 µg/d iodide.

[9] http://wwwn.cdc.gov/nchs/nhanes/2007-2008/THYROD_E.htm
[10] http://wwwn.cdc.gov/nchs/nhanes/2009-2010/THYROD_F.htm

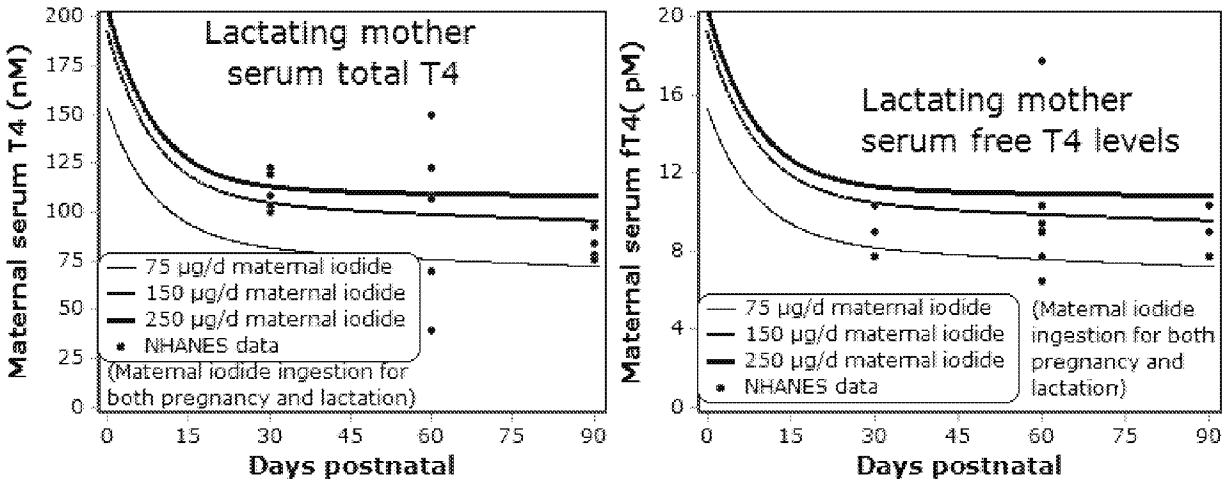

Figure 8. Comparison of model-simulated plasma T4 concentrations with experimental data for the lactating mother.

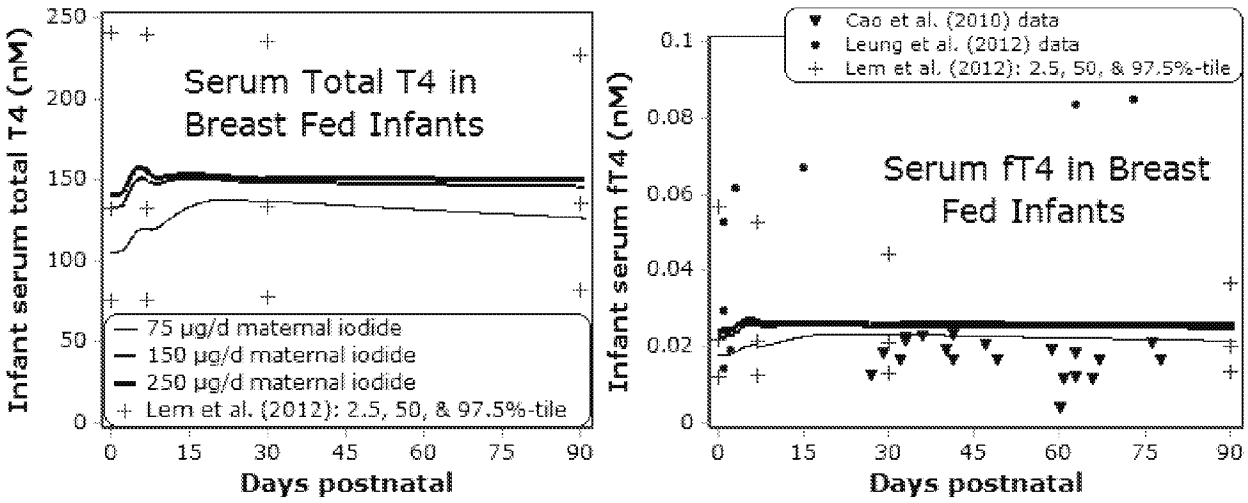

Figure 9. Comparison of model-simulated plasma T4 concentrations with experimental data for the breast-fed infant.

Data are from observational studies where the iodine and perchlorate exposures are unknown. Simulated iodine intakes are the same for both plots.

SUMMARY OF CALIBRATION RESULTS

Overall, the model predictions were considered adequate based on the comparison of model simulations to data in **Figure 4**-**Figure 9**. Because the only source of variability evaluated was iodide and perchlorate dietary intake, it is not expected that the model will capture the entire observed variability. Variability in physiological factors, such as urinary excretion rate and body

weight, and biochemical factors, such as NIS expression and thyroid hormone synthesis activity, would also have significant effects on the metrics evaluated. However model predictions should roughly match average values in each data set and variability in ingestion should account for some of the observed variability. Variability should be more completely described for perchlorate than iodide, since there is a wider range of possible perchlorate ingestion levels and perchlorate metabolism is not a source of variability.

The urinary iodide concentrations for formula-fed infants were within the upper range of literature-reported values (**Figure 4**, top panel). Compared with iodide, fewer urinary perchlorate data were available; however, the model-predicted perchlorate urinary concentrations were within the range of reported values for birth to PND 90 for both formula-fed and breast-fed infants (**Figure 4**, bottom panel, and **Figure 6**). The model-predicted urinary iodide concentrations were within the range of reported values for PND 45 to PND 90 for breast-fed infants, although the predicted values were generally in the upper range of reported values for earlier postnatal days (**Figure 5**). The model-predicted iodide concentrations in milk for maternal intake of ≥ 150 µg/d iodine were within the observed range of literature-reported values; although the majority of values were lower than model predictions (**Figure 7**, top panel).

The model-predicted concentrations of serum T4 and fT4 in both lactating mothers and breast-fed infants were within the range of literature reported values (**Figure 8** and **Figure 9**). The model-predicted concentrations of serum T4 and fT4 for maternal intake of 75 µg/d iodide were clearly lower than those predicted for maternal iodine intakes of 150-250 µg/d, but were still within the reported ranges for normal infants, PND 7 to 90 (**Figure 9**).

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DOSE-RESPONSE EVALUATION

This section provides the results for the model's behavior, specifically dose-response predictions for the effect of perchlorate on thyroid hormones. Because the model sets the ratio of T4 to fT4 in serum at a constant proportion, the dose-response for one will parallel the other. Therefore, serum fT4 levels were evaluated as a measure of response. Provisional reference and hypothyroxinemic ranges for each life stage, and postnatal day, were identified as described in the Technical Introduction. Hypothyroxinemia is assumed to ensue when fT4 drops to the 10th population percentile for each life stage (and PND), and hypothyroidism to ensue at the 2.5th percentile. These ranges, including the specific values for the percentiles, require evaluation during the peer review, and revision may occur. They are used here to illustrate how the BBDR model can be used in conjunction with identified fT4 levels that mark the boundary of adversity.

The range of perchlorate exposures over which the model is valid was discussed in the Technical Introduction, and can be evaluated based on both model predictions of radio-iodide uptake inhibition and perchlorate-induced changes in T4 or fT4. Comparing the predictions with the perchlorate sub-model to the radio-iodide uptake data of Greer et al. (2002) (for adults, the lactating mother, see Appendix B) indicated that the model is only accurate for prediction of iodide uptake inhibition up to an exposure level of ~ 100 µg/kg/d perchlorate. However, dose-response analyses were restricted to 0-20 µg/kg/d perchlorate, since that is the range of interest and likely application, and because predicted ft4 levels for many of the life-stages and iodine intake levels fell below their respective reference levels in this range. Predicted fT4 levels below the hypothyroxinemic range (i.e., to the point that TSH is expected to be significantly elevated) will be shown with grey, to emphasize their uncertainty. *When model predictions fall below that*

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range, hypothyroid conditions are assumed to occur and TSH feedback is activated, at which point the model is no longer valid.

For this evaluation, perchlorate doses were set at specific normalized ingestion rates, µg/kg/d of perchlorate for the formula fed infant or breast feeding mother (the amount of perchlorate transferred to the breast-fed infant is a model prediction), rather than being set based on a given water concentration and water ingestion rate. The analysis provided here is a straightforward dose-response evaluation separated from the issues of water ingestion or exposure. This approach allows the results to be compared directly with the existing RfD (0.7 µg/kg/d) and point-of-departure (7 µg/kg/d).

Pregnant Mother and Fetus

First, dose-response results for the pregnant mother and fetus at gestation week 40 are shown in **Figure 10**. As described previously, the Km for perchlorate interaction with NIS was changed in the pregnancy model to match the value now used for the lactating mother and infant. Therefore these results do not match those reported by Lumen et al. (2013).

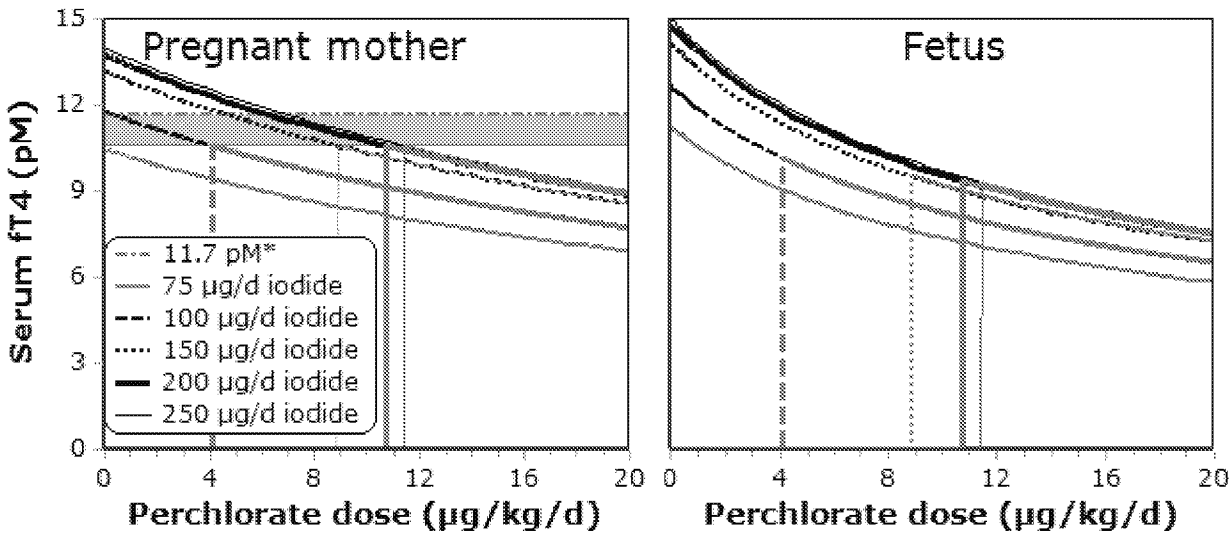

Figure 10. Free T4 (fT4) levels as a function of perchlorate exposure and dietary iodine ingestion in the gestation-week 40 pregnant mother and fetus.

The horizontal line at 11.7 pM marks the bottom of the applied reference range for the pregnant mother. The grey band (11.7-10.6 pM) shows the assumed range for maternal hypothyroxinemia. (There is not a defined range for the fetus.) Results are shown in grey for both mother and fetus when the maternal level is below 10.6 and vertical lines mark the points at which maternal fT4 falls below 10.6 ppm, hence the model becomes uncertain. These are drawn at the same perchlorate concentrations for the fetus as the mother, since the predictions for the fetus also become uncertain at those concentrations. Simulation results are shown in grey for both the mother and fetus when the maternal level is below 10.6 pM.

Formula Fed Infant

The next set of results are for the formula-fed infant (**Figure 11** and **Figure 12**) with an iodine concentration of 108 µg/L (the lowest level measured among 8 infant formulas by Pearce et al. (2004). In **Figure 11** the maternal iodide refers to the level of the mother's ingestion during pregnancy, which was used to set the initial condition. For these simulations, the pregnant mother also was assumed to have received the same level of perchlorate exposure (in µg/kg/d) as the infant did after birth; for example the results for 4 µg/kg/d perchlorate exposure in infants in **Figure 11** assumed that the mother was exposed to 4 µg/kg/d perchlorate during pregnancy, and the results from that pregnancy simulation were used to set the initial condition for these infant simulations. The bottom of the reference range is defined as the predicted 10th percentile using the lambda-mu-sigma (LMS) transformed Gaussian (normal) distribution derived from Lem et al. (2012) as described in the Technical Introduction (Table 1) and Appendix E. The age-specific mean was defined by model predictions for a formula-fed infant ingesting formula with an average concentration of 159 µg/L iodine (average of 8 formulas reported by Pearce et al. (2004)), born to a mother who ingested 200 µg/d iodide during pregnancy; i.e., an infant with fully sufficient iodine levels and intake. The skewness (λ) and age-specific variance (σ) estimated from the results of Lem et al. (2012) (**Table 1**) were applied with this model-derived age-dependent mean.

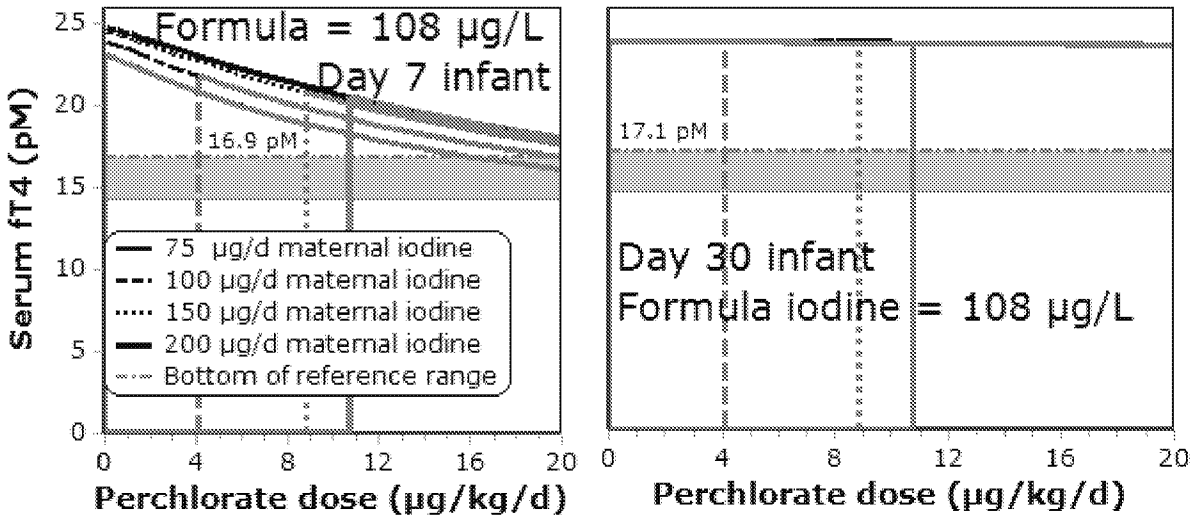

Figure 11. Free T4 in the formula fed infant at 7 and 30 days of age, given 108 µg/L iodine in formula, as a function of perchlorate exposure and maternal iodine ingestion during gestation.

The vertical lines mark the perchlorate exposure from Figure 10 where the pregnant mother's fT4 levels were predicted to fall below 10.6 pM (below the range of hypothyroxinemia) and model simulations are shown in grey beyond those points to emphasize their uncertainty. For 75 ug/d maternal iodine the pregnant mother's fT4 is below 10.6 pM with no perchlorate exposure, so the vertical line is at the y axis and the entire line is grey. For day 30 the results for all maternal iodine levels are indistinguishable, so the uncertainty with regard to the initial condition is reduced, assuming there is no long-term effect on the infant's thyroid function if the mother becomes hypothyroid during pregnancy. The grey band shows the assumed range for infant hypothyroxinemia.

However, as seen in **Figure 10**, a range of perchlorate exposures was predicted to cause the pregnant mother's fT4 levels to fall below the range of hypothyroxinemia for iodine intake between 100 and 250 µg/d, at which point the BBDR model is not valid. Hence the initial conditions for an infant whose mother ingested 150 µg/d iodine and was exposed to 9 µg/kg/d perchlorate, for example, is uncertain. The vertical lines in **Figure 11** mark the corresponding perchlorate exposure levels, and the model curves for 100-250 µg/d iodide to the right of these points are colored grey to emphasize this uncertainty. Since the pregnant mother is predicted to be hypothyroid when ingesting only 75 µg/d iodine, even with zero perchlorate exposure, the entire corresponding infant curve in **Figure 11** is colored grey.

Assuming that there are no longer term effects on infant's thyroid function, **Figure 11** illustrates that maternal iodine ingestion during pregnancy has only a modest effect on the results for the formula-fed infant at day 7, and by day 30 the initial iodide condition has almost no effect. The simulation results are sufficiently above the bottom of the infant's reference range to accommodate some uncertainty. Results for days 60 and 90 are likewise independent of initial iodide (not shown). For consistency with the analyses for other life stages, the subsequent plots (for higher formula iodine levels) used the 200 µg/d maternal iodine initial condition to set the initial condition for the formula-fed infant.

Figure 12 shows that the range of iodine levels in formula is sufficient to almost entirely offset the effects of perchlorate exposure at 30, 60, and 90 days: the predicted serum concentrations of fT4 were essentially independent of perchlorate exposure and there was very little difference among predictions for 108-384 µg/L iodine. As in **Figure 11**, the initial condition in these simulations was set assuming the same perchlorate exposure in the pregnant mother, hence to the fetus, as to the formula-fed infant, but with a fully sufficient maternal iodine intake (200 µg/d). The steeper dose-response for the day 7 infant was therefore the result of that in utero exposure with border-line inadequate maternal iodine ingestion.

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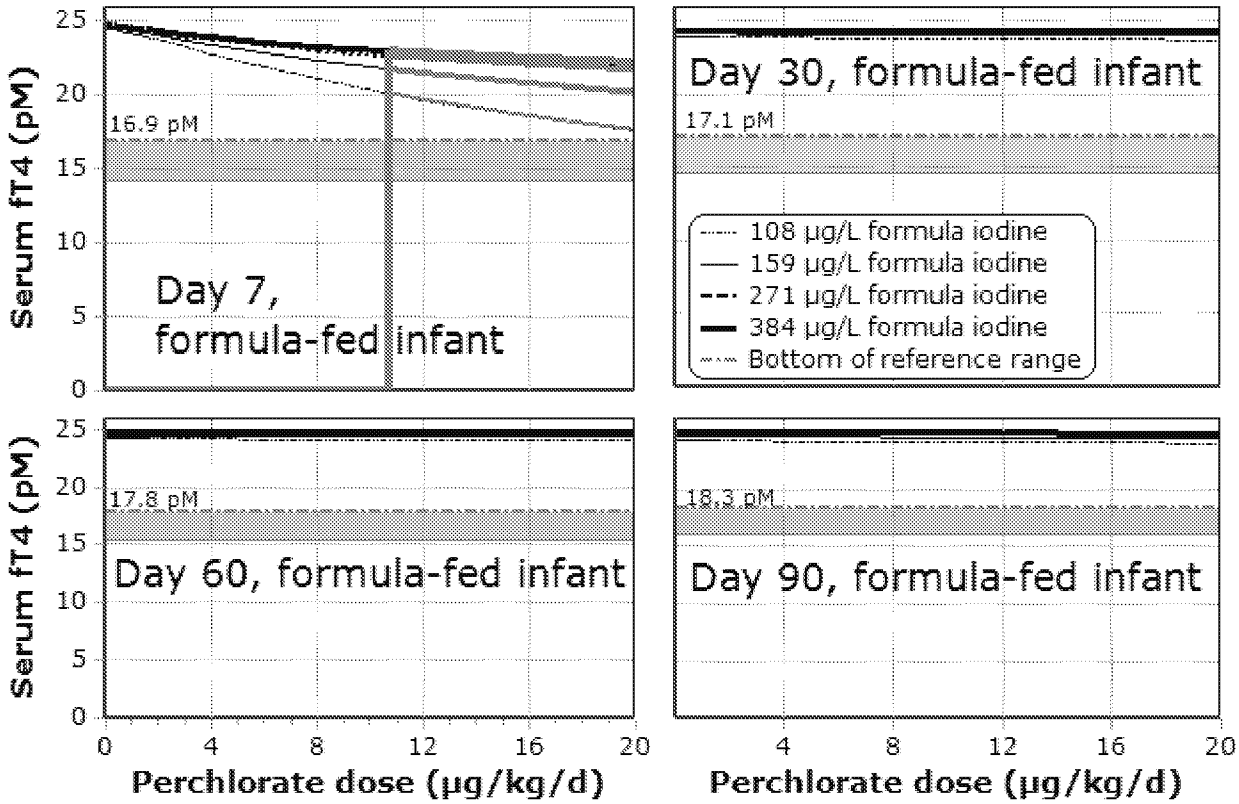

Figure 12. Free T4 in the formula fed infant, given varying iodine in formula, as a function of perchlorate exposure.

Levels of perchlorate, iodine, and thyroid hormones at birth are set assuming the same perchlorate dose to the pregnant mother and 200 µg/d iodine. The vertical line at 8.85 µg/kg/d on the day 7 plot is the level at which the pregnant mother's fT4 level falls below her reference range (i.e., below 10.6 pM), making model predictions for higher perchlorate levels uncertain. So predictions for higher perchlorate levels above that point are shown in grey. Initial conditions had little effect on model predictions for days 30-90. The grey band shows the assumed range for infant hypothyroxinemia.

Breast-Fed Infant

Results for the breast-fed infant are shown in **Figure 13**. The iodine (and perchlorate) levels given are for the mother both during pregnancy and lactation.

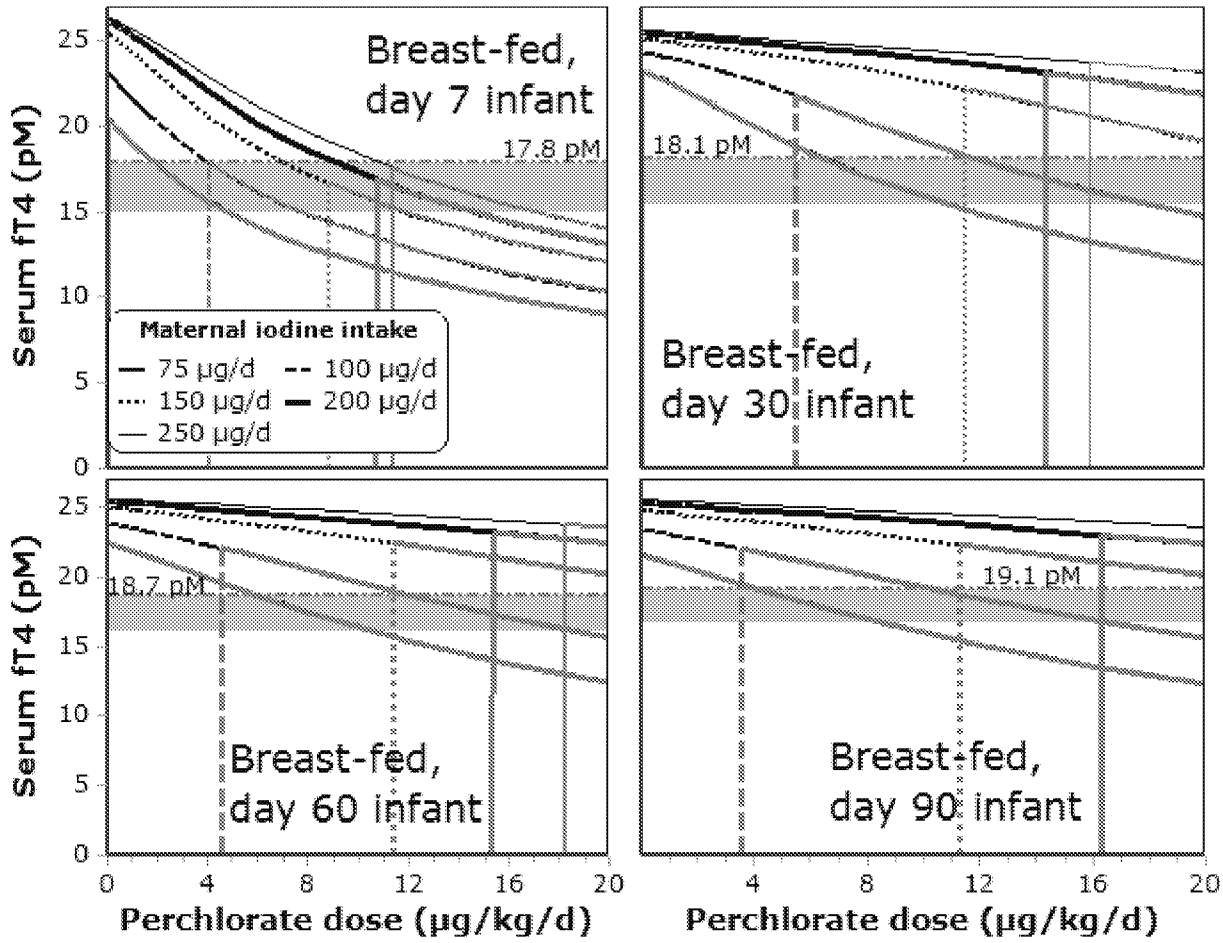

Figure 13. Free T4 (fT4) in the breast-fed infant, as a function of maternal perchlorate exposure and iodine ingestion.

Levels of perchlorate, iodide, and thyroid hormones at birth were set assuming the same perchlorate and iodide doses to the mother (and fetus) when pregnant. The grey horizontal band shows the range over which hypothyroxinemia is assumed to occur, from the 10th percentile of the age-dependent reference distribution (labelled by that fT4 level) down to 2.5th percentile. The vertical lines mark the perchlorate level beyond which the lactating mother falls below her hypothyroxinemic range, making the model predictions uncertain for perchlorate exposure above that point. Simulation results for the out-of-range conditions (perchlorate levels higher than the vertical lines) are in grey.

For the day 7 infant the vertical lines mark the perchlorate exposures above which model predictions become uncertain, because fT4 levels in the pregnant mother are predicted to be below the hypothyroxinemic range. For days 30-90 the vertical lines are the exposure levels at which the lactating mother's fT4 levels fall below her hypothyroxinemic range (details in next section). Hence the predicted iodide (and T4) levels in the ingested breast milk become uncertain above those perchlorate levels, and the model curves are shown in grey. Since the infant's fT4 levels are predicted to fall into the infant's hypothyroxinemic range (and below) for some of the iodine and perchlorate levels that were simulated, the potential impact of this uncertainty is more significant.

To better understand why the breast-fed infant shows such a strong dose-response compared to the formula-fed infant, the time-evolution of serum fT4 levels in the breast- and formula-fed infants for various levels of maternal and formula iodine ingestion, respectively, with no perchlorate exposure is shown in **Figure 14**. The formula-fed infant simulations assumed a low level of iodine nutrition during pregnancy, 75 µg/d. Despite that low initial value, even the lowest formula iodine that was simulated (108 µg/L) is sufficient for a quick recovery of the infants to levels near those for breast-fed infants whose mothers consume higher iodine levels: 150-250 µg/d (**Figure 14**). But breast-fed infants of mothers who consume 100 µg/d or less iodine have slightly lower predicted levels of fT4, which declines incrementally from about PND 30 onward (**Figure 14**). The predicted decrement is modest when the mother consumes 100 µg/d iodine, but the iodide levels are no longer saturating thyroid hormone production: the decrease from 150 to 100 µg/d iodine (50 µg/d difference) has very little effect on fT4, but a further decrease of only 25 µg/d iodine (from 100 to 75 µg/d) more significantly affects the infant's fT4 levels (**Figure 14**). Thus, the iodide levels and thyroidal stores for infants whose mothers ingest only 100 µg/d iodine are already compromised (though they may be sufficient for healthy

development in the absence of perchlorate), hence these infants are more sensitive to any further decrease due to inhibition by perchlorate (Figure 13).

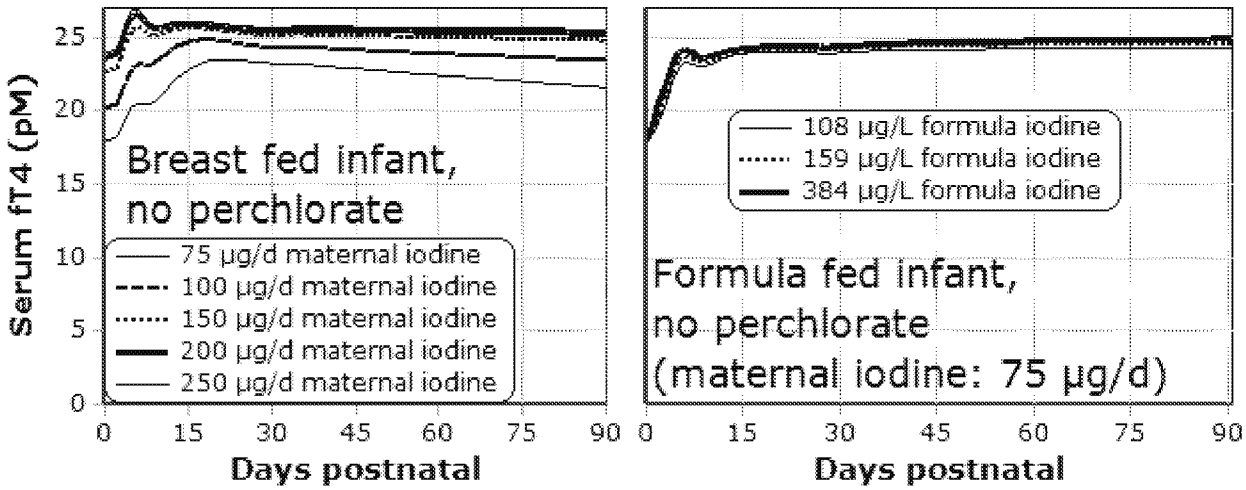

Figure 14. Evolution of serum fT4 over time in breast-fed and formula fed infants with no perchlorate exposure, but varying levels of iodine ingestion.

Another way to understand the sensitivity of formula- vs. breast-fed infants is to compare their intake of iodine vs. perchlorate on a µg/kg/d basis. For infants ingesting formulas with iodine concentrations of 108 µg /L or 384 µg/L the average iodine dose over the 90 day postnatal period is predicted to be 20 or 69 µg/kg/d, respectively. This iodine dose rate is equal or > 3 times a perchlorate exposure of 20 µg/kg/d (maximum evaluated here). For breast-fed infants whose mothers ingested 20 µg/kg/d perchlorate, the predicted average daily doses of perchlorate is 183 µg/kg/d; i.e., 9 times higher than direct maternal exposure to 20 µg/kg/d. Further, while the predicted iodide concentration in breast milk was ~ 140 µg/L for a mother ingesting 250 µg/d iodine averaged, comparable to the infant formulas, when a mother ingests only 75 µg/d iodine, the predicted iodide concentration in her milk was only ~ 50 µg/L, much lower than available in formulas.

Perchlorate exposure in the range of 0-20 µg/kg/d also reduces breast milk iodide levels by a few percent. When the mother ingests 75 µg/d iodine and 20 µg/kg/d perchlorate, the model predicts that the infant only gets an average of 7.6 µg/kg/d iodine and that between birth and day 90 the infant ingests 25 ng perchlorate per µg of iodide. When the mother ingests 200 µg/d iodine and 20 µg/kg/d perchlorate, the model predicts that the infant gets an average of 17.5 µg/kg/d iodine and that the infant ingests 11 ng perchlorate per µg of iodide between birth and day 90. While this difference in relative dose is only a bit more than 2-fold, for breast milk or formula iodine concentrations above 100 µg/L infant T3 and T4 production becomes saturated, so a small decrement in iodine uptake by the formula- or breast-fed infant thyroid has little effect on T3 and T4 levels. But when the milk (or formula) concentration is below 100 µg/L iodine, the infant's TH levels become more sensitive to perchlorate.

Lactating Mother

Predicted changes in free T4 in the lactating mother due to perchlorate exposure are shown in **Figure 15**. The control maternal fT4 levels (i.e., for zero perchlorate exposure) in **Figure 15**, depended on maternal iodide intake, including iodine ingestion during pregnancy. There was a clear decrease in control maternal fT4 between day 7 and 30; control maternal fT4 levels increased somewhat between days 30, 60, and 90, but remained below the day 7 levels. This reduction in control levels was likely due to iodide excretion in breast-milk.

Figure 15 also shows that iodine ingestion levels during pregnancy and lactation (75-250 µg/d) have a larger effect on maternal fT4 levels than the range of formula iodine concentrations (108-384 µg/L) had on the PND 30-90 formula-fed infant (**Figure 12**). This difference in sensitivity to the iodine ranges exists across the range of perchlorate doses analyzed. (Recall that the internal iodide and TH levels at PND 0 are determined by ingestion during pregnancy.) The

predicted sensitivity of the lactating mother to perchlorate exposure exists precisely because her fT4 levels are sensitive to iodine intake, hence iodide availability to the thyroid.

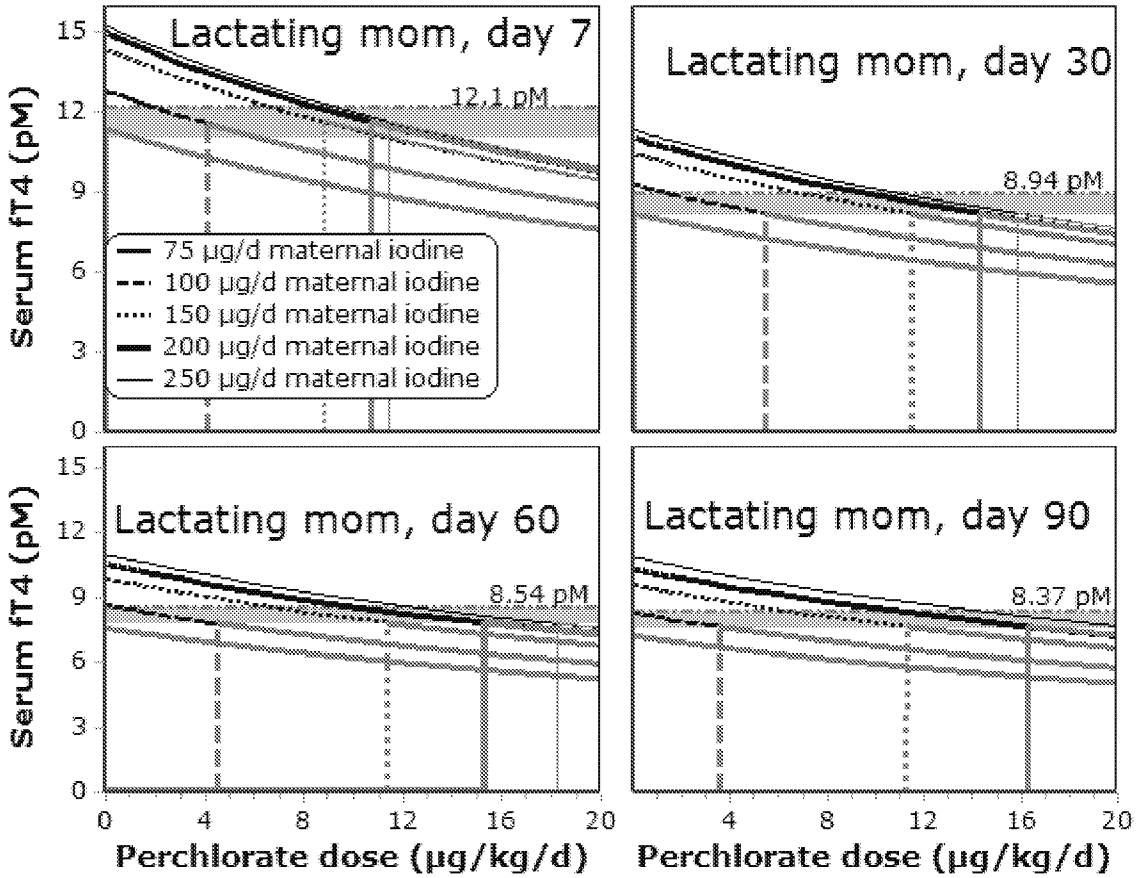

Figure 15. Free T4 (fT4) in the lactating mother, as a function of maternal perchlorate exposure and iodine ingestion.

Levels of perchlorate, iodide, and thyroid hormones at birth were set assuming the same perchlorate and iodine doses to the mother when pregnant. The annotated horizontal lines mark the bottom of the provisional reference range for each PND. They grey bar marks the assumed range for hypothyroxinemia.

For day 7 the vertical lines mark the perchlorate levels for which the *pregnant mother* is predicted to fall below 10.6 pM, i.e., below the range of hypothyroxinemia, making the initial conditions for the lactation model uncertain. This uncertainty is assumed to impact the day 7 predictions, so simulation curves above the corresponding perchlorate levels are shown in grey.

For days 30-90 the vertical lines mark perchlorate levels where the *lactating mother* falls below the range of hypothyroxinemia (varies with PND), so the model is no longer valid and simulations become uncertain. Simulations above those levels are shown in grey.

While the pregnant mother's predicted fT4 levels in the absence of perchlorate (**Figure 10**) were higher than the lactating mother, except for PND 7 (**Figure 15**), the dose-response curve was steeper for the pregnant mother than for the lactating mother on PND 30-90. The dependence on iodine intake (i.e., the degree to which the curves for different iodine levels are separated in **Figure 10** and **Figure 15**) was similar for both life-stages, however. Hence it is not immediately clear whether the pregnant mother or the lactating woman is the more "sensitive" life-stage. The dose-response for the lactating mother on PND 7 likely reflects the effect of exposure during pregnancy to a large extent, hence the similarity in the shape of the relationship to perchlorate exposure: it is transitional between that for pregnancy and later in lactation. The fetus' dose-response was slightly stronger than the mother. This likely occurred because the model contains three avenues of impact on fetal T4: 1) perchlorate inhibits the uptake of iodide and hence T4 production by the fetal thyroid; 2) perchlorate inhibits the transfer of iodine into the placenta (hence iodine availability to the fetus); and 3) perchlorate reduces T4 in mother and thus the transfer of T4 to the fetus[11].

A detailed analysis of the dosimetry of perchlorate and iodide to the lactating mother and breast-fed infant vs. the formula-fed infant is provided in Appendix C. In summary, the µg/kg/d of iodine ingested by the lactating mother at her intake levels (less than 250 µg/d) is about 7-fold lower than the formula-fed infant. The much stronger effect predicted for the breast-fed infant compared with a formula fed infant receiving the same perchlorate dose (µg/kg/d) as the breast-feeding mother is assumed to be due to two factors: 1) significant concentration of perchlorate

[11] The pregnancy model predicts that the late-gestation fetus gets ~ 20% of its T4 from the mother.

in breast-milk; and 2) lower iodine ingestion by the infant from breast-milk when maternal iodine ingestion is low (which is further reduced by perchlorate, Figure 16) vs. from formula.

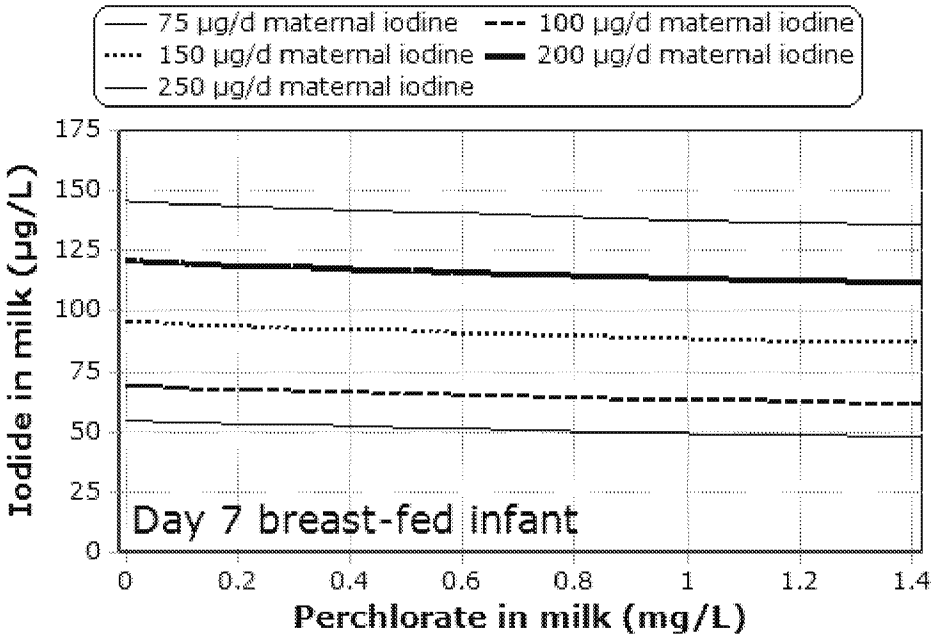

Figure 16. Predicted perchlorate vs. iodide concentrations in breast milk for varying maternal iodide ingestion levels.

While the dose-effect of perchlorate on lactational transfer of iodide described in Appendix C (Figure C-2; **Figure 16**) may seem stronger than expected, compared to epidemiological sampling studies (e.g., Leung et al. (2012)), it should be noted that the perchlorate dose-range evaluated here is much larger than most environmental exposures.

To confirm that the model predicts reasonable levels of breast milk iodide and is consistent with epidemiological studies, model results were compared to the data of Leung et al. (2012) who measured perchlorate and iodide in breast milk samples from breast-feeding mothers, mostly in the range of 30-90 days postnatal in **Figure 17**. The left-hand panel of Figure 17 shows milk iodide vs. milk perchlorate, with simulations for PND 60. In the range of perchlorate levels observed by Leung et al. (2012) the model predicts almost no effect of perchlorate on milk iodide levels, which agrees with the lack of trend obtained for those data.

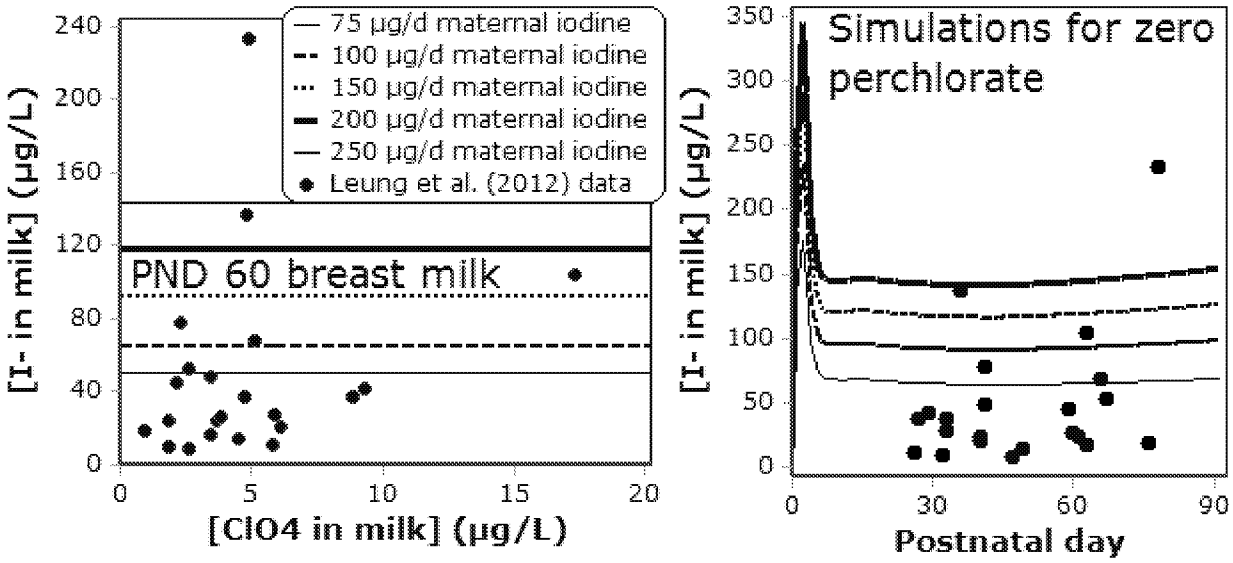

Figure 17. Model predictions of iodide vs. perchlorate concentrations in breast milk at low perchlorate exposure levels (left panel) and iodide in milk vs. PND with zero perchlorate exposure (right panel), for varying levels of maternal iodide ingestion.

The right-hand panel of **Figure 17** shows model predictions vs. PND for zero perchlorate, to illustrate that neither do the data show nor does the model predict a significant time-dependence in milk iodide after ~ PND 7. The median urinary iodine level of the subjects was 101.9 µg/L, but the range (27-570 µg/L) (Leung et al., 2012) indicates that many of the subjects were well below the level of sufficiency, 100 µg/L. It is possible that the subjects were more iodine deficient than the levels evaluated with the model. Alternatively, the stability of iodine in breast milk declined as much as 50% after three days of storage at 4°C (observed by Dr. Benjamin Blount, CDC; personal communications) which may explain the reported low iodide levels (**Figure 17**).

LOCAL SENSITIVITY ANALYSIS OF BBDR MODEL WITH PERCHLORATE

A local sensitivity analysis was conducted for the formula-fed and breast-fed infant to determine which parameters were most influential on the plasma concentrations of T4 and fT4 in the infant. The infant and mother's initial iodide and perchlorate concentrations were set based on the end of pregnancy values for the Lumen et al. (2013) model according to the maternal exposure conditions given in **Table 9** and **Table 10**, but the limited exposure-dependence in the values obtained for the cases evaluated here indicates that the additional information one might gain from further analyses is limited. Therefore analyses were limited to either low maternal iodine with low infant iodide exposure or high maternal iodine with high infant iodide exposure (**Table 9** and **Table 10**,). The BBDR model described in this report was used to run exposure scenarios in the infant from birth through 90 days, and the normalized sensitivity coefficients were calculated for all parameters in the model based on a 1% change from default values.

Table 9: Initialization conditions and formula-fed exposures for sensitivity analysis

Maternal Pregnancy Exposure for Initial Values		Infant exposure	
Iodide (µg/d)	Perchlorate (µg/kg/d)	Iodide formula (µg/L)	Perchlorate (µg/kg/d)
75	0	108	0
75	1	108	1
75	20	108	20
200	0	384	0
200	1	384	1
200	20	384	20

Table 10: Initialization conditions and breast-fed exposure scenarios for sensitivity analysis

Maternal Pregnancy Exposure for Initial Values		Breastfeeding Mother Exposure	
Iodide (µg/d)	Perchlorate (µg/kg/d)	Iodide (µg/d)	Perchlorate (µg/kg/d)
75	0	75	0
75	1	75	1
75	20	75	20
200	0	200	0
200	1	200	1
200	20	200	20

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The sensitivity analysis results for total T4 and fT4 are identical, except that total T4 is not sensitive to FRCONVT4 (mother) or FRCONVT4_N (infant), which define the relationship between fT4 and T4. Therefore only results for fT4 are explicitly described here. The sensitivity coefficients for fT4 in the formula- and breast-fed infants are listed in **Table 11** and **Table 12**, respectively, and shown graphically in **Figure 18** (formula-fed infant), **Figure 19** (infant parameters' influence on breast-fed infant), and **Figure 20** (maternal parameters' influence in breast-fed infant). As seen in these graphs, the influential parameters were time-dependent, iodine-dependent, and perchlorate-dependent. The influence of perchlorate on iodine was more significant in the breast-fed exposures because the low predicted iodide transferred in milk at the lower maternal exposure of 75 μg/d.

Figure 18. Normalized sensitivity coefficients for fT4 in the formula-fed infant at Day 7, 30, 60, and 90.

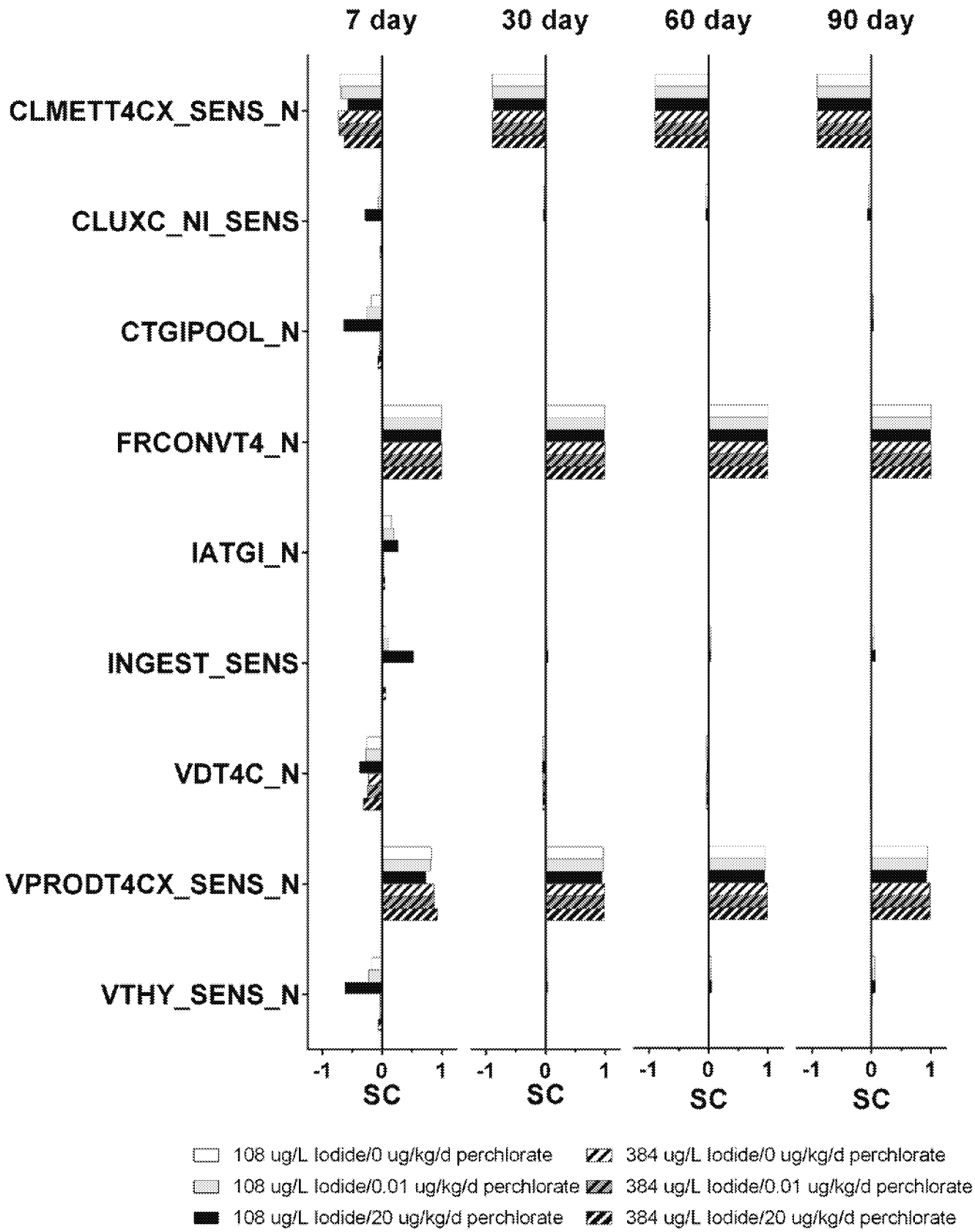

Figure 19. Normalized sensitivity coefficients for infant parameters on fT4 in the breast-fed infant at Day 7, 30, 60, and 90.

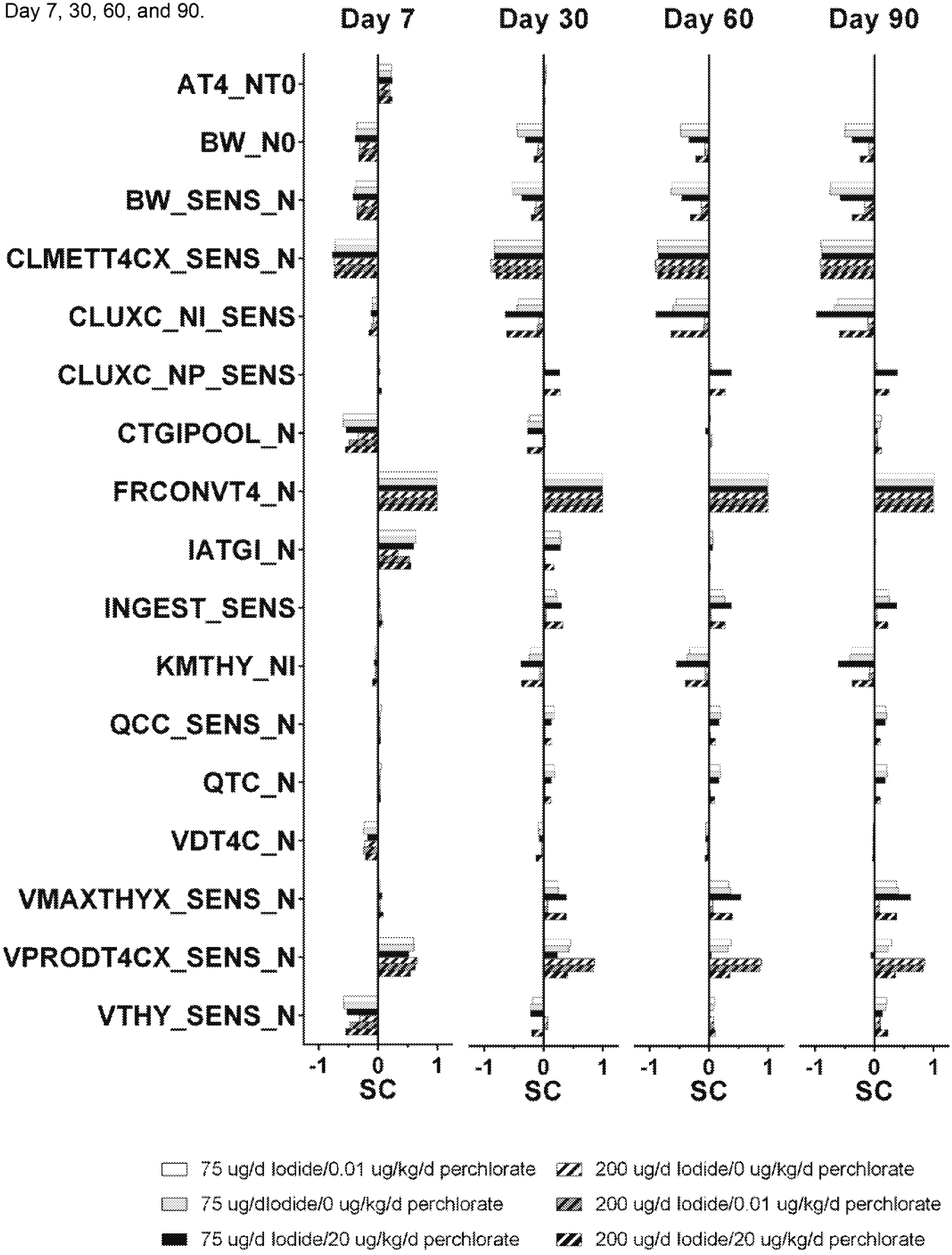

Figure 20. Normalized sensitivity coefficients for maternal parameters on fT4 in the breast-fed infant at Day 7, 30, 60, and 90.

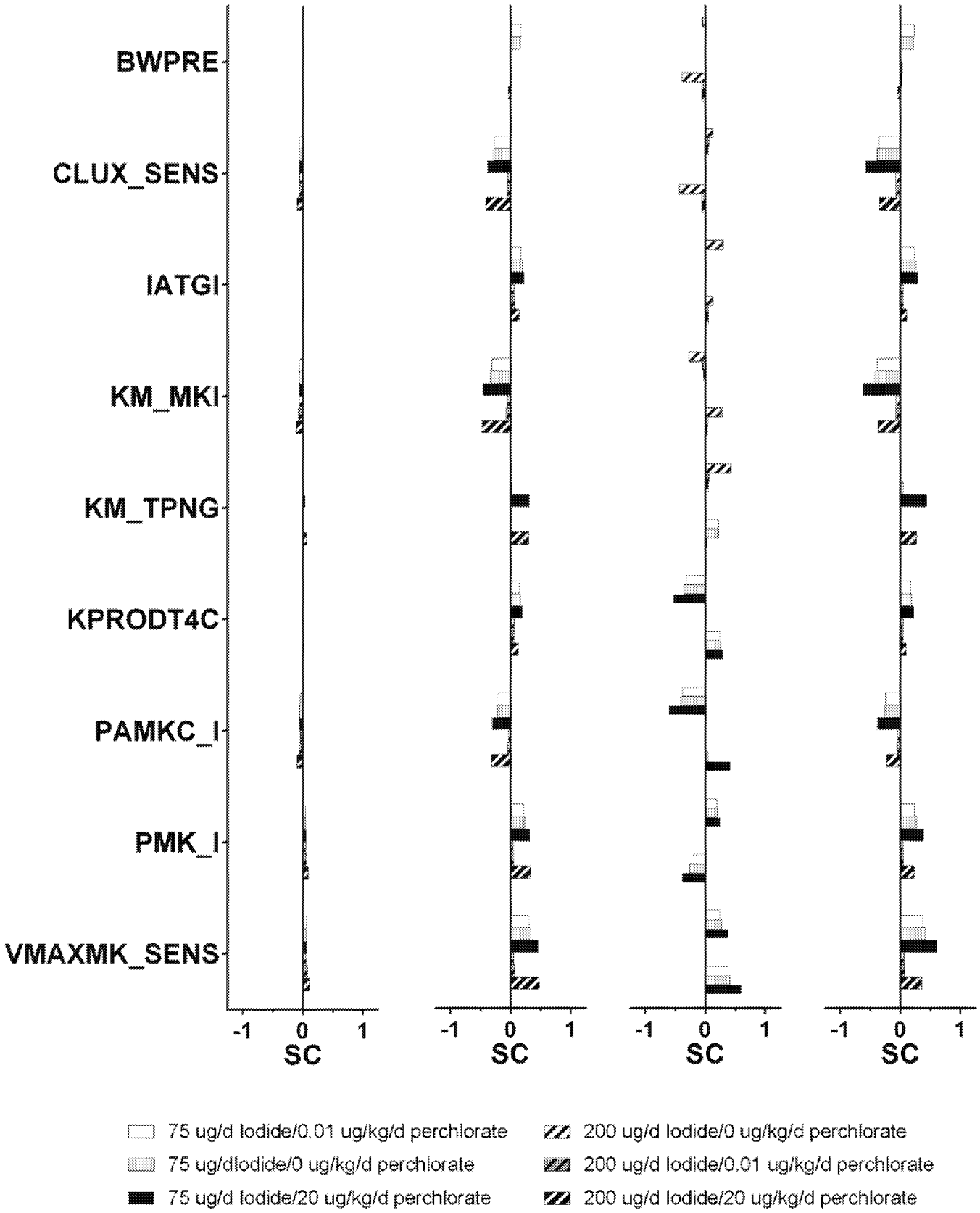

Table 11: Normalized sensitivity coefficients for fT4 in formula fed infant

Parameter	Normalized Sensitivity Coefficients						
		Formula iodide concentration (µg/L)					
		108			384		
		Infant perchlorate dose (µg/kg/d)					
	Day	0	0.01	20	0	0.01	20
CLMETT4CX_SENS_N	7	-0.71	-0.69	-0.58	-0.73	-0.72	-0.64
	30	-0.89	-0.89	-0.88	-0.89	-0.89	-0.89
	60	-0.9	-0.9	-0.9	-0.9	-0.9	-0.9
	90	-0.91	-0.91	-0.91	-0.91	-0.91	-0.91
CLUXC_NI_SENS	7	-0.05	-0.06	-0.29	0	0	-0.03
	30	-0.03	-0.03	-0.04	0	0	0
	60	-0.04	-0.04	-0.05	0	0	0
	90	-0.05	-0.05	-0.07	0	0	0
CTGIPOOL_N	7	-0.18	-0.25	-0.65	-0.03	-0.04	-0.07
	30	0.02	0.02	0.02	0	0	0
	60	0.03	0.03	0.03	0	0	0
	90	0.03	0.03	0.04	0	0	0
FRCONVT4_N	7	1	1	1	1	1	1
	30	1	1	1	1	1	1
	60	1	1	1	1	1	1
	90	1	1	1	1	1	1
IATGI_N	7	0.16	0.2	0.28	0.03	0.03	0.05
	30	0	0	0	0	0	0
	60	0	0	0	0	0	0
	90	0	0	0	0	0	0
INGEST_SENS	7	0.07	0.11	0.54	0	0.01	0.06
	30	0.03	0.03	0.05	0	0	0
	60	0.04	0.04	0.05	0	0	0
	90	0.05	0.05	0.07	0	0	0
VDT4C_N	7	-0.25	-0.27	-0.38	-0.23	-0.24	-0.31
	30	-0.04	-0.04	-0.06	-0.04	-0.04	-0.04
	60	-0.03	-0.03	-0.03	-0.03	-0.03	-0.03
	90	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02
VPRODT4CX_SENS_N	7	0.83	0.82	0.74	0.87	0.88	0.93
	30	0.97	0.97	0.96	0.99	0.99	0.99
	60	0.96	0.96	0.95	0.99	0.99	0.99
	90	0.95	0.95	0.93	0.99	0.99	0.99
VTHY_SENS_N	7	-0.17	-0.23	-0.62	-0.03	-0.03	-0.06
	30	0.03	0.03	0.04	0	0	0
	60	0.05	0.05	0.06	0.01	0.01	0.01
	90	0.06	0.06	0.07	0.01	0.01	0.01

Table 12: Normalized sensitivity coefficients for fT4 in the breast fed infant

Parameter	Day	Normalized Sensitivity Coefficients					
		Maternal iodide intake (µg/d)					
		75			200		
		Maternal perchlorate dose (µg/kg/d)					
		0	0.01	20	0	0.01	20
AT4_NT0	7	0.22	0.23	0.25	0.19	0.21	0.24
	30	0.04	0.05	0.04	0	0	0.02
	60	0	0.01	0.01	0	0	0
	90	0	0	0	0	0	0
BWPRE	7	0.01	0.02	0	0	0.01	-0.01
	30	0.17	0.16	0	0.01	0.01	-0.04
	60	0.22	0.22	0.03	0.01	0.01	-0.04
	90	0.23	0.22	0.02	0.02	0.02	-0.04
BW_N0	7	-0.37	-0.35	-0.39	-0.31	-0.33	-0.33
	30	-0.45	-0.44	-0.31	-0.09	-0.1	-0.16
	60	-0.48	-0.48	-0.34	-0.07	-0.08	-0.22
	90	-0.49	-0.5	-0.38	-0.09	-0.09	-0.24
BW_SENS_N	7	-0.39	-0.37	-0.42	-0.33	-0.35	-0.35
	30	-0.53	-0.52	-0.37	-0.13	-0.15	-0.21
	60	-0.63	-0.64	-0.46	-0.13	-0.13	-0.31
	90	-0.74	-0.75	-0.58	-0.16	-0.17	-0.38
CLMETT4CX_SENS_N	7	-0.72	-0.72	-0.77	-0.74	-0.72	-0.74
	30	-0.83	-0.82	-0.84	-0.9	-0.89	-0.8
	60	-0.87	-0.86	-0.86	-0.9	-0.9	-0.86
	90	-0.9	-0.89	-0.89	-0.91	-0.91	-0.9
CLUXC_NI_SENS	7	-0.09	-0.09	-0.12	-0.08	-0.1	-0.15
	30	-0.42	-0.45	-0.65	-0.08	-0.1	-0.62
	60	-0.55	-0.61	-0.9	-0.08	-0.09	-0.64
	90	-0.62	-0.68	-0.98	-0.11	-0.12	-0.59
CLUXC_NP_SENS	7	0	0.03	0.04	0	0	0.06
	30	0	0.03	0.28	0	0	0.28
	60	0	0.04	0.38	0	0	0.27
	90	0	0.04	0.4	0	0	0.25
CLUX_SENS	7	-0.05	-0.05	-0.06	-0.05	-0.06	-0.09
	30	-0.26	-0.28	-0.39	-0.05	-0.06	-0.41
	60	-0.32	-0.36	-0.53	-0.05	-0.06	-0.39
	90	-0.36	-0.39	-0.58	-0.07	-0.08	-0.35
CTGIPOOL_N	7	-0.59	-0.59	-0.54	-0.33	-0.49	-0.55
	30	-0.24	-0.27	-0.27	0.03	0.03	-0.27
	60	0.02	0	-0.06	0.04	0.05	0
	90	0.12	0.11	0.06	0.05	0.06	0.12

Table 12: Normalized sensitivity coefficients for fT4 in the breast fed infant (cont'd)

FRCONVT4_N	7	1	1	1	1	1	1
	30	1	1	1	1	1	1
	60	1	1	1	1	1	1
	90	1	1	1	1	1	1
IATGI	7	0.03	0.03	0.03	0.02	0.03	0.03
	30	0.18	0.2	0.23	0.06	0.07	0.14
	60	0.24	0.26	0.3	0.05	0.06	0.13
	90	0.24	0.26	0.29	0.05	0.05	0.11
IATGI_N	7	0.65	0.65	0.61	0.34	0.53	0.56
	30	0.28	0.3	0.29	0	0.02	0.17
	60	0.06	0.07	0.07	0	0	0.02
	90	0.01	0.02	0.02	0	0	0
INGEST_SENS	7	0.04	0.04	0.05	0.04	0.06	0.08
	30	0.21	0.23	0.31	0.04	0.05	0.32
	60	0.24	0.27	0.38	0.03	0.04	0.27
	90	0.24	0.26	0.38	0.04	0.05	0.23
KMTHY_NI	7	-0.04	-0.05	-0.07	-0.04	-0.05	-0.09
	30	-0.23	-0.25	-0.39	-0.06	-0.07	-0.37
	60	-0.33	-0.37	-0.55	-0.06	-0.07	-0.4
	90	-0.38	-0.42	-0.61	-0.08	-0.09	-0.38
KM_MKI	7	-0.06	-0.06	-0.07	-0.06	-0.07	-0.11
	30	-0.31	-0.33	-0.46	-0.05	-0.07	-0.48
	60	-0.37	-0.41	-0.6	-0.05	-0.06	-0.43
	90	-0.39	-0.42	-0.62	-0.06	-0.07	-0.37
KM_TPNG	7	0	0	0.05	0	0	0.07
	30	0	0.02	0.31	0	0	0.3
	60	0	0.04	0.42	0	0	0.3
	90	0	0.04	0.44	0	0	0.27
KPRODT4C	7	0.02	0.02	0.03	0.02	0.02	0.03
	30	0.15	0.16	0.2	0.06	0.06	0.13
	60	0.19	0.21	0.25	0.05	0.05	0.12
	90	0.18	0.19	0.23	0.04	0.05	0.1
PAMKC_I	7	-0.04	-0.05	-0.06	-0.04	-0.06	-0.09
	30	-0.21	-0.23	-0.31	-0.04	-0.05	-0.32
	60	-0.23	-0.26	-0.38	-0.03	-0.04	-0.27
	90	-0.24	-0.26	-0.38	-0.04	-0.05	-0.23
PMK_I	7	0.05	0.05	0.06	0.05	0.06	0.09
	30	0.21	0.23	0.32	0.04	0.05	0.33
	60	0.24	0.27	0.39	0.03	0.04	0.28
	90	0.24	0.27	0.39	0.04	0.05	0.23
QCC_SENS_N	7	0.05	0.06	0.04	0.03	0.05	0.05
	30	0.18	0.18	0.14	0.02	0.02	0.13
	60	0.18	0.2	0.17	0.01	0.02	0.11
	90	0.2	0.21	0.19	0.02	0.02	0.1

Table 12: Normalized sensitivity coefficients for fT4 in the breast fed infant (cont'd)

QTC_N	7	0.05	0.06	0.04	0.03	0.05	0.05
	30	0.18	0.19	0.14	0.02	0.02	0.12
	60	0.19	0.2	0.17	0.01	0.02	0.1
	90	0.21	0.22	0.19	0.02	0.02	0.1
VDT4C_N	7	-0.24	-0.23	-0.18	-0.22	-0.24	-0.21
	30	-0.09	-0.09	-0.08	-0.03	-0.04	-0.13
	60	-0.05	-0.05	-0.06	-0.03	-0.03	-0.07
	90	-0.02	-0.02	-0.03	-0.02	-0.02	-0.03
VMAXMK_SENS	7	0.06	0.06	0.07	0.06	0.08	0.11
	30	0.31	0.34	0.46	0.05	0.07	0.48
	60	0.37	0.41	0.6	0.05	0.06	0.43
	90	0.38	0.42	0.62	0.06	0.07	0.36
VMAXTHYX_SENS_N	7	0.04	0.04	0.07	0.04	0.05	0.09
	30	0.23	0.25	0.39	0.06	0.07	0.38
	60	0.33	0.37	0.55	0.06	0.07	0.4
	90	0.38	0.41	0.62	0.08	0.09	0.38
VPRODT4CX_SENS_N	7	0.61	0.6	0.52	0.66	0.63	0.55
	30	0.46	0.43	0.24	0.86	0.84	0.41
	60	0.38	0.32	0.05	0.88	0.86	0.36
	90	0.3	0.24	-0.06	0.85	0.83	0.36
VTHY_SENS_N	7	-0.58	-0.58	-0.53	-0.31	-0.48	-0.54
	30	-0.19	-0.22	-0.22	0.07	0.07	-0.2
	60	0.1	0.08	0.02	0.08	0.09	0.11
	90	0.21	0.2	0.15	0.1	0.11	0.23

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Appendices

Biologically Based Dose Response Models for the Effect of Perchlorate on Thyroid Hormones in the Infant, Breast Feeding Mother, Pregnant Mother, and Fetus: Model Development, Revision, and Preliminary Dose-Response Analyses

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Appendix A: Parameter Definitions

PARAMETER		DEFINITION
Maternal	Infant	
(n/a)	IABODY_NI	Birth amount of iodide in rest of body
AT3_T0	AT3_NT0	Initial amt. of T3 in distribution volume
AT4_T0	AT4_NT0	Initial amt. of T4 in distribution volume
IATGI	IATGI_N	Initial thyroidal organified iodide stores
CTGIPOOL	CTGIPOOL_N	Euthyroid organified iodide stores
BWPRE	BW_N0	Pre-pregnancy body weight; initial infant BW
(n/a)	BW_SENS_N	SC for body weight over time
CLUX_SENS*	CLUXC_NI_SENS*, CLUXC_NP_SENS*	SC for urinary clearance of iodide in mother and infant, and for perchlorate in infant
CLUC_P	(n/a)	Scaled urinary clearance rate for perchlorate
CLFECEST3C	CLFECEST3C_N	Fecal clearance of T3
CLFECESFT4C	CLFECEST4C_N	Fecal clearance of T4
CLMILKT3C	(n/a)	Clearance of T3 to milk
CLMILKFT4C	(n/a)	Clearance of T4 to milk
CLUT3C	(n/a)	Scaled urinary clearance of T3
CLUFT4C	CLUFT4C_N	Scaled urinary clearance of T4
FRCONVT4	FRCONVT4_N	Ratio of free T4 to total T4
IABIND_P	IABIND_NP	Initial amount of bound perchlorate in plasma
IAF_P	IAF_NP	Initial amt. of fat perchlorate
IAG_P	IAG_NP	Initial amt. of gastric tissue perchlorate
IAGB_P	IAGB_NP	Initial amt. of gastric plasma perchlorate
IAGJ_P	IAGJ_NP	Initial amt. of gastric juice perchlorate
IAK_P	IAK_NP	Initial amt. of kidney perchlorate
IAL_P	IAL_NP	Initial amt. of liver perchlorate
IAMB_I, IAMB_P	(n/a)	Initial amt. of mammary blood iodide, perchlorate
IAMK_I, IAMK_P	(n/a)	Initial amt. of milk iodide, perchlorate
IAPLAS_I, IAPLAS_P	IAPLAS_NI, IAPLAS_NP	Initial plasma iodide, FREE (unbound) perchlorate
IAR_I, IAR_P	(n/a), IAR_NP	Initial amt. of richly perfused iodide, perchlorate
IARBC_P	IARBC_NP	Initial amt. of red blood cell perchlorate
IAS_I, IAS_P	(n/a), IAS_NP	Initial amt. of slowly perfused iodide, perchlorate
IASK_P	IASK_NP	Initial amt. of skin tissue perchlorate
IASKB_P	IASKB_NP	Initial amt. of skin plasma perchlorate
IAT_FI	IAT_NFI	Initial amount of FREE thyroid iodide
IAT_P	IAT_NP	Initial amt. of thyroid tissue perchlorate
IATB_I, IATB_P	IATB_NI, IATB_NP	Initial amt. of thyroid plasma iodide, perchlorate
(n/a)	INGEST_SENS*	Sensitivity constant for ingested volume of formula (VFORMULAC) or breast milk (KTRANS)
KBINDC_I	KBINDC_NI	2nd order rate constant for organification of free iodide in thyroid

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Maternal parameter	Infant parameter	Definition
KMETT3C	CLMETT3CX_SENS_N	1st order(mom) T3 degradation rate; SC infant clearance(CLMETT3CX_N)
KMETT4C	CLMETT4CX_SENS_N	1st order (mom) T4 degradation rate; SC infant clearance (CLMETT4CX_N)
KM_BPNG	KM_BNPNG	Michaelis Menten (M-M) constant for plasma binding
KM_GPNG	KM_GNPNG	M-M constant for gastric uptake (set = KM_TPNG)
KM_MKI, KM_MKPNG	(n/a)	M-M constant for iodide, perchlorate uptake into milk = KM_TPNG)
KM_SPNG	KM_SNPNG	M-M constant for skin NIS transport (= KM_TPNG)
KMTHY_I, KM_TPNG	KMTHY_NI, KM_TNPNG	M-M constant for NIS iodide, perchlorate transport in thyroid
KPRODT3C	VPRODT3F_N	1st order rate constant for production of T3; ratio of T3:T4 production in infant* VPRODT3F = VPRODT3F_N* VPRODT4CX(t)*BW_N
KPRODT4C	VPRODT4CX_SENS_N*	1st order rate constant for production of T4; SC for infant zero order T4 production (VPRODT4CX_N)
KUNBC_P	KUNBC_NP	Diffusion rate constant for perchlorate from bound compartment to plasma
PATHYC_I, PATC_P	PATHYC_NI, PATC_NP	Scaled permeability-surface area (PA) product for iodide, perchlorate diffusion between thyroid blood and thyroid tissue
PAGC_P	PAGC_NP	PA product between GI blood and tissue
PAGJC_P	PAGJC_NP	PA product for perchlorate between gastric tissue and gastric juice
PAMKC_I, PAMKC_P	(n/a)	PA product for iodide, perchlorate between milk and mammary plasma
PARBCC_P	PARBCC_NP	PA product for perchlorate into red blood cells
PASKC_P	PASKC_NP	PA product for perchlorate into skin
(n/a)	PBODY_NI	Rest of body tissue: plasma partition coefficient (PC) for iodide
PF_P	PF_NP=PF_P	Fat tissue: plasma partition PC for perchlorate
PG_P	PG_NP	Gastric tissue: plasma PC for perchlorate
PGJ_P	PGJ_NP	Gastric juice: tissue PC for perchlorate
PK_P	PK_NP=PK	Kidney: plasma PC for perchlorate
PL_P	PL_NP = PL_P	Liver: plasma PC for perchlorate
PMK_I, PMK_P	(n/a)	Milk: mammary plasma PC for iodide, perchlorate
PRP_I , PR_P	(n/a),PR_NP = PR_P	Richly perfused: plasma PC for perchlorate, iodide
PRBC_P	PRBC_NP = PRBC_P	Red blood cells: plasma PC for perchlorate
PSP_I , PS_P	(n/a), PS_NP = PS_P	Slowly perfused: plasma PC for perchlorate, iodide
PSK_P	PSK_NP = PSK_P	Skin: plasma PC for perchlorate
PTHY_I, PT_P	PTHY_NI, PT_NP=PT_P	Thyroid tissue: plasma PC for iodide, perchlorate
QCC	QCC_SENS_N*	Scaled cardiac output; SC for absolute cardiac output
QFC	(QFC*VFC_N/VFC)	Fractional plasma flow to fat

Maternal parameter	Infant parameter	Definition
QGC	(QGC*VGC_N/VGC)	Fractional plasma flow to gastric tissue
QKC	(QKC*VKC_N/VKC)	Fractional plasma flow to kidney
QLC	(QLC*VLC_N/VLC)	Fractional plasma flow to liver
QMC	(n/a)	Fractional plasma flow to mammary gland
QRC	QRC_N	Fractional plasma flow to richly perfused
QSC	QSC_N	Fractional plasma flow to slowly perfused
QSKC	(QSKC*VSKC_N/VSKC)	Maternal fractional plasma flow to skin
QTC	QTC_N	Fractional plasma flow to thyroid
T3FRAC	T3FRAC_SENS_N*	Fraction of T4 metabolized to T3, SC (T3FRAC _N)
(n/a)	VBODYC_N	Rest of body volume fraction
VDT3C	VDT3C_N	Fractional volume of distribution for T3
VDT4C	VDT4C_N	Fractional volume of distribution for T4
VF_SENS*	VF_SENS_N*	Fat volume fraction SC
VFC	(n/a)	Fat volume fraction in nonpregnant, non-lactating
VGBC	VGBC_N	GI blood (plasma + blood cells) volume fraction
VGC	VGIT_SENS_N*	GI tissue volume fraction (*SC)
VGJC	VGJC	Gastric juices volume fraction
VKC	VK_SENS_N*	Kidney volume fraction (*SC)
VLC	VL_SENS_N*	Liver volume fraction (*SC)
VMAM_SENS*	(n/a)	Mammary tissue volume SC (VmamC)
VMAXC_BP	VMAXC_BNP	Scaled Vmax for blood binding of perchlorate
VMAXC_GP	VMAXC_GNP	Scaled Vmax for NIS (active uptake of): perchlorate into gastric juice
VMAXMK_SENS	(n/a)	SC for scaled Vmax for NIS: iodide into milk (VMAXCx_MKI)
VMAXC_MKP	(n/a)	Scaled Vmax for NIS: perchlorate into milk
VMAXC_SP	VMAXC_SNP	Scaled Vmax for NIS: perchlorate into skin
VMAXC_TP	(n/a)	Scaled Vmax for NIS: perchlorate into thyroid
VMAXTHYX_SENS*	VMAXTHYX_SENS_N*	SC for NIS: iodide into thyroid (VMAXTHYCx in mother; VMAXTHYCx_N in infant) - also assesses Vmax for perchlorate in infant because VMAXTNP = VMAXTHY_NI/25
VMBC	(n/a)	Mammary gland blood fraction
VMK	(n/a)	Volume of milk
VPLASC	VPLASC_N	Plasma volume fraction
VRC	VR_SENS_N*	Richly perfused volume fraction (remaining), SC
VRBCC	VRBCC_N	Red blood cells in body volume fraction
VSC	VS_SENS_N*	Slowly perfused tissues volume fraction, SC
VSKC	VSK_SENS_N*	Skin volume fraction, SC
VSKBC	VSKBC_N	Skin blood (plasma +blood cells) volume fraction
VTBC	VTBC_N	Thyroid blood (plasma + cells) volume fraction
VTTOTC	VTHY_SENS_N*	Total thyroid volume fraction, SC
VURINE	VURINE_SENS_N*	Daily urine volume, SC for infant urine volume

* Constant multiplier with a value of one, except when used to assess sensitivity coefficients for parameters specified as table function or calculated as equation.

Appendix B: Model Structure Changes and Additional Evaluation

In developing the current iodide BBDR model for the lactating mother and infant, Dr. Fisher made two structural changes that required parallel changes in the perchlorate PBPK model for consistency. First, the lumen compartment was removed from the thyroid sub-model; in the previous models there was active transport of iodide and perchlorate from the thyroid follicle/tissue to the lumen and passive diffusion between them, so the lumen acted as a storage compartment for inorganic iodide. While it is known that a separate lumen compartment exists in the thyroid with active transport as in the previous model, Dr. Fisher deemed this to be an unnecessary complication of the model.

Second, the transfer of iodide and perchlorate into breast milk had previously been described as a two-step process: 1) transport from mammary blood plasma to mammary tissue and 2) transport from mammary tissue to the milk gland. While biological data has shown that the mammary gland actively concentrates iodide in milk by transporters that can be inhibited by perchlorate, the cellular location (apical versus basolateral) is not certain (Rillema and Hill, 2003a, b; Perron et al., 2001; Rillema et al., 2000; Brown-Grant, 1961, 1957); therefore transport was simplified in the model to occur directly from mammary blood plasma to the milk.

Along with these structural changes, the blood flow to the mammary was changed to the average rate measured by Geddes et al. (2012), and the Vmax and permeability-area diffusion constant (PAMKC_I) for iodide transfer into milk adjusted to better describe iodide dosimetry in lactating mothers and breast-fed infants. In particular PAMKC_I was reduced from 0.002 to 0.001 L/h/kg$^{0.75}$, which allowed for a greater difference between milk and plasma concentration of iodide to be obtained, hence higher iodide transfer to the infant.

As indicated above, the perchlorate PBPK model structure was therefore changed in parallel to match the iodide model structure. After making these changes, it was not clear if the previous tuning of the perchlorate model to match human perchlorate-exposure radio-iodide data (Greer et al., 2002) or breast milk data still held. Also, preliminary results with the lactating mother/breastfeeding infant model indicated that higher levels of perchlorate than previously considered could be reasonably safe, but performance of the model at these higher exposure levels had not been evaluated. The following sections describe current analyses with the model and resulting updates in parameter values made to address these concerns.

B-1: Alignment of Perchlorate-NIS Affinity Values

The perchlorate PBPK sub-model used in conjunction with the iodide/thyroid hormone (TH) BBDR model was developed in a series of steps and extrapolations, beginning with the adult male rat (Merrill et al., 2003), the lactating maternal rat and neonate (Clewell et al., 2003a), the adult "average" human (Merrill et al., 2005), and the human "Life Stage" model, which included lactation (Clewell et al., 2007). In Merrill et al. (2003) the Km for perchlorate binding to NIS ($Km_{NIS,ClO4}$; 1/affinity) was given virtually the same value for thyroid, skin, and GI tissues, 1.7 or 1.8 x 10^5 ng/L.

For the rat lactation model, Clewell et al. (2003a) adjusted these values somewhat, beginning with the Km as reported by Kosugi et al. (1996), 1.5 x 10^5 ng/L. The text of that paper states, "This value was adjusted slightly to obtain the best fit of thyroid perchlorate to the drinking water data, resulting in a Km of 2.0 x 10^5 ng/L." However in the code as obtained from Dr. Harvey Clewell the thyroid $Km_{NIS,ClO4}$ still

had a value of 1.5 x 10^5 ng/L while that for skin, GI tract, and mammary tissue was 2.0 x 10^5 ng/L. Considering that the evaluation by Kosugi et al. (1996) was in a cellular environment not native to the molecule (i.e., Chinese hamster ovarian cells in culture), it is not surprising that some adjustments were required to match the rat PK data. However this adjustment is inconsistent with the almost identical values across tissues that did fit the dosimetry in the adult male rat (Merrill et al., 2003), given just above. The molecular form of NIS would not be altered by pregnancy or lactation, while other parameters such as permeability and the partition coefficient, which depend on tissue thickness and lipid content, respectively, would be much more likely to change. Thus we suggest here that the differential adjustment of $Km_{NIS,ClO4}$ for the lactating rat may not have been necessary to fit the perchlorate PK data.

Even if adjustment of $Km_{NIS,ClO4}$ to match rat lactation data may have been appropriate, it is not clear that using different values for $Km_{NIS,ClO4}$ in different tissues is appropriate for human predictions. In particular the U.S. EPA questions use of a higher value for transfer to breast milk and competition with iodide in that process, when the Km for *iodide* on NIS is assumed to be the same for transfer to breast milk as for uptake into thyroid. The extrapolation of the difference in perchlorate $Km_{NIS,ClO4}$-values from the Clewell et al. (2003a) rat lactation model is also questionable because the current model structure no longer matches that model. Lastly, this choice results in a lower estimated effect on the breast-fed infant than assuming equality of $Km_{NIS,ClO4}$ for milk and thyroid transfer (i.e., that the milk transfer $Km_{NIS,ClO4}$ is as low as the thyroid value). Therefore, for use in EPA's analysis, $Km_{NIS,ClO4}$ was given a common value in all tissues of the lactating mother and infant models. Since the molecular form of NIS is the same across the tissues in which it is expressed, the Km for perchlorate should also be the same.

The value of $Km_{NIS,ClO4}$ for the GI tract and skin are not expected to significantly impact model predictions at or near steady-state, but given the change in value considered in the next section, it seemed appropriate and easiest to set all values the same prior to any adjustment in the thyroidal value.

Like the lactation model, the Clewell et al. (2007) pregnancy model used a lower $Km_{NIS,ClO4}$ for the maternal and fetal thyroid (1.6 x 10^5 ng/L) than other tissues (2 x 10^5 ng/L). Since the end-of-pregnancy analysis is conducted at steady-state, values of $Km_{NIS,ClO4}$ for the skin and GI tract will have little or no effect on risk predictions. For the pregnant rat Clewell et al. (2003b) used identical values for $Km_{NIS,ClO4}$ in all tissues. Thus any data from the rat on disposition of perchlorate across the rat placenta vs. thyroid do not indicate a differential value of $Km_{NIS,ClO4}$ from the thyroid. Therefore, for human predictions it again seems appropriate to assume consistency, equality of $Km_{NIS,ClO4}$ across all tissues of the pregnant mother and fetus, and in particular to use the value for the adult thyroid, whose value can be evaluated against predictions of iodide uptake inhibition in adult humans, as described next.

B-2: Update in Affinity of Perchlorate for the Sodium-Iodide Symporter (NIS) Based on Predictions for the "Average Adult" Perchlorate/Iodide PBPK Model and In Vitro NIS Kinetics

The analysis and conclusions described here have now also been reported by Schlosser (2016).

The original models for perchlorate and iodide dosimetry, and the initial effect of perchlorate, iodide uptake inhibition, from which the other life-stages and subsequent modeling have largely been extrapolated, was that of Merrill et al. (2005) for an "average" human adult, which should be predictive of healthy young men and women, outside of pregnancy and lactation. The previous evaluation of this model (U.S. EPA, 2009) focused on results at the then-established reference dose (RfD), for which the point-of-departure (POD) was the dose of 7 µg/kg/d, at which Greer et al. (2002) observed radio-iodide uptake (RAIU) of 98.2 ± 8.3% of controls. The Greer et al. (2002) data set is of primary importance because controlled perchlorate exposures were administered to a set of human volunteers and the

impact on radio-iodide uptake by the subjects' thyroids was measured during and after the 2-week exposure. Thus it provides a direct observation of this key initial endpoint in humans with known exposure levels. Hence the ability of the model to fit these data is considered an important validation measure.

The Merrill et al. (2005) model recapitulated the effect measured by Greer et al. (2002) at 7 µg/kg/d exactly, predicting radio-iodide uptake at 98.2% of control, as shown in Figure B-1. The U.S. EPA did make several minor modifications to the Merrill et al. (2005) model (described in U.S. EPA (2009)), and the results shown here and below are with that modified model. Therefore, for accuracy, it is henceforth referred to as the EPA model (U.S. EPA, 2009).

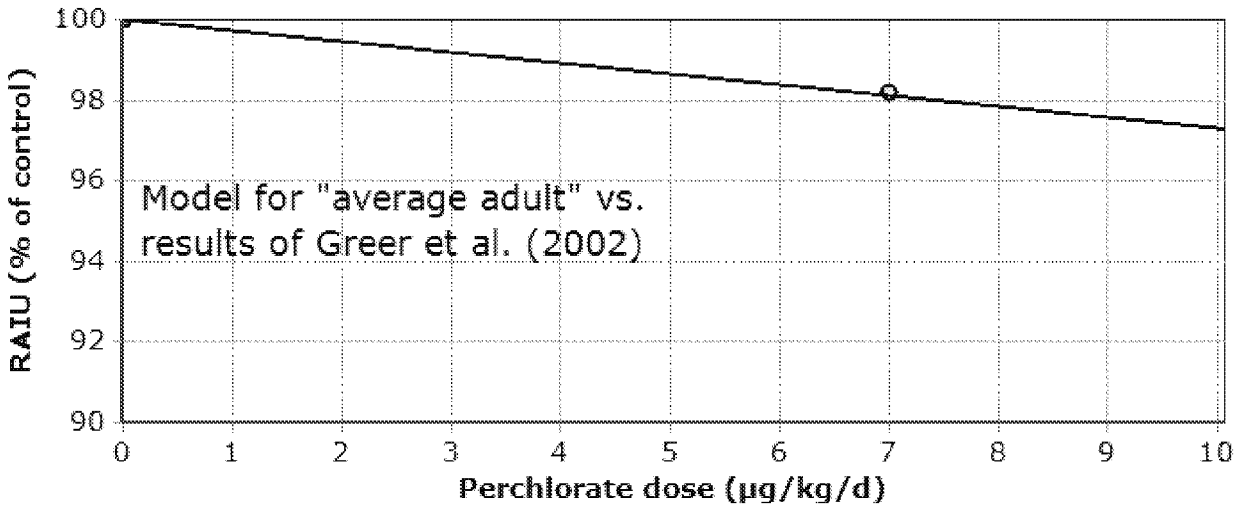

Figure B-1: Predicted low-dose Radio-Iodide Uptake (RAIU) as a % of control using the EPA perchlorate PBPK model (U.S. EPA, 2009) for the average adult (line) with lowest data point from Greer et al. (2002). Simulation and measurement are for the last day of a two-week human study.

The prior review, however, did not evaluate model behavior at higher doses because the focus was on doses below the POD (7 µg/kg/d). With the predictions of the new BBDR modeling indicating that response of T3 and T4 might not be significant at 7 µg/kg/d, the U.S. EPA (2009) model has now been compared to the higher-dose Greer et al. (2002) data, since it is a basis for the current model: Figure B-2. The results show that the model significantly under-predicts the effect (over-predicts the RAIU as % of control) at the next two higher doses. That the prior EPA review failed to evaluate the full dose-response range and recognize the model's failure in this regard was an oversight.

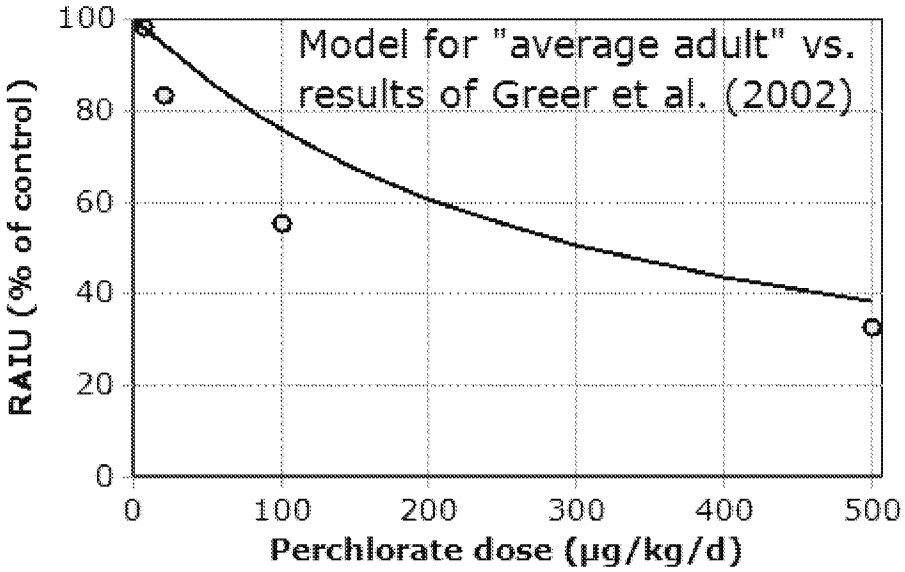

Figure B-2: Predicted Radio-Iodide Uptake (RAIU) as a % of control using the U.S. EPA (2009) perchlorate PBPK model for the average adult (line) with data from Greer et al. (2002) for all dose levels. Simulation and measurement are for the last day of a two-week human study.

To understand why this failure in prediction occurs, the ability of the U.S. EPA (2009) model to predict the blood perchlorate levels in the Greer et al. (2002) study was evaluated. While the perchlorate blood levels were not matched exactly, the difference between model predictions and measured perchlorate blood levels in the study subjects were not sufficient to explain the discrepancy in fits to RAIU inhibition shown above (results not shown). Since blood perchlorate levels were predicted reasonably well, the next most important parameter which might explain this discrepancy is the affinity of perchlorate for the NIS, $Km_{NIS,ClO4}$, which is central to the prediction of iodide uptake.

As described above, $Km_{NIS,ClO4}$ had been given a value of $1.6x10^5$ ng/L by Merrill et al. (2005) based on an in vitro study by Kosugi et al. (1996), who expressed *rat* NIS in Chinese hamster ovary (CHO) cells. Given that the results of Kosugi et al. (1996) were for a non-human form of NIS expressed outside of its normal tissue and cellular environment, it is possible that the measured affinity might not be exactly predictive of the effects of perchlorate in humans. Initial tests with the U.S. EPA (2009) adult human model indicated that increasing the affinity (reducing the Km) about 3-fold would provide a better fit to the data. Thus, simply re-fitting the Km to the human *in vivo* data seemed justified.

However, we first examined the manner in which Kosugi et al. (1996) estimated $Km_{NIS,ClO4}$. Kosugi et al. (1996) used a classical but inexact approach for estimating kinetic parameters where (first) reciprocal plots of 1/velocity vs. 1/[substrate] are used to estimate an apparent Km for each level of inhibitor, and then the slope of that relationship is plotted against the inhibitor (perchlorate) concentration, and a final linear regression used to estimate the inhibition constant (see Figure B-3). This approach was used when computational power and tools were limited and direct non-linear regression was difficult. But it also distorts the signal-noise ratio in the data, by magnifying the results with the lowest substrate concentration and velocity and discounting the results for which those are highest.

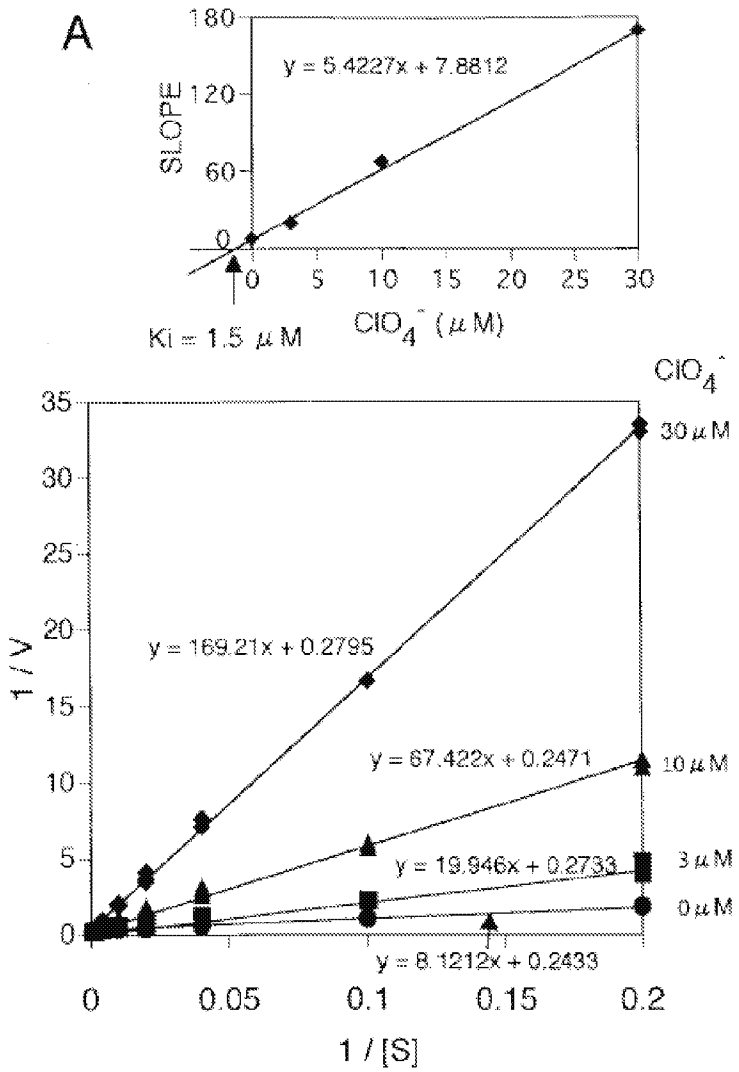

Figure B-3: Panel A of Fig. 3 from Kosugi et al. (1996), showing double-reciprocal plots (lower plot) used to estimate an effective Km for iodide at each perchlorate concentration, and estimation of x-intercept (upper plot) to identify the inhibition constant for perchlorate, assumed to be its Km.

To see if this outdated parameter estimation method could have resulted in errors, and evaluate whether a Km which is consistent with the Greer et al. (2002) human data might also be consistent with these in vitro data, the data of Kosugi et al. (1996) were digitized and re-converted to original (non-inverse) units, plotted, and used in a nonlinear regression. In Figure B-4 the upper set of data and model fit is the Michaelis-Menten fit obtained by nonlinear regression for the iodide uptake data in the absence of perchlorate. The value of $Km_{NIS,iodide}$ (i.e., affinity for iodide) obtained is 33.4 µM, reasonably close to the value obtained using the inverse/linear-regression method by Kosugi et al. (1996) 34.7 µM. *However*, the value $Km_{NIS,ClO4}$ obtained in this re-analysis, which yields the model fits shown to the 3, 10, and 30 µM ClO4 data, was 0.59 µM ($6x10^4$ ng/L), not 1.5 µM as estimated by Kosugi et al. (1996).

Note: this value of $Km_{NIS,ClO4}$ was estimated *without* using the top-most point in each of the perchlorate-exposed data sets shown, since these were the most difficult to digitize from the published plot. (An

email was sent to the corresponding author of the paper to see if the original data could be obtained, but no response was received.) When those top-most points are included the result value of $Km_{NIS,ClO4}$ is 0.57 µM, slightly lower. In either case the value would be rounded to $6x10^4$ ng/L for use in the model.

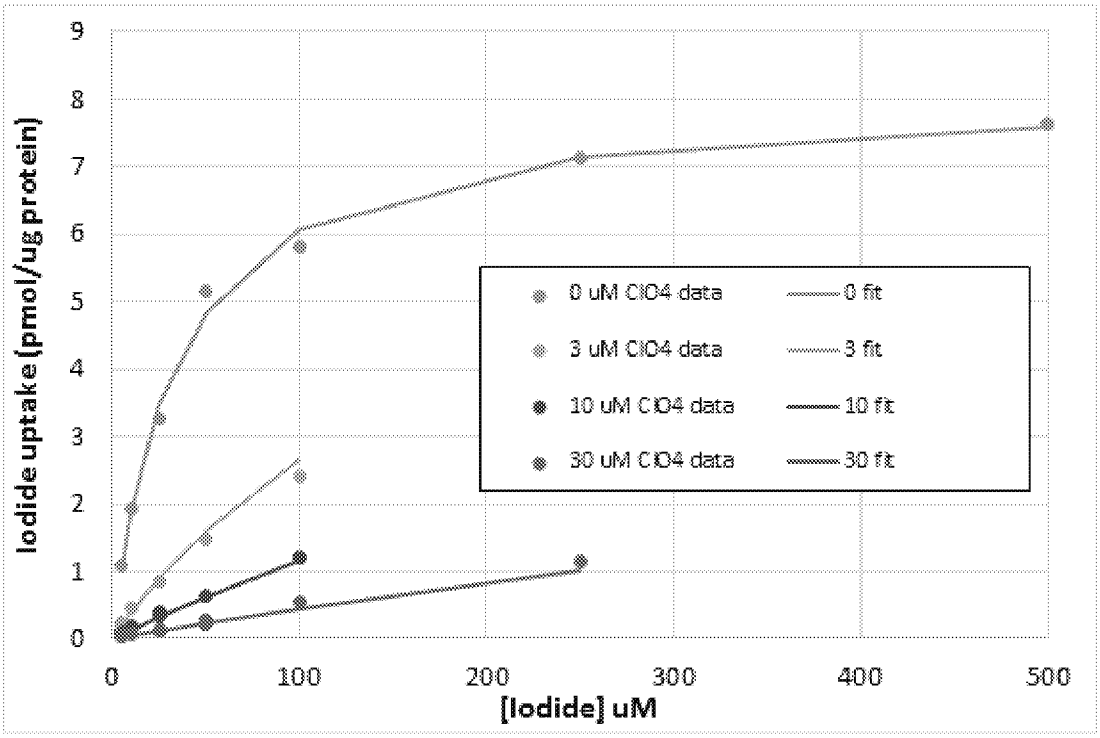

Figure B-4: Fit of competitive-inhibition Michaelis-Menten kinetic model to data of Kosugi et al. (1996) for NIS-mediated iodide uptake, with inhibition by perchlorate.

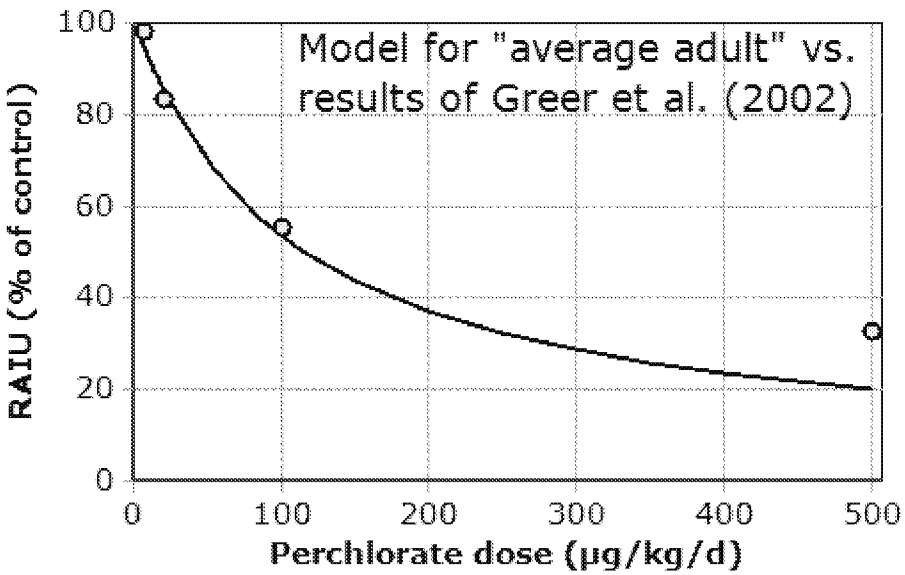

Figure B-5: Predicted RAIU levels (curve) for the average adult using the U.S. EPA (2009) PBPK model, but with $Km_{NIS,ClO4}$ = $6x10^4$ ng/L. Data points from Greer et al. (2002).

When this lower value of $Km_{NIS,ClO4}$ is applied in the U.S. EPA (2009) perchlorate model, it provides much better agreement with the Greer et al. (2002) data as high as 100 µg/kg/d (Figure B-5). While the predicted uptake at 7 µg/kg/d is thereby reduced from 98% to 95% (inhibition increased from 2 to 5%), this is still within one standard deviation of the value reported by Greer et al. (2002), 98.2 ± 8.3% of controls. While the response at 500 µg/kg/d is over-predicted, that is a very high level of perchlorate that could well have resulted in up-regulation of NIS or other compensatory changes in the thyroid which are not included in the model. Hence the model with the revised $Km_{NIS,ClO4}$ will only be used to simulate dose levels lower than 100 µg/kg/d. All subsequent results use this value for the Km.

B-3: Comparison of Lactating Mother BBDR Model to Greer et al. (2002) Data

There are many, significant physiological differences between a lactating or pregnant mother and an adult woman peer (or man) who is neither, which are accounted for in the pharmacokinetic/BBDR models for those life-stages. On the other hand, EPA's previous analysis showed that the two key parameters which determine the effect of perchlorate on RAIU are clearance of perchlorate and its affinity for the NIS. In the previous review of the data U.S. EPA (2009) it was determined that while there was uncertainty regarding the urinary clearance of perchlorate in lactating women vs. non-lactating women, there were no data or known biological reasons to expect it to be significantly different, hence the same urinary clearance scaling constant was used as for the average adult from Merrill et al. (2005). The exact clearance rate will differ slightly due to differences in BW.

However, lactation itself has the potential to be a significant additional avenue of perchlorate clearance (see below). Therefore, for the purpose of comparing iodide-uptake predictions of the revised BBDR lactation model to the Greer et al. (2002) data, to test how similar they might be, simulations were run with lactation excretion of iodide, perchlorate, T3, and T4 turned off, by setting the corresponding Vmax and permeability constants to zero. This "non-lactating woman" model then has nearly the same total clearance of perchlorate as the Merrill et al. (2005) average adult model.

For the analysis of perchlorate effects using the pregnancy and lactation BBDR models, a range of values of dietary iodide ingestion as well as perchlorate exposure are simultaneously being varied. Because the woman at the start of lactation is assumed to have similar dietary and perchlorate exposures as during pregnancy, for these analyses the estimated tissue levels of iodide, thyroid hormones, and perchlorate from the pregnancy model (which predicts dosimetry and response at the end of pregnancy) were used as initial conditions for lactation simulations. In particular the iodide ingestion rate in µg/d was assumed to be the same during pregnancy and lactation, so when a lactation simulation was conducted for 150 µg/d iodide ingestion, for example, the initial value was set using the predicted levels for the pregnant woman ingesting 150 µg/d. Likewise perchlorate exposure levels were generally assumed consistent between the two phases in order to set the initial condition for lactation.

However, since the Greer et al. (2002) involved deliberate dosing with perchlorate for only two weeks, to compare lactation model predictions with those data simulations were run with perchlorate only being ingested in the last two weeks before evaluating iodide uptake by the thyroid. Since the lactating woman at 3 months (90 days) after birth is expected to be closer to the average adult in physiological parameters than at earlier times, the simulation was run with perchlorate exposure beginning 14 days prior to that (postnatal day 76) and iodide uptake evaluated over the 89th day postnatal. Since the Greer et al. (2002) subjects are presumed to have no (or only environmental) perchlorate exposure prior to the experiment, for these simulations the initial perchlorate level was assumed to be zero.

A set of parallel model equations which exactly replicated the BBDR thyroid iodide/hormone sub-model was introduced, but with distinct variable names and the assumption that an iodide "pulse" equal to 1% of the dietary iodide level in blood (predicted by the BBDR model) occurred only on the last day of simulation, to mimic a 24-hour radio-iodide exposure of that duration. The "radio-iodide" uptake during this exposure was assumed to be inhibited by the perchlorate present and to compete with the endogenous iodide. The total amount of this tracer iodide, both inorganic and organified, predicted to be in the thyroid at the end of the last simulation day (postnatal day 89 for this exercise) was then used as a measure of iodide uptake under various exposure conditions, and assumed to be comparable to 24-hour RAIU data.

The resulting predicted iodide uptake levels for 0-500 µg/kg/d perchlorate and a range of dietary iodine levels, for the "non-lactating woman" are shown in Figure B-6.

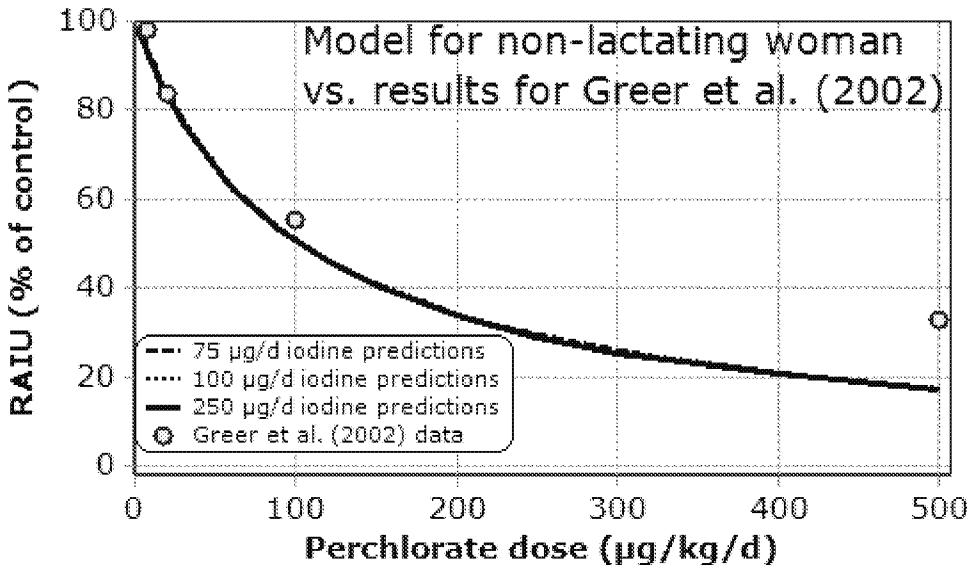

Figure B-6: PBPK/BBDR model predictions for RAIU (% of control) vs. perchlorate dose for a non-lactating woman 90 days after childbirth.

Comparing these results to Figure B-5, one can see that the results for 75-250 µg/d dietary iodine are virtually indistinguishable from each other, and are similar to those for the U.S. EPA (2009) average adult (when both use $Km_{NIS,ClO4} = 6x10^4$ ng/L), even though that model does not track dietary iodide, only a radio-tracer pulse. Higher levels of dietary iodide are seen to reduce the impact of a given perchlorate exposure slightly with regard to iodide uptake. Note that each of the curves shown uses the model simulation for zero perchlorate with the iodide-uptake predicted for that level of dietary iodine ingestion as its own control; e.g., the model prediction for 100 µg/d with 0 µg/kg/d perchlorate is used as the control for the 100 µg/d curve, etc.

Finally a similar comparison was made but with lactation excretion turned on for perchlorate, iodide, and the thyroid hormones. This simulation used an adjusted Vmax for perchlorate expression in breast milk, the derivation of which is described in the next section. But for continuity of presentation the results are presented here (Figure B-7). Not surprisingly, the excretion of perchlorate into breast milk reduces the mother's plasma levels of perchlorate and hence the predicted effect on iodide uptake. However an unexpected result is that the dietary iodide levels now appear to have very little influence

on the outcome (again, when each response is quantified vs. its own control). This may occur because perchlorate inhibits both thyroid uptake of iodide and its excretion into breast-milk

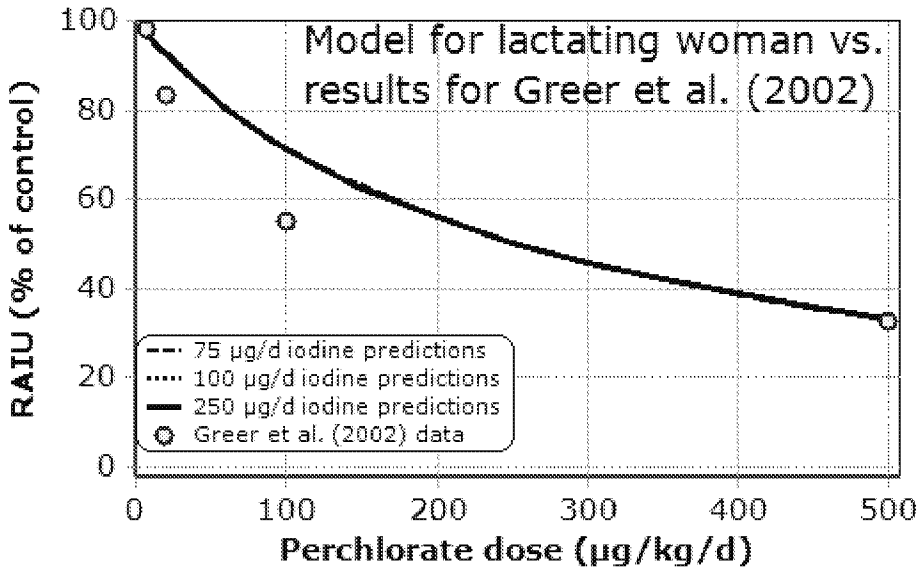

Figure B-7. PBPK/BBDR model predictions for RAIU (% of control) vs. perchlorate dose for a lactating woman 90 days after childbirth.

B-4: Calibration of Vmax for Transfer of Perchlorate into Breast Milk

As summarized above, due to the model structure change for transfer of perchlorate and iodide into breast milk, the model's prediction of perchlorate transfer should be checked against existing data. Also a much more robust data set is now available, Kirk et al. (2013), compared to that used previously (Téllez Téllez et al., 2005). In particular Kirk et al. (2013) measured breast milk concentrations and 24-hour urinary perchlorate levels in 13 subjects. For each subject the measurements were made on (up to) three three-day periods (a total of 9 days). Since urinary output can vary considerably and total dietary ingestion is difficult to estimate, the 24-hour urine data, in particular, provided an excellent measure of total perchlorate exposure for the subjects. In order not to bias the data with assumptions about volumetric flows, it was used essentially as is: the average milk concentration of perchlorate measured on a particular day was simply associated with the corresponding 24-hour urinary excretion of perchlorate on that day, with results for all subjects on all days of analysis shown.

In contrast, Téllez Téllez et al. (2005) only reported the average breast milk concentration measured in each of three cities, average spot urine concentrations, and the average drinking water concentration of perchlorate in those cities. However, Téllez Téllez et al. (2005) did provide average maternal body weights, from which one can estimate daily urine excretion rates assuming an average of 22.0 ml/kg/d urine excreted, which then allowed 24-hour urinary excretion rates to be estimated from the spot urine concentrations. The city-average breast-milk concentrations of Téllez Téllez et al. (2005) could then be plotted against these estimated city-average urinary rates on the same plot as the Kirk et al. (2013) data (Figure B-8). While the Téllez Téllez et al. (2005) data indicate higher perchlorate concentrations than the Kirk et al. (2013) data, the ratio of breast-milk concentration to estimated urinary output is seen to be consistent with the range observed by Kirk et al. (2013).

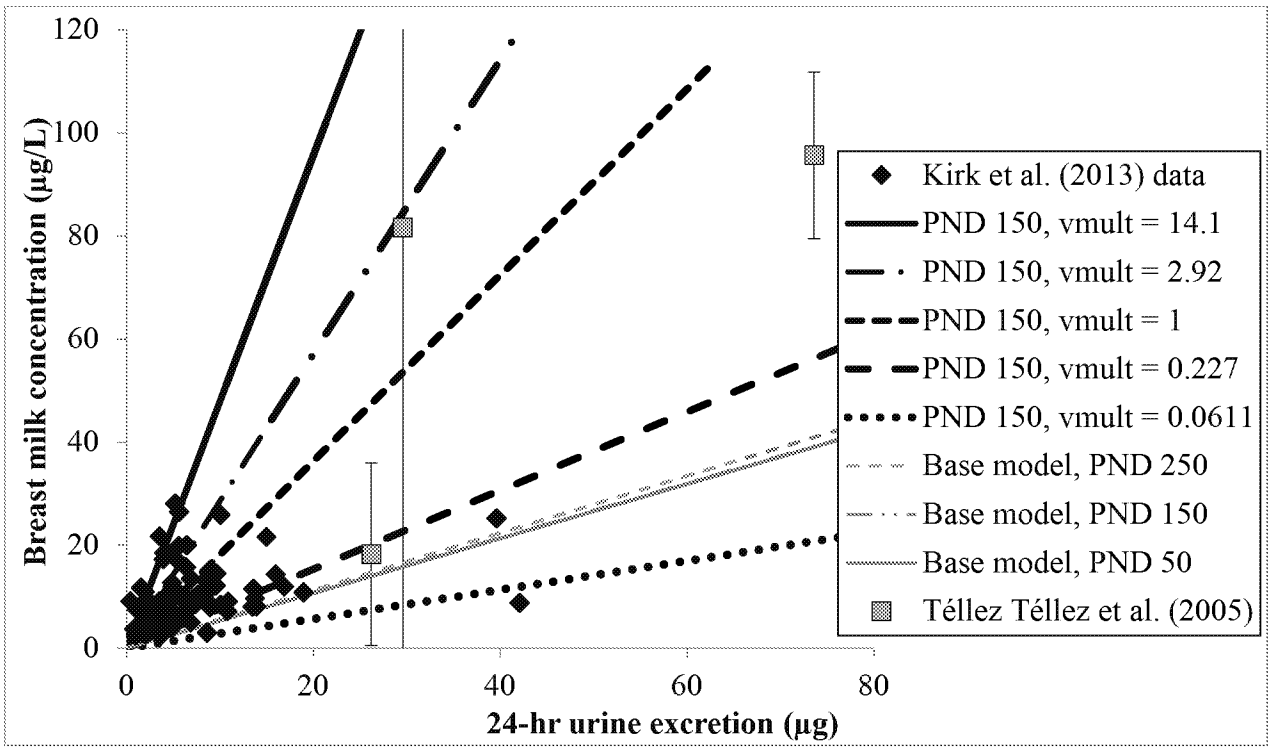

Figure B-8: Perchlorate concentrations in breast milk vs. 24-hr excretion rates from Kirk et al. (2013), Téllez Téllez et al. (2005) (average urine volume/day assumed), and model predictions for a range of $Vmax_{NIS,ClO4}$ values (Vmax for NIS-mediated transport of ClO_4). See text for further details.

In Figure B-8 the Kirk et al. (2013) data are shown without error bars because each represents a single day's measurement for one individual (y-axis are perchlorate concentrations in breast milk; for Kirk et al. (2013) an average of measurements made on a given day). The three lines described as "Base model" are results using perchlorate plasma-milk transfer parameters from the U.S. EPA (2009) lactation model (modified from Clewell et al. (2007)), for postnatal days (PNDs) 50, 150, and 250. Since there is almost no difference between predictions for the different PNDs all other results are just shown for PND 150.

In particular, the base model uses the model structure revised by Dr. Fisher, U.S. FDA, and $Km_{NIS,ClO4}$ = $6x10^4$ ng/L, as described above. Despite this change in the Km, it can be seen that most of the data lie above these lines. While increasing the Vmax for perchlorate transfer to breast-milk (VMAXC_MKP in the model code) alone increased the simulations somewhat, it was found that as was the case for iodide, the permeability-area constant for diffusional transfer had to be reduced to match the data: PAMKC_P was also reduced to 0.001 L/h/kg$^{0.75}$, matching the value for iodide. Using this value for PAMKC_I and increasing the Vmax for NIS-mediated transfer of perchlorate (VMAXC_MKP) from $2x10^4$ to $4.52x10^4$ ng/h/kg$^{0.75}$, the average milk:urine ratio of perchlorate observed by Kirk et al. (2013) was matched. This prediction corresponds to the line identified as "PND 150, vmult = 1" in Figure B-8.

While reducing PAMKC_P and increasing VMAXC_MKP as described just above was sufficient to match model predictions to the average milk:urine ratio of perchlorate observed by Kirk et al. (2013), further increases in VMAXC_MKP alone were not sufficient to match the higher levels obtained for some subjects; i.e., variation in that parameter alone could not describe the full range of the data. The reason

for this is that as VMAXC_MKP is increased, mammary blood flow (QMWB) becomes rate-limiting. Since Geddes et al. (2012) observed significant variation in mammary blood flow among their subjects, QMWB was allowed to vary in concert with VMAXC_MKP in order to determine what range was needed to describe the data. However, since the fold-variation in QMWB between individuals is expected to be less than the potential variation in NIS levels, an empirical relationship was selected to reduce its variation compared to VMAXC_MKP. In particular, VMAXC_MKP was varied using a multiplicative constant vmult; i.e., VMAXC_MKP = vmult*VMAXC_MKP0, where VMAXC_MKP0 = 4.52×10^4 ng/h/kg$^{0.75}$; but QMWB was only varied as vmult$^{0.25}$. The result is that a 16-fold increase in VMAXC_MKP is matched with only a 2-fold increase in QMWB, for example.

To be clear, this relationship between mammary blood-flow and the Vmax for NIS-mediated transport of perchlorate is simply an empirical convenience used to allow an easy demonstration that the model can describe the range of data from Kirk et al. (2013). Whether there is any biological relationship between these two and the possible mechanisms are not known. However it is clear that to match the highest milk:urine ratio observed by Kirk et al. (2013), both QMWB and VMAXC_MKP must be increased significantly above their average values, if other parameters are kept constant.

The various lines in Figure B-8, are results with the identified values of vmult, both VMAXC_MKP and QMWB varying as described above.

For each individual in Kirk et al. (2013), the slope of the milk concentration: urine excretion was estimated by linear regression among the days of observation for that individual. The resulting set of 13 slopes were treated as 13 individual measurements (an average milk: urine ratio for each subject), and primary statistics (mean, mx, min, 5th, and 95th percentile) computed. The vmult values correspond to percentiles of the milk concentration: urine excretion ratio from the Kirk et al. (2013) data are given in Table B-1. In particular simulations with vmult = 1, hence VMAXC_MKP = 4.52×10^4 ng/(hr-kg$^{0.75}$) yield a milk: urine slope equal to the mean slope obtained from the Kirk et al. (2013) data. This value was used for subsequent simulations of the breast-feeding mother and breast-fed infant, provided in the main body of the report.

Table B-1: Changes in the Vmax for NIS-mediated transfer of perchlorate to breast milk (VMAXC_MKP) and mammary blood flow (QMWB) vs. the average values (vmult = 1), obtained from data of Kirk et al. (2013) and Geddes et al. (2012).

Milk: urine value/percentile	vmult	VMAXC_MKP (ng/h/kg$^{0.75}$)	QMWB (L/h)
Maximum individual	14.0	6.36×10^5	19.0
95th %tile	2.91	1.32×10^5	12.9
mean	1	4.52×10^4	9.8
5th %tile	0.227	1.03×10^4	6.8
Minimum individual	0.061	2.76×10^3	4.9

Appendix C: Supplemental Analyses of Differences among the Lactating Mother, Breast Fed Infant, and Formula Fed Infant

Some further analysis is needed to understand why, even with an iodine intake of 250 µg/d, the lactating mother is so much more sensitive to perchlorate than the formula fed infant. At PND 7 the simulated mother's BW is 74.4 kg, so 250 µg iodine/d is 3.36 µg/kg/d. On the other hand, an infant ingesting 0.199 L/kg/d of formula (Fomon et al., 1964) containing 108 µg/L iodide is ingesting 21.5 µg/kg/d iodide. Also, the lactating mother has the demands of lactation, which clears iodide and T4 from her system. The mother's basal levels of fT4 (i.e., with zero perchlorate) are seen to decline 23% from postnatal day 7 to 90, which may be in part because the rate of milk ingestion, hence clearance of iodide and T4, is increasing. There are also known changes in TBG levels and alterations in several thyroid-function-specific components, where the relatively hyperactive thyroid during gestation declines back to its pre-pregnancy state in these postnatal days, which have been incorporated into the model via a time-dependence in the Vmax for maternal thyroidal uptake of iodide (Vmaxthyx_I).

There are two other interesting features of the maternal dose-response: 1) the somewhat steeper response predicted at PND 7 vs. 30-90 (for lower perchlorate dose rates); and 2) the increasing sensitivity to maternal iodide ingestion (wider spacing of simulation curves) with PND. The first result is due to the fact that maternal clearance of perchlorate is assumed to be lower during pregnancy and is assumed to return to average adult levels immediately at birth. Between this increased urinary clearance and the clearance of perchlorate via lactation, the lactating mother's perchlorate levels are predicted to drop very quickly after birth, so while the levels still increase with exposure the relationship is less steep. While infant breast-milk ingestion increases with time, as the infant grows, the maternal fT4 levels stabilize after 2-3 weeks. The changes in maternal serum perchlorate and fT4 for three levels of perchlorate and 150 µg/d iodine are shown in Figure C-1.

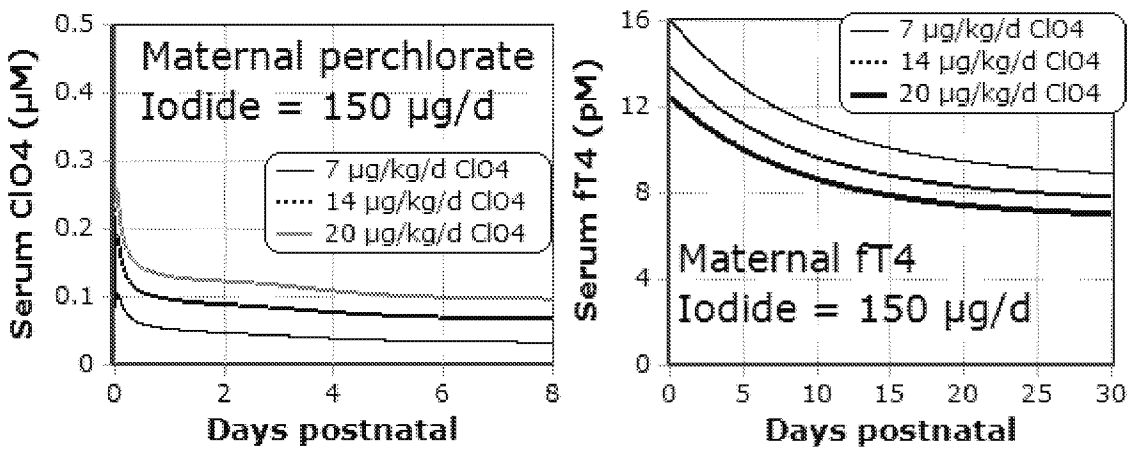

Figure C-1: Serum perchlorate and fT4 levels in the lactating mother as a function of time for three levels of perchlorate exposure.

The increasing maternal sensitivity to iodide intake with postnatal time – note that the curves for 200 and 250 µg/d iodine in Figure 15 are nearly indistinguishable on day 7 but clearly separated on day 90 – is likely due to the increasing demand due to lactation, as the infant's milk ingestion increases with time. This is reflected by the decreasing maternal serum fT4 with time, shown in the right panel of Figure D-4.

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The model-predicted iodide concentration in breast-milk is shown for the breast fed infant at 7 days in the upper-left panel of Figure C-2, and iodide ingestion rate in the lower-left plot, as a function of maternal perchlorate exposure and iodide ingestion. Even for a mother ingesting 250 µg/d iodine with no perchlorate exposure, the breast fed infant is receiving less than 15 µg/kg/d iodide, and the levels fall roughly in proportion with maternal iodide ingestion. While the effect of perchlorate on iodide delivered is not as strong as the effect on maternal fT4, it is also a significant contributor to the total impact on the breast fed infant.

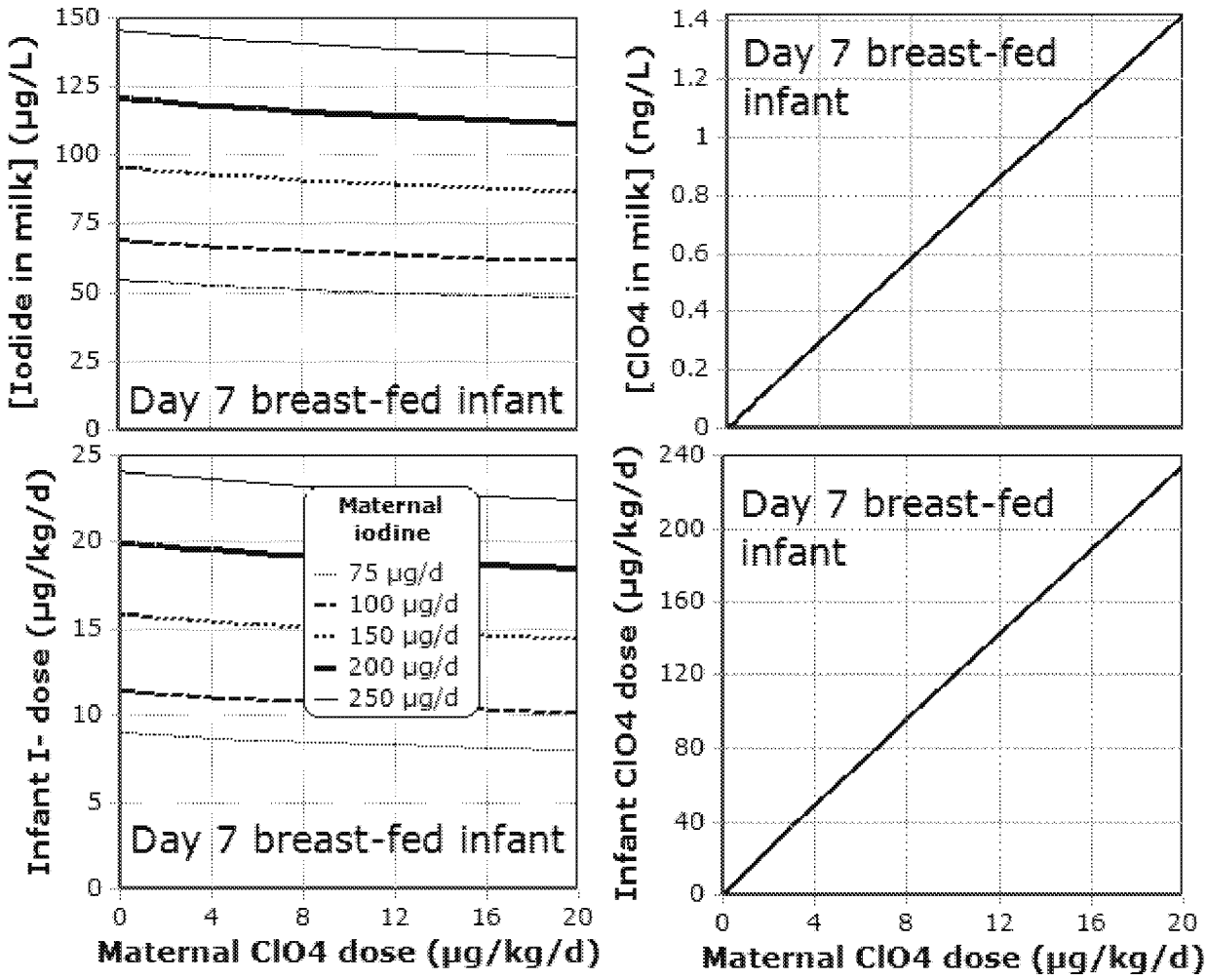

Figure C-2: Iodide and perchlorate concentrations in breast milk (upper panels, respectively) and infant ingestion rates (lower panels) as a function of maternal iodide and perchlorate ingestion.

Finally, the perchlorate concentration in breast milk and the dose-rate received by the (7-day) breast-fed infant are shown in the upper- and lower-right panels of Figure C-2, respectively, as a function of the mother's dose-rate. (The results for different maternal iodide ingestion rates are indistinguishable.) Keep in mind that the ratio of perchlorate in breast-milk to exposure (as estimated by urinary excretion) showed a wide range, ~ 13-fold (see Appendix B-4), and this prediction is for the mean of the study population evaluated. The relationship is slightly nonlinear, but across the perchlorate dose range shows a significant concentration of perchlorate (increase in dose-rate) from the maternal dose-rate to the infant, ranging from 12-fold at the lowest perchlorate levels to 10-fold at 20 µg/kg/d.

Thus the much stronger effect predicted for the breast-fed infant at a given maternal dose of perchlorate, compared to a bottle-fed infant receiving the same dose (µg/kg/d) as the breast-feeding mother, results from three factors: a significant concentration of perchlorate into breast-milk, lower iodide transfer via breast-milk (which is further reduced by perchlorate) vs. formula, and reduced T4 transfer into breast-milk. It should be noted that the analysis at this point does not factor in differences in water (or formula) ingestion. A 65-kg mother ingesting 2 L of water per day ingests 0.03 L/kg/d of water, while the 7-day bottle-fed infant ingests 0.2 L/kg/d, almost 7 times more on a BW basis.

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Appendix D: Perchlorate Clearance Analysis

In order to evaluate perchlorate kinetics, simulations were conducted with continuous perchlorate exposure until the system is close to or at steady state, followed by cessation of exposure, for dose rates of 1, 10 and 100 µg/kg/d. The terminal half-life of clearance was then calculated from the model results during the simulated decay period. The first results shown in Figure D-1 are from the Merrill et al. (2005) model for an average adult, with adjustments as described in Mclanahan et al. (2014) and U.S. EPA (2009), and the additional change of the Km for perchlorate on NIS in all tissues set to the value obtained in Appendix C, 1.6×10^5 ng/L. The model results are fairly linear in with respect to the perchlorate exposure and exhibit a terminal clearance half-life of about 11 hours.

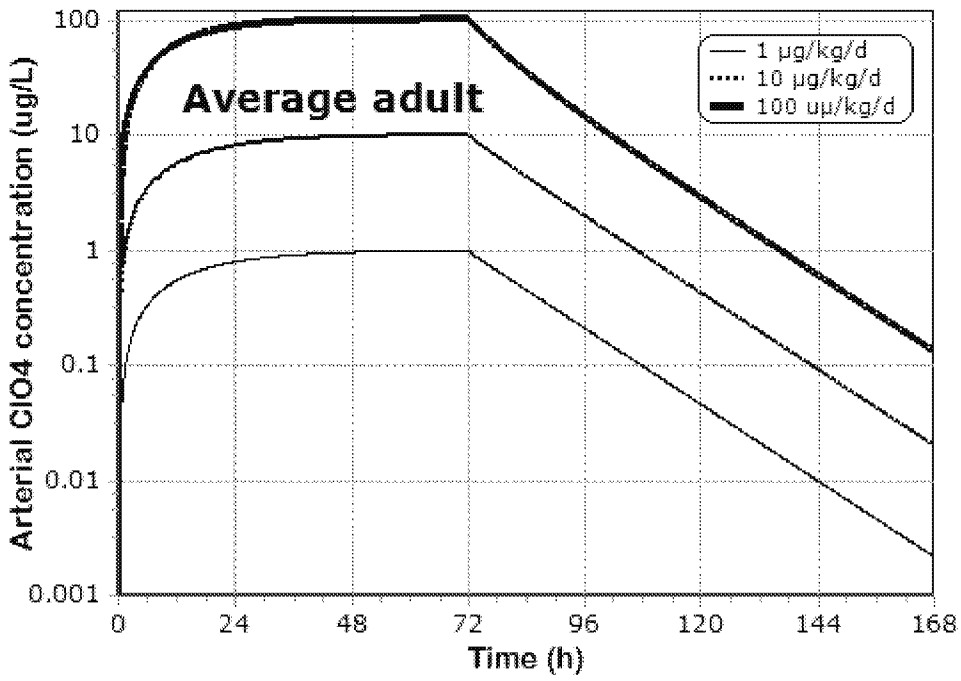

Figure D-1: Simulations of perchlorate kinetics in the average adult (Merrill et al., 2005; Merrill et al., 2003) given 72-h exposures to perchlorate.

Next results are shown in Figure D-2 for the non-lactating mother model described here; i.e., the lactating mother model with both active and diffusional transfer into breast-milk turned off. Note the difference in time scale from Figure D-1. For this model the infusion was run for 10 days (240 hours) which was necessary for the lower two exposures to reach steady state, or at least close to it. The clearance phase is also much slower, with a terminal half-life around 60 hours.

A number of key perchlorate transport parameters differ between the lactation PBPK model and the average adult model, listed in Table D-1, based on the original modeling by Clewell et al. (2007). These changes (between the "average adult" and "previous lactation" models in Table D-1) were made based on the common "parallelogram" approach, where differences in rodent models, in particular differences the perchlorate dosimetry in the adult male rate (Merrill et al., 2003) and the lactating maternal rat (Clewell et al., 2003b). The largest difference in perchlorate parameters between the two life-stages is the Vmax for NIS-mediated perchlorate excretion from the GI tissue to the GI lumen (or "juices"), VmaxC_GP. In order to match the available PK data, which included the amount of perchlorate in the GI

contents after 4 days of dosing to the lactating rat dam, VmaxC_GP had to be increased by a factor of 50 from the value which was fitted to the adult male rat data. Hence the same factor for increase in this Vmax was assumed to occur in humans between average adults and lactating women. Unfortunately there are no human PK data for perchlorate in lactating women against which to test this assumption, but in the absence of such data it is considered the best possible assumption.

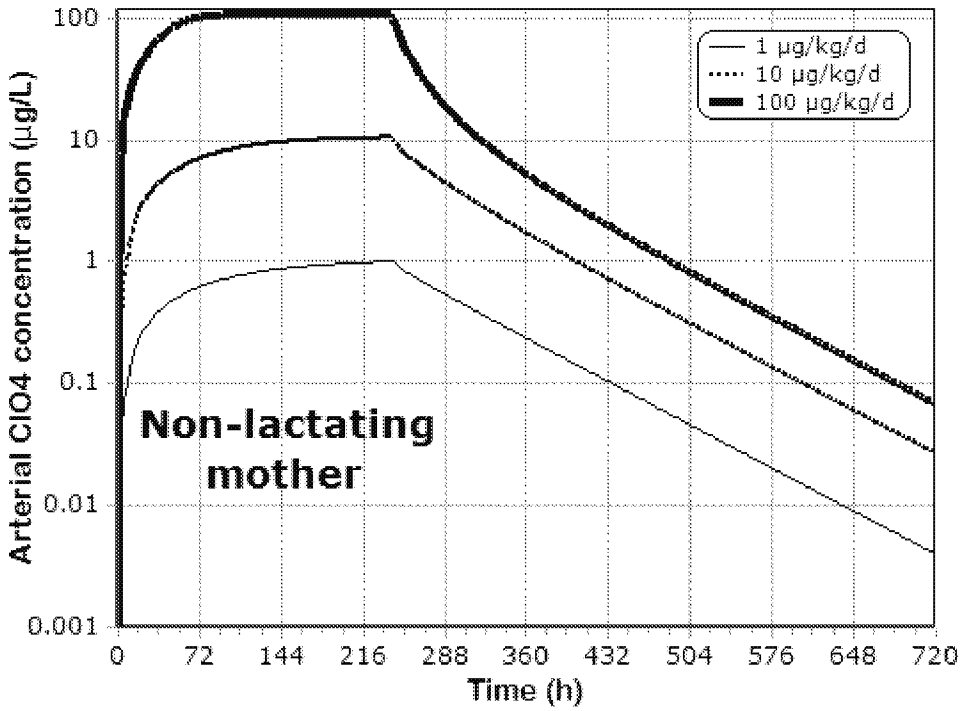

Figure D-2: Simulations of perchlorate kinetics in the "non-lactating mother" (current lactation model, but transfer to breast milk turned off), given 240-h exposures to perchlorate.

Table D-1: Perchlorate transport and binding parameters which differ between the average adult and lactation PBPK models

Parameter	Average adult model [a]	Previous lactation [b]	(Non-)Lactating mother model [c]
VMAXC_GP (GI tissue → lumen) (ng/h/kg$^{0.75}$)	10^5	5×10^6	5×10^6
VMAXC_TP (thyroid blood → tissue) (ng/h/kg$^{0.75}$)	8000	9000	9000
VMAXC_SP (skin blood → tissue) (ng/h/kg$^{0.75}$)	10^6	1.6×10^6	1.6×10^6
VMAXC_BP (blood binding) (ng/h/kg$^{0.75}$)	700	1230	1230
PATC_P (thyroid blood ↔ tissue) (L/h/kg$^{0.75}$)	3×10^{-4}	6.67×10^{-5}	6.67×10^{-5}
PASKC_P (skin blood ↔ tissue) (L/h/kg$^{0.75}$)	1	0.63	0.63
PARBCC_P (plasma ↔ red blood cells) (L/h/kg$^{0.75}$)	5	10	10
KUNBC_P (un-binding in blood) (L/h/kg$^{0.75}$)	0.07	0.01	0.03

[a] Merrill et al. (2005)
[b] Clewell et al. (2007)
[c] Current model with transfer of perchlorate to breast milk turned off

The result of increasing VmaxC_GP is that the amount of perchlorate "held" in the stomach lumen compartment at steady state is increased significantly, and the net rate of transfer from this compartment to the blood becomes (in part) rate limiting for overall clearance, slowing clearance down

significantly. But of significant importance to this analysis, it should also be noted that the steady-state blood levels in Figures D-1 and D-2 are virtually identical. This is because, while VmaxC_GP and other transport parameters alter the approach to and decline from steady-state blood levels, the steady-state concentration in blood is determined by only two factors: the dose rate and the urinary clearance constant, which is assumed to be the same in the lactating woman as the average adult.

The last column in Table D-1 shows that of these parameters, only KUNBC_P was further changed during development of the current model, from 0.01 to 0.03 L/h/kg$^{0.75}$.

In order to further evaluate the source of the kinetic differences, simulations were run again with the non-lactating mother model, but with the perchlorate transport VmaxC values and permeability-area (PA) constants set identical to those for the average adult. The results are shown in Figure D-3. Perchlorate clearance with this hybrid model is much faster than with the non-lactating mother, though slower than with the average adult. The terminal half-life for clearance is 22 h. Thus some of the difference in clearance between the average adult and lactating mother must be due to physiological parameters or other model differences. However again it can be seen that the steady state levels are not altered by the changes in these kinetic parameters.

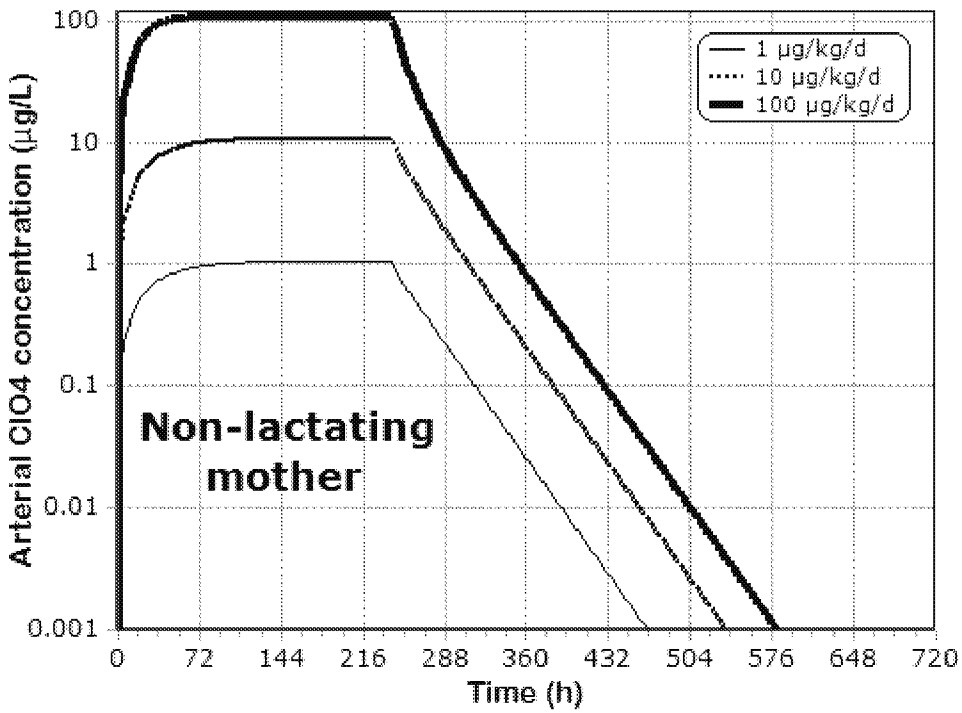

Figure D-3: Simulations of perchlorate kinetics in the "non-lactating mother", with perchlorate transport parameters set to those of the average adult, given 240-h exposures to perchlorate.

To evaluate the significance of the specific parameter differences in Table D-1, perchlorate dose-response results for the lactating mother and breast-fed infant were obtained using the current model parameters vs. the average adult values in Table D-1. The results for fT4 levels in the lactating mother and breast fed infant on PNDs 7, 30, 60, and 90 were virtually indistinguishable (results not shown). The reason is that, given the assumption of continuous exposure and initial conditions set using the end-of-pregnancy levels, this change in model parameters only affects maternal perchlorate levels for the first few days after birth, as shown in Figure D-4. With perchlorate levels being only transiently affected by

the difference in parameters for a few days, the overall impact on thyroidal iodine stores and TH levels is negligible. Hence the uncertainty regarding these parameter differences has little impact on risk predictions and should not require further analysis for this purpose.

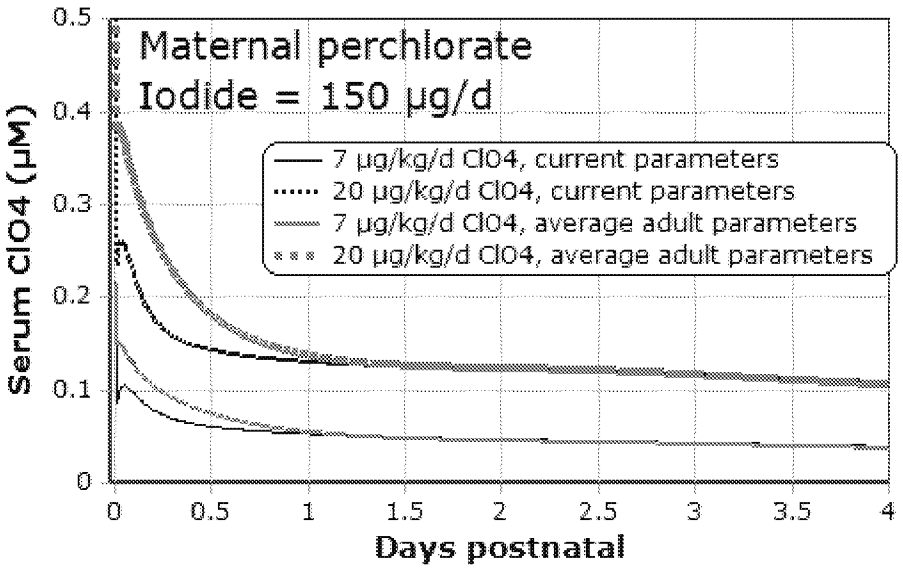

Figure D-4: Model simulations of perchlorate kinetics in the lactating mother immediately after birth, with either current model parameters or average adult parameters for perchlorate transport and binding as listed in Table D-1.

Appendix E: Population Percentiles for fT4 During Pregnancy

Table E-1 summarizes population distribution data for fT4 during pregnancy, primarily for the 3rd trimester. In order to combine these results into an overall estimate of 2.5th and 10th percentiles for late gestation, which can be applied to the BBDR model-predicted mean, as discussed in the Technical Introduction.

The analysis reflects an assumption that differences between observations in different studies (for late gestation women) are primarily due to differences in analytic methods and the population mean, but the distribution shape and coefficient of variation will be similar. Therefore the data on population percentiles were first normalized to the study-specific median (or mean if available). The resulting normalized percentiles (Table E-1, right columns) were similar enough that this assumption was considered reasonable.

Most of the studies in Table E-1 only provided median and the 95-percent population range (i.e., 2.5th and 97.5th percentile). From these results, the data appeared to be consistently right-skewed, but as these extreme percentile estimates have associated uncertainty, the analysis of distribution shape was focused on the two study which also provided information on the 25th and 75th percentiles, Gong and Hoffman (2008) and Wang et al. (2011).

Wang et al. (2011), provided trimester-specific percentiles estimated by two different methods: self-sequential longitudinal reference intervals and general gestation-specific reference intervals. (The 25th and 75th percentiles were shown in plots and digitized for this analysis.) The results for the general gestation-specific reference intervals were used to estimate the statistical model parameters, since these were assumed most comparable to the results for the other studies included. Gong and Hoffman (2008) also gave tabulated results for the 2.5th and 97.5th percentiles, with the 25th and 75th percentiles show in plots, so those quartiles were also digitized.

Each reported percentile was normalized to the corresponding trimester- and method-specific median and the results simulated in Excel by calculating the corresponding percentiles using the lambda-mu-sigma (LMS) transformed normal distribution (Box and Cox, 1964) with the mean (μ) set equal to one, the shape (λ) and variance (σ) set to values in specific cells in the Excel spreadsheet: $x(z) = (1 + \lambda \cdot \sigma \cdot z)^{1/\lambda}$, for z corresponding to the 2.5th, 25th, 75th, and 97.5th percentiles. The sum of square errors between the predicted percentiles and reported/normalized percentiles was calculated, and the solver function in Excel used to identify the λ and σ which minimized the error. The resulting value for the shape parameter is $\lambda = 0.169$.

With λ fixed at 0.169, the modeled 2.5th and 97.5th percentiles were then fit to the 3rd trimester (only) data from all studies listed in Table F-1, by calculating the sum of square errors between the reported percentiles and the statistical model. (For studies which reported values on multiple weeks within the 3rd trimester, an arithmetic mean of the normalized percentiles for those weeks was calculated first, and that average used in the total model error.) The Excel solver function was then used again to re-estimate σ by minimizing the error across the 3rd trimester results, and obtained $\sigma = 0.139$. Using these values for λ and σ, the 10th and 2.5th percentiles of the fT4 distribution are predicted to be 83.5% and 75.7% of the mean, respectively.

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Table E-1: summarizes population distribution data for fT4 during pregnancy

Reference Source		Reported fT4 serum concentrations (pmol/L)*						Ratio of percentile:median or mean†			
			Percentile					Percentile			
	n	mean	2.5th	25th	50th	75th	97.5th	2.5th	25th	75th	97.5th
Gong and Hoffman (2008)	211		8.1	10.4	11.7	12.9	15.3	0.692	0.892	1.105	1.31
Gong and Hoffman (2008), 1st trimester	*224*		*11.0*	*13.0*	*15.0*	*16.1*	*19.0*	*0.733*	*0.868*	*1.072*	*1.27*
Gong and Hoffman (2008), 2nd trimester	*240*		*9.7*	*12.5*	*13.5*	*14.7*	*17.5*	*0.719*	*0.923*	*1.092*	*1.30*
Khalid et al. (2014)	NS[a]		8.7		11.4		15.1	0.765			1.33
Panesar et al. (2001)	76[b]		8.5		11.4		14.6	0.745			1.28
Stricker et al. (2007)	501	11.2	8.6		11.1		13.6	0.771			1.21
Wang et al. (2011)[c]	*301*		*9.3*	*11.5*	*12.6*	*13.9*	*17.1*	*0.737*	*0.908*	*1.103*	*1.36*
Wang et al. (2011), 1st trimester[c]	*301*		*11.9*	*14.0*	*15.5*	*17.2*	*21.5*	*0.766*	*0.903*	*1.113*	*1.39*
Wang et al. (2011), 2nd trimester[c]	*301*		*9.5*	*11.0*	*12.1*	*13.2*	*16.3*	*0.782*	*0.913*	*1.090*	*1.35*
Wang et al. (2011)[d]	661		9.4	11.8	12.9	14.2	17.1	0.729	0.915	1.101	1.33
Wang et al. (2011), 1st trimester[d]	*406*		*12.0*	*14.2*	*16.0*	*17.4*	*24.6*	*0.749*	*0.887*	*1.085*	*1.53*
Wang et al. (2011), 2nd trimester[d]	*388*		*9.5*	*12.2*	*12.6*	*14.3*	*16.9*	*0.754*	*0.964*	*1.133*	*1.34*
Yan et al. (2011)	169		9.2		12.1		16.7	0.760			1.38
LMS statistical model[e]								***0.757***	***0.910***	***1.097***	***1.305***

*Values are for 3rd trimester unless otherwise indicated (for Gong et al. (2008) and Wang et al. (2011))

† Median are listed as 50th Only Stricker et al. (2011) reported the mean.

[a] Average of values given for weeks 28 to 40. Total number of women included over course of pregnancy was 351. The reference did not specify the number of women sampled only during the third trimester

[b] Average of values given for weeks 31, 35, and 39. The number of subjects per week were 26 (31 weeks), 25 (31 weeks), and 25 (31 weeks)

[c] Reference intervals were determined longitudinally during pregnancy from same individual; these are shown for comparison but were not used to estimate parameters of the statistical model.

[d] Reference intervals were determined in a general random sample of women

[e] Percentile(z) = $\mu\cdot(1 + \lambda\cdot\sigma\cdot z)^{1/\lambda}$, with $\mu = 1$, $\lambda = 0.169$, and $\sigma = 0.139$, and z being the normal distribution z-score for the corresponding percentile.

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Appendix F: Summary of fT4 Levels in Lactating, Pregnant, and All Other Women Based on NHANES 2007-2012

memorandum

Environment & Natural Resources Division

Date January 28, 2016

To Erik Helm, EPA

From Anna Belova, Jacky Haskell, Lauren Brown, Abt Associates

Subject Summary of fT4 levels in lactating, pregnant, and all other women based on NHANES 2007-2012

Abt Associates is assisting EPA with the perchlorate drinking water rule analysis. In this process, Abt is supporting EPA's derivation of a perchlorate maximum contaminant level goal (MCLG) that addresses sensitive life stages with biologically based dose response (BBDR) modeling based upon perchlorate's mode of action. The Science Advisory Board (SAB) recommended this approach in 2013.[1] To assist EPA in their model interpretation, Abt Associates compared the distribution of free thyroxine (fT4) levels (in ng/dL) for lactating women, pregnant women, and all other women based on EPA technical direction. This was done using data from CDC's National Health and Nutrition Examination Survey (NHANES). This memorandum summarizes our approach for this analysis.

Method for Analyzing NHANES Data

NHANES is a nationally representative data source that gathered biomonitoring data, including thyroid hormone (TH) levels, in several survey cycles. Three recent NHANES cycles (2007-2008, 2009-2010, 2011-2012) collected TH data.[2] In 2007-2008, all participants aged 12 years and older were eligible for the thyroid level data collection. In 2009-2010 and 2011-2012, the thyroid level data were collected for a one-third subsample of participants aged 12 years and older. Subjects' pregnancy status is provided in NHANES demographic files, as determined by self-reporting and the results of a urine pregnancy test. Additionally, lactation status is determined by self-reporting in the reproductive health questionnaire.

We pooled the data across the three survey cycles to create a 6-year dataset. We followed the NHANES Analytic and Reporting Guidelines (CDC, 2013) to adjust the survey weights for each sample to estimate parameters that are representative of the U.S. civilian noninstitutionalized population at the midpoint of

[1] SAB report available here: http://yosemite.epa.gov/sab/sabproduct.nsf/d21b76bff879fa0a8525735a00766807/86E44EE7F27EEC1A85257B7B0060F364/$File/EPA-SAB-13-004-unsigned2.pdf

[2] At the time of Abt Associates' previous analysis of thyroid hormone data for this effort, data from NHANES 2011-2012 were unavailable due to errors in the sampling weights. A new file with corrected weights has since been released and were incorporated into the current analysis.

those 6 years. Using the adjusted weights, we generated point estimates of the following population parameters on fT4 levels for each group of women:

- Population means (arithmetic and geometric);
- Population variance; and
- A set of population quantiles. Specifically, we estimated 0.05-quantile and 0.10-quantile per EPA technical direction. We also estimated 100 quantiles from 0.005-quantile to 0.995-quantile, with an increment of 0.01, to characterize the entire distribution of fT4 levels in each group.

We characterized sampling uncertainty surrounding these estimates by computing the standard error (SE) and a 95% confidence interval (CI) for each. For the estimates of means, we used Stata MP12 <svy> procedures that compute the SEs via Taylor series linearization (Kreuter and Valliant, 2007).[3] For the estimates of variance and quantiles, we used an open-source R package <survey> (R Core Team, 2015; Lumley, 2014). In that, we computed SEs for the population variances were computed using Taylor series linearization; we estimated the CIs for the population quantiles based on Woodruff (1952).

Results

Table F-1 provides summary statistics for fT4 levels in the three groups of women. Lactating women and pregnant women have lower mean and lower variance estimates compared to other women.

Table F-1: Characteristics of fT4 Level (ng/dL) Distribution in Three Populations of U.S. Women (NHANES 2007-2012)

Population Parameter	Lactating Women [a] (N=48)			Pregnant Women [b] (N=87)			All Other Women [c] (N=5,181)		
	Estimate (SE) [d]	95% CI		Estimate (SE) [d]	95% CI		Estimate (SE) [d]	95% CI	
Geometric mean	0.713 (0.015)	0.683	0.745	0.689 (0.015)	0.659	0.720	0.797 (0.005)	0.786	0.807
Arithmetic mean	0.721 (0.016)	0.688	0.753	0.714 (0.022)	0.669	0.759	0.812 (0.006)	0.801	0.823
Variance	0.012 (0.005)	-	-	0.057 (0.039)	-	-	0.030 (0.003)	-	-
2.5th quantile	0.536	0.505	0.555	0.400	0.400	0.500	0.600	0.554	0.600
10th quantile	0.583	0.527	0.600	0.502	0.400	0.600	0.640	0.600	0.660

Source: NHANES 2007-2012, Thyroid Profile datasets. Statistics were estimated using information on survey design and weights. Survey weights were adjusted to generate estimates that are representative of the U.S. civilian noninstitutionalized population at the midpoint of the 6-year period.

a. Lactating women are those who answered "yes" to the question "Now breastfeeding a child?" (Variable RHQ 200, NHANES Reproductive Health dataset).
b. Pregnant women were identified based on the RIDEXPRG variable ("Pregnancy Status at Exam – Recode") from the NHANES Demographics File.
c. All women in the Thyroid Profile datasets, except those who were lactating or pregnant, as defined above.
d. SEs and CIs characterize the sampling uncertainty surrounding the point estimates.

[3] We computed geometric means (and the SEs and CIs for these estimates) by taking the natural logarithm of the fT4 variable, calculating the mean, and using the Stata <ereturn> command to obtain the exponentiated form of the mean estimates, SEs, and CIs.

The point estimates and 95% CIs for the quantiles of fT4 levels are provided in the spreadsheet "fT4 summary statistics_1-19-16.xlsx." The plots in Figure F-1 compare the quantiles for each group of women. These plots demonstrate the differences in the quantile estimates for lactating and pregnant women compared to all other women, particularly in the tails of the distributions. The point estimates and CIs for the cumulative distribution function (CDF) for each group of women are presented in Figure F-2. As shown in Figure F-2, the uncertainty that surrounds the quantile estimates for lactating women and pregnant women are much higher than the uncertainty for all other women, due to much smaller sample sizes.

The quality of the direct fT4 quantile estimates for lactating women is likely to be poor due to the small sample size. On the other hand, the quality of fT4 quantile estimates for all other women (i.e., non-lactating, non-pregnant women) is arguably higher due to a much larger sample. If the distribution of fT4 levels in lactating women is similar in shape to the distribution of fT4 levels in all other women, then quantile estimates for lactating women can be obtained by adjusting the quantile estimates for all other women for differences in the mean and the variance between the two distributions.

In addition to evaluating the summary statistics provided in **Table F-1** we also assessed the validity of the assumption that the shape of the fT4 level distribution is same for lactating women and all other women based on EPA direction. To this end, we adjusted the quantile point estimates for all other women for the differences in the mean and the variance between the two populations using:

$$\tilde{q} = s_L \frac{q - m_O}{s_O} + m_L, \qquad (1)$$

Where

$\tilde{q}$ = adjusted point estimate of a quantile of fT4 levels in all other women;
q = point estimate of a quantile of fT4 levels in all other women;
s_L = point estimate of the standard deviation in fT4 levels for lactating women;[4]
m_L = point estimate of the arithmetic mean of fT4 levels for lactating women;
s_O = point estimate of the standard deviation in fT4 levels for all other women;[5] and
m_O = point estimate of the arithmetic mean of fT4 levels for all other women.

We then compared these adjusted quantile point estimates to the CIs for lactating women's fT4 quantiles[6]. Even after the adjustment for the differences in the mean and the variance, other women's quantile estimates fell outside the quantile CIs for lactating women below 0.15-quantile and above 0.785-quantile. Furthermore, for the lower tail of the fT4 level distribution (i.e., below 0.15-quantile), the adjusted quantile point estimates for all other women were higher than the corresponding quantile point for lactating women. This indicates that there are potentially significant differences[7] in shape in the upper and lower tails of the distribution, and that the assumption of shape similarity is likely invalid.

[4] Computed as a square root of variance point estimate, as reported in Table 1 for lactating women.
[5] Computed as a square root of variance point estimate, as reported in Table 1 for all other women.
[6] See excel file sent to EPA on January 21, 2016, file name "Copy of fT4 summary statistics_rev3_1-21-16.xlsx"
[7] We note that there is uncertainty that surrounds estimates of adjusted fT4 quantiles for all other women, for which we did not account in this informal assessment. A better approach would be determine whether there is an overlap in CIs for the corresponding quantiles, which could attenuate the apparent significance of some of the differences in the lower and upper tails of the fT4 level distributions. However, a custom-designed Monte Carlo procedure would be needed to develop CIs for the adjusted quantiles.

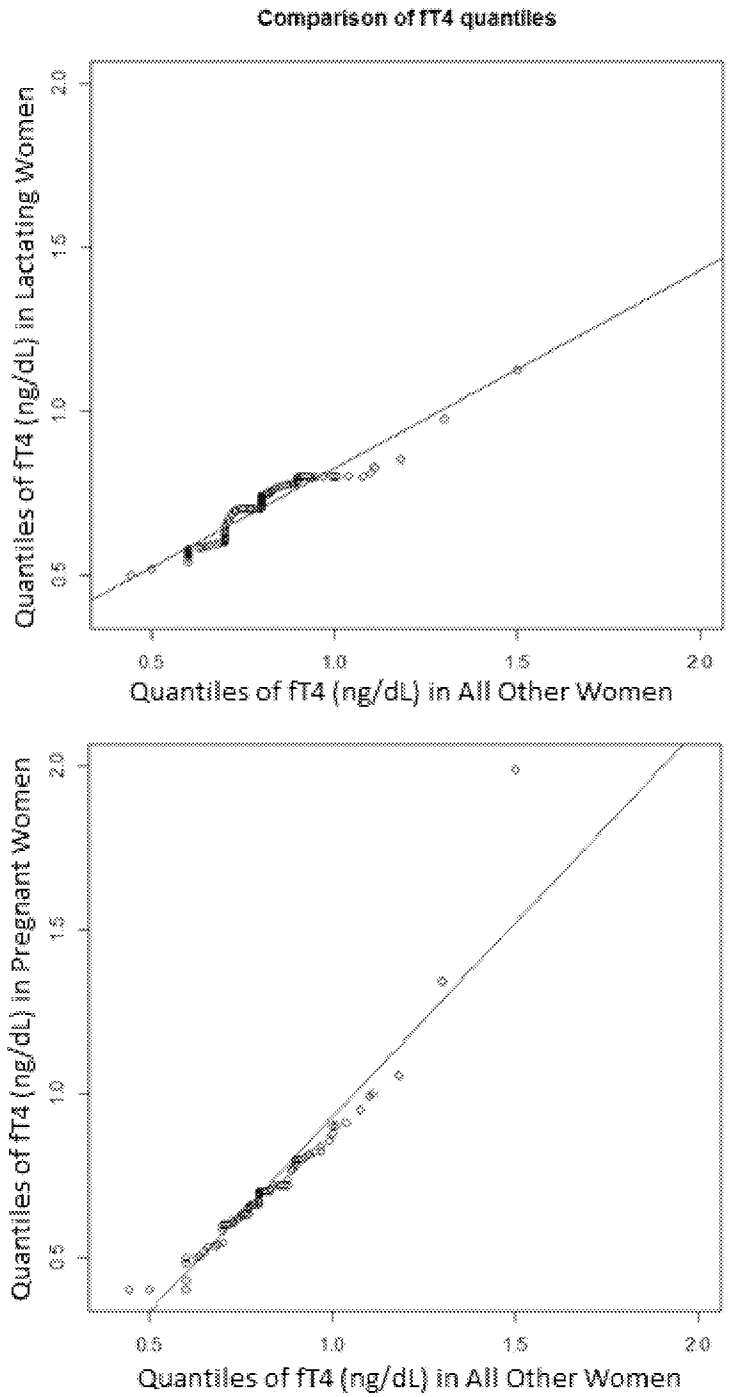

Figure F-1. Comparison of fT4 Level Quantiles for Lactating Women (n=48) and Pregnant Women (n=87) to those for All Other Women (n=5,181), based on NHANES 2007-2012.

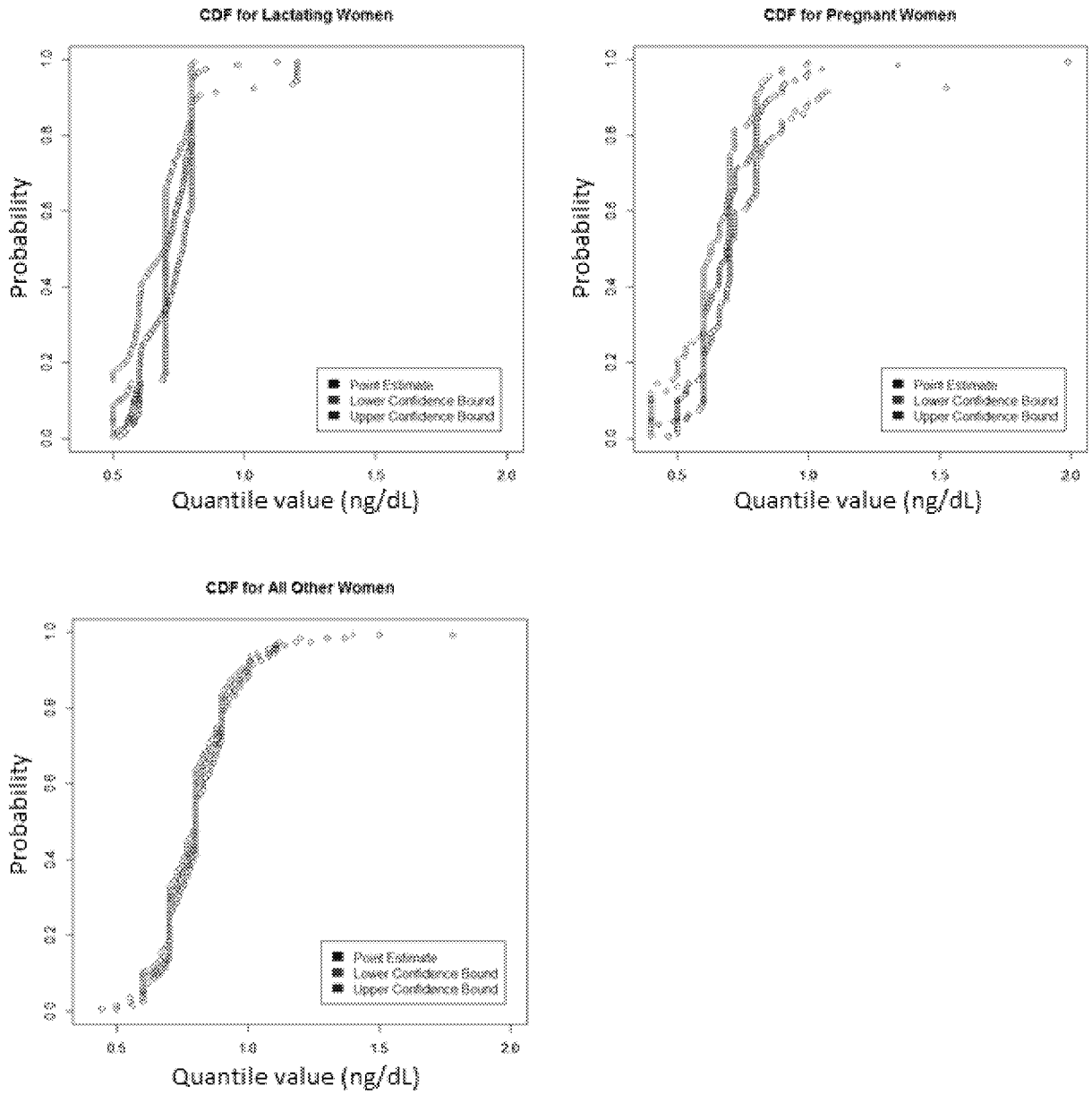

Figure F-2: CDFs of fT4 Levels in Lactating Women (n=48), Pregnant Women (n=87), and All Other Women (n=5,181), based on NHANES 2007-2012

References for Appendix F

Johnson, C.L., Paulose-Ram, R., Ogden, C.L., Carroll, M.D., Kruszon-Moran, D., Dohrmann, S.M., & Curtin, L.R. (2013). National Health and Nutrition Examination Survey: Analytic Guidelines, 1999-2010. National Center for Health Statistics. *Vital Health Stat, 2*(161).

Kreuter. F., & Valliant, R. (2007). A survey on survey statistics: What is done and can be done in Stata. *The Stata Journal, 7*(1), 1-21.

Lumley, T. (2014). Survey: analysis of complex survey samples [Software]. R package, version 3.30. Available from https://cran.r-project.org/web/packages/survey/index.html

R Core Team. (2015). R: A language and environment for statistical computing [Software]. R Foundation for Statistical Computing, Vienna, Austria. Available from https://www.R-project.org/

Woodruff, R.S. (1952). Confidence intervals for medians and other position measures. *Journal of the American Statistical Association 57*, 622- 627.

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Appendix G: Thyroid Hormone Model Equations

Pregnant Mother

A subset of the equations is provided here, which capture the key parts of the iodide and TH equations.

Iodide in thyroid

$d\,A_{thyB,i}/dt = Q_{thy}\times(C_{a,i} - C_{thyB,i}) - R_{NIS,thy,i} - R_{PAthy,i}$! Amount of iodide in thyroid blood

$C_{thyB,i} = A_{thyB,i}/V_{thyB}$

$R_{NIS,thy,i} = Vmax_{NIS,thy}\times C_{thyB,i} /[C_{thyB,i} + Km_{NIS,i}\times (1.0 + C_{thyB,p}/Km_{NIS,p})]$! Active Iodide uptake

$R_{PAthy,i} = PA_{thy,i}\times(C_{thyB,i} - C_{thyT,free,i}/P_{thy,i})$! Bidirectional diffusional uptake

The amount of free iodide in the thyroid was assumed to be a very small quantity, negligible compared to the flux in and out. In the original version of the model it was set up with its own mass balance equation, but this created extreme numerical stiffness. Therefore it was decided to simply set the rate of binding equal to the net rate of uptake from the blood.

$R_{bind,i} = R_{NIS,thy,i} + R_{PAthy,i}$

$d\,A_{bound,i}/dt = R_{bind,i} - R_{ioduT4} - R_{ioduT3}$! Rate of change of bound Iodide pool

$C_{bound,i} = A_{bound,i} / V_{thyT}$

$R_{prodT4} = K_{prodT4} \times A_{bound,i}$! Production rate of T4

$R_{ioduT4} = 4.0*R_{prodT4}$! Rate of Iodide usage for the production of T4

$R_{prodT3} = K_{prodT3} \times A_{bound,i}$! Production rate of T3

$R_{ioduT3} = 3.0\times R_{prodT3}$! Rate of Iodide usage for the production of T3

Thyroxine (T4) (T3 is similar): Two compartments for T4's Volume of distribution (VD and Placenta)

$d\,T4/dt = R_{prodT4} - R_{degT4} + Q_{plc}\times(Cv_{T4,plcB} - C_{T4}) - R_{elimT4}$! Rate of change of T4 in volume of distribution

$fT4 = Frconv_{T4} \times T4$! Total amount of free T4

$C_{T4} = T4/VD_{T4}$; $C_{fT4} = fT4/VD_{T4}$! Concentration of total & free T4

$R_{degT4} = K_{degT4} \times T4$! Degradation rate of T4

$R_{elimT4} = CL_{T4} \times C_{T4}$! Rate of urinary T4 elimination from VD

Half of T4 degradation is assumed to be conversion to T3, which releases one iodide per reaction and the other half is assumed to be complete degradation, which releases 4 iodides per reaction. Hence the rates of iodide released from those processes are as follows (blood iodine mass balance not shown here, since it is otherwise straight-forward).

$R_{deiodT4} = 0.5 \times (R_{degT4} + 4.0\times R_{degT4})$! Rate of iodide released from T4 deiodination

$R_{degT3} = K_{degT3} \times T3$! Degradation rate of T3

$R_{deiodT3} = 3.0 \times R_{degT3}$! Rate of Iodide released from T3 deiodination

Fetus

Model equations for the fetus are almost identical. One difference is that free T4 in the fetus is not assumed to be a constant fraction of total T4 but has its own mass balance equation.

$d\ fT4_f/dt = Q_{cord} \times (Cv_{fT4,plcT} - C_{fT4,f}) + (R_{prodT4,f} - R_{degT4,f}) \times FrconvT4$! Free T4 in fetus

$C_{fT4,f} = fT4_f/VD_{T4,f}$

Lactating Mother

<u>Iodide in thyroid</u>

Unlike the pregnant mother and fetus, free iodide in the thyroid for the lactating mother is tracked with its own differential equation. Here VT is the volume of thyroid tissue.

$d\ AthyT_{f,i}/dt = R_{NIS,thy,i} + R_{PA,thy,i} - R_{bind,i}$! Free iodide amount in maternal thyroid

$CthyT_{f,i} = AthyT_{f,i}/VT$! Free iodide concentration in maternal thyroid tissue

$R_{NIS,thy,i} = Vmax_{thy,i} \times C_{thyBl,i}/[C_{thyBl,i} + Km_{NIS,i} \times (1.0 + CV_{thyBl,p}/Km_{NIS.p})]$

! Rate of active uptake of Iodide into thyroid

$R_{PA,thy,i} = PA_{thy,i} \times (C_{thyBl,i} - CthyT_{f,i}/P_{thy,i})$! Rate of diffusion of iodide from plasma to tissue

The rate of binding/organification of iodide, $R_{bind,I}$, depends on the "unused capacity" for organified iodide in the thyroid, which is ultimately characterized as a concentration:

$Atgi_{pool}$, Atgi ! Total capacity (pool); amount of organified iodide

$A_{remain} = Atgi_{pool} - Atgi$! Amount of unused capacity for organified iodide in thyroid

$Fractionalpool = Atgi/Atgi_{pool}$! Ratio of amount of organified iodide to total capacity

$C_{remain} = A_{remain}/VT$! Concentration of unused capacity for organified iodide

$R_{bind,i} = k_{bind,i} \times VT \times CthyT_{fi} \times C_{remain}$! Rate of iodide binding in thyroid

$R_{prod,T4} = K_{prod,T4} \times Fractionalpool$! Rate of T4 production

$R_{prod,T3} = K_{prod,T4} \times Fractionalpool$! Rate of T3 production

$d\ Atgi/dt = R_{bind,i} - 4.0 \times R_{prod,T4} - 3.0 \times R_{prod,T4}$! Rate of organified iodide accumulation

<u>Thyroxine (T4) (T3 is similar, but urinary clearance assumed negligible/not included for T3)</u>

$d\ T4/dt = R_{prod,T4} - R_{met,T4} - R_{urine,T4} - R_{feces,T4} - R_{Mk,fT4}$! Rate of change of total T4

$fT4 = FrconV_{T4} \times T4$! Free T4

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$C_{T4} = T4/VD_{T4}$; $C_{fT4} = fT4/VD_{T4}$! Concentration of total and free T4

$R_{met,T4} = K_{met,T4} \times T4$! Rate of T4 metabolism/degredation

$R_{deiod,T4} = (4.0 - 3.0*T3_{FRAC})*R_{met,T4}$! Rate of Iodide released from T4 metabolism

! $T3_{FRAC}$ is the fraction of T4 metabolism that is converted to T3.

! 1 iodide released from T3 formation, 4 released from remaining fraction (via rT3)

$R_{urine,T4} = CL_{U,fT4} \times C_{fT4}$! Rate of T4 urinary elimination

$R_{feces,T4} = CL_{feces,fT4} \times C_{fT4}$! Rate of T4 fecal elimination

$R_{Mk,fT4} = CL_{Milk,fT4} \times C_{fT4}$! Rate of T4 elimination to milk

Infant

The equations for iodide uptake and disposition in the thyroid, and T4 and T3 production and elimination in the infant are identical to the mother (including the fact that urinary elimination of T3 is assumed negligible) with the exception that there's no excretion to milk in the infant and that the production of T3 is zero-order:

$R_{prod,T3,N} = V_{prodT3,N}$! Rate of T3 production in the infant

$V_{prod,T3,N} = V_{prod,T4,N} \times V_{prod,T3,F,N}$! T3 max production in the infant (nmol/hr)

! $V_{prod,T3,F,N}$ is the ratio of T3 to T4 (maximal) production rates, hence is unitless.

From the model code of Fisher et al. (2016), "Assume that for the range of iodide intake of interest.. T3 production increase relative to decrease in T4 for low iodide intake." In other words, this is based on an assumption that over the iodide range of interest, there is a compensatory increase in the relative rate of T3 production in the infant as organified iodide in the thyroid and T4 production decline, such that T3 production remains constant. This mechanism is not assumed to apply in the mother.

Also, a minor difference is that fecal elimination in the infant uses total T4 concentration rather than C_{fT4}.

Fisher, W; Wang, J; George, NI; Gearhart, JM; McLanahan, ED. (2016). Dietary Iodine Sufficiency and Moderate Insufficiency in the Lactating Mother and Nursing Infant: A Computational Perspective. PLoS ONE 11: e0149300. http://dx.doi.org/10.1371/journal.pone.0149300

Draft Perchlorate Biologically Based Dose-Response (BBDR) Model

EPA, with contributions from Food and Drug Administration (FDA) scientists, developed a BBDR (also known as a PBPK/PD) model. The BBDR model was developed by integrating PBPK models for perchlorate and iodide with BBDR models for thyroid hormones to predict the effect of perchlorate on the thyroid gland in formula-fed and breast-fed infants for the postnatal period from days 7 to 90, as well as lactating women. The draft model is focused on the condition of hypothyroxinemia as an indicator of the potential adverse health effects. This integrated draft model predicts the effects of perchlorate on serum thyroid hormone concentrations in the pregnant and lactating mother exposed to perchlorate in the diet and in infants exposed via ingestion of perchlorate in formula or breast milk.

The model was developed in acslX and was subsequently translated to R/MCSIM. The draft BBDR model code files can be accessed at:
https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/3352518

The files include the following:

3352514 – Perchlorate-Iodide-Thyroid-Hormone BBDR Model for the Pregnant Mother and Fetus – acslX version

3352515 – Perchlorate-Iodide-Thyroid-Hormone BBDR Model for the Lactating Mother and Infant – acslX version

3352516 – Perchlorate-Iodide-Thyroid-Hormone BBDR Model for the Pregnant Mother and Fetus – R/MCSIM

3352517 – Perchlorate-Iodide-Thyroid-Hormone BBDR Model for the Lactating Mother and Infant – R/MCSIM

Message

From: Kim, Jim H. EOP/OMB [Ex. 6 EOP (PP)]
Sent: 9/28/2016 5:20:00 PM
To: Christ, Lisa [Christ.Lisa@epa.gov]
CC: Dorjets, Vlad EOP/OMB [Ex. 6 EOP (PP)]; Grevatt, Peter [Grevatt.Peter@epa.gov]; Greene, Ashley [Greene.Ashley@epa.gov]; Burneson, Eric [Burneson.Eric@epa.gov]; Schwab, Margo EOP/OMB [Ex. 6 EOP (PP)]
Subject: Re: Perchlorate Peer Review Advance Materials

Hi Lisa,

Just wanted to note that Margo's email was mistyped on your original email. The correct address is now CC'd.

Best regards,
Jim

On Sep 28, 2016, at 10:36 AM, Christ, Lisa <Christ.Lisa@epa.gov<mailto:Christ.Lisa@epa.gov>> wrote:

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

Lisa Christ, Chief
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USEPA
1200 Pennsylvania Ave NW
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phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M

<1. Draft Charge Questions for Perchlorate BBDR Model Peer Review 9-28-16.pdf>
<2. Draft Perchlorate BBDR Model Report for Peer Review 9-28-16.pdf>
<3. Draft Perchlorate BBDR Model Report for Peer Review - Appendices 9-28-16.pdf>
<4. Link to Obtain Draft Perchlorate BBDR Model for Peer Review 9-28-16.pdf>

Message

From: Christ, Lisa [Christ.Lisa@epa.gov]
Sent: 9/28/2016 5:06:12 PM
To: Grevatt, Peter [Grevatt.Peter@epa.gov]; Burneson, Eric [Burneson.Eric@epa.gov]
CC: Greene, Ashley [Greene.Ashley@epa.gov]
Subject: RE: Perchlorate Peer Review Advance Materials

On the call we agreed to Ex. 5 Deliberative Process (DP) but as I recall, Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) You explained that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)

From: Grevatt, Peter
Sent: Wednesday, September 28, 2016 12:44 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Cc: Greene, Ashley <Greene.Ashley@epa.gov>
Subject: Fwd: Perchlorate Peer Review Advance Materials

I'm good with this.

Sent from my iPhone

Begin forwarded message:

> **From:** "Kim, Jim H. EOP/OMB" < Ex. 6 EOP (PP)
> **Date:** September 28, 2016 at 12:00:32 PM EDT
> **To:** "Christ, Lisa" <Christ.Lisa@epa.gov>, "Dorjets, Vlad EOP/OMB" < Ex. 6 EOP (PP) >, " Ex. 6 EOP (PP) >
> **Cc:** "Grevatt, Peter" <Grevatt.Peter@epa.gov>, "Greene, Ashley" <Greene.Ashley@epa.gov>, "Burneson, Eric" <Burneson.Eric@epa.gov>
> **Subject: RE: Perchlorate Peer Review Advance Materials**
>
> Lisa,
>
> Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.
>
> I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) the second would be to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) and the third would Ex. 5 Deliberative Process (DP)
>
> Can we start looking at dates/times for the end of next week?
>
> Best regards,
> Jim
>
> ---
>
> **From:** Christ, Lisa [mailto:Christ.Lisa@epa.gov]
> **Sent:** Wednesday, September 28, 2016 10:36 AM
> **To:** Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) ; Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP)
> **Cc:** Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric

<Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

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Predicted Effect of Perchlorate on Thyroid Hormone Levels in the Pregnant & Lactating Mother, Fetus, and Breast- and Bottle-Fed Infant: Model Dose-Response Application

Paul M Schlosser

U.S. Environmental Protection Agency, ORD, NCEA-Washington

September 27, 2016

Background

- In 2012, as required by the Safe Drinking Water Act, EPA sought guidance from the Science Advisory Board on how best to consider and interpret life stage information, epidemiologic and biomonitoring data, physiologically-based pharmacokinetic (PBPK) analyses and the totality of perchlorate health information to derive an MCLG for perchlorate.
- In 2013, the SAB recommended that:

  "… EPA derive a perchlorate MCLG that addresses sensitive life stages through physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling based upon its mode of action rather than the default MCLG approach using the reference dose and specific chemical exposure parameters"

  "…the following MOA-based approach for using PBPK/PD modeling and additional clinical and toxicological data to inform the derivation of a health-protective MCLG recognizing that the sensitive populations for perchlorate exposure are the fetuses of hypothyroxinemic pregnant women, and infants exposed to perchlorate through either water-based formula preparations or the breast milk of lactating women"
- A team of scientists lead by Dr. Jeff Fisher, U.S. FDA, developed the Biologically Based Dose-Response (BBDR) model
  - Integrates PBPK models for perchlorate and iodide with BBDR models for thyroid hormones
  - Predicts effect of perchlorate on thyroid hormone levels in formula-fed and breast-fed infants, and lactating women, for postnatal days 7 to 90

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Background II

- June 2013: SAB Recommendations – EPA should:
  - Use hypothyroxinemia (hX) as immediate endpoint vs. Radio-Iodide Uptake (RAIU) Inhibition suggested by NAS in 2005
  - hX = moderate TH (T4 and T3) reduction, with thyroid stimulating hormone (TSH) in normal range
- Excluding TSH feedback makes the modeling less complex, but limits range for which it is valid
- Report includes statistical analyses to define range for hX
  - Range defined *relative to model-predicted fT4 levels for each life-stage*, given suggested iodine nutrition levels (i.e., euthyroid state)
  - This approach used to control for bias due to possible over- or under-prediction of the model vs. actual population mean TH levels
- Study populations providing data for model calibration, hence "controls" for perchlorate exposure, are assumed to have similar exposures to other goitrogens (SCN^-, NO_3^-) as populations of concern
  - Hence no specific correction for exposure to these other common goitrogens

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General Picture

- **Idealized view:**

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Bottle/formula-fed Infant: Days 7-90
200 µg/d iodine during pregnancy

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Breast-fed infant: maternal nutrition & perchlorate exposure = pregnancy levels

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Summary: Formula-/Breast-Fed Infant & Lactating Mom (Perchlorate ≤ 20 µg/kg/d)

Ex. 5 Deliberative Process (DP)

Predicted Effect of Perchlorate on Thyroid Hormone Levels in the Pregnant & Lactating Mother, Fetus, and Breast- and Bottle-Fed Infant: Model Dose-Response Application

Paul M Schlosser

U.S. Environmental Protection Agency, ORD, NCEA-Washington

September 27, 2016

Background

- In 2012, as required by the Safe Drinking Water Act, EPA sought guidance from the Science Advisory Board on how best to consider and interpret life stage information, epidemiologic and biomonitoring data, physiologically-based pharmacokinetic (PBPK) analyses and the totality of perchlorate health information to derive an MCLG for perchlorate.
- In 2013, the SAB recommended that:

  "… EPA derive a perchlorate MCLG that addresses sensitive life stages through physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling based upon its mode of action rather than the default MCLG approach using the reference dose and specific chemical exposure parameters"

  "…the following MOA-based approach for using PBPK/PD modeling and additional clinical and toxicological data to inform the derivation of a health-protective MCLG recognizing that the sensitive populations for perchlorate exposure are the fetuses of hypothyroxinemic pregnant women, and infants exposed to perchlorate through either water-based formula preparations or the breast milk of lactating women"
- A team of scientists lead by Dr. Jeff Fisher, U.S. FDA, developed the Biologically Based Dose-Response (BBDR) model
  - Integrates PBPK models for perchlorate and iodide with BBDR models for thyroid hormones
  - Predicts effect of perchlorate on thyroid hormone levels in formula-fed and breast-fed infants, and lactating women, for postnatal days 7 to 90

Background II

- June 2013: SAB Recommendations – EPA should:
  - Use hypothyroxinemia (hX) as immediate endpoint vs. Radio-Iodide Uptake (RAIU) Inhibition suggested by NAS in 2005
  - hX = moderate TH (T4 and T3) reduction, with thyroid stimulating hormone (TSH) in normal range
- Excluding TSH feedback makes the modeling less complex, but limits range for which it is valid
- Report includes statistical analyses to define range for hX
  - Range defined *relative to model-predicted fT4 levels for each life-stage*, given suggested iodine nutrition levels (i.e., euthyroid state)
  - This approach used to control for bias due to possible over- or under-prediction of the model vs. actual population mean TH levels
- Study populations providing data for model calibration, hence "controls" for perchlorate exposure, are assumed to have similar exposures to other goitrogens (SCN^-, NO_3^-) as populations of concern
  - Hence no specific correction for exposure to these other common goitrogens

General Picture

- **Idealized view:**

Ex. 5 Deliberative Process (DP)

Pregnancy Dose-Response: 1st View

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

EPA

Bottle/formula-fed Infant: Day 7

Ex. 5 Deliberative Process (DP)

Bottle/formula-fed Infant: Days 7-90
200 µg/d iodine during pregnancy

Ex. 5 Deliberative Process (DP)

Breast-feeding mother: maternal nutrition & perchlorate exposure = pregnancy levels

Ex. 5 Deliberative Process (DP)

Breast-fed infant: maternal nutrition & perchlorate exposure = pregnancy levels

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Summary: Formula-/Breast-Fed Infant & Lactating Mom (Perchlorate ≤ 20 µg/kg/d)

Ex. 5 Deliberative Process (DP)

Message

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 9/28/2016 4:42:35 PM
To: Grevatt, Peter [Grevatt.Peter@epa.gov]
CC: Christ, Lisa (Christ.Lisa@epa.gov) [Christ.Lisa@epa.gov]
Subject: FW: Perchlorate Peer Review Advance Materials

Peter:
I think we need to talk about how best to respond to this request.

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 EOP (PP)
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) ; Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) the second would be to discuss Ex. 5 Deliberative Process (DP) and the third would Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, September 28, 2016 10:36 AM
To: Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) >; Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) ; 'schwab.margo@omb.eop.gov' < Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

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Treatment of Subclinical Hypothyroidism or Hypothyroxinemia in Pregnancy

B.M. Casey, E.A. Thom, A.M. Peaceman, M.W. Varner, Y. Sorokin, D.G. Hirtz, U.M. Reddy, R.J. Wapner, J.M. Thorp, Jr., G. Saade, A.T.N. Tita, D.J. Rouse, B. Sibai, J.D. Iams, B.M. Mercer, J. Tolosa, S.N. Caritis, and J.P. VanDorsten, for the Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal–Fetal Medicine Units Network*

ABSTRACT

BACKGROUND

Subclinical thyroid disease during pregnancy may be associated with adverse outcomes, including a lower-than-normal IQ in offspring. It is unknown whether levothyroxine treatment of women who are identified as having subclinical hypothyroidism or hypothyroxinemia during pregnancy improves cognitive function in their children.

METHODS

We screened women with a singleton pregnancy before 20 weeks of gestation for subclinical hypothyroidism, defined as a thyrotropin level of 4.00 mU or more per liter and a normal free thyroxine (T_4) level (0.86 to 1.90 ng per deciliter [11 to 24 pmol per liter]), and for hypothyroxinemia, defined as a normal thyrotropin level (0.08 to 3.99 mU per liter) and a low free T_4 level (<0.86 ng per deciliter). In separate trials for the two conditions, women were randomly assigned to receive levothyroxine or placebo. Thyroid function was assessed monthly, and the levothyroxine dose was adjusted to attain a normal thyrotropin or free T_4 level (depending on the trial), with sham adjustments for placebo. Children underwent annual developmental and behavioral testing for 5 years. The primary outcome was the IQ score at 5 years of age (or at 3 years of age if the 5-year examination was missing) or death at an age of less than 3 years.

RESULTS

A total of 677 women with subclinical hypothyroidism underwent randomization at a mean of 16.7 weeks of gestation, and 526 with hypothyroxinemia at a mean of 17.8 weeks of gestation. In the subclinical hypothyroidism trial, the median IQ score of the children was 97 (95% confidence interval [CI], 94 to 99) in the levothyroxine group and 94 (95% CI, 92 to 96) in the placebo group (P=0.71). In the hypothyroxinemia trial, the median IQ score was 94 (95% CI, 91 to 95) in the levothyroxine group and 91 (95% CI, 89 to 93) in the placebo group (P=0.30). In each trial, IQ scores were missing for 4% of the children. There were no significant between-group differences in either trial in any other neurocognitive or pregnancy outcomes or in the incidence of adverse events, which was low in both groups.

CONCLUSIONS

Treatment for subclinical hypothyroidism or hypothyroxinemia beginning between 8 and 20 weeks of gestation did not result in significantly better cognitive outcomes in children through 5 years of age than no treatment for those conditions. (Funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development and the National Institute of Neurological Disorders and Stroke; ClinicalTrials.gov number, NCT00388297.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Casey at the Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235, or at brian.casey@utsouthwestern.edu.

*A complete list of the investigators in the Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal–Fetal Medicine Units Network is provided in the Supplementary Appendix, available at NEJM.org.

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Observational studies spanning almost three decades suggest that subclinical thyroid disease during pregnancy is associated with adverse outcomes.[1-5] In 1999, interest in undiagnosed maternal thyroid dysfunction was heightened by studies suggesting an association between subclinical thyroid hypofunction and impaired fetal neuropsychological development.[6,7] In one report, children of women whose serum thyrotropin levels during pregnancy were greater than the 98th percentile had a lower IQ than children of matched controls who had a normal thyrotropin level.[6] In another study, children whose mothers had a serum free thyroxine (T_4) level of less than the 10th percentile in early pregnancy had impaired psychomotor development at 10 months of age, as compared with children whose mothers had a higher free T_4 level.[7] Subclinical hypothyroidism has also been associated with increased risks of preterm birth, placental abruption, admission to the intensive care nursery (or neonatal intensive care unit), and other adverse pregnancy outcomes that could explain neurodevelopmental delay.[8-11] However, the risks of these adverse outcomes are not increased among women with hypothyroxinemia in pregnancy.[12,13]

These findings led several professional organizations to recommend routine prenatal screening for and treatment of subclinical thyroid disease during pregnancy.[14] This recommendation could affect more than 15% of pregnant women, depending on the thyrotropin and free T_4 thresholds used.[15] However, the American College of Obstetricians and Gynecologists has maintained that recommendations for routine screening are premature in the absence of trials showing an improvement in these outcomes with levothyroxine treatment.[16] The Controlled Antenatal Thyroid Screening (CATS) study showed that cognitive function in 3-year-old children was not better than that in controls when mothers who had been identified with subclinical hypothyroidism or hypothyroxinemia were treated with levothyroxine.[17] Despite this evidence, the treatment of subclinical thyroid dysfunction is still recommended by several organizations in their clinical practice guidelines.[18,19] The primary objective of our trials involving women with either subclinical hypothyroidism or hypothyroxinemia was to assess the effect of screening and thyroxine replacement during pregnancy on the IQ of children at 5 years of age.

METHODS

STUDY POPULATION

We conducted two multicenter, randomized, placebo-controlled trials in parallel at 15 centers within the Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal–Fetal Medicine Units Network. The protocol, available with the full text of this article at NEJM.org, was approved by the institutional review board at each center. All the women with a singleton pregnancy who presented for prenatal care before 20 weeks of gestation were invited to undergo thyroid screening for serum thyrotropin and free T_4 values. The serum samples obtained from women who provided written informed consent were analyzed at a centralized laboratory (see the Supplementary Appendix, available at NEJM.org).

The criteria that were used to diagnose subclinical hypothyroidism at the origin of the trial were a thyrotropin level of 3.00 mU or more per liter (which was presumed to correspond to the 97.5th percentile) and a free T_4 level in the normal range (0.86 to 1.90 ng per deciliter [11 to 24 pmol per liter]). After 10 months of screening, during a planned evaluation, the prevalence of subclinical hypothyroidism was found to exceed 6%. On the basis of an analysis of the 97.5th percentile in the first 15,000 women who underwent screening, the cutoff for the thyrotropin level was subsequently increased to 4.00 mU per liter. Hypothyroxinemia was defined as a thyrotropin level in the normal range (0.08 to 3.99 mU per liter) and a free T_4 level of less than 0.86 ng per deciliter.

Women with either subclinical hypothyroidism or hypothyroxinemia and an ultrasonographically verified singleton pregnancy between 8 weeks 0 days of gestation and 20 weeks 6 days of gestation met the inclusion criteria. The complete eligibility criteria are listed in the Supplementary Appendix. Women who were found to have overt hypothyroidism or hyperthyroidism at the time of screening were excluded, and obstetrical providers were notified of abnormal test results for subsequent follow-up.

TRIAL REGIMENS

Eligible women who provided written informed consent were given a 7-day supply of placebo capsules (adherence run-in phase). Women who took 50% or more of the capsules and returned within 2 weeks were randomly assigned, in a 1:1

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ratio, to receive either levothyroxine or placebo in one of two trials, the subclinical hypothyroidism trial or the hypothyroxinemia trial, according to their results on the thyrotropin and free T_4 tests. Separate randomization sequences were prepared at the independent data coordinating center with the use of the simple urn method,[20] with stratification according to clinical site. Numbered trial-regimen kits were prepared, and at randomization, each patient was assigned to the next sequentially numbered kit. Blood and urine samples were obtained from all the participants for analysis, at the central laboratory, of thyroid peroxidase antibodies and urinary iodine concentration.

Participants with subclinical hypothyroidism began taking 100 μg of levothyroxine or matching placebo daily. Participants with hypothyroxinemia began taking 50 μg of levothyroxine or matching placebo daily; the lower dose in the hypothyroxinemia trial was intended to avoid overtreatment in women with mild suppression of free T_4 at trial entry. Women in the two trials were seen monthly, and blood samples for thyrotropin and free T_4 testing were sent to the same laboratory. Results were reported to the coordinating center, which notified the clinical center whether a dose adjustment was required according to the algorithm shown in Table S2 in the Supplementary Appendix. Sham adjustments were communicated for the placebo group. Adjustments were made within 7 days after the blood test. The goal for women with subclinical hypothyroidism was a thyrotropin level between 0.1 and 2.5 mU per liter, with a maximum daily dose of 200 μg of levothyroxine. The goal for participants with hypothyroxinemia was a free T_4 level between 0.86 and 1.90 ng per deciliter, with the same maximum dose of levothyroxine.

Pregnancy and neonatal outcomes were abstracted from the medical records by certified research staff. The children underwent developmental testing annually for 5 years. Examiners were trained to administer each test, and they submitted a videotaped encounter to two expert psychologists for initial certification. Annual recertification was required. Research staff, examiners, and participants were unaware of the trial-group assignments.

TRIAL OUTCOMES

The primary outcome was the full-scale IQ as assessed with the use of the Wechsler Preschool and Primary Scale of Intelligence III (WPPSI-III) at 5 years of age, or with the overall (general conceptual ability) score from the Differential Ability Scales–II (DAS) at 3 years of age if the WPPSI-III score was not available, or death before 3 years of age (because it was a competing event for IQ score). Results are expressed as age-standardized scores, with an expected population mean of 100 and a standard deviation of 15. The DAS and WPPSI-III scores correlate well ($r=0.89$). Prespecified subgroup analyses for the primary outcome were performed according to gestational age at randomization, race or ethnic group, and baseline thyroid peroxidase antibody, thyrotropin, free T_4, and iodine levels.

Secondary outcomes in infants and children included the cognitive, motor, and language scores on the Bayley Scales of Infant Development, Third Edition (Bayley-III), at 12 months and 24 months of corrected age; DAS overall scores at 36 months of age; specific scores on the DAS (subtests regarding recall of digits forward and recognition of pictures) plus the Conners' Rating Scales–Revised at 48 months of age for assessment of attention; and scores on the Child Behavior Checklist at 36 months and 60 months of age for assessment of behavioral and social competency. Maternal and neonatal secondary outcomes included preterm delivery, pregnancy complications, fetal death, and neonatal morbidity and mortality. A complete list of secondary outcomes is provided in the Supplementary Appendix.

STATISTICAL ANALYSIS

Assuming an analysis that would be based on a Wilcoxon test with a 5-point difference between the group median IQ scores, a death rate before the age of 3 years (including spontaneous abortions, stillbirths, and neonatal and infant deaths) of 2 to 5%, and 15% loss to follow-up, we initially calculated that a sample of 500 patients in each trial (250 women per group) would provide the trial with a power of at least 80%, at a two-sided type I error rate of 5%. When the eligibility criteria for the subclinical hypothyroidism trial changed, the sample was adjusted to 670 participants under the assumption that there would be no between-group difference in the children's IQ scores if the mothers' thyrotropin levels were between 3.00 and 4.00 mU per liter, but there would be a between-group difference of 5 points if the mothers' thyrotropin levels were 4.00 mU or more per liter.

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The analysis was performed according to the intention-to-treat principle. The primary outcome and other continuous variables were compared with the use of the Wilcoxon test or van Elteren's test for stratified analysis. For the primary outcome, death before 3 years of age was assigned a score of 0 (lowest possible rank) and was included in the estimation of the median. Differences between groups were estimated with the use of the Hodges–Lehmann estimator, and 95% confidence intervals were reported. Categorical variables were analyzed with the use of the chi-square test or Fisher's exact test, as appropriate. To test for interaction in the prespecified subgroup analyses, we used regression with normal-order scores.

An independent data and safety monitoring committee monitored the trials. Since recruitment was completed before any 5-year outcomes were available, there was no interim analysis of the primary outcome. For secondary outcomes, nominal P values of less than 0.05 were considered to indicate statistical significance. No adjustments were made for multiple comparisons.

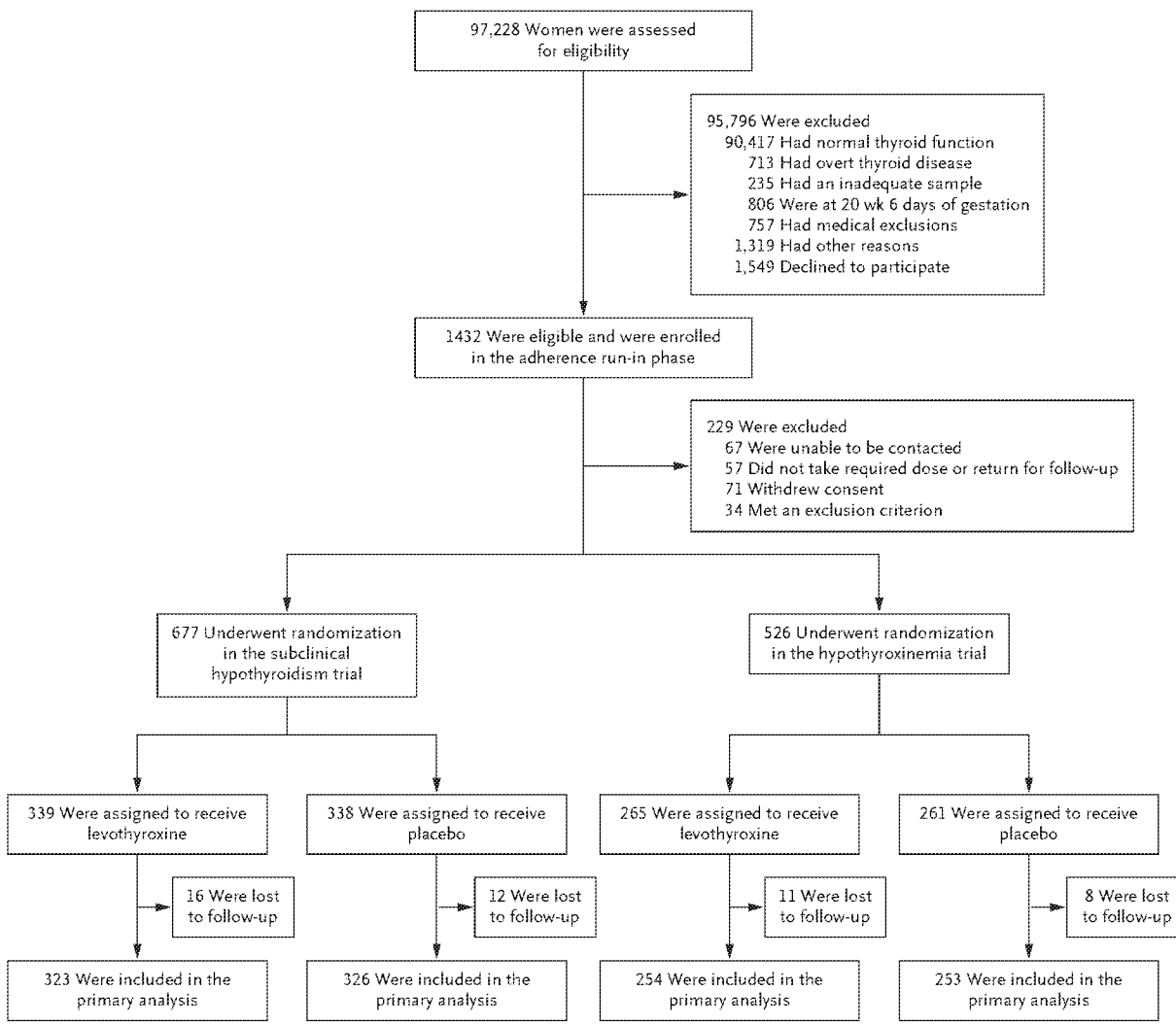

Figure 1. Screening and Enrollment.
Two women with hypothyroxinemia underwent randomization in error in the subclinical hypothyroidism trial, and one with normal thyroid function underwent randomization in error in the hypothyroxinemia trial.

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RESULTS

TRIAL POPULATIONS

From October 2006 through October 2009, a total of 97,228 pregnant women underwent thyroid screening. A total of 90,417 women (93%) had results that were considered to be normal, 463 (<1%; prevalence, 5 cases per 1000 pregnant women) had overt hypothyroidism, and 250 (<1%; prevalence, 3 cases per 1000 pregnant women) had overt hyperthyroidism. Of the 3057 women (3%) with subclinical hypothyroidism, 800 were eligible and consented to participate in the adherence run-in phase, and 677 underwent randomization. Of the 2805 women (3%) with hypothyroxinemia, 632 were eligible and consented to participate in the adherence run-in phase, and 526 underwent randomization (Fig. 1). IQ scores were not available for 4% of the offspring in each trial (28 offspring in the subclinical hypothyroidism trial and 19 in the hypothyroxinemia trial).

There were no significant differences at baseline between the levothyroxine group and the placebo group in either trial (Table 1). The population in each trial was iodine-sufficient (median urinary iodine concentration, ≥150 μg per liter).[21] On average, women in the subclinical hypothyroidism trial underwent randomization before 17 weeks of gestation, and 93% of the women in the levothyroxine group had a thyrotropin level between 0.1 and 2.5 mU per liter by a median gestational age of 21 weeks. In the hypothyroxinemia trial, women underwent randomization

Table 1. **Maternal Characteristics at Baseline.***

Characteristic	Subclinical Hypothyroidism		Hypothyroxinemia	
	Levothyroxine (N=339)	Placebo (N=338)	Levothyroxine (N=265)	Placebo (N=261)
Age — yr	27.7±5.7	27.3±5.7	27.8±5.7	28.0±5.8
Race or ethnic group — no. (%)†				
Black	27 (8)	25 (7)	61 (23)	65 (25)
Hispanic	195 (58)	185 (55)	131 (49)	125 (48)
White	109 (32)	117 (35)	69 (26)	69 (26)
Other	8 (2)	11 (3)	4 (2)	2 (1)
Body-mass index‡	28.1±6.4	28.2±6.4	30.3±6.4	30.2±7.1
Nulliparous — no. (%)	124 (37)	134 (40)	69 (26)	64 (25)
Baseline thyrotropin — mU/liter				
Median	4.5	4.3	1.5	1.4
95% CI	4.4–4.7	4.2–4.5	1.4–1.6	1.3–1.5
Baseline free thyroxine — ng/dl§				
Median	1.01	1.02	0.83	0.83
95% CI	1.00–1.02	1.01–1.04	0.82–0.83	0.82–0.83
Urinary iodine — μg/liter¶				
Median	199	196	185	191
95% CI	184–238	172–229	167–219	164–208
No. of weeks of gestation at randomization	16.6±3.0	16.7±3.0	18.0±2.8	17.7±2.9

* Plus–minus values are means ±SD. There were no significant differences at baseline between the levothyroxine group and the placebo group in either trial (P>0.05). CI denotes confidence interval.
† Race and ethnic group were determined by the research nurses.
‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.
§ To convert thyroxine values to picomoles per liter, multiply by 12.87.
¶ One patient in the levothyroxine group and one in the placebo group in the subclinical hypothyroidism trial were missing the urinary iodine measurement.

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at a mean gestational age of 18 weeks, and 83% of the women in the levothyroxine group met the treatment goal (free T_4 level between 0.86 and 1.90 ng per deciliter) by a median gestational age of 23 weeks.

PREGNANCY AND NEONATAL OUTCOMES

The frequencies of adverse pregnancy and neonatal outcomes did not differ significantly between the groups in either trial (Table 2). After neonatal discharge, there was one death in the subclinical hypothyroidism trial and none in the hypothyroxinemia trial. Two women were lost to follow-up before delivery. There was no significant difference between the levothyroxine group and the placebo group in either trial with regard to the mean gestational age at delivery (subclinical hypothyroidism trial: 39.1±2.5 weeks and

Table 2. **Pregnancy and Neonatal Outcomes.***

Outcome	Subclinical Hypothyroidism			Hypothyroxinemia		
	Levothyroxine (N=339)	Placebo (N=338)	P Value	Levothyroxine (N=263)	Placebo (N=261)	P Value
Maternal						
Week of gestation at delivery	39.1±2.5	38.9±3.1	0.57	39.0±2.4	38.8±3.1	0.46
Preterm birth — no. (%)						
At <34 wk	9 (3)	10 (3)	0.81	10 (4)	7 (3)	0.47
At <37 wk	31 (9)	37 (11)	0.44	31 (12)	20 (8)	0.11
Placental abruption — no. (%)	1 (<1)	5 (1)	0.12	3 (1)	2 (1)	1.00
Gestational hypertension — no. (%)	33 (10)	36 (11)	0.69	20 (8)	24 (9)	0.51
Preeclampsia — no. (%)	22 (6)	20 (6)	0.76	9 (3)	11 (4)	0.64
Gestational diabetes — no. (%)	25 (7)	22 (7)	0.66	21 (8)	24 (9)	0.62
Fetal or neonatal†						
Stillbirth or miscarriage — no. (%)	4 (1)	7 (2)	0.36	2 (1)	5 (2)	0.28
Neonatal death — no. (%)	0	1 (<1)	0.50	1 (<1)	1 (<1)	1.00
Apgar score at 1 min <4 — no. (%)	6 (2)	7 (2)	0.76	6 (2)	7 (3)	0.76
Apgar score at 5 min <7 — no. (%)	2 (1)	3 (1)	0.69	2 (1)	4 (2)	0.45
Admission to NICU — no. (%)	29 (9)	21 (6)	0.24	31 (12)	31 (12)	0.97
Birth weight <10th percentile — no. (%)	33 (10)	27 (8)	0.45	23 (9)	20 (8)	0.68
Head circumference — cm	33.9±1.8	33.9±1.7	0.46	33.9±1.8	34.2±1.6	0.19
Respiratory distress syndrome — no. (%)	9 (3)	6 (2)	0.45	4 (2)	5 (2)	0.75
Retinopathy of prematurity — no. (%)	1 (<1)	0	1.00	0	0	—
Necrotizing enterocolitis — no. (%)	1 (<1)	1 (<1)	1.00	2 (1)	0	0.50
Bronchopulmonary dysplasia — no. (%)	0	1 (<1)	0.50	0	1 (<1)	0.49
Composite neonatal outcome — no. (%)‡	7 (2)	12 (4)	0.24	5 (2)	7 (3)	0.55
Respiratory therapy ≥1 day — no. (%)	11 (3)	11 (3)	0.99	13 (5)	12 (5)	0.85
No. of days in hospital nursery			0.43			0.39
Median	2	2		2	2	
95% CI	2–2	2–2		2–2	2–2	

* Plus–minus values are means ±SD. NICU denotes neonatal intensive care unit.

† Analyses of neonatal outcomes of the respiratory distress syndrome, retinopathy of prematurity, necrotizing enterocolitis, and bronchopulmonary dysplasia did not include stillbirths or miscarriages in the levothyroxine group (four offspring) or the placebo group (seven) in the subclinical hypothyroidism trial or in the levothyroxine group (two) or the placebo group (four) in the hypothyroxinemia trial. One infant in the placebo group in the hypothyroxemia trial was born out of network and was also not included in the analyses.

‡ The composite neonatal outcome was defined as periventricular leukomalacia, intraventricular hemorrhage of grade III or IV, necrotizing enterocolitis (stage ≥II), severe retinopathy of prematurity (stage ≥III), the severe respiratory distress syndrome, bronchopulmonary dysplasia, neonatal death, stillbirth, or serious infectious complication.

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38.9±3.1 weeks, respectively; P=0.57; and hypothyroxinemia trial: 39.0±2.4 weeks and 38.8±3.1 weeks, respectively; P=0.46). There were no significant differences in overall or serious adverse events between the two groups in either trial; serious adverse events were rare in the two trials (Tables S4 and S5 in the Supplementary Appendix).

NEURODEVELOPMENTAL AND BEHAVIORAL OUTCOMES

In the subclinical hypothyroidism trial, data on the primary outcome were available for 649 offspring (96%) (Table 3). A total of 11 children had DAS scores at 3 years that were substituted for the WPPSI-III IQ score, and 13 offspring died

Table 3. Developmental and Behavioral Outcomes in Offspring of Mothers with Subclinical Hypothyroidism.*

Outcome	Levothyroxine		Placebo		Difference (95% CI)†	P Value
	No. of Children	Median Value (95% CI)	No. of Children	Median Value (95% CI)		
Primary outcome‡	323	97 (94 to 99)	326	94 (92 to 96)	0 (−3 to 2)	0.71
Bayley-III score§						
At 12 mo						
Cognitive	311	100 (95 to 100)	315	100 (95 to 100)	0 (0 to 0)	0.63
Motor	312	97 (97 to 97)	314	97 (97 to 97)	0 (0 to 3)	0.83
Language	309	94 (94 to 97)	312	94 (94 to 97)	0 (0 to 3)	0.48
At 24 mo						
Cognitive	308	90 (90 to 90)	302	90 (90 to 90)	0 (0 to 0)	0.59
Motor	304	97 (97 to 97)	300	97 (97 to 100)	0 (0 to 3)	0.31
Language	300	89 (89 to 91)	296	91 (89 to 94)	0 (0 to 3)	0.30
Differential Ability Scales–II scores						
Overall at 36 mo	304	90 (88 to 93)	308	90 (87 to 93)	0 (−2 to 3)	0.90
Recall of digits forward at 48 mo	298	84 (76 to 91)	299	84 (76 to 91)	0 (−5 to 7)	0.60
Recognition of pictures at 48 mo	298	74 (74 to 80)	302	74 (74 to 80)	0 (−6 to 0)	0.52
Child Behavior Checklist T score¶						
At 36 mo	306	46 (45 to 48)	309	46 (45 to 48)	0 (−2 to 2)	0.99
At 60 mo	314	44 (43 to 46)	313	44 (42 to 46)	0 (−2 to 2)	0.96
Conners' Rating Scales–Revised ADHD score at 48 mo‖	302	48 (47 to 49)	303	49 (47 to 51)	0 (−1 to 2)	0.37
WPPSI-III at 60 mo	311	97 (95 to 99)	314	95 (93 to 97)	0 (−3 to 2)	0.89

* For all outcomes except the primary outcome, the potential follow-up cohort consisted of 335 children in the levothyroxine group and 329 in the placebo group (offspring who were not lost to follow-up at maternal delivery, who were discharged alive after birth, and who did not die before 1 year of age).

† Shown is the Hodges–Lehmann estimate of the absolute difference between the placebo group and the levothyroxine group. The Hodges–Lehmann estimate is the median of all paired differences between the observations in the two samples, and negative numbers reflect lower scores in the placebo group.

‡ The primary outcome was death or IQ score at 5 years of age (or at 3 years of age if the 5-year examination was missing). The full-scale IQ was assessed with the use of the Wechsler Preschool and Primary Scale of Intelligence III (WPPSI-III) at 5 years of age or the overall (general conceptual ability) score from the Differential Ability Scales–II at 3 years of age if the WPPSI-III score was not available. Results are expressed as an age-standardized score, with an expected population mean of 100 and a standard deviation of 15. Death before 3 years of age was assigned a score of 0 (lowest possible rank) and was included in the estimation of the median.

§ Results on the Bayley Scales of Infant Development, Third Edition (Bayley-III) are expressed as an age-standardized score, with an expected population mean of 100 and a standard deviation of 15.

¶ A Child Behavior Checklist T score of less than 60 is considered to be in the normal range, a T score of 60 to 63 is a borderline score, and a T score of more than 63 is in the clinical range.[17]

‖ The Conners' Rating Scales–Revised were used to assess attention deficit–hyperactivity disorder (ADHD). A T score of 45 to 55 is considered to be typical or average; a T score of 44 or less is not a concern, a T score of 56 to 60 is considered to be a borderline score, and a T score of 61 or higher indicates a possible or clinically significant problem.

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before 3 years of age (fetal death, neonatal death, or infant death), including 4 in the levothyroxine group and 9 in the placebo group (P=0.16). The median IQ score was 97 (95% confidence interval [CI], 94 to 99) in the levothyroxine group and 94 (95% CI, 92 to 96) in the placebo group (P=0.71). Annual developmental-testing scores and the results of the behavioral and attention assessments (the Child Behavior Checklist and Connors' Rating Scales–Revised, respectively) did not differ significantly between the groups for any test.

In the hypothyroxinemia trial, data on the primary outcome were available for 507 offspring (96%) (Table 4). A total of 12 children had a DAS score substituted for the WPPSI-III IQ score, and 9 offspring died before 3 years of age, including 3 in the levothyroxine group and 6 in the placebo group (P=0.34). The median IQ score was 94 (95% CI, 91 to 95) in the levothyroxine group and 91 (95% CI, 89 to 93) in the placebo group (P=0.30). There were no significant between-group differences in any of the annual measures.

The median T scores for the Child Behavior Checklist and the Conners' Rating Scales–Revised in all comparison groups (in the two trials) were within the normal range.[22,23] None of the subgroup interaction tests were significant (Table S6 in the Supplementary Appendix). Stratification according to clinical center did not materially alter the results in either trial.

Table 4. Developmental and Behavioral Outcomes in Offspring of Mothers with Hypothyroxinemia.*

Outcome	Levothyroxine		Placebo		Difference (95% CI)†	P Value
	No. of Children	Median Value (95% CI)	No. of Children	Median Value (95% CI)		
Primary outcome	254	94 (91 to 95)	253	91 (89 to 93)	−1 (−4 to 1)	0.30
Bayley-III score						
At 12 mo						
Cognitive	247	100 (100 to 100)	238	100 (100 to 100)	0 (0 to 0)	0.89
Motor	246	97 (94 to 97)	236	97 (94 to 97)	0 (0 to 3)	0.54
Language	246	94 (91 to 94)	237	94 (91 to 97)	0 (−3 to 3)	0.92
At 24 mo						
Cognitive	235	90 (85 to 90)	235	90 (85 to 90)	0 (0 to 0)	0.70
Motor	233	97 (94 to 100)	232	97 (94 to 97)	0 (−3 to 0)	0.20
Language	232	89 (89 to 94)	229	89 (89 to 91)	0 (−3 to 2)	0.71
Differential Ability Scales–II scores						
Overall at 36 mo	244	90 (87 to 92)	235	89 (87 to 91)	−1 (−3 to 2)	0.64
Recall of digits forward at 48 mo	236	91 (84 to 99)	224	84 (84 to 91)	0 (−8 to 0)	0.22
Recognition of pictures at 48 mo	234	74 (74 to 80)	226	74 (74 to 80)	0 (−4 to 0)	0.91
Child Behavior Checklist T score						
At 36 mo	244	48 (46 to 50)	237	48 (45 to 49)	0 (−2 to 2)	0.65
At 60 mo	244	45 (43 to 46)	243	43 (42 to 45)	−1 (−3 to 1)	0.44
Conner's Rating Scales–Revised ADHD score at 48 mo	238	50 (49 to 51)	228	49 (48 to 51)	0 (−2 to 2)	0.98
WPPSI-III score at 60 mo	243	94 (91 to 95)	243	92 (90 to 95)	−1 (−3 to 2)	0.48

* For all outcomes except the primary outcome, the potential follow-up cohort consisted of 260 children in the levothyroxine group and 255 children in the placebo group (those who were not lost to follow-up at maternal delivery, who were discharged alive after birth, and who did not die before 1 year of age).

† Shown is the Hodges–Lehmann estimate of the absolute difference between the placebo group and the levothyroxine group. The Hodges–Lehmann estimate is the median of all paired differences between the observations in the two samples, and negative numbers reflect lower scores in the placebo group.

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DISCUSSION

These two parallel, randomized, placebo-controlled trials involving women with subclinical hypothyroidism or hypothyroxinemia in the first half of pregnancy showed no significant effect of thyroid hormone–replacement therapy on the cognitive function of the children or on other indexes of neurodevelopment through 5 years of age. There were no significant differences in measures of behavior, attention deficits, or hyperactivity in either trial. Moreover, treatment of women who had either an elevated thyrotropin level or a low free T_4 level had no significant effect on pregnancy or neonatal outcomes.

A previous study indicated that children who were born to untreated women who had a thyrotropin value above the 98th percentile had diminished school performance and an average IQ that was 7 points lower than the average IQ of control children.[6] These results are often offered as evidence that the offspring of women with subclinical hypothyroidism during pregnancy are at risk for subnormal brain function. However, most women who were included in this previous study had a thyrotropin level greater than 10 mU per liter and a free T_4 level that was more consistent with the diagnosis of overt hypothyroidism.[6] Studies involving children of women with hypothyroxinemia that was identified before 12 weeks of gestation also showed significantly lower scores on the Bayley mental and psychomotor subscales at 2 years of age than infants of euthyroid women.[7,24] These studies suggested that offspring would benefit from maternal treatment for subclinical thyroid disease and resulted in recommendations by some medical organizations for routine maternal screening and treatment to prevent subnormal cognitive development in offspring.[14]

The results of our trials are consistent with those of the CATS study, which was a thyroid-screening trial involving 21,846 pregnant women, primarily from the United Kingdom. Women in that trial were either screened immediately and treated with levothyroxine if they were identified as having subclinical hypothyroidism or hypothyroxinemia or had their serum frozen to be analyzed on completion of the pregnancy. The results of IQ testing of the children at 3 years of age did not differ significantly between children whose mothers had been immediately treated during the pregnancy and those whose mothers had treatment that was deferred. Thyroid hormone–replacement therapy in the CATS study was initiated at a median gestational age of 13 weeks 3 days, as compared with 16 weeks 4 days and 18 weeks in our two trials.[17] However, 24% of the children in the CATS study were lost to follow-up. In the current trials, the follow-up rate at 5 years of age was more than 92%.

Several studies have also suggested that either a high maternal thyrotropin level or hypothyroxinemia is associated with externalizing behavioral problems such as attention deficit–hyperactivity disorder (ADHD).[25,26] In the Generation R Study, 8-year-old children of women from an iodine-deficient geographic area who had been identified with hypothyroxinemia before 20 weeks of gestation were found to have higher ADHD index scores than children of women without hypothyroxinemia.[26] This association persisted after adjustment for IQ. We did not identify any significant between-group differences in ADHD index scores in either of our trials, and the scores were well within the normal range. Moreover, the Child Behavior Checklist scores did not reveal any evidence of behavioral problems in children whose mothers with subclinical hypothyroidism or hypothyroxinemia were in the placebo group. The CATS study also did not detect any behavioral improvements in children who had been exposed to thyroid hormone replacement.[17]

Subclinical hypothyroidism has been associated with several obstetrical complications,[8-11] but there has been no direct evidence that levothyroxine therapy reduces these risks.[19] One study involving women with thyroid peroxidase antibodies showed a lower rate of preterm delivery among women treated with levothyroxine during pregnancy than among those who were untreated[27]; however, these women had normal thyroid-function tests. We did not detect any significant improvement in pregnancy or neonatal outcomes that was associated with levothyroxine therapy in women with subclinical thyroid hypofunction.

A limitation of the two trials is the relatively late time during gestation at which women were randomly assigned to the trial groups. The fetal thyroid gland begins producing thyroid hormone between 10 weeks and 12 weeks of gestation, and on average, women underwent randomization in our trials several weeks after this time (at a mean of 16.7 weeks of gestation in mothers with subclinical hypothyroidism and 17.8 weeks

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of gestation in those with hypothyroxinemia). However, in a previous study, a small cohort of children whose mothers had a low free T_4 level in the first trimester but whose level increased before 24 weeks of gestation had scores that were similar to those of children of euthyroid mothers, which suggests a possible benefit with the initiation of supplementation after the first trimester.[24]

In our trials, the mothers who were treated with levothyroxine met therapy goals by a median gestational age of less than 24 weeks. We also found no significant interaction according to the time of initiation of therapy (<17 weeks of gestation vs. ≥17 weeks of gestation). Furthermore, post hoc analyses of the CATS study did not suggest any significant differences in IQ scores between the treated group and the control group among women who were screened before 14 weeks of gestation or who met the target thyrotropin levels within 6 weeks after screening.[17] Although we did not enroll women earlier than at 8 weeks of gestation in order to avoid enrolling women who might have an early miscarriage, we believe that these trials, which were performed at centers that were actively screening women on presentation for prenatal care, probably reflect what could be accomplished by means of routine thyroid screening during pregnancy in the United States.

In conclusion, on the basis of a comprehensive battery of tests through 5 years of age, we did not find significantly better neurodevelopmental outcomes in children whose mothers had received thyroxine treatment for subclinical hypothyroidism or hypothyroxinemia during pregnancy than in children whose mothers did not receive such treatment. Our trials also showed no significant effect of thyroxine treatment on pregnancy and neonatal outcomes.

The views expressed in this article are those of the authors and do not necessarily represent the views of the National Institutes of Health.

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No potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

The authors' full names and academic degrees are as follows: Brian M. Casey, M.D., Elizabeth A. Thom, Ph.D., Alan M. Peaceman, M.D., Michael W. Varner, M.D., Yoram Sorokin, M.D., Deborah G. Hirtz, M.D., Uma M. Reddy, M.D., M.P.H., Ronald J. Wapner, M.D., John M. Thorp, Jr., M.D., George Saade, M.D., Alan T.N. Tita, M.D., Ph.D., Dwight J. Rouse, M.D., Baha Sibai, M.D., Jay D. Iams, M.D., Brian M. Mercer, M.D., Jorge Tolosa, M.D., Steve N. Caritis, M.D., and J. Peter VanDorsten, M.D.

The authors' affiliations are as follows: the University of Texas Southwestern Medical Center, Dallas (B.M.C.), the University of Texas Medical Branch, Galveston (G.S.), and the University of Texas Health Science Center at Houston, McGovern Medical School–Children's Memorial Hermann Hospital, Houston (B.S.) — all in Texas; George Washington University Biostatistics Center, Washington, DC (E.A.T.); Northwestern University, Chicago (A.M.P.); the University of Utah Health Sciences Center, Salt Lake City (M.W.V.); Wayne State University, Detroit (Y.S.); the National Institute of Neurological Disorders and Stroke (D.G.H.) and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (U.M.R.), Bethesda, MD; Columbia University, New York (R.J.W.); the University of North Carolina at Chapel Hill, Chapel Hill (J.M.T.); the University of Alabama at Birmingham, Birmingham (A.T.N.T.); Brown University, Providence, RI (D.J.R.); Ohio State University, Columbus (J.D.I.), and MetroHealth Medical Center–Case Western Reserve University, Cleveland (B.M.M.) — both in Ohio; Oregon Health and Science University, Portland (J.T.); University of Pittsburgh, Pittsburgh (S.N.C.); and the Medical University of South Carolina, Charleston (J.P.V.).

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Message

From: Christ, Lisa [Christ.Lisa@epa.gov]
Sent: 10/6/2016 7:20:26 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]; Oshida, Phil [Oshida.Phil@epa.gov]
CC: Huff, Lisa [Huff.Lisa@epa.gov]
Subject: FW: Perchlorate Peer Review Advance Materials

Can we discuss at TAB updates please?

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 EOP (PP)]
Sent: Thursday, October 06, 2016 2:56 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) >; Schwab, Margo EOP/OMB < Ex. 6 EOP (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks. Also – I wanted to confirm that if Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)? That would give EPA a little more than 2 weeks to consider interagency comments. So our goal will be for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Also – I wanted to confirm that our comments on the peer review charge would be submitted to you, while comments on the BBDR draft report and model will be submitted to the docket.

I am thinking that a 3rd meeting prior to the end of November would be useful to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)

Please let me know your thoughts.

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Thursday, October 6, 2016 7:35 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) >
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) >; Schwab, Margo EOP/OMB < Ex. 6 EOP (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Hi Jim
Of course. I held the time on EPA participants calendars, so I'll send confirmation. I'll set up logistics and send you the information soon.
Lisa

Sent from my iPhone

On Oct 5, 2016, at 4:40 PM, Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) > wrote:

Hi Lisa,

Thanks for the info. Sorry for the late response. Can we go ahead and schedule a meeting for October 20? Thanks for your patience.

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 11:04 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim
The closing date for public comments on the charge questions is October 21. The public comment period for the model and report ends November 14. We intend to have our contractor identify the peer reviewers and we'll finalize charge questions between October 21 and November 14. [potential call dates are Oct 20 & 27]

Public comments will be summarized and provided to the peer reviewers at the end of November around the same time we publish the next federal register notice announcing a meeting date sometime in early January (based on availability of peer reviewers and at least 30 days before the panel meeting). [potential call dates are Nov 7 & 8]

Lisa

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 EOP (PP)
Sent: Friday, September 30, 2016 10:27 AM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks for the dates and times. I was hoping to get something on our calendars earlier to ensure that Ex. 5 Deliberative Process (DP) Can you tell me the timeline that is planned in regards to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)?

Thanks,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 9:42 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim,
I'd like to suggest we schedule two meetings and see if an additional meeting, as suggested, is needed. I looked at calendars for relevant EPA folks and can offer the following dates/times.
1st meeting: October 20 from 4-5pm OR October 27 from 405pm
2nd meeting: November 7 from 11-11am OR November 8 from 11a-noon

I am unable to find availability sooner than Oct 20. However, that provides time for review of materials before discussions begin.
Please let me know at your earliest convenience which times work and I will set up a conference call.
Lisa

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 EOP (PP)
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) ; Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) the second would be to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) and the third would Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, September 28, 2016 10:36 AM
To: Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

~~~~~~
Lisa Christ, Chief
~~~~~~

Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M

Message

From: Grevatt, Peter [Grevatt.Peter@epa.gov]
Sent: 10/8/2016 12:20:49 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: Re: Perchlorate Peer Review Advance Materials

Yes. It would be great if you could provide that clarification. I'm headed to C'Ville now and will be mostly out of pocket today. Let me know if we need to connect later.

Thanks for all you worked through yesterday. I spoke with Jennifer and we'll have her help soon!

Sent from my iPhone

On Oct 7, 2016, at 4:14 PM, Burneson, Eric <Burneson.Eric@epa.gov> wrote:

> Let me know if you want me to reply to Kevin.
>
> What we agreed to was a meeting on the 20th about the charge questions and a meeting around the 7th on the BBDR model. That's what Jim Kim laid out in his Sept 28 note below (plus he asked for a third meeting to follow up on remaining issues, but we said Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)
> Jim came back yesterday and asked for Ex. 5 Deliberative Process (DP) We presume he will still want Ex. 5 Deliberative Process (DP)
>
> ---
>
> **From:** Rennert, Kevin
> **Sent:** Friday, October 07, 2016 4:10 PM
> **To:** Grevatt, Peter <Grevatt.Peter@epa.gov>
> **Cc:** Burneson, Eric <Burneson.Eric@epa.gov>
> **Subject:** RE: Perchlorate Peer Review Advance Materials
>
> Hi Peter,
>
> I may be misunderstanding you here (or in a previous email chain), but the original request from Jim that I forwarded on to you and Joel was specifically to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) The response I got from you and Joel on that request was that this was something that Ex. 5 Deliberative Process (DP) I relayed that Ex. 5 Deliberative Process (DP) and it looks from this email chain like Lisa Christ was looking to set up times on both of those days for that purpose. So if we're now looking to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) If you're saying that we don't need to do Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Thanks.
>
> Kevin
>
> ---
>
> **From:** Grevatt, Peter
> **Sent:** Thursday, October 06, 2016 5:28 PM
> **To:** Rennert, Kevin <Rennert.Kevin@epa.gov>
> **Cc:** Burneson, Eric <Burneson.Eric@epa.gov>
> **Subject:** FW: Perchlorate Peer Review Advance Materials

Kevin, FYI. I propose that Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 EOP (PP)
Sent: Thursday, October 06, 2016 2:56 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) ; Schwab, Margo EOP/OMB < Ex. 6 EOP (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks. Also – I wanted to confirm that if Ex. 5 Deliberative Process (DP)
Ex. 5 Deliberative Process (DP)
Ex. 5 Deliberative Process (DP) That would give EPA a little more than 2 weeks to consider interagency comments. So our goal will be for interagency scientists to provide comments on the peer review charge shortly after the October 20 call, and then have a discussion on November 7/8. Also – I wanted to confirm that our comments on the peer review charge would be submitted to you, while comments on the BBDR draft report and model will be submitted to the docket.

I am thinking that a 3rd meeting prior to the end of November would be useful to discuss Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Please let me know your thoughts.

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Thursday, October 6, 2016 7:35 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) >
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) ; Schwab, Margo EOP/OMB < Ex. 6 EOP (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Hi Jim
Of course. I held the time on EPA participants calendars, so I'll send confirmation. I'll set up logistics and send you the information soon.
Lisa

Sent from my iPhone

On Oct 5, 2016, at 4:40 PM, Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) > wrote:

Hi Lisa,

Thanks for the info. Sorry for the late response. Can we go ahead and schedule a meeting for October 20? Thanks for your patience.

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 11:04 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim
The closing date for public comments on the charge questions is October 21. The public comment period for the model and report ends November 14. We intend to have our contractor identify the peer reviewers and we'll finalize charge questions between October 21 and November 14. [potential call dates are Oct 20 & 27]

Public comments will be summarized and provided to the peer reviewers at the end of November around the same time we publish the next federal register notice announcing a meeting date sometime in early January (based on availability of peer reviewers and at least 30 days before the panel meeting). [potential call dates are Nov 7 & 8]

Lisa

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 EOP (PP)
Sent: Friday, September 30, 2016 10:27 AM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks for the dates and times. I was hoping to get something on our calendars earlier to ensure that our feedback on the peer review charge questions is considered. Can you tell me the timeline that is planned in regards to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)

Thanks,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 9:42 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>

Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim,
I'd like to suggest we schedule two meetings and see if an additional meeting, as suggested, is needed. I looked at calendars for relevant EPA folks and can offer the following dates/times.
1st meeting: October 20 from 4-5pm OR October 27 from 405pm
2nd meeting: November 7 from 11-11am OR November 8 from 11a-noon

I am unable to find availability sooner than Oct 20. However, that provides time for review of materials before discussions begin.
Please let me know at your earliest convenience which times work and I will set up a conference call.
Lisa

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 EOP (PP)
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)
<Ex. 6 EOP (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) the second would be to Ex. 5 Deliberative Process (DP) and the third would Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, September 28, 2016 10:36 AM
To: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)
<Ex. 6 EOP (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,

Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

Lisa Christ, Chief
Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

Message

From: Flowers, Lynn [Flowers.Lynn@epa.gov]
Sent: 10/20/2016 7:44:18 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: RE: Perchlorate Peer Review Advance Materials

Ok. I am familiar with this line of discussion as this is Ex. 5 Deliberative Process (DP) Focus on closure! Got it!

Lynn Flowers, PhD, DABT
Office of Science Policy
US EPA
Washington, DC
202-564-6293

From: Burneson, Eric
Sent: Thursday, October 20, 2016 3:18 PM
To: Flowers, Lynn <Flowers.Lynn@epa.gov>
Subject: FW: Perchlorate Peer Review Advance Materials

FYI

From: Rennert, Kevin
Sent: Thursday, October 20, 2016 3:10 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Cc: Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Short summary is that Jim understands that our position is that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) What they really want is to be able to have us Ex. 5 Deliberative Process (DP) To the extent that we can use today's call to do so, Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Thanks.

On Oct 20, 2016, at 1:33 PM, Burneson, Eric <Burneson.Eric@epa.gov> wrote:

> Kevin: The interagency call about the perchlorate peer review charge is today at 4:00. We have not responded to Jim Kim's request for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) I assume that our answer is that Ex. 5 Deliberative Process (DP)
>
> Ex. 5 Deliberative Process (DP)
>
> Let us know if we should take a different approach to responding.
>
> Eric G. Burneson, P.E.
> Director of Standards and Risk Management
> Office of Ground Water and Drinking Water
> U.S. Environmental Protection Agency

From: Rennert, Kevin
Sent: Monday, October 17, 2016 2:00 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Eric – thanks for flagging. I thought we had actually put this to bed on our call with Jim on Friday, as I'd stated that we were planning Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) He thought that sounded fine, and in alignment with Jim Kim, which is what he cared about. It looks from the below email like that's not the case. I'll call Jim L. and discuss, and will follow up afterwards. Thanks. Kevin

---

From: Burneson, Eric
Sent: Monday, October 17, 2016 10:11 AM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: Fw: Perchlorate Peer Review Advance Materials

Kevin: Apologies if we have already worked through a path forward on the interagency review of the perchlorate peer review charge but OMB continues to ask for Ex. 5 Deliberative Process (DP)
Ex. 5 Deliberative Process (DP)
Ex. 5 Deliberative Process (DP) We would appreciate any guidance as to how to respond this request from Jim.

I am at the ASDWA meeting in Milwaukee but am happy to discuss if needed
Eric Burneson Ex. 6 Personal Privacy (PP)

From: Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>
Sent: Monday, October 17, 2016 2:02 PM
To: Christ, Lisa
Cc: Dorjets, Vlad EOP/OMB; Schwab, Margo EOP/OMB; Grevatt, Peter; Greene, Ashley; Burneson, Eric
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

I forgot to ask – can we schedule a teleconference for interagency discussion of the charge questions prior to them being finalized? I think it is important to understand EPA's perspectives on the charge and to clear up any confusion on interagency comments (if any).

Jim

From: Kim, Jim H. EOP/OMB
Sent: Monday, October 17, 2016 9:58 AM
To: 'Christ, Lisa' <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB

<Ex. 6 EOP (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thank you so much!

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Monday, October 17, 2016 9:56 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Jim,
I wanted to provide the information for the October 20th call on the perchlorate BBDR model charge questions.
Conference # Ex. 6 Personal Privacy (PP) access code Ex. 6 Personal Privacy (PP)

Interagency comments on the charge questions will be provided to EPA and comments on the BBDR model and report will be submitted to the docket. We respectfully request comments on the charge as soon as possible after the meeting -- October 27 would be ideal to allow ample time for peer reviewers consideration.

Sincerely,
Lisa

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 EOP (PP)
Sent: Thursday, October 06, 2016 2:56 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

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I am thinking that a 3rd meeting prior to the end of November would be useful to Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Please let me know your thoughts.

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Thursday, October 6, 2016 7:35 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) >
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) >; Schwab, Margo EOP/OMB < Ex. 6 EOP (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
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Sent from my iPhone

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> Hi Lisa,
>
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>
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> **To:** Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) >; Dorjets, Vlad EOP/OMB Ex. 6 EOP (PP) Schwab, Margo EOP/OMB < Ex. 6 EOP (PP) >
> **Cc:** Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
> **Subject:** RE: Perchlorate Peer Review Advance Materials
>
> Jim
> The closing date for public comments on the charge questions is October 21. The public comment period for the model and report ends November 14. We intend to have our contractor identify the peer reviewers and we'll finalize charge questions between October 21 and November 14. [potential call dates are Oct 20 & 27]
>
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Lisa

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Sent: Friday, September 30, 2016 10:27 AM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB
< Ex. 6 EOP (PP) ; Schwab, Margo EOP/OMB
Ex. 6 EOP (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley
<Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
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Hi Lisa,

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< Ex. 6 EOP (PP) ; Schwab, Margo EOP/OMB
< Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley
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Lisa

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Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB
Ex. 6 EOP (PP)
< Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley
<Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
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Can we start looking at dates/times for the end of next week?

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Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
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Thank you,
Lisa

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Lisa Christ, Chief
Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M
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Message

From: Tiago, Joseph [Tiago.Joseph@epa.gov]
Sent: 9/21/2017 9:45:05 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: RE: EPA Perchlorate Peer Review follow up

Thanks, Eric. Peter now has a conflict with the previous staff meeting time so we'll likely keep the current schedule.

Joe.

-----Original Message-----
From: Burneson, Eric
Sent: Thursday, September 21, 2017 5:33 PM
To: Tiago, Joseph <Tiago.Joseph@epa.gov>
Subject: RE: EPA Perchlorate Peer Review follow up

Thanks Joe. We knew we would either have to have the call without Jennifer or without me and OMB made that decision for us. Can you please move the staff meeting back and I wills schedule the Federal partners meeting tomorrow.

-----Original Message-----
From: Tiago, Joseph
Sent: Thursday, September 21, 2017 3:31 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: EPA Perchlorate Peer Review follow up

Hi Eric,

I can move the staff meeting. Note Jennifer is not here on Oct 2, not sure if that matters.

Thanks,

Joe.

-----Original Message-----
From: Burneson, Eric
Sent: Thursday, September 21, 2017 3:15 PM
To: Grevatt, Peter <Grevatt.Peter@epa.gov>; Mclain, Jennifer <Mclain.Jennifer@epa.gov>
Cc: Tiago, Joseph <Tiago.Joseph@epa.gov>; Mason, Paula <Mason.Paula@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: FW: EPA Perchlorate Peer Review follow up

Peter and Jennifer:
Per the response below, OMB has a preference for holding the Federal Partners perchlorate call on Oct 2nd vs the 5th. ORD has better availability at 3:00 on that date.

Can we ask Paula to postpone the OGWDW Staff meeting to start at 4:00 on this date to enable the Federal partners call to take place from 3:00 to 4:00?

Once I hear back from you I will schedule the Federal Partners call.

Eric

-----Original Message-----
From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 Personal Privacy (PP)
Sent: Thursday, September 21, 2017 1:56 PM
To: Dorjets, Vlad EOP/OMB [Ex. 6 Personal Privacy (PP)]; Burneson, Eric <Burneson.Eric@epa.gov>
Cc: Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Johnson, Ann <Johnson.Ann@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>; Mclain, Jennifer <Mclain.Jennifer@epa.gov>
Subject: RE: EPA Perchlorate Peer Review follow up

I can make October 2, but not October 5 at that time.

Jim

-----Original Message-----
From: Dorjets, Vlad EOP/OMB
Sent: Thursday, September 21, 2017 1:38 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>

Cc: Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Johnson, Ann <Johnson.Ann@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>; Mclain, Jennifer <Mclain.Jennifer@epa.gov>; Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)>
Subject: RE: EPA Perchlorate Peer Review follow up

Eric - Sorry for not replying sooner and thanks for offering to set this up. Unless Jim Kim (who I've copied) has any conflicts, all three times work for me. I realize I'm the one that has been the cause of the delay here but if we can get the invitation to reviewers tomorrow or Monday that would be great so that they will have a week notice to read the report before the call.

-----Original Message-----
From: Burneson, Eric [mailto:Burneson.Eric@epa.gov]
Sent: Tuesday, September 19, 2017 3:27 PM
To: Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>
Cc: Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Johnson, Ann <Johnson.Ann@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>; Mclain, Jennifer <Mclain.Jennifer@epa.gov>
Subject: RE: EPA Perchlorate Peer Review follow up

Vlad:

I wanted to check back with you on potential times for calls with the Federal Agency partners to discuss questions /concerns with the charge. Here are three possible times

October 2, 11:00 to 12:00
October 2, 3:00 - 4:00
October 5: 2:00 - 3:00

Please let me know which of these times works for the critical folks from OMB and I will send out a meeting request to the Federal Partners for that time. Thanks for your continued assistance with this important action.

Eric Burneson, P.E.
Director of Standards and Risk Management Office of Ground Water and Drinking Water U.S. Environmental Protection Agency
202 564 5250

On Sep 13, 2017, at 12:11 PM, Burneson, Eric <Burneson.Eric@epa.gov<mailto:Burneson.Eric@epa.gov>> wrote:

Federal Agency Partners:
Thank you again for your input on the peer review charge for the materials to inform the Safe Drinking Water Act Decision Making on Perchlorate. I am writing to inform you that the following Federal Register announcements are scheduled for publication on Friday, September 15 (and will be available on line for public inspection tomorrow, Sept 14)

1. Federal Register.
Title: Request for Public Comments to be sent to Versar, Inc., on an Interim List of Perchlorate in Drinking Water Expert Peer Reviewers and Draft Peer Review Charge Questions FRL #: 9967-70-OW Docket #: EPA-HQ-OW-2016-0439

1. Federal Register.
Title: Request for Public Comments to be sent to EPA on Peer Review Materials to Inform the Safe Drinking Water Act Decision Making on Perchlorate FRL #: 9967-69-OW Docket #: EPA-HQ-OW-2016-0438

As we stated in our previous discussions, EPA is committed to working with you to assure we have a robust peer review and scientifically sound analysis to inform decision making. We will be scheduling a teleconference during the week of October 2, 2017, to answer any questions you have about the charge and to discuss your preliminary feedback on the charge. We are asking for your feedback on the revised charge questions via email by October 6, 2017.

As we discussed, in order for your comments on the draft report to be considered by the peer reviewers, they will need to be submitted to the docket through the processes identified in the second Federal Register notice above (comment due date will be October 30, 2017). However we will also be open to any input that you wish to provide directly to us via email and can schedule a second teleconference to discuss questions and preliminary comments during the week of October 23 if there is interest.

Thanks again for your continued involvement in this important scientific analysis.

Eric Burneson, P.E.
Director of Standards and Risk Management Office of Ground Water and Drinking Water U.S. Environmental Protection Agency
202 564 5250

Message

From: Rennert, Kevin [Rennert.Kevin@epa.gov]
Sent: 10/20/2016 6:12:06 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]; Owens, Nicole [Owens.Nicole@epa.gov]; Muellerleile, Caryn [Muellerleile.Caryn@epa.gov]
CC: Grevatt, Peter [Grevatt.Peter@epa.gov]; Christ, Lisa [Christ.Lisa@epa.gov]
Subject: RE: Perchlorate Peer Review Advance Materials

Eric – if you have a second, please give me a call at 202-564-3155. Thanks.

From: Burneson, Eric
Sent: Thursday, October 20, 2016 1:33 PM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Kevin: The interagency call about the perchlorate peer review charge is today at 4:00. We have not responded to Jim Kim's request for a second call and expect that he will raise this question in our discussion. I assume that our answer is that Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Let us know if we should take a different approach to responding.

Eric G. Burneson, P.E.
Director of Standards and Risk Management
Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency

From: Rennert, Kevin
Sent: Monday, October 17, 2016 2:00 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Eric – thanks for flagging. I thought we had actually put this to bed on our call with Jim on Friday, as I'd stated that we were planning Ex. 5 Deliberative Process (DP). He thought that sounded fine, and in alignment with Jim Kim, which is what he cared about. It looks from the below email like that's not the case. I'll call Jim L. and discuss, and will follow up afterwards. Thanks. Kevin

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From: Burneson, Eric
Sent: Monday, October 17, 2016 10:11 AM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: Fw: Perchlorate Peer Review Advance Materials

Kevin: Apologies if we have already worked through a path forward on the interagency review of the perchlorate peer review charge but OMB continues to ask for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) We think this sets up Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) We would appreciate any guidance as to how to respond this request from Jim.

I am at the ASDWA meeting in Milwaukee but am happy to discuss if needed
Eric Burneson Ex. 6 Personal Privacy (PP)

From: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) >
Sent: Monday, October 17, 2016 2:02 PM
To: Christ, Lisa
Cc: Dorjets, Vlad EOP/OMB; Schwab, Margo EOP/OMB; Grevatt, Peter; Greene, Ashley; Burneson, Eric
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

I forgot to ask – can we schedule a teleconference for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)? I think it is important to understand EPA's perspectives on the charge and to clear up any confusion on interagency comments (if any).

Jim

From: Kim, Jim H. EOP/OMB
Sent: Monday, October 17, 2016 9:58 AM
To: 'Christ, Lisa' <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thank you so much!

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Monday, October 17, 2016 9:56 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) >
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Jim,
I wanted to provide the information for the October 20th call on the perchlorate BBDR model charge questions.
Conference Ex. 6 Personal Privacy (PP) access code Ex. 6 Personal Privacy (PP)

Interagency comments on the charge questions will be provided to EPA and comments on the BBDR model and report will be submitted to the docket. We respectfully request comments on the charge as soon as possible after the meeting -- October 27 would be ideal to allow ample time for peer reviewers consideration.

Sincerely,
Lisa

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 Personal Privacy (PP)
Sent: Thursday, October 06, 2016 2:56 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks. Also – I wanted to confirm that if Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)? That would give EPA a little more than 2 weeks to consider interagency comments. So our goal will be for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Also – I wanted to confirm that our comments on the peer review charge would be submitted to you, while comments on the BBDR draft report and model will be submitted to the docket.

I am thinking that a 3rd meeting prior to the end of November would be useful to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)

Please let me know your thoughts.

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Thursday, October 6, 2016 7:35 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) >
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB Ex. 6 Personal Privacy (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Hi Jim
Of course. I held the time on EPA participants calendars, so I'll send confirmation. I'll set up logistics and send you the information soon.
Lisa

Sent from my iPhone

On Oct 5, 2016, at 4:40 PM, Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) > wrote:

> Hi Lisa,

Thanks for the info. Sorry for the late response. Can we go ahead and schedule a meeting for October 20? Thanks for your patience.

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 11:04 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) ; Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB Ex. 6 Personal Privacy (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim
The closing date for public comments on the charge questions is October 21. The public comment period for the model and report ends November 14. We intend to have our contractor identify the peer reviewers and we'll finalize charge questions between October 21 and November 14. [potential call dates are Oct 20 & 27]

Public comments will be summarized and provided to the peer reviewers at the end of November around the same time we publish the next federal register notice announcing a meeting date sometime in early January (based on availability of peer reviewers and at least 30 days before the panel meeting). [potential call dates are Nov 7 & 8]

Lisa

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 Personal Privacy (PP)
Sent: Friday, September 30, 2016 10:27 AM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) ; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks for the dates and times. I was hoping to get something on our calendars earlier to ensure that Ex. 5 Deliberative Process (DP) Can you tell me the timeline that is planned in regards to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) ?

Thanks,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 9:42 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) ; Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) Schwab, Margo EOP/OMB Ex. 6 Personal Privacy (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim,

I'd like to suggest we schedule two meetings and see if an additional meeting, as suggested, is needed. I looked at calendars for relevant EPA folks and can offer the following dates/times.
1st meeting: October 20 from 4-5pm OR October 27 from 405pm
2nd meeting: November 7 from 11-11am OR November 8 from 11a-noon

I am unable to find availability sooner than Oct 20. However, that provides time for review of materials before discussions begin.
Please let me know at your earliest convenience which times work and I will set up a conference call.
Lisa

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 Personal Privacy (PP)
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP)
' Ex. 6 Personal Privacy (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) the second would be to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) and the third would Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, September 28, 2016 10:36 AM
To: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) , Kim, Jim H. EOP/OMB
< Ex. 6 Personal Privacy (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

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Attention Deficit and Hyperactivity Disorders in the Offspring of Mothers Exposed to Mild-Moderate Iodine Deficiency: A Possible Novel Iodine Deficiency Disorder in Developed Countries

F. VERMIGLIO, V. P. LO PRESTI, M. MOLETI, M. SIDOTI, G. TORTORELLA, G. SCAFFIDI, M. G. CASTAGNA, F. MATTINA, M. A. VIOLI, A. CRISÀ, A. ARTEMISIA, AND F. TRIMARCHI

Dipartimento Clinico-Sperimentale di Medicina e Farmacologia-Sezione di Endocrinologia (F.V., V.P.L.P., M.M., G.S., M.G.C., F.M., M.A.V., F.T.), Dipartimento di Scienze Pediatriche-Sezione di Neuropsichiatria Infantile (M.S., G.T., A.C.), and Dipartimento di Diagnostica di Laboratorio-Servizio di Biochimica Clinica (A.A.), University of Messina, Messina 98125, Italy

Over a period of almost 10 yr, we carried out a prospective study of the neuropsychological development of the offspring of 16 women from a moderately iodine-deficient area (area A) and of 11 control women from a marginally iodine-sufficient area (area B) whose thyroid function had been monitored during early gestation.

Attention deficit and hyperactivity disorder (ADHD) was diagnosed in 11 of 16 area A children (68.7%) but in none from area B. Total intelligence quotient score was lower in area A than in area B children (92.1 ± 7.8 *vs.* 110 ± 10) and in ADHD children when compared with both non-ADHD children from the same area and control children (88.0 ± 6.9 *vs.* 99.0 ± 2.0 and 110 ± 10, respectively). Seven of 11 ADHD children (63.6%) were born to the seven of eight area A mothers who became hypothyroxinemic at early gestation, whereas only one of five non-ADHD children was born to a woman who was hypothyroxinemic at 20 wk of gestation.

So far, a similar prevalence of ADHD has been reported only in children with generalized resistance to thyroid hormones. This might suggest a common ADHD pathogenetic mechanism consisting either of reduced sensitivity of the nuclear receptors to thyroid hormone (generalized resistance to thyroid hormones) or reduced availability of intracellular T_3 for nuclear receptor binding. The latter would be the ultimate consequence of maternal hypothyroxinemia (due to iodine deficiency), resulting in a critical reduction of the source of the intracellular T_3 available to the developing fetal brain. (*J Clin Endocrinol Metab* 89: 6054–6060, 2004)

NONSYSTEMATIC OBSERVATIONS IN a moderately iodine deficiency (ID)-endemic goiter area in Northeastern Sicily, where we described children's defective neuromotor and cognitive ability (1) along with transient maternal thyroid failure during early (2) and late (3) gestation, revealed an unexpectedly high occurrence in schoolchildren of a developmental disorder whose symptoms, which included difficulties in sustaining attention and hyperactivity, were suggestive of attention deficit and hyperactivity disorder (ADHD). This syndrome has been reported to be strongly associated with generalized resistance to thyroid hormone (GRTH) (4).

The present long-term prospective study was carried out on the offspring of a series of women, all born and living in the same moderately ID area, whose thyroid function was studied during early pregnancy with the aim of identifying the long-term effects of ID-related maternal hypothyroxinemia on the behavioral, psychoneurological, and intellectual development of children.

Abbreviations: ADHD, Attention deficit and hyperactivity disorder; ADHD−ve, without ADHD; ADHD+ve, with ADHD; FT_3, free T_3; FT_4, free T_4; GRTH, generalized resistance to thyroid hormones; ID, iodine deficiency; IQ, intelligence quotient; t-IQ, total IQ; TBG, T_4-binding globulin; WISC-III, Wechsler Intelligence Scale for Children, 3rd edition.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Subjects and Methods

Participants

The study group included 16 children born to 16 healthy mothers living in the moderately ID and extensively studied area (area A) and 11 control children born to 11 age-matched women from an area with marginally sufficient iodine intake (area B).

Table 1 shows the characteristics of both areas when the children were conceived (1990–1992) and at the present time.

Neuropsychological and neurological assessment

To rule out other psychiatric or primary disorders that would more adequately explain the clinical picture of ADHD, we combined staff from the departments of endocrinology, neurology, developmental pediatrics, and psychology (5). The first behavioral and neuropsychological evaluation (1994) was performed on a clinical observation basis when the children were 18–36 months old by two independent examiners (M.S. and G.T.), who were unaware of their mothers' thyroid function during pregnancy. All children from both areas A and B were subsequently reevaluated (2001–2002), by the same examiners, when they were 8–10 yr old. Besides neurological examination and ADHD screening, the second evaluation included intelligence testing to rule out mental retardation, in which inattention and hyperactivity symptoms are commonly found.

Neurological evaluation, carried out according to the directions of Touwen (6) when the children were 18–36 months and 8–10 yr old, included assessment of the child while sitting, standing, walking, and lying, as well as examination of muscle tone, reflexes, and general data (dominance, fine motor coordination, sensory examination, speech, and language).

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TABLE 1. Epidemiological characteristics of the two areas where the study was carried out

	1990–1992		2000	
	Area A	Area B	Area A	Area B
Urinary iodine excretion (μg/d)	48.1 ± 38.2	95.2 ± 55.8	63.0 ± 35.4	115.2 ± 61.4
% goiter in schoolchildren	24.7	7.8	16.3	3.5

Area A refers to the iodine-deficient area where the 16 pregnant women lived and bore their offspring. Area B is a sea-level small town where the 11 control mothers resided.

Screening for ADHD

The reference scale for inattention and hyperactivity derives from the Diagnostic and Statistical Manual of Mental Disorders, 4th edition-Text Revision (DSM-IV-TR) (7), validated by subscales for the Italian population (8). The essential feature of ADHD is a persistent pattern of inattention and/or hyperactivity-impulsivity. Analysis of the structured psychiatric interviews makes it possible to identify different subtypes of the disorder, depending on the prevalence (and persistence for at least 6 months) of different symptoms (nine for the attention deficit variant and nine for the hyperactive variant): 1) ADHD, combined type subtype (6 of 9 or more inattention symptoms and six of nine or more hyperactivity/impulsivity symptoms); 2) ADHD, predominantly inattentive type subtype (six of nine or more inattention symptoms but fewer than six of nine hyperactivity/impulsivity symptoms); 3) ADHD, predominantly hyperactive-impulsive type subtype (six of nine or more hyperactivity-impulsivity symptoms but fewer than six of nine inattention symptoms). The minimum score for a child to be considered to be affected with ADHD was, therefore: six of nine for both ADHD predominantly inattentive type, and ADHD predominantly hyperactive-impulsive type subtypes; and at least 12 of 18 for the ADHD combined type subtype.

The questionnaires with the items listed in the DSM-IV-TR were distributed both to parents and to teachers when the children's ages ranged between 8 and 10 yr, with directions to mark each item "often", "sometimes", "rarely", or "never". An item was considered positive when both parents and teachers marked it "often".

Intelligence testing

Intelligence was measured by the most widely used intelligence test, the Wechsler Intelligence Scale for Children, 3rd edition (WISC-III) (9), which provides a full-scale intelligence quotient (IQ) score and subscale scores (range, 40–160) for verbal skills and performances in the following areas: general information, general comprehension, arithmetic, similarities, vocabulary, digit span, picture completion, picture arrangement, block design, object assembly, coding, and mazes.

The WISC-III full-scale IQ score comprises three subscores devoted to items considered free from distractibility (arithmetic, digit span, and coding—WISC-III freedom-from-distractibility score); to verbal items (general information and comprehension, arithmetic, similarities, vocabulary, and digit span—WISC-III verbal IQ score) and to performance items (picture completion and arrangement, block design, object assembly, coding, and mazes—WISC-III performance IQ score).

According to this scale, a child is considered to be mentally retarded when his total IQ (t-IQ) score is less than 75 points (10).

Children's thyroid function tests

All children were euthyroid at delivery, as proved by the results of neonatal screening for congenital hypothyroidism. Serum free T_3 (FT_3), free T_4 (FT_4), and TSH were measured in all children when they were 18–36 months old (January-June 1994) using the same commercial kits as used to study their mothers (see below) and at 8–10 yr (November 2001-May 2002) using commercial kits supplied by Diagnostic Products Corporation, Los Angeles, CA (Immulite 2000 Analyzer).

Maternal thyroid function tests

We measured serum T_3, T_4, FT_3, FT_4, TSH, and T_4-binding globulin (TBG) values at three different points in time (between 5 and 10, 11 and 14, and18 and 20 wk of gestation). These correspond approximately to times before (8–13 wk) and subsequent to (20 wk) the onset of secretion of thyroid hormones by the fetal thyroid and will be referred to as 8, 13, and 20 wk of pregnancy, respectively. TBG saturation by T_4 was calculated as the molar T_4/TBG ratio, assuming 57 kDa to be the molar mass for TBG.

Maternal circulating total T_4 and T_3 (RIA), TSH (immunoradiometric assay), FT_3 and FT_4 (Amerlex-MAB, a one-step radiolabeled analog RIA) were determined (1990–1994) using commercial kits supplied by Kodak Clinical Diagnostic Ltd., Amersham, UK. Precision profiles showed inter- and intraassay coefficients of variation not exceeding 5%, over the entire measurement range, for both free thyroid hormones.

Serum antithyroid antibodies were measured using a two-step immunoenzymometric assay supplied by Tosoh Corporation, Tokyo, Japan (AIA-PACK TgAb and TPOAb). TBG concentration was measured using a commercial RIA kit (Behring, Marburg, Germany).

Definition of hypothyroxinemia

The term hypothyroxinemic is used here and elsewhere to indicate pregnant women with normal TSH concentrations (0.4–4.0 μU/ml) but low serum FT_4 values as compared with the range values calculated (mean ± 2 SD) at the same stage of pregnancy (8, 13, and 20 wk) in a series of 50 healthy women with moderately adequate iodine intake (150–200 μg iodine/d). Accordingly, normal values at 8 wk were: 1.02–1.72 ng/dl; mean ± SD, 1.36 ± 0.17; median, 1.39 (13.1–22.2 pmol/liter; mean ± SD, 17.6 ± 2.25; median, 17.9). Normal values at 13 wk were: 0.98–1.49 ng/dl; mean ± SD, 1.24 ± 0.12; median, 1.25 (12.7–19.2 pmol/liter; mean ± SD, 16.0 ± 1.64; median, 16.2). At 20 wk, values were: 0.85–1.39 ng/dl; mean ± SD, 1.12 ± 0.13; median, 1.11 (11.0–17.9 pmol/liter; mean ± SD, 14.5 ± 1.7; median, 14.3).

Statistical analysis

Unless otherwise indicated, data are expressed as mean ± SD. Statistical analysis was performed by two-way ANOVA, linear regression, Student's t test for unpaired data, and the χ^2 test.

Informed written consent was obtained from all the mothers recruited and from both the children's parents.

Results

Endocrine and neuromotor evaluation in children

All the children from both areas were euthyroid at neonatal screening and afterward (18–36 months and 8–10 yr) (data not shown). At clinical neuromotor evaluation, no child showed any neurological signs typically belonging to the spectrum of ID disorders (11).

ADHD testing

In nine of 16 children from area A, clinical observation in 1994 had revealed the presence of psychoneurological features suggestive of ADHD. This diagnosis was confirmed in 2001–2002 (DSM-IV-TR) in all of them and in a further two area A children in whom ADHD had not been suspected in 1994. None of the 11 area B children were affected with ADHD, because the tentative diagnosis made in 1994 for one of them was not confirmed in 2001–2002.

Therefore, ADHD affected 11 of 16 (68.7%) children from area A (5 of 11 ADHD, combined type subtype; five of 11

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ADHD, predominantly hyperactive-impulsive type subtype; and one of 11 ADHD, predominantly inattentive type subtype).

To improve the readability of the further results, the area A children and (their respective) mothers will henceforth be referred to as ADHD+ve and ADHD−ve area A subgroups, according to the presence or absence of ADHD, respectively.

Intelligence testing

Full-scale IQ score and subscale scores for each item are summarized in Table 2.

The WISC-III was completed for all children except one from area A, in whom extreme inattention and hyperactivity made WISC-III administration impossible to complete.

Mean t-IQ score was lower in area A than in area B children by approximately 18 points (92.1 ± 7.8 *vs.* 110 ± 10, $P < 0.00005$).

When we compared the intelligence test results of the ADHD+ve, ADHD−ve, and control children (Table 2), we observed that t-IQ score was lower in ADHD+ve than in ADHD−ve children by 11 points; and in both subgroups, it was lower than in the control group by 22 and 11 points, respectively. The attention WISC-III freedom-from-distractibility subscore was lower only in ADHD+ve children than in the controls, whereas the WISC-III verbal IQ subscore was lower in ADHD+ve than in either ADHD−ve or control children (14.4 and 21.4 points, respectively). The WISC-III performance IQ subscore was lower in both ADHD+ve children and ADHD−ve children than in controls by 19.3 and 12.5 points, respectively.

Of the ADHD+ve children, three of 10 had a t-IQ score ≥ 90, six of 10 ranged between 90 and 75, and only one of 10 had a score less than 75. Both the ADHD−ve and the control children had a t-IQ score more than 90.

Maternal thyroid function and psychoneurological impairment

For the purposes of our study, we compared the changes in maternal thyroid function of both ADHD+ve (n = 11) and ADHD−ve (n = 5) area A subgroups with those of the control area mothers (n = 11) over the first half of gestation.

Average values of maternal T_3, T_4, FT_3, FT_4, TSH, and TBG saturation by T_4 at 8, 13, and 20 wk of gestation, in both area A ADHD+ve and ADHD−ve subgroups and in area B control mothers, are illustrated in Fig. 1.

Neither difference nor significant changes were observed at any time in serum total T_3 levels among the three groups. Serum FT_3 levels progressively dropped in both ADHD+ve and ADHD−ve subgroups by almost 20% at midgestation ($F = 9.5$, $P < 0.005$; and $F = 8.4$, $P < 0.05$, respectively), related to pregnancy progression ($r = 0.55$, $P < 0.001$; and $r = 0.59$, $P < 0.05$, respectively). Serum total T_4 values increased about 10% at the 13th wk in all three groups and remained at a plateau until midgestation. TBG saturation by T_4 was lower

TABLE 2. Neuropsychological test scores in ADHD+ve and ADHD−ve children from iodine-deficient area (area A) as compared with iodine-sufficient matched children (area B)

Neuropsychological test	Area A ADHD+ve children (n = 10)[a]	Area A ADHD−ve children (n = 5)	Area B control children (n = 11)	*P* value
Intelligence WISC-III full-scale IQ score (t-IQ)	88.0 ± 6.9	99.0 ± 2.0	110 ± 10	<0.0001[b] <0.05[c] <0.005[d]
WISC-III freedom-from-distractibility score	8.8 ± 2.5	8.9 ± 2.5	10.3 ± 3.1	<0.05[b]
WISC-III verbal IQ score	88.6 ± 11.4	103 ± 2.6	110 ± 12.3	<0.001[b] <0.05[d]
WISC-III verbal section				
General information	9.0 ± 2.6	9.3 ± 3.0	9.6 ± 2.5	NS
General comprehension	7.6 ± 2.4	10.3 ± 2.9	10.9 ± 3.4	<0.05[b]
Arithmetic	8.0 ± 1.5	10.6 ± 3.2	10.7 ± 2.1	<0.005[b] <0.05[d]
Similarities	7.1 ± 4.9	11.3 ± 2.5	12.7 ± 2.9	<0.005[b]
Vocabulary	9.1 ± 2.8	11.0 ± 2.0	14.2 ± 2.5	<0.005[b] <0.05[d]
Digit span	9.7 ± 2.3	8.0 ± 2.6	9.1 ± 2.3	NS
WISC-III performance IQ score	87.8 ± 8.2	94.6 ± 7.1	107.1 ± 8.9	<0.0001[b] <0.05[c]
WISC-III performance section				
Picture completion	5.9 ± 1.2	4.3 ± 2.3	11.3 ± 2.2	<0.00001[b] <0.0005[c]
Picture arrangement	9.0 ± 2.4	11.0 ± 3.6	10.5 ± 1.7	NS
Block design	9.5 ± 3.2	8.7 ± 1.5	11.4 ± 1.9	<0.05[c]
Object assembly	9.5 ± 5.0	14.6 ± 3.5	11.1 ± 2.5	<0.05[c]
Coding	8.7 ± 3.4	8.0 ± 1.0	11.0 ± 4.3	NS
Mazes	8.7 ± 2.6	5.3 ± 3.5	11.0 ± 2.8	<0.05[b] <0.01[c]

NS, Not significant.

[a] Results refer to 10/11 ADHD+ve children due to the fact that in 1/11, extreme inattention and hyperactivity made WISC administration impossible.

[b] ADHD+ve *vs.* controls.

[c] ADHD−ve *vs.* controls.

[d] ADHD+ve *vs.* ADHD−ve.

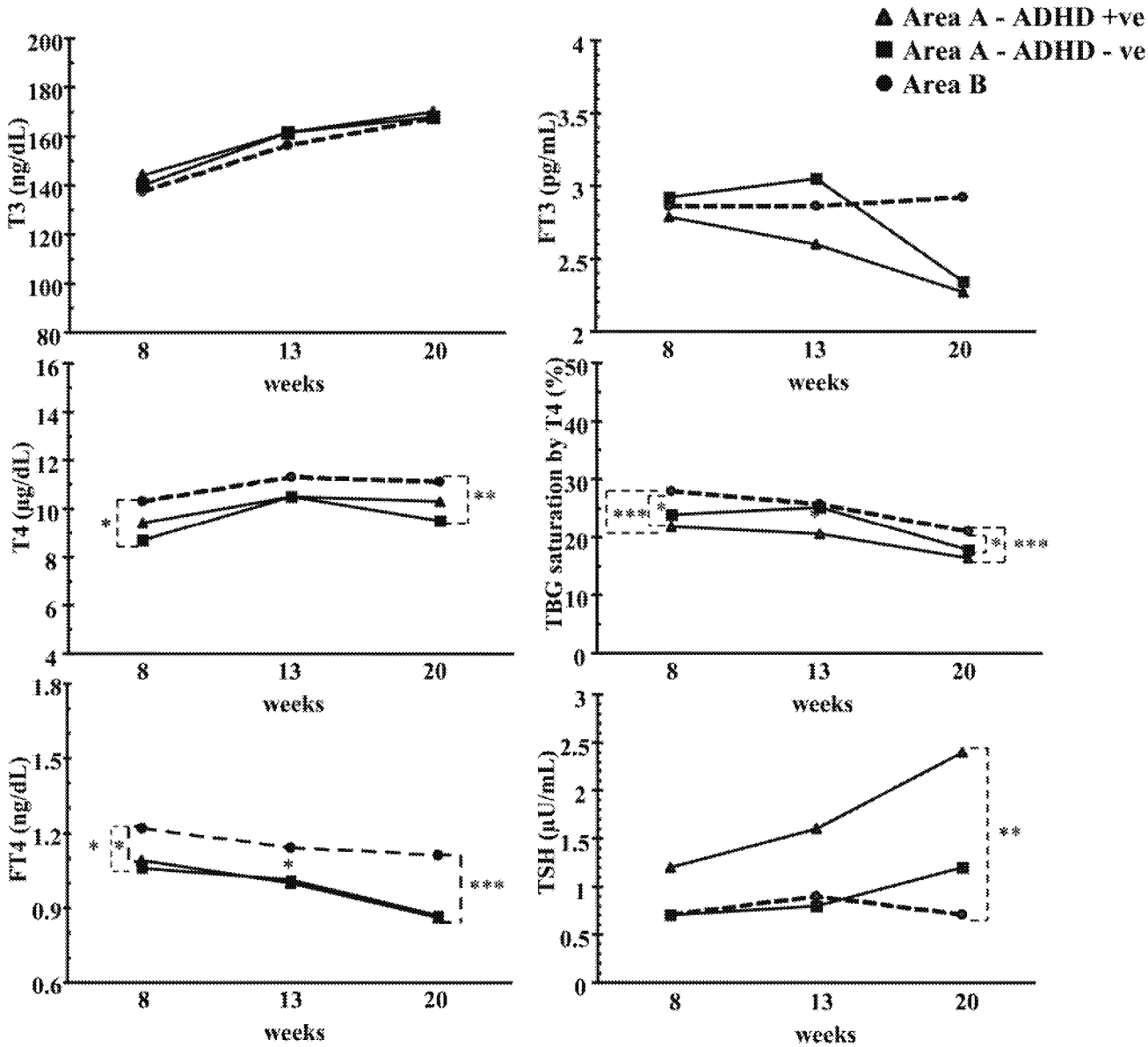

FIG. 1. Average values of maternal T_3, T_4, FT_3, FT_4, TSH, and TBG saturation by T_4 at 8, 13, and 20 wk of gestation in both area A ADHD+ve and ADHD−ve subgroups and in area B control mothers. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$.

in both ADHD+ve and ADHD−ve subgroups than in the area B mothers (t = 3.1 and 2.0, respectively) from the 8th wk onwards and further declined by about 25% with pregnancy progression (r = 0.39, $P < 0.05$; and r = 0.63, $P < 0.05$, respectively), ultimately remaining lower in both A subgroups than in the area B mothers (t = 3.27 and 1.97, respectively) at midgestation. Serum FT_4 values decreased by about 20% with gestational age in both A subgroups (r = 0.44, $P = 0.01$; r = 0.76, $P < 0.001$, respectively) but by only 8% in the control area, being always lower in both ADHD+ve and ADHD−ve subgroups than in area B pregnant women at any time (t = 1.8 and 2.6, respectively, at 8 wk; t = 2.17 and 1.98 at 13 wk; t = 3.0 and 3.57 at 20 wk). Serum TSH mean values increased over the first half of gestation about 100% (F = 4.9, $P < 0.05$) in the ADHD+ve subgroup and about 30% (F = 10.5, $P < 0.01$) in the ADHD−ve subgroup, remaining unmodified in the control mothers. At 20 wk, TSH levels were higher in the ADHD+ve subgroup than in the control area (t = 2.4).

Figure 2 shows individual maternal FT_4 and TSH data for both area A and area B subgroups. Of the 16 area A women, eight had serum FT_4 values that were lower than normal for the gestational week. In fact, hypothyroxinemia occurred at 8 wk in one ADHD+ve mother, at 13 wk in two other ADHD+ve mothers, and at 20 wk in one ADHD−ve and in four more ADHD+ve mothers. In two of these eight women, hypothyroxinemia was accompanied by a slight increase in TSH concentration, one from the second sampling on and the other at 20 wk. In the mothers from area B, both FT_4 and TSH values were consistently found to fall within the normal range in all but one woman, who was found (at the 13th wk of gestation) to be transiently hypothyroxinemic.

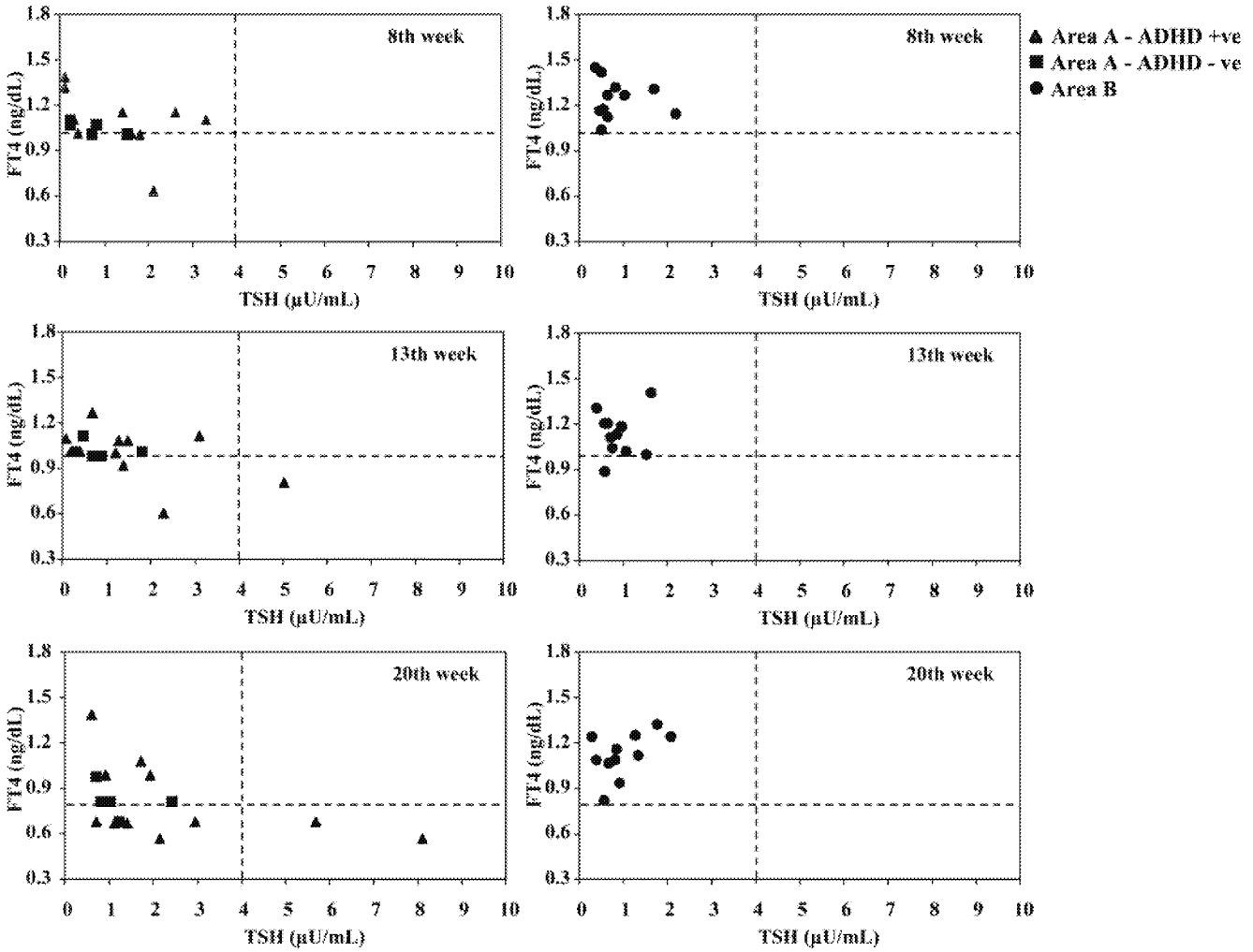

FIG. 2. Serum FT_4 and TSH levels in the 16 area A studied mothers (n = 11 ADHD+ve and n = 5 ADHD−ve subgroups) (*left*) and in the 11 area B pregnant women (*right*) measured at 8, 13, and 20 wk of gestation. The *vertical dotted lines* indicate the highest normal TSH value (4.0 μU/ml). The *horizontal dotted lines* indicate the lowest normal FT_4 values for the gestational week [1.02 ng/dl (13.1 pmol/liter) at 8 wk, 0.98 ng/dl (12.7 pmol/liter) at 13 wk, and 0.85 ng/dl (11.0 pmol/liter) at 20 wk].

Therefore, thyroid failure occurred in early pregnancy in eight of the 16 (50%) women from the ID area and only transiently in one of 11 (9.1%) from the control area.

Seven of eight (87.5%) women who experienced hypothyroxinemia during early gestation generated seven of the 11 ADHD+ve children, whereas the remaining four of 11 were born to mothers who remained euthyroid throughout the first half of their pregnancies. Conversely, of the five ADHD−ve children, four (80%) were born to mothers who had remained euthyroid throughout the study period, whereas only one was born to a hypothyroxinemic mother.

Finally, of the 11 of 16 area A children with neuropsychological disorders, seven of 11 (63.6%) were born to the eight iodine-deficient women who had become hypothyroxinemic during early gestation. As a consequence, the overall prevalence of ADHD among the area A children studied was significantly higher (χ^2, 2.34; $P = 0.001$) in children born to mothers whose FT_4 concentration was below the reference range at midgestation.

Similarly, the children's t-IQ score was directly ($r = 0.56$, $P < 0.005$) related to maternal FT_4 and inversely ($r = 0.63$, $P < 0.001$) related to maternal TSH values at midgestation, when both area A and B were considered as a whole (Fig. 3).

Discussion

ADHD is a developmental disorder involving difficulties with sustained attention, distractibility, poor impulse control, and hyperactivity or inability to regulate activity level to situational demands. The disorder is believed to arise early in childhood (3–7 yr) and is considered organic in pathology (12). The current view is that dysfunctions of both the frontal and prefrontal lobes and of cortical and subcortical striatal areas, involved in impulsivity and motor activity control (13) and in inhibition of irrelevant responses and executive functions (14), respectively, are responsible for the disorder

A high prevalence (70%) of ADHD has been reported in children with GRTH (4), a disease caused by mutations in the thyroid receptor-β gene and characterized by reduced responsiveness of peripheral and pituitary tissues to the actions of the thyroid hormone.

The present study was based on long-term observation of

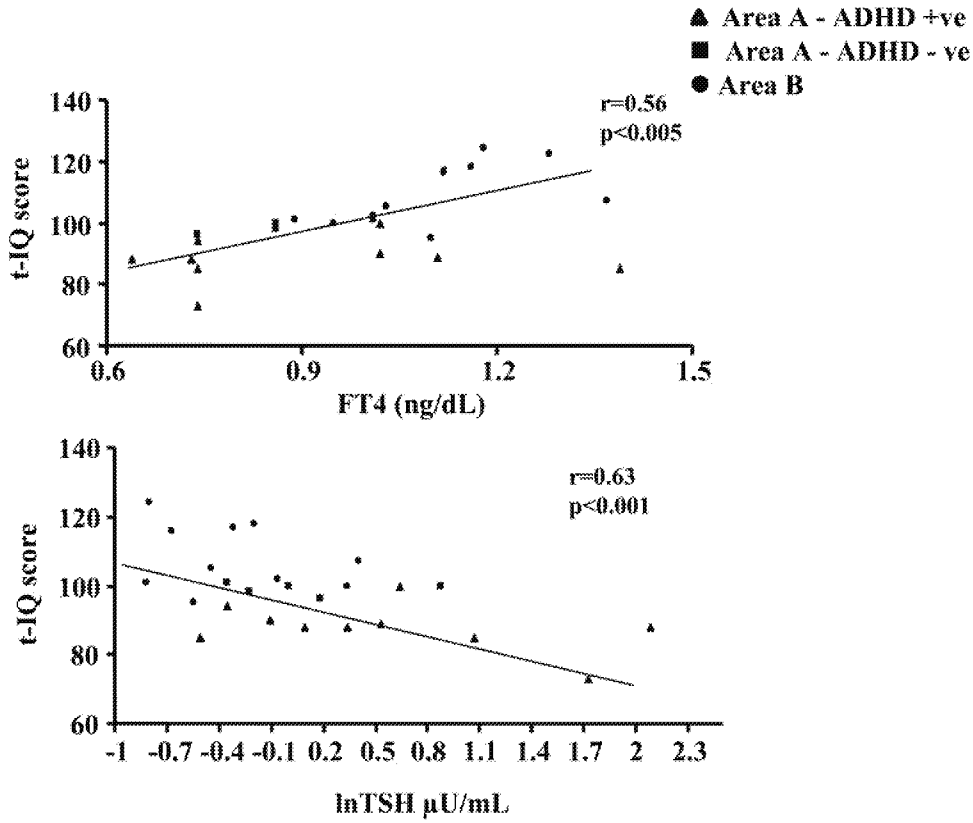

FIG. 3. Linear regression between t-IQ score of both groups A and B children and FT_4 (*upper*) and TSH (*lower*) levels of their mothers at midgestation. TSH values are presented as: ln TSH (natural logarithm).

the neuropsychological and intelligence performances of children born to mothers from a mild-moderate ID area, some of whom developed hypothyroxinemia during early pregnancy. At the time of initial neurological observation, we reported clinical findings that were consistent with a diagnosis of ADHD, but we were not able to confirm this suspicion because the children studied were thought to be still too young (18–36 months). It was for this reason that we prolonged our observation until the children were 8–10 yr old, when conclusive diagnosis of ADHD was made in 11 of 16 (68.7%) children born to mothers from this mild-moderate ID area in whom the disorder was associated with some degree of intelligence failure. In fact, ADHD+ve children's mean IQ scores (although ≥75 points in all but one) were 12 and 23 points lower than those of the ADHD−ve and control children, respectively.

When neurological results were related to early-pregnancy maternal thyroid function, it was seen that all 11 ADHD+ve children were born to ID area mothers whose thyroid function proved more heavily compromised than that of the five ADHD−ve mothers and that seven of eight (87.5%) ID area mothers who experienced thyroid failure generated ADHD+ve children. It is worth noting, however, that individual TSH levels fell consistently within the normal range in all but two of these women, whose TSH concentration slightly increased toward the end of the study period. Therefore, most of the ADHD+ve children (seven of 11) were born to mothers who had become hypothyroxinemic during early gestation, whereas four of five (80%) ADHD−ve children were born to mothers who remained consistently euthyroid during the first half of gestation.

The ADHD prevalence of almost 70% in our ID area (rising to 87.5% in hypothyroxinemic mothers), so surprisingly similar to that reported in children with GRTH, seems to point to a strong association of this neuropsychological disorder with both the GRTH syndrome and the ID-induced early gestation maternal hypothyroxinemia. ADHD syndrome associated with GRTH could be the result of the developing brain being deprived of the biological effect of the thyroid hormone, whereas ADHD in children from our ID area could be due to an inadequate supply of maternal thyroid hormone to the fetal brain, development of which therefore suffered from varying (and subtle) degrees of maternal thyroid failure during the first phases of organogenesis.

The role of T_4 transfer in human beings in early pregnancy is key to ensuring the normal neurological development of the fetus, which is particularly important before the onset of thyroid hormone secretion by the fetal thyroid (15) (20th wk). The existence of T_4 transfer from mother to fetus has been demonstrated by several conclusive studies in both rats (16–21) and human beings (22). First-trimester fetal tissues are exposed to concentrations of FT_4, which have recently been demonstrated in human fetuses to ultimately depend on the circulating maternal levels of T_4 or FT_4 (23).

The relationship between maternal thyroid hormone deficiency and neurological damage in offspring has already been established by several epidemiological studies in areas with severe ID (24) or normal dietary iodine intake (25, 26) and in animal models (27–28). Maternal hypothyroxinemia, also observed in areas with adequate iodine intake, has been regarded, so far, as a normal condition, even though reduced intellectual performances have been reported in children

born to iodine-sufficient hypothyroxinemic mothers (29). The growing concern that hypothyroxinemia during early gestation could be harmful to the fetus (30–32) has recently been reinforced by the first experimental evidence of a permanent alteration of cortical cytoarchitecture in the progeny of ID-induced hypothyroxinemic dams (33).

In conclusion and for the first time in a mild-moderate ID area, our data seem to vindicate the hypothesis of a direct causal relationship between ID-induced early gestational maternal hypothyroxinemia and ADHD, which might therefore be considered as a new ID disorder to be systematically screened in the progeny. A recent report indicating that neuropsychological development does not seem to be adversely affected in children whose maternal hypothyroxinemia is corrected within the 24th wk of pregnancy (34) is a strong indication of the need for routine screening and monitoring of thyroid function in early pregnancy. An inadequate dietary supply of iodine, still a widespread problem all over the world (including European countries and the United States) (32), further emphasizes the need for intensive programs of iodine prophylaxis to promptly prevent/correct gestational hypothyroxinemia and the resulting permanent and invalidating neurological damage to progeny.

Acknowledgments

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Message

From: Wennerberg, Linda S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
Sent: 10/6/2017 9:24:41 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
CC: denise.r.thaller@nasa.gov; Mcneill, Mike A (HQ-LD020) [mike.a.mcneill@nasa.gov]
Subject: RE: EPA Perchlorate Peer Review Comment, NASA Response
Attachments: Perchlorate draft charge and peer review list, NASA final comments EPAdocx.docx

Eric:
Attached are the NASA comments, as requested on the Interim List and Draft Charge Questions.

In support of a robust interagency process, NASA appreciates the opportunity to comment and hold dialogue with EPA and other agencies on scientific and technical issues. We concur with the submissions provided by the participating agencies and suggestions to improve these documents.

If you have any questions, please let me know.

Many thanks.

Linda

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From: Burneson, Eric [mailto:Burneson.Eric@epa.gov]
Sent: Friday, October 06, 2017 4:56 PM
To: Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov>
Subject: RE: EPA Perchlorate Peer Review Comment Deadlines

Linda: Thanks for the update. I look forward to your comments.
Eric

From: Wennerberg, Linda S. (HQ-LD020) [mailto:linda.s.wennerberg@nasa.gov]
Sent: Friday, October 06, 2017 4:26 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: EPA Perchlorate Peer Review Comment Deadlines

Eric
I will be submitting NASA's comments to you today, as requested. I am awaiting the final input from my management as they indicate that they have minor edits. I should be getting them back and finalized in an hour or so. Please let me know if that is a problem.

Thanks for your update so the schedule for comments.

Linda Wennerberg

Sent from my iPhone

On Oct 6, 2017, at 4:03 PM, Burneson, Eric <Burneson.Eric@epa.gov> wrote:

> Federal Partners:
> As we have discussed previously, EPA currently has two pending requests for comment related to the perchlorate peer review.
>
> 1. The deadline for comments on the charge question and potential peer reviewers is **today**. **The comment deadline for this request remains unchanged.** Note that you have the option of sending your comments directly to me via email.
> 2. The current deadline for the comments on the report is October 30, 2017. **EPA will be extending the deadline for the comments on the Draft Report and Model by 21 days**. We expect to publish a comment deadline extension (to Nov. 20) in the Federal Register next week.
>
> Thank you for your continued input on this important project.
>
> Eric Burneson, P.E.
> Director of Standards and Risk Management
> Office of Ground Water and Drinking Water
> U.S. Environmental Protection Agency
> 202 564 5250

EPA MEMO to FILE

SUBJECT: Letter from Dr. Pleus to Dr. Barton as a follow up to the perchlorate BBDR model peer review meeting held January 10th and 11th

Date: January 30, 2017

On September 30, 2016, EPA published in the Federal Register (81 FR 67350), a notice announcing the release of materials for public comment. The notice provided for written comments to be submitted to EPA on or before November 14, 2016, (a 45-day public comment period). EPA extended the public comment period for eleven days until November 25, 2016.

On January 10 and 11 EPA's contractor held a public peer review meeting on EPA's BBDR model for perchlorate. EPA considers the model highly influential scientific information.

Both EPA and the peer review contractor received the attached letter from Dr. Pleus dated January 24, 2017 encouraging Dr. Barton, Chair of the peer review panel, to include his recommendations into the peer review panel report.

The letter was not transmitted to the Chair because the comment period had closed. However, the letter is provided in the docket for public review and to assure transparency.

INTERTOX

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January 24, 2017

Hugh A. Barton, PhD
Chair, Perchlorate Peer Review Panel
c/o Versar
6850 Versar Center
Springfield, VA 22151

RE: PERCHLORATE PEER REVIEW PROCESS

Dear Dr. Barton:

Thank you for carefully reading our report and listening to my comments at your recent public meeting on EPA's current BBDR model for perchlorate. Applying BBDR methodology holds great promise for informing the perchlorate regulatory process.

Computational models are the future direction of our field. There are many benefits from using such models. It must also be acknowledged that there are limitations as well. Without a doubt, any BBDR model must be based on solid scientific information, particularly for key physiological and toxicological matters. It's essential that the model be transparent. This can ensure that the model's operation is consistent with known physiology and empirical evidence.

At the public meeting, EPA staff emphasized that this model is considered highly influential information. Highly influential scientific information must maintain corresponding credibility. When the Office of Management and Budget (OMB) released its Final Information Quality Bulletin for Peer Review in 2004, OMB's expressed intent was to "...improve the quality of the scientific information upon which policy decisions are based."

I attended both days of the peer review meeting on January 10th and 11th. I was impressed by the candid discourse among panelists. Your committee's work is important—this is the first peer review of an EPA BBDR model for use in this capacity. While the peer review committee might be focused on perchlorate, other models will be used in a similar manner for other chemicals. As the OMB Bulletin states, "Peer review is one of the important procedures used to ensure that the quality of published information meets the standards of the scientific and technical community." Thus, your critical task as peer reviewers is to ensure the Agency applies a rigorous, scientifically-based process in developing these models.

I would request you carefully consider, in light of the OMB Bulletin, that although the perchlorate BBDR model may be the "best" model available on the subject (since it is the ONLY model to predict fT4 values for specific populations), it is not currently adequate to inform an important regulatory decision that will impact other agencies, industry, water purveyors, and the public. To be sure, EPA's model is a good start. Nonetheless, the Agency must continue improving this model. I want to encourage you to not "rubber stamp" the current iteration. Doing so would leave the Agency on the shaky ground of unstable science.

Such a result would be as unnecessary as it would be unfortunate. There is no doubt that EPA has the scientific talent to make this an acceptable model.

Here is a brief summary of the key comments I heard from the Peer Review that must be addressed to ensure the best available science is used:

- First and foremost, the model must evaluate the first trimester pregnant woman, not the end of pregnancy. The most sensitive life stage for neurodevelopmental outcomes is the first trimester (and into the second to some degree). A valid scientific extrapolation of late stage pregnancy to support the first term association with neurodevelopmental outcomes is difficult based on the available literature. The previous Science Advisory Board (SAB), of which you were a member, recommended use of hypothyroxinemia in first trimester pregnant women as the most sensitive endpoint. Dr. Joanne Rovet repeatedly expressed her disappointment in EPA on this issue.
- Second, hypothyroxinemia is not an adverse endpoint and evaluating only fT4 without TSH is not clinically valid. Drs. Elizabeth Pearce and Angela Leung, both respected clinical endocrinologists, stated that hypothyroxinemia is not clinically relevant and is at best a "research construct." On the second day of the meeting, Dr. Pearce reviewed the single paper EPA used to support the assumption that TSH does not change in the hypothyroxinemic range defined by EPA (Silva and Silva, 1981), and found it unsuitable. The model should include TSH to demonstrate, at a minimum, that TSH is not changing in response to changes in fT4. Similarly, we note that hypothyroxinemia and neurodevelopmental outcomes are only equivocally associated in the literature. There is no association for pregnant women, lactating women, or infants.
- Third, the model must reflect the known mechanism of action. Although the model was developed mechanistically, the predicted results are inconsistent with the known mechanism of action: 1) doses causing changes in fT4 are orders of magnitude lower than those that have been reported to cause iodide uptake inhibition—a necessary precursor event; 2) the model predicts changes in thyroid hormones at doses that would not inhibit iodide uptake; and 3) the model has not been demonstrated to accurately predict iodide deficiency without perchlorate, a fundamental aspect of this modeling exercise.
- Finally, the panel was also unanimous that other goitrogen exposures must be modeled in addition to perchlorate. The purpose of this model is to inform development of the MCL, a national regulation. EPA's Office of the Inspector General reported in 2010 that,

> In the OIG Analysis, we conducted a cumulative risk assessment and determined that the risk from each of the four NIS stressors is not equal. The OIG Analysis also independently confirmed that EPA's perchlorate RfD is conservative and protects human health, but limiting perchlorate exposure does not effectively address this public health issue. Potentially lowering the perchlorate drinking water limit from 24.5 ppb to 6 ppb does not provide a meaningful opportunity to lower the public's risk.

I respectfully encourage you to include these recommendations in your report. As a member of EPA's SAB on perchlorate, you likely support the use of a model to inform the MCLG. It

represents a major step forward over the lack of precision provided by the algebraic approach. The changes that I and the peer reviewers have noted can be made to the model. By contrast, moving forward without such necessary adjustments would be scientifically unsupportable.

To best inform the regulatory process—and make effective use of the resources before you—it would be valuable to link the current peer review panel with the upcoming, second panel. This can be accomplished readily by having several members of the current panel, including endocrinologists, serve on the second panel.

Finally, we emphatically urge EPA to continue to meet its commitment to transparency in this process. To be effective, transparency must also be timely. We request that the peer review comments be made public following finalization of the assessment. So, too, EPA's response to the comments should be made public.

Please don't hesitate to let me know if I can provide additional information as you continue your important work.

Sincerely,
INTERTOX, INC.

Richard C. Pleus, PhD
Managing Director and Toxicologist

January 24, 2017

Message

From: Christ, Lisa [Christ.Lisa@epa.gov]
Sent: 9/30/2016 11:54:57 AM
To: Grevatt, Peter [Grevatt.Peter@epa.gov]
CC: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: RE: Perchlorate Peer Review Advance Materials

Will do.

From: Grevatt, Peter
Sent: Thursday, September 29, 2016 8:50 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Fwd: Perchlorate Peer Review Advance Materials

Please reach out to Jim to get 2 meetings on the calendar as he suggests and then we can see where we go from there.

Thx.
Sent from my iPhone

Begin forwarded message:

> **From:** "Kim, Jim H. EOP/OMB" < Ex. 6 EOP (PP) >
> **Date:** September 28, 2016 at 12:00:32 PM EDT
> **To:** "Christ, Lisa" <Christ.Lisa@epa.gov>, "Dorjets, Vlad EOP/OMB" < Ex. 6 EOP (PP) >, < Ex. 6 EOP (PP) >
> **Cc:** "Grevatt, Peter" <Grevatt.Peter@epa.gov>, "Greene, Ashley" <Greene.Ashley@epa.gov>, "Burneson, Eric" <Burneson.Eric@epa.gov>
> **Subject: RE: Perchlorate Peer Review Advance Materials**
>
> Lisa,
>
> Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.
>
> I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP), the second would be to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) and the third would Ex. 5 Deliberative Process (DP)
>
> Can we start looking at dates/times for the end of next week?
>
> Best regards,
> Jim
>
> ---
>
> **From:** Christ, Lisa [mailto:Christ.Lisa@epa.gov]
> **Sent:** Wednesday, September 28, 2016 10:36 AM
> **To:** Dorjets, Vlad EOP/OMB Ex. 6 EOP (PP) Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) >
> **Cc:** Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric

<Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

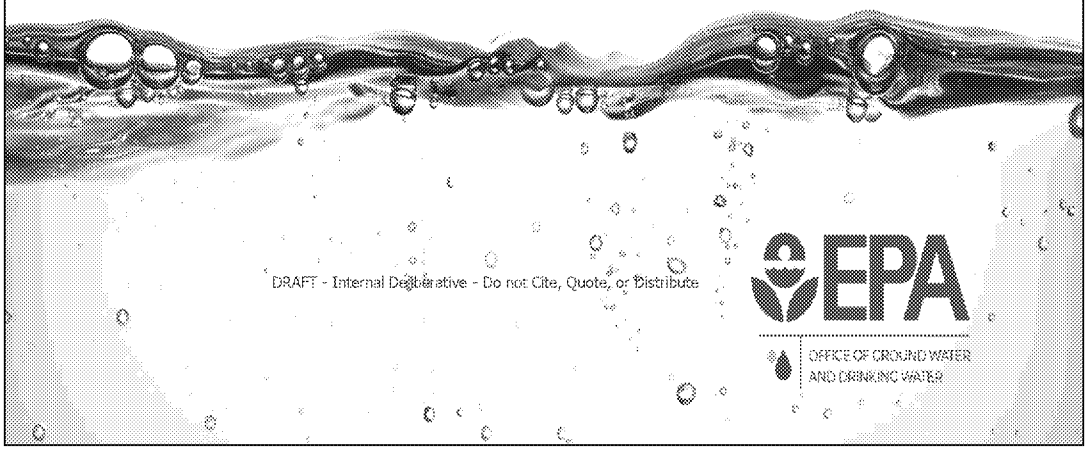
Thank you,
Lisa

~~~~~~

Lisa Christ, Chief
Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M
~~~~~~

EPA's Draft Approach to Inform the Derivation of an MCLG for Perchlorate in Drinking Water

Inter-Agency Briefing | August 9, 2017



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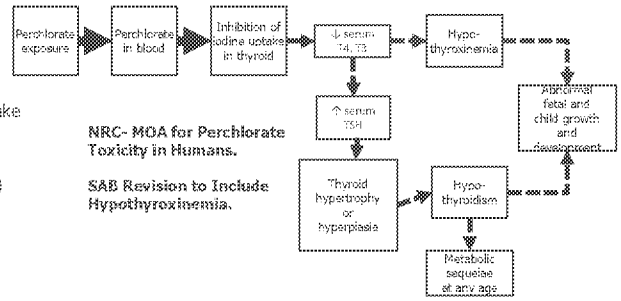
EPA

OFFICE OF GROUND WATER AND DRINKING WATER

Approach to Inform the Derivation of an MCLG for Perchlorate in Drinking Water

- **2005 NAS/NRC :**
  - Pregnant women and their fetuses are particularly sensitive populations. Some pregnant women may have a low iodide intake increasing susceptibility.
  - Recommend inhibition of iodide uptake (IUI) by the thyroid be used as the basis for RfD derivation
- **2013 SAB recommended:**
  - The EPA should extend the PBPK/PD-IUI model to describe changes in thyroid hormone levels during sensitive life stages. This will provide a key tool to link IUI, thyroid hormone, and neurodevelopmental outcome as reported in the scientific and clinical literature.



NRC- MOA for Perchlorate Toxicity in Humans.

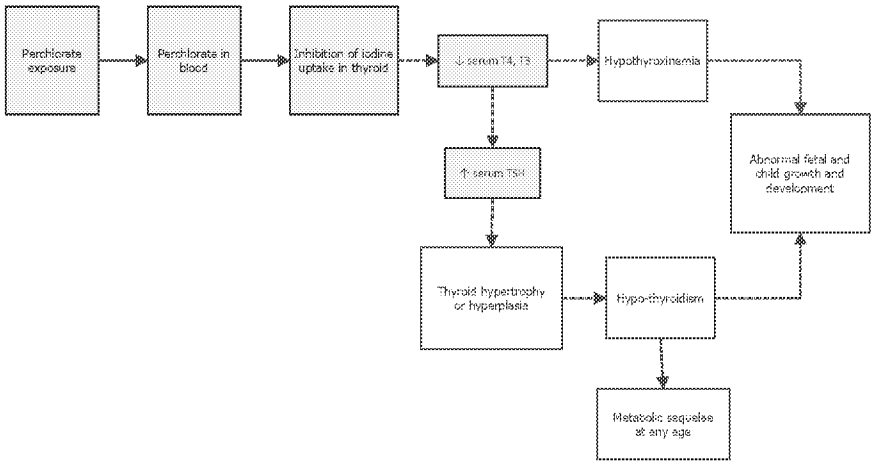
SAB Revision to Include Hypothyroxinemia.

: Perchlorate-induced outcomes NRC 2005 determined were observed in humans

: Outcomes determined by NRC to not be clearly demonstrated in humans exposed to perchlorate, but biologically possible in the absence of adequate compensation

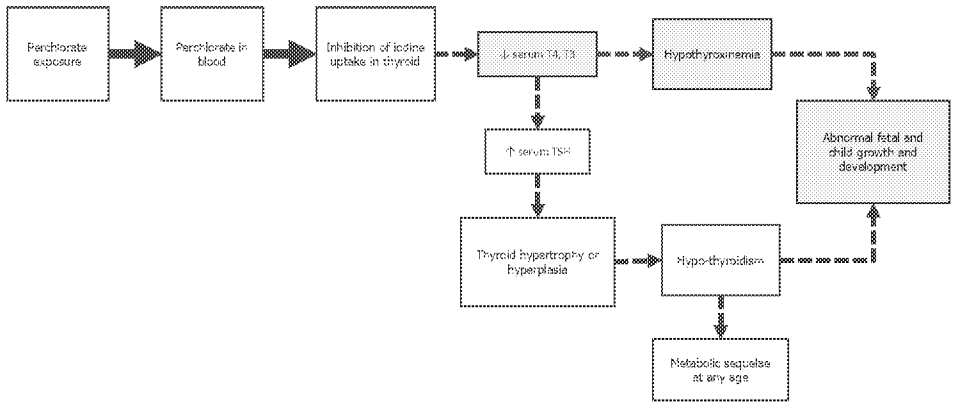
: Augmented mode of action determined by SAB 2013

Part 1: BBDR Model Predicts the Relationship Between Perchlorate Exposure and Changes in Thyroid Hormones



Expert peer review conducted January, 2017; Subsequent revisions to BBDR model included in this next peer review

Part 2: Connect Alterations in Thyroid Hormones to Alterations in Neurodevelopment



Subject of current peer review

Objective and Protocol Used to Identify Literature

- Based on the recommendations of previous peer review panels and a body of data covering years of research, EPA assumed that changes in thyroid hormone levels would be expected to lead to neurodevelopmental outcomes.
- EPA conducted a focused literature review to identify, select, assess, and summarize literature that provided empirical linkages between thyroid hormone changes and neurodevelopmental outcomes.
- The review was conducted in PubMed and Google Scholar.
  - Search strings were developed
  - Searches were restricted to studies published from 2000 to January 2017
- 3 step process to categorize the literature
  - Step 1: Identify studies that would not inform empirical linkage between thyroid hormone and neurodevelopment (Group 3)
  - Step 2: Distinguish between studies containing categorical (Group 2) and regression analysis (Group 1) information
  - Step 3: Characterized Group 1 studies using the following:
    - The population for which the dose-response information is available.
    - The endpoint evaluated.
    - The availability of iodine intake data for the study population.
    - The method of thyroid hormone sampling.
    - The timing of the thyroid hormone measurement
    - The size of the study population

Process for Identifying Literature

Search Category	Search Strings[a]
Endpoint: Neurodevelopment	• Neurodevelopment AND hypothyroxinemia[b] • "Neurodevelopmental effects" AND hypothyroxinemia[b] • Hypothyroxinemia AND behavior • Hypothyroxinemia AND behavior AND infant • Hypothyroxinemia AND thyroxine AND behavior AND infant • (Thyroxine OR T4 OR hypothyroxinemia) AND (neurodevelopment OR "mental ability" OR intelligence OR cognition OR cognitive OR motor OR language)
Endpoint: Attention Deficit/Hyperactivity Disorder	• "Maternal hypothyroxinemia" AND thyroxine AND ADHD AND infant
Endpoint: Autism	• Hypothyroxinemia AND autism[b] • Hypothyroxinemia AND thyroxine AND autism AND infant

[a] Searches were restricted to studies published from 2000 to January 2017.

[b] This search string resulted in more articles than we could reasonably evaluate. Therefore, we constrained the search so that databases searched for those terms only in the title.

- Identified studies assessing thyroid hormone levels and neurodevelopmental outcomes using PubMed/Google Scholar of epidemiology literature and search strings
- Also reviewed SAB report to confirm literature review captured all studies cited as potential references related to the relationship between thyroid hormone and neurodevelopment, including some published prior to 2000. This step yielded 6 additional papers to evaluate
- 55 studies identified

Group 3 Studies

- Studies that do not inform the relationship between thyroid hormone changes and neurodevelopmental outcomes
- All studies are discussed and summarized in the report
- Studies did not provide information on maternal T4 or fT4
- Studies evaluated pregnant women with existing disease (e.g., hypothyroidism)
- Studies were not primary literature (e.g. review articles) and did not provide data on T4 or fT4 or neurodevelopmental outcomes

Group 2 Studies

- Studies that provided categorical analyses
  - Example: Analysis of neurodevelopmental outcomes in offspring of mothers with hypothyroxinemia, compared to non-hypothyroxinemic mothers
- Many studies provided multiple neurodevelopmental endpoints for various age groups
- Results for all studies are presented in the report regardless of statistical significance or association between thyroid hormone levels and neurodevelopmental endpoints

Group 1 Studies

- Studies that provided data allowing for regression analysis relating thyroid hormone levels to a defined change in neurodevelopment.
- As in Group 2, many studies provided multiple neurodevelopmental endpoints for various age groups including:
  - IQ, Bayley Scales, reaction time, expressive language delay, schizophrenia, arithmetic performance, and visual recognition memory
- Results for all studies are summarized in text and presented regardless of statistical significance or relationship
- See attached summary table

Characterization of Uncertainty and Variability in the Report

- Some study results did not show statistical significance
- Relationships analyzed using fT4 levels at only a single point during pregnancy
- Several of the regression analysis functions are based on data digitized from figures
- True fT4 levels at various percentiles in the distribution around the median output on the BBDR model (shape of the distribution)
- None of the current studies used to quantify the defined change in neurodevelopment are based on a U.S. population
- Iodine intake of study populations are not always known
- Thyroid hormone assay results obtained at different times or in different countries

Predicted Dose of Perchlorate Associated with a Change in Neurodevelopmental Measure

Study	Endpoint	Change in fT4 in pmol/L (Percentage change in fT4)	Dose of Perchlorate (µg/kg/day)
Korevaar et al. (2016)	1 point change in IQ	1.30 – 1.67 (19.3%)	27.3 – 77.2[a]
Vermiglio et al. (2004)	1 point change in IQ	0.33 – 0.47 (3.9 – 5.5%)	6.5 – 14.9[a]
Pop et al. (2003)	1 point change in MDI	0.12 – 0.15 (1.9 – 2.3%)	2.3 – 5.2[a]
Pop et al. (2003)	1 point change in PDI	0.08 – 0.12 (0.9 – 1.8%)	1.9 – 3.8[a]
Pop et al. (1999)	1 point change in PDI	0.08 – 0.12 (0.9 – 1.8%)	1.4 – 3.8[a]
Finken et al. (2013)	1 millisecond change in Standard Deviation of Reaction Time	0.12 – 0.28 (1.3 – 4.1%)	2.6 – 6.8[a]
N/A	1% or 5% increase in proportion of hypothyroxinemic pregnant women	N/A	0.7 – 0.8[b] [1%] 3.8 – 3.9[b] [5%]

[a]Low Iodide intake (= 75 µg/day); Range based on using the central and outer bound 95% CI Beta.
[b]Range based on gestational week used to perform the analysis (12 to 16 weeks).

APPENDIX A: BBDR MODEL

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Overview of January 2017 Draft BBDR Model

". . . EPA undertook the development of biologically based dose-response models for the effect of perchlorate on thyroid hormones in women and their offspring during end of term pregnancy and lactation."

"The model represents a major effort to utilize quantitative computational modeling to capture available scientific information describing perchlorate inhibition of the sodium iodide symporter (NIS) and the relationships between iodine intake and circulating levels of thyroid hormones including T4 and T3."

"Thyroid hormones, in turn, are major determinants of neurodevelopment during pregnancy, infancy, and childhood."

"Notable strengths of this effort are that it is based upon the consensus mode of action (MOA) for perchlorate inhibition of iodine uptake, uses human iodine and perchlorate toxicokinetic data, addresses sensitive life stages, and is designed to be protective of serious, unambiguous adverse health effects known to occur in humans (i.e., neurodevelopmental deficits) associated with thyroid hormone deficiencies."

(Peer Review Summary Report, 2017)

Peer Review Recommendations

EPA contractor conducted an independent, external peer review of EPA's draft BBDR model and draft model report.

Peer reviewers were responsible only for evaluating the quality of the science and were not asked to make any regulatory recommendations or to reach consensus in either their deliberations or written comments.

Summary of recommendations based on 7 charge questions (See appendix for complete text of charge, panelist responses and EPA actions.):

1. BBDR Model structure
   - Model should focus on predicting maternal iodide and hormone levels in early pregnancy
   - Inclusion of TSH feedback process would be ideal; mixed opinions on necessity
   - Inclusion of other goitrogens; mixed opinions on whether they should be included or are essentially already accounted for
2. Modifications to existing models
   - Parameters scientifically supported
3. Model calibration and sensitivity analysis
   - A more statistical approach along with a focus on explicitly characterizing the uncertainty in model outputs
   - Sensitivity analysis was limited
   - Present a qualitative evaluation of support for important model parameters
   - Conduct a statistical comparison of model predictions and empirical observations

Peer Review Recommendations (cont.)

Summary of recommendations:

4. Hypothyroxinemic reference levels and model predictions
   - Concern for assessing health effects in contrast to defining regions of model applicability
   - Model the continuous exposure-response relationship
5. Robustness of model predictions and sensitivity at lower perchlorate dose levels
   - Model predictions were likely to be more robust with higher iodine intake and no or low perchlorate
6. Empirical functions to predict thyroid hormone levels
   - Necessary and reasonable if the functions allow the model to better match data
7. Characterization of model predictions
   - Major and important effort to quantitatively characterize the biological relationships between iodine and thyroid hormones
   - Desirable to have a model that predicts the full range of population behaviors

Response to BBDR Model Peer Review Comments

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Message

From: Grevatt, Peter [Grevatt.Peter@epa.gov]
Sent: 10/11/2016 8:20:38 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: RE: Perchlorate Peer Review Advance Materials

All good. I'm glad you're still able participate on calls!

From: Burneson, Eric
Sent: Tuesday, October 11, 2016 4:18 PM
To: Grevatt, Peter <Grevatt.Peter@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Just spoke with Kevin call me on my cell if you want to discuss.

Sent from my iPhone

On Oct 11, 2016, at 4:15 PM, Grevatt, Peter <Grevatt.Peter@epa.gov> wrote:

> Kevin – my mistake that I did not recall that Eric was in NC on a panel with Marc Edwards today. I would like to do what we can to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) The schedule that Jim initially proposed below is Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Perhaps Jim confused the purpose of the 3 meetings that he originally suggested?
>
> One further development that is important to make sure you are aware of is that our intent is to Ex. 5 Deliberative Process (DP)
>
> **Ex. 5 Deliberative Process (DP)**
>
> Apologies again for causing a delay in getting this information to you more quickly!!
>
> **From:** Burneson, Eric
> **Sent:** Tuesday, October 11, 2016 4:07 PM
> **To:** Rennert, Kevin <Rennert.Kevin@epa.gov>
> **Cc:** Grevatt, Peter <Grevatt.Peter@epa.gov>
> **Subject:** Re: Perchlorate Peer Review Advance Materials
>
> Sorry Kevin
> I am at a conference in NC and I just got off the dais. I am available via cell phone Ex. 6 Personal Privacy (PP) Ex. 6 Personal Privacy (PP) if it would still be helpful.
>
> Sent from my iPhone
>
> On Oct 11, 2016, at 1:39 PM, Rennert, Kevin <Rennert.Kevin@epa.gov> wrote:
>
> > Thanks, Eric. Are you in today to discuss? Peter is tied up until 4, which is when we have our weekly with OIRA. I'm available from 3-4 if there's a time in that window that works for you. Kevin

From: Burneson, Eric
Sent: Saturday, October 08, 2016 10:27 AM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>
Subject: Fw: Perchlorate Peer Review Advance Materials

Kevin
Sorry but what we thought we agreed to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) That's what Jim Kim laid out in his Sept 28 note below. We were open to Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

We are concerned that:

1.
2.
3.

Ex. 5 Deliberative Process (DP)

We recognize we need to respond in a manner consistent with how others in the Agency are addressing interagency coordination. That's why we are checking in with you prior to responding to Jim Kim's request.

Eric Burneson

From: Rennert, Kevin
Sent: Friday, October 07, 2016 4:10 PM
To: Grevatt, Peter <Grevatt.Peter@epa.gov>
Cc: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Peter,

I may be misunderstanding you here (or in a previous email chain), but the original request from Jim that I forwarded on to you and Joel was specifically to do an initial meeting around the 20th and then have a followup meeting around the 7th to discuss Ex. 5 Deliberative Process (DP)
The response I got from you and Joel on that request was that this

was something that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) and it looks from this email chain like Lisa Christ was looking to set up times on both of those days for that purpose. So if we're now looking to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) and we should probably discuss how to approach that. If you're saying that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) that may be a different story. Thanks.

Kevin

From: Grevatt, Peter
Sent: Thursday, October 06, 2016 5:28 PM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>
Cc: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: FW: Perchlorate Peer Review Advance Materials

Kevin, FYI. I propose that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) As we discussed with OMB, I don't think Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Please let me know what you think.

From: Kim, Jim H. EOP/OMB
[mailto: Ex. 6 Personal Privacy (PP)
Sent: Thursday, October 06, 2016 2:56 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks. Also – I wanted to confirm that if Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)? That would give EPA a little more than 2 weeks to consider interagency comments. So our goal will be for

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP) Also – I wanted to confirm that our comments on the peer review charge would be submitted to you, while comments on the BBDR draft report and model will be submitted to the docket.

I am thinking that a 3rd meeting prior to the end of November would be useful to discuss Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Please let me know your thoughts.

Best regards,

Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Thursday, October 6, 2016 7:35 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Hi Jim

Of course. I held the time on EPA participants calendars, so I'll send confirmation. I'll set up logistics and send you the information soon.

Lisa

Sent from my iPhone

On Oct 5, 2016, at 4:40 PM, Kim, Jim H. EOP/OMB
<Ex. 6 Personal Privacy (PP)> wrote:

Hi Lisa,

Thanks for the info. Sorry for the late response. Can we go ahead and schedule a meeting for October 20? Thanks for your patience.

Best regards,

Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 11:04 AM
To: Kim, Jim H. EOP/OMB
<Ex. 6 Personal Privacy (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)v>; Schwab, Margo EOP/OMB
<Ex. 6 Personal Privacy (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim

The closing date for public comments on the charge questions is October 21. The public comment period for the model and report ends November 14. We intend to have our contractor identify the peer reviewers and we'll finalize charge questions between October 21 and November 14. [potential call dates are Oct 20 & 27]

Public comments will be summarized and provided to the peer reviewers at the end of November around the same time we publish the next federal register notice announcing a meeting date sometime in early January (based on availability of peer reviewers and at least 30 days before the panel meeting). [potential call dates are Nov 7 & 8]

Lisa

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 Personal Privacy (PP)
Sent: Friday, September 30, 2016 10:27 AM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Doriets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks for the dates and times. I was hoping to get something on our calendars earlier to ensure that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Can you tell me the timeline that is planned in regards to Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Thanks,

Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 9:42 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim,

I'd like to suggest we schedule two meetings and see if an additional meeting, as suggested, is needed. I looked at calendars for relevant EPA folks and can offer the following dates/times.

1st meeting: October 20 from 4-5pm OR October 27 from 405pm

2nd meeting: November 7 from 11-11am OR November 8 from 11a-noon

I am unable to find availability sooner than Oct 20. However, that provides time for review of materials before discussions begin.

Please let me know at your earliest convenience which times work and I will set up a conference call.

Lisa

From: Kim, Jim H. EOP/OMB [mailto:Ex. 6 Personal Privacy (PP)
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Ex. 6 Personal Privacy (PP)' Ex. 6 Personal Privacy (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>;

Greene, Ashley <Greene.Ashley@epa.gov>;
Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) the second would be to discuss Ex. 5 Deliberative Process (DP) and the third would Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,

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Vlad, Jim and Margo,

Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,

Lisa

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

Lisa Christ, Chief

Targeting and Analysis Branch

Office of Ground Water and Drinking Water

USEPA

1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760

Mail Code: 4607M
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Message

From: Rennert, Kevin [Rennert.Kevin@epa.gov]
Sent: 10/11/2016 8:18:10 PM
To: Grevatt, Peter [Grevatt.Peter@epa.gov]; Burneson, Eric [Burneson.Eric@epa.gov]
Subject: RE: Perchlorate Peer Review Advance Materials

Thanks, Peter. We just talked with Eric by cell phone as well, so I think we're good on this front now. Jim just rescheduled our weekly, but I'll raise this with him when we talk tomorrow.

From: Grevatt, Peter
Sent: Tuesday, October 11, 2016 4:16 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>; Rennert, Kevin <Rennert.Kevin@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Kevin – my mistake that I did not recall that Eric was in NC on a panel with Marc Edwards today. I would like to do what we can to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) The schedule that Jim initially proposed below is Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Perhaps Jim confused the purpose of the 3 meetings that he originally suggested?

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> Kevin

Sorry but what we thought we agreed to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) That's what Jim Kim laid out in his Sept 28 note below. We were open to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)

We are concerned that:

1.
2.
3.

Ex. 5 Deliberative Process (DP)

We recognize we need to respond in a manner consistent with how others in the Agency are addressing interagency coordination. That's why we are checking in with you prior to responding to Jim Kim's request.

Eric Burneson

From: Rennert, Kevin
Sent: Friday, October 07, 2016 4:10 PM
To: Grevatt, Peter <Grevatt.Peter@epa.gov>
Cc: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Peter,

I may be misunderstanding you here (or in a previous email chain), but the original request from Jim that I forwarded on to you and Joel was specifically to

Ex. 5 Deliberative Process (DP)

The response I got from you and Joel on that request was that this was something that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) So if we're now looking to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) and we should probably discuss how to approach that. If you're saying that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Thanks.

Kevin

From: Grevatt, Peter
Sent: Thursday, October 06, 2016 5:28 PM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>
Cc: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: FW: Perchlorate Peer Review Advance Materials

Kevin, FYI. I propose that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) As we discussed with OMB, I don't think Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Please let me know what you think.

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 Personal Privacy (PP)
Sent: Thursday, October 06, 2016 2:56 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks. Also – I wanted to confirm that if Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)? That would give EPA a little more than 2 weeks to consider interagency comments. So our goal will be for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Also – I wanted to confirm that our comments on the peer review charge would be submitted to you, while comments on the BBDR draft report and model will be submitted to the docket.

I am thinking that a 3rd meeting prior to the end of November would be useful to discuss Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Please let me know your thoughts.

Best regards,

Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Thursday, October 6, 2016 7:35 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Hi Jim

Of course. I held the time on EPA participants calendars, so I'll send confirmation. I'll set up logistics and send you the information soon.

Lisa

Sent from my iPhone

On Oct 5, 2016, at 4:40 PM, Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)> wrote:

> Hi Lisa,
>
> Thanks for the info. Sorry for the late response. Can we go ahead and schedule a meeting for October 20? Thanks for your patience.
>
> Best regards,

Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 11:04 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim

The closing date for public comments on the charge questions is October 21. The public comment period for the model and report ends November 14. We intend to have our contractor identify the peer reviewers and we'll finalize charge questions between October 21 and November 14. [potential call dates are Oct 20 & 27]

Public comments will be summarized and provided to the peer reviewers at the end of November around the same time we publish the next federal register notice announcing a meeting date sometime in early January (based on availability of peer reviewers and at least 30 days before the panel meeting). [potential call dates are Nov 7 & 8]

Lisa

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 Personal Privacy (PP)]
Sent: Friday, September 30, 2016 10:27 AM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks for the dates and times. I was hoping to get something on our calendars earlier to ensure that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Can you tell me the timeline that is planned in regards to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)

Thanks,

Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 9:42 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim,

I'd like to suggest we schedule two meetings and see if an additional meeting, as suggested, is needed. I looked at calendars for relevant EPA folks and can offer the following dates/times.

1st meeting: October 20 from 4-5pm OR October 27 from 405pm

2nd meeting: November 7 from 11-11am OR November 8 from 11a-noon

I am unable to find availability sooner than Oct 20. However, that provides time for review of materials before discussions begin.

Please let me know at your earliest convenience which times work and I will set up a conference call.

Lisa

From: Kim, Jim H. EOP/OMB
[mailto: Ex. 6 Personal Privacy (PP)
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB
< Ex. 6 Personal Privacy (PP)
< Ex. 6 Personal Privacy (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) the second would be to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP), and the third would Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,

Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, September 28, 2016 10:36 AM
To: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >;
Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) >;

Ex. 6 Personal Privacy (PP)

Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,

Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,

Lisa

~~~~~~

Lisa Christ, Chief

Targeting and Analysis Branch

Office of Ground Water and Drinking Water

USEPA

1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760

Mail Code: 4607M
~~~~~~

September 26, 2017

SUBMITTED VIA EMAIL
Mr. Samuel Hernandez
U.S. Environmental Protection Agency
Office of Ground Water and Drinking Water
1200 Pennsylvania Ave., NW
Washington, D.C. 20460

RE: Request for comment period extension on the draft report titled, "Proposed Approaches to Inform the Derivation of a Maximum Contaminant Level Goal for Perchlorate in Drinking Water" (draft MCLG Approaches Report; 82 FR 43354; EPA-HQ-OW-2016-0438)

Mr. Hernandez:

The American Chemistry Council (ACC) respectfully requests that EPA extend the comment period on the draft report, "Proposed Approaches to Inform the Derivation of a Maximum Contaminant Level Goal for Perchlorate in Drinking Water," by 45 days to December 14, 2017. We appreciate EPA's responsiveness in addressing the recommendations made by the first peer review panel in January 2017. However, given the complexity and length of the revisions in the Biologically Based Dose-Response (BBDR) model and supporting documentation being used to develop the MCLG, a 90-day comment period is necessary to allow sufficient time for public review and comment.

All parties, but especially the second peer review panel, will benefit from additional time to evaluate the changes and technical assumptions in the updated BBDR model.

We would appreciate a response to this request at your earliest convenience. Should you have questions or would like to discuss this request, please contact me at judith_nordgren@americanchemistry.com or Mark Gibson at mark_gibson@americanchemistry.com.

Sincerely,

Judith Nordgren

Judith Nordgren
Managing Director
Chlorine Chemistry Division

cc:
Michael Shapiro – EPA, OW
Lee Forsgren – EPA, OW
Peter Grevatt – EPA, OGWDW
Eric Burneson – EPA, OGWDW

Message

From: Wennerberg, Linda S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
Sent: 1/24/2017 5:29:36 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: RE: Last week

Eric:
Let's plan to talk by phone this Thursday at 3:30. I will plan to be in my office (number provided below). Suggest that you call me as I know that I will be in my office at that time.

Please let me know if that works for you or you have an alternative option.

Thanks.
Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89
300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

From: Burneson, Eric [mailto:Burneson.Eric@epa.gov]
Sent: Tuesday, January 24, 2017 11:56 AM
To: Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov>
Subject: RE: Last week

Linda
I could call you Thursday at 3:30 or we could meet for coffee Friday at 10 (we could also talk by phone at that time too). Let me know your preference and I will lock it on my calendar.

Eric Burneson, P.E.
Director of Standards and Risk Management
Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency
202 564 5250

From: Wennerberg, Linda S. (HQ-LD020) [mailto:linda.s.wennerberg@nasa.gov]
Sent: Tuesday, January 24, 2017 11:53 AM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Last week

Eric

I am finally back in front of my computer.

I am free the morning until 1:30pm and then from 3:30-5pm on this Thursday. I also have got a relatively light Friday as I am teleworking. Any option to meet up over coffee or on the phone, given my schedule this week?

Thanks.

Linda

Linda S. Wennerberg, Ph.D.
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300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

From: Burneson, Eric [mailto:Burneson.Eric@epa.gov]
Sent: Monday, January 23, 2017 3:10 PM
To: Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov>
Subject: Last week

Linda:
I am sorry that I did not follow up with you last week as we had discussed at the perchlorate peer review meeting. With the pre-inaugural security measures and the other activities underway I neglected to reach out. Let me know if you want to get together for coffee or just have a phone conversation.

Eric Burneson, P.E.
Director of Standards and Risk Management
Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency
202 564 5250

Appointment

From: Hafez, Ahmed [Hafez.Ahmed@epa.gov]
Sent: 10/19/2017 1:07:04 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]; Miller, Gregory [Miller.Gregory@epa.gov]; Flowers, Lynn [Flowers.Lynn@epa.gov]; Christ, Lisa [Christ.Lisa@epa.gov]
CC: Hernandez-Quinones, Samuel [Hernandez.Samuel@epa.gov]

Subject: Canceled: Finalizing Perchlorate Charge Questions
Attachments: Proposed Draft SBA Edits to Percholorate Charge Questions v2 October 5.docx; RE: Perchlorate Charge Question - question about Lisa's email discussion below; DRAFT FINAL MCLG Approach Charge 9-5-17.docx
Location: Call-in or Eric's Office (WJC-E 2331A)

Start: 10/19/2017 3:30:00 PM
End: 10/19/2017 4:30:00 PM
Show Time As: Free

Importance: High

Hi All,

Meeting to discuss comments on the charge questions and finalize revisions.

Attached:
SBA email and proposed changes
EPA email response to SBA questions
Current version of the Charge

Call-in number to follow.

Thanks,
Ahmed

Message

From: Wennerberg, Linda S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
Sent: 10/27/2016 7:25:33 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
CC: Olson, Daniel [Olson.Daniel@epa.gov]; Mcneill, Mike A (HQ-LD020) [mike.a.mcneill@nasa.gov]
Subject: RE: NASA Comments, Draft Perchlorate Model Peer Review Charge Questions
Attachments: Perchlorate BBDR Model External Peer Review.NASA 10_26_16.docx

Importance: High

Eric and Dan
Attached are NASA's comments on the draft Perchlorate Model Charge Questions. We are submitting them today in accordance with your request.

NASA thanks EPA for the opportunity to review and comment. We utilized a redline/strikeout format as it provided specific language for EPA's consideration. This is a Word Document and you need to review this with ALL MARKUP showing to see the specific changes, under the Review Tab. If you have any questions or need additional information from us, please let me know. If you have any difficulty seeing the redline markup, please let me know and I will send you a pdf version of this.

Please let me know that you received this and can see the redline comments.

Thanks again.

Linda

Linda S. Wennerberg, Ph.D.
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300 E Street SW
Washington, DC 20546-0001

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Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

From: Burneson, Eric [mailto:Burneson.Eric@epa.gov]
Sent: Thursday, October 27, 2016 11:05 AM
To: Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Olson, Daniel <Olson.Daniel@epa.gov>
Subject: Re: URGENT:: Please provide POC to receive comments on Perchlorate Draft Charge Questions

Linda
Please send them to me and to Dan Olson

Sent from my iPhone

On Oct 27, 2016, at 10:45 AM, Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov> wrote:

Peter and Eric:

I am planning to submit the NASA comments today, as per your request. However, the POC I received to send them to (Ms. Lisa Christ) is out of the office until November 4th. I got an out of office email from her.

Please send me the name and email for your staff person who is to receive our comments as Ms. Christ is away.

Your attention to this matter is much appreciated.

Thanks.

Linda

Linda S. Wennerberg, Ph.D.
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300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

Message

From: Wennerberg, Linda S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
Sent: 1/23/2017 8:29:51 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: Re: Last week

Eric
No worries. I was also slammed with work etc. last week

Am running out to a meeting now but check out my Calendar for options when I am back online.

Thanks.
Linda

Sent from my iPhone

On Jan 23, 2017, at 3:10 PM, Burneson, Eric <Burneson.Eric@epa.gov> wrote:

> Linda:
> I am sorry that I did not follow up with you last week as we had discussed at the perchlorate peer review meeting. With the pre-inaugural security measures and the other activities underway I neglected to reach out. Let me know if you want to get together for coffee or just have a phone conversation.
>
> Eric Burneson, P.E.
> Director of Standards and Risk Management
> Office of Ground Water and Drinking Water
> U.S. Environmental Protection Agency
> 202 564 5250

Message

From: Wennerberg, Linda S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
Sent: 10/27/2016 6:15:45 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
CC: Grevatt, Peter [Grevatt.Peter@epa.gov]; Olson, Daniel [Olson.Daniel@epa.gov]
Subject: RE: URGENT:: Please provide POC to receive comments on Perchlorate Draft Charge Questions

Eric:
Thanks. Will do.

Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89
300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

From: Burneson, Eric [mailto:Burneson.Eric@epa.gov]
Sent: Thursday, October 27, 2016 11:05 AM
To: Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Olson, Daniel <Olson.Daniel@epa.gov>
Subject: Re: URGENT:: Please provide POC to receive comments on Perchlorate Draft Charge Questions

Linda
Please send them to me and to Dan Olson

Sent from my iPhone

On Oct 27, 2016, at 10:45 AM, Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov> wrote:

> Peter and Eric:
>
> I am planning to submit the NASA comments today, as per your request. However, the POC I received to send them to (Ms. Lisa Christ) is out of the office until November 4th. I got an out of office email from her.
>
> Please send me the name and email for your staff person who is to receive our comments as Ms. Christ is away.
>
> Your attention to this matter is much appreciated.
>
> Thanks.
>
> Linda

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linda.s.wennerberg@nasa.gov

Perchlorate Peer Review

Inter-Agency Meeting

August 9, 2017

- Roll Call
- Introduction
- Briefing: Draft Approach to Inform the Derivation of an MCLG
- Review of the Group 1 Study Characterization Table
- Review of the Revised Peer Review Charge
- Inter - Agency discussion

Message

From: Dorjets, Vlad EOP/OMB Ex. 6 Personal Privacy (PP)
Sent: 9/25/2017 1:04:06 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: Re: EPA Perchlorate Peer Review Charge follow up

No problem. Thanks for checking though.

Sent from my iPhone

> On Sep 25, 2017, at 8:56 AM, Burneson, Eric <Burneson.Eric@epa.gov> wrote:
>
> I assume you are aware that Jim will not be able to participate but I am forwarding just in case. I don't plan to reschedule at this point based on his note below.
>
> -----Original Appointment-----
> From: Laity, Jim A. EOP/OMB [mailto Ex. 6 Personal Privacy (PP)]
> Sent: Friday, September 22, 2017 7:38 PM
> To: Burneson, Eric
> Subject: Tentative: EPA Perchlorate Peer Review Charge follow up
> When: Monday, October 02, 2017 3:00 PM-4:00 PM (UTC-05:00) Eastern Time (US & Canada).
> Where: Teleconference # Ex. 6 Personal Privacy (PP) code Ex. 6 Personal Privacy (PP) or EPA WJC East Room 2123
>
> I will be out on annual leave from Oct 2-5, but you do not need to reschedule for me if this works for others.
>
>

Message

From: Wennerberg, Linda S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
Sent: 10/27/2016 3:03:13 PM
To: Grevatt, Peter [Grevatt.Peter@epa.gov]
CC: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: RE: URGENT:: Please provide POC to receive comments on Perchlorate Draft Charge Questions

Peter
Thanks so much for getting back to me. I will send them to Eric.

Eric:
I am running out to an offsite meeting but will be returning to my office at or just after 2PM. I will send the NASA comments to you then. They are in a redline/strikeout format from the draft Charge Questions for ease of reading.

Many thanks.

Linda

Linda S. Wennerberg, Ph.D.
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MS-2T89
300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

From: Grevatt, Peter [mailto:Grevatt.Peter@epa.gov]
Sent: Thursday, October 27, 2016 11:00 AM
To: Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov>
Cc: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: URGENT:: Please provide POC to receive comments on Perchlorate Draft Charge Questions

Thanks Linda. Please send these directly to Eric Burneson.

Sent from my iPhone

On Oct 27, 2016, at 10:45 AM, Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov> wrote:

> Peter and Eric:
>
> I am planning to submit the NASA comments today, as per your request. However, the POC I received to send them to (Ms. Lisa Christ) is out of the office until November 4th. I got an out of office email from her.
>
> Please send me the name and email for your staff person who is to receive our comments as Ms. Christ is away.

Your attention to this matter is much appreciated.

Thanks.

Linda

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300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

Message

From: Dorjets, Vlad EOP/OMB Ex. 6 Personal Privacy (PP)
Sent: 9/22/2017 6:41:14 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
CC: Muellerleile, Caryn [Muellerleile.Caryn@epa.gov]
Subject: Re: EPA Perchlorate Peer Review Charge follow up

Maybe we can create an excel file with people on distribution list by agency? That would also make my life easier when this gets to rule making phase.

Sent from my iPhone

On Sep 22, 2017, at 2:25 PM, Burneson, Eric <Burneson.Eric@epa.gov<mailto:Burneson.Eric@epa.gov>> wrote:

Caryn and Vlad:
I don't have any problem accommodating the request below but, since I am working off of invitation lists that you developed I just wanted to check in be sure there is not a more formal protocol for deciding who is or is not on the invite.
Eric Burneson

-----Original Appointment-----
From: O'Conor, Letitia [mailto:Letitia.O'Conor@em.doe.gov]
Sent: Friday, September 22, 2017 11:42 AM
To: Burneson, Eric
Subject: Accepted: EPA Perchlorate Peer Review Charge follow up
When: Monday, October 02, 2017 3:00 PM-4:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: Teleconference # Ex. 6 Personal Privacy (PP) code Ex. 6 Personal Privacy (PP) or EPA WJC East Room 2123

Hi Eric,
Please include beth.moore@em.doe.gov<mailto:beth.moore@em.doe.gov> to your list and delete gareth.buckland@em.doe.gov<mailto:gareth.buckland@em.doe.gov> and delete Emily.adamo@em.doe.gov<mailto:Emily.adamo@em.doe.gov>
Beth is working with Sarah Jensen both from DOE.
Thank you,
Tish O'Conor

Appointment

From: Laity, Jim A. EOP/OMB Ex. 6 Personal Privacy (PP)
Sent: 9/22/2017 11:38:04 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]

Subject: Tentative: EPA Perchlorate Peer Review Charge follow up
Location: Teleconference # Ex. 6 Personal Privacy (PP) code Ex. 6 Personal Privacy (PP) or EPA WJC East Room 2123

Start: 10/2/2017 7:00:00 PM
End: 10/2/2017 8:00:00 PM
Show Time As: Busy

Recurrence: (none)

I will be out on annual leave from Oct 2-5, but you do not need to reschedule for me if this works for others.

Message

From: Muellerleile, Caryn [Muellerleile.Caryn@epa.gov]
Sent: 9/22/2017 7:55:13 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]; Vlad Dorjets Ex. 6 Personal Privacy (PP)
Ex. 6 Personal Privacy (PP)
Subject: RE: EPA Perchlorate Peer Review Charge follow up

On my end, no particular protocol. The only thing I have done in the past is refuse a reschedule that one agency staff person requested after EPA & EOP had carefully coordinated the best dates for all of us.

Caryn

From: Burneson, Eric
Sent: Friday, September 22, 2017 2:26 PM
To: Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Vlad Dorjets Ex. 6 Personal Privacy (PP)
< Ex. 6 Personal Privacy (PP) >
Subject: FW: EPA Perchlorate Peer Review Charge follow up

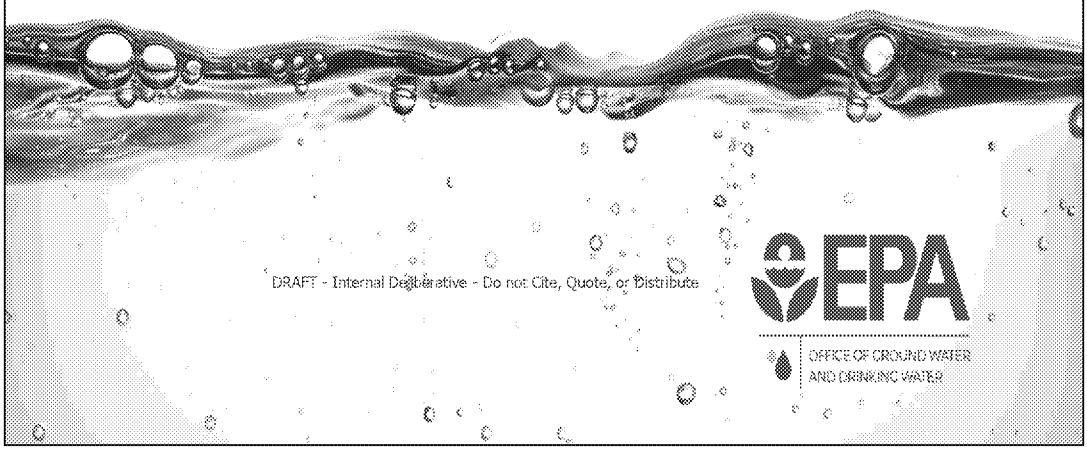
Caryn and Vlad:
I don't have any problem accommodating the request below but, since I am working off of invitation lists that you developed I just wanted to check in be sure there is not a more formal protocol for deciding who is or is not on the invite.
Eric Burneson

-----Original Appointment-----
From: O'Conor, Letitia [mailto:Letitia.O'Conor@em.doe.gov]
Sent: Friday, September 22, 2017 11:42 AM
To: Burneson, Eric
Subject: Accepted: EPA Perchlorate Peer Review Charge follow up
When: Monday, October 02, 2017 3:00 PM-4:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: Teleconference # Ex. 6 Personal Privacy (PP) code Ex. 6 Personal Privacy (PP) or EPA WJC East Room 2123

Hi Eric,
Please include beth.moore@em.doe.gov to your list and delete gareth.buckland@em.doe.gov and delete Emily.adamo@em.doe.gov
Beth is working with Sarah Jensen both from DOE.
Thank you,
Tish O'Conor

EPA's Draft Approach to Inform the Derivation of an MCLG for Perchlorate in Drinking Water

OMB Briefing | August 3, 2017



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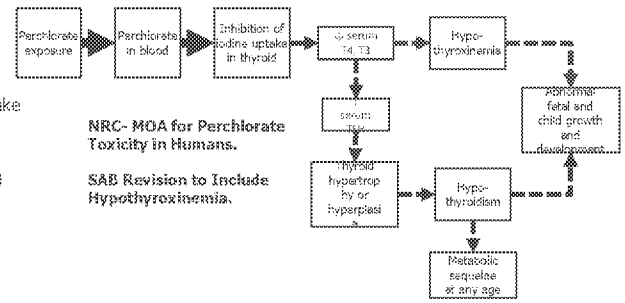
EPA

OFFICE OF GROUND WATER AND DRINKING WATER

Approach to Inform the Derivation of an MCLG for Perchlorate in Drinking Water

- **2005 NAS/NRC :**
  - Pregnant women and their fetuses are particularly sensitive populations. Some pregnant women may have a low iodide intake increasing susceptibility.
  - Recommend inhibition of iodide uptake (IUI) by the thyroid be used as the basis for RfD derivation
- **2013 SAB recommended:**
  - The EPA should extend the PBPK/PD-IUI model to describe changes in thyroid hormone levels during sensitive life stages. This will provide a key tool to link IUI, thyroid hormone, and neurodevelopmental outcome as reported in the scientific and clinical literature.



NRC- MOA for Perchlorate Toxicity in Humans.

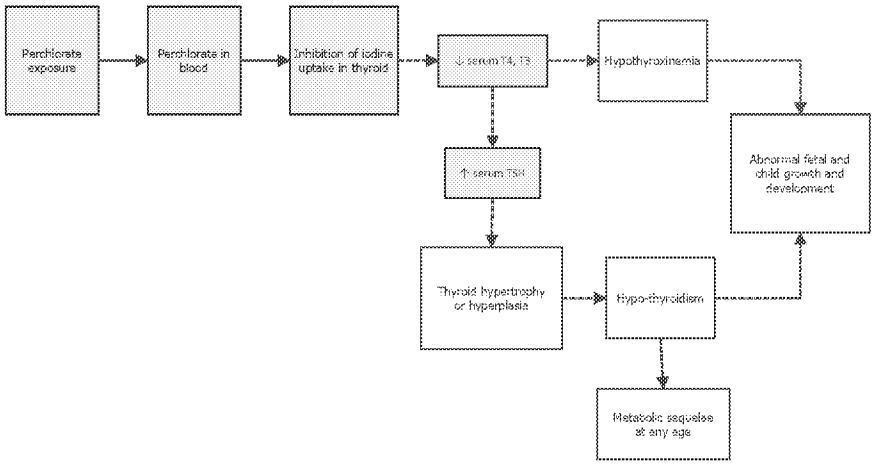
SAB Revision to Include Hypothyroxinemia.

: Perchlorate-induced outcomes NRC 2005 determined were observed in humans

: Outcomes determined by NRC to not be clearly demonstrated in humans exposed to perchlorate, but biologically possible in the absence of adequate compensation

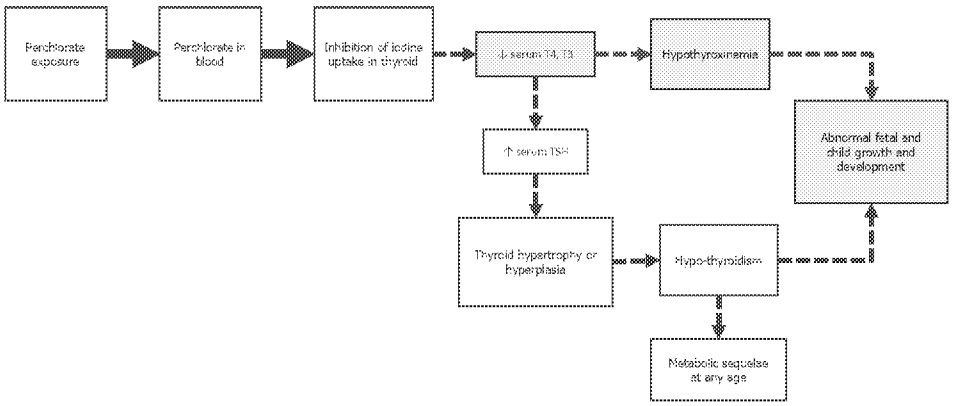
: Augmented mode of action determined by SAB 2013

BBDR Model Predicts the Relationship Between Perchlorate Exposure and Changes in Thyroid Hormones



Expert peer review conducted January, 2017; final report published March 2017

Approach to Connect Alterations in Thyroid Hormones to Alterations in Neurodevelopment



Subject of current peer review

Objective and Protocol Used to Identify Literature

- EPA conducted a review to identify, select, assess, and summarize literature that provided empirical linkages between thyroid hormone changes and neurodevelopmental outcomes as recommended by the SAB.
- The review was conducted in PubMed and Google Scholar.
  - Search strings were developed
  - Searches were restricted to studies published from 2000 to January 2017
- 3 step process to categorize the literature
  - Step 1: Identify studies that would not inform empirical linkage between thyroid hormone and neurodevelopment (Group 3)
  - Step 2: Distinguish between studies containing categorical (Group 2) and regression analysis (Group 1) information
  - Step 3: Characterized Group 1 studies using the following:
    - The population for which the dose-response information is available.
    - The endpoint evaluated.
    - The availability of iodine intake data for the study population.
    - The method of thyroid hormone sampling.
    - The timing of the thyroid hormone measurement
    - The size of the study population

Process for Identifying Literature

Search Category	Search Strings[a]
Endpoint: Neurodevelopment	• Neurodevelopment AND hypothyroxinemia[b] • "Neurodevelopmental effects" AND hypothyroxinemia[b] • Hypothyroxinemia AND behavior • Hypothyroxinemia AND behavior AND infant • Hypothyroxinemia AND thyroxine AND behavior AND infant • (Thyroxine OR T4 OR hypothyroxinemia) AND (neurodevelopment OR "mental ability" OR intelligence OR cognition OR cognitive OR motor OR language)
Endpoint: Attention Deficit/Hyperactivity Disorder	• "Maternal hypothyroxinemia" AND thyroxine AND ADHD AND infant
Endpoint: Autism	• Hypothyroxinemia AND autism[b] • Hypothyroxinemia AND thyroxine AND autism AND infant

[a] Searches were restricted to studies published from 2000 to January 2017.

[b] This search string resulted in more articles than we could reasonably evaluate. Therefore, we constrained the search so that databases searched for those terms only in the title.

- Identified studies assessing thyroid hormone levels and neurodevelopmental outcomes using PubMed/Google Scholar of epidemiology literature and search strings
- Also reviewed SAB report to confirm literature review captured all studies cited as potential references related to the relationship between thyroid hormone and neurodevelopment, including some published prior to 2000. This step yielded 6 additional papers to evaluate
- 55 studies identified

Group 3 Studies

- Studies that do not inform the relationship between thyroid hormone changes and neurodevelopmental outcomes
- All studies are discussed and summarized in the report
- Studies did not provide information on maternal T4 or fT4
- Studies evaluated pregnant women with existing disease (e.g., hypothyroidism)
- Studies were not primary literature (e.g. review articles) and did not provide data on T4 or fT4 or neurodevelopmental outcomes

Group 2 Studies

- Studies that provided categorical analyses
  - Example: Analysis of neurodevelopmental outcomes in offspring of mothers with hypothyroxinemia, compared to non-hypothyroxinemic mothers
- Many studies provided multiple neurodevelopmental endpoints for various age groups
- Results for all studies are presented in the report regardless of statistical significance or association between thyroid hormone levels and neurodevelopmental endpoints

Group 1 Studies

- Studies that provided data allowing for regression analysis relating thyroid hormone levels to a defined change in neurodevelopment.
- As in Group 2, many studies provided multiple neurodevelopmental endpoints for various age groups including:
  - IQ, Bayley Scales, reaction time, expressive language delay, schizophrenia, arithmetic performance, and visual recognition memory
- Results for all studies are summarized in text and presented regardless of statistical significance or relationship
- See attached summary table

Predicted Dose of Perchlorate Associated with a Change in Neurodevelopmental Measure

Study	Endpoint	Change in fT4 in pmol/L (Percentage change in fT4)	Dose of Perchlorate (µg/kg/day)
Korevaar et al. (2016)	1 point change in IQ	1.30 – 1.67 (19.3%)	27.3 – 77.2[a]
Vermiglio et al. (2004)	1 point change in IQ	0.33 – 0.47 (3.9 – 5.5%)	6.5 – 14.9[a]
Pop et al. (2003)	1 point change in MDI	0.12 – 0.15 (1.9 – 2.3%)	2.3 – 5.2[a]
Pop et al. (2003)	1 point change in PDI	0.08 – 0.12 (0.9 – 1.8%)	1.9 – 3.8[a]
Pop et al. (1999)	1 point change in PDI	0.08 – 0.12 (0.9 – 1.8%)	1.4 – 3.8[a]
Finken et al. (2013)	1 millisecond change in Standard Deviation of Reaction Time	0.12 – 0.28 (1.3 – 4.1%)	2.6 – 6.8[a]
N/A	1% or 5% increase in proportion of hypothyroxinemic pregnant women	N/A	0.7 – 0.8[b] [1%] 3.8 – 3.9[b] [5%]

[a]Low Iodide intake (= 75 µg/day); Range based on using the central and outer bound 95% CI Beta.
[b]Range based on gestational week used to perform the analysis (12 to 16 weeks).

Characterization of Uncertainty and Variability in the Report

- Some study results did not show statistical significance
- Relationships analyzed using fT4 levels at only a single point during pregnancy
- Several of the regression analysis functions are based on data digitized from figures
- True fT4 levels at various percentiles in the distribution around the median output on the BBDR model (shape of the distribution)
- None of the current studies used to quantify the defined change in neurodevelopment are based on a U.S. population
- Iodine intake of study populations are not always known
- Thyroid hormone assay results obtained at different times or in different countries

APPENDIX A: BBDR MODEL

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Overview of January 2017 Draft BBDR Model

". . . EPA undertook the development of biologically based dose-response models for the effect of perchlorate on thyroid hormones in women and their offspring during end of term pregnancy and lactation."

"The model represents a major effort to utilize quantitative computational modeling to capture available scientific information describing perchlorate inhibition of the sodium iodide symporter (NIS) and the relationships between iodine intake and circulating levels of thyroid hormones including T4 and T3."

"Thyroid hormones, in turn, are major determinants of neurodevelopment during pregnancy, infancy, and childhood."

"Notable strengths of this effort are that it is based upon the consensus mode of action (MOA) for perchlorate inhibition of iodine uptake, uses human iodine and perchlorate toxicokinetic data, addresses sensitive life stages, and is designed to be protective of serious, unambiguous adverse health effects known to occur in humans (i.e., neurodevelopmental deficits) associated with thyroid hormone deficiencies."

(Peer Review Summary Report, 2017)

Peer Review Recommendations

EPA contractor conducted an independent, external peer review of EPA's draft BBDR model and draft model report.

Peer reviewers were responsible only for evaluating the quality of the science and were not asked to make any regulatory recommendations or to reach consensus in either their deliberations or written comments.

Summary of recommendations based on 7 charge questions (See appendix for complete text of charge, panelist responses and EPA actions.):

1. BBDR Model structure
   - Model should focus on predicting maternal iodide and hormone levels in early pregnancy
   - Inclusion of TSH feedback process would be ideal; mixed opinions on necessity
   - Inclusion of other goitrogens; mixed opinions on whether they should be included or are essentially already accounted for
2. Modifications to existing models
   - Parameters scientifically supported
3. Model calibration and sensitivity analysis
   - A more statistical approach along with a focus on explicitly characterizing the uncertainty in model outputs
   - Sensitivity analysis was limited
   - Present a qualitative evaluation of support for important model parameters
   - Conduct a statistical comparison of model predictions and empirical observations

Peer Review Recommendations (cont.)

Summary of recommendations:

4. Hypothyroxinemic reference levels and model predictions
   - Concern for assessing health effects in contrast to defining regions of model applicability
   - Model the continuous exposure-response relationship
5. Robustness of model predictions and sensitivity at lower perchlorate dose levels
   - Model predictions were likely to be more robust with higher iodine intake and no or low perchlorate
6. Empirical functions to predict thyroid hormone levels
   - Necessary and reasonable if the functions allow the model to better match data
7. Characterization of model predictions
   - Major and important effort to quantitatively characterize the biological relationships between iodine and thyroid hormones
   - Desirable to have a model that predicts the full range of population behaviors

Response to BBDR Model Peer Review Comments

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Message

From: Dorjets, Vlad EOP/OMB Ex. 6 Personal Privacy (PP)
Sent: 9/13/2017 7:11:10 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: Automatic reply: EPA Perchlorate Peer Review follow up

I will be out of the office on work travel from Monday, September 9 to Friday, September 15. I will do my best to check email regularly and will get back to you as soon as I can.

Message

From: Wennerberg, Linda S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
Sent: 1/5/2017 11:00:51 PM
To: Ex. 6 Personal Privacy (PP)
CC: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: Re: URGENT: Registration for the Perchlorate Peer Review public meeting

Jim
I will share my notes. Is there anyone else attending from OMB, such as Margo or Jim?

Eric
Thanks again for your help,

Linda

Sent from my iPhone

On Jan 5, 2017, at 5:42 PM, Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)> wrote:

> Eric,
>
> Thanks for this. Unfortunately, I will be unable to attend. Though I will be keenly interested in how the meeting goes ☺
>
> Jim
>
> **From:** Burneson, Eric [mailto:Burneson.Eric@epa.gov]
> **Sent:** Thursday, January 5, 2017 4:38 PM
> **To:** Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov>
> **Cc:** Kim, Jim H. EOP/OMB <James_H_Kim@omb.eop.gov>
> **Subject:** Re: URGENT: Registration for the Perchlorate Peer Review public meeting
>
> Linda
> I have asked my staff managing the Versar Contract to make certain that both you and Jim can attend as observers.
>
> Sent from my iPhone
>
> On Jan 5, 2017, at 4:03 PM, Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov> wrote:
>
>> Eric;
>> Just spoke with Jim Kim of OMB. Neither he nor I saw the FR Notice (12/5/16) about the Perchlorate Peer Review meeting and registration info. As you can imagine, the hectic time of Administration transition, heavy work loads of interagency review, and the Holidays swamped us both and we were not aware of the meeting or the registration deadline. I have just sent off an urgent request to Versar to permit our registration but have not heard back from them.
>>
>> Please let me know if our registering now is an issue and what we will need to do to properly sign up and participate in the public meeting.

Thanks for your patience with all this.

Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89
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Washington, DC 20546-0001

Tel: 202/358-4558
Cell Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

Message

From: Steve Via [SVia@awwa.org]
Sent: 3/4/2019 4:14:46 PM
To: Brozena, Sarah [Sarah_Brozena@americanchemistry.com]; Burneson, Eric [Burneson.Eric@epa.gov]; Andes, Fredric P. [fandes@btlaw.com]; Fischer, David [David_Fischer@americanchemistry.com]; Brendan_Mascarenhas@americanchemistry.com
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call
Attachments: 2019 03 GlobalChem Via.pptx

Attached is revised per Eric and Sarah's observations.

Steve

Steve Via
Director Federal Relations, AWWA | 202.326.6130

From: Brozena, Sarah <Sarah_Brozena@americanchemistry.com>
Sent: Monday, March 04, 2019 8:59 AM
To: Steve Via <SVia@awwa.org>; 'Burneson.eric@Epa.gov' <Burneson.eric@Epa.gov>; Andes, Fredric P. <fandes@btlaw.com>; Fischer, David <David_Fischer@americanchemistry.com>; Mascarenhas, Brendan <Brendan_Mascarenhas@americanchemistry.com>
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

Hey Steve,
Thanks for the opportunity to review. Very informative presentation. I had a couple of comments.

Slide 2: I'm a little confused about how to read the concentric circles on public water systems and community water systems. Perhaps it'll be clearer as you speak to the slides. In particular, what do the percentages in each of the two circles refer to – population served (millions) or number of systems that are groundwater vs. surface water? I thought I understood what you were saying in the PWS circle, but then the CWS circle only includes percentages for the surface water CWSs.

Slide 3 appears to be using PFOA/PFOS as a case example for how something can become a contaminant under the SDWA. Obviously not all chemicals will follow this precise set of events. So perhaps it would be clearer if you revised the title of the slide to something like "Case Example of How Something Might Become a "Contaminant" . Also, this slide includes MCL regulatory determinations in 2019 and effective in 2027. Is this just a forecast of when these would be done? These aren't set in stone yet are they? Again, if the title of the slide were revised a bit to qualify the information, that would help.

Slide 9: I'd suggest the title of this slide NOT include the phrase "other than PFAS". You use PFOA and PFOS as an example in slide 3, but that's not the main focus of your presentation. So I think a more neutral title such as "Other Contaminants of Emerging Concern that Water Systems are Thinking About" would be better.

Just some suggestions.
Sarah

From: Steve Via [mailto:SVia@awwa.org]
Sent: Sunday, March 3, 2019 5:50 PM
To: Brozena, Sarah <Sarah_Brozena@americanchemistry.com>; 'Burneson.eric@Epa.gov' <Burneson.eric@Epa.gov>; Andes, Fredric P. <fandes@btlaw.com>; Fischer, David <David_Fischer@americanchemistry.com>; Mascarenhas,

Brendan <Brendan_Mascarenhas@americanchemistry.com>
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

Any feedback on attached w/r/t Friday's panel?

Steve

Steve Via
Director Federal Relations, AWWA | 202.326.6130

-----Original Appointment-----
From: Brozena, Sarah <Sarah_Brozena@americanchemistry.com>
Sent: Tuesday, February 26, 2019 3:33 PM
To: Brozena, Sarah; 'Burneson.eric@Epa.gov'; Andes, Fredric P.; Fischer, David; Steve Via; Mascarenhas, Brendan
Subject: GlobalChem Panel on Drinking Water Organizing Call
When: Wednesday, February 27, 2019 9:30 AM-10:00 AM (UTC-05:00) Eastern Time (US & Canada).
Where: Ex. 6 Personal Privacy (PP) Pin# Ex. 6 Personal Privacy (PP)

TO: Eric Burneson, EPA
Fred Andes, Barnes and Thornburg
David Fischer, IBEX Partners
Steve Via, American Water Works Association
FROM: Sarah Brozena and Brendan Mascarenhas, ACC
RE: Organizing Call
DATE: Feb. 26, 2019

Brendan and I hope to talk to you all tomorrow, Wed., Feb. 27 at 9:30 a.m. about the GlobalChem panel next week. Thanks

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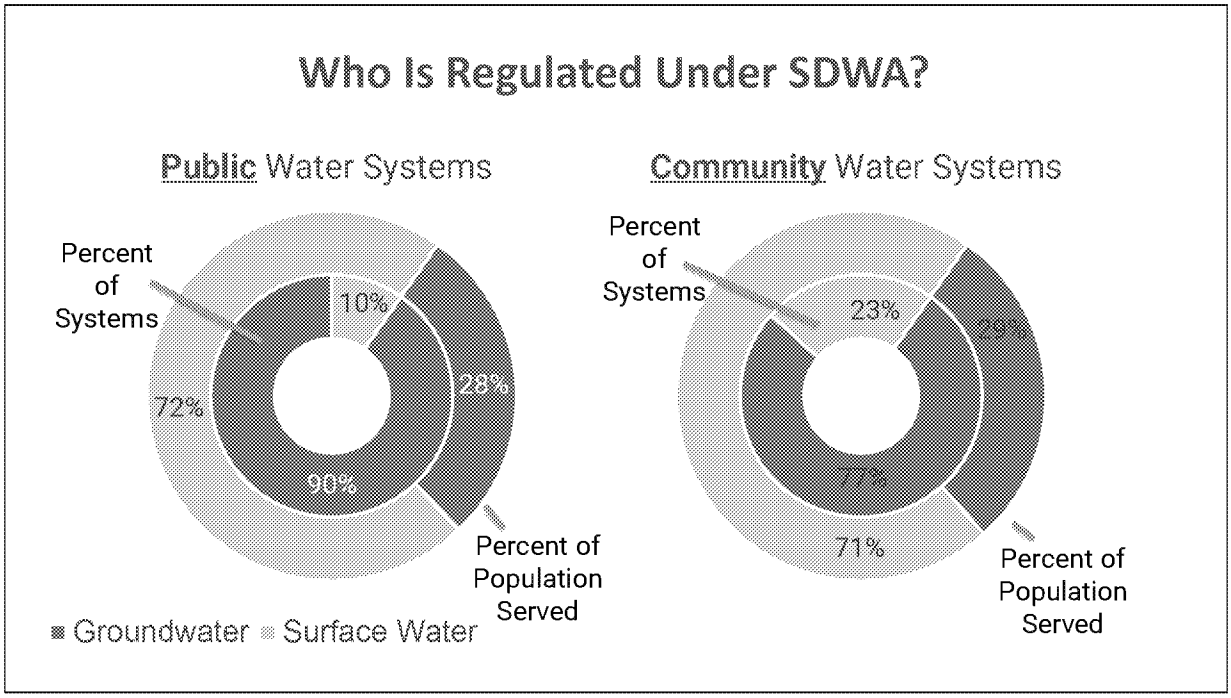
CONTAMINANTS OF EMERGING CONCERN

STEVE VIA

AT GLOBALCHEM, WASHINGTON DC

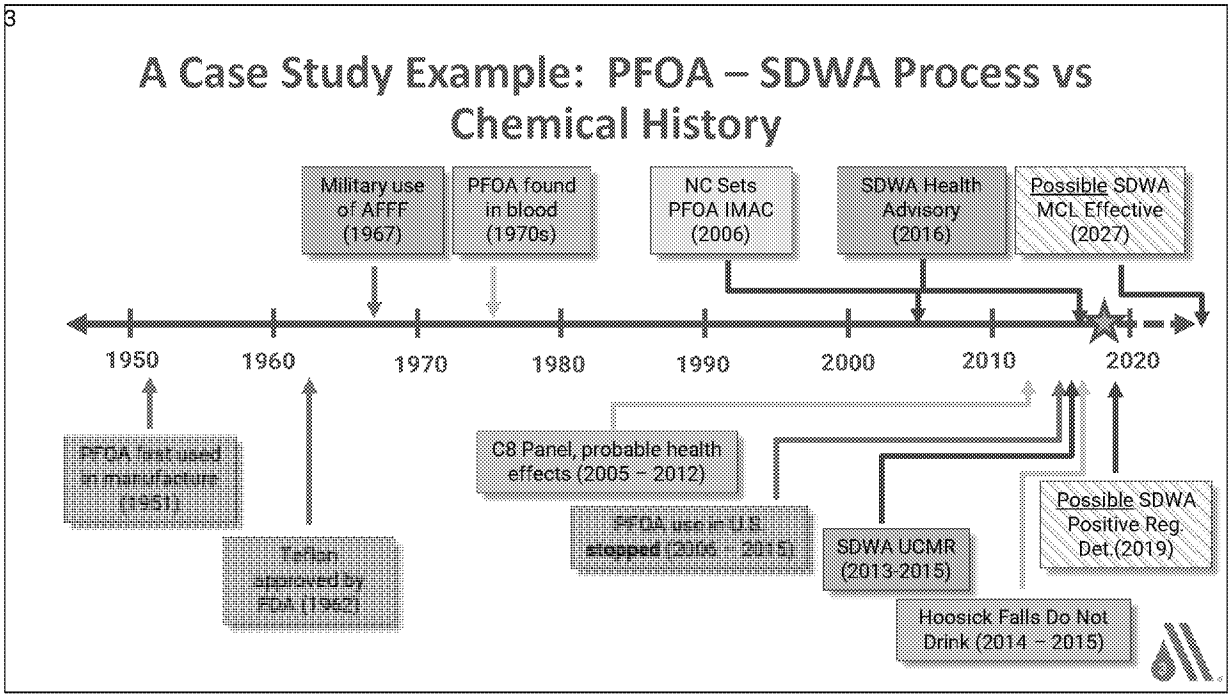
MARCH 8, 2019



Population served is on the order of 300 million
Roughly 150,000 PWSs
Roughly 50,000 CWSs
PWSs - "a system for the provision to the public of water for human consumption through pipes or other constructed conveyances, if such system has at least fifteen service connections, or regularly serves at least twenty-five individuals."

CWSs include public water systems that serve 25 people or 15 connections year-round.

NCWSs are PWSs that do not serve a permanent resident population



Every chemical history is different
The most salient points in this figure are not limited to PFAS, they apply equally well to:
Hexavalent chromium
Fire retardants that now replace PCBs
Nanomaterials
When environmental fate is not adequately considered early on
In the instance of PFAS there is significant public concern when occurrence is limited to very low concentrations at a limited number of systems
At present less than 2% of water systems have PFOA or PFOS above 70 ng/L
Individual state surveys are not turning up higher levels of occurrence.
There is limited occurrence of replacement chemicals in the same family though levels are seen in some locations at 100 ng/L levels.
PFAS is also indicative of other large groups chemicals that can be presented scientifically or to the public as being similar (e.g., pharmaceuticals)

Entire SDWA Process Impacts Utilities

	Contaminant Candidate List	Health Advisory / Reference Level	Unregulated Contaminant Monitoring Rule	Regulatory Determination	Proposed Rule	Final Rule
Duration	5 – 20 years or longer	No precursor, permanent until replaced	New list every 5 years; <30 analytes	New list every 5 years; ≥5 decisions	2 – 3 years	2 – 3 years; permanent until replaced
Regulatory Action	None	States / EPA use in site-specific situations	Monitoring & publication of observations (see HA/RL implications)	None	None	Any necessary treatment, ongoing monitoring, enforceable & public notice
Planning	Enters planning considerations	Enters planning considerations	Considered in planning	Actively considered in planning	Active response	Reliable compliance with margin of safety
Public	Impacts awareness	Elevates awareness	Drives awareness	Focuses awareness	Focuses awareness	Engaged

Analytical methods are key
Consistency in the health effects data
Degree to which contaminant is an anthropogenic, imposed contaminant concern matters in terms of public awareness and concern

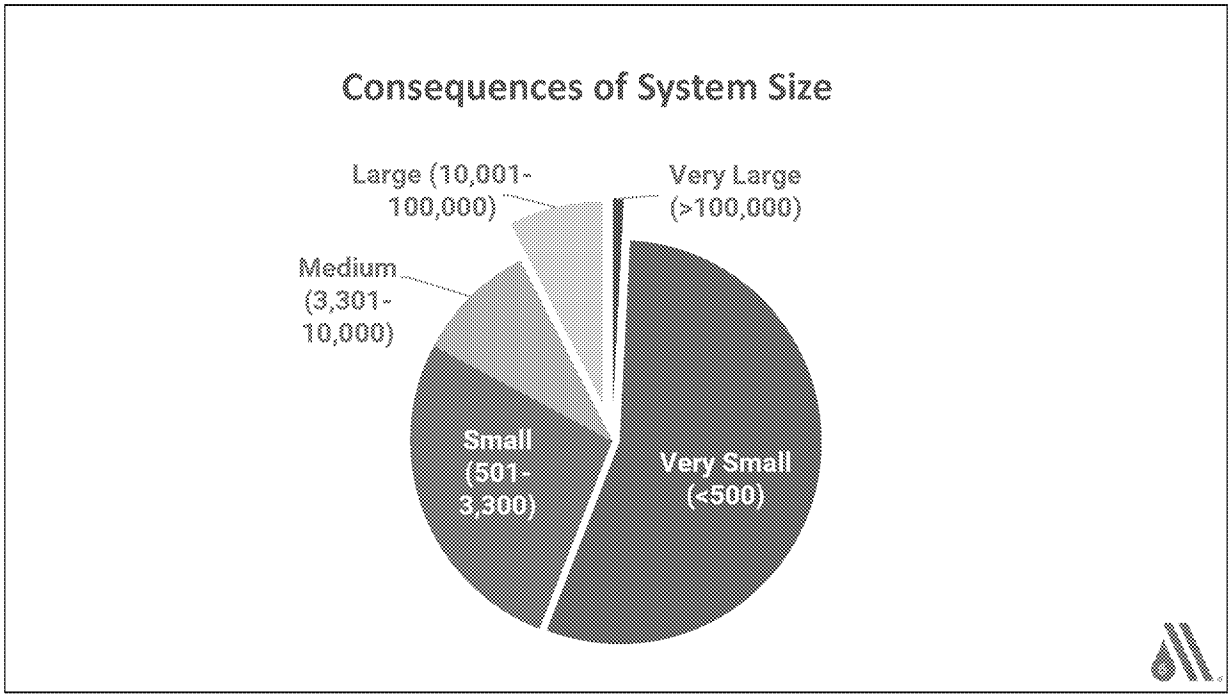
Cost Consequences

- More than 75% of community water systems are ground water systems

- Treatment (example theoretical 10 MGD facility)
  - Conventional treatment , $ 30 – 40 million
  - Addition of granular activated carbon or ozone, another $10 million
  - Addition of reverse osmosis, $40 million

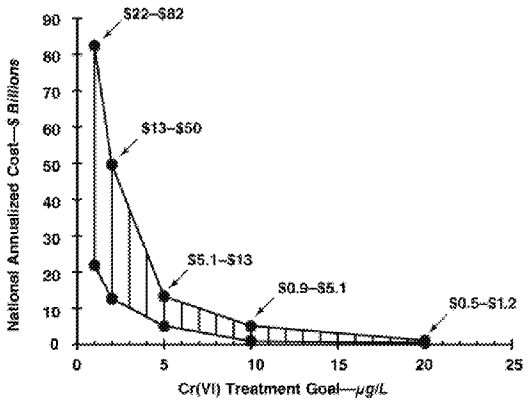
- In addition to treatment
  - Preparatory studies, piloting, design, construction oversight, etc.
  - Related costs (intakes, pipelines, storage, electrical, SCADA, personnel, etc.)
  - Unanticipated consequences (e.g., corrosion control)
  - Monitoring
  - Residual disposal

Loss of supply

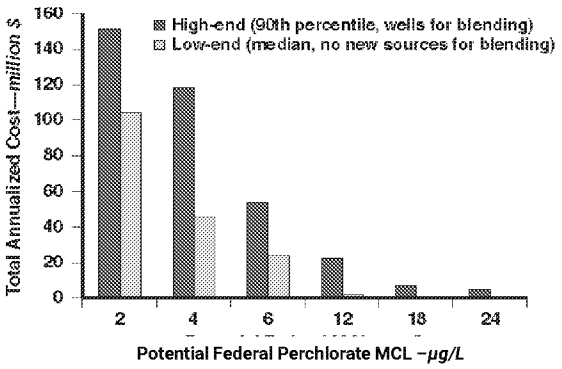


There are 150,000 public water systems
There are 50,000 community water systems (15 connections, same 25 people all year)
Less than 10 percent of community water systems serve more than 10,000 persons
Approximately 10% (about 4,200 systems) serve 10,000 or more people and provide drinking water to more than 80% of the U.S. population.

National Consequences --Treatment Objectives Matter

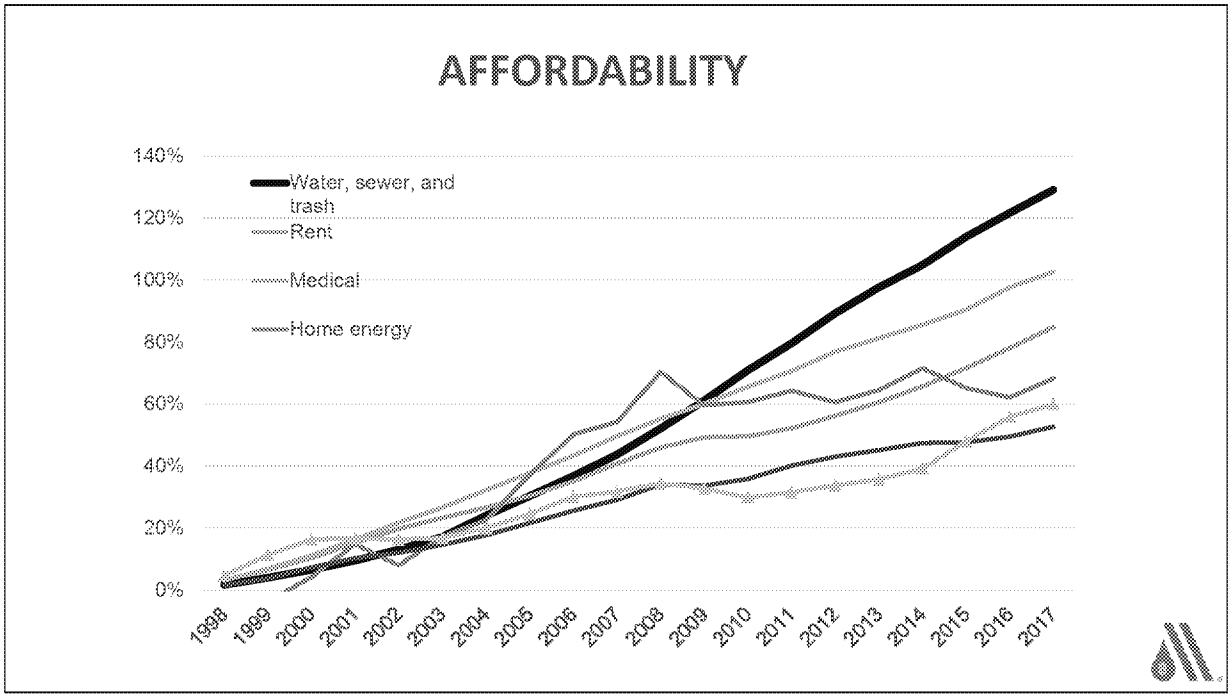


Journal - American Water Works Association, Volume: 105, Issue: 6, Pages: E320-E336, First published: 01 June 2013, DOI: (10.5942/jawwa.2013.105.0080)



Journal - American Water Works Association, Volume: 109, Issue: 2, Pages: E25-E36, First published: 01 February 2017, DOI: (10.5942/jawwa.2017.109.0009)

These slides are AWWA sponsored cost analyses of contaminants on different stages in the regulatory process at present
Determines appropriate treatment – GAC, IX, RO, etc.
Determines the number of facilities impacted



Infrastructure replacement
Additional water supply
Resilience
Bimodal income distribution

Some Other Emerging Contaminants that Water Systems Are Thinking About

- Cyanotoxins
  - Microcystins
  - Cylindrospermopsin
  - Saxotoxin
- Manganese
- Brominated and iodinated disinfection byproducts
- 1,2,3-Trichloropropane (TCP)
- 1,4-Dioxane
- Hexavalent chromium
- Pharmaceuticals
- Personal care products
- Endocrine Disruptors
- Microplastics

Message

From: Brozena, Sarah [Sarah_Brozena@americanchemistry.com]
Sent: 3/4/2019 2:15:57 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]; svia@awwa.org; Andes, Fredric P. [fandes@btlaw.com]; Fischer, David [David_Fischer@americanchemistry.com]; Brendan_Mascarenhas@americanchemistry.com
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

I have no questions or comments on your slides, Eric.
Thanks,
Sarah

From: Burneson, Eric [mailto:Burneson.Eric@epa.gov]
Sent: Monday, March 4, 2019 8:28 AM
To: Via, Steve <svia@awwa.org>; Brozena, Sarah <Sarah_Brozena@americanchemistry.com>; Andes, Fredric P. <fandes@btlaw.com>; Fischer, David <David_Fischer@americanchemistry.com>; Mascarenhas, Brendan <Brendan_Mascarenhas@americanchemistry.com>
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

Thanks Steve for sharing your presentation. I would ask that when delivering slide 7 you note that you are speculating on Agency decisions which have not been made with respect to the potential MCL's and treatment goals and MCLs for Hexavalent Chromium and Perchlorate. The Agency has not made a decision to revise the total chromium standard or to issue a separate hexavalent chromium standard and we have yet to propose a perchlorate standard (but must do so this spring).

Also please find the slide deck I plan to use in Friday's panel discussion. Let me know if you have any questions or concerns. With the attached slides.
Eric Burneson, P.E.
Director of Standards and Risk Management
Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency
202 564 5250

From: Steve Via <SVia@awwa.org>
Sent: Sunday, March 03, 2019 5:50 PM
To: Brozena, Sarah <Sarah_Brozena@americanchemistry.com>; Burneson, Eric <Burneson.Eric@epa.gov>; Andes, Fredric P. <fandes@btlaw.com>; Fischer, David <David_Fischer@americanchemistry.com>; Brendan_Mascarenhas@americanchemistry.com
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

Any feedback on attached w/r/t Friday's panel?

Steve

Steve Via
Director Federal Relations, AWWA | 202.326.6130

-----Original Appointment-----
From: Brozena, Sarah <Sarah_Brozena@americanchemistry.com>
Sent: Tuesday, February 26, 2019 3:33 PM
To: Brozena, Sarah; 'Burneson.eric@Epa.gov'; Andes, Fredric P.; Fischer, David; Steve Via; Mascarenhas, Brendan
Subject: GlobalChem Panel on Drinking Water Organizing Call
When: Wednesday, February 27, 2019 9:30 AM-10:00 AM (UTC-05:00) Eastern Time (US & Canada).
Where: Ex. 6 Personal Privacy (PP) Pin# Ex. 6 Personal Privacy (PP)

TO: Eric Burneson, EPA
Fred Andes, Barnes and Thornburg
David Fischer, IBEX Partners
Steve Via, American Water Works Association
FROM: Sarah Brozena and Brendan Mascarenhas, ACC
RE: Organizing Call
DATE: Feb. 26, 2019

Brendan and I hope to talk to you all tomorrow, Wed., Feb. 27 at 9:30 a.m. about the GlobalChem panel next week. Thanks

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American Water Works Association
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Message

From: Steve Via [SVia@awwa.org]
Sent: 3/4/2019 1:59:30 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]; Brozena, Sarah [Sarah_Brozena@americanchemistry.com]; Andes, Fredric P. [fandes@btlaw.com]; Fischer, David [David_Fischer@americanchemistry.com]; Brendan_Mascarenhas@americanchemistry.com
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

Thanks Eric.

My thought on slide 7 is only to illustrate how lower standards drive cost consequences. No intention of speaking to what action EPA is taking on either contaminant.

No worries with your talk from my point of view.

Have a great day.
Steve

Steve Via
Director Federal Relations, AWWA | 202.326.6130

From: Burneson, Eric <Burneson.Eric@epa.gov>
Sent: Monday, March 04, 2019 8:28 AM
To: Steve Via <SVia@awwa.org>; Brozena, Sarah <Sarah_Brozena@americanchemistry.com>; Andes, Fredric P. <fandes@btlaw.com>; Fischer, David <David_Fischer@americanchemistry.com>; Brendan_Mascarenhas@americanchemistry.com
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

Thanks Steve for sharing your presentation. I would ask that when delivering slide 7 you note that you are speculating on Agency decisions which have not been made with respect to the potential MCL's and treatment goals and MCLs for Hexavalent Chromium and Perchlorate. The Agency has not made a decision to revise the total chromium standard or to issue a separate hexavalent chromium standard and we have yet to propose a perchlorate standard (but must do so this spring).

Also please find the slide deck I plan to use in Friday's panel discussion. Let me know if you have any questions or concerns. With the attached slides.
Eric Burneson, P.E.
Director of Standards and Risk Management
Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency
202 564 5250

From: Steve Via <SVia@awwa.org>
Sent: Sunday, March 03, 2019 5:50 PM
To: Brozena, Sarah <Sarah_Brozena@americanchemistry.com>; Burneson, Eric <Burneson.Eric@epa.gov>; Andes, Fredric P. <fandes@btlaw.com>; Fischer, David <David_Fischer@americanchemistry.com>; Brendan_Mascarenhas@americanchemistry.com
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From: Brozena, Sarah <Sarah_Brozena@americanchemistry.com>
Sent: Tuesday, February 26, 2019 3:33 PM
To: Brozena, Sarah; 'Burneson.eric@Epa.gov'; Andes, Fredric P.; Fischer, David; Steve Via; Mascarenhas, Brendan
Subject: GlobalChem Panel on Drinking Water Organizing Call
When: Wednesday, February 27, 2019 9:30 AM-10:00 AM (UTC-05:00) Eastern Time (US & Canada).
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TO: Eric Burneson, EPA
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FROM: Sarah Brozena and Brendan Mascarenhas, ACC
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Message

From: Steve Via [SVia@awwa.org]
Sent: 3/3/2019 10:49:41 PM
To: Brozena, Sarah [Sarah_Brozena@americanchemistry.com]; Burneson, Eric [Burneson.Eric@epa.gov]; Andes, Fredric P. [fandes@btlaw.com]; Fischer, David [David_Fischer@americanchemistry.com]; Brendan_Mascarenhas@americanchemistry.com
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call
Attachments: 2019 03 GlobalChem Via.pptx

Any feedback on attached w/r/t Friday's panel?

Steve

Steve Via
Director Federal Relations, AWWA | 202.326.6130

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To: Brozena, Sarah; 'Burneson.eric@Epa.gov'; Andes, Fredric P.; Fischer, David; Steve Via; Mascarenhas, Brendan
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When: Wednesday, February 27, 2019 9:30 AM-10:00 AM (UTC-05:00) Eastern Time (US & Canada).
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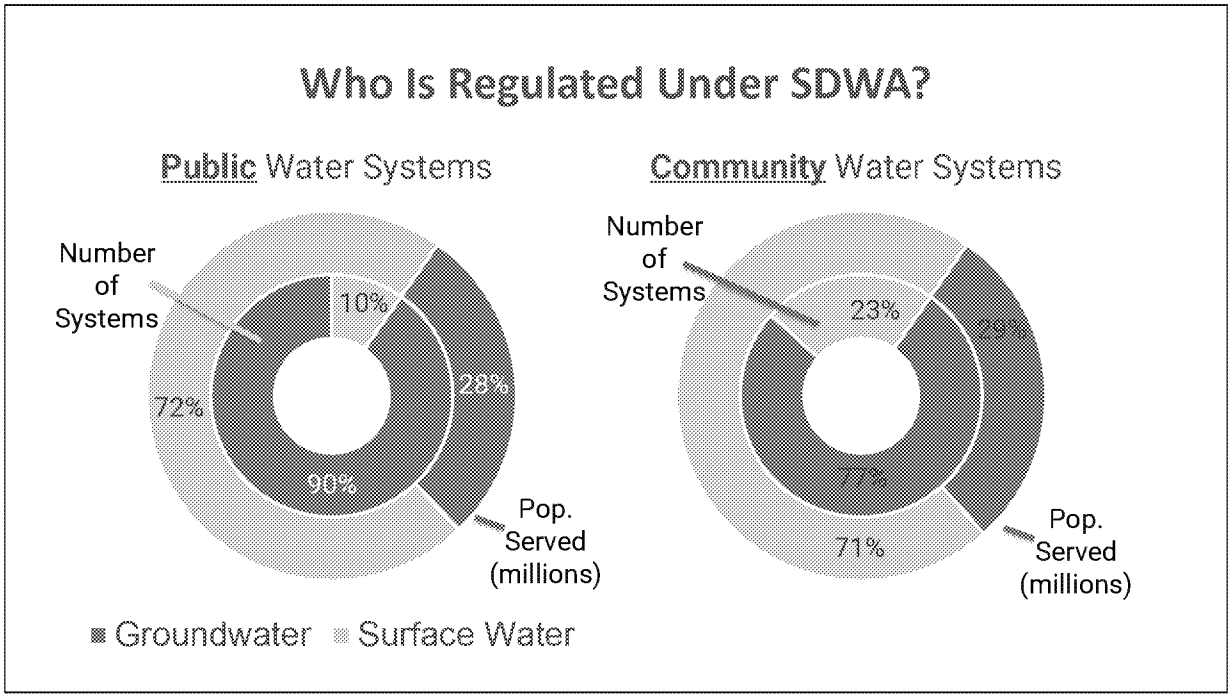
American Water Works Association

CONTAMINANTS OF EMERGING CONCERN

STEVE VIA

AT GLOBALCHEM, WASHINGTON DC

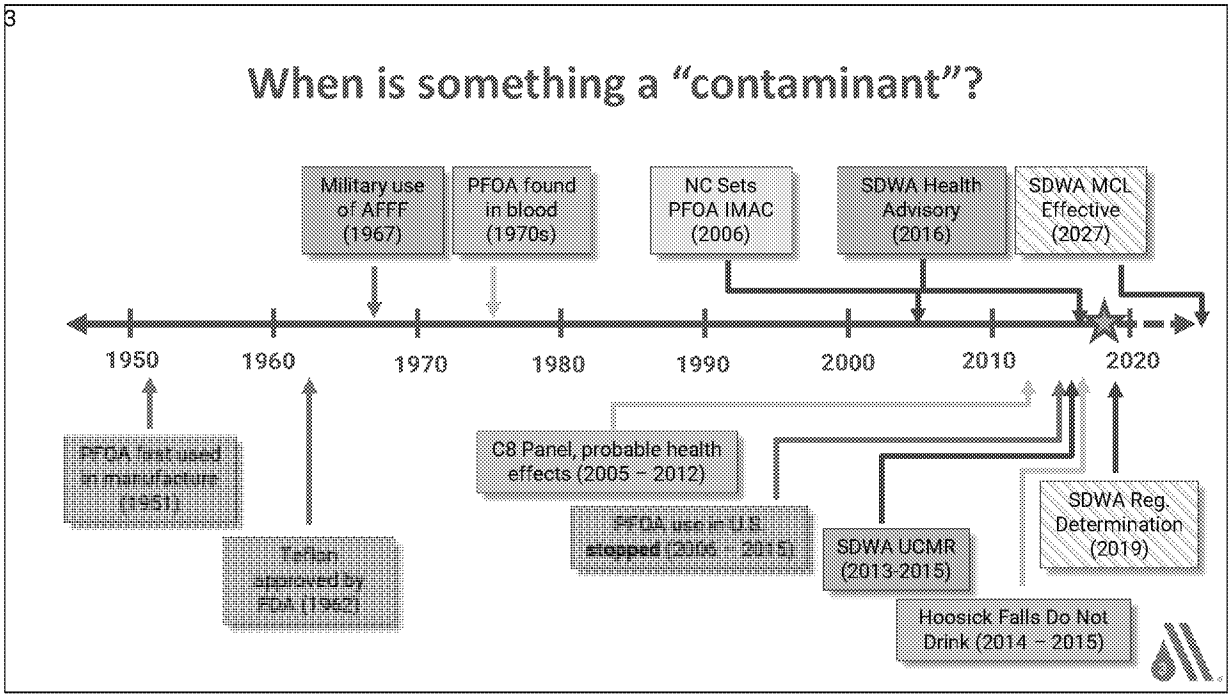
MARCH 8, 2019



PWSs - "a system for the provision to the public of water for human consumption through pipes or other constructed conveyances, if such system has at least fifteen service connections, or regularly serves at least twenty-five individuals."

CWSs include public water systems that serve 25 people or 15 connections year-round.

NCWSs are PWSs that do not serve a permanent resident population



The most salient points in this figure are not limited to PFAS, they apply equally well to:
Hexavalent chromium
Fire retardants that now replace PCBs
When environmental fate is not adequately considered
In the instance of PFAS there is significant public concern when occurrence is limited to very low concentrations at a limited number of systems
At present less than 2% of water systems have PFOA or PFOS above 70 ng/L
Individual state surveys are not turning up higher levels of occurrence.
There is limited occurrence of replacement chemicals in the same family though levels are seen in some locations at 100 ng/L levels.

Entire SDWA Process Impacts Utilities

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Analytical methods are key
Consistency in the health effects data
Degree to which contaminant is an anthropogenic, imposed contaminant concern matters in terms of public awareness and concern

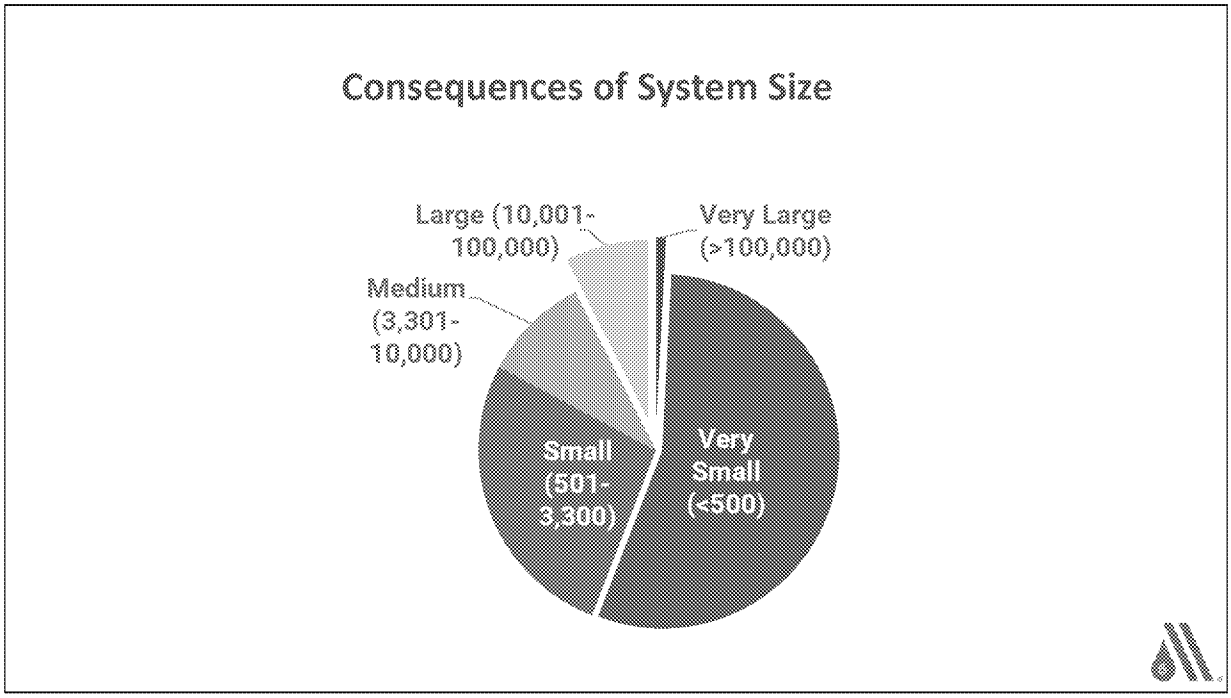
Cost Consequences

- More than 75% of community water systems are ground water systems

- Treatment (example theoretical 10 MGD facility)
  - Conventional treatment , $ 30 – 40 million
  - Addition of granular activated carbon or ozone, another $10 million
  - Addition of reverse osmosis, $40 million

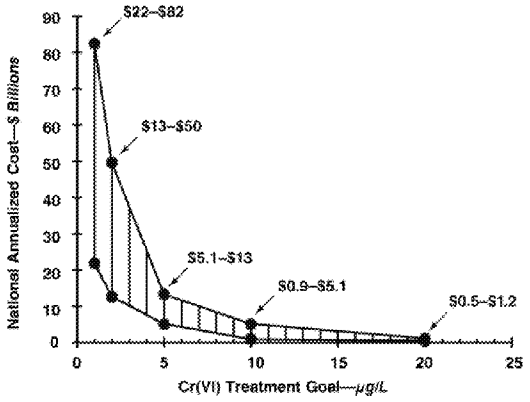
- In addition to treatment
  - Preparatory studies, piloting, design, construction oversight, etc.
  - Related costs (intakes, pipelines, storage, electrical, SCADA, personnel, etc.)
  - Unanticipated consequences (e.g., corrosion control)
  - Monitoring
  - Residual disposal

Loss of supply

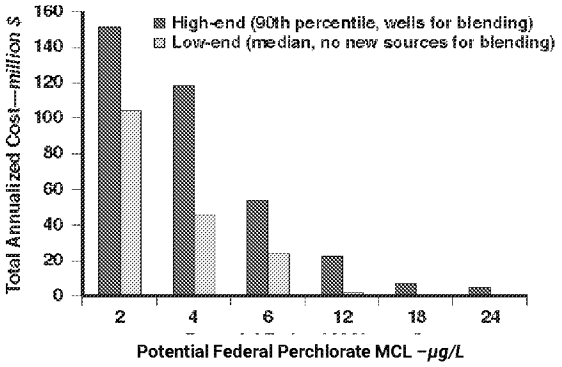


There are 150,000 public water systems
There are 50,000 community water systems (15 connections, same 25 people all year)
Less than 10 percent of community water systems serve more than 10,000 persons
Approximately 10% (about 4,200 systems) serve 10,000 or more people and provide drinking water to more than 80% of the U.S. population.

National Consequences --Treatment Objectives Matter

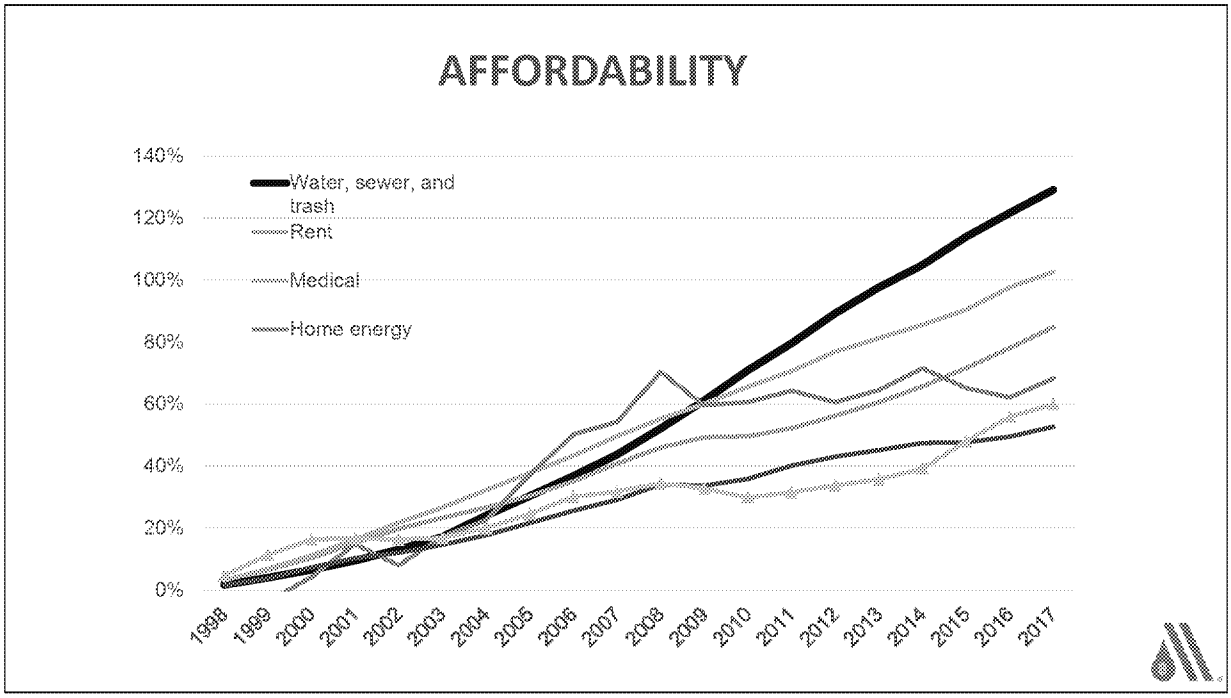


Journal - American Water Works Association, Volume: 105, Issue: 6, Pages: E320-E336, First published: 01 June 2013, DOI: (10.5942/jawwa.2013.105.0080)



Journal - American Water Works Association, Volume: 109, Issue: 2, Pages: E25-E36, First published: 01 February 2017, DOI: (10.5942/jawwa.2017.109.0009)

Determines appropriate treatment – GAC, IX, RO, etc.
Determines the number of facilities impacted



Infrastructure replacement
Additional water supply
Resilience
Bimodal income distribution

CONTAMINANTS OF EMERGING CONCERN

… OTHER THAN PFAS WATER SYSTEMS ARE THINKING ABOUT

- *Legionella*
- Cyanotoxins
  - Microcystins
  - Cylindrospermopsin
  - Saxotoxin
- Manganese
- Brominated and iodinated disinfection byproducts
- 1,2,3-Trichloropropane (TCP)
- 1,4-Dioxane
- Hexavalent chromium
- Pharmaceuticals
- Personal care products
- Endocrine Disruptors
- Microplastics

Message

From: Steve Via [SVia@awwa.org]
Sent: 3/8/2019 10:23:15 PM
To: Brendan_Mascarenhas@americanchemistry.com; Brozena, Sarah [Sarah_Brozena@americanchemistry.com]
CC: Burneson, Eric [Burneson.Eric@epa.gov]; Farris, Erika D. [Farris.Erika@epa.gov]
Subject: Presentation for packaging for distribution
Attachments: 2019 03 GlobalChem Via 3 8 .pptx

Sarah / Brendan,

Thank you for inviting me to participate in today's panel.

Please use the attached presentation when distributing presentations from today's event.

Have a great weekend.

Best regards,
Steve

Steve Via
Director Federal Relations, AWWA | 202.326.6130

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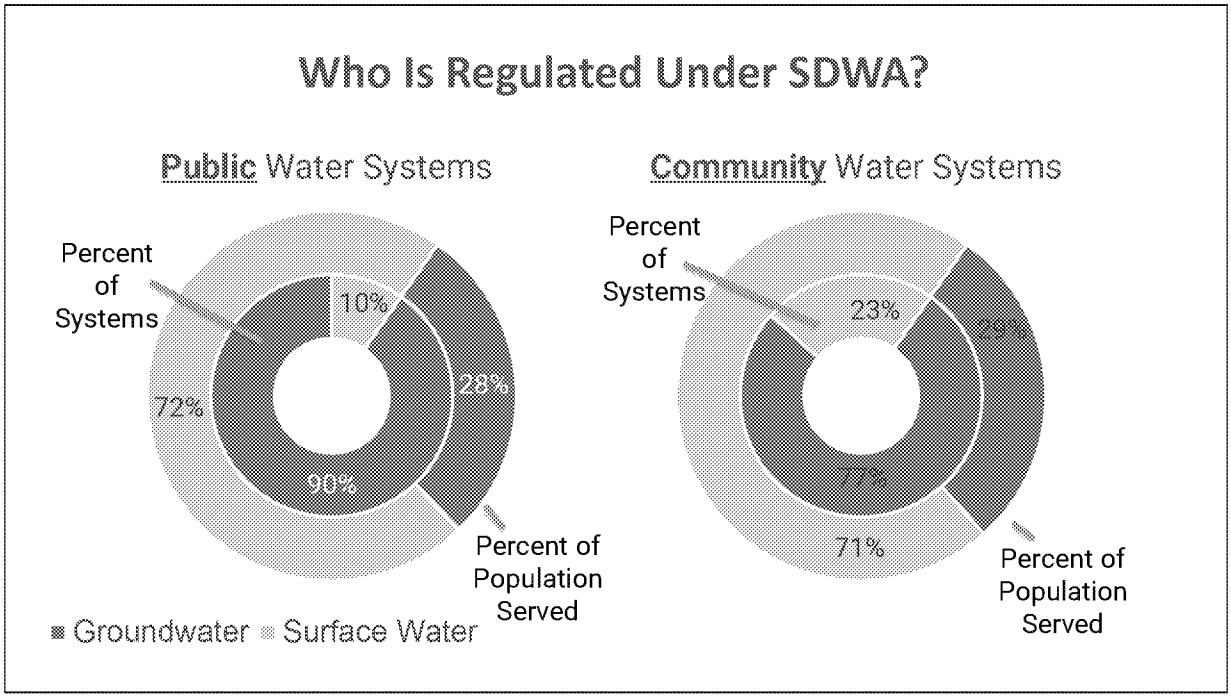
CONTAMINANTS OF EMERGING CONCERN

STEVE VIA

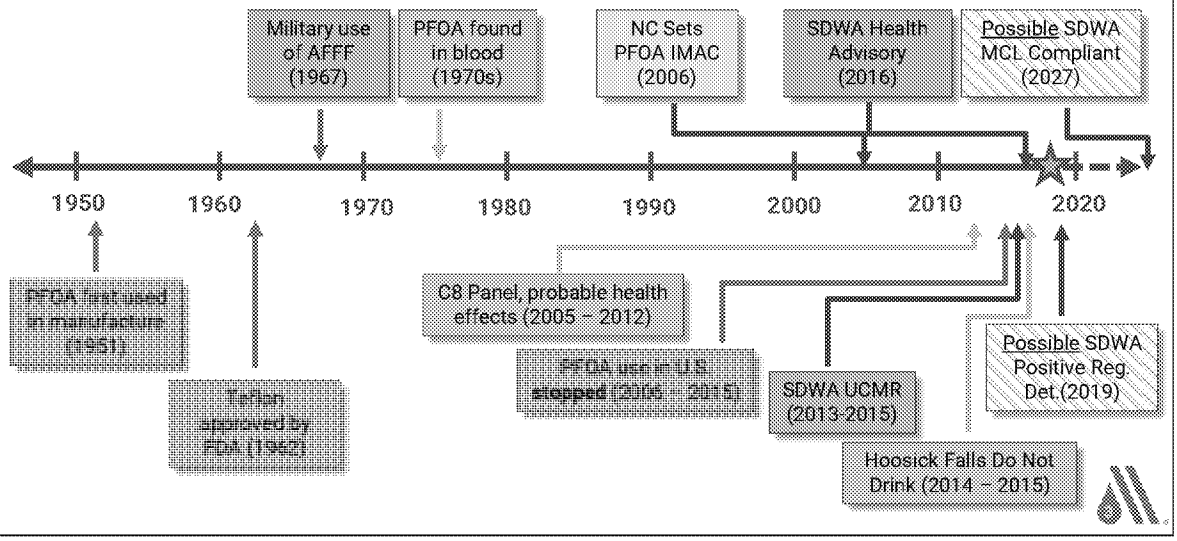
AT GLOBALCHEM, WASHINGTON DC

MARCH 8, 2019


Who Is Regulated Under SDWA?
Public Water Systems
Percent of Systems
10%
72%
28%
90%
Percent of Population Served
Groundwater
Surface Water
Community Water Systems
Percent of Systems
23%
29%
77%
71%
Percent of Population Served

3

A Case Study Example: PFOA – SDWA Process vs Chemical History



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  - Conventional treatment , $ 30 – 40 million
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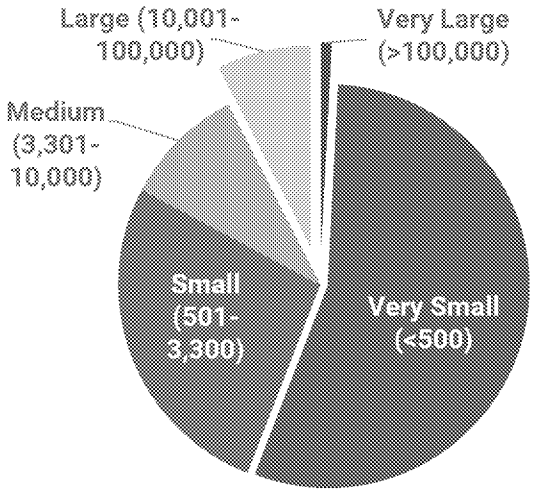
  - Unanticipated consequences (e.g., corrosion control)
  - Monitoring
  - Residual disposal

Consequences of System Size



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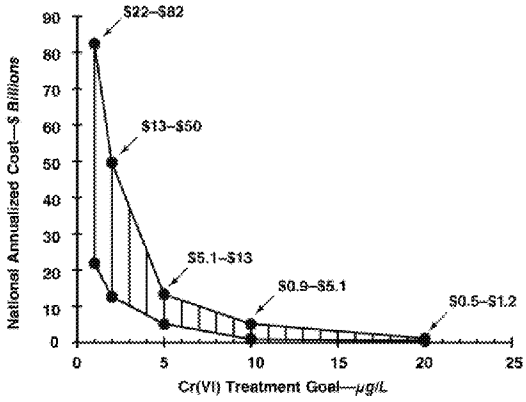

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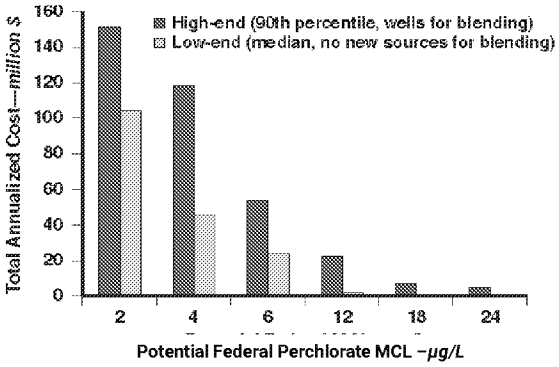

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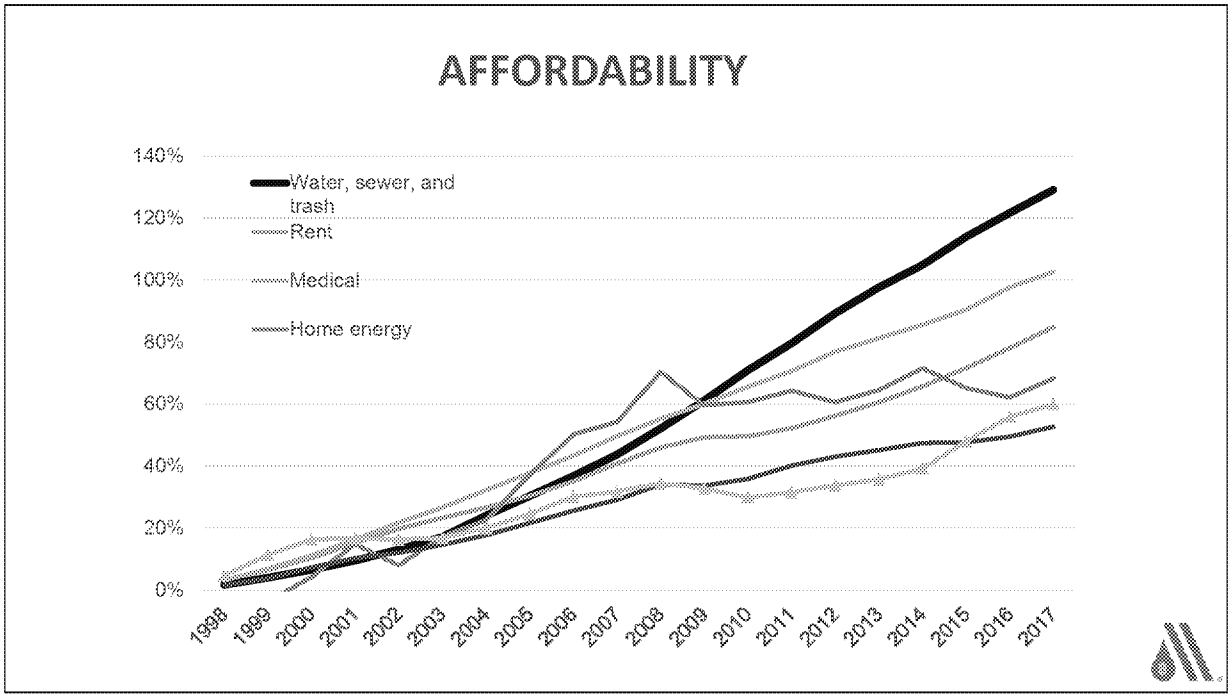
AFFORDABILITY
140%
120%
100%
80%
60%
40%
20%
0%
Water, sewer, and trash
Rent
Medical
Home energy
1998
1999
2000
2001
2002
2003
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2017

Some Other Emerging Contaminants that Water Systems Are Thinking About

- Cyanotoxins
  - Microcystins
  - Cylindrospermopsin
  - Saxotoxin
- Manganese
- Brominated and iodinated disinfection byproducts
- 1,2,3-Trichloropropane (TCP)
- 1,4-Dioxane
- Hexavalent chromium
- Pharmaceuticals
- Personal care products
- Endocrine Disruptors
- Microplastics

Message

From: Brozena, Sarah [Sarah_Brozena@americanchemistry.com]
Sent: 3/6/2019 10:59:15 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]; svia@awwa.org; Andes, Fredric P. [fandes@btlaw.com]; Fischer, David [David_Fischer@americanchemistry.com]
CC: Brendan_Mascarenhas@americanchemistry.com
Subject: GlobalChem presentations
Attachments: BURNESON_GlobalChem DW contaminants of emerging concern 3 19.pptx; VIA_2019 03 GlobalChem Via.pptx; ANDES_globalchemandesslides030819.ppt; FISCHER ppt deck for GlobalChem.pptx

Dear Eric, Steve, Fred and David,

Attached are the presentations each of you sent in for Friday's GlobalChem panel discussion on Emerging Contaminants in Drinking Water. I thought each of you would like to see what the other would be covering. The session will be held on Friday, March 8 starting at 2:00 p.m. in the Palladian Ballroom at the Omni Shoreham Hotel in DC.

If we could meet up around the stage/podium a few minutes before the session on Friday, that would be great.

Let me know if you have any questions.
Thanks,
Sarah

Sarah H. Brozena
Senior Director, Regulatory & Technical Affairs
American Chemistry Council
700 Second St., NE
Washington, DC 20002
202-249-6403
Sarah_Brozena@americanchemistry.com

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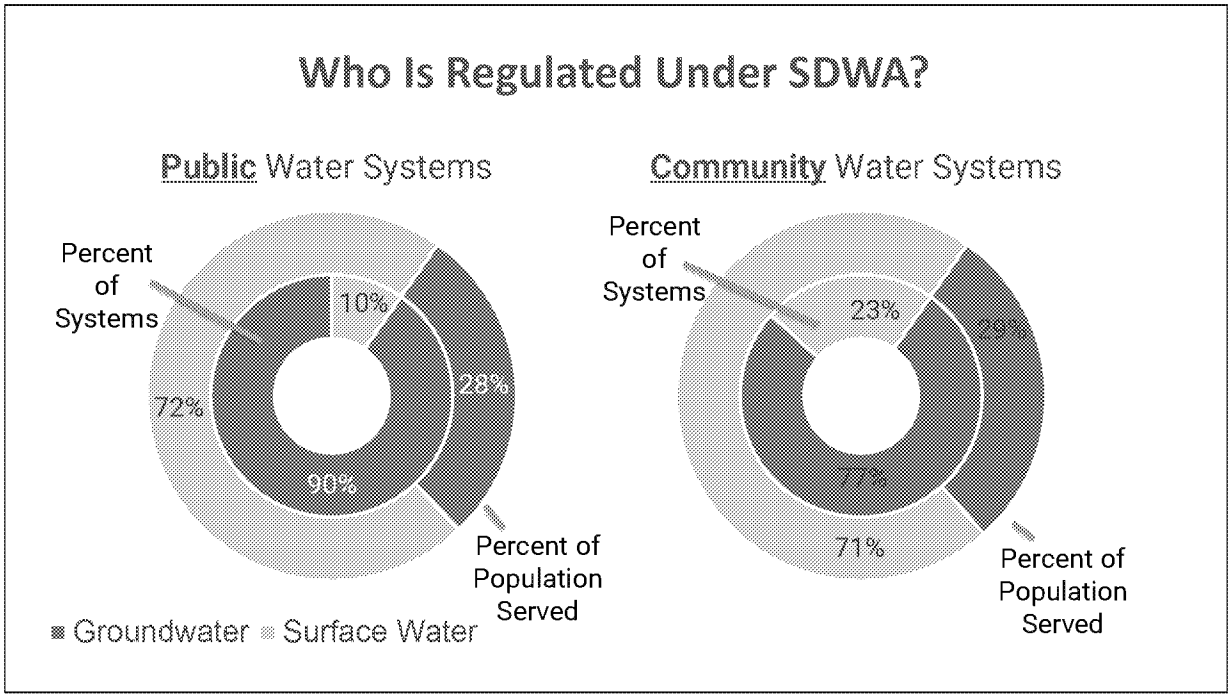

Population served is on the order of 300 million
Roughly 150,000 PWSs
Roughly 50,000 CWSs
PWSs - "a system for the provision to the public of water for human consumption through pipes or other constructed conveyances, if such system has at least fifteen service connections, or regularly serves at least twenty-five individuals."

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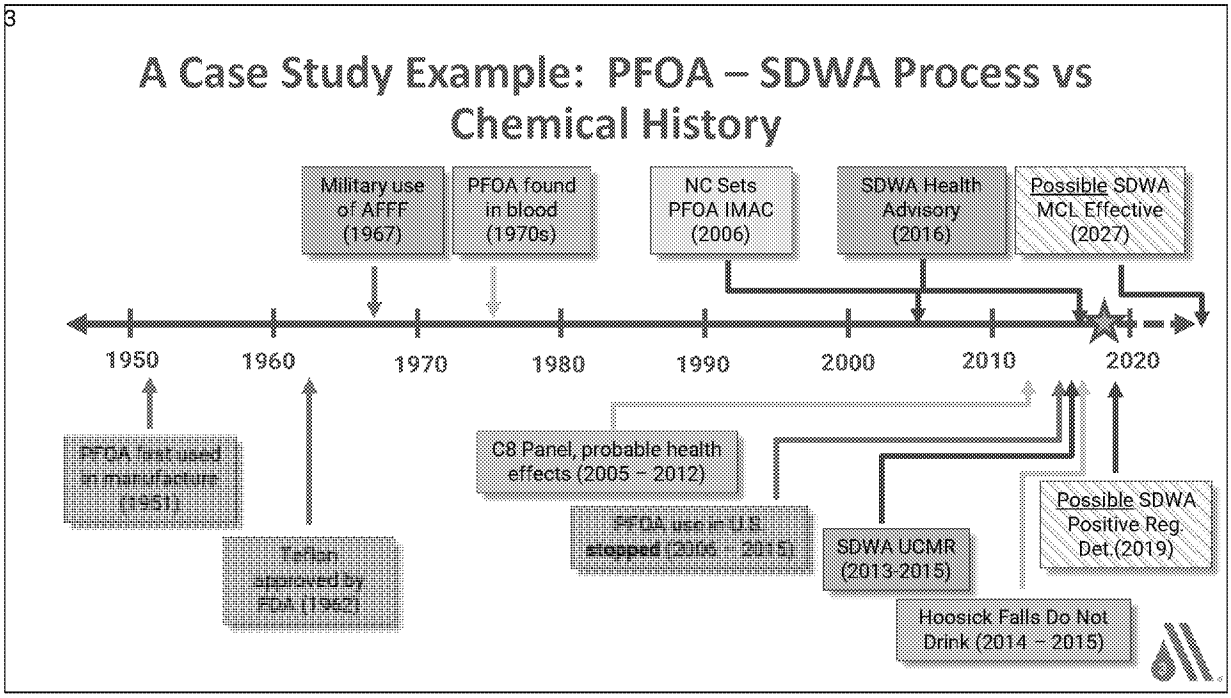

Every chemical history is different
The most salient points in this figure are not limited to PFAS, they apply equally well to:
Hexavalent chromium
Fire retardants that now replace PCBs
Nanomaterials
When environmental fate is not adequately considered early on
In the instance of PFAS there is significant public concern when occurrence is limited to very low concentrations at a limited number of systems
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PFAS is also indicative of other large groups chemicals that can be presented scientifically or to the public as being similar (e.g., pharmaceuticals)

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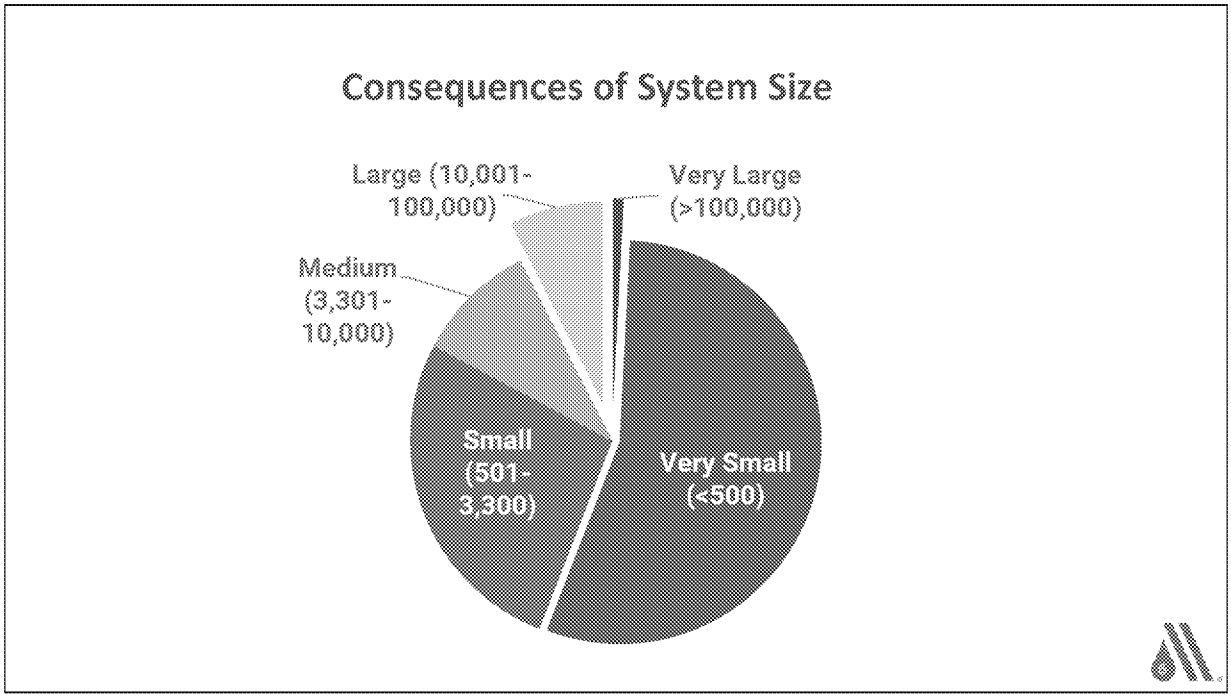
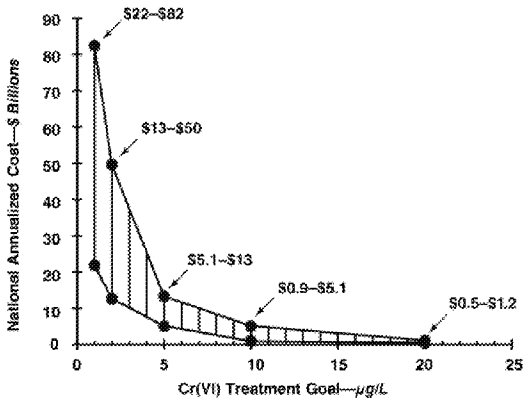
  - Monitoring
  - Residual disposal

Loss of supply

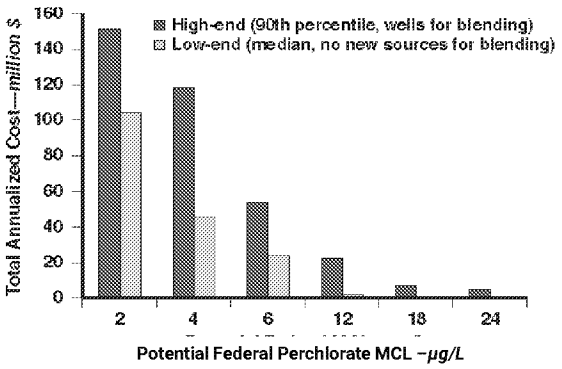


There are 150,000 public water systems
There are 50,000 community water systems (15 connections, same 25 people all year)
Less than 10 percent of community water systems serve more than 10,000 persons
Approximately 10% (about 4,200 systems) serve 10,000 or more people and provide drinking water to more than 80% of the U.S. population.

National Consequences --Treatment Objectives Matter

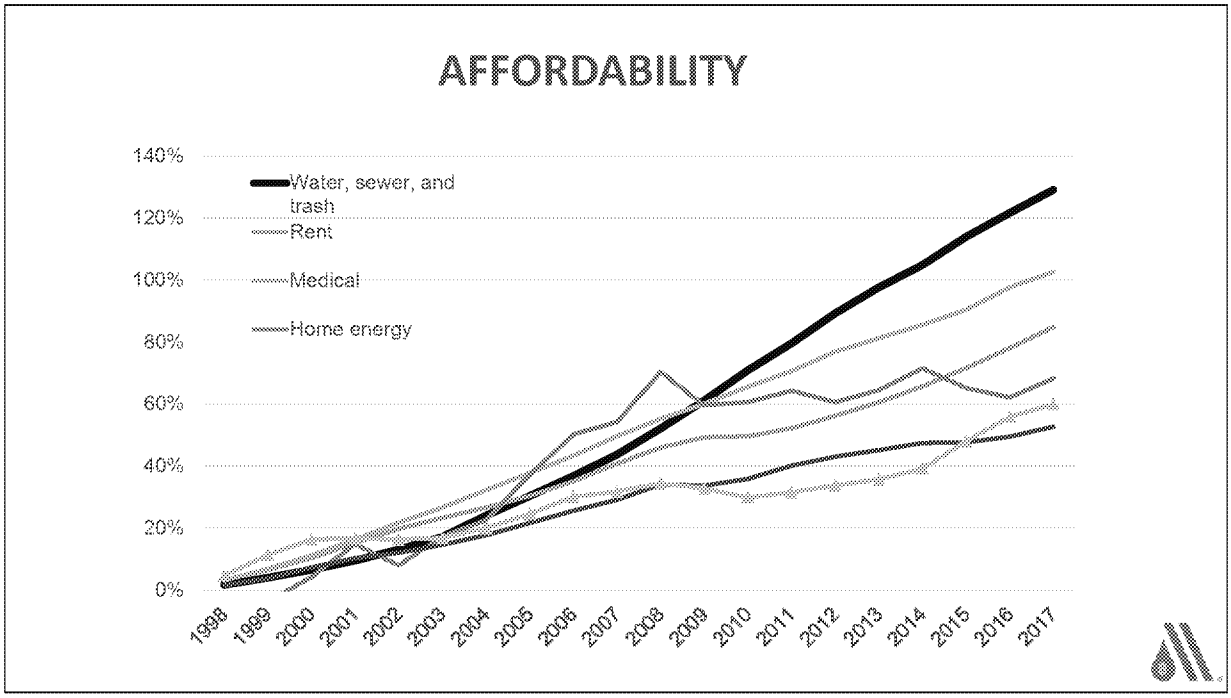


Journal - American Water Works Association, Volume: 105, Issue: 6, Pages: E320-E336, First published: 01 June 2013, DOI: (10.5942/jawwa.2013.105.0080)



Journal - American Water Works Association, Volume: 109, Issue: 2, Pages: E25-E36, First published: 01 February 2017, DOI: (10.5942/jawwa.2017.109.0009)

These slides are AWWA sponsored cost analyses of contaminants on different stages in the regulatory process at present
Determines appropriate treatment – GAC, IX, RO, etc.
Determines the number of facilities impacted



Infrastructure replacement
Additional water supply
Resilience
Bimodal income distribution

Some Other Emerging Contaminants that Water Systems Are Thinking About

- Cyanotoxins
  - Microcystins
  - Cylindrospermopsin
  - Saxotoxin
- Manganese
- Brominated and iodinated disinfection byproducts
- 1,2,3-Trichloropropane (TCP)
- 1,4-Dioxane
- Hexavalent chromium
- Pharmaceuticals
- Personal care products
- Endocrine Disruptors
- Microplastics

Message

From: Barringer, Jody M. EOP/OMB Ex. 6 EOP (PP)
Sent: 7/9/2018 9:03:05 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: Automatic reply: TWG - Perchlorate

I will be out of the office until Wednesday, July 11th. If you need immediate assistance, please contact Leah Fine at Ex. 6 EOP (PP)
Otherwise, I will respond to your email when I return.

Thank you.

Message

From: Falk Curtin, Edna T. EOP/OMB [Ex. 6 EOP (PP)]
Sent: 7/9/2018 9:03:05 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: Automatic reply: TWG - Perchlorate

Hello,

I will be out of the office until Monday, July 16th. I will not be checking my e-mail. If your matter is urgent, please contact Josh Glazer at: Ex. 6 EOP (PP). Otherwise, I will get back to you when I return on Monday.

Have a great day!
Edna

Message

From: Kim, Jim H. EOP/OMB Ex. 6 Personal Privacy (PP)
Sent: 4/5/2018 2:01:54 PM
To: Burneson, Eric [Burneson.Eric@epa.gov]
Subject: Automatic reply: EPA Perchlorate Peer Review Report

I am currently out of the office. If you need immediate assistance, please contact Margo Schwab at Ex. 6 Personal Privacy (PP) I will be back in the office on April 9.

Thanks,
Jim

Message

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 9/22/2017 12:51:28 PM
To: Mason, Paula [Mason.Paula@epa.gov]
CC: Bradford, Charlotte [Bradford.Charlotte@epa.gov]
Subject: FW: EPA Perchlorate Peer Review follow up

Paula: Can you please book room 2123 for this perchlorate call on Oct. 2 from 3:00 to 4:00? I will send a calendar invitation shortly.
Thanks
Eric

-----Original Message-----
From: Grevatt, Peter
Sent: Friday, September 22, 2017 7:29 AM
To: Burneson, Eric <Burneson.Eric@epa.gov>; Mclain, Jennifer <Mclain.Jennifer@epa.gov>
Cc: Tiago, Joseph <Tiago.Joseph@epa.gov>; Mason, Paula <Mason.Paula@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: RE: EPA Perchlorate Peer Review follow up

Of course!

-----Original Message-----
From: Burneson, Eric
Sent: Thursday, September 21, 2017 3:15 PM
To: Grevatt, Peter <Grevatt.Peter@epa.gov>; Mclain, Jennifer <Mclain.Jennifer@epa.gov>
Cc: Tiago, Joseph <Tiago.Joseph@epa.gov>; Mason, Paula <Mason.Paula@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: FW: EPA Perchlorate Peer Review follow up

Peter and Jennifer:
Per the response below, OMB has a preference for holding the Federal Partners perchlorate call on Oct 2nd vs the 5th. ORD has better availability at 3:00 on that date.

Can we ask Paula to postpone the OGWDW Staff meeting to start at 4:00 on this date to enable the Federal partners call to take place from 3:00 to 4:00?

Once I hear back from you I will schedule the Federal Partners call.

Eric

-----Original Message-----
From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 Personal Privacy (PP)
Sent: Thursday, September 21, 2017 1:56 PM
To: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) Burneson, Eric <Burneson.Eric@epa.gov>
Cc: Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Johnson, Ann <Johnson.Ann@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>; Mclain, Jennifer <Mclain.Jennifer@epa.gov>
Subject: RE: EPA Perchlorate Peer Review follow up

I can make October 2, but not October 5 at that time.

Jim

-----Original Message-----
From: Dorjets, Vlad EOP/OMB
Sent: Thursday, September 21, 2017 1:38 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Cc: Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Johnson, Ann <Johnson.Ann@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>; Mclain, Jennifer <Mclain.Jennifer@epa.gov>; Kim, Jim H. EOP/OMB
< Ex. 6 Personal Privacy (PP)
Subject: RE: EPA Perchlorate Peer Review follow up

Eric - Sorry for not replying sooner and thanks for offering to set this up. Unless Jim Kim (who I've copied) has any conflicts, all three times work for me. I realize I'm the one that has been the cause of the delay here but if we can get the invitation to reviewers tomorrow or Monday that would be great so that they will have a week notice to read the report before the call.

-----Original Message-----
From: Burneson, Eric [mailto:Burneson.Eric@epa.gov]
Sent: Tuesday, September 19, 2017 3:27 PM

To: Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)
Cc: Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Johnson, Ann <Johnson.Ann@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>; Mclain, Jennifer <Mclain.Jennifer@epa.gov>
Subject: RE: EPA Perchlorate Peer Review follow up

Vlad:

I wanted to check back with you on potential times for calls with the Federal Agency partners to discuss questions /concerns with the charge. Here are three possible times

October 2, 11:00 to 12:00
October 2, 3:00 - 4:00
October 5: 2:00 - 3:00

Please let me know which of these times works for the critical folks from OMB and I will send out a meeting request to the Federal Partners for that time. Thanks for your continued assistance with this important action.

Eric Burneson, P.E.
Director of Standards and Risk Management Office of Ground Water and Drinking Water U.S. Environmental Protection Agency
202 564 5250

On Sep 13, 2017, at 12:11 PM, Burneson, Eric <Burneson.Eric@epa.gov<mailto:Burneson.Eric@epa.gov>> wrote:

Federal Agency Partners:
Thank you again for your input on the peer review charge for the materials to inform the Safe Drinking Water Act Decision Making on Perchlorate. I am writing to inform you that the following Federal Register announcements are scheduled for publication on Friday, September 15 (and will be available on line for public inspection tomorrow, Sept 14)

1. Federal Register.
Title: Request for Public Comments to be sent to Versar, Inc., on an Interim List of Perchlorate in Drinking Water Expert Peer Reviewers and Draft Peer Review Charge Questions FRL #: 9967-70-OW Docket #: EPA-HQ-OW-2016-0439

1. Federal Register.
Title: Request for Public Comments to be sent to EPA on Peer Review Materials to Inform the Safe Drinking Water Act Decision Making on Perchlorate FRL #: 9967-69-OW Docket #: EPA-HQ-OW-2016-0438

As we stated in our previous discussions, EPA is committed to working with you to assure we have a robust peer review and scientifically sound analysis to inform decision making. We will be scheduling a teleconference during the week of October 2, 2017, to answer any questions you have about the charge and to discuss your preliminary feedback on the charge. We are asking for your feedback on the revised charge questions via email by October 6, 2017.

As we discussed, in order for your comments on the draft report to be considered by the peer reviewers, they will need to be submitted to the docket through the processes identified in the second Federal Register notice above (comment due date will be October 30, 2017). However we will also be open to any input that you wish to provide directly to us via email and can schedule a second teleconference to discuss questions and preliminary comments during the week of October 23 if there is interest.

Thanks again for your continued involvement in this important scientific analysis.

Eric Burneson, P.E.
Director of Standards and Risk Management Office of Ground Water and Drinking Water U.S. Environmental Protection Agency
202 564 5250

Message

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 10/6/2016 9:20:44 PM
To: Grevatt, Peter [Grevatt.Peter@epa.gov]
Subject: FW: Draft Reply on Interagency meeting perchlorate

Below is the response I would like to provide to OMB on their request to have two meetings discussing the charge. Is this consistent with the discussion you had with OP?

From: Christ, Lisa
Sent: Thursday, October 06, 2016 5:05 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Draft Reply on Interagency meeting perchlorate

Draft Reply:

Ex. 5 Deliberative Process (DP)

From: Kim, Jim H. EOP/OMB [mailto **Ex. 6 EOP (PP)**
Sent: Thursday, October 06, 2016 2:56 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB < **Ex. 6 EOP (PP)** >; Schwab, Margo EOP/OMB < **Ex. 6 EOP (PP)** >; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks. Also – I wanted to confirm that if **Ex. 5 Deliberative Process (DP)** **Ex. 5 Deliberative Process (DP)** **Ex. 5 Deliberative Process (DP)**? That would give EPA a little more than 2 weeks to consider interagency comments. So our goal will be for **Ex. 5 Deliberative Process (DP)** **Ex. 5 Deliberative Process (DP)** Also – I wanted to confirm that our comments on the peer review charge would be submitted to you, while comments on the BBDR draft report and model will be submitted to the docket.

I am thinking that a 3rd meeting prior to the end of November would be useful to discuss Ex. 5 Deliberative Process (DP)
Ex. 5 Deliberative Process (DP)

Please let me know your thoughts.

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Thursday, October 6, 2016 7:35 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Hi Jim
Of course. I held the time on EPA participants calendars, so I'll send confirmation. I'll set up logistics and send you the information soon.
Lisa

Sent from my iPhone

On Oct 5, 2016, at 4:40 PM, Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)> wrote:

> Hi Lisa,
>
> Thanks for the info. Sorry for the late response. Can we go ahead and schedule a meeting for October 20? Thanks for your patience.
>
> Best regards,
> Jim
>
> ---
>
> **From:** Christ, Lisa [mailto:Christ.Lisa@epa.gov]
> **Sent:** Friday, September 30, 2016 11:04 AM
> **To:** Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>
> **Cc:** Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
> **Subject:** RE: Perchlorate Peer Review Advance Materials
>
> Jim
> The closing date for public comments on the charge questions is October 21. The public comment period for the model and report ends November 14. We intend to have our contractor identify the peer reviewers and we'll finalize charge questions between October 21 and November 14. [potential call dates are Oct 20 & 27]
>
> Public comments will be summarized and provided to the peer reviewers at the end of November around the same time we publish the next federal register notice announcing a meeting date sometime in early January (based on availability of peer reviewers and at least 30 days before the panel meeting). [potential call dates are Nov 7 & 8]
>
> Lisa

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 EOP (PP)]
Sent: Friday, September 30, 2016 10:27 AM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) >; Schwab, Margo EOP/OMB < Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks for the dates and times. I was hoping to get something on our calendars earlier to ensure that Ex. 5 Deliberative Process (DP) Can you tell me the timeline that is planned in regards to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) ?

Thanks,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 9:42 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) >; Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) >; Schwab, Margo EOP/OMB < Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim,
I'd like to suggest we schedule two meetings and see if an additional meeting, as suggested, is needed. I looked at calendars for relevant EPA folks and can offer the following dates/times.
1st meeting: October 20 from 4-5pm OR October 27 from 405pm
2nd meeting: November 7 from 11-11am OR November 8 from 11a-noon

I am unable to find availability sooner than Oct 20. However, that provides time for review of materials before discussions begin.
Please let me know at your earliest convenience which times work and I will set up a conference call.
Lisa

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 EOP (PP)]
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) ; Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) the second would be to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) and the third would Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, September 28, 2016 10:36 AM
To: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

~~~~~~

Lisa Christ, Chief
Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M
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Message

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 10/5/2016 12:40:33 PM
To: Christ, Lisa [Christ.Lisa@epa.gov]
Subject: Re: Perchlorate Charge Questions Interagency Comments

---

From: Grevatt, Peter
Sent: Wednesday, October 5, 2016 11:00 AM
To: Rennert, Kevin
Cc: Burneson, Eric
Subject: RE: Perchlorate Charge Questions Interagency Comments

Good. Thanks.

From: Rennert, Kevin
Sent: Wednesday, October 05, 2016 6:33 AM
To: Grevatt, Peter <Grevatt.Peter@epa.gov>
Subject: Re: Perchlorate Charge Questions Interagency Comments

No worries. I did close the loop with Jim, and he was happy to hear that we're going to work with them on this. Thanks.

On Oct 4, 2016, at 10:52 PM, Grevatt, Peter <Grevatt.Peter@epa.gov> wrote:

> Kevin, apologies that I am only now catching up with the day's email. My iPhone has been out for a couple of days (no thanks EZ-Tech!) which is making it more difficult to keep up. We had agreed to Ex. 5 Deliberative Process (DP) I don't believe we had yet closed the loop with Jim, so it will be good to know whether you were able to do so today. Thanks.
>
> ---
>
> **From:** Rennert, Kevin
> **Sent:** Tuesday, October 4, 2016 10:54:57 AM
> **To:** Grevatt, Peter
> **Subject:** RE: Perchlorate Charge Questions Interagency Comments
>
> Great – thank you. Do you know if that's already been signaled to OMB? If not, I'll bring it up with Jim later today. Thanks.
>
> ---
>
> **From:** Grevatt, Peter
> **Sent:** Tuesday, October 04, 2016 10:51 AM
> **To:** Rennert, Kevin <Rennert.Kevin@epa.gov>
> **Subject:** RE: Perchlorate Charge Questions Interagency Comments
>
> Thanks Kevin. We're happy to do this, we're just trying to work schedules to get a meeting time that will work for all of the participants.

From: Rennert, Kevin
Sent: Tuesday, October 04, 2016 10:48 AM
To: Grevatt, Peter <Grevatt.Peter@epa.gov>
Subject: FW: Perchlorate Charge Questions Interagency Comments

Peter – any thoughts on this? We have our weekly with OIRA today, and I'm certain it will come up, so it would be good to know if Ex. 5 Deliberative Process (DP) Thanks. -Kevin

---

From: Beauvais, Joel
Sent: Monday, October 03, 2016 9:29 AM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>
Cc: Vaught, Laura <Vaught.Laura@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>
Subject: RE: Perchlorate Charge Questions Interagency Comments

Thanks, Kevin – I think Ex. 5 Deliberative Process (DP) this, but I'll let Peter connect with his folks and with me and get back to you (and then Jim)

From: Rennert, Kevin
Sent: Sunday, October 02, 2016 10:48 PM
To: Beauvais, Joel <Beauvais.Joel@epa.gov>
Cc: Vaught, Laura <Vaught.Laura@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>
Subject: Fwd: Perchlorate Charge Questions Interagency Comments

Joel, Peter, see below from Jim on Ex. 5 Deliberative Process (DP) I'll follow your lead. Thanks. Kevin

Begin forwarded message:

> **From:** "Laity, Jim A. EOP/OMB" < Ex. 6 Personal Privacy (PP)
> **Date:** September 30, 2016 at 7:01:26 PM EDT
> **To:** "Rennert, Kevin" <Rennert.Kevin@epa.gov>
> **Subject: Perchlorate Charge Questions Interagency Comments**
>
> Hi Kevin, I'm back in the office today. OMB folks were Ex. 5 Deliberative Process (DP)
>
> Ex. 5 Deliberative Process (DP)
>
> You will see below that EPA has tentatively offered October 20 as a date to discuss Ex. 5 Deliberative Process (DP) The team here asks if we could possibly have a meeting about a week before the end of the 21 day comment period to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) and then a second meeting something between Oct 20 and early November to discuss Ex. 5 Deliberative Process (DP)
>
> Ex. 5 Deliberative Process (DP)
>
> ch
>
> I can ask this of the DW office directly but thought I would start with you. Feel free to call if you would like to discuss. Jim

From: Kim, Jim H. EOP/OMB
Sent: Friday, September 30, 2016 2:27 PM
To: Laity, Jim A. EOP/OMB <Ex. 6 Personal Privacy (PP)
Subject: FW: Perchlorate Peer Review Advance Materials

Jim

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Sent: Friday, September 30, 2016 11:04 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP); Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)
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Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley

<Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
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Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

~~~~~~

Lisa Christ, Chief
Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M
~~~~~~

Message

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 10/7/2017 10:49:23 AM
To: Christ, Lisa [Christ.Lisa@epa.gov]; Hernandez-Quinones, Samuel [Hernandez.Samuel@epa.gov]
Subject: Fwd: EPA Perchlorate Peer Review Comment, NASA Response
Attachments: Perchlorate draft charge and peer review list, NASA final comments EPAdocx.docx; ATT00001.htm

Sent from my iPhone

Begin forwarded message:

From: "Wennerberg, Linda S. (HQ-LD020)" <linda.s.wennerberg@nasa.gov>
Date: October 6, 2017 at 5:24:41 PM EDT
To: "Burneson, Eric" <Burneson.Eric@epa.gov>
Cc: "Thaller, Denise R. (KSC-SIE00)" <denise.r.thaller@nasa.gov>, "Mcneill, Mike A (HQ-LD020)" <mike.a.mcneill@nasa.gov>
Subject: RE: EPA Perchlorate Peer Review Comment, NASA Response

Eric:
Attached are the NASA comments, as requested on the Interim List and Draft Charge Questions.

In support of a robust interagency process, NASA appreciates the opportunity to comment and hold dialogue with EPA and other agencies on scientific and technical issues. We concur with the submissions provided by the participating agencies and suggestions to improve these documents.

If you have any questions, please let me know.

Many thanks.

Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89
300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

From: Burneson, Eric [mailto:Burneson.Eric@epa.gov]
Sent: Friday, October 06, 2017 4:56 PM
To: Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov>
Subject: RE: EPA Perchlorate Peer Review Comment Deadlines

Linda: Thanks for the update. I look forward to your comments.

Eric

From: Wennerberg, Linda S. (HQ-LD020) [mailto:linda.s.wennerberg@nasa.gov]
Sent: Friday, October 06, 2017 4:26 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: EPA Perchlorate Peer Review Comment Deadlines

Eric
I will be submitting NASA's comments to you today, as requested. I am awaiting the final input from my management as they indicate that they have minor edits. I should be getting them back and finalized in an hour or so. Please let me know if that is a problem.

Thanks for your update so the schedule for comments.

Linda Wennerberg

Sent from my iPhone

On Oct 6, 2017, at 4:03 PM, Burneson, Eric <Burneson.Eric@epa.gov> wrote:

> Federal Partners:
> As we have discussed previously, EPA currently has two pending requests for comment related to the perchlorate peer review.
>
> 1. <!--[if !supportLists]--><!--[endif]-->The deadline for comments on the charge question and potential peer reviewers is **today**. **The comment deadline for this request remains unchanged.** Note that you have the option of sending your comments directly to me via email.
> 2. <!--[if !supportLists]--><!--[endif]-->The current deadline for the comments on the report is October 30, 2017. **EPA will be extending the deadline for the comments on the Draft Report and Model by 21 days**. We expect to publish a comment deadline extension (to Nov. 20) in the Federal Register next week.
>
> Thank you for your continued input on this important project.
>
> Eric Burneson, P.E.
> Director of Standards and Risk Management
> Office of Ground Water and Drinking Water
> U.S. Environmental Protection Agency
> 202 564 5250

Message

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 9/19/2017 8:19:12 PM
To: Flowers, Lynn [Flowers.Lynn@epa.gov]; Bussard, David [Bussard.David@epa.gov]
Subject: FW: EPA Perchlorate Peer Review follow up

FYI here are the potential dates for the next Federal Partners call.

-----Original Message-----
From: Burneson, Eric
Sent: Tuesday, September 19, 2017 3:29 PM
To: 'Dorjets, Vlad EOP/OMB' <Ex. 6 EOP (PP)>
Cc: Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Johnson, Ann <Johnson.Ann@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>; Mclain, Jennifer <Mclain.Jennifer@epa.gov>
Subject: RE: EPA Perchlorate Peer Review follow up

Vlad:

I wanted to check back with you on potential times for calls with the Federal Agency partners to discuss Ex. 5 Deliberative Process (DP) Here are three possible times

October 2, 11:00 to 12:00
October 2, 3:00 - 4:00
October 5: 2:00 - 3:00

Please let me know which of these times works for the critical folks from OMB and I will send out a meeting request to the Federal Partners for that time. Thanks for your continued assistance with this important action.

Eric Burneson, P.E.
Director of Standards and Risk Management Office of Ground Water and Drinking Water U.S. Environmental Protection Agency
202 564 5250

On Sep 13, 2017, at 12:11 PM, Burneson, Eric <Burneson.Eric@epa.gov<mailto:Burneson.Eric@epa.gov>> wrote:

Federal Agency Partners:
Thank you again for your input on the peer review charge for the materials to inform the Safe Drinking Water Act Decision Making on Perchlorate. I am writing to inform you that the following Federal Register announcements are scheduled for publication on Friday, September 15 (and will be available on line for public inspection tomorrow, Sept 14)

1. Federal Register.
Title: Request for Public Comments to be sent to Versar, Inc., on an Interim List of Perchlorate in Drinking Water Expert Peer Reviewers and Draft Peer Review Charge Questions FRL #: 9967-70-OW Docket #: EPA-HQ-OW-2016-0439

1. Federal Register.
Title: Request for Public Comments to be sent to EPA on Peer Review Materials to Inform the Safe Drinking Water Act Decision Making on Perchlorate FRL #: 9967-69-OW Docket #: EPA-HQ-OW-2016-0438

As we stated in our previous discussions, EPA is committed to working with you to assure we have a robust peer review and scientifically sound analysis to inform decision making. We will be scheduling a teleconference during the week of October 2, 2017, to answer any questions you have about the charge and to discuss your preliminary feedback on the charge. We are asking for your feedback on the revised charge questions via email by October 6, 2017.

As we discussed, in order for your comments on the draft report to be considered by the peer reviewers, they will need to be submitted to the docket through the processes identified in the second Federal Register notice above (comment due date will be October 30, 2017). However we will also be open to any input that you wish to provide directly to us via email and can schedule a second teleconference to discuss questions and preliminary comments during the week of October 23 if there is interest.

Thanks again for your continued involvement in this important scientific analysis.

Eric Burneson, P.E.

Director of Standards and Risk Management Office of Ground Water and Drinking Water U.S. Environmental Protection Agency
202 564 5250

Message

From: Burneson, Eric [Burneson.Eric@epa.gov]
Sent: 1/5/2017 9:30:36 PM
To: Christ, Lisa [Christ.Lisa@epa.gov]; Perkinson, Russ [Perkinson.Russ@epa.gov]
Subject: Fwd: URGENT: Registration for the Perchlorate Peer Review public meeting

Please assure that Linda and Jim are registered and can attend the meeting as observers.

Sent from my iPhone

Begin forwarded message:

From: "Wennerberg, Linda S. (HQ-LD020)" <linda.s.wennerberg@nasa.gov>
Date: January 5, 2017 at 3:17:24 PM EST
To: "Burneson, Eric" <Burneson.Eric@epa.gov>
Subject: URGENT: Registration for the Perchlorate Peer Review public meeting

Eric;
Just spoke with Jim Kim of OMB. Neither he nor I saw the FR Notice (12/5/16) about the Perchlorate Peer Review meeting and registration info. As you can imagine, the hectic time of Administration transition, heavy work loads of interagency review, and the Holidays swamped us both and we were not aware of the meeting or the registration deadline. I have just sent off an urgent request to Versar to permit our registration but have not heard back from them.

Please let me know if our registering now is an issue and what we will need to do to properly sign up and participate in the public meeting.

Thanks for your patience with all this.

Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89
300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

Message

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 9/26/2017 1:57:04 PM
To: Messier, Dawn [Messier.Dawn@epa.gov]; Wehling, Carrie [Wehling.Carrie@epa.gov]
Subject: FW: ACC request for comment period extension: perchlorate draft MCLG Approaches Report
Attachments: ACC request for comment period extension_perchlorate 9-26-17.pdf

FYI per my earlier email, one of two comment period extension requests.

From: Gibson, Mark [mailto:mark_gibson@americanchemistry.com]
Sent: Tuesday, September 26, 2017 8:42 AM
To: Hernandez-Quinones, Samuel <Hernandez.Samuel@epa.gov>
Cc: Nordgren, Judith <Judith_Nordgren@americanchemistry.com>; Shapiro, Mike <Shapiro.Mike@epa.gov>; Forsgren, Lee <Forsgren.Lee@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: ACC request for comment period extension: perchlorate draft MCLG Approaches Report

Good morning Mr. Hernandez.

I am sending this email and attached letter requesting a 45-day extension to the comment period on "Proposed Approaches to Inform the Derivation of a Maximum Contaminant Level Goal for Perchlorate in Drinking Water" on behalf of Judith Nordgren, Managing Director of ACC's Chlorine Chemistry Division.

Thank you.

Mark C. Gibson | American Chemistry Council
Director, Chlorine Issues
Mark_Gibson@americanchemistry.com
700 2nd Street NE | Washington, DC | 20002
O: 202-249-6738 | C: Ex. 6 Personal Privacy (PP)
www.americanchemistry.com

++++++++++++++++++++++++++++++ This message may contain confidential information and is intended only for the individual named. If you are not the named addressee do not disseminate, distribute or copy this email. Please notify the sender immediately by email if you have received this email by mistake and delete this email from your system. E-mail transmission cannot be guaranteed to be secure or error-free as information could be intercepted, corrupted, lost, destroyed, arrive late or incomplete, or contain viruses. The sender therefore does not accept liability for any errors or omissions in the contents of this message which arise as a result of email transmission. American Chemistry Council, 700 – 2nd Street NE, Washington, DC 20002, www.americanchemistry.com

Message

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 9/26/2017 12:48:15 PM
To: Mclain, Jennifer [Mclain.Jennifer@epa.gov]; Christ, Lisa [Christ.Lisa@epa.gov]
Subject: FW: ACC request for comment period extension: perchlorate draft MCLG Approaches Report
Attachments: ACC request for comment period extension_perchlorate 9-26-17.pdf

FYI

From: Gibson, Mark [mailto:mark_gibson@americanchemistry.com]
Sent: Tuesday, September 26, 2017 8:42 AM
To: Hernandez-Quinones, Samuel <Hernandez.Samuel@epa.gov>
Cc: Nordgren, Judith <Judith_Nordgren@americanchemistry.com>; Shapiro, Mike <Shapiro.Mike@epa.gov>; Forsgren, Lee <Forsgren.Lee@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: ACC request for comment period extension: perchlorate draft MCLG Approaches Report

Good morning Mr. Hernandez.

I am sending this email and attached letter requesting a 45-day extension to the comment period on "Proposed Approaches to Inform the Derivation of a Maximum Contaminant Level Goal for Perchlorate in Drinking Water" on behalf of Judith Nordgren, Managing Director of ACC's Chlorine Chemistry Division.

Thank you.

Mark C. Gibson | American Chemistry Council
Director, Chlorine Issues
Mark_Gibson@americanchemistry.com
700 2nd Street NE | Washington, DC | 20002
O: 202-249-6738 | C: Ex. 6 Personal Privacy (PP)
www.americanchemistry.com

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Message

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 9/25/2017 12:54:40 PM
To: Grevatt, Peter [Grevatt.Peter@epa.gov]; Mclain, Jennifer [Mclain.Jennifer@epa.gov]
Subject: FW: EPA Perchlorate Peer Review Charge follow up

FYI

-----Original Appointment-----
From: Laity, Jim A. EOP/OMB [mailto: Ex. 6 Personal Privacy (PP)
Sent: Friday, September 22, 2017 7:38 PM
To: Burneson, Eric
Subject: Tentative: EPA Perchlorate Peer Review Charge follow up
When: Monday, October 02, 2017 3:00 PM-4:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: Teleconference # Ex. 6 Personal Privacy (PP) code Ex. 6 Personal Privacy (PP) or EPA WJC East Room 2123

I will be out on annual leave from Oct 2-5, but you do not need to reschedule for me if this works for others.

Message

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 10/20/2016 7:35:55 PM
To: Rennert, Kevin [Rennert.Kevin@epa.gov]
Subject: RE: Perchlorate Peer Review Advance Materials

No we have not received any input from them yet. We will double check to see if they submitted anything through the formal process.

From: Rennert, Kevin
Sent: Thursday, October 20, 2016 3:21 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Have they not sent us any suggestions prior to the call?

On Oct 20, 2016, at 3:17 PM, Burneson, Eric <Burneson.Eric@epa.gov> wrote:

Got it. We will endeavor to be clear in responding to the input that they provide to us today and will take their suggestions to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)
Thanks

From: Rennert, Kevin
Sent: Thursday, October 20, 2016 3:10 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Cc: Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Short summary is that Jim understands that our position is that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) What they really want is to be able to have us Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) To the extent that we can use today's call to do so, the less friction we'll have on this point moving forward, so I'd encourage you to use the call for that as much as possible. Thanks.

On Oct 20, 2016, at 1:33 PM, Burneson, Eric <Burneson.Eric@epa.gov> wrote:

Kevin: The interagency call about the perchlorate peer review charge is today at 4:00. We have not responded to Jim Kim's request for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) I assume that our answer is that Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Let us know if we should take a different approach to responding.

Eric G. Burneson, P.E.

Director of Standards and Risk Management
Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency

From: Rennert, Kevin
Sent: Monday, October 17, 2016 2:00 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Eric – thanks for flagging. I thought we had actually put this to bed on our call with Jim on Friday, as I'd stated that we were planning Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) He thought that sounded fine, and in alignment with Jim Kim, which is what he cared about. It looks from the below email like that's not the case. I'll call Jim L. and discuss, and will follow up afterwards. Thanks. Kevin

---

From: Burneson, Eric
Sent: Monday, October 17, 2016 10:11 AM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: Fw: Perchlorate Peer Review Advance Materials

Kevin: Apologies if we have already worked through a path forward on the interagency review of the perchlorate peer review charge but OMB continues to Ex. 5 Deliberative Process (DP) We think this sets up a dynamic in which Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) We would appreciate any guidance as to how to respond this request from Jim.

I am at the ASDWA meeting in Milwaukee but am happy to discuss if needed
Eric Burneson Ex. 6 Personal Privacy (PP)

From: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) >
Sent: Monday, October 17, 2016 2:02 PM
To: Christ, Lisa
Cc: Dorjets, Vlad EOP/OMB; Schwab, Margo EOP/OMB; Grevatt, Peter; Greene, Ashley; Burneson, Eric
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

I forgot to ask – can we schedule a teleconference for interagency discussion of the Ex. 5 Deliberative Process (DP) ? I think it is important to understand Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)

Jim

From: Kim, Jim H. EOP/OMB
Sent: Monday, October 17, 2016 9:58 AM
To: 'Christ, Lisa' <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB Ex. 6 Personal Privacy (PP); Schwab, Margo EOP/OMB Ex. 6 Personal Privacy (PP); Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thank you so much!

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Monday, October 17, 2016 9:56 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Jim,
I wanted to provide the information for the October 20th call on the perchlorate BBDR model charge questions.
Conference Ex. 6 Personal Privacy (PP), access code Ex. 6 Personal Privacy (PP)

Interagency comments on the charge questions will be provided to EPA and comments on the BBDR model and report will be submitted to the docket. We respectfully request comments on the charge as soon as possible after the meeting -- October 27 would be ideal to allow ample time for peer reviewers consideration.

Sincerely,
Lisa

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 Personal Privacy (PP)
Sent: Thursday, October 06, 2016 2:56 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks. Also – I wanted to confirm that if Ex. 5 Deliberative Process (DP)
Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP) That would give EPA a little more than 2 weeks to consider interagency comments. So our goal will be for Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP). Also – I wanted to confirm that our comments on the peer review charge would be submitted to you, while comments on the BBDR draft report and model will be submitted to the docket.

I am thinking that a 3rd meeting prior to the end of November would be useful to discuss

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Please let me know your thoughts.

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Thursday, October 6, 2016 7:35 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)>
Cc: Dorjets, Vlad EOP/OMB Ex. 6 Personal Privacy (PP); Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Hi Jim
Of course. I held the time on EPA participants calendars, so I'll send confirmation. I'll set up logistics and send you the information soon.
Lisa

Sent from my iPhone

On Oct 5, 2016, at 4:40 PM, Kim, Jim H. EOP/OMB <James_H_Kim@omb.eop.gov> wrote:

> Hi Lisa,
>
> Thanks for the info. Sorry for the late response. Can we go ahead and schedule a meeting for October 20? Thanks for your patience.
>
> Best regards,
> Jim
>
> ---
>
> **From:** Christ, Lisa [mailto:Christ.Lisa@epa.gov]
> **Sent:** Friday, September 30, 2016 11:04 AM
> **To:** Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>
> **Cc:** Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
> **Subject:** RE: Perchlorate Peer Review Advance Materials
>
> Jim

The closing date for public comments on the charge questions is October 21. The public comment period for the model and report ends November 14. We intend to have our contractor identify the peer reviewers and we'll finalize charge questions between October 21 and November 14. [potential call dates are Oct 20 & 27]

Public comments will be summarized and provided to the peer reviewers at the end of November around the same time we publish the next federal register notice announcing a meeting date sometime in early January (based on availability of peer reviewers and at least 30 days before the panel meeting). [potential call dates are Nov 7 & 8]

Lisa

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 Personal Privacy (PP)
Sent: Friday, September 30, 2016 10:27 AM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) ; Schwab, Margo EOP/OMB < >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks for the dates and times. I was hoping to get something on our calendars earlier to ensure that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Can you tell me the timeline that is planned in regards to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) ?

Thanks,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 9:42 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) ; Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim,
I'd like to suggest we schedule two meetings and see if an additional meeting, as suggested, is needed. I looked at calendars for relevant EPA folks and can offer the following dates/times.
1st meeting: October 20 from 4-5pm OR October 27 from 405pm
2nd meeting: November 7 from 11-11am OR November 8 from 11a-noon

I am unable to find availability sooner than Oct 20. However, that provides time for review of materials before discussions begin.

Please let me know at your earliest convenience which times work and I will set up a conference call.
Lisa

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 Personal Privacy (PP)
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)
Ex. 6 Personal Privacy (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) the second would be to discuss Ex. 5 Deliberative Process (DP) and the third would Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, September 28, 2016 10:36 AM
To: Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP);
Ex. 6 Personal Privacy (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday.
Please let me know if you have questions or need additional information.

Thank you,
Lisa

~~~~~~

Lisa Christ, Chief
Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
~~~~~~

1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M

Message

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 10/19/2016 12:43:42 PM
To: Christ, Lisa [Christ.Lisa@epa.gov]
Subject: Re: let me know if I should reply to Jim Kim -- RE: Perchlorate Peer Review Advance Materials -

Let's wait for Kevin to get back to us

Sent from my iPhone

On Oct 19, 2016, at 6:42 AM, Christ, Lisa <Christ.Lisa@epa.gov> wrote:

Let me know if you want me to follow up.

From: Burneson, Eric
Sent: Monday, October 17, 2016 10:11 AM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: Fw: Perchlorate Peer Review Advance Materials

Kevin: Apologies if we have already worked through a path forward on the interagency review of the perchlorate peer review charge but OMB continues to ask for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) We think this sets up a dynamic in which Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) We would appreciate any guidance as to how to respond this request from Jim.

I am at the ASDWA meeting in Milwaukee but am happy to discuss if needed
Eric Burneson Ex. 6 Personal Privacy (PP)

From: Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>
Sent: Monday, October 17, 2016 2:02 PM
To: Christ, Lisa
Cc: Dorjets, Vlad EOP/OMB; Schwab, Margo EOP/OMB; Grevatt, Peter; Greene, Ashley; Burneson, Eric
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

I forgot to ask – can we Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)? I think it is important to understand EPA's perspectives on the charge and to clear up any confusion on interagency comments (if any).

Jim

From: Kim, Jim H. EOP/OMB
Sent: Monday, October 17, 2016 9:58 AM
To: 'Christ, Lisa' <Christ.Lisa@epa.gov>

Cc: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thank you so much!

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Monday, October 17, 2016 9:56 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Jim,
I wanted to provide the information for the October 20th call on the perchlorate BBDR model charge questions.
Conference Ex. 6 Personal Privacy (PP), access code Ex. 6 Personal Privacy (PP)

Interagency comments on the charge questions will be provided to EPA and comments on the BBDR model and report will be submitted to the docket. We respectfully request comments on the charge as soon as possible after the meeting -- October 27 would be ideal to allow ample time for peer reviewers consideration.

Sincerely,
Lisa

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 EOP (PP)
Sent: Thursday, October 06, 2016 2:56 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks. Also – I wanted to confirm that if Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)? That would give EPA a little more than 2 weeks to consider interagency comments. So our goal will be for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Also – I wanted to confirm that our comments on the peer review charge would be submitted to you, while comments on the BBDR draft report and model will be submitted to the docket.

I am thinking that a 3rd meeting prior to the end of November would be useful to discuss interagency comments on the BBDR draft report and model, prior to submission of EPA's summary of public comments to the peer review panel.

Please let me know your thoughts.

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Thursday, October 6, 2016 7:35 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Hi Jim
Of course. I held the time on EPA participants calendars, so I'll send confirmation. I'll set up logistics and send you the information soon.
Lisa

Sent from my iPhone

On Oct 5, 2016, at 4:40 PM, Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)> wrote:

> Hi Lisa,
>
> Thanks for the info. Sorry for the late response. Can we go ahead and schedule a meeting for October 20? Thanks for your patience.
>
> Best regards,
> Jim
>
> ---
>
> **From:** Christ, Lisa [mailto:Christ.Lisa@epa.gov]
> **Sent:** Friday, September 30, 2016 11:04 AM
> **To:** Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>
> **Cc:** Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
> **Subject:** RE: Perchlorate Peer Review Advance Materials
>
> Jim
> The closing date for public comments on the charge questions is October 21. The public comment period for the model and report ends November 14. We intend to have our contractor identify the peer reviewers and we'll finalize charge questions between October 21 and November 14. [potential call dates are Oct 20 & 27]
>
> Public comments will be summarized and provided to the peer reviewers at the end of November around the same time we publish the next federal register notice announcing a meeting date sometime in early January (based on availability of peer reviewers and at least 30 days before the panel meeting). [potential call dates are Nov 7 & 8]

Lisa

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 EOP (PP)
Sent: Friday, September 30, 2016 10:27 AM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB
< Ex. 6 EOP (PP) Schwab, Margo EOP/OMB
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley
<Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks for the dates and times. I was hoping to get something on our calendars earlier to ensure that Ex. 5 Deliberative Process (DP) Can you tell me the timeline that is planned in regards to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)?

Thanks,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 9:42 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) >; Dorjets, Vlad EOP/OMB
< Ex. 6 EOP (PP) Schwab, Margo EOP/OMB
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley
<Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim,
I'd like to suggest we schedule two meetings and see if an additional meeting, as suggested, is needed. I looked at calendars for relevant EPA folks and can offer the following dates/times.
1st meeting: October 20 from 4-5pm OR October 27 from 405pm
2nd meeting: November 7 from 11-11am OR November 8 from 11a-noon

I am unable to find availability sooner than Oct 20. However, that provides time for review of materials before discussions begin.
Please let me know at your earliest convenience which times work and I will set up a conference call.
Lisa

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 EOP (PP)
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB
< Ex. 6 EOP (PP)
< Ex. 6 EOP (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley
<Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) the second would be to discuss Ex. 5 Deliberative Process (DP) and the third would Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, September 28, 2016 10:36 AM
To: Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) ; Kim, Jim H. EOP/OMB Ex. 6 EOP (PP) Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

~~~~~~

Lisa Christ, Chief
Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M
~~~~~~

Message

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 3/4/2019 4:52:10 PM
To: Steve Via [SVia@awwa.org]
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

Agreed.

From: Steve Via <SVia@awwa.org>
Sent: Monday, March 04, 2019 11:51 AM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

Probably not the best audience for general observations. They might go the wrong direction with the information.

Steve

Steve Via
Director Federal Relations, AWWA | 202.326.6130

From: Burneson, Eric <Burneson.Eric@epa.gov>
Sent: Monday, March 04, 2019 11:49 AM
To: Steve Via <SVia@awwa.org>
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

Assuming you mean EPA's cost, yes it would be difficult to articulate but I could make some general observations about the Agency's resources.

From: Steve Via <SVia@awwa.org>
Sent: Monday, March 04, 2019 9:00 AM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

It would be entertaining but probably impossible to articulate what it costs to get through on or more step (CCL, UCMR, Reg Det, Proposal, Final, initial implementation/outreach / SDWIS).

Steve

Steve Via
Director Federal Relations, AWWA | 202.326.6130

From: Burneson, Eric <Burneson.Eric@epa.gov>
Sent: Monday, March 04, 2019 8:28 AM
To: Steve Via <SVia@awwa.org>; Brozena, Sarah <Sarah_Brozena@americanchemistry.com>; Andes, Fredric P. <fandes@btlaw.com>; Fischer, David <David_Fischer@americanchemistry.com>; Brendan_Mascarenhas@americanchemistry.com
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

Thanks Steve for sharing your presentation. I would ask that when delivering slide 7 you note that you are speculating on Agency decisions which have not been made with respect to the potential MCL's and treatment goals and MCLs for Hexavalent Chromium and Perchlorate. The Agency has not made a decision to revise the total chromium standard or to issue a separate hexavalent chromium standard and we have yet to propose a perchlorate standard (but must do so this spring).

Also please find the slide deck I plan to use in Friday's panel discussion. Let me know if you have any questions or concerns. With the attached slides.

Eric Burneson, P.E.
Director of Standards and Risk Management
Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency
202 564 5250

From: Steve Via <SVia@awwa.org>
Sent: Sunday, March 03, 2019 5:50 PM
To: Brozena, Sarah <Sarah_Brozena@americanchemistry.com>; Burneson, Eric <Burneson.Eric@epa.gov>; Andes, Fredric P. <fandes@btlaw.com>; Fischer, David <David_Fischer@americanchemistry.com>; Brendan_Mascarenhas@americanchemistry.com
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

Any feedback on attached w/r/t Friday's panel?

Steve

Steve Via
Director Federal Relations, AWWA | 202.326.6130

-----Original Appointment-----
From: Brozena, Sarah <Sarah_Brozena@americanchemistry.com>
Sent: Tuesday, February 26, 2019 3:33 PM
To: Brozena, Sarah; 'Burneson.eric@Epa.gov'; Andes, Fredric P.; Fischer, David; Steve Via; Mascarenhas, Brendan
Subject: GlobalChem Panel on Drinking Water Organizing Call
When: Wednesday, February 27, 2019 9:30 AM-10:00 AM (UTC-05:00) Eastern Time (US & Canada).
Where: Ex. 6 Personal Privacy (PP) Pin# Ex. 6 Personal Privacy (PP)

TO: Eric Burneson, EPA
Fred Andes, Barnes and Thornburg
David Fischer, IBEX Partners
Steve Via, American Water Works Association
FROM: Sarah Brozena and Brendan Mascarenhas, ACC
RE: Organizing Call
DATE: Feb. 26, 2019

Brendan and I hope to talk to you all tomorrow, Wed., Feb. 27 at 9:30 a.m. about the GlobalChem panel next week. Thanks

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Message

From: Burneson, Eric [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2CACB9A8D49F49AF80531E9E2CCB9018-EBURNESO]
Sent: 3/4/2019 4:49:03 PM
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Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

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Steve

Steve Via
Director Federal Relations, AWWA | 202.326.6130

From: Burneson, Eric <Burneson.Eric@epa.gov>
Sent: Monday, March 04, 2019 8:28 AM
To: Steve Via <SVia@awwa.org>; Brozena, Sarah <Sarah_Brozena@americanchemistry.com>; Andes, Fredric P. <fandes@btlaw.com>; Fischer, David <David_Fischer@americanchemistry.com>; Brendan_Mascarenhas@americanchemistry.com
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Eric Burneson, P.E.
Director of Standards and Risk Management
Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency
202 564 5250

From: Steve Via <SVia@awwa.org>
Sent: Sunday, March 03, 2019 5:50 PM
To: Brozena, Sarah <Sarah_Brozena@americanchemistry.com>; Burneson, Eric <Burneson.Eric@epa.gov>; Andes,

Fredric P. <fandes@btlaw.com>; Fischer, David <David_Fischer@americanchemistry.com>; Brendan_Mascarenhas@americanchemistry.com
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

Any feedback on attached w/r/t Friday's panel?

Steve

Steve Via
Director Federal Relations, AWWA | 202.326.6130

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From: Brozena, Sarah <Sarah_Brozena@americanchemistry.com>
Sent: Tuesday, February 26, 2019 3:33 PM
To: Brozena, Sarah; 'Burneson.eric@Epa.gov'; Andes, Fredric P.; Fischer, David; Steve Via; Mascarenhas, Brendan
Subject: GlobalChem Panel on Drinking Water Organizing Call
When: Wednesday, February 27, 2019 9:30 AM-10:00 AM (UTC-05:00) Eastern Time (US & Canada).
Where: Ex. 6 Personal Privacy (PP) Pin# Ex. 6 Personal Privacy (PP)

TO: Eric Burneson, EPA
Fred Andes, Barnes and Thornburg
David Fischer, IBEX Partners
Steve Via, American Water Works Association
FROM: Sarah Brozena and Brendan Mascarenhas, ACC
RE: Organizing Call
DATE: Feb. 26, 2019

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Message

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Sent: 3/4/2019 1:28:22 PM
To: Steve Via [SVia@awwa.org]; Brozena, Sarah [Sarah_Brozena@americanchemistry.com]; Andes, Fredric P. [fandes@btlaw.com]; Fischer, David [David_Fischer@americanchemistry.com]; Brendan_Mascarenhas@americanchemistry.com
Subject: RE: GlobalChem Panel on Drinking Water Organizing Call
Attachments: GlobalChem DW contaminants of emerging concern 3 19.pptx

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Subject: RE: GlobalChem Panel on Drinking Water Organizing Call

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Message

From: Christ, Lisa [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=10DBD8E424704E43B5A50F74A4DAC626-LCHRIST]
Sent: 7/28/2016 6:36:52 PM
To: Olson, Daniel [Olson.Daniel@epa.gov]
Subject: FW: URGENT: Perchlorate actions

FYI – more details on the OMB interagency briefing

From: Burneson, Eric
Sent: Thursday, July 28, 2016 2:31 PM
To: Grevatt, Peter <Grevatt.Peter@epa.gov>; Beauvais, Joel <Beauvais.Joel@epa.gov>
Cc: Shapiro, Mike <Shapiro.Mike@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: RE: URGENT: Perchlorate actions

Just had a conversation with Linda W. and clarified the approach that we had described to OMB.

Ex. 5 Deliberative Process (DP)

Linda stated that she understood this process and that many of her concerns were relieved but that she would still have concerns about Ex. 5 Deliberative Process (DP) And she also stated that we need to be more proactive in Ex. 5 Deliberative Process (DP)

Eric

From: Grevatt, Peter
Sent: Thursday, July 28, 2016 1:44 PM
To: Beauvais, Joel <Beauvais.Joel@epa.gov>
Cc: Shapiro, Mike <Shapiro.Mike@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: FW: URGENT: Perchlorate actions
Importance: High

Joel, FYI. This misrepresents our process. I'll work with Mike and Eric to develop a response.

From: WENNERBERG, LINDA S. (HQ-LD020) [mailto:linda.s.wennerberg@nasa.gov]
Sent: Thursday, July 28, 2016 12:35 PM
To: Shapiro, Mike <Shapiro.Mike@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>
Cc: Laity, James A. Ex. 6 Personal Privacy (PP); Dorjets, Vlad Ex. 6 Personal Privacy (PP); Ex. 6 Personal Privacy (PP) Mcneill, Mike A (HQ-LD020) <mike.a.mcneill@nasa.gov>; james.leatherwood-1@nasa.gov; Ex. 6 Personal Privacy (PP)

Subject: URGENT: Perchlorate actions
Importance: High

Mike and Peter;

I just received a call from a senior colleague at DoD regarding proposed EPA action on Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP) I am writing to dispel any confusion or misrepresentation.

I, on behalf of NASA, am most concerned about Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP). I request that you get back to me on Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP) The federal family has long worked together to ensure sound science is utilized for perchlorate.

Ex. 5 Deliberative Process (DP)

Please contact me as soon as possible to communicate EPA's plans.

Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89
300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

Message

From: Burneson, Eric [Burneson.Eric@epa.gov]
Sent: 9/22/2017 1:17:08 PM
To: Ex. 6 Personal Privacy (PP) Dorjets, Vlad EOP/OMB Ex. 6 Personal Privacy (PP)
CC: Muellerleile, Caryn [Muellerleile.Caryn@epa.gov]; Johnson, Ann [Johnson.Ann@epa.gov]; Christ, Lisa [Christ.Lisa@epa.gov]; Mclain, Jennifer [Mclain.Jennifer@epa.gov]
Subject: RE: EPA Perchlorate Peer Review follow up

Thanks Jim and Vlad. I have schedule the call for 3:00 pm on October 2. You should have the invitation in your in box. I used the distribution list that we had for the last call to identify the invitees.

-----Original Message-----
From: Kim, Jim H. EOP/OMB [mailto Ex. 6 Personal Privacy (PP)
Sent: Thursday, September 21, 2017 1:56 PM
To: Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Burneson, Eric <Burneson.Eric@epa.gov>
Cc: Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Johnson, Ann <Johnson.Ann@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>; Mclain, Jennifer <Mclain.Jennifer@epa.gov>
Subject: RE: EPA Perchlorate Peer Review follow up

I can make October 2, but not October 5 at that time.

Jim

-----Original Message-----
From: Dorjets, Vlad EOP/OMB
Sent: Thursday, September 21, 2017 1:38 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Cc: Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Johnson, Ann <Johnson.Ann@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>; Mclain, Jennifer <Mclain.Jennifer@epa.gov>; Kim, Jim H. EOP/OMB
Ex. 6 Personal Privacy (PP)>
Subject: RE: EPA Perchlorate Peer Review follow up

Eric - Sorry for not replying sooner and thanks for offering to set this up. Unless Jim Kim (who I've copied) has any conflicts, all three times work for me. I realize I'm the one that has been the cause of the delay here but if we can get the invitation to reviewers tomorrow or Monday that would be great so that they will have a week notice to read the report before the call.

-----Original Message-----
From: Burneson, Eric [mailto:Burneson.Eric@epa.gov]
Sent: Tuesday, September 19, 2017 3:27 PM
To: Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>
Cc: Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>; Johnson, Ann <Johnson.Ann@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>; Mclain, Jennifer <Mclain.Jennifer@epa.gov>
Subject: RE: EPA Perchlorate Peer Review follow up

Vlad:

I wanted to check back with you on potential times for calls with the Federal Agency partners to discuss questions /concerns with the charge. Here are three possible times

October 2, 11:00 to 12:00
October 2, 3:00 - 4:00
October 5: 2:00 - 3:00

Please let me know which of these times works for the critical folks from OMB and I will send out a meeting request to the Federal Partners for that time. Thanks for your continued assistance with this important action.

Eric Burneson, P.E.
Director of Standards and Risk Management Office of Ground Water and Drinking Water U.S. Environmental Protection Agency
202 564 5250

On Sep 13, 2017, at 12:11 PM, Burneson, Eric <Burneson.Eric@epa.gov<mailto:Burneson.Eric@epa.gov>> wrote:

Federal Agency Partners:
Thank you again for your input on the peer review charge for the materials to inform the Safe Drinking Water Act Decision Making on Perchlorate. I am writing to inform you that the following Federal Register

announcements are scheduled for publication on Friday, September 15 (and will be available on line for public inspection tomorrow, Sept 14)

1. Federal Register.
Title: Request for Public Comments to be sent to Versar, Inc., on an Interim List of Perchlorate in Drinking Water Expert Peer Reviewers and Draft Peer Review Charge Questions FRL #: 9967-70-OW Docket #: EPA-HQ-OW-2016-0439

1. Federal Register.
Title: Request for Public Comments to be sent to EPA on Peer Review Materials to Inform the Safe Drinking Water Act Decision Making on Perchlorate FRL #: 9967-69-OW Docket #: EPA-HQ-OW-2016-0438

As we stated in our previous discussions, EPA is committed to working with you to assure we have a robust peer review and scientifically sound analysis to inform decision making. We will be scheduling a teleconference during the week of October 2, 2017, to answer any questions you have about the charge and to discuss your preliminary feedback on the charge. We are asking for your feedback on the revised charge questions via email by October 6, 2017.

As we discussed, in order for your comments on the draft report to be considered by the peer reviewers, they will need to be submitted to the docket through the processes identified in the second Federal Register notice above (comment due date will be October 30, 2017). However we will also be open to any input that you wish to provide directly to us via email and can schedule a second teleconference to discuss questions and preliminary comments during the week of October 23 if there is interest.

Thanks again for your continued involvement in this important scientific analysis.

Eric Burneson, P.E.
Director of Standards and Risk Management Office of Ground Water and Drinking Water U.S. Environmental Protection Agency
202 564 5250

Message

From: Wennerberg, Linda S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
Sent: 10/27/2016 2:34:24 PM
To: Christ, Lisa [Christ.Lisa@epa.gov]
Subject: NASA comments on perchlorate charge questions

Ms. Christ:

I will be submitting NASA's comments on the draft Peer Review Charge Questions for Perchlorate later this afternoon to meet EPA's submission deadline. The comments will be in a redline/strikeout version on the Charge Questions to make it easier for EPA to see our input. I now have to run to an out of office meeting but plan to return and submit our comments later this afternoon.

If you have any questions or need me to send these comments to someone else, please let me know.

Thanks.

Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89
300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

Message

From: Grevatt, Peter [Grevatt.Peter@epa.gov]
Sent: 10/6/2016 11:45:48 AM
To: Christ, Lisa [Christ.Lisa@epa.gov]
Subject: RE: Perchlorate Peer Review Advance Materials

Thx

From: Christ, Lisa
Sent: Thursday, October 06, 2016 7:35 AM
To: Ex. 6 EOP (PP)
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Hi Jim
Of course. I held the time on EPA participants calendars, so I'll send confirmation. I'll set up logistics and send you the information soon.
Lisa

Sent from my iPhone

On Oct 5, 2016, at 4:40 PM, Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)> wrote:

> Hi Lisa,
>
> Thanks for the info. Sorry for the late response. Can we go ahead and schedule a meeting for October 20? Thanks for your patience.
>
> Best regards,
> Jim
>
> ---
>
> **From:** Christ, Lisa [mailto:Christ.Lisa@epa.gov]
> **Sent:** Friday, September 30, 2016 11:04 AM
> **To:** Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>
> **Cc:** Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
> **Subject:** RE: Perchlorate Peer Review Advance Materials
>
> Jim
> The closing date for public comments on the charge questions is October 21. The public comment period for the model and report ends November 14. We intend to have our contractor identify the peer reviewers and we'll finalize charge questions between October 21 and November 14. [potential call dates are Oct 20 & 27]
>
> Public comments will be summarized and provided to the peer reviewers at the end of November around the same time we publish the next federal register notice announcing a meeting date sometime in early January (based on availability of peer reviewers and at least 30 days before the panel meeting). [potential call dates are Nov 7 & 8]
>
> Lisa

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 EOP (PP)
Sent: Friday, September 30, 2016 10:27 AM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) >; Schwab, Margo EOP/OMB < Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks for the dates and times. I was hoping to get something on our calendars earlier to ensure that Ex. 5 Deliberative Process (DP) Can you tell me the timeline that is planned in regards to Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP))?

Thanks,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 9:42 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) >; Dorjets, Vlad EOP/OMB Ex. 6 EOP (PP) >; Schwab, Margo EOP/OMB < Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim,
I'd like to suggest we schedule two meetings and see if an additional meeting, as suggested, is needed. I looked at calendars for relevant EPA folks and can offer the following dates/times.
1st meeting: October 20 from 4-5pm OR October 27 from 405pm
2nd meeting: November 7 from 11-11am OR November 8 from 11a-noon

I am unable to find availability sooner than Oct 20. However, that provides time for review of materials before discussions begin.
Please let me know at your earliest convenience which times work and I will set up a conference call.
Lisa

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 EOP (PP)
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) >; Ex. 6 EOP (PP) < Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) the second would be to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) and the third would cover Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, September 28, 2016 10:36 AM
To: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

~~~~~~

Lisa Christ, Chief
Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M
~~~~~~

Message

From: Christ, Lisa [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=10DBD8E424704E43B5A50F74A4DAC626-LCHRIST]
Sent: 10/23/2017 4:32:31 PM
To: Hafez, Ahmed [Hafez.Ahmed@epa.gov]; Miller, Gregory [Miller.Gregory@epa.gov]
Subject: FW: EPA Perchlorate Peer Review Comment, NASA Response
Attachments: Perchlorate draft charge and peer review list, NASA final comments EPAdocx.docx; ATT00001.htm

---

From: Burneson, Eric
Sent: Saturday, October 07, 2017 6:49 AM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Hernandez-Quinones, Samuel <Hernandez.Samuel@epa.gov>
Subject: Fwd: EPA Perchlorate Peer Review Comment, NASA Response

Sent from my iPhone

Begin forwarded message:

> **From:** "Wennerberg, Linda S. (HQ-LD020)" <linda.s.wennerberg@nasa.gov>
> **Date:** October 6, 2017 at 5:24:41 PM EDT
> **To:** "Burneson, Eric" <Burneson.Eric@epa.gov>
> **Cc:** "Thaller, Denise R. (KSC-SIE00)" <denise.r.thaller@nasa.gov>, "Mcneill, Mike A (HQ-LD020)" <mike.a.mcneill@nasa.gov>
> **Subject: RE: EPA Perchlorate Peer Review Comment, NASA Response**
>
> Eric:
> Attached are the NASA comments, as requested on the Interim List and Draft Charge Questions.
>
> In support of a robust interagency process, NASA appreciates the opportunity to comment and hold dialogue with EPA and other agencies on scientific and technical issues. We concur with the submissions provided by the participating agencies and suggestions to improve these documents.
>
> If you have any questions, please let me know.
>
> Many thanks.
>
> Linda
>
> Linda S. Wennerberg, Ph.D.
> Environmental Management Division
> NASA Headquarters
> MS-2T89
> 300 E Street SW
> Washington, DC 20546-0001
>
> Tel: 202/358-4558
> Cell: Ex. 6 Personal Privacy (PP)
> Fax: 202/358-3948
> linda.s.wennerberg@nasa.gov

From: Burneson, Eric [mailto:Burneson.Eric@epa.gov]
Sent: Friday, October 06, 2017 4:56 PM
To: Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov>
Subject: RE: EPA Perchlorate Peer Review Comment Deadlines

Linda: Thanks for the update. I look forward to your comments.
Eric

From: Wennerberg, Linda S. (HQ-LD020) [mailto:linda.s.wennerberg@nasa.gov]
Sent: Friday, October 06, 2017 4:26 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: EPA Perchlorate Peer Review Comment Deadlines

Eric
I will be submitting NASA's comments to you today, as requested. I am awaiting the final input from my management as they indicate that they have minor edits. I should be getting them back and finalized in an hour or so. Please let me know if that is a problem.

Thanks for your update so the schedule for comments.

Linda Wennerberg

Sent from my iPhone

On Oct 6, 2017, at 4:03 PM, Burneson, Eric <Burneson.Eric@epa.gov> wrote:

> Federal Partners:
> As we have discussed previously, EPA currently has two pending requests for comment related to the perchlorate peer review.
>
> 1. The deadline for comments on the charge question and potential peer reviewers is **today**. **The comment deadline for this request remains unchanged.** Note that you have the option of sending your comments directly to me via email.
> 2. The current deadline for the comments on the report is October 30, 2017. **EPA will be extending the deadline for the comments on the Draft Report and Model by 21 days**. We expect to publish a comment deadline extension (to Nov. 20) in the Federal Register next week.
>
> Thank you for your continued input on this important project.
>
> Eric Burneson, P.E.
> Director of Standards and Risk Management
> Office of Ground Water and Drinking Water
> U.S. Environmental Protection Agency
> 202 564 5250

Message

From: Christ, Lisa [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=10DBD8E424704E43B5A50F74A4DAC626-LCHRIST]
Sent: 9/15/2017 4:53:06 PM
To: Hernandez-Quinones, Samuel [Hernandez.Samuel@epa.gov]
Subject: track change charge
Attachments: EPA Perchlorate Peer Review follow up ; RE: EPA Perchlorate Peer Review follow up

Hi Sam,
Kevin requested a redline version of the revised charge. I thik the attached email from Caryn contains the last version feds commented on after our 8/9 face to face meeting. Can you do a document compare with this and the final charge?
Thanks-
Lisa

Appointment

From: Penman, Crystal [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=93662678A6FD4D4695C3DF22CD95935A-PENMAN, CRYSTAL]
Sent: 7/25/2017 1:42:49 PM
To: Forsgren, Lee [Forsgren.Lee@epa.gov]; linda.s.wennerberg@nasa.gov

Subject: Perchlorate Conference call
Location: Call in Ex. 6 Personal Privacy (PP) passcode Ex. 6 Personal Privacy (PP)

Start: 7/26/2017 8:00:00 PM
End: 7/26/2017 8:30:00 PM
Show Time As: Busy

Message

From: Wennerberg, Linda S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
Sent: 7/19/2017 7:01:12 PM
To: Forsgren, Lee [Forsgren.Lee@epa.gov]
CC: Penman, Crystal [Penman.Crystal@epa.gov]
Subject: RE: Perchlorate

Lee
I understand completely.

Crystal;
Could you please let me know several options when Lee is available for a telecom this Friday before 11AM, next Monday after noon or Tuesday.

Many thanks.

Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89
300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

From: Forsgren, Lee [mailto:Forsgren.Lee@epa.gov]
Sent: Wednesday, July 19, 2017 2:47 PM
To: Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov>
Cc: Penman, Crystal <Penman.Crystal@epa.gov>
Subject: Fwd: Perchlorate

Linda,

Like the AA's at NASA I don't control my schedule. Crystal Penman can find some time for us to talk either Friday or early next week.

I would love to chat with you.

Regards,
Lee

Sent from I phone

From: "Forsgren, Lee" <Forsgren.Lee@epa.gov>
Date: July 19, 2017 at 12:55:39 PM EDT
To: "Beck, Nancy" <Beck.Nancy@epa.gov>

Cc: "linda.s.wennerberg@nasa.gov" <linda.s.wennerberg@nasa.gov>, "Davis, Patrick" <davis.patrick@epa.gov>
Subject: Re: Perchlorate

Linda,

As former NASA official myself I would be happy talk/meet with you regarding perchlorate.

Regards,

Lee

Sent from my iPhone

On Jul 19, 2017, at 12:47 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:

> Linda,
>
> Regarding your perchlorate concerns, I suggest you contact Lee Forsgren or Patrick Davis regarding concerns with the interagency process for the EPA review.
>
> I'm sure they would try to assist you.
>
> Regards,
>
> Nancy
>
> **********
> **********
>
> Nancy B. Beck, Ph.D., DABT
>
> Deputy Assistant Administrator
>
> Office of Chemical Safety and Pollution Prevention
>
> P: 202-564-1273
>
> M: Ex. 6 Personal Privacy (PP)
>
> beck.nancy@epa.gov

Message

From: Wennerberg, Linda S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
Sent: 7/19/2017 5:08:02 PM
To: Beck, Nancy [Beck.Nancy@epa.gov]; Forsgren, Lee [Forsgren.Lee@epa.gov]
CC: Davis, Patrick [davis.patrick@epa.gov]
Subject: RE: Perchlorate

Nancy
Thanks so much for the introduction.

Lee
I welcome talking with you about this. And Nancy is correct, I have been working perchlorate so long that it is almost old enough to vote.....

My issue now is the Ex. 5 Deliberative Process (DP) Please let me know if you have some time Friday morning or later afternoon or early next week to touch base by phone. Sadly today and tomorrow are already jammed.

Many thanks.

Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89
300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

From: Beck, Nancy [mailto:Beck.Nancy@epa.gov]
Sent: Wednesday, July 19, 2017 1:01 PM
To: Forsgren, Lee <Forsgren.Lee@epa.gov>
Cc: Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov>; Davis, Patrick <davis.patrick@epa.gov>
Subject: RE: Perchlorate

Linda has quite the story for you--- she's been working this issue since 2002 (the good old days...)!

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
M: Ex. 6 Personal Privacy (PP)
beck.nancy@epa.gov

From: Forsgren, Lee
Sent: Wednesday, July 19, 2017 12:56 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>

Cc: linda.s.wennerberg@nasa.gov; Davis, Patrick <davis.patrick@epa.gov>
Subject: Re: Perchlorate

Linda,

As former NASA official myself I would be happy talk/meet with you regarding perchlorate.

Regards,
Lee

Sent from my iPhone

On Jul 19, 2017, at 12:47 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:

> Linda,
> Regarding your perchlorate concerns, I suggest you contact Lee Forsgren or Patrick Davis regarding concerns with **Ex. 5 Deliberative Process (DP)**
> I'm sure they would try to assist you.
>
> Regards,
> Nancy
> **********
> Nancy B. Beck, Ph.D., DABT
> Deputy Assistant Administrator
> Office of Chemical Safety and Pollution Prevention
> P: 202-564-1273
> M: Ex. 6 Personal Privacy (PP)
> beck.nancy@epa.gov

Non Delivery Report

From: Microsoft Outlook [MicrosoftExchange329e71ec88ae4615bbc36ab6ce41109e@usepa.onmicrosoft.com]
Sent: 7/19/2017 5:01:40 PM
To: margaret.kieffer@nasa.giv
Subject: Undeliverable: Fwd: Perchlorate
Attachments: Fwd: Perchlorate

Your message did not reach some or all of the intended recipients.

Subject:Fwd: Perchlorate
Sent:7/19/2017 5:01:40 PM

The following recipient(s) cannot be reached:

margaret.kieffer@nasa.giv on 7/19/2017 5:01:40 PM
Diagnostic code = MtsCongested; Reason code = TransferFailed; Status code = 540
< #5.4.310 smtp;550 5.4.310 DNS domain does not exist [Message=InfoDomainNonexistent] [LastAttemptedServerName=nasa.giv] [CY1GCC01FT008.eop-gcc01.prod.protection.outlook.com]>

Delivery has failed to these recipients or groups:

margaret.kieffer@nasa.giv (margaret.kieffer@nasa.giv)
Your message couldn't be delivered. The Domain Name System (DNS) reported that the recipient's domain does not exist.

Contact the recipient by some other means (by phone, for example) and ask them to tell their email admin that it appears that their domain isn't properly registered at their domain registrar. Give them the error details shown below. It's likely that the recipient's email admin is the only one who can fix this problem.

For more information and tips to fix this issue see this article:
http://go.microsoft.com/fwlink/?LinkId=389361.

Diagnostic information for administrators:

Generating server: CY4PR09MB1400.namprd09.prod.outlook.com

margaret.kieffer@nasa.giv
Remote Server returned '550 5.4.310 DNS domain does not exist [Message=InfoDomainNonexistent] [LastAttemptedServerName=nasa.giv] [CY1GCC01FT008.eop-gcc01.prod.protection.outlook.com]'

Original message headers:

DKIM-Signature: v=1; a=rsa-sha256; c=relaxed/relaxed; d=usepa.onmicrosoft.com;

s=selector1-epa-gov;
h=From:Date:Subject:Message-ID:Content-Type:MIME-Version;
bh=d2L1BNH7VZlsPMS4hpGY48Hf6NKbPhK1ULgoi5yoamg=;

b=NLO4O+0xqVUDEx7a4xM7U5L/P6UHToiikZAh1kRQdiF7QDtMoPdl+zUOqGSlCF+icXtVUKu2YymSTejcNoDBPt3
XvGl+HTHgc9mvM24rZQ2gyhHDbwlG9Rj3dih9MIX17YkGhaMdvT1IWo4fDcA334Oq9UhWRXFMtrLZH+lmncQ=
Received: from CY4PR09MB1398.namprd09.prod.outlook.com (10.172.67.20) by
CY4PR09MB1400.namprd09.prod.outlook.com (10.172.67.22) with Microsoft SMTP
Server (version=TLS1_2, cipher=TLS_ECDHE_RSA_WITH_AES_128_CBC_SHA256_P256) id
15.1.1261.13; Wed, 19 Jul 2017 17:01:39 +0000
Received: from CY4PR09MB1398.namprd09.prod.outlook.com ([10.172.67.20]) by
CY4PR09MB1398.namprd09.prod.outlook.com ([10.172.67.20]) with mapi id
15.01.1261.024; Wed, 19 Jul 2017 17:01:39 +0000
From: "Forsgren, Lee" <Forsgren.Lee@epa.gov>
To: "margaret.kieffer@nasa.giv" <margaret.kieffer@nasa.giv>
Subject: Fwd: Perchlorate
Thread-Topic: Perchlorate
Thread-Index: AdMArnzKlcTzfHAoRGWW6urO8dvoKgAAVyNPAAAlr6s=
Date: Wed, 19 Jul 2017 17:01:39 +0000
Message-ID: <813A2FE2-FDCC-4D8A-9BC4-140494A32865@epa.gov>
References:
<CY1PR09MB0553BB85127BA516DF6F526584A60@CY1PR09MB0553.namprd09.prod.outlook.com>,<290B58D
8-80E2-4DAA-9025-6BA7E1C756D8@epa.gov>
In-Reply-To: <290B58D8-80E2-4DAA-9025-6BA7E1C756D8@epa.gov>
Accept-Language: en-US
Content-Language: en-US
X-MS-Has-Attach:
X-MS-TNEF-Correlator:
authentication-results: nasa.giv; dkim=none (message not signed)
header.d=none;nasa.giv; dmarc=none action=none header.from=epa.gov;
x-originating-ip: [2600:1003:b007:daff:4826:a282:ae3:a17b]
x-ms-publictraffictype: Email
x-microsoft-exchange-diagnostics:
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MIME-Version: 1.0
X-OriginatorOrg: epa.gov
X-MS-Exchange-CrossTenant-originalarrivaltime: 19 Jul 2017 17:01:39.3466
 (UTC)
X-MS-Exchange-CrossTenant-fromentityheader: Hosted
X-MS-Exchange-CrossTenant-id: 88b378b3-6748-4867-acf9-76aacbeca6a7
X-MS-Exchange-Transport-CrossTenantHeadersStamped: CY4PR09MB1400

Message

From: Forsgren, Lee [Forsgren.Lee@epa.gov]
Sent: 7/19/2017 5:01:39 PM
To: margaret.kieffer@nasa.giv
Subject: Fwd: Perchlorate

Margaret,
Where is Linda Wennerbergin NASA organization?

Lee

Sent from my iPhone

Begin forwarded message:

> **From:** "Forsgren, Lee" <Forsgren.Lee@epa.gov>
> **Date:** July 19, 2017 at 12:55:39 PM EDT
> **To:** "Beck, Nancy" <Beck.Nancy@epa.gov>
> **Cc:** "linda.s.wennerberg@nasa.gov" <linda.s.wennerberg@nasa.gov>, "Davis, Patrick" <davis.patrick@epa.gov>
> **Subject: Re: Perchlorate**
>
> Linda,
>
> As former NASA official myself I would be happy talk/meet with you regarding perchlorate.
>
> Regards,
> Lee
>
> Sent from my iPhone
>
> On Jul 19, 2017, at 12:47 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:
>
>> Linda,
>> Regarding your perchlorate concerns, I suggest you contact Lee Forsgren or Patrick Davis regarding concerns with the interagency process for the EPA review.
>> I'm sure they would try to assist you.
>>
>> Regards,
>> Nancy
>> **********
>> Nancy B. Beck, Ph.D., DABT
>> Deputy Assistant Administrator
>> Office of Chemical Safety and Pollution Prevention
>> P: 202-564-1273
>> M: Ex. 6 Personal Privacy (PP)
>> beck.nancy@epa.gov

Message

From: Forsgren, Lee [Forsgren.Lee@epa.gov]
Sent: 7/19/2017 7:59:51 PM
To: Kieffer, Margaret (HQ-TH000) [margaret.kieffer@nasa.gov]
Subject: Re: Perchlorate

Darn. Was hoping to get someone on the issue who wouldn't accuse me of killing babies on one hand or preventing space exploration on the other.

Sent from my iPhone

On Jul 19, 2017, at 3:56 PM, Kieffer, Margaret (HQ-TH000) <margaret.kieffer@nasa.gov> wrote:

> Here is her bio. No thank you (re perchlorate). Very familiar w/ it from a remediation stand point. Nasty stuff to clean up, very expensive.
>
> https://www.nasa.gov/feature/krista-c-paquin-associate-administrator-mission-support
>
> ---
>
> **From:** Forsgren, Lee [mailto:Forsgren.Lee@epa.gov]
> **Sent:** Wednesday, July 19, 2017 3:35 PM
> **To:** Kieffer, Margaret (HQ-TH000) <margaret.kieffer@nasa.gov>
> **Subject:** Re: Perchlorate
>
> Now that you mention it I think she may have been at GSFC working for Al Diaz at the time. Does this sound right?
>
> BTW - do you want to become a perchlorate expert? ☺
>
> Sent from my iPhone
>
> On Jul 19, 2017, at 3:30 PM, Kieffer, Margaret (HQ-TH000) <margaret.kieffer@nasa.gov> wrote:
>
>> Not sure. She had a longish career at GSFC at the time, but also did a stint in industry for a several years before coming back to NASA/HQ.
>>
>> M
>>
>> ---
>>
>> **From:** Forsgren, Lee [mailto:Forsgren.Lee@epa.gov]
>> **Sent:** Wednesday, July 19, 2017 3:03 PM
>> **To:** Kieffer, Margaret (HQ-TH000) <margaret.kieffer@nasa.gov>
>> **Subject:** Re: Perchlorate
>>
>> Didn't have the pleasure of working with her when I was at NASA did I ?
>>
>> Sent from my iPhone
>>
>> On Jul 19, 2017, at 2:59 PM, Kieffer, Margaret (HQ-TH000) <margaret.kieffer@nasa.gov> wrote:
>>
>>> Krista Paquin.

From: Forsgren, Lee [mailto:Forsgren.Lee@epa.gov]
Sent: Wednesday, July 19, 2017 2:50 PM
To: Kieffer, Margaret (HQ-TH000) <margaret.kieffer@nasa.gov>
Subject: Re: Perchlorate

Margaret,

Who is the AA in Mission Support now? I have completely lost the bubble.

Lee

Sent from my iPhone

On Jul 19, 2017, at 2:18 PM, Kieffer, Margaret (HQ-TH000) <margaret.kieffer@nasa.gov> wrote:

> She is a chemical engineer in the Mission Support Directorate, Office of Strategic Investment, Environmental Management Div.
>
> https://www.nasa.gov/content/nasas-environmental-management-division
>
> **From:** Forsgren, Lee [mailto:Forsgren.Lee@epa.gov]
> **Sent:** Wednesday, July 19, 2017 1:04 PM
> **To:** Kieffer, Margaret (HQ-TH000) <margaret.kieffer@nasa.gov>
> **Subject:** Fwd: Perchlorate
>
>> Margaret,
>> Where is Linda Wennerbergin NASA organization?
>>
>> Lee
>>
>> Sent from my iPhone
>>
>> Begin forwarded message:
>>
>>> **From:** "Forsgren, Lee" <Forsgren.Lee@epa.gov>
>>> **Date:** July 19, 2017 at 12:55:39 PM EDT
>>> **To:** "Beck, Nancy" <Beck.Nancy@epa.gov>
>>> **Cc:** "linda.s.wennerberg@nasa.gov"

<linda.s.wennerberg@nasa.gov>, "Davis, Patrick" <davis.patrick@epa.gov>

Subject: Re: Perchlorate

Linda,

As former NASA official myself I would be happy talk/meet with you regarding perchlorate.

Regards,
Lee

Sent from my iPhone

On Jul 19, 2017, at 12:47 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:

> Linda,
> Regarding your perchlorate concerns, I suggest you contact Lee Forsgren or Patrick Davis regarding concerns with the interagency process for the EPA review.

I'm sure they would try to assist you.

Regards,
Nancy
****
*

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator
Office of Chemical Safety and Pollution Prevention
P: 202-564-1273
M: [redacted]
Ex. 6 Personal Privacy (PP)
beck.nancy@epa.gov

Message

From: Forsgren, Lee [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A055D7329D5B470FBAA9920CE1B68A7D-FORSGREN, D]
Sent: 10/6/2017 9:40:45 PM
To: Wennerberg, Linda S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
CC: denise.r.thaller@nasa.gov; Mcneill, Mike A (HQ-LD020) [mike.a.mcneill@nasa.gov]
Subject: Re: NASA update

Thanks Linda. Appreciate your working cooperatively within the Federal Family.

Sent from my iPhone

On Oct 6, 2017, at 5:38 PM, Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov> wrote:

> Lee:
> Just a quick note to let you know that NASA submitted comments today on the proposed Interim List for Perchlorate Peer Review and the proposed Draft Charge Questions. We have shared our comments with your senior staff on this matter. EPA's continued support of the interagency process supports good science in decision making and is much appreciated.
>
> NASA also plans to submit comments to the public docket on the Draft BBDR Model Report that is also out for public comment. We understand that other Federal agencies intend to also submit technical and scientific comments to the docket on that Report.
>
> I am sending you this quick update in the spirit of improved communication with the EPA Office Of Water, yourself, and your staff. We welcome continued discussions with you and your team. If you have any questions or need information from the NASA team, please let me know.
>
> Respectfully,
>
> Linda
>
> Linda S. Wennerberg, Ph.D.
> Environmental Management Division
> NASA Headquarters
> MS-2T89
> 300 E Street SW
> Washington, DC 20546-0001
>
> Tel: 202/358-4558
> Cell: Ex. 6 Personal Privacy (PP)
> Fax: 202/358-3948
> linda.s.wennerberg@nasa.gov

Message

From: Forsgren, Lee [Forsgren.Lee@epa.gov]
Sent: 7/19/2017 6:40:21 PM
To: Kieffer, Margaret (HQ-TH000) [margaret.kieffer@nasa.gov]
Subject: Re: Perchlorate

Thanks.

Sent from my iPhone

On Jul 19, 2017, at 2:18 PM, Kieffer, Margaret (HQ-TH000) <margaret.kieffer@nasa.gov> wrote:

> She is a chemical engineer in the Mission Support Directorate, Office of Strategic Investment, Environmental Management Div.
>
> https://www.nasa.gov/content/nasas-environmental-management-division
>
> ---
>
> **From:** Forsgren, Lee [mailto:Forsgren.Lee@epa.gov]
> **Sent:** Wednesday, July 19, 2017 1:04 PM
> **To:** Kieffer, Margaret (HQ-TH000) <margaret.kieffer@nasa.gov>
> **Subject:** Fwd: Perchlorate
>
>> Margaret,
>> Where is Linda Wennerbergin NASA organization?
>>
>> Lee
>>
>> Sent from my iPhone
>>
>> Begin forwarded message:
>>
>>> **From:** "Forsgren, Lee" <Forsgren.Lee@epa.gov>
>>> **Date:** July 19, 2017 at 12:55:39 PM EDT
>>> **To:** "Beck, Nancy" <Beck.Nancy@epa.gov>
>>> **Cc:** "linda.s.wennerberg@nasa.gov" <linda.s.wennerberg@nasa.gov>, "Davis, Patrick" <davis.patrick@epa.gov>
>>> **Subject: Re: Perchlorate**
>>>
>>> Linda,
>>>
>>> As former NASA official myself I would be happy talk/meet with you regarding perchlorate.
>>>
>>> Regards,
>>> Lee
>>>
>>> Sent from my iPhone
>>>
>>> On Jul 19, 2017, at 12:47 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:

Linda,
Regarding your perchlorate concerns, I suggest you contact Lee Forsgren or Patrick Davis regarding concerns with the interagency process for the EPA review.
I'm sure they would try to assist you.

Regards,
Nancy
****
****

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator
Office of Chemical Safety and Pollution Prevention
P: 202-564-1273
M: Ex. 6 Personal Privacy (PP)
beck.nancy@epa.gov

Message

From: Barringer, Jody M. EOP/OMB [Ex. 6 Personal Privacy (PP)]
Sent: 7/8/2018 9:37:08 PM
To: Grevatt, Peter [Grevatt.Peter@epa.gov]
Subject: Automatic reply: Technical Working Group - EPA - perchlorate

I will be out of the office until Wednesday, July 11th. If you need immediate assistance, please contact Leah Fine at lfine@omb.eop.gov. Otherwise, I will respond to your email when I return.

Thank you.

Message

From: Wennerberg, Linda S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
Sent: 7/8/2018 9:36:35 PM
To: Grevatt, Peter [Grevatt.Peter@epa.gov]
Subject: Automatic reply: Technical Working Group - EPA - perchlorate

I am out on leave, returning on Monday, July 9th. If your matter is urgent, please contact Ex. 6 Personal Privacy (PP) Ex. 6 Personal Privacy (PP)

Message

From: WENNERBERG, LINDA S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]
Sent: 7/28/2016 6:57:30 PM
To: Shapiro, Mike [Shapiro.Mike@epa.gov]; Grevatt, Peter [Grevatt.Peter@epa.gov]
CC: Laity, James A. Ex. 6 EOP (PP); Dorjets, Vlad Ex. 6 EOP (PP); Ex. 6 EOP (PP); Mcneill, Mike A (HQ-LD020) [mike.a.mcneill@nasa.gov]; james.leatherwood-1@nasa.gov; Ex. 6 EOP (PP)
Subject: RE: URGENT: EPA update on Perchlorate actions

Importance: High

All:

I appreciate that EPA has quickly responded to clarify the proposed plans to conduct Peer Review and public comment in the Perchlorate MCL process. I just got a very clear and informative call from Eric Burneson who walked me through a general outline of proposed next steps. His input addressed my questions and the underlying confusion among federal agencies on the next steps and the role of interagency review.

As I understand it, EPA plans to Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

EPA plans that there will be Ex. 5 Deliberative Process (DP)
Ex. 5 Deliberative Process (DP). NASA plans to build our team to facilitate our review and the submission of comments to the public docket.

I now understand the proposed public comment process and opportunity for document review. I appreciate EPA's response to clarify and explain the proposed next steps and will share this information among my interagency colleagues. My concern remains that Ex. 5 Deliberative Process (DP)
Ex. 5 Deliberative Process (DP) Please consider our requests for meetings and information sharing to minimize confusion and promote information sharing among the federal agencies on this and other Office of Water efforts.

Again, thanks to EPA for providing me a clearer picture of proposed events. We at NASA are committed to promoting sound science and interagency dialogue. We will participate in the next round of public review and comments.

Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89
300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948

linda.s.wennerberg@nasa.gov

From: WENNERBERG, LINDA S. (HQ-LD020)
Sent: Thursday, July 28, 2016 12:35 PM
To: 'Shapiro, Mike' <Shapiro.Mike@epa.gov>; Grevatt, Peter <Grevatt.Peter@epa.gov>
Cc: Laity, James A. <Ex. 6 EOP (PP)>; Dorjets, Vlad <Ex. 6 EOP (PP)>; Kim, Jim <Ex. 6 EOP (PP)>; McNeill, Mike A (HQ-LD020) <mike.a.mcneill@nasa.gov>; Leatherwood, James (HQ-LD020) <james.leatherwood-1@nasa.gov>; Rodan, Bruce <Ex. 6 EOP (PP)>
Subject: URGENT: Perchlorate actions
Importance: High

Mike and Peter;

I just received a call from a senior colleague at DoD regarding Ex. 5 Deliberative Process (DP). I am led to believe that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP). I am writing to dispel any confusion or misrepresentation.

I, on behalf of NASA, am most concerned about Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) I request that you get back to me on EPA's plans and strongly request that you consider Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) The federal family has long worked together to ensure sound science is utilized for perchlorate.

NASA requested Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Please contact me as soon as possible to communicate EPA's plans.

Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89
300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

Appointment

From: Hafez, Ahmed [Hafez.Ahmed@epa.gov]
Sent: 10/18/2017 3:39:19 PM
To: Hafez, Ahmed [Hafez.Ahmed@epa.gov]; Burneson, Eric [Burneson.Eric@epa.gov]; Miller, Gregory [Miller.Gregory@epa.gov]; Flowers, Lynn [Flowers.Lynn@epa.gov]; Christ, Lisa [Christ.Lisa@epa.gov]
CC: Hernandez-Quinones, Samuel [Hernandez.Samuel@epa.gov]

Subject: Canceled: Finalizing Perchlorate Charge Questions
Attachments: Proposed Draft SBA Edits to Percholorate Charge Questions v2 October 5.docx; RE: Perchlorate Charge Question - question about Lisa's email discussion below
Location: Call-in or Eric's Office (WJC-E 2331A)

Start: 10/19/2017 3:30:00 PM
End: 10/19/2017 4:30:00 PM
Show Time As: Free

Importance: High

Appointment

From: Hafez, Ahmed [Hafez.Ahmed@epa.gov]
Sent: 10/18/2017 3:39:19 PM
To: Hafez, Ahmed [Hafez.Ahmed@epa.gov]; Burneson, Eric [Burneson.Eric@epa.gov]; Miller, Gregory [Miller.Gregory@epa.gov]; Flowers, Lynn [Flowers.Lynn@epa.gov]; Christ, Lisa [Christ.Lisa@epa.gov]
CC: Hernandez-Quinones, Samuel [Hernandez.Samuel@epa.gov]

Subject: Canceled: Finalizing Perchlorate Charge Questions
Attachments: Proposed Draft SBA Edits to Percholorate Charge Questions v2 October 5.docx; RE: Perchlorate Charge Question - question about Lisa's email discussion below; DRAFT FINAL MCLG Approach Charge 9-5-17.docx
Location: Call-in or Eric's Office (WJC-E 2331A)

Start: 10/19/2017 3:30:00 PM
End: 10/19/2017 4:30:00 PM
Show Time As: Free

Importance: High

Hi All,

Meeting to discuss comments on the charge questions and finalize revisions.

Attached:
SBA email and proposed changes
EPA email response to SBA questions
Current version of the Charge

Call-in number to follow.

Thanks,
Ahmed

Message

From: Gibson, Mark [mark_gibson@americanchemistry.com]
Sent: 9/3/2019 7:16:00 PM
To: Hernandez-Quinones, Samuel [Hernandez.Samuel@epa.gov]
Subject: RE: Posting status of ACC's comments on proposed perchlorate NPDWR?
Attachments: ATT00001.txt

Hello Sam.

Thanks for the timely response.

Cheers,

Mark Gibson

From: Hernandez-Quinones, Samuel [mailto:Hernandez.Samuel@epa.gov]
Sent: Tuesday, September 3, 2019 2:52 PM
To: Gibson, Mark <mark_gibson@americanchemistry.com>
Subject: RE: Posting status of ACC's comments on proposed perchlorate NPDWR?

Hi Mark

The comment letter was posted on the public website today. We received ACC's letter on August 26, 2019.

The letter is available at https://www.regulations.gov/document?D=EPA-HQ-OW-2018-0780-0263

Thanks
Sam
==========
Samuel Hernández Quiñones, P.E.
Environmental Engineer
Office of Water
Environmental Protection Agency
1200 Pennsylvania Ave. NW
Washington, DC 20460
202-564-1735

"USEPA Protecting Human Health and the Environment"

From: Gibson, Mark <mark_gibson@americanchemistry.com>
Sent: Tuesday, September 03, 2019 11:31 AM
To: Hernandez-Quinones, Samuel <Hernandez.Samuel@epa.gov>
Subject: Posting status of ACC's comments on proposed perchlorate NPDWR?

Good morning Mr. Hernandez.

My name is Mark Gibson and I work for ACC's Chlorine Chemistry Division. I am writing to ask if you can tell me the posting status of ACC's comments to regulations.gov per forwarded email below. If you can suggest an alternative person to contact, please do so.

Thank you,

Mark

Mark C. Gibson | American Chemistry Council
Director, Chlorine Issues
Mark_Gibson@americanchemistry.com
700 2nd Street NE | Washington, DC | 20002
O: 202-249-6738 | C: Ex. 6 Personal Privacy (PP)
www.americanchemistry.com

From: no-reply@regulations.gov [mailto:no-reply@regulations.gov]
Sent: Monday, August 26, 2019 4:21 PM
To: Gibson, Mark <mark_gibson@americanchemistry.com>
Subject: Your Comment Submitted on Regulations.gov (ID: EPA-HQ-OW-2018-0780-0001)

Please do not reply to this message. This email is from a notification only address that cannot accept incoming email.

Your comment was submitted successfully!

Comment Tracking Number: 1k3-9btw-97ub

Your comment may be viewable on Regulations.gov once the agency has reviewed it. This process is dependent on agency public submission policies/procedures and processing times. Use your tracking number to find out the status of your comment.

Agency: Environmental Protection Agency (EPA)
Document Type: Rulemaking
Title: National Primary Drinking Water Regulations: Perchlorate
Document ID: EPA-HQ-OW-2018-0780-0001

Comment:
Please see the attached comments of the American Chemistry Council.

Uploaded File(s):

- ACC Comments_perchlorate in DW_8-26-19_FINAL.pdf

For further information about the Regulations.gov commenting process, please visit https://www.regulations.gov/faqs.

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Message

From: Gibson, Mark [mark_gibson@americanchemistry.com]
Sent: 9/3/2019 3:30:43 PM
To: Hernandez-Quinones, Samuel [Hernandez.Samuel@epa.gov]
Subject: Posting status of ACC's comments on proposed perchlorate NPDWR?

Good morning Mr. Hernandez.

My name is Mark Gibson and I work for ACC's Chlorine Chemistry Division. I am writing to ask if you can tell me the posting status of ACC's comments to regulations.gov per forwarded email below. If you can suggest an alternative person to contact, please do so.

Thank you,

Mark

Mark C. Gibson | American Chemistry Council
Director, Chlorine Issues
Mark_Gibson@americanchemistry.com
700 2nd Street NE | Washington, DC | 20002
O: 202-249-6738 | C: Ex. 6 Personal Privacy (PP)
www.americanchemistry.com

From: no-reply@regulations.gov [mailto:no-reply@regulations.gov]
Sent: Monday, August 26, 2019 4:21 PM
To: Gibson, Mark <mark_gibson@americanchemistry.com>
Subject: Your Comment Submitted on Regulations.gov (ID: EPA-HQ-OW-2018-0780-0001)

regulations.gov
Your Voice in Federal Decision-Making

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Your comment was submitted successfully!

Comment Tracking Number: 1k3-9btw-97ub

Your comment may be viewable on Regulations.gov once the agency has reviewed it. This process is dependent on agency public submission policies/procedures and processing times. Use your tracking number to find out the status of your comment.

Agency: Environmental Protection Agency (EPA)
Document Type: Rulemaking
Title: National Primary Drinking Water Regulations: Perchlorate
Document ID: EPA-HQ-OW-2018-0780-0001

Comment:
Please see the attached comments of the American Chemistry Council.

Uploaded File(s):

- ACC Comments_perchlorate in DW_8-26-19_FINAL.pdf

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Message

From: Kim, Jim H. EOP/OMB Ex. 6 EOP (PP)
Sent: 4/5/2018 1:05:28 PM
To: Hernandez-Quinones, Samuel [Hernandez.Samuel@epa.gov]
Subject: Automatic reply: Peer Review Final Report - Perchlorate

I am currently out of the office. If you need immediate assistance, please contact Margo Schwab at Ex. 6 EOP (PP)). I will be back in the office on April 9.

Thanks,
Jim

Message

From: Hernandez-Quinones, Samuel [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=4C46D56B82F143DF82F81D322BD109D7-HERNANDEZ-QUINONES, SAMUEL]
Sent: 9/3/2019 6:52:14 PM
To: Gibson, Mark [mark_gibson@americanchemistry.com]
Subject: RE: Posting status of ACC's comments on proposed perchlorate NPDWR?

Hi Mark

The comment letter was posted on the public website today. We received ACC's letter on August 26, 2019.

The letter is available at https://www.regulations.gov/document?D=EPA-HQ-OW-2018-0780-0263

Thanks
Sam
==========
Samuel Hernández Quiñones, P.E.
Environmental Engineer
Office of Water
Environmental Protection Agency
1200 Pennsylvania Ave. NW
Washington, DC 20460
202-564-1735

"USEPA Protecting Human Health and the Environment"

From: Gibson, Mark <mark_gibson@americanchemistry.com>
Sent: Tuesday, September 03, 2019 11:31 AM
To: Hernandez-Quinones, Samuel <Hernandez.Samuel@epa.gov>
Subject: Posting status of ACC's comments on proposed perchlorate NPDWR?

Good morning Mr. Hernandez.

My name is Mark Gibson and I work for ACC's Chlorine Chemistry Division. I am writing to ask if you can tell me the posting status of ACC's comments to regulations.gov per forwarded email below. If you can suggest an alternative person to contact, please do so.

Thank you,

Mark

Mark C. Gibson | American Chemistry Council
Director, Chlorine Issues
Mark_Gibson@americanchemistry.com
700 2nd Street NE | Washington, DC | 20002
O: 202-249-6738 | C: Ex. 6 Personal Privacy (PP)
www.americanchemistry.com

From: no-reply@regulations.gov [mailto:no-reply@regulations.gov]
Sent: Monday, August 26, 2019 4:21 PM

To: Gibson, Mark <mark_gibson@americanchemistry.com>
Subject: Your Comment Submitted on Regulations.gov (ID: EPA-HQ-OW-2018-0780-0001)

Please do not reply to this message. This email is from a notification only address that cannot accept incoming email.

Your comment was submitted successfully!

Comment Tracking Number: 1k3-9btw-97ub

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Agency: Environmental Protection Agency (EPA)
Document Type: Rulemaking
Title: National Primary Drinking Water Regulations: Perchlorate
Document ID: EPA-HQ-OW-2018-0780-0001

Comment:
Please see the attached comments of the American Chemistry Council.

Uploaded File(s):

- ACC Comments_perchlorate in DW_8-26-19_FINAL.pdf

For further information about the Regulations.gov commenting process, please visit https://www.regulations.gov/faqs.

NOTICE: This email originated from a source outside of the American Chemistry Council. Do not click any links or access attachments unless you are expecting them, and know that the content is safe.
++++++++++++++++++++++++++++ This message may contain confidential information and is intended only for the individual named. If you are not the named addressee do not disseminate, distribute or copy this email. Please notify the sender immediately by email if you have received this email by mistake and delete this email from your system. E-mail transmission cannot be guaranteed to be secure or error-free as information could be intercepted, corrupted, lost, destroyed, arrive late or incomplete, or contain viruses. The sender therefore does not accept liability for any errors or omissions in the contents of this message which arise as a result of email transmission. American Chemistry Council, 700 – 2nd Street NE, Washington, DC 20002, www.americanchemistry.com

Message

From: Olson, Daniel [Olson.Daniel@epa.gov]
Sent: 10/28/2016 7:50:06 PM
To: Schlosser, Paul [Schlosser.Paul@epa.gov]; Flowers, Lynn [Flowers.Lynn@epa.gov]; Perkinson, Russ [Perkinson.Russ@epa.gov]
CC: Huff, Lisa [Huff.Lisa@epa.gov]
Subject: RE: NASA Comments, Draft Perchlorate Model Peer Review Charge Questions

Got it, thanks. Have a great weekend everyone!

From: Schlosser, Paul
Sent: Friday, October 28, 2016 3:48 PM
To: Olson, Daniel <Olson.Daniel@epa.gov>; Flowers, Lynn <Flowers.Lynn@epa.gov>; Perkinson, Russ <Perkinson.Russ@epa.gov>
Cc: Huff, Lisa <Huff.Lisa@epa.gov>
Subject: RE: NASA Comments, Draft Perchlorate Model Peer Review Charge Questions

Monday is my CDO, though I do plan to come on-line from ~ 11-noon to participate in a PCB assessment team meeting... they are covering how to best use SharePoint for that. But otherwise I have a pretty full personal day lined up.

So I'll likely check my in-box and can respond briefly to urgent/big things in that late morning slot. Otherwise please do proceed with further edits.

-Paul

From: Olson, Daniel
Sent: Friday, October 28, 2016 3:36 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Flowers, Lynn <Flowers.Lynn@epa.gov>; Perkinson, Russ <Perkinson.Russ@epa.gov>
Cc: Huff, Lisa <Huff.Lisa@epa.gov>
Subject: RE: NASA Comments, Draft Perchlorate Model Peer Review Charge Questions

A herculean effort, thanks!

I haven't looked at it closely, but do you want to take another look at it on Monday or would you like us to pick it up?

From: Schlosser, Paul
Sent: Friday, October 28, 2016 3:26 PM
To: Olson, Daniel <Olson.Daniel@epa.gov>; Flowers, Lynn <Flowers.Lynn@epa.gov>; Noyes, Pamela <Noyes.Pamela@epa.gov>; Perkinson, Russ <Perkinson.Russ@epa.gov>
Subject: RE: NASA Comments, Draft Perchlorate Model Peer Review Charge Questions

I've done my best to incorporate the changes suggested by NASA, with modifications as indicated in my previous emails, into the last draft from Lynn. Ex. 5 Deliberative Process (DP) Also I put in a bit in Ex. 5 Deliberative Process (DP)

There's one thing they suggest that Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) That is noted with a comment.

This may be too much now, but it's a starting point for us to further edit.

-Paul

From: Olson, Daniel
Sent: Friday, October 28, 2016 1:04 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Flowers, Lynn <Flowers.Lynn@epa.gov>; Noyes, Pamela <Noyes.Pamela@epa.gov>; Perkinson, Russ <Perkinson.Russ@epa.gov>
Subject: FW: NASA Comments, Draft Perchlorate Model Peer Review Charge Questions

Passing along the invitation to call NASA regarding questions or clarifications.

From: Wennerberg, Linda S. (HQ-LD020) [mailto:linda.s.wennerberg@nasa.gov]
Sent: Friday, October 28, 2016 1:00 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>
Cc: Olson, Daniel <Olson.Daniel@epa.gov>; Mcneill, Mike A (HQ-LD020) <mike.a.mcneill@nasa.gov>
Subject: RE: NASA Comments, Draft Perchlorate Model Peer Review Charge Questions

Eric:
You are most welcome. NASA appreciated the opportunity to review and comment on these important Charge Questions.

I sent these changes in redline form to make it easier for EPA to see and consider NASA's comments. I hope that you all can see all the input, including Ex. 5 Deliberative Process (DP) when viewed in All Markup format. Again, you or your team have any questions or need clarification on the NASA comments, please contact me.

Thanks,

Linda

Linda S. Wennerberg, Ph.D.
Environmental Management Division
NASA Headquarters
MS-2T89
300 E Street SW
Washington, DC 20546-0001

Tel: 202/358-4558
Cell: Ex. 6 Personal Privacy (PP)
Fax: 202/358-3948
linda.s.wennerberg@nasa.gov

From: Burneson, Eric [mailto:Burneson.Eric@epa.gov]
Sent: Friday, October 28, 2016 12:56 PM
To: Wennerberg, Linda S. (HQ-LD020) <linda.s.wennerberg@nasa.gov>
Cc: Olson, Daniel <Olson.Daniel@epa.gov>; Mcneill, Mike A (HQ-LD020) <mike.a.mcneill@nasa.gov>
Subject: RE: NASA Comments, Draft Perchlorate Model Peer Review Charge Questions

Thanks Linda we received your comments and appreciate you sending them in track changes to enable us to understand precisely where and how you recommend we modify the charge.

Eric G. Burneson, P.E.
Director of Standards and Risk Management
Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency

Appointment

From: Johnson, Ann [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=BF046C29B1604A0E9F712B671C4F3CE5-JOHNSON, ANN]
Sent: 7/10/2018 7:06:25 PM
To: Ex. 6 EOP (PP)

Subject: Accepted: FW: TWG - Perchlorate
Location: Conference Call

Start: 7/11/2018 6:00:00 PM
End: 7/11/2018 7:00:00 PM
Show Time As: Busy

Message

From: Muellerleile, Caryn [Muellerleile.Caryn@epa.gov]
Sent: 10/18/2016 1:17:44 PM
To: Johnson, Ann [Johnson.Ann@epa.gov]
Subject: FW: Perchlorate Peer Review Advance Materials

From: Rennert, Kevin
Sent: Monday, October 17, 2016 2:00 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Eric – thanks for flagging. I thought we had actually put this to bed on our call with Jim on Friday, as I'd stated that we were planning Ex. 5 Deliberative Process (DP) He thought that sounded fine, and in alignment with Jim Kim, which is what he cared about. It looks from the below email like that's not the case. I'll call Jim L. and discuss, and will follow up afterwards. Thanks. Kevin

---

From: Burneson, Eric
Sent: Monday, October 17, 2016 10:11 AM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: Fw: Perchlorate Peer Review Advance Materials

Kevin: Apologies if we have already worked through a path forward on the interagency review of the perchlorate peer review charge but OMB continues to ask for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) We think this sets up a dynamic in which Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) We would appreciate any guidance as to how to respond this request from Jim.

I am at the ASDWA meeting in Milwaukee but am happy to discuss if needed
Eric Burneson Ex. 6 Personal Privacy (PP)

From: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP)
Sent: Monday, October 17, 2016 2:02 PM
To: Christ, Lisa
Cc: Dorjets, Vlad EOP/OMB; Schwab, Margo EOP/OMB; Grevatt, Peter; Greene, Ashley; Burneson, Eric
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

I forgot to ask – can we schedule a teleconference for interagency discussion of Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) I think it is important to understand Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)

Jim

From: Kim, Jim H. EOP/OMB
Sent: Monday, October 17, 2016 9:58 AM
To: 'Christ, Lisa' <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thank you so much!

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Monday, October 17, 2016 9:56 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Jim,
I wanted to provide the information for the October 20th call on the perchlorate BBDR model charge questions. Conference Ex. 6 Personal Privacy (PP) access code Ex. 6 Personal Privacy (PP)

Interagency comments on the charge questions will be provided to EPA and comments on the BBDR model and report will be submitted to the docket. We respectfully request comments on the charge as soon as possible after the meeting -- October 27 would be ideal to allow ample time for peer reviewers consideration.

Sincerely,
Lisa

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 Personal Privacy (PP)
Sent: Thursday, October 06, 2016 2:56 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks. Also – I wanted to confirm that if Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) That would give EPA a little more than 2 weeks to consider interagency comments. So our goal will be for interagency scientists to provide comments on the peer review charge shortly after the October 20 call, and then have a discussion on November 7/8. Also – I wanted to confirm that our comments on the

peer review charge would be submitted to you, while comments on the BBDR draft report and model will be submitted to the docket.

I am thinking that a 3rd meeting prior to the end of November would be useful to discuss Ex. 5 Deliberative Process (DP)
Ex. 5 Deliberative Process (DP)

Please let me know your thoughts.

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Thursday, October 6, 2016 7:35 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Re: Perchlorate Peer Review Advance Materials

Hi Jim
Of course. I held the time on EPA participants calendars, so I'll send confirmation. I'll set up logistics and send you the information soon.
Lisa

Sent from my iPhone

On Oct 5, 2016, at 4:40 PM, Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)> wrote:

> Hi Lisa,
>
> Thanks for the info. Sorry for the late response. Can we go ahead and schedule a meeting for October 20? Thanks for your patience.
>
> Best regards,
> Jim
>
> ---
>
> **From:** Christ, Lisa [mailto:Christ.Lisa@epa.gov]
> **Sent:** Friday, September 30, 2016 11:04 AM
> **To:** Kim, Jim H. EOP/OMB <Ex. 6 Personal Privacy (PP)>; Dorjets, Vlad EOP/OMB <Ex. 6 Personal Privacy (PP)>; Schwab, Margo EOP/OMB <Ex. 6 Personal Privacy (PP)>
> **Cc:** Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
> **Subject:** RE: Perchlorate Peer Review Advance Materials
>
> Jim
> The closing date for public comments on the charge questions is October 21. The public comment period for the model and report ends November 14. We intend to have our contractor identify the peer reviewers and we'll finalize charge questions between October 21 and November 14. [potential call dates are Oct 20 & 27]
>
> Public comments will be summarized and provided to the peer reviewers at the end of November around the same time we publish the next federal register notice announcing a meeting date sometime in early January (based on availability of peer reviewers and at least 30 days before the panel meeting). [potential call dates are Nov 7 & 8]
>
> Lisa

From: Kim, Jim H. EOP/OMB [mailto: Ex. 6 Personal Privacy (PP)]
Sent: Friday, September 30, 2016 10:27 AM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) ;
Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thanks for the dates and times. I was hoping to get something on our calendars earlier to ensure that Ex. 5 Deliberative Process (DP) Can you tell me the timeline that is planned in regards to the Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) ?

Thanks,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 9:42 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Schwab, Margo EOP/OMB < Ex. 6 Personal Privacy (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim,
I'd like to suggest we schedule two meetings and see if an additional meeting, as suggested, is needed. I looked at calendars for relevant EPA folks and can offer the following dates/times.
1st meeting: October 20 from 4-5pm OR October 27 from 405pm
2nd meeting: November 7 from 11-11am OR November 8 from 11a-noon

I am unable to find availability sooner than Oct 20. However, that provides time for review of materials before discussions begin.
Please let me know at your earliest convenience which times work and I will set up a conference call.
Lisa

From: Kim, Jim H. EOP/OMB [mailto Ex. 6 Personal Privacy (PP)]
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) Ex. 6 Personal Privacy (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) the second would be to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) and the third would Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, September 28, 2016 10:36 AM
To: Dorjets, Vlad EOP/OMB < Ex. 6 Personal Privacy (PP) >; Kim, Jim H. EOP/OMB < Ex. 6 Personal Privacy (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

Lisa Christ, Chief
Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

Message

From: Owens, Nicole [Owens.Nicole@epa.gov]
Sent: 10/17/2016 8:21:20 PM
To: Johnson, Ann [Johnson.Ann@epa.gov]
Subject: RE: Perchlorate Peer Review Advance Materials

I think Jim and Kevin spoke about it.

From: Johnson, Ann
Sent: Monday, October 17, 2016 4:21 PM
To: Owens, Nicole <Owens.Nicole@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Thanks so much.

Do you know if this came up in the regular OMB call, or in another call with OMB on Friday?

From: Owens, Nicole
Sent: Monday, October 17, 2016 4:10 PM
To: Johnson, Ann <Johnson.Ann@epa.gov>
Subject: FW: Perchlorate Peer Review Advance Materials

From: Rennert, Kevin
Sent: Monday, October 17, 2016 2:00 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Eric – thanks for flagging. I thought we had actually put this to bed on our call with Jim on Friday, as I'd stated that we were planning Ex. 5 Deliberative Process (DP) He thought that sounded fine, and in alignment with Jim Kim, which is what he cared about. It looks from the below email like that's not the case. I'll call Jim L. and discuss, and will follow up afterwards. Thanks. Kevin

---

From: Burneson, Eric
Sent: Monday, October 17, 2016 10:11 AM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: Fw: Perchlorate Peer Review Advance Materials

Kevin: Apologies if we have already worked through a path forward on the interagency review of the perchlorate peer review charge but OMB continues to ask for Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) We think this sets up a dynamic in which Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) We would appreciate any guidance as to how to respond this request from Jim.

I am at the ASDWA meeting in Milwaukee but am happy to discuss if needed
Eric Burneson Ex. 6 Personal Privacy (PP)

From: Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)
Sent: Monday, October 17, 2016 2:02 PM
To: Christ, Lisa
Cc: Dorjets, Vlad EOP/OMB; Schwab, Margo EOP/OMB; Grevatt, Peter; Greene, Ashley; Burneson, Eric
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

I forgot to ask – can we schedule a teleconference for interagency discussion of Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) I think it is important to understand EPA's perspectives on Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP)

Jim

From: Kim, Jim H. EOP/OMB
Sent: Monday, October 17, 2016 9:58 AM
To: 'Christ, Lisa' <Christ.Lisa@epa.gov>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Lisa,

Thank you so much!

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Monday, October 17, 2016 9:56 AM
To: Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>
Cc: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Schwab, Margo EOP/OMB <Ex. 6 EOP (PP)>; Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Hi Jim,
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Conference Ex. 6 Personal Privacy (PP) access code Ex. 6 Personal Privacy (PP).

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Lisa Christ, Chief
Targeting and Analysis Branch
Office of Ground Water and Drinking Water
USEPA
1200 Pennsylvania Ave NW
Washington, DC 20460-0001
phone: 202.564.8354
fax: 202.564-3760
Mail Code: 4607M
~~~~~~

Message

From: Johnson, Ann [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=BF046C29B1604A0E9F712B671C4F3CE5-JOHNSON, ANN]
Sent: 6/13/2017 12:26:20 AM
To: Rees, Sarah [rees.sarah@epa.gov]
Subject: FW: Perchlorate Peer Review Advance Materials

From: Muellerleile, Caryn
Sent: Thursday, October 20, 2016 1:34 PM
To: Johnson, Ann <Johnson.Ann@epa.gov>
Subject: FW: Perchlorate Peer Review Advance Materials

From: Burneson, Eric
Sent: Thursday, October 20, 2016 1:33 PM
To: Rennert, Kevin <Rennert.Kevin@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Christ, Lisa <Christ.Lisa@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Kevin: The interagency call about the perchlorate peer review charge is today at 4:00. We have not responded to Jim Kim's request for a second call and expect that he will raise this question in our discussion. I assume that our answer is that Ex. 5 Deliberative Process (DP)
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Let us know if we should take a different approach to responding.

Eric G. Burneson, P.E.
Director of Standards and Risk Management
Office of Ground Water and Drinking Water
U.S. Environmental Protection Agency

From: Rennert, Kevin
Sent: Monday, October 17, 2016 2:00 PM
To: Burneson, Eric <Burneson.Eric@epa.gov>; Owens, Nicole <Owens.Nicole@epa.gov>; Muellerleile, Caryn <Muellerleile.Caryn@epa.gov>
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Eric – thanks for flagging. I thought we had actually put this to bed on our call with Jim on Friday, as I'd stated that we were planning Ex. 5 Deliberative Process (DP) He thought that sounded fine, and in alignment with Jim Kim, which is what he cared about. It looks from the below email like that's not the case. I'll call Jim L. and discuss, and will follow up afterwards. Thanks. Kevin

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From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Friday, September 30, 2016 9:42 AM
To: Kim, Jim H. EOP/OMB < Ex. 6 EOP (PP) ; Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) ; Schwab, Margo EOP/OMB < Ex. 6 EOP (PP) >
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Jim,
I'd like to suggest we schedule two meetings and see if an additional meeting, as suggested, is needed. I looked at calendars for relevant EPA folks and can offer the following dates/times.
1st meeting: October 20 from 4-5pm OR October 27 from 405pm
2nd meeting: November 7 from 11-11am OR November 8 from 11a-noon

I am unable to find availability sooner than Oct 20. However, that provides time for review of materials before discussions begin.
Please let me know at your earliest convenience which times work and I will set up a conference call.
Lisa

From: Kim, Jim H. EOP/OMB [mailto:J Ex. 6 EOP (PP)
Sent: Wednesday, September 28, 2016 12:01 PM
To: Christ, Lisa <Christ.Lisa@epa.gov>; Dorjets, Vlad EOP/OMB < Ex. 6 EOP (PP) ; Ex. 6 EOP (PP)
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: RE: Perchlorate Peer Review Advance Materials

Lisa,

Thank you for the peer review materials, and for the informative briefing yesterday. We will distribute to the other interagency scientists that were on the call yesterday, and provide input on the charge questions within the 21 days that agreed on.

I would also like to get 3 interagency meetings on our calendars during the public comment and peer review process to continue the dialogue from yesterday. The first would be in the next week or so to discuss Ex. 5 Deliberative Process (DP) the second would be to discuss Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) and the third would Ex. 5 Deliberative Process (DP)

Can we start looking at dates/times for the end of next week?

Best regards,
Jim

From: Christ, Lisa [mailto:Christ.Lisa@epa.gov]
Sent: Wednesday, September 28, 2016 10:36 AM
To: Dorjets, Vlad EOP/OMB <Ex. 6 EOP (PP)>; Kim, Jim H. EOP/OMB <Ex. 6 EOP (PP)>
Cc: Grevatt, Peter <Grevatt.Peter@epa.gov>; Greene, Ashley <Greene.Ashley@epa.gov>; Burneson, Eric <Burneson.Eric@epa.gov>
Subject: Perchlorate Peer Review Advance Materials

Vlad, Jim and Margo,
Attached are the perchlorate peer review materials we discussed yesterday. Please let me know if you have questions or need additional information.

Thank you,
Lisa

~~~~~~

Lisa Christ, Chief
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A LITERATURE REVIEW OF THE CURRENT STATE OF THE SCIENCE REGARDING SPECIES DIFFERENCES IN THE CONTROL OF, AND RESPONSE TO, THYROID HORMONE PERTURBATIONS. PART 1: A HUMAN HEALTH PERSPECTIVE

Report No:

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Report prepared for Sponsor:

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12TH AUGUST 2019

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THIS REPORT IS APPROVED AND ISSUED BY:

AUTHOR: _____ **DATE:** 12TH AUGUST 2019

Prof John R Foster BSc, PhD, DipRCPath, FRCPath, FIATP. Regulaatory Science Ltd

REVIEWER: _____ **DATE:** 12th AUGUST 2019

Dr M Provan BSc PhD. Regulatory Science Ltd

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1. Executive Summary

This review was commissioned by the European Crop Protection Association to address a series of questions raised following the issue of the draft guidance document (GD) from the joint ECHA/EFSA/JRC recommendations. The questions asked specifically related to the literature used by the draft guidance and whether or not it represented a state of the current science regarding chemical thyroid disruption. The scope of this review is only related to that part of the guidance that refers to the thyroid and does not include reference to the non-thyroid hormonal effects.

While the intention was that this document would present the laboratory animal data related to chemical induced disruption, and a second document would cover the human evidence, it was apparent in beginning the review that a document that ignored the human data was not going to be possible without seriously compromising the scientific content. This was because of the enormous amount of basic information, derived from human thyroid diseases and the clinical treatment of those diseases, that was available and its incorporation was considered integral to understanding the laboratory animal data in order to place animal findings into the context of human relevance and risk.

Although there are numerous publications detailing the basic control of thyroid hormones it was decided that the first part of the review would require an introduction to the normal control and functioning of thyroid hormones in order for the reader to understand the subsequent sections dealing with perturbations in thyroid homeostasis. The review is structured to address the ECPA questions in order and covers species differences in the functional morphology and biology of the thyroid gland always with an eye to comparing the most common laboratory species for toxicity testing, the rat, with what we know about human thyroid responses. In terms of the effects of thyroid hormone perturbation there are two major endpoints of concern, namely neurodevelopmental effects on the foetus and neonate, and thyroid cancer. These have generally been separated where possible since the critical features of both, in terms of thyroid effects and the consequences of dysfunction in one part of the thyroid control process, are different between those acting in carcinogenesis and those determining foetal and postnatal development.

Section 4 of this review is an appraisal of the GD and, on the whole, it provides an excellent roadmap for prospective registrants of new products and describes in detail what will be expected in terms of levels of proof, what it terms "lines of evidence". Throughout, the GD illustrates its points with relevant literature references to clarify where necessary. However, it is highly selective in its use of the literature and in no way does it aspire to be a "state of the science" review of the area of endocrine disruption and neither is this within the stated scope of the document. Appendix A of the final document is a summary of the information regarding thyroid physiology and provides a high-level opinion on how chemicals could secondarily affect the thyroid predominantly through a primary effect on the liver and it is this section of the GD that this review is primarily aimed. There are a number of other appendices dealing with the design, conduct and interpretation of hormonal studies, available software tools for SAR, an Excel template for reporting data, suggestions for developing a literature search strategy and an example of a MOA for a non-target organism, amongst others, but a discussion of these is beyond the scope of this document,

While the GD does mention alternative modes of action, such as effects on the sodium-iodide symporter, as potential thyroid targets, the degree of coverage is superficial in the extreme and would not permit any reader from understanding these processes, let alone the other thyroid targets that are present. It is clearly not a state of the science document, as regards the thyroid gland, but it does take the opportunity of repeating a fallacy of there being effects on thyroid hormones in the absence of histopathology on the gland and while they have a heading in the appendix entitled "Investigations of perturbations of circulating thyroid hormone in the absence of histological changes in adults" this section fails to support the assumptions of the title without providing any literature evidence that this occurs and, considering how important this would be if proven, it is very disappointing that, what appears to be an anecdotal comment made, is allowed remain and to go unchallenged in the final GD. It would have been most helpful to the critical reader if a relevant mammalian literature reference could have been included to substantiate this statement since it has the potential to undermine one of the most sensitive and regularly conducted endpoints for most of the in vivo assays. While this is

extremely disappointing it simply displays the perils of straying off the stated scope of the document into what appears, at face value, to be an uninformed, and frankly dangerous, foray into prejudice. The heading at the very least should be removed as it lends credence to an unsubstantiated statement in a document by a group that until now has been held in high esteem by its peers.

Section 6 of this review document details the literature evidence for species differences between human and rodents addressing each function in thyroid hormone synthesis, metabolism and excretion separately. It then covers the respective responses to chemicals known to affect thyroid homeostasis in the rodent using classic examples of rodent thyroid disruptors to illustrate the points made. Where possible, such as is the case for the sulphonamide drugs, the responses in rodents and humans can be directly compared under similar exposure conditions. Throughout the review statements are illustrated with clear chemical examples, such as those compounds operating through induction of hepatic metabolism and excretion that show qualitative differences between human and rat thyroid effects while for others, such as those chemical inhibiting thyroperoxidase which show significant quantitative differences in responses with humans being considerably more resistant to the thyroid disruption than rodents.

Section 6.10 discusses the underlying principles of carcinogenesis as they apply to thyroid cancer in both human and the rodent, and addresses similarities and differences (summarized in Table 2), in particular the pivotal role of thyroid stimulating hormone (TSH) in rodent thyroid cancer and its secondary role in human cancer. There are a number of additional significant differences between the rodent and human in their basic thyroid biology and in their responses to the same or similar chemicals and the differences are exemplified with particular reference to iodine deficiency which induces goitre in humans under the sustained driving influence of TSH, even though thyroid cancer under these circumstances doesn't normally occur. In contrast non-genotoxic rodent thyroid cancer appears exquisitely dependent upon sustained TSH stimulus alone without the need for additional factors. The section concludes with a discussion of the overall relevance of chemically-induced rodent thyroid cancer to humans. Section 7 discusses the presence of thresholds for thyroid hormone changes for both neurodevelopmental and carcinogenic outcomes again by reference to literature examples of situations where threshold shave been clearly demonstrated for both outcomes.

Section 8 is a discussion of the effects on extra-thyroidal changes such as concurrent disease, concurrent systemic toxicity in other organs such as the liver and kidney, starvation, and heat and cold in affecting the concentrations of circulating thyroid hormones and thyroid morphology. All of these environmental changes can profoundly affect the turnover of thyroid hormones and at the very least organ toxicity, and food and water consumption, are situations that can occur regularly in routine toxicity studies as a consequence of the maximum tolerated dose (MTD) approaches that are applied in the conduct of such studies. A consideration of these factors will be critical if thyroid hormone measurements are routinely incorporated into toxicity studies.

Throughout the review, where possible, the discussion has been undertaken with reference to the WHO/IPCS MoA/human relevance framework and the draft GD also embraces the principles outlined in the numerous publications that have arisen out of the initiative. This is a welcome move to introduce more objective approaches to the regulatory process and to lay solid factual foundations to regulatory decisions.

The final part of the current review is questioning whether or not the current testing strategies for detecting thyroid disrupting chemicals are adequate for the purpose, and alternative animal models, including in vitro screens, are discussed. In light of the two-tier approaches advocated in the GD, additional assays that specifically target potential molecular initiating events, are to be welcomed as part of the second tier for establishing MoA and determining human relevance of the particular events. This review makes use of an extensive list of references, both current and some considerably older, in making the points throughout.

2. Introduction

This document was commissioned by the European Crop Protection Association (ECPA) to appraise the Association of the current ECHA/EFSA/JRC (2017) draft guidance document (GD) in terms of its fitness for purpose and its use of the current science in arriving at its conclusions. In order to carry out this remit, the current document has summarised the current state of understanding regarding the control of thyroid hormone homeostasis, in both human and animal species, and incorporates a critical review of the publicly available literature regarding the chemical perturbation of thyroid hormones particularly in those animal species that are used in the non-clinical safety evaluation studies required for the determination of human safety.

The publication of the outcome of the Thyroid European Commission-ANSES Workshop (European Union 2017) and the ECHA EFSA JRC Endocrine Disruptor Draft guidance (European Food Safety Authority/European Chemicals Agency 2017) posed a number of critical questions regarding the entirety of the information used to arrive at their conclusions, with particular emphasis on the sensitivity and specificity of the assays currently used to detect thyroid effects and the relevance of the species used in the bioassays for subsequent human risk extrapolation. This paper attempts to address those questions, by providing a comprehensive review of the available literature and to place this into the appropriate context with regard to the value, or otherwise, of current testing strategies. It also proposes an approach by which the effects seen can be objectively used in providing appropriate assessment of the risks associated with exposure of the human population to chemical entities that could potentially perturb thyroid hormone function.

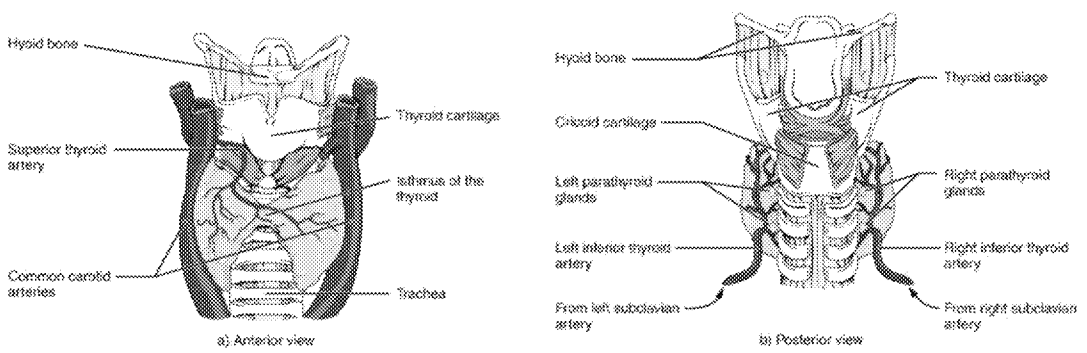
ECPA requested a focus on certain specific questions that are highlighted in italics before the respective relevant parts of the review.

3. Thyroid hormone function – the established understanding

This section summarises what is known about thyroid hormone production and secretion and is generally an amalgamation of the current knowledge derived from human and animal species. Where species differences exist, these are mentioned within the text.

The thyroid gland in mammals and birds (Fig. 1) is normally a bilobed organ with the two lobes being connected by an isthmus which lies on, and is located ventro-laterally to, the trachea (McNabb and Darrass 2015). Histologically the gland consists of a number of follicles lined by low cuboidal epithelial cells and a follicular lumen containing colloid, composed of thyroglobulin, which appears pink in haematoxylin and eosin stained sections.

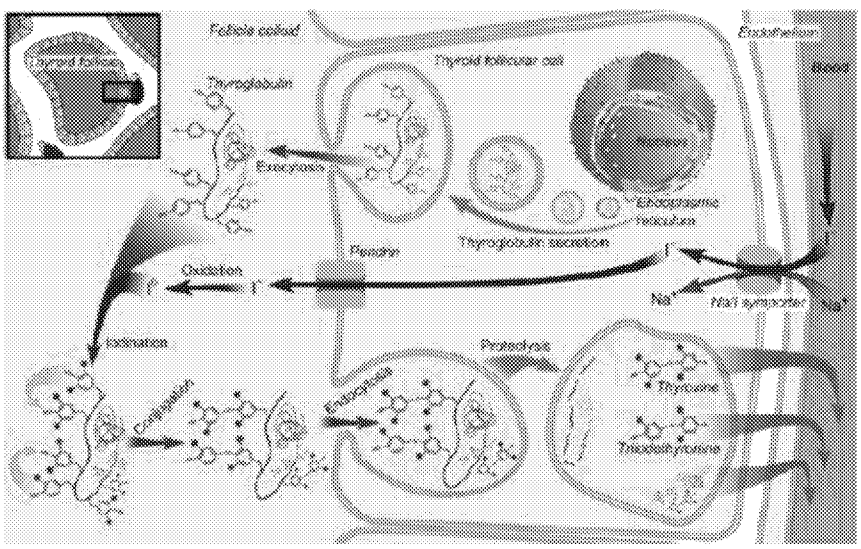
Fig. 1: Gross anatomy of the thyroid gland in mammals and birds - from https://courses.lumenlearning.com/ap2/chapter/the-thyroid-gland/



3.1. Thyroid hormone synthesis

Thyroglobulin is a glycoprotein made up of 134 tyrosine residues that is stored in the follicular lumen and which is the starting molecule for the subsequent synthesis of triiodothyronine (T3) and thyroxine (T4). Binding of thyroid stimulating hormone (TSH) to its receptor on the follicular cells triggers the up-regulation of the sodium-iodide symporter (NIS) on the basolateral membrane of follicular cells, resulting in an increase in the intracellular concentrations of iodine through a process known as iodine trapping (Rousset et al 2015). Once inside the cell the iodide is transported to the apical membrane of the follicular cell where it is acted upon by the enzyme thyroid peroxidase (TPO), an integral membrane protein present in the apical plasma membrane that catalyzes the sequential reactions needed for the formation of the respective thyroid hormones. TPO first oxidizes iodide to iodine, then iodinates tyrosine residues on thyroglobulin to produce mono- and diiodotyrosine, and finally links two tyrosine molecules together to produce T3 and T4. The peptide linkage between the thyroid hormones and thyroglobulin is then enzymatically cleaved, thyroid hormones are internalized at the apical surface of the thyroid epithelial cells by endocytosis, and hydrolytic enzymes within lysosomes fuse with the endosomes to release the hormones which are subsequently actively exported into the circulation, through the basolateral membrane of the follicular cells via the monocarboxylate transporter 8 (Di Cosmo et al 2010). Once in the circulation the majority of the hormones reversibly complex with liver-derived binding proteins for transport to other tissues although a small proportion remains free in the plasma.

Fig. 2: Formation of hormones in the thyroid follicular cells – *from* https://courses.lumenlearning.com/boundless-ap/chapter/the-thyroid-gland/



T3 is the biologically active hormone and T4, the major thyroid hormone that is secreted from the thyroid gland, is considered a precursor or prohormone. Although all of the T4 is synthesised within the thyroid, in human beings between 40 - 80% of T3 (Bianco and Kim 2006; Bianco et al 2002; Gereben et al 2008; Gereben et al 2015) is made through deiodination of T4 in peripheral tissues, especially the liver, kidney, pituitary gland and muscle. An additional, inactive molecule, reverse T3 (rT3), is also made in peripheral tissue by the action of deiodinases on T4. T3 is produced by deiodination on the outer ring of the T4 molecule, while the inactive form, rT3 is produced through deiodination of the inner ring of the T4 molecule (Visser et al 2016). Production of rT3 is thought to be a means of controlling excess circulating T4 since it has no known biological activity. Approximately 20% of all of the bound T4 is converted to rT3 on a daily basis in the human liver and other extra-thyroidal tissues.

The deiodination reaction is catalysed by one of three enzymes called type 1, type 2 and type 3 iodothyronine deiodinases (Visser, 1988; Leonard and Visser 1986; Chanoine et al 1993). Type 1 deiodinase is the main enzyme expressed in the liver, kidney and thyroid, and the liver is considered to be the most important extra-thyroidal site for the production of T3 and for removal or rT3. Both T3 and rT3 are metabolized within the liver by 5- and 5'-deiodination respectively to produce the inactive 3,3'-di-iodothyronine (T2). In situations, such as illness, increased metabolic demand in general, or administration of drugs such as amiodarone or β-adrenergic medication, the proportions of T3 and rT3 made from T4 can change to accommodate the altered demands (Hackney et al 1995a; Narayana et al 2011; Barbesino 2014).

3.2. Tissue uptake, signal transduction, and metabolism of thyroid hormones

Tissue thyroid status depends not only on thyroid hormone uptake and secretion but also on metabolism, delivery of T3 to the nuclear receptors, and receptor expression and distribution within the target cell populations.

It had generally been assumed that thyroid hormone, due to its hydrophobicity, enters cells via passive diffusion but *in vitro* studies identified several membrane transporters belonging to the monocarboxylate, and organic anion, transporter families with the ability to actively transport thyroid hormones into cells (Janson et al 2005). A human genetic disorder, known as Allan-Herndon-Dudley Syndrome, was instructive in indicating the critical role of the monocarboxylate transporter 8 (MCT8) protein in actively sequestering hormones into target cells. (Dumitrescu et al 2004; Friesema et al 2004; Schwartz and Stevenson 2007), and severe neurologic deficits. The syndrome is due to a mutation in the MCT8 gene and it was shown that active thyroid hormone transport is required in certain tissues, especially the brain for normal functioning (Kersseboom and Visser 2011; Visser et al 2011).

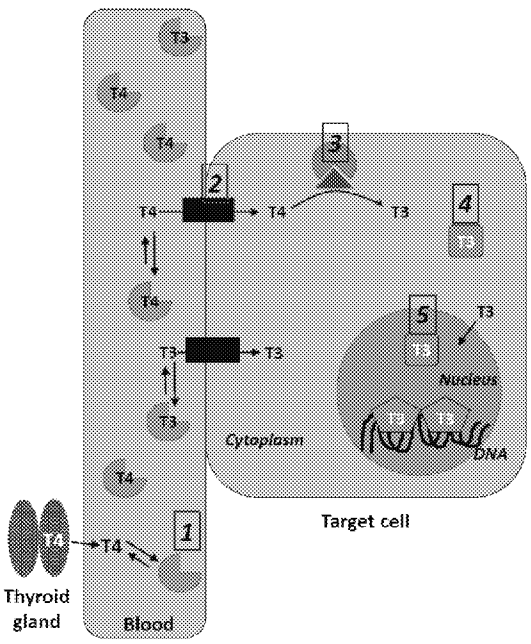
Table 1: Types of thyroid hormone transporters and their iodothyronine derivatives (Ahmed 2012)

Transporter	Iodothyronine derivatives	Specificity
MCT8	T3, T4, rT3, T2	+++
MCT10	T3. T4	++
OATP1A1	T3, T4, rT3, T2, T4S, T3S, rT3S, T2S	+
OATP1A2	T4, T3, rT3	
OATP1A3	T4, T3	
OATP1A4		
OATP1A5		
OATP1B1	T4, T3, T3S, T4S, rT3S	
OATP1B2	T3, T4	
OATP1B3	rT3, T4S, T3S, rT3S	
OATP1C1	T4, rT3, T3, T4S	++
OATP2B1	T4	+
OATP3A1 (V1/V2)		++
OATP4A1	T3, T4, rT3	+
OATP4C1	T3, T4	
OATP6B1		
OATP6C1		
LAT1	T3, T4, rT3, T2	
LAT2		
NTCP	T4, T3, T4S, T3S	++

Notes: The specificity is defined as high (+++) if the transporter only transports iodothyronine hormones, moderate (++) if it transports fewer than 5 other ligands, and low (+) if more than 5 ligands are known.

The MCT8 transporter has also been localised on the basolateral membrane of follicular epithelial cells suggesting that this protein might also act in the export of T4/T3 following its synthesis in the follicular epithelial cells (Friesma et al 2006). There are many known intracelllular thyroid hormone transporters, in addition to MCT8, and these are listed in Table 1 above (Ahmed 2012).

Fig. 3: Thyroid hormone secretion and transfer into cells (from Alshehri et al 2015)



1. = Plasma binding proteins (TBG, TTR, albumin); 2 = thyroid hormone transporters (MCT8, OCT14); 3. = iodothyronine deiodinases; 4. = cytosolic thyroid hormone binding proteins; 5. = thyroid hormone nuclear receptors.

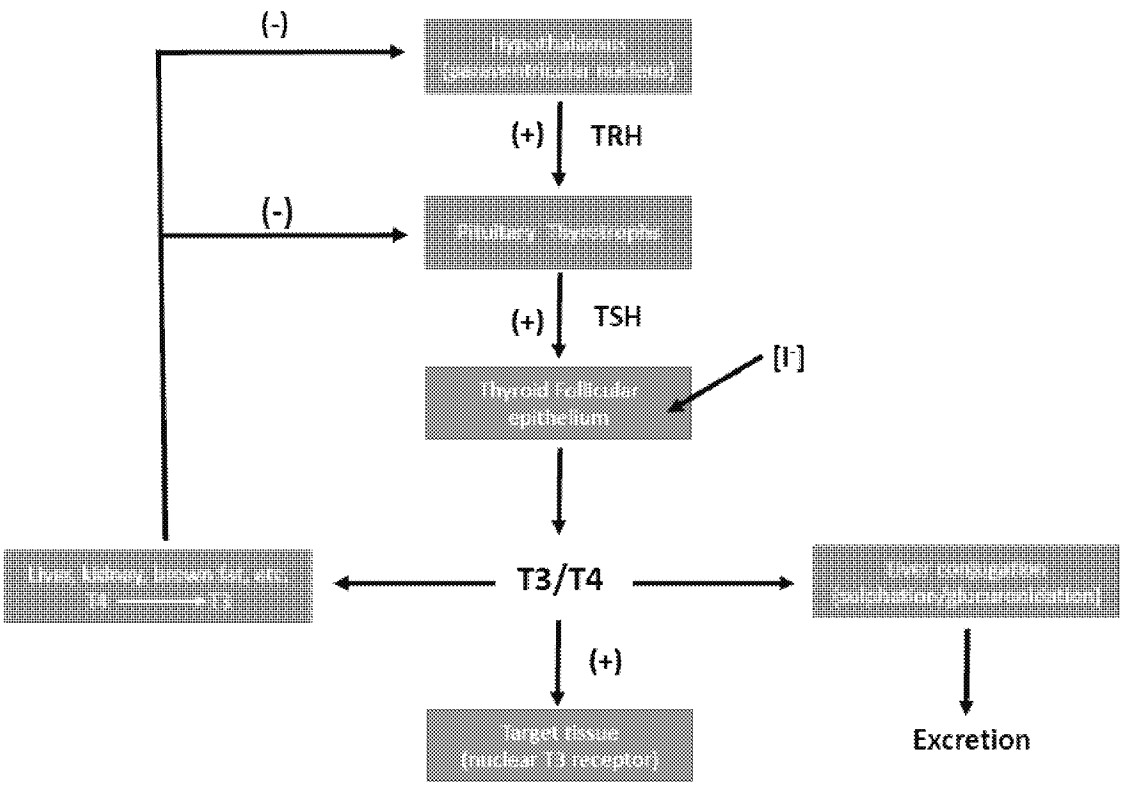
Once across the cell membrane and inside their target cells, cytosolic thyroid hormone binding proteins transfer the hormones to the nucleus where they dissociate from the carrier protein and bind to the nuclear T3 hormone receptor for subsequent cellular responses (Fig 3). Several different cytosolic proteins can act as thyroid transfer proteins including glutathione s-transferases (Ishigaki et al 1989; Kato et al 1989). It has been found that the thyroid nuclear receptor exists in several different forms, depending primarily on tissue type, some of which are able to bind T3 while others act to supress the activity of T3 (Mullur et al 2014). The different isoforms of the thyroid nuclear receptor are thought to exist to provide a pathway of tissue specific thyroid hormone action. The thyroid hormone receptor is part of the nuclear superfamily group that includes the retinoic acid receptor, retinoid X receptor, vitamin D, and the peroxisome proliferator activated receptor (Evans 1988). These receptors, on binding their respective ligand, are able to bind as monomers to thyroid response elements (TREs) on DNA, but the majority bind in the form of a heterodimer with the retinoid X receptor (RXR). Heterodimer formation is thought to enhance DNA binding affinity as well as providing target gene specificity (Kliewer et al 1992). The thyroid hormone/RXR heterodimer complex, on binding to its thyroid response element on DNA, is then able to stimulate or inhibit subsequent gene transcription within the target cell. There is considerable opportunity for crosstalk between the thyroid hormone receptor/RXR complex and other nuclear hormone receptors including the alpha and gamma forms of the peroxisome proliferator activated receptors (PPARα and PPARγ), and the liver X receptor (LXR) (Liu and Brent 2010). Since these nuclear receptors all form heterodimers with RXR, there is potential for competition for the limited amounts of RXR available (Hsu et al 1995; Liu et al 2007; Fattori et al 2015) that would result in depression of receptor activation by thyroid hormone and a possible inability or reduced ability to respond to elevated levels of thyroid hormone.

3.3. The hypothalamus/pituitary/thyroid axis (Fig. 4)

Thyroid releasing hormone, (TRH), secreted by the tanycytes within the hypothalamus, acts upon the pituitary gland, binding to G protein-coupled TRH receptors, resulting in an increase in intracellular cAMP, and subsequent thyrotropin (TSH) release from the thyrotrophs (Hershman 1974). Hormone signals that have modulatory effects on pituitary TSH secretion include dopamine, somatostatin, and leptin, and these molecules help to regulate thyroid hormone release at the level of the CNS (Scanlon et al 1979; Tanjasiri et al 1976; Seoane et al 2000; Ghamari-Langroudi et al 2010).

Thyroidectomy in rats results in a marked increase in serum TSH concentrations in response to the failure to produce T4 and T3. In experiments with thyroidectomised rats, where exogenous T4 was administered in the presence of selenium deficiency, TSH levels continued to remain high despite the maintenance of serum T4 concentrations identical to those observed in the serum of intact rats. In this experiment although serum T4 levels were normal, serum T3 levels remained low due to the diet-induced depression in deiodinase enzyme activities (selenium containing enzymes). This data confirmed the hypothesis that circulating T3, rather than T4, plays the critical role in regulating TSH secretion (Chanoine et al 1992; Abend et al 1991; Emerson et al 1989; Scanlon and Toft, 2000).

Fig. 4: Feedback control of thyroid hormone synthesis and release (adapted from Hill et al 1989).



Local tissue conversion of T4 to T3, by iodothyronine deiodinase type 2, provides negative feedback at the level of the thyrotrophs in the pituitary, and via the TRH secreting tanycytes in the hypothalamus (Fonseca et al 2013; Gereben et al 2008; Larsen and Zavacki 2012. High circulating thyroid hormone levels result in a reduction in TRH and TSH secretion, whereas low thyroid hormone levels stimulate TRH release from the hypothalamus and TSH release from the pituitary which act to increase thyroid production of the hormones.

On release from the pituitary gland, TSH binds to G protein-coupled TSH receptors on the thyroid follicular cells, stimulating the production and secretion of T4 and lower levels of T3, and when required to be increased, e.g. by certain chemicals and drugs, TSH stimulation will lead to hypertrophy of the follicular cells to meet the extra demand (Parmentier et al 1989; Tani et al 2004). If increased thyroid hormone production by the existing follicular cell population alone is insufficient to correct the decreased circulating hormone levels, continued TSH stimulation results in a stimulation of follicular cell proliferation, hyperplasia of the follicles, and ultimately can result in the development of follicular cell neoplasms (Smith et al 1991). Although originally it was thought that T3 and T4 simply diffused into target cells it is now clear that there are several ion-coupled, membrane transporters that are able to actively transport the thyroid hormones into target cells (Visser 2011). The MCT8 protein is involved in transporting T3 into cells throughout the body and mutations in the gene are associated with serious neurodevelopmental abnormalities in humans. Mouse models of MCT8 gene knockout show thyroid function study changes similar to those in patients with so-called Allan-Herndon-Dudley Syndrome, a condition brought about by a mutation of the transporter, but these mice show only minor changes in brain function in comparison with the human syndrome (Dumitrescu et al 2006; Trajkovic et al 2007; Visser et al 2011). The discrepancy between the response of inactivation of the MCT8 transporter in mice, and the serious consequences that result from the same mutation of the transporter in humans, is most likely due to the former having redundant thyroid hormone transporters that are able to compensate for the loss of the MCT8 gene. MCT8 is highly expressed in the hypothalamus and certain mutations have shown impairment of central hormone regulation and an abnormal thyroid hormone feedback (Alkemade et al 2011). Without a functioning MCT8 transporter in the brain, specific brain areas are unable to absorb T3 and become hypothyroid although the liver is still able to respond through the use of alternative thyroid uptake systems. Mutated MCT8 also means that the excess TRH/TSH is produced due to impaired negative feedback in the hypothalamus results in tissue-specific hyperthyroidism, hyper-metabolism and profound weight loss (Heuer et al 2009). Treatment with the thyroid hormone analogue, diiodothyropropionic acid (DITPA), in both animal models and humans with inactivated MCT8 gene, results in a reduction in both serum TSH and serum T3, with a consequent improvement in weight gain and decreased metabolic rates (Di Cosmo et al 2009; Verge et al 2012).

3.4. Physiological function of thyroid hormones

Thyroid hormones regulate the metabolic processes essential for normal growth and differentiation in the developing organism, as well as determining the metabolic rate in the adult (Brent 2012b; Cheng et al 2010; Oetting and Yen 2007) particularly with regard to the regulation of energy metabolism within cells (Malik and Hodgson 2002; Iwen et al 2013; Liu and Brent 2010). In healthy humans, thyroid hormone status correlates with body weight and energy expenditure (Fox et al 2008; Iwen et al 2013; Knudsen et al 2005) and hyperthyroidism promotes a hypermetabolic state characterized by increased resting energy expenditure, weight loss, reduced cholesterol levels, increased lipolysis, and gluconeogenesis (Brent 2008; Motomura and Brent 1998). In contrast, hypothyroidism induces reductions in metabolic rate that are characterized by chronic fatigue, weight gain, increased cholesterol levels, reduced lipolysis, and reduced gluconeogenesis (Brent 2012a; Oppenheimer et al 1991). The effect of thyroid hormones on metabolism is achieved through interactions with receptors in the brain, white fat, brown fat, skeletal muscle, liver, and pancreas (Mullur et al 2014).

3.5. Thyroid hormones in neurodevelopment

During the initial stages of gestation/pregnancy, the foetus relies on maternal thyroid hormones for normal brain development and growth. Deprivation of the maternal thyroid hormones, in hypothyroidism, can have devastating effects on the foetus and in humans, dysfunction of thyroid hormones in brain development is most often mediated through mutations in thyroid hormone transporters or in the deiodinases that degrade excess T3 (Ahmed 2015). Human hypothyroidism is most commonly monitored through assessment of circulating TSH levels and where needed, maternal thyroid hormone supplementation (levothyroxine) given during pregnancy corrects for any deficit (Maraka et al 2017). The recommended fixed upper threshold for TSH concentration in humans in

January 2017 was 2.5 mIU/L during the first trimester and 3.0 mIU/L during the second and third trimesters (Maraka et al 2017). According to these diagnostic criteria, subclinical hypothyroidism, defined as an elevated TSH concentration with concurrent normal thyroid hormone concentrations, was estimated to affect up to 15% of pregnancies in the US and 14% in Europe. This represented a fivefold increase in the apparent prevalence of hypothyroidism compared with the 2-3% prevalence of subclinical hypothyroidism before these criteria were established, raising the possibility of overdiagnosis of subclinical hypothyroidism and subsequent discussions by the American Thyroid Association (Alexander et al 2017) have revised these estimations by increasing the TSH cut-off limit to 4.0 mIU/L.

Because of increased thyroid hormone production, increased renal iodine excretion, and foetal iodine requirements, dietary iodine requirements are higher in pregnancy than they are for non-pregnant adults (Glinoer 2007).

Patients with the human genetic disorder, Allan-Herndon-Dudley Syndrome, show low levels of serum T4 and rT3, elevated T3, and normal or slightly elevated serum thyrotropin (TSH) (Dumitrescu et al 2004; Friesema et al 2004; Schwartz and Stevenson 2007), and severe neurologic developmental deficits that develop before birth. The mutated MCT8 gene translates a dysfunctional protein that is unable to adequately transport sufficient T3 into the developing brain and prevents the normal formation and growth of nerve cells and its discovery was instrumental in showing the critical requirement of thyroid hormones in the developing brain and that active thyroid hormone transport was required in the brain to ensure normal development (Kersseboom and Visser 2011; Visser et al 2011).

Studies in MCT8 knockout mice show a dramatically reduced uptake of T3 into the brain in comparison with wild type mice with an intact MCT8 system (Dumitrescu et al 2006; Trajkovic et al 2007). Because mutations in the MCT8 transporter are associated with multiple neurologic abnormalities in humans, with developmental delays, and progression to quadriplegia (Bernal 2011), the transporter is thought to have a critical role in normal brain development, and thyroid hormone transporters in general show both a specific temporal, but also spatial, pattern of expression in the developing brain (Sharlin et al 2011; Van der Deure et al 2010, Visser et al 2011).

Experimental data on thyroid hormone transport suggests significant species differences exist between humans and experimental animals, especially with regard to the uptake of thyroid hormones by the brain. In contrast to the situation in man knockout mice, lacking the MCT8 transporter, fail to show motor deficits suggesting that alternate pathways for transporting thyroid hormones into the brain exist in this species (Di Cosmo et al 2010; Roberts et al 2008). It is now known that the organic ion transporter polypeptide-14 (OATP14) is the primary thyroid hormone transporter expressed in the endothelial cells at the blood-brain barrier, whereas MCT8 mediates thyroid hormone uptake into neurons (Friesma et al 2005). OATP14 mRNA and protein is strongly expressed in both rat and mouse cerebral microvessels, but not in human, while both MCT8 and OATP14 is present in mouse and rat tanycytes, the cells in the hypothalamus that are responsible for the secretion of TRH (Roberts et al 2008). While MCT8 is primarily concerned with transporting T3 into the brain, OATP14 has been found to primarily transport T4 (Pizzagalli et al 2002; Sugiyama et al 2003; Tohyama et al 2004), and the high microvessel expression of OATP14 in the rodent, as compared with the human brain, may explain the relatively mild neurophysiological consequences of deletion of the MCT8 transporter in Mct8-null mice. In Mct8 KO mice, OATP14 is thought to be able to compensate for the loss of MCT8 in transporting thyroid hormone into the brain without loss of function, in contrast to humans lacking functional MCT8, where the absence of an alternate transporter results in serious neurodevelopmental consequences.

MCT8-mediated T3 transport itself, rather than being generated following uptake of T4 into the brain is also consistent with the finding that brain development and function is normal in type 2 deiodinase knockout mice (Schneider et al 2001). Type 2 deiodinase is responsible for converting T4 to T3 and the fact that type 2 deiodinase knockout mice are phenotypically normal indicates that direct brain uptake of T3 from the circulation can compensate for the inability of the neurones themselves to generate T3 by deiodination of T4.

Conversion of the pre-hormone T4 to the active thyroid hormone, T3, occurs mainly in peripheral tissue via the enzymatic activity of a group of iodothyronine deiodinases. Deiodinases generally are present at low levels in the brain, and while the type 1 deiodinase is found at high concentrations in the liver, within the brain the type 3 deiodinase generally mediates the degradation of thyroid hormones to inactive metabolites rather than deiodinases catalysing the conversion of T4 to T3. Especially high type 3 deiodinase activity has been demonstrated in the placenta and the pregnant uterus, as well as in different foetal tissues. This is considered to be a protective mechanism to prevent exposure of foetal tissues to high T3 levels at inappropriate times in development thus allowing the normal growth of these tissues. Paradoxically, T3 is only required at the differentiation stage of tissue development, and its presence at earlier stages, in conditions of maternal hyperthyroidism, has been linked with developmental abnormalities (Batra 2013). The critical role of type 2 and 3 deiodinases, and their differential expression at different stages in the developing foetus, is exemplified in cochlear development, since mice carrying either a type 2 or a type 3 deiodinase knockout have severe hearing loss (Ng et al 2004). At immature stages of foetal development, the type 3 enzyme limits stimulation by T3 whereas postnatally, a double switch occurs with a decline in the activity of the type 3 enzyme and a concomitant increase in the activity of type 2 deiodinase, resulting in a local T3 surge which is independent of serum T3 levels and which triggers the onset of auditory function (Ng et al 2004; Visser et al 2016).

4. ECPA Question: Consider the references in the draft guidance document – is this the state of the science? If not, what is?

The GD is intended to provide guidance for applicants and risk assessors on the implementation of the draft scientific criteria for determining endocrine disrupting chemicals and as such it appears to excellently provide "point by point" guidance using relevant literature references but it clearly is not, and does not claim to be, a state of the science document. It does introduce the entire area of endocrine disruption and does a good job of introducing those assays having OECD guidelines, together with other assays not currently covered by guidelines but it clearly does not incorporate the most up to date scientific research in the area as a whole and in the thyroid gland in particular. It does address its stated scope and provides an invaluable source of information for assessors and dossier submitters that provides clear guidance on what evidence will currently constitute a chemical being classified as an endocrine disruptor of relevance to the human exposure situation.

The finalised GD (Andersson et al 2018) outlines the principles used to set out the guidance are detailed for assessing whether a substance meets the hazard based ED criteria. The strategy is based on the requirements outlined in the ED-criteria

'[…] that a substance shall be considered as having endocrine disrupting properties […] if:

(1) It shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;
(2) It has an endocrine mode of action. i.e. it alters the function(s) of the endocrine system;
(3) The adverse effect is a consequence of the endocrine mode of action.'

The link between the adverse effect and the endocrine mode of action (MoA) addressed in point (3) shall be established based on biological plausibility. The biological plausibility shall be determined in the light of current scientific knowledge and all available relevant scientific data by using a WoE approach".

The last sentence above in the GD is a critical one since its whole precept is dependent upon the scientific database being not only comprehensive, but also being critically balanced between the academic understanding of thyroid hormone disruption, obtained from non-guideline, *in vivo* and *in*

vitro, laboratory studies, but also on practical experience gained through the evaluation of a broad range of chemical classes conducted following the strict guidelines laid down by the OECD TGs.

Appendix A of the draft guidance specifically addressed the problem of potential thyroid hormone disruption and was a pragmatic description of the current problems of species extrapolation between the rat and human in particular, but it also described the evidence that would be taken to arrive at a conclusion of a relevant MoA to human risk, and how a registrant might approach disproving this human link, making excellent use of the WHO/IPCS MoA/human relevance framework approach advocated by Meek et al (2014b) and others. The finally issued GD (Andersson 2018) has thankfully removed much of the previous discussion regarding thyroid toxicity, which was superficial and in places factually wrong) although there are still some misconceptions that remain (detail to be explained subsequently in this document).

Appendix A of the draft GD undertook to aid the interpretation of any potential thyroid disrupting effect by setting out clearly stated decisions points based upon the statement "Using the current understanding of thyroid physiology and toxicology13 it is proposed that the following be applied when interpreting data from experimental animals:

1. It is presumed that substances that alter the circulating levels of T3 and/or T4 with concurrent histopathological findings in the thyroid would pose a hazard for human thyroid hormone insufficiency in adults as well as pre- and post-natal neurological development of offspring.
2. It is presumed that substances that alter the circulating levels of T3 and/or T4 without histopathological findings would still present a potential concern for neurodevelopment.
3. In the absence of substance-specific data which provide proof of the contrary, humans and rodents are presumed to be equally sensitive to thyroid-disruption (including cases where liver enzyme induction is responsible for increased TH clearance)."

Point 2 is an important statement if proven to be true. As such it warrants a literature reference to justify its inclusion as one of the three most important points in the decision-making process. It seems to suggest that only neurodevelopmental endpoints would be relevant in such circumstances and also that the control of thyroid hormones is somehow different in the thyroid carcinogenesis process than it is in the neurodevelopmental ones. There are many reasons why a histopathological investigation of thyroid glands, in the presence of decreased thyroid hormones, may not detect follicular hypertrophy/hyperplasia and the reasoning behind this is explained in more detail in section 7.1 of this review. However this misconception of histopathology as an insensitive indicator of thyroid dysfunction is repeated again in the 2018 issued GD (Andersson et al 2018) but this time in the absence of any clarification as to why this might be the case, merely making the statement as an emboldened section header in Appendix A. Bizarrely, the finalised GD (Andersson et al 2018) goes on to say on page 73 of the finalised GD that "Thyroid histology is a valuable and sensitive diagnostic parameter for detecting the ability of a substance to interact with the HPT axis, particularly for thyroid system antagonism (Grim et al., 2009)" with regard to the frog, and considering how conserved thyroid physiology is across different species it begs the question as to why they consider histopathology perfectly adequate for the frog while insensitive for rodents! I believe this apparent contradiction occurs with a number of key points that include histopathology but that also includes the acceptance of the transthyretin argument that has been used to undermine the species difference argument in terms of thyroid hormone binding differences. The writers of the GD appear to have uncritically accepted, on occasions, single publications as being authoritative in guiding their recommendations without a critical appraisal of the value and quality of the publication. This is contrary to EFSA's own published criteria for critical appraisal of scientific literature (European Food Safety Authority, 2015) and leads the document, which will be read and followed by a large number of individuals open to accusations of bias and poor scientific credibility.

An objective discussion of the balance of pure and applied research was not within the stated scope of the draft GD and clearly is not within the scope of the finalized GD but it is clearly one of the objectives of this current review document.

5. ECPA Question: Is the way in which the references are used in the GD appropriate?

The literature references presented in the main text of the draft GD do appear to be appropriate for the intent and stated scope of the document. The literature listings are not extensive but the majority are relevant, and appear in the appropriate parts of the document to help clarify some of the more complex thinking behind the proposals. They are a pragmatic listing that does not attempt to introduce the reader to the most current scientific data being generated particularly in the human, but also in the experimental animal, field of thyroid research. As stated above there is a tendency for the finally issued GD, and it was certainly there for the draft GD, to accept, in an uncritical way, published papers without the stringency that is currently expected from literature reviews, this leading to misleading and in some cases, such as those relevant to transthyretin, erroneous statements that can undermine many years of research proving the opposite to what a recent paper (accepted uncritically by the GD) may be proposing.

The GD is lacking particularly in its inclusion of the current state of thyroid scientific literature and, while heavily biased towards oestrogen and androgen disruption, it is still not comprehensive in its coverage of even these areas. However, a state of the science review is not the intended scope of the GD.

The literature references used do appear to support the sections throughout the GD and offer clarification in those areas requiring it. They do not provide a "get-out" clause for industrial submitters to refute decisions of human relevance, but the GD does suggest appropriate actions, via their Tier 2 list of approaches, that can usefully be adopted to support MoA/human relevance cases that could possibly challenge suggestions of thyroid hormone disruption in other Tier tests.

6. ECPA Question: Comparison between human and key laboratory animal species (rodent, dog, monkey)

The physiological functioning and regulation of the pituitary-hypothalamic-thyroid system in all known mammalian species, birds and humans is qualitatively extremely similar (Choksi et al 2003; Bianco et al. 2002) and correct thyroid functioning and maturation during foetal organogenesis are essential for the development of critical organ systems, including the nervous system and reproductive tract (Jannini et al 1995; Metz et al 1996; Krassas 2000). However, the dynamics of the thyroid hormone control and turnover do differ substantially between the different mammalian species even though there are significant structural homologies between the various hormones (Imamura et al 1991; Tsykin and Schreiber 1993; Power et al 2000). Table 2 summarises the main thyroid features where differences are seen between human and rodent thyroid hormone control.

6.1. Species comparison of the functional morphology of the thyroid gland

Compared to the thyroid follicles in humans and primates, which are large with abundant colloid and with follicular cells that are relatively flattened (low cuboidal) (Sorenson and Brelje 2014), rodent follicles are considerably smaller, contain less thyroglobulin colloid and are most often lined by cuboidal, basophilic-staining epithelium indicative of a higher content of mRNA production, and by inference, higher rate of hormone synthesis, than the equivalent cells in those species having low eosinophilic epithelium such as primates including human. Within any single gland in both rats and mice, a small number of follicles will be large, lined by an attenuated epithelial layer and contain large amounts of thyroglobulin, in similarity with the majority of follicles in the primate and human gland, while other rodent follicles, the majority, will normally contain much smaller amounts of thyroglobulin colloid and have taller lining epithelium considered to represent more actively synthesising, and hormone secreting, cells (Suttie 2017). During times of increased hormone demand the number of active follicles, in the thyroid gland of rodents, increases while the number of resting "cold" follicles

decreases. Because of this plasticity in the follicular response in rodents, the morphological differences in the appearances of the follicles in the normal thyroid gland between control primates and rodents is supportive of the faster rates of thyroid hormone synthesis and turnover in the rodent (US EPA 1998).

6.2. Species comparison of thyroid hormone binding proteins in the plasma

Once secreted into the plasma from the thyroid follicular cells, the vast majority (>90%) of the T3 and T4 are transported through the blood bound to thyroid hormone binding proteins. These liver-derived binding proteins, and the proportion of T3 and T4 that they are able to bind, varies significantly among animal species. T3 and T4, in different species, have been found to be able to reversibly bind to three different liver-derived binding proteins: thyroxine-binding globulin (TBG), transthyretin (TTR), also called thyroid-binding prealbumin, and albumin (Schussler 2000; Bartalena and Robbins 1993). Lipoproteins also bind a small fraction of the available thyroid hormones. TBG is a monomer and a member of the serine protease inhibitor (serpin) superfamily of proteins (Flink et al., 1986; Robbins, 2000) while TTR is a tetramer composed of four identical subunits each composed of 127 amino acids (Power et al., 2000). Albumin is a monomer that has substantial sequence homology with α-fetoprotein and vitamin D-binding proteins (Robbins, 2000). There is little overall amino acid sequence homology between the three major binding proteins.

In normal human plasma, there are three T4 binding proteins (Fig. 3) with respective hormone distribution being approximately 80% bound to TBG, 15% to TTR, and 5% to albumin and lipoproteins, while for T3 the respective proportions are 90% bound to TBG and the remainder to albumin and lipoproteins. The binding distribution of T4 and T3 appears to correlate exactly with the binding affinity of these hormones to their respective proteins in humans, and the affinity of both T4 and T3 for TBG is much higher than are their affinities for either albumin or TTR (Kaneko, 1989; Robbins, 2000). Although only about 0.3% or less of T3 and T4 circulates unbound, it is this free hormone fraction that is metabolically active at the tissue and cellular level although the presence of deiodinases within target tissues means that local intracellular production of T3 from T4 can occur and most probably is responsible for the majority of T3 required by these tissues.

Table 2: A comparison of thyroid function and control between humans, rats and mice (adapted from Choksi et al 2003; Jahnke et al 2004; Colnot & Dekant 2017; Lewandowski et al 2003).

Parameter	Human	Rat	Mouse
Half-life of T4	5-9 days	0.5-1 day	0.5-0.75 days
Half-life of T3	1 day	0.25 days	0.45 days
High affinity TBG	Present	Absent	Absent
Primary serum binding protein	TBG	Albumin	Albumin
Serum TSH levels (ng/ml)	0.05-0.5	0.6-6.0	unknown
Sex difference in serum TSH level?	Males = females	males>females	males>females
Sex ratio for thyroid cancer	Females>males	Males>females	Males>females
Effect of chronic TSH stimulation?	Goitre	Cancer	Cancer
Amount of T4 supplementation required in absence of functioning thyroid?	2.2 mg/kg bw/day	20 mg/kg bw/day	Unknown
Development of foetal HPT	TSH/T3 by week 20 of gestation	TH & TSH by day 17 gestation	Unknown

Effect of mutant/KO MCT8	Multiple severe neurological deficits	unknown	Normal
Morphology of the follicular epithelium	Low epithelium	Tall cuboidal epithelium	Tall cuboidal epithelium
Morphology of the follicles	Large, lots of colloid	Small, little colloid	Small, little colloid
T4 glucuronidation	Minor route	Major route	unknown
Type 2 deiodinase expression in thyroid	High	Very low/Absent	Very low/Absent
% of T4 eliminated in bile	10-15%	~50%	Unknown
Timing of thyroid nuclear receptor binding in foetus	Week 10-16 (of 39 weeks total)	Day 10-15 (of 21 days total)	Unknown

In humans, inherited or acquired variations in the concentration and/or affinity of these thyroid hormone binding proteins may produce substantial changes in serum total thyroid hormone levels but these changes do not result in hypothyroidism or hyperthyroidism because the concentration of the free thyroid hormone does not change. A deficiency in thyroid hormone binding proteins in humans is suspected when abnormally low serum total thyroid hormone concentrations are present in clinically normal (euthyroid) subjects in the presence of normal serum TSH. More specifically, low TBG is suggested to be the cause of the low total T4 levels in these circumstances as it is this protein that carries the majority of the serum hormones. Under these circumstances the assay of free T4/T3 is more diagnostic of a thyroid gland effect than measures of total hormone where decreases could occur during liver pathologies where decreased production of binding proteins could be the cause of the decreased total T4/T3 levels measured.

Compared to humans, albumin is the major thyroid hormone binding protein in adult rodents and while they do carry a TBG gene, the TBG protein is expressed at very low levels in adult animals (Vranckx et al 1990; Rouaze-Romet et al 1992; Tani et al 1994). Developmental studies in the rat have shown that TBG protein expression increases briefly postnatally in rodents, but then declines to very low levels during weaning to remain at these low levels for the remainder of the rat's life (Savu et al 1987, 1991; Vranckx et al 1990). Although the binding affinity of T3 and T4 for TBG in the adult rat is still higher than it is for TTR and albumin in the rat, the fact that serum TBG levels in the rat are so low means that both thyroid hormones, to all intents and purposes, bind only to TTR and albumin in the serum of adult rodents.

6.3. The role of transthyretin (TTR)

The protein that is currently known as transthyretin (TTR) was first described in 1942 in human serum (Kabat et al 1942) and cerebrospinal fluid (CSF) and as a result of its mobility during electrophoresis at pH 8.6, where it was the only serum protein that migrated ahead of albumin, it was named at the time as 'prealbumin' (Seibert and Nelson 1942).

TTR is a thyroid hormone binding protein that is synthesised in the liver, is secreted into the bloodstream and is able to supplement the major transport proteins (TBG in humans and albumin in rodents) in distributing thyroid hormones around the body (Alshehri et al 2015). A second source of TTR is the choroid plexus and at this site it is thought to be involved in the movement of thyroxine from the blood into the cerebrospinal fluid and the subsequent distribution of thyroid hormones in the brain. Adequate uptake of thyroid hormone into the developing brain is essential for normal maturation and differentiation of the nervous system in the foetus in both animals and humans. In human plasma

thyroxine binding globulin has the highest affinity and greatest capacity for binding and transporting T3 and T4 followed by transthyretin and finally albumin, and they carry respectively 75%, 15% and 10% of the thyroid hormones in human blood (Alshehri et al 2015). It has been argued that TTR is the main protein responsible for transporting T4 in rodent blood (Palha et al 1994) although this has been challenged in the light of data from TTR knockout mice where the mice are perfectly viable without phenotypic changes (see discussion below).

The vast majority of *in vivo* studies, assessing the potential for chemically-induced thyroid hormone disruption, are carried out in the rat and it is this species where most attention has been focussed in determining the relevance of any observed rodent thyroid effect for the human population. While humans have all three major binding proteins, healthy adult rodents have only albumin and TTR (Vranckx et al 1990a; 1990b; Savu et al 1991; Lewandowski et al., 2004). If TTR is the major binding protein in the rat then it is clear that factors other than hormone binding affinity and dissociation rates must explain the clear differences demonstrated between the hormone kinetics in the rat with T4 half-life being around 24h, versus 5-6 days in humans. It is equally true that the enhanced clearance rate in the rat is compensated for by a correspondingly higher production rate of thyroid hormones, with an equally greater basal TSH levels in the plasma, and a more active thyroid gland as a consequence (Lewandowski et al., 2004).

It is a perfectly viable hypothesis, supported by data, that the differing thyroid hormone kinetics between rats and humans make the former considerably more sensitive to the effects of many, if not the majority, of chemical disruptors of thyroid homeostasis (McClain 1995; Colnot and Dekant 2017). A comparison of the data on the affinities and dissociation rates of thyroid hormones for the TTRs from rodents, humans and other animals have admittedly shown little variation (Chang et al 1999) but TTR is one, and a minor one at that, of the thyroid binding proteins in rodents. Albumin is the main carrier of thyroid hormones in the rat (Jahnke et al 2004) and differences in thyroid hormone binding is only one of the determining factors that result in the major differences in the kinetics of thyroid hormone turnover between humans and rodents.

There are several well described examples of both qualitative and quantitative differences in the responses of humans and rats to drug induced thyroid hormone disrupters, such as with the sulphonamide antibiotics, where controlled exposures in both humans and rats have shown qualitative and significant quantitative differences in the thyroid responses (Capen 1999; McClain 1995; Colnot and Dekant 2017). Perhaps the best understood examples of drugs affecting thyroid hormone homeostasis in rodents and not in man are the rodent hepatic enzyme inducing drugs (Curran and DeGroot 1991) where a considerable amount of controlled human exposure has been monitored for thyroid effects with drugs such as sodium phenobarbitone and has failed to find such a relationship even though other anti-epileptics, such as carbamazepine and rifampin have been shown to be goitrogenic.

In an attempt to better understand the importance of TTR in rodents, TTR knockout mice have been produced and shown to be perfectly viable (Episkopou et al 1993; Richardson 2007) without neurodevelopmental abnormalities. If mice are like rats in that they make TBG in the foetus, then this thyroid hormone binding protein could, along with albumin, substitute for TTR at critical developmental stages in the knockout foetus, which are taken over exclusively by albumin postnatally. The mouse TTR knockout data therefore suggest that TTR, rather than being a key thyroid hormone transporter, as is claimed in the draft GD (although dropped from the final GD) and in the publication by Alshehri et al (2015), has a secondary, or backup, role at most in the transport of thyroid hormones in rodents, and supports a conclusion that, in the adult rat/mouse albumin, with its relatively low affinity but high dissociation rates for carrying T4 and T3, is the main thyroid binding protein in rodents (Palha et al 1994; 2000; 2002; Sousa et al 2005; Alshehri et al 2015). Considering the critical importance of thyroid hormones to brain development it is inconceivable that the absence of the major thyroid hormone transporter, as is claimed for TTR, in knockout mice would permit survival without significant neurological disorders. These results support the conclusion that albumin is the most important thyroid hormone transporters in the mouse, and most probably also in the rat, under these circumstances.

6.4. Species differences in thyroid hormone metabolism

Thyroid hormones are metabolised in peripheral tissues by three enzyme systems, the iodothyronine deiodinases, by sulphotransferase (SULT) mediated conjugation with sulphate, and by conjugation with glucuronic acid catalysed by glucuronosyltransferases (UGT). The deiodinases and UGT enzymes are localised intracellularly within the endoplasmic reticulum while the SULTs are cytosolic enzymes (Dentice et al 2013; Radominska-Pandya et al 2005; Teubner et al 2007). Both pathways are responsible for the excretion of thyroid hormones in the rat, and the liver and kidney are major sites for this process (Vansell and Klaassen 2002; Visser et al. 1993). In the rat liver, there are a large number of UGT enzymes involved in conjugating various substrates, but with regards to the thyroid hormones, UGT1A1, UGT1A6 and UGT1A7 are reported to conjugate T4 while a number of different UGT2 enzymes conjugate T3 (Vansell and Klaassen 2002; Emi et al 2007).

T3 is not glucuronidated significantly in human liver or kidney although it is induced in certain diseases where circulating thyroid hormones increase, such as in hyperthyroidism, and where glucuronidation becomes more important (Findlay et al 2000; Visser 1996). The lack of hepatic T3 glucuronidation in humans suggests that either human liver doesn't express an enzyme homologous to rat UGT2B2, the enzyme primarily, although not solely, responsible for glucuronidating T3 in the rat, or that it does not accept T3 as a substrate. In humans therefore T3 appears to be metabolized predominantly by deiodination and sulfation. In contrast to the situation in humans, normal rat liver has been shown to have substantial T3 glucuronidation (Findlay et al 2000).

Laboratory animal studies show that there are both species-and gender-dependent variations in enzyme activity (Kelly, 2000) that can explain some of the gender specific thyroid hormone effects seen with enzyme inducing agents. The UGT enzymes have especial importance in the biology of thyroid hormones since they can be upregulated in the liver by a number of xenobiotics as part of the pleiotropic response that occurs following activation of several nuclear hormone receptors, including the constitutive androstane receptor (CAR) and PXR, and the impact of altered thyroid hormone homeostasis is considerable in terms of both thyroid cancer and neonatal and postnatal development in animal species susceptible to the induction of these enzymes (McClain et al 1988; 1989). Since the capacity for the hepatic induction of these enzymes in humans is low (Richardson et al 2014) while rodents are almost exquisitely responsive to chemically-induced activation of these nuclear hormone receptors, disruption of thyroid hormone homeostasis via induction of hepatic UDP-GT enzymes, as a MoA, is not thought to be relevant to human situation (McClean 1995).

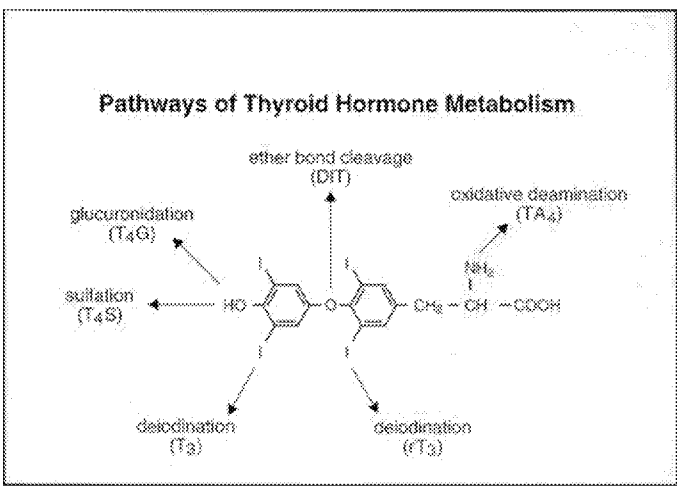
As with the UGTs, there are a number of SULT enzymes present in the liver that are involved with conjugation of thyroid hormones (Kester et al 1999). Whereas glucuronidation appears to facilitate the faecal excretion of thyroid hormones, sulfation initiates its degradation, allowing reutilization of the iodide for *de novo* thyroid hormone synthesis. In healthy humans, the sulphation pathway does not appear to contribute significantly to thyroid hormone metabolism, although its role increases in importance when Type I deiodinase activity is depressed (Visser 1994). Animal studies indicate that activation of the sulphation pathway inhibits T3 formation and increases the degradation of T4 and the inactive rT3 to additional inactive metabolites (Kelly, 2000). While minor in the adult human, T3-sulphate activity may be more important in the human foetus where, in the absence of foetal iodothyronine deiodinase to generate T3 from T4, sulphated T3 (T3S) can serve as a vital source of foetal T3 (Brucker-Davis, 1998).

Although type 2 deiodinase is normally expressed at high levels in human thyroid, and both mRNA and activity of type 2 deiodinase are induced by TSH, and agonistic TSH receptor antibodies circulating in patients with Graves' disease (Imai et al 2001; Murakami et al 2001), this enzyme is not expressed in normal rat or mouse thyroid (Wagner et al 2003). The consequences of a lack of expression of type 2 deiodinase in the rat are that intracellular conversion of T4 to T3 will not occur within the follicular cells, possibly as a result of the fact that rat follicular cells are already normally in a higher state of enzyme production than those present in humans and lack the need to increase their production rate of T3. Type 2 deiodinase can however be induced in conditions of hypothyroidism in both human and the rats in an attempt to increase the conversion of T4 to T3 (Wagner et al 2003).

6.5. Hepatic metabolism of thyroid hormones (Fig. 5).

Studies using ^{131}I labelled T4 have shown that in humans, the liver extracts between 5–10% of plasma T4 passing through the organ at any one time, a value that is considerably higher than could be accounted for by the amount of free T4 in the plasma delivered to the organ. This discrepancy shows that a substantial amount of protein bound T4, in addition to free T4, is absorbed directly into the cells (Mendel et al 1988). MCT8, a major thyroid hormone transporter has been localised to the membranes of hepatocytes and the thyroid hormone uptake occurs in a saturatable, energy and sodium-dependent, manner that enables the protein bound T4 and T3 to cross the hepatocyte membrane and to concentrate free intracellular hormones to a much higher level than those present in the plasma (Nishimura and Naito 2008). Although MCT8 is an important thyroid hormone transporter, several other proteins, such as the organic anion transporter protein 1C1 (OATP1C1), have been shown to transport thyroid hormones into cells throughout the body although they do tend to show a tissue specific pattern of expression (Abe et al 1998; Visser et al 2011).

Fig. 5: Thyroid hormone metabolism (from Visser et al 2016)



Thyroid function in a healthy individual is critically dependent upon a normally functioning liver to thyroid axis (Malik and Hodgson 2002) and while the major route for metabolism of T3 is through deiodination, significant removal of T3, rT3 and T4 occurs in the liver through conjugation reactions with sulphate and glucuronic acid (Fig. 2) catalysed respectively by iodothyronine sulfotransferase and iodothyronine glucuronyltransferase enzymes with the conjugates of the latter in particular, being excreted into the intestine through the bile (Visser 1996; Wu et al 2005).

There are some differences in the metabolism of T4 by the liver between rats and humans with approximately 50% of the T4 being eliminated via bile in rats, but only 10-15% in humans (Hill et al. 1989). However this difference does not appear to reflect a qualitative difference in metabolism, because the major metabolite in bile (glucuronide conjugate) remains the same in both species (Hard 1998). It does however suggest a kinetic difference in metabolism between the two species with the rat turning over T4 at an appreciably faster rate than in humans. This is consistent with the proposed biological differences in the transport of free and bound thyroid hormones between the two species. Iodothyronine glucuronides are rapidly excreted in large quantities in the bile with approximately 20% of human daily T4 production appearing in the faeces. This is not an end stage process as the conjugates are readily hydrolysed within the intestine by bacterial ß-glucuronidases, and some of the liberated hormone can be reabsorbed through enterohepatic cycling (Visser et al 1988).

Sulphated iodothyronine (T3S) levels are normally present at very low concentrations in plasma, bile and urine, because these conjugates are rapidly degraded by type 1 deiodinase. This has been taken to indicate that sulphate conjugation is a primary step leading to the irreversible inactivation of thyroid hormone (Visser 1994; Peeters et al 2005). Plasma concentrations, and biliary excretion, of T3S is significantly increased following inhibition of type 2 deiodinase activity with PTU or the iodine-

containing radiocontrast medium, iopanoic acid, both during foetal development, and following fasting (Wu et al 2005; Visser 1994). Under these conditions, T3S may be acting as a reservoir of inactive hormone from which active T3 may be regenerated.

6.6. The importance of thyroid hormone sulphation

Serum concentrations of T3S are normally very low in healthy human subjects but are very high in the blood of the foetus and umbilical cord, and high in patients treated with the type 1 deiodinase inhibitor, triac, an acetic acid derivative of T4 (Eelkman et al 1989; Wu et al 2005). Similar high T3S/T3 ratios are also seen in some human cases of hypothyroidism, with the high sulphated T3 levels being due to a low peripheral type 1 deiodinase activity (Visser 1994; Peeters et al 2005).

An increase in T3S levels is also seen in rats when hepatic and renal type 1 deiodinase activities are decreased following exposure to enzyme inhibitors of the type 1 deiodinase enzymes, or in the presence of selenium deficiency, where marked increases in both serum and bile concentrations of iodothyronine sulphates occur (Visser 1994). These changes result from decreased clearance of the sulphated iodothyronines due to the absence of the type 1 deiodinases, but under conditions of hypothyroidism the inactivation of thyroid hormone by sulphation has been found to be reversible due to the presence of sulphatases in different tissues and in intestinal bacteria (Kester et al 2002). It is supposed therefore that the presence of relatively high concentrations of T3S in the foetus has an important function as a reservoir from which active T3 may be released in a tissue-specific, and time-dependent, manner (Santini et al 1992; Wu et al 1992; Darras et al 1999).

6.7. Species comparison of thyroid hormone and TSH half-life

The serum half-life of T4 and T3 in normal human adults is 5–9 days and 1 day, respectively (Choksi et al 2003) while in rats, the comparative values are 0.5–1 and 0.25 days, for T4 and T3 respectively. The plasma half-life of T4 and T3 in the dog has been estimated to be 8 –16 hours and 5-6 hours respectively (Kaptein et al 1993; 1994). The basis for the difference in half-lives is not completely understood, but it is proposed that the lack of the high-affinity T4 binding protein, TBG, in the adult rat plays a critical role since this leads to a higher serum level of free T4 in the rat and a greater tendency for the bound hormones to dissociate as a consequence of the lower affinity binding that albumin and TTR has for the thyroid hormones. These increased concentrations of free hormones are thought to make the hormones far more susceptible to metabolism and excretion (U.S. EPA, 1998; Capen 1999), and to explain the very short half-lives that the thyroid hormones have in the rat and mouse in comparison to humans. The binding affinity of TBG for T4 in humans is approximately 1000 times greater than it is of TTR for T4, and it is well known that the % of free T4 is appreciably lower in species with high levels of TBG than it is in species without TBG (Capen 1999). Experiments have shown that thyroidectomised rats require 10 times more T4, equating to 20 μg/kg body weight (Frumess and Larsen 1975; Dohler et al 1979; Schlenker et al 2008), for full substitution as compared to an adult human requiring 2.2 μg/kg body weight (McAninch and Bianco 2016).

Comparative studies on the effects of perchlorate in the rat and human thyroid gland have also shown significant biochemical and physiologic differences in the relative responses. In an experiment by Yu et al. (2002) with perchlorate, inhibition of radiolabelled iodide uptake was 15%, 55%, and 65% at 1.0, 3.0, and 10 mg/kg perchlorate respectively at 1 day following perchlorate administration to the rat. However, by day 5, inhibition of iodide uptake had decreased to 0, 10%, and 30% at each respective dose level and after 14 days, inhibition of iodide uptake was only observed only at the top dose levels of 10 mg/kg. The data showed that the initial inhibition of iodide uptake by perchlorate in rats was similar to that observed in humans but that rats were able to compensate for the inhibition within 5 days of administration, most likely by increasing the expression of the sodium-iodide symporter on the follicular cells of the thyroid. A similar response was not observed in a 14-day human study with perchlorate administration (Greer et al. 2002). It was thought that compensation occurred in rats because of their smaller reserve capacity of thyroid hormones than humans and their more rapid turnover of circulating hormones.

The mitogenic hormone responsible for the normal production of thyroid hormones, and for the chemically induced thyroid hypertrophy and hyperplasia is TSH. It is this hormone that, on chronic stimulation in rodents, can lead to follicular neoplasia of the thyroid due to chemicals that interfere with thyroid hormone homeostasis (Hard 1998). The plasma levels of TSH in the male rat are approximately 6 ng/ml (Helmreich and Tylee 2011) which are about 3-fold higher than they are in the female rat (Kieffer et al 1976). This compares with values of 0.05 – 0.5 ng/ml in adult humans (Lewandowski et al 2003). In addition, production of both T4 and T3, in the rat, is appreciably higher than it is in man, due to their shorter half-life, and since production is driven by TSH levels, the higher plasma levels of TSH in rodents are thought to be responsible for the differences in follicle morphology seen between rodent and primates, including humans, with rodents having a follicular appearance consistent with a higher rate of thyroid hormone production and turnover.

6.8. Comparison of thyroid modifying chemicals in human and animal species

There is no doubt that adequate thyroid hormones are essential for the development of the foetus in both humans and laboratory animal species, as well as other vertebrate species (Williams 2008). It is also evident that thyroid hormone deficiency will result in neurodevelopmental deficits in humans and animals, the severity of which appears to be related directly to the severity of the hypothyroidism and the degree of thyroid hormone depression (Glinoer 2000; Haddow et al 1999; Klein et al 2001).

6.8.1. Thyroid modifying chemicals in humans

There are a small number of environmental chemicals that have been associated with decreasing circulatory and urinary levels of thyroid hormones in human epidemiological studies that include polychlorinated biphenols (PCBs), perchlorate, tetrachlorodibenzo-p-dioxin (TCDD), polychlorinated dibenzofuran (PCDF), pentachlorophenol (a breakdown product of hexachlorobenzene), triclosan, polybrominated and tetrabrominated diphenyl ethers (PBDEs) and thiocyanates in cruciferous vegetables (Miller et al 2009). Perchlorate has been used therapeutically to treat hyperthyroidism by inhibiting the sodium iodide symporter and because of its ubiquitous distribution in the environment, there has been intensive study of the potential for perchlorate to cause human thyroid effects and, more importantly, neurodevelopmental deficits in the human population at the concentrations present in the environment. While a clear association with decreased T4 and increased TSH has been described in exposed women, no such association was found in men (Blount et al 2006) and more significantly, no neurodevelopmental effects of decreased TH were found in the epidemiological studies with perchlorate (Leung et al 2010).

There are a variety of drugs that have also been shown to inhibit peripheral production of T3 in humans including propylthiouracil (PTU), dexamethasone, propranolol, lithium, iodinated compounds such as the radiographic agents, iopanoic acid and ipodate, and the anti-arrhythmic drug amiodarone. PTU is a specific non-competitive inhibitor of type 1 deiodinase, while iopanoic acid and ipodate are competitive inhibitors not only of type 1 deiodinase but also of the type 2 enzyme. In addition, the radiographic agents have been shown to inhibit hepatic uptake of thyroid hormone (Dentice et al 2013). Amiodarone, and its metabolite desethylamidarone, may also interfere with peripheral thyroid hormone levels by a combination of inhibition of deiodinase activities and of tissue thyroid hormone uptake (Narayana et al 2011; Rosene et al 2010). The therapeutic use of lithium in the treatment of manic-depressive psychosis in humans has been known to be associated with the development of goitre for many years, and decreases in the rate of degradation of T4, and a decrease in serum T3 is seen in patients receiving high doses of lithium carbonate through a variety of mechanisms including enhancement of iodide induced inhibition of the NIS, inhibition of the deiodinases, and an exaggerated response of pituitary TSH release to TRH (Shopsin et al 1973; Andersen 1973; Carlson et al 1973). Little is known about the mechanisms by which propranolol and dexamethasone inhibit peripheral T3 production. In recognition of the thyroid inhibiting properties of these drugs, combinations of PTU, ipodate, dexamethasone and/or propranolol have been used to acutely

decrease plasma T3 levels in patients with severe hyperthyroidism, known as a "thyrotoxic storm" (Carroll and Matfin 2010).

While a link has been established between the urinary concentrations of PCBs and human neurodevelopmental deficiencies, a study by Longnecker et al (2003) failed to establish a link between serum thyroid hormone deficits and PCB exposure even though this study was, wrongly, cited by Crofton (2008) as an example of a chemical-induced thyroid hormone effects operating in humans. Nevertheless, PCBs have been shown to alter thyroid hormone homeostasis in rats and to be associated with neurodevelopmental deficits (Gauger et al 2004).

6.8.2. Thyroid modifying chemicals in rodents

In comparison to the situation in humans, there are a far greater proportion of tested chemicals that have been shown to alter thyroid homeostasis in animals, mostly rodents, that include a number of phthalate esters, pregnenolone-16α-carbonitrile, benzodiazepines, calcium channel blockers, steroids, chlorinated hydrocarbons, such as chlordane and DDT, and polyhalogenated hydrocarbons such as PCBs and PBBs (Curran and DeGroot 1991; Capen 1999). Despite considerable variation in chemical structure and classes, for those chemicals that cause thyroid hormone effects in rodents, there are a limited number of ways (Fig. 5) in which they can affect thyroid hormone homeostasis either by direct effects on the thyroid gland itself or by effects on extra-thyroidal tissues, such as the liver, brain and kidney, responsible for the deiodination of T4 to T3 (Capen 1999).

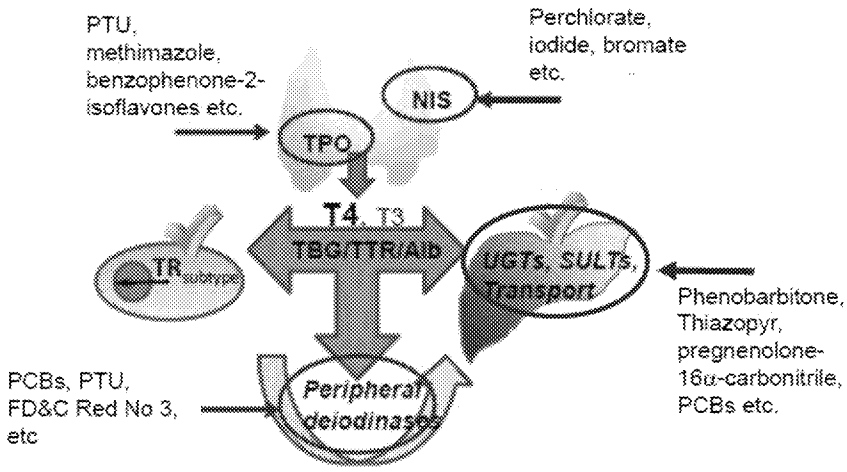
6.8.2.1 Competitive inhibitors of the sodium iodide symporter (NIS)

Chemicals such as pertechnetate, thiocyanate and perchlorate ions can directly affect the thyroid gland by acting as competitive inhibiters of the follicular cell NIS (Fig 5). This leads to a decrease in the availability of inorganic iodide in the thyroid gland and a consequential decrease in the synthesis and release of thyroid hormones (Merrell et al 2003; Capen 1999). When circulating levels of T4 and T3 decrease, compensatory release of TSH from the pituitary gland leads to hypertrophy, hyperplasia, thyroid gland growth and increases the chances of developing cancer (Fisher et al 2012).

6.8.2.2 Thyroperoxidase inhibitors

Certain chemical classes including thionamides such as 6-propylthiouracil and ethylene thiourea, some of the sulphonamide drug classes, and miscellaneous compounds such as amitrole, reversibly or irreversibly inhibit the thyroperoxidase catalysed incorporation of active iodide into thyroglobulin (Sarne 2016). Inhibition of thyroperoxidase reduces the production and release of T4 and T3 into the circulation, provoking a compensatory release of TSH from the pituitary gland and causing hypertrophy and hyperplasia in the thyroid follicular epithelium (Hard 1998).

Fig. 6: Known sites of action of chemicals in disrupting thyroid hormone homeostasis in rodents (from Paul 2014)



Notes: Abbreviations are TPO = thyroperoxidase; NIS = sodium/iodine symporter; TBG = thyroxine binding globulin; TTR = transthyretin; Alb = albumin; UGTs = UDP glucuronyltransferase; SULT = sulphotransferases; TR = thyroid hormone nuclear receptor; PCBs = polychlorinated biphenyls; PTU = propylthiourea;

6.8.2.3 Direct toxicity to follicular epithelium

Direct chemical toxicity to the thyroid follicular cells has been seen with PCBs (Collins et al 1977), which reduce the output of thyroid hormones, while lithium (Kibirge et al 2013; Bocchetta and Loviselli 2006) and excess iodide (Leung and Braverman 2014) inhibit the secretion of thyroid hormones from the follicular cells, induce retention of the hormones within the follicular lumen, seen as increased colloid, and produce a decrease in circulatory thyroid hormones (Green, 1978; Capen and Martin 1989). Both of these effects have been seen in humans. Fipronil is a phenylpyrazole class insecticide which has been shown to reduce TH in the rat through direct toxicity to the follicular cells (Tukhtaev et al 2013; Leghait et al 2010), and another pyrazole fungicide, bixafen has also been shown to be directly toxic to the thyroid follicular epithelium in rats (WHO 2013) Amiodarone is an iodine-rich drug used to manage ventricular and supraventricular tachyarrhythmias, and is also associated with thyroid dysfunction, most probably through its effect as an iodide source (Minelli et al 1992).

6.8.2.4 Inhibition/induction of iodothyronine 5'-deiodinases

There are a number of chemicals that alter thyroid hormone homeostasis either by inhibiting the 5' deiodinase catalysed conversion of T4 to T3 in peripheral tissues (Capen 1999) or, in rarer cases, by enhancing the conversion of T4 to T3 through induction of the deiodinases (Morse et al 1993).

The dye erythrosine, also known as FD & C Red No 3, was shown to increase thyroid follicular adenomas in rats in lifetime bioassays (Borzelleca et al 1987) and the compound induces thyroid gland hypertrophy and hyperplasia in shorter duration studies in the rat. The thyroid morphological changes are accompanied by decreases in serum T3 and increases in rT3, T4 and TSH, due to inhibition of 5'-deiodinase (Capen 1997; 1999; Capen and Martin 1989).

PTU has also been shown to inhibit 5'deiodination (Cavalieri and Pitt-Rivers 1981) in addition to inhibiting thyroperoxidase (TPO), and its potency in inducing thyroid hypertrophy and hyperplasia is most probably a product of a combination of effects rather than down to any single MoA.

A common component of sun-cream, octyl-methoxycinnamate, when given over a five day period, has also induced a dose-dependent decrease in serum T3, T4, TSH, and in hepatic type 1 5'-deiodinase

(Klammer et al 2007). In this study TSH receptor expression in the thyroid was also increased while hypothalamic TRH expression, and the activities of TPO and NIS in the thyroid were unaffected. Oral administration of the PCB, 3,3',4,4',5,5'-hexachlorobiphenyl (HCB), or a combination of HCB with 3,3',4,4'-tetrachlorobiphenyl (TCB), to pregnant rats from day 1 to day 18 post-gestation induced significant decreases in plasma thyroid hormones in the dams, and significant increases in brain type 2 5' deiodinase activity in foetuses (Day 20 of gestation) and neonates (Days 7 and 21 postpartum), and an associated reduction in circulating total, and free, T4 levels. To complicate matters further, treatment also induced hepatic T4 glucuronidation in both 20 day gestation foetuses, and in neonates (Morse et al 1993).

In the Morse et al (1993) study as described above, the induced decreases in plasma thyroid hormones were accompanied by a highly significant induction of type 2 thyroxine 5'-deiodinase activity in brain homogenates from 20 day post-gestation foetuses, and day 7 and 21 postpartum neonates (Morse et al 1993). The increase in deiodinase activity was interpreted as indicating that local hypothyroidism had occurred in the brains of the foetal and neonatal rats exposed to HCB. Since these effects occurred during a period in which thyroid hormones play an important role in brain maturation, the data may be relevant in explaining the mechanism of developmental neurotoxicity induced by PCBs. As this group of chemicals also induce hepatic glucuronidation, that increase clearance of thyroid hormones, this group of compounds, in common with many of the well worked chemical examples of thyroid hormone disrupters, exhibits more than simply one single molecular initiating event that could operate in concert (an additive effect) to magnify the resulting thyroid hormone perturbation seen with this chemical class (Crofton 2008).

6.8.2.5 Displacement of hormones binding to thyroid transport proteins/receptors

There is some evidence from *in vitro* competitive binding studies that certain chemicals are able to displace T4 from one of the serum thyroid binding proteins, TTR (Meerts et al 2000). Although opinion differs as to the relative importance of this binding protein in the rat the findings, if substantiated *in vivo,* would have the potential to stress the thyroid system, making it more likely that dysfunction could occur (Kohrle et al., 1989; Lueprasitsakul et al., 1990; Lans et al., 1993; Cheek et al., 1999; Chauhan et al., 2000; Ishihara et al., 2003). Because of the redundancy in thyroid hormone binding proteins in humans and rodents, it is questionable whether or not such displacement of T4 from TTR alone would lead to functional consequences. *In vivo* evidence with the flavonoid, EMD 49209, which appears to displace T4 from TTR *in vitro*, shows that it reduces tissue levels of T3 *in vivo,* not through any MoA involving displacement of T4 from TTR, but as a result of a shortage of T4 as a substrate for deiodinases (Schroder-van der Elst et al 1998). Until *in vivo* evidence is produced to support this hypothetical MoA it has to be concluded that, of the time of writing, this *in vitro* phenomenon has not been reciprocated in any known *in vivo* chemical-induced thyroid disrupter.

6.8.2.6 Induction of hepatic turnover/biliary excretion of thyroid hormones

At least in rodents, certain chemicals have been shown to induce the activity of the hepatic UGT and SULT enzymes responsible for the conjugation and removal, through biliary excretion, of thyroid hormones (Oppenheimer et al 1968; McClain et al 1989; Visser et al 1993; Hood and Klaassen 2000; Liu and Klaassen 1996). This results in decreased circulating free and bound thyroid hormones, a higher turnover and decreased half-life of circulating hormones, and a compensatory TRH and TSH increased release from the hypothalamus/pituitary acts on the follicular cells of the thyroid to induce hypertrophy and hyperplasia, and if sustained, thyroid follicular neoplasia (McClaIn 1989; Hill et al 1989; Thomas and Williams 1991; Williams 1995). In addition to chemical induction of hepatic SULTs, a number of drugs and environmental chemicals, such as salicylic acid, clomiphene, naturally occurring chemicals such as flavonoids and phytoestrogens, and environmental chemicals such as the disinfectant, pentachlorophenol, and the antibacterial product, triclosan, have been shown to inhibit hepatic SULTs and to have the potential to increase circulating levels of thyroid hormones (Schuur et al 1998; Wang et al 2004; Wang & James 2006). There is evidence that thyroid hormone perturbations, specifically those mediated via hepatic enzyme induction and increased biliary excretion, are a rodent only phenomenon that is not able to work in humans for a number of reasons

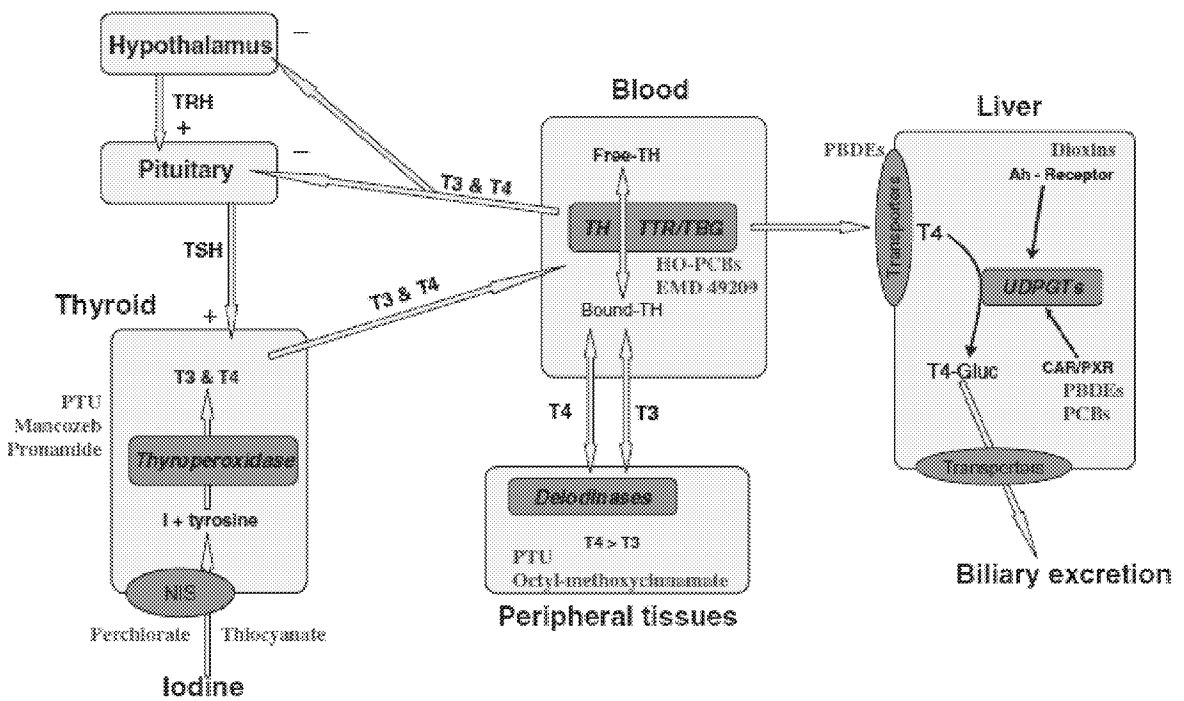
relating to the significantly greater sensitivity of hepatic enzyme induction in rodents versus humans, and the much shorter half-life, and hence greater turnover, of thyroid hormones in rodents in comparison to humans (McClean 1995; McClean et al 1989; Colnot and Dekant 2017).

The other modes of action of chemicals in affecting thyroid hormone homeostasis are not inherently species specific but pharmacokinetic and metabolism differences may render some species more sensitive to the thyroid effects than others (Zimmermann and Galetti 2015).

6.8.2.7 Miscellaneous modes of action in disrupting thyroid hormone homeostasis

There is considerable debate as to whether or not chemical disrupters are able to bind directly to thyroid receptors *in vivo* but while it is clear that certain chemicals, such as tetrabromo-bisphenol A, bisphenol A and hydroxyl PCBs, can alter thyroid hormone-responsive genes in various tissues including liver and brown fat (Moriyama et al 2002; Gauger et al 2004; Bansal et al 2005; Kitamura et al 2005), the evidence exists that the changes observed are not induced by binding to the nuclear thyroid receptors (Cheek et al 1999; Ishihara et al 2003; Gauger et al 2004). In an *in vitro* thyroid receptor binding assay, Ishihara et al (2003) showed that while several chemicals, including diethylstilboestrol, pentachlorophenol and ioxynil, were able to displace thyroid hormone from TTR, none were able to displace thyroid hormone from its receptor. Marsh et al (1998) found that two synthesised polybrominated diphenyl ethers were able to bind, *in vitro,* to both the alpha and beta forms of the thyroid hormone receptor but this has not been repeated by other groups investigating competitive displacement of thyroid hormones from the receptors. However, most *in vitro* screens for thyroid hormone receptor binding have relied upon the ligand-binding domain of the receptor, and while clearly this is the site for activation of the receptor, binding to other parts of the receptor could potentially result in allosteric hindrance that would alter the subsequent functioning of the receptor. Indeed this has been shown at least *in vitro* to be the case for PCBs (Miyazaki et al 2008).

Fig. 7: Known MoAs (in blue boxes) for disruption of thyroid hormone production and release (from Crofton 2008)



6.9. Comparison of thyroid hormone involvement in neurodevelopment in humans and rodents

6.9.1. Thyroid hormone and neurodevelopment in humans

It has been over 120 years since a committee of the UK Royal College of Physicians in London linked the pivotal role of the thyroid gland to normal human brain development (Ord 1898). Many countries have now instigated a neonatal hypothyroidism screening test for the early diagnosis of hypothyroidism, in recognition of the latter as the most common preventable cause of mental retardation in the young (Bhatara et al 2002). Thyroid hormones are known to be essential for the normal maturation and functioning of the central nervous system although they also modulate a broad range of effects on virtually all tissues (Karapanou and Papadimitriou 2011). It was originally thought that thyroid hormones were only important for human neurodevelopment postnatally. This was based on the observation that circulating foetal levels of these hormones were very low, together with the fact that the placenta was thought to present a very efficient barrier to the transfer of thyroid hormones from the mother. This prejudice was supported by the findings that post-natal thyroid support therapy could correct many of the thyroid dependent conditions that were present at birth following maternal hypothyroidism due to low iodine (Glendenning 2008). However, it is now clear that thyroid hormones are essential for both foetal and post-natal neurodevelopment, and for the regulation of neuropsychological function in children and adults (Williams 2008).

Thyroid hormones have no influence on the very early developmental events in the human foetus, such as neural induction and the establishment of cellular polarity, but they are essential to regulate later processes, including neurogenesis, myelination, dendritic proliferation and synapse formation (Bernal et al 2003; Bernal 2007; Zoeller and Rovet 2004). There are large numbers of genes transcribed following activation of the thyroid hormone nuclear receptor and the timing of the onset of thyroid hormone action is crucial for the correct development of the brain (Zoeller and Rovet 2004; Hindmarsh 2002). A condition known as endemic neurological cretinism in humans is due to maternal iodine deficiency, and resulting maternal hypothyroxinaemia, with low maternal T4 levels causing neurological hypothyroidism in the foetus, which results in profound mental retardation, cerebral spastic diplegia, deaf-mutism and squint in the absence of general signs of hypothyroidism (Porterfield and Hendrich 1993). Although iodine dependent neurodegenerative conditions, in neonates, can be prevented by iodine supplementation, iodine deficiency remains the commonest endocrine disorder in the human population and has been estimated to be the most frequent cause of preventable mental retardation (de Escobar et al 2004).

6.9.2. Comparison of the stages of thyroid hormone dependency in the rat and human foetus

There are three stages of thyroid hormone dependent neurological development in the foetus, with the first occurring between 16-20 weeks post-conception in humans, equivalent to day E17.5-18 in the rat, and before the foetus is able to synthesise its own thyroid hormones (Fig. 8). During this first stage thyroid hormones can only be delivered to the foetus via the placenta from maternally synthesised hormone (De Escobar et al 2000; 2004; Obregon et al 2007) and its presence stimulates neuronal proliferation and migration in the cerebral cortex, hippocampus, and medial ganglionic eminence (Narayanan and Narayanan 1985; Lucio et al 1997; Cuevas et al 2005; Auso et al 2004). The second stage occupies the remainder of pregnancy after the initiation of foetal thyroid hormone synthesis when the developing brain receives a dual supply of thyroid hormones from both a foetal and maternal origin. During this stage processes dependent upon thyroid hormones include neurogenesis, neurone migration, axonal outgrowth, dendritic branching and synaptogenesis, glial cell differentiation and migration, and the onset of myelination (de Escobar et al 2000; Porterfield and Hendrich 1993).

The third stage occurs between the neonatal and post-natal period when thyroid hormone supplies to the brain are entirely derived from the child (Fig. 8). During this stage, thyroid hormone is needed for the continuing maturation of the central nervous system that involves migration of the granule cells in

the dentate gyrus and cerebellum, migration of the pyramidal cells in the cerebral cortex and the Purkinje cells of the cerebellum, and the maturation of the glial system.

Fig 8: Contribution of human maternal and foetal thyroid hormones to development (from Williams 2008).

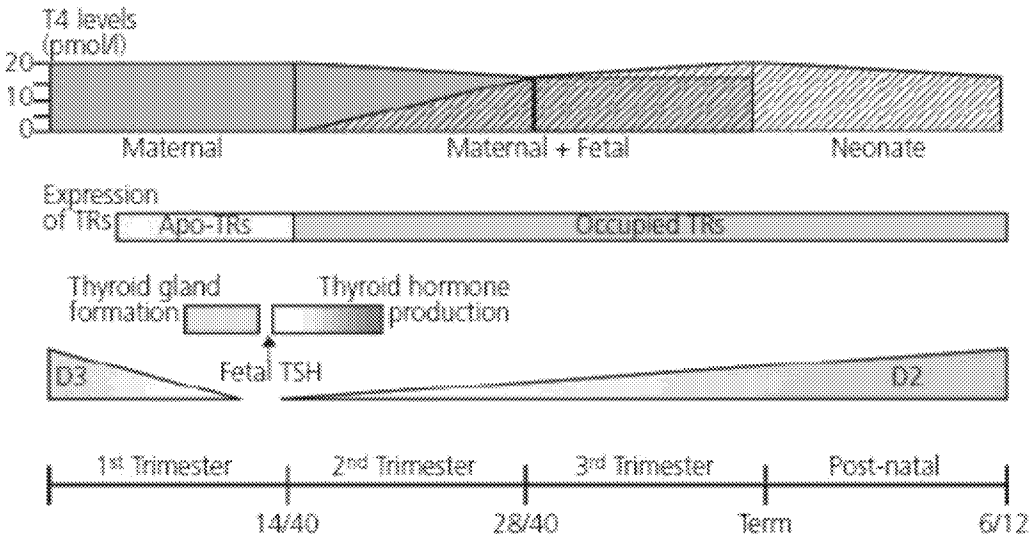


Fig. 8 above illustrates the respective contribution of foetal and maternal thyroid hormones at the respective times during gestation/pregnancy. The foetus is entirely dependent upon maternal thyroid hormone during the first trimester and entirely dependent upon its own hormone after birth. Foetal thyroid hormone production begins at the end of the first trimester peaking at term. The thyroid gland begins development midway through the first trimester and begins production of foetal hormone following a surge in foetal TSH at the end of the first trimester. D3 is an inactivating type 3 iodothyronine deiodinase which decreases during the first trimester allowing the initiation of production of the foetal hormone as concentrations of type 2 deiodinase (D2) increase after this period. TR represents thyroid hormone nuclear receptor which is able to bind thyroid hormone at the end of the first trimester. Type 3 iodothyronine deiodinase is thought to act to prevent thyroid hormone access to specific tissues at certain critical times and to prevent saturation of the thyroid hormone nuclear receptor (Bianco and Kim 2006; Bianco et al 2002). Type 3 deiodinase works by removing iodine from the inner ring of T4 or T3 and hence inactivates the hormone. It is expressed at its highest levels in the foetus whereas it is present only at low levels in adult tissue (Williams 2008).

The findings of neurodevelopmental problems following thyroid hormone deficiency in humans were subsequently confirmed and shown in animal studies (Koibuchi and Chin 2000; Thompson and Potter 2000; Morte et al 2002; Singh et al 2003). This data provided the critical evidence that thyroid hormones are essential in early (foetal) brain development, and that the associated neurological deficits depended upon the timing and severity of thyroid hormone insufficiency. Hence for chemicals that showed dose-response relationships in terms of their depression in circulating thyroid hormones, a threshold for the initiation of defects, based upon the threshold for thyroid hormone dependent effects, existed (Narayanan and Narayanan 1985). In the case of hypothyroidism occurring in adult rats, the neurological defects could be reversed by T4 supplementation (Ruiz-Marcos et al 1988) although this was not the case for perinatal and prenatal thyroid deficiency, where neurological deficits were generally permanent.

The order of development of the thyroid gland, during organogenesis, is similar for rodents, sheep, and humans, but the timing of various perinatal developmental events differs significantly between the species, with rats being born relatively immature, and hence late developmental events, that occur *in utero* in humans, only occur postnatally in rats. Because of their advanced state of development at

birth, thyroid development in sheep, by comparison, occurs almost exclusively in utero (Hombach-Klonisch et al 2013).

The timing of the onset of thyroid receptor (TR) binding also differs significantly between species independent of their differing gestational times and can be first observed in the rat during mid-to late-gestation (average gestation is 21 days), in sheep during the latter two-thirds of gestation (average gestation is 20.5 weeks), and in humans between gestational weeks 10 and 16 (average gestation is 39 weeks) (Fisher and Brown, 2000).

6.10. Comparison of thyroid cancer in human and animal species

Thyroid cancer is the most common endocrine malignancy in the human population and incidence rates in most countries have been steadily increasing over the past few decades, particularly in women (Zimmermann and Galetti 2015). Known risk factors for human thyroid cancer are radiation exposure during childhood, exposure from nuclear accidents (Mazonakis et al 2007; Bounacer et al 1997), natural radiation, or medical imaging (Bard et al 1997), but suspected risk factors include obesity and the metabolic syndrome (Renehan, et al 2008; Rinaldi et al 2012), environmental pollutants (Hallgren and Darnerud 2002; Zhang et al 2008), a family history of thyroid cancer, or thyroid disorders (Franceschi et al 1999) and, for certain susceptible individuals, iodine intake (Zimmermann and Galetti 2015). While iodine deficiency together with a small number of drugs are known to induce thyroid growth (goitrogen) in humans, the only confirmed human thyroid carcinogen is ionizing radiation, and the available evidence would suggest that chemicals that induce thyroid growth alone, in the absence of additional mutational events, will not lead to human thyroid cancer (McTiernan et al 1984; Holm et al 1988; Daminet and Ferguson 2003).

Chemically-induced rodent thyroid follicular cell cancer arises from one of two different processes that ultimately converge under the influence of continued TSH stimulation. The first process is found with chemicals that induce DNA damage/mutations specific to the thyroid, while the second is seen with chemicals that induce perturbations of hypothalamus/pituitary/thyroid axis with subsequent increased stimulation of thyroid cell growth by TSH. An example of a chemical that induces thyroid follicular cancer in rodents exclusively through a mutagenic MoA is N-Bis-(2-hydroxypropyl) nitrosamine as it is entirely devoid of effects on thyroid hormone production or activity (Hiasa et al 1991).

6.10.1. The association between iodide deficiency, goitre and thyroid cancer in the human population

The epidemiology of goitre and thyroid cancer is confusing, and confused, and the appropriate interpretation requires an in depth understanding of the molecular biology, underlying causes of thyroid cancer, and the clinical development and treatment of both goitre and cancer. One of the most scientifically enlightening causes of human thyroid disease is that resulting from iodine deficiency since it has been known for centuries that in such conditions, there will be a high incidence of thyroid hypertrophy in the local population. Humans living in areas of chronic iodide deficiency develop thyroid hypertrophy/hyperplasia manifest in the appearance of goitre (Zimmerman 2009; Kotwal et al 2007; Li and Eastman 2012), due to chronic deficiency in the production of thyroid hormones, and the continued stimulation of the thyroid gland by TSH released from the pituitary gland. Supplementation of dietary iodine is normally able to reverse the clinical symptoms of both goitre and the other physiological and neurological effects of hypothyroidism (Kotwal et al 2007; van der Haar 2007; Liesenkötter et al 1995).

6.10.1.1. The role of TSH in human thyroid cancer

The data for the role of TSH in human thyroid cancer contrasts dramatically with the situation in rats. There is a large volume of epidemiological data regarding thyroid cancer rates in areas of endemic goitre, where iodine deficiency would be expected to lead to chronic TSH stimulation of the thyroid gland. In the United States an extensive study compared regional rates of thyroid cancer mortality and

endemic goitre over a 20-yr period both before, and after, the generalized introduction of iodinated salt (Pendergrast et al 1961). While the incidence of goitre significantly decreased in the areas previously affected by endemic goitre, there was no similar change in thyroid cancer mortality rates and hence no association between the two diseases. Similar results were obtained in Austria (Riccabona 1986) where large areas of the Tyrol have historically shown an abnormally high incidence of goitre due to iodine deficiency. While there are regions of the world with endemic goitre and a high incidence of thyroid cancer, there are also similar regions with a high incidence of goitre in the absence of similar high thyroid cancer rates, as well as areas without endemic goitre where the incidence of thyroid malignancies is higher than normal (Clements 1954, Riccabona 1987).

In contrast to the previous examples however, patients with goitres that arise, not from iodide deficiency but from congenital thyroid gland metabolic defects (i.e. mutations of various kinds), have been noted to have a markedly increased incidence of thyroid malignancies (Kitahara and Sosa 2016; Cooper et al 1981; McGirr et al 1959; Elman 1958). There is also an increased incidence of thyroid carcinoma in patients with thyrotoxicosis (Siegel and Lee, 1998; Verburg and Reiners 2010; Pendergrast et al 1961; Clements 1954). There are, however, no reports of an increased incidence of thyroid cancer in patients with thyroid hormone resistance where chronically high levels of circulating TSH exist, due to a non-functional negative feedback control in the hypothalamus/pituitary gland, and an inability to react to normal levels of circulating T3 (Yen 2003).

In those situations where there is an increased risk of thyroid malignancy in patients with glands showing metabolic defects, mutations, or in subjects with thyrotoxicosis, the thyroid cancers do not arise simply due to the hyperplastic stimulus of TSH alone but are complex combinations of genetic and hormonal factors. The finding that simple goitre can be fully reversed by supplementing the diet with iodine shows that while the goitre is undoubtedly produced under a chronic TSH stimulus, the thyroid gland nevertheless retains its ability to return to normal despite many years of TSH stimulation. This is very different to the situation in rodents where the evidence points to the ability of sustained TSH stimulation alone, as can happen with chemically induced thyroid insufficiency, being able to induce neoplasia (Capen 1999). Indeed the implantation of TSH secreting pituitary tumours alone into recipient rats has been shown to be able to induce thyroid follicular neoplasms in the absence of any further treatment (Hill et al 1989).

6.10.1.2. The role of TSH in rodent cancer

Rats have higher basal levels of circulating TSH than are present in humans (Table 2; Lewandowski et al 2003) and in contrast to the situations in the human population, where plasma levels of TSH are roughly equal between men and women, male rats have higher normal levels of TSH than are present in female rats (Hill et al 1998). This finding correlates well with the observation that the height of the follicular epithelium is greater in males than in female rats, reflecting the greater TSH-induced activity in males versus females (Capen 1996). Male rats also show a higher spontaneous incidence of thyroid follicular cancer than do female rats (IARC 1999; Haseman et al 1985). In a survey of the outcome of mouse and rat carcinogenicity studies (Hurley et al 1998), twenty-four of the approximately 240 pesticides tested were found to induce thyroid follicular cell cancer, and of these twenty-two induced thyroid tumours only in rats, with the remaining two inducing thyroid cancer in both rats and mice. Additionally, chemically-induced thyroid neoplasms occurred far more frequently in male rats than in female rats, both with respect to the proportion of chemicals that induced thyroid tumours, and to final tumour incidence. In contrast to the situation in the rat, thyroid cancer incidences are greater in human females than in males, while TSH levels between men and women are the same suggesting some fundamental differences both in basal thyroid control and physiology but also in their responses to situations that cause thyroid cancer (Hill et al 1998; Parker et al 1997).

6.10.2. Comparative cancer studies in laboratory animals and humans for goitrogenic chemicals

The goitrogenic effects of sulphonamide drugs have been known for many years since the first demonstration of rat thyroid hypertrophy by the prototype sulphonamide, sulphaguanidine (MacKenzie and MacKenzie 1943; Mackenzie et al 1941; Astwood et al 1943). The sulphonamide drugs, as a

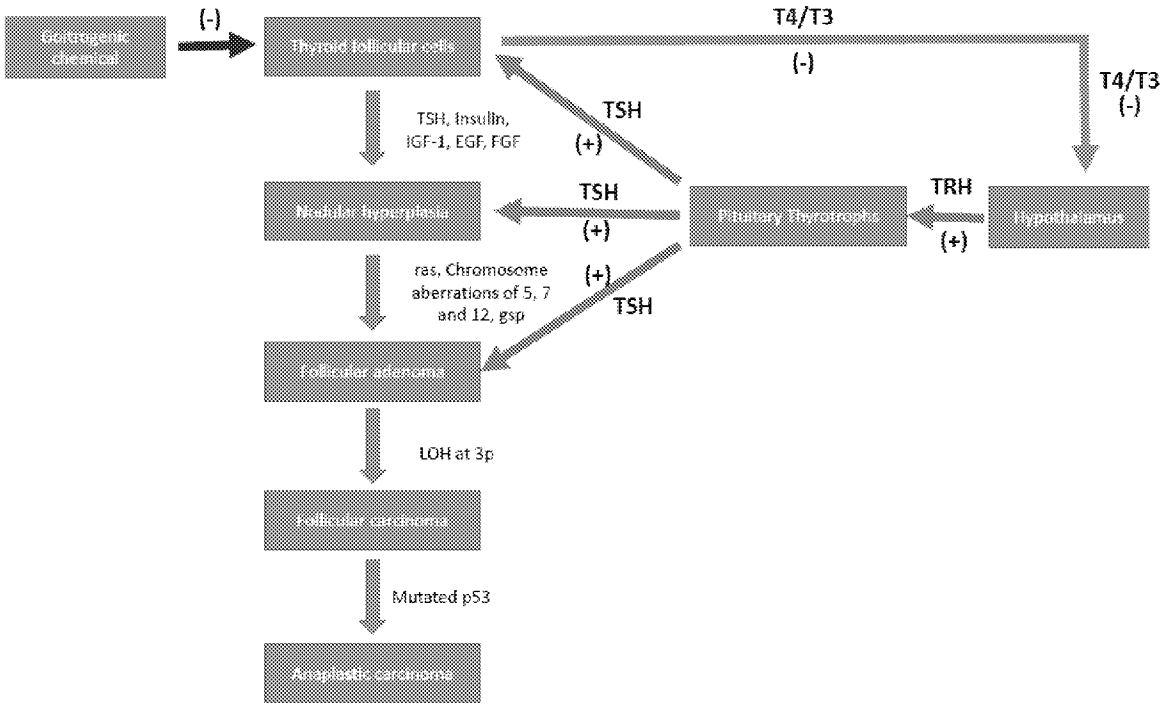
class, induce thyroid changes in the rat and dog through inhibition of thyroperoxidase-catalysed binding of iodine to thyroglobulin and the resulting decrease in circulating thyroid hormones (Nishikawa 1983).The next generation of sulphonamides also turned out to be potent goitrogens in the rat, when a combination of sulfamethoxazole and trimethoprim was shown to cause dramatic decreases in T3 and T4, with compensatory increases in both circulating TSH and in the weight of the thyroid gland. While similar effects were also seen in the dog with this drug (Nishikawa 1983), monkeys and humans did not show the thyroid inhibitory effects, and when rhesus monkeys were given doses of sulfamethoxazole up to 300 mg/kg/day for 52 weeks there were no changes in either thyroid gland weight or histology (Swarm et al 1973). In contrast long-term administration of sulphonamides, at dose levels that result in prolonged stimulation of the thyroid gland by TSH, induces thyroid neoplasia in rats (Doerge and Decker 1994).

A similar species difference in sensitivity to the inhibitory effects of these drugs was found following *ex vivo* experiments in a thyroid peroxidase assay using PTU (another thyroperoxidase inhibitor) and the sulphonamide, sulphamonomethoxine. In this study, the concentration of PTU required to inhibit thyroperoxidase sourced from monkey thyroid was approximately 50x higher than that required for the same degree of inhibition in rat thyroperoxidase, while for sulphamonomethoxine the concentration required to inhibit 50% of the thyroperoxidase activity was 500 fold greater in the monkey enzyme than in rat thyroperoxidase (Takayama et al 1986). This data was strongly supportive of an intrinsic sensitivity difference in the enzyme between that derived from rat thyroid and that from monkey thyroid. These studies further demonstrated that, at least with thyroperoxidase inhibitors, significant differences in potency existed between sensitive species, such as rat, mouse and dog, and more resistant species that included non-human primates, human, guinea pig and chicken (Capen 1999). In support of this data, only mild effects of sulfonamides have ever been observed on human thyroid function (Cohen et al 1980).

6.10.3. Thyroid hormone perturbation and thyroid cancer in rodents

In mammals in general, including humans, when demands for more thyroid hormone are small, existing thyroid follicular cells can meet any extra demand by increasing their synthesis and output, often becoming larger in size (hypertrophic). At least in rodents, inhibition of thyroid hormone synthesis and/or secretion, such as that occurring following the administration of certain chemicals, is responded to by increasing the number (hyperplasia) and size (hypertrophy) of thyroid follicular cells to enhance thyroid hormone output. This response is mediated through feedback release of TSH from the pituitary gland, and in the presence of continued thyroid hormone deficiency, and chronic TSH stimulation, there is an increase in the thyroid weight as a result of a combination of thyroid follicular hypertrophy and hyperplasia. Since the TSH-producing thyrotrophs in the pituitary gland are also producing, and secreting, at a greater rate than normal, they will also undergo hypertrophy and hyperplasia and where looked for, these are invariably seen in the adenohypophysis of the pituitary gland under conditions of moderate to severe thyroid hypertrophy (Norford et al 1993; Russfield 1967; Moriarty and Tobin 1976; Akosa et al 1982).

Fig. 9: The postulated progression of changes in rat thyroid follicular epithelium in the development of cancer from chronic exposure to a goitrogenic chemical



Notes: The red arrow indicates a thyroid disrupting chemical, the green arrows indicate a positive effect on the target cells while the blue arrow indicates the negative feedback response of the hypothalamus to low circulating T3/T4 levels.

In rodents, there is adequate evidence, with a comparatively large number of chemicals of various classes and diverse pharmacological, that continued thyroid stimulation by TSH alone eventually leads to neoplasia of the thyroid follicular cells (Hill et al 1998; Hurley et al 1998; Capen 1999). The proposed MoA for the production of rat thyroid cancers by goitrogenic chemicals is illustrated in Fig. 9. Although TSH is the main hormonal driver for follicular cell hypertrophy and hyperplasia, several other factors, including insulin, epidermal growth factor (EGF), fibroblast growth factor (FGR) and insulin-like growth factor (IGF), have been shown *in vitro* to also be mitogenic for the follicular thyroid epithelial cells (Maviel et al 1988; Logan et al 1992; Żerek-Meleń et al 1987).

The data therefore support a conclusion that intrinsic sensitivity differences occur between species, in terms of their sensitivity to thyroid hormone inhibition, and also strongly suggests that qualitative differences may also determine a cancer outcome with prolonged TSH stimulation being the main driver for rodent thyroid non-genotoxic cancer, whereas TSH is only able to promote cancer in humans in the presence of underlying thyroid problems such as inherited mutation associated metabolic disorders or thyrotoxicosis (Curran and DeGroot 1991).

6.10.4. Is chemically-induced rodent thyroid cancer relevant to man?

For regulatory purposes, and in the absence of MoA data to the contrary, chemical induced rodent thyroid tumours is presumed to be relevant to humans, and when information on differences in inter-species MoA is lacking, the default is to assume comparable carcinogenic sensitivity in rodents and humans (Hill et al 1998; Hard 1998). Where there are follicular neoplasms in the absence of evidence of follicular hyperplasia/ hypertrophy, or evidence of disruption of the thyroid-pituitary axis, such neoplasms are presumed to be relevant to humans and in terms of their dose-response relationships, the Environmental Protection Agency applies linearity when estimating the risk to thyroid cancer

induced by chemical substances that either do not disrupt thyroid functioning, are mutagenic, or that lack MoA information. In terms of a neoplastic MoA for rodent thyroid tumours both IARC (1999; 2000) and the US Environmental Protection Agency (US EPA 1998) currently have established specific guidance for evaluating the human relevance of these tumours.

In order to show a thyroid-pituitary MoA for any particular chemical, the US EPA (1998) asks for evidence in the following five areas:

1. Increases in cellular growth (e.g., increased thyroid weight, hypertrophy or hyperplasia, proliferation detected by DNA labelling or mitotic indices)
2. Changes in thyroid and pituitary hormones (T3/T4, TSH)
3. Location of site of action (e.g., thyroid, liver, or peripheral, and enzyme target within the target organ);
4. Dose correlations among thyroid effects (cell proliferation/hypertrophy) and cancer;
5. Reversibility of effects when chemical dosing stops.

For those chemicals that are non-mutagenic and non-genotoxic, that reduce thyroid hormones acutely/chronically, and that increase TSH levels, in the absence of a proven MoA having no relevance to humans, the outcome is still considered to represent a human risk.

The increasing adoption of the WHO/IPCS principles of establishing MoA and human relevance for these findings have, more recently introduced a more stringent list of requirements that consider all possible alternative MoAs and that apply modified Bradford Hill criteria for estimating the strength of association of between the proposed MoA and the adverse outcome (Dellarco et al 2006; Boobis et al 2006; Meek 2008; Meek et al 2014). This has been a welcome step in applying objective scientific data to support arguments of non-adversity of effects, and non-relevance to the human population of animal toxicity results and has applied an objective approach to regulatory decision making that takes into account non-guideline investigative study data in addition to GLP conducted guideline study data to arrive at their final conclusions.

6.11. Cross-species extrapolation for thyroid effects

Inter-species extrapolation of adverse thyroid effects, normally detected in laboratory animal studies, to human exposures, is an exacting science requiring firstly a thorough demonstration of the MoA of the toxicity in the target laboratory animal species, most often the rat. Once accepted as a plausible explanation a reasoned argument would then be made, based upon sound principles, as to the human relevance of the laboratory animal MoA. Acceptable approaches to this problem have been widely publicised in the WHO/IPCS guidelines for establishing a weight of evidence conclusion to the exercise (Meek et al 2003; Boobis et al 2006) and this approach has been included in the draft GD (ECHA/EFSA/JRC 2017). There are clearly situations in which the effects of a chemical in the rat are similar to what would be predicted in humans, and perchlorate is a clear example of this principle (Wolff 1998).

For some chemicals that affect thyroid function however the situation may be very different and there may be little data to support cross-species extrapolation (Crofton 2004). Both *in vivo* and *in vitro* studies show that sodium phenobarbitone, pregnenolone-16α-carbonitrile (PCN), acetochlor and PCBs, activate nuclear receptors such as the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) either individually, or as dimers and that administration of these chemicals to rodents leads to an up-regulation of hepatic catabolic enzymes, stimulation of the biliary elimination of thyroid hormones, a decrease in circulating thyroid hormones (Schuetz et al. 1998; Liu et al 1996; Hood and Klaassen 2000), and thyroid hypertrophy and hyperplasia. Although human liver does contain both CAR and PXR (Moore et al 2000; Omiecinski et al 2011) there are significant species

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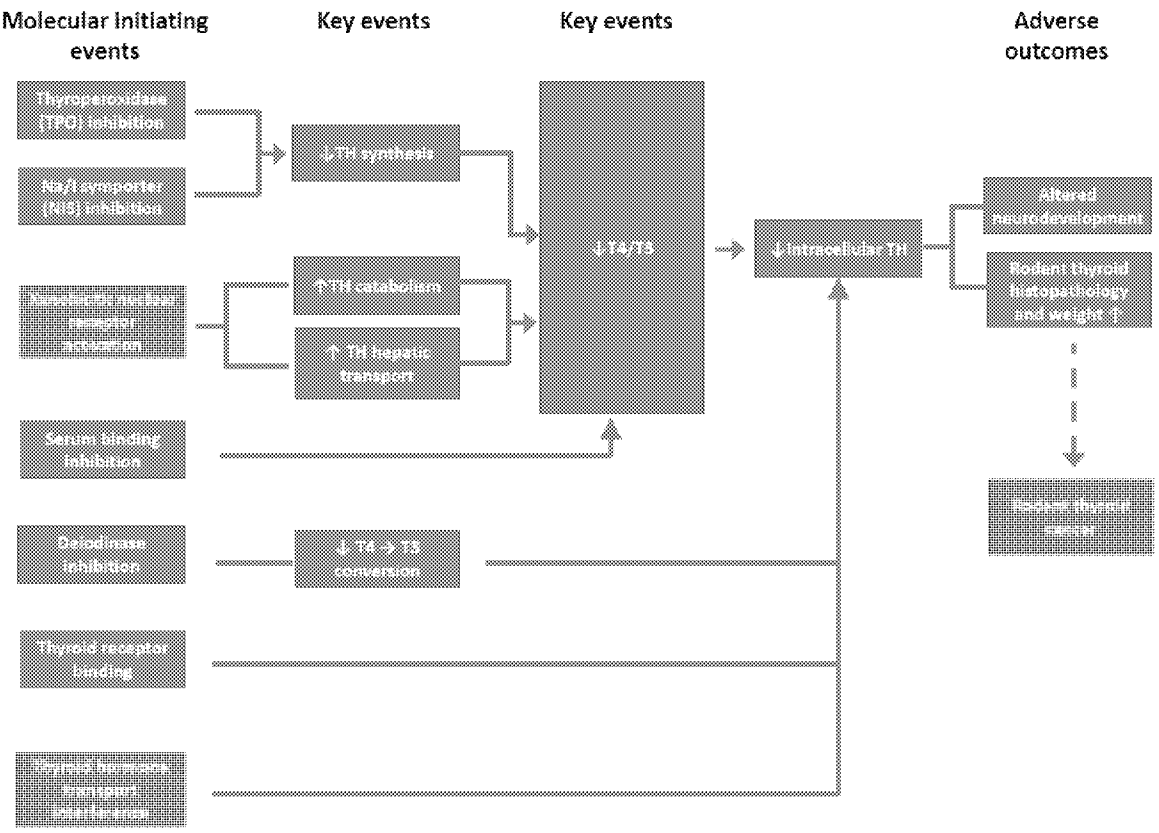
differences in response to those chemicals that activate these nuclear receptors both in terms of sensitivity of response (Elcombe et al 2014) and in terms of specificity with rodent PXR being activated by PCN, but not by rifampicin, whereas human PXR is activated by rifampicin but not by PCN (Kliewer et al. 2002). Additionally, while PCBs in general are agonistic to PXR in rats, *in vitro* data in human hepatocytes show that high concentrations of PCB-153 are antagonists to the human PXR (Tabb et al. 2004).

Not to make too much of a point about it, significant species differences exist between human and rodents in terms of the thyroid binding proteins in the circulation where the relative importance of TTR, TBG and albumin for transporting thyroid hormones accounts for significant differences in thyroid hormone half-life and turnover, with rodents showing appreciably higher rates of production and loss of thyroid hormones than do humans, a situation that leads to increased overall sensitivity, and in some cases species specific differences, in chemical perturbation of the HPT axis (Capen 1997; Hill et al 1998).

Fig. 10: An adverse outcome pathway analysis of chemically-induced thyroid hormone disruption (from Paul 2014).



7. ECPA Question: Is there evidence for a threshold/correlation for adversity linked to serum thyroid hormone levels (T3, T4, TSH and sulphated thyroid hormone), between species

Thyroid hormone disruption has been the subject of adverse outcome pathway (AOP) analysis (Paul 2014). Fig 10 above is taken from a presentation that clearly outlines the current understanding of the molecular initiating events (MIEs) that lead to altered hormone homeostasis (Crofton 2008; Murk et al 2013). Although the AOP above might suggest that any single chemical would act through a single molecular initiating event, many rodent thyroid-acting chemicals disrupt multiple MIEs and for several the ultimate thyroid disruption is thought to be considerably greater as a result of these multiple hits on

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its function. PCBs exemplify this principle where they have been shown to displace thyroid hormone from its binding protein, to inhibit deiodinase activity, and to increase thyroid hormone catabolism by inducing hepatic glucuronidation (Barter and Klaassen 1992; 1994; Brouwer 1989; Hood and Klaassen 2000).

While each of the MIEs in Fig 10 will operate essentially independent of the administered dose of a thyroid disrupting chemical, activation of subsequent key events would be expected to follow classical dose-response relationships with higher dose levels inducing greater changes in each key event, and a threshold dose level below which each respective key event will not be triggered (Dellarco et al 2006). Hence each key event would be expected to follow conventional dose-response relationships dependent upon the degree of change in the previous key event with clearly defined exposure thresholds for triggering subsequent events. In the absence of a sufficient change in the previous key event the downstream key event would not occur. An alternative way of looking at this is in terms of chemically induced depressions in thyroid hormones where TSH release from the pituitary gland only occurs when circulating levels of T3 achieve a threshold level that stimulates the receptors in the hypothalamus to release TRH.

Comparative studies on the effects of perchlorate in the rat and human thyroid gland (Yu et al. 2002) have shown dose related inhibition of uptake of radiolabelled iodide of 15%, 55%, and 65% at 1.0, 3.0, and 10 mg/kg perchlorate respectively on day 1 following initiation of perchlorate administration to the rat but that the homeostasis was reset in animals killed after 28 days where inhibition of iodide uptake was only observed at the top dose levels of 10 mg/kg. This adaptation of the thyroid system to prolonged administration is somewhat different to the way in which other organs deal with toxicity but shows clear adaptation probably by increasing the expression of the sodium-iodide symporter on the follicular cells of the thyroid. A similar response was not observed in a 14-day study with perchlorate administration in human volunteers (Greer et al. 2002) and the species difference exhibited was thought to have occurred in rats because of their smaller reserve capacity of thyroid hormones than humans.

7.1. Thyroid hormone changes in the absence of histological changes

The negative feedback control of circulating free T3 and T4 on the hypothalamus and pituitary plays an essential role in ensuring that there is sufficient circulating thyroid hormones to satisfy the daily needs of the body. The circulating T3 concentrations are a product of the deiodination of T4 through the action of hepatic 5' deiodinases together with small amounts of T3 being released directly from the thyroid gland. Hence circulatory T3 levels are maintained by a large pool of protein bound T4 which can be deiodinised in peripheral tissues, mostly the liver, when the concentration of free circulating T3 falls below acceptable limits. In addition to TSH controlling the production and release of T4/T3 from the thyroid gland, it is also responsible for the control of the activity of peripheral tissue deiodinases to modulate the conversion of T3 from T4 (Hoermann et al 2015) and hence TSH acts to integrate the peripheral and central elements of thyroid hormone homeostasis into one overarching control system. This is the normal physiological control of thyroid hormones and it operates within defined limits of circulating hormones, but not within a narrow band of concentrations since it is clear that release of the master controller, TSH, follows a circadian rhythm of peaks and troughs in the circulation throughout the day (Fisher 1996). Indeed, the differences between peak and nadir levels of TSH in humans have been shown to differ by as much as ±50% around a mean value (Hoermann et al 2015; Fisher 1996). Despite these daily fluctuations in serum TSH levels, those of T3 and T4 remain essentially within acceptable limits underlining the critical importance of an adequate concentration of thyroid hormones in maintaining the normal physiology in the body (Bianco and Kim 2006).

It was originally stated in the draft GD (page 96; Appendix A) that "A decrease in T4 (total or free) in the absence of other histological changes and/or hormonal evidence of hypothyroidism is a relatively frequent observation in experimental toxicological studies, particularly in rodents." (ECHA/EFSA/JRC 2018). This statement has subsequently been removed from the final issued GD (Andersson et al 2018) but still appears as a sub-heading in Appendix A entitled "Investigations of perturbations of circulating thyroid hormone in the absence of histological changes in adults" although without any

clarification it is unclear exactly where this statement has come from, and within what context it is meant since it does have some significant consequences if true.. Even within the final GD there is a contradiction in this statement in that a literature reference is quoted with regard to frog thyroid gland whereby the document contends that "Thyroid histology is a valuable and sensitive diagnostic parameter for detecting the ability of a substance to interact with the HPT axis, particularly for thyroid system antagonism (Grim et al., 2009).". While there are differences between frog, human and rodent, the control of thyroid gland physiology is remarkably similar across species and it is surprising that frog histopathology can be adequate whereas rodent histopathology, with considerably more experience of examination than anything done in the frog, can be any less so. The document throughout appears to have been heavily influenced by certain literature references (and possibly by anecdotal comments) that require considerably more critical evaluation before acceptance than has been done, with potentially significant repercussions as a consequence.

Sporadic changes in single parameters, in the multitude of clinical chemistry endpoints measured in routine toxicity studies, is indeed a common observation and, in the absence of expected accompanying changes in linked parameters, is normally discounted (Hamada et al 1998; Lewis et al 2002). As with all parameters in such studies, the difficulty lies in linking the effects, in this case a decrease in T4, to any functional consequence since thyroid control, like most physiological systems in the body, operates within tolerances rather than being dependent upon excursions outside of narrow ranges. Considering the critical importance of thyroid hormones, there is an inbuilt redundancy in terms of high circulating T4 levels and increased intracellular conversion rates of T4 to T3 by induction of deiodinase enzymes, which allows the maintenance of T3 homeostasis within the limits required for adequate physiological control. Hence a degree of decrease in T4 will still permit adequate levels of T3 to be maintained without triggering compensatory TSH release and thyroid histological changes. Therefore, in the absence of supporting evidence of histopathological effects in the thyroid gland or pituitary, or expected associated changes in TSH and T3, isolated changes in T4 need to be placed into the context of the study as a whole, and interpreted with an eye to the duration of the study and dose-response relationships for the observed change, as it would for any other potentially adverse endpoint.

While decreases in T4 can be diagnostic of an endocrine effect, there are a number of non-physiological, and non-endocrinological, reasons why a decrease in a single parameter should not be considered biologically/toxicologically relevant (Lewis et al 2002). Despite the fact that decreases in T4 in human cases can be adverse with regard to pre- and post-natal neurological development and thyroid pathology, they are always accompanied by concomitant hormonal and/or tissue changes that may or may not be described due to the constraints placed upon clinical studies (Colnot and Dekant 2017). There are no such restrictions on laboratory animal toxicity studies and complete tissue histopathology and a broad range of hormonal and clinical chemistry assessments will accompany any appropriately OECD guide-lined test, such that expected accompanying pathophysiological endpoints will be present, alongside the change in the measured parameter, to enable that change to be placed into context.

In a situation where a chemical is suspected of affecting thyroid homeostasis then specialised studies to establish any functional consequences of the changes, including MoA and human relevance focussed experiments, might help clarify whether or not the observed change was accompanied by functional consequences. Studies such as the EOGRTS or DNT would help to clarify the biological significance of particular hormonal changes, but since hormonal disruption generally follows classical dose response relationships (Klaassen and Hood 2001; Ren et al 1988; Liu et al 1995), small changes in circulating hormones, that occur at relatively low doses of chemicals, would not be expected to result in functional consequences when the considerable redundancy in function that exists for the thyroid control system is taken into account.

A discussion of the topic of isolated thyroid hormone changes on studies has been covered in an excellent recent publication by Colnot and Dekant (2017) where they outline the considerations that would normally be taken when arriving at a decision regarding the treatment-relatedness or otherwise of a change in thyroid hormones. Hence a difference in thyroid hormone levels between controls and treated animals is unlikely to represent a treatment-related effect if it is transient and occurs in the

absence of dose-response or if it occurs in one or a few animals. A difference in thyroid hormone levels is less likely related to treatment even if statistically significant if it remains within the range of hormone levels present in the serum of control animals from other studies conducted in the same laboratory (historical controls).

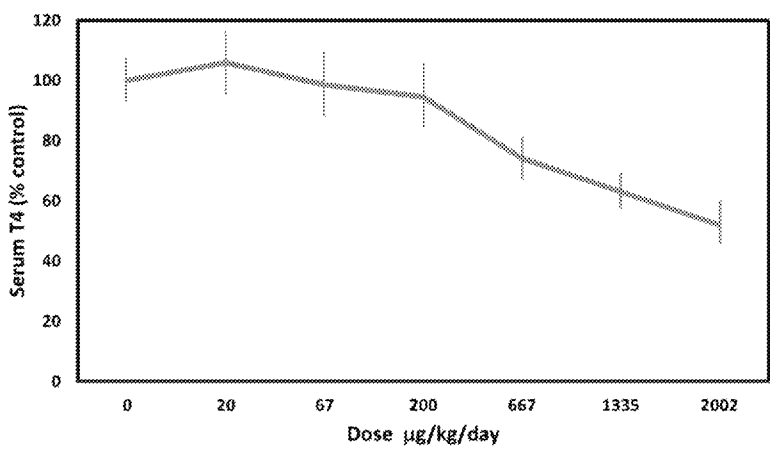
Since it is well known that circulating thyroid hormone levels are subject to a number of confounders such as generalised stress including body weight and food consumption effects, altered thyroid hormone levels are less likely to be specific and more likely to be secondary to systemic stress effects if there is no alteration in the general function of the thyroid as determined by thyroid weights and histology (DeVito et al., 1999; Choksi et al., 2003) since these endpoints are less sensitive to these secondary effects (St Germain and Galton, 1985; Cavalieri, 1991). Altered caloric intake is known to affect thyroid hormones, and reduced food and water intake is commonly observed on toxicity studies in rodents, some of the thyroid hormone changes observed, when unaccompanied by supportive changes in histology and thyroid weight may be secondary to inanition induced by the use of maximum tolerated doses or, considering the impact of disease on thyroid hormone homeostasis, be secondary to toxicity in other organs such as the kidney or liver.

7.2. Evidence for dose response/threshold relationships for thyroid hormone effects

7.2.1. Evidence in animal studies

A series of dose response studies on a range of chemical mixtures, known to disrupt thyroid hormone homeostasis, were carried out on female Long-Evans rats dosed via gavage with a number of poly-halogenated aromatic hydrocarbons for 4 consecutive days and serum total T4 was measured in samples collected 24 hr after the last dose (Crofton et al 2005). These studies clearly showed a threshold dose for the mixtures (Fig. 11), below which no effects on serum T4 were observed (Crofton et al 2005).

Fig 11: Dose dependent decrease in serum T4 concentrations following exposure to a mixture of polycyclic halogenated hydrocarbons (PHAH) – redrawn from Crofton et al 2005)



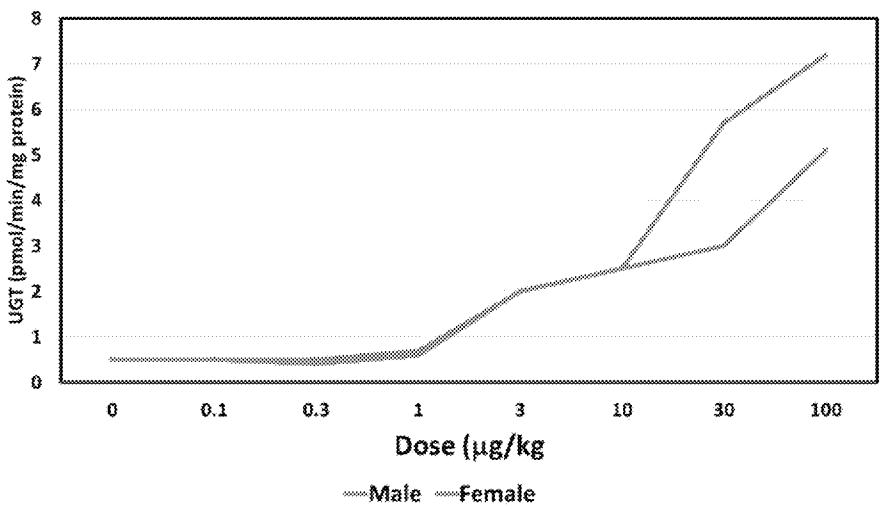
A dose response study was also carried out under the auspices of the National Toxicology Program where 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) was dosed by oral gavage to female Harlan Sprague-Dawley rats at dose levels of 10, 100, 300, 1,000, or 3,000 µg/kg 5 days per week for periods of up to 105 weeks (NTP 2006). Assessments carried out on this study included free and total thyroid hormones and potential cancer outcomes, but not thyroid organ weight.

Serum total T4, free T4, and total T3 concentrations in the 3,000 µg/kg group only were significantly lower than those in the vehicle controls at the 14-week interim evaluation. At the 53-week interim evaluation, serum total T4 and free T4 concentrations in the 3,000 µg/kg group only were significantly lower than in the vehicle controls but there were no changes in total or free T3 levels. At 2 years, the incidences of minimal to mild follicular cell hypertrophy were significantly increased in the 300 µg/kg and 3,000 µg/kg (core and stop-exposure) groups although follicular hypertrophy was recorded at all dose levels including the controls (incidences of 5/51, 9/52, 9/53, 12/53, 10/53, 17/51, 12/49 at the 0, 10, 100, 300, 1000 and 3000 µg/kg).

Once again this study clearly illustrates a threshold for thyroid hormone disruption at all time points measured (NOEL at 1000 µg/kg), but not for histopathology of follicular hypertrophy at the 2 year time period. The proposed AOP for thyroid changes predicts that hormone changes would be a more sensitive endpoint than histopathology and that the latter should occur following activation of the respective MIE. The fact that neither of these was seen in this study is most probably explained as an artefact of the time points where the various endpoints were measured and emphasises the fact that bolting on thyroid hormone measure to a standard study design is most probably not going to give supportive information for establishing MoA. This data strongly supports the conclusion that specially designed, preferably short term, studies would most likely provide the information that would give the most informative data on which to address prospective MoAs.

A similar study was conducted with another polyhalogenated hydrocarbon, PCB 126, which was dosed by oral gavage to rats daily for four days at a range of dose levels (Fig. 12) from 0.1 to 100 µg/kg/day (Craft et al 2002). The study measured effects on hepatic glucuronyltransferase and on serum T4 levels, and while showing clear thresholds for dose levels where reductions in serum T4 occurred, it also demonstrated a concomitant induction of hepatic UGT which showed a LOEL at 3 µg/kg which corresponded to a similar LOEL for reductions in serum T4 (Fig. 11).

Fig. 12: Hepatic UGT levels in rats following dosing with PCB 126 for 4 days (Craft et al 2002)



Studies in rapidly growing, prepubertal, cynomolgus monkeys, made hypothyroid by the administration of methimazole in drinking water, showed a clear dose response in the concentration of exogenous T4 required to correct the inhibition of lower leg growth rate, and to stabilise serum levels of T3, T4 and TSH. (Ren et al 1988). Hypothyroid monkeys showed a 65% decrease in lower leg growth rate, decreased T3, T4 and IGF-1, and increased TSH compared to non-hypothyroid monkeys of similar age. Following administration of exogenous T4 in this experiment, lower leg growth rate increased significantly, in a dose dependent manner, such that T4, given at dose levels of 4 and 8 µg/kg/day had 56% and 73% increases in lower leg growth rate respectively compared to controls, this change being accompanied by a return of the circulating thyroid hormone levels to normal.

7.2.2. Evidence in human studies

The evidence for thresholds in the effects of T4/T3 is also seen with TSH in human studies. In a study where PTU was administered to hypothyroid patients receiving 0.2 mg/day of T4 (Saberi et al 1974), PTU induced a fall in mean serum T3 concentrations from a mean value of 84 ng/ 100 ml to 70 ng/100 ml (a 17% decrease) after 1 day, and to 63 ng/100 ml (a 25% decrease) after 2 days of PTU. Under these conditions, and following these drops in serum T3, serum TSH concentrations did not increase. Hypothyroid patients receiving T4 at a lower dose of 0.1 mg/day showed a similar decrease in serum T3 following PTU administration which was accompanied by increases in TSH from pre-study levels of 29.6 μU/ml to a peak of 40 μU/ml on day 5-6 of administration of PTU. These results suggest that at least in hypothyroid patients, there is a tolerance before a TSH response occurs following decreases in circulating T3, and a threshold for decreasing circulatory thyroid hormones, above which no compensatory release of TSH will occur from the pituitary.

7.3. Evidence for dose responses/thresholds for neurodevelopmental disorders of thyroid homeostasis

It is clear from both human and animal studies that foetal development is critically dependent upon the correct concentration of thyroid hormones at the right phase in the foetal development. For many years human hypothyroidism during pregnancy has been known to produce severe neurological abnormalities in the offspring of affected individuals that cannot be corrected by thyroid hormone supplementation post-partum. However, the severity of the neurological deficits of neonatal hypothyroidism in humans are dependent upon the severity of the maternal hypothyroidism, and when detected are largely preventable by immediate thyroid hormone replacement (Williams 2008; Alexander et al 2017). However, untreated human neonates exhibit growth retardation and general features of hypothyroidism with mental retardation, tremor, spasticity, and speech and language deficits.

In human beings, pregnancy has a significant impact on the thyroid gland and its function. During pregnancy, the thyroid gland increases in size by 10% in countries with healthy iodine levels in the diets, but by 20% to 40% in areas of iodine deficiency. Production of T4 and T3 has been reported to increase by nearly 50% on non-pregnant women, and dietary demand for iodide increases proportionally to meet these needs (Alexander et al 2017). Subclinical thyroid disease can become clinical during pregnancy and it is for these reasons that TSH levels are monitored in women during pregnancy. Because thyroid hormone supply to the foetus during the first trimester is dependent entirely on the mother thyroid hormone deficiency at this time can have severe effects on the developing nervous system (Bath et al 2013). Results of the Avon Longitudinal Study of Parents and Children (ALSPAC) survey showed that a higher proportion of children born to women with an iodine status of less than 150 μg/g/day had sub-optimum cognitive outcomes than did those born to women in the 150 μg/g/day or more group (Bath et al 2013). Since iodine is essential for the production of the thyroid hormones and iodine deficiency is known to result in hypothyroidism, these data demonstrate a clear threshold for dietary iodine intake below which an increased risk to the developing foetus results.

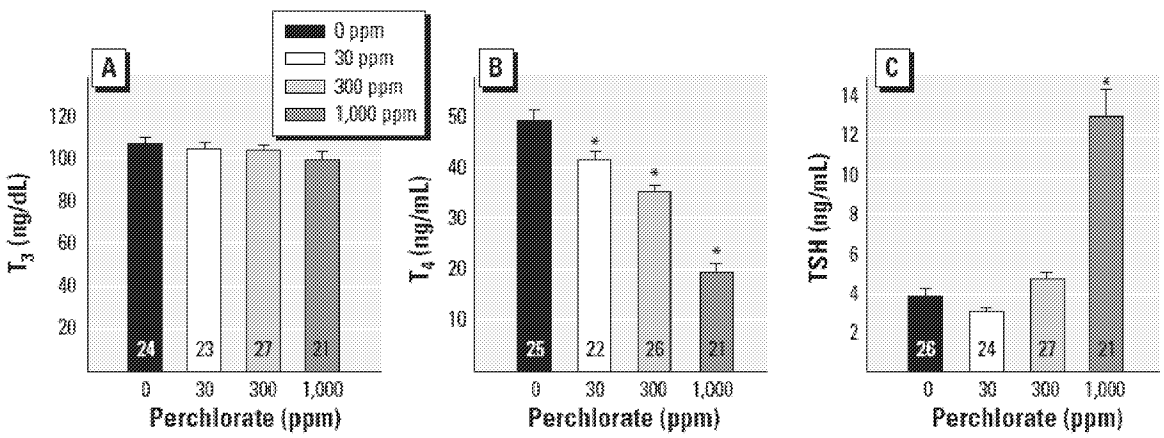
Subclinical hypothyroidism in pregnant humans has been internationally defined as an upper limits of serum TSH levels of 4 mIU/L (Alexander et al 2017). Levels of TSH higher than this incur recommended therapeutic intervention, typically using the soluble thyroxine substitute, levothyroxine or LT4 (Maraka et al 2017). A typical dose of LT4 is 50 μg/day. This was shown to correct thyroid hormone deficiencies, to minimise the risk of neurodeficiencies in children, and to decrease pregnancy loss associated with low thyroid hormone levels. Adequate iodine intake is essential for the production of thyroid hormones and the World Health Organisation recommends a daily intake of 250 μg for pregnant and lactating women (Alexander et al 2017). There are many parts of the world where iodine deficiency is endemic and associated with increased pregnancy loss and cretinism in offspring and goitre in adults (de Benoist et al 2004). There have been two non-randomised trials in which neurodevelopmental outcomes were improved in children from mildly to moderately iodine-

deficient areas whose mothers received iodine supplementation early in pregnancy (Berbel et al 2009; O'Donnell et al 2002)

Data in rats on chemicals that disrupt the thyroid hormone homeostasis has shown that while high doses of potent thyrotoxic chemicals will produce profound neurodevelopment abnormalities, lower doses, where decreases in circulating thyroid hormones are less, are also associated with less severe developmental defects (Auso et al. 2004; Crofton 2004; Crofton et al. 2000; Goldey et al. 1995a, 1995b; Goodman and Gilbert 2007; Morreale de Escobar 2003). These results support the concept of conventional dose response relationships of cause and effect, and although MIEs would be expected to be activated on exposure, irrespective of dose, downstream key events would be expected to follow normal dose-response relationships which would only be triggered once the previous key event reached a particular threshold.

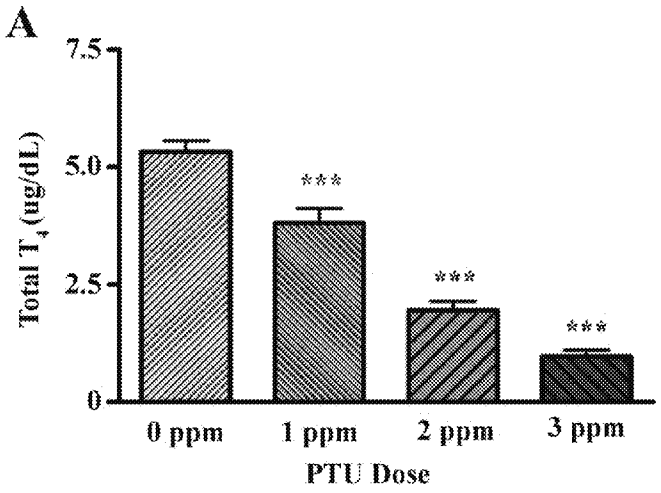
Experimental data supports the contention of thresholds for developmental abnormalities and in a study in pregnant rats, a 50% decrease in maternal T4 was shown to affect cochlear maturation in rats resulting in them being born deaf (Crofton 2004). A study in which pregnant rats were made hypothyroid by perchlorate administered in the drinking water, at dose levels up to 1000 ppm beginning on GD6 and sacrificed on PND30 (Gilbert and Sui 2008), found that T4 in the dams was reduced by 16%, 28%, and 60% in the 30, 300, and 1,000-ppm dose groups respectively accompanied by compensatory increases in TSH at the high-dose only supporting the threshold concept of preceding key events where the reductions in T4 (preceding key event), at the two lower dose levels were insufficient to trigger the subsequent key event, TSH release. Interestingly, in this study there were no changes in T3 values at any of the dose levels illustrating the degree of redundancy in the system that allows sufficient levels of T3 to be maintained despite decreases in T4. This study illustrates the expected dose-response relationships in terms of decreasing T4 and increasing TSH although the relationship was less obvious for T3 levels (Fig. 13).

Fig. 13: Changes in circulating hormone levels in rats dosed with perchlorate (Gilbert and Sui 2008)



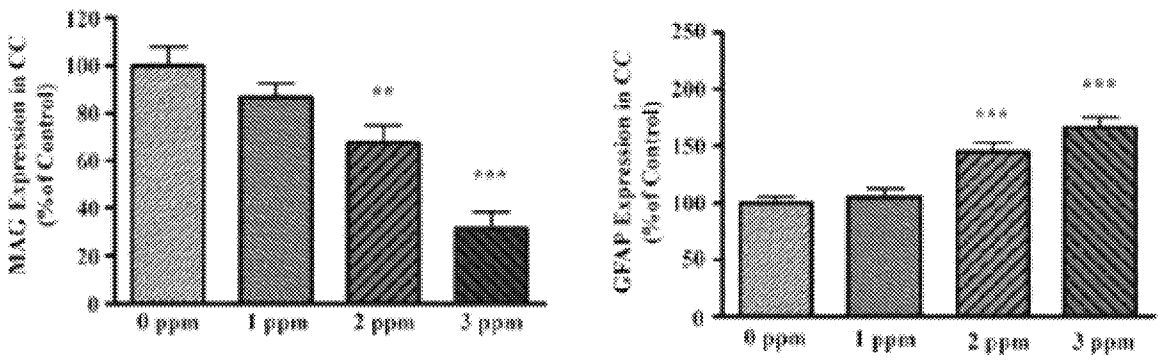
Another study where two week old rats were given PTU in the drinking water at dose levels of 0, 1, 2 and 3 ppm (Sharlin et al 2008) decreased T4 levels at all administered doses in a dose-dependent manner producing a 28%, a 63% and a 82% decrease in total T4 at the 1, 2, and 3 ppm dose levels respectively in PND 15 pups (Fig. 14).

Fig. 14: Dose dependent decrease in total T4 in the serum (from Sharlin et al 2008)



The decrease in T4 showed a direct correlation with a dose-dependent decrease in the density of oligodendrocytes, and an increase in the density of glial fibrillary acidic protein-positive astrocytes in both the corpus callosum and anterior commissure (Fig. 15). Although linear regression analyses demonstrated a strong positive correlation with each of these parameters, the 1 ppm dose level of PTU was a NOEL for both oligonucleotide and astrocytic responses even though T4 decreases were seen at all exposure levels. This again supports the concept of a threshold degree of decrease in total T4 levels below which there are no neurodevelopmental deficits presumably because of the maintenance of T3 values sufficient to satisfy the requirements of the developing foetuses.

Fig. 15: Dose dependent decrease in oligonucleocytes (identified by presence of myelin associated glycoprotein expression - MAG) and increase in GFAP astrocytes in the corpus callosum (CC) in rats exposed to PTU (from Sharlin et al 2008)



An interesting chemical example which appears to demonstrate thresholds for decreases in circulating maternal thyroid hormones not affecting the offspring is seen with the fungicide, mancozeb in two developmental toxicity studies and one EOGRTS study (Axelstad et al 2011). This compound was extensively discussed in the thyroid disruption workshop that took place in April 2017, and an account of the discussion was published in the final report of the workshop (Kortenkamp et al 2017). Mancozeb is thought to be thyrotoxic via a degradation product, ethylene thiourea, which is a known rodent goitrogen and carcinogen acting through inhibition of thyroperoxidase (Marinovich et al 1997; National Toxicology Program 1992). The maximum dose level of mancozeb permissible in the

developmental neurotoxicity study was limited by maternal toxicity consisting of significant body weight loss and hind limb paralysis but the top dose levels used were still considered adequate because it depressed maternal thyroid hormone levels by ~50% and dose levels lower than those used in the study had been shown to induce thyroid weight increases, decreased thyroid hormones and thyroid gland hypertrophy and hyperplasia, in a previous ninety day rat study. None of the three studies showed effects on the developmental and behavioural parameters assessed in the pups and foetuses on the study and mancozeb is not a developmental neurotoxicant despite clear evidence of maternal thyroid effects. This data supports the concept of a threshold for depression of circulating thyroid hormones in the dam below which there are no toxicological consequences for the pups.

In summary it is apparent that both the decreases in thyroid hormones and any subsequent neurodevelopmental abnormalities arising from the altered thyroid hormone homeostasis show expected dose response relationships with clear NOELs and thresholds for responses below which no biological effect is seen.

7.4. Is there evidence for a threshold for chemicals inducing thyroid cancer through thyroid hormone disruption?

The presence or absence of a threshold for the induction of rodent thyroid cancer by chemical exposure is dependent upon the MoA of the particular chemical in inducing thyroid hormone dysfunction (McClain 1995). Clearly it would be difficult to argue any thresholds for chemicals inducing thyroid cancer through mechanisms involving mutational events but for most other non-genotoxic modes of action for producing thyroid cancer, it is the chronic effect of high TSH levels that stimulate the thyroid gland to undergo growth and sustained cell hyperplasia, the latter outcome classically increasing the chances of the hyper-proliferative follicular epithelial cells acquiring spontaneous mutations that lead eventually, through promotional and progressional changes, to cancer (Cohen and Ellwein 1991; Foster 1997).

Since the hypothalamus/pituitary sensing system for thyroid hormones is considered to be responsive only to free T3, and that this represents <10% of the total circulating T3, there is also considerable redundancy in terms of measuring any reduction in total serum thyroid hormones before a TSH response is invoked. This latter comment argues for the importance of measuring both total and free thyroid hormones when assessing potential thyroid modulating effects of a particular chemical (Kioukia et al 2000; Dayan 2001; van der Watt 2008; Li et al 2014). Furthermore, the whole postulate regarding non-genotoxic MoAs for cancer demands a sustained condition of stimulation of the target cell population (Paynter et al 1988), and the degree of thyroid dysfunction produced by a chemical over a prolonged period of time would present a significant clinical problem to affected individuals before such exposure would increase the risk for neoplasia in humans.

In an EPA series of case examples of thyroid carcinogens (EPA 2014), a thionamide chemical that inhibited both thyroperoxidase and deiodinase activity was found to cause an increase in thyroid follicular cancer in both rats (Table 3) and mice (Table 4), and a corresponding dose related increase in serum TSH. In the article the chemicals were anonymised so that their exact structure could not be obtained. The compound was stated to be non-mutagenic by standard assays and to inhibit the synthesis of T4 within the thyroid, and the subsequent conversion of T4 to the active T3 form in the peripheral tissues. The compound also induced an increase in TSH secreting pituitary adenomas in the rat oncogenicity study. The compound has also been dosed to humans and monkeys and, with regard to altered TSH levels, exposed humans showed no TSH imbalance, while monkeys showed minor TSH increases but were considerably less sensitive on a mg/kg basis than were rats (EPA 2014). Table 3 shows the outcome of a two-year carcinogenicity study with the compound and demonstrated a clear threshold for development of thyroid follicular cancer at 120 mg/kg/day for both male and female rats. A two-year study in B6C3F1 mice was also carcinogenic for the compound but the NOEL was considerably higher than was seen in the rat study (Table 4) suggesting a differential sensitivity between the two, rodent, species.

Table 3: Incidence of thyroid follicular cell tumours in F344 rats in a 2-year study of compound 1 (EPA 2014).

Dose	0		120		240		480	
Sex	M	F	M	F	M	F	M	F
Total No. rats	49	50	49	50	47	48	45	46
Follicular adenoma	1	0	1	0	10*	14*	34*	28*
Follicular carcinoma	1	0	0	0	4*	3*	8*	7*
Adenoma+carcinoma	2	0	1	0	14*	17*	42*	35*

* $p<0.05$

Table 4: Incidence of thyroid follicular neoplasms in B6C3F1 mice in a 2-year study of compound 1 (EPA 2014).

Dose	0		120		240		480	
Sex	M	F	M	F	M	F	M	F
Total No. rats	48	50	46	47	44	47	47	46
Follicular adenoma	1	2	1	1	2	0	16*	11*
Follicular carcinoma	0	0	0	0	0	0	0	1
Adenoma+carcinoma	1	2	1	1	2	1	16*	11*

* $p<0.05$

In summary, since the mitogenic stimulus for any chemical that targets the rodent thyroid gland is sustained TSH stimulation, there would be no cancer risk from non-genotoxic chemicals at exposure levels that fail to decrease circulating thyroid hormone levels sufficiently to provoke the TSH release from the pituitary gland (McClain 1995). For those rodent thyroid gland carcinogens, a threshold dose for thyroid hormone depression will exist based upon a lack of TSH induced cell proliferation in the thyroid follicular cells, below which there will be no hazard for the development of thyroid cancer in the rodent.

8. ECPA Question: Are there non-endocrine causes of the same adverse effects e.g. stress, starvation, environmental factors such as temperature?

The sensitive and tightly regulated thyroid hormone feedback control system, thyroid gland autoregulation, and the large intra- and extra-thyroidal storage pools of thyroid hormone under normal conditions are able to provide a constant supply of free thyroid hormone to peripheral tissues in the face of perturbations imposed by the external environment, chemicals and drugs, and a variety of diseases processes. However, under certain situations the homeostatic control is disturbed and compensatory regulation of synthesis and secretion can occur. This section details the secondary response of the thyroid to particular stresses unrelated to a direct effect of the particular stress on the thyroid gland itself.

It is especially important, in the context of laboratory animal studies, to understand those responses that affect thyroid hormones under stress situations since many animal safety evaluation studies are conducted at a range of dose levels that might include, at the top dose level, exposures that can compromise the normal physiology of the rat. The use of a maximum tolerated dose level can

significantly influence the normal hormonal homeostasis (Carr and Kolbye 1991; Downes and Foster 2015; Apostolou 1990; Carr and Kolbye 1991; Haseman and Seilkop 1992; McConnell 1989), not as a primary effect of the chemical, but as a secondary effect of being exposed to concentrations of chemicals that inhibit food consumption, or cause disease in organs in addition to, or other than, direct toxicity to the thyroid gland. Hence the interpretation of any thyroid hormone changes under circumstances of multi-organ pathology, or significant effects on food and water consumption, will need to be very carefully evaluated to eliminate the secondary consequences of these confounding influences on circulating thyroid hormone levels.

TSH secretion by the pituitary gland thyrotrophs, and their sensitivity to TRH stimulation, has been shown in humans to be affected by renal failure, starvation, sleep deprivation, depression, and hormones, including cortisol, growth hormone, and sex steroids (Gary et al 1996; Jackson 1982).

8.1. Thyroid hormone changes in disease states

In many chronic illnesses in humans, defects occur in thyroid hormone metabolism, resulting in the "sick euthyroid syndrome" characterized by a normal total T4, normal/high free T4, low total T3, low free T3 and an elevated rT3 (Malik and Hodgson 2002). These changes reflect a reduction in type 1 deiodinase activity, an increase in type 3 deiodinase activity (Bianco et al 2002) and changes in the plasma concentration of thyroid-binding proteins and free fatty acids which displace thyroid hormones from binding proteins. There are also non-thyroidal influences on the hypothalamic-pituitary-thyroid axis such as cortisol inhibiting TSH secretion (Camacho and Dwarkanathan 1999).

Increased levels of rT3, indicating greater conversion of T4, have been observed in humans in starvation, anorexia nervosa, severe trauma and haemorrhagic shock, hepatic dysfunction, postoperative states, severe infection, and in burn patients (Chopra 1976; Boelaert and Franklyn 2005; Jabbar et al 2017). Since rT3 is an inactive form of thyroid hormone, an increased conversion of T4 suggests that certain disease states induce a lowering of the metabolic rates and recuing caloric requirements as part of the natural physiological processes involved in coping with the disease.

8.2. Thyroid hormone changes in pregnancy in humans

Pregnancy has a variety of effects on maternal thyroid function, and thyroid gland enlargement, commonly referred to as "pregnancy goitre", is common in areas of relative iodine deficiency (Glinder and Lemone 1992; Feely 1979). The renal clearance of iodine is increased in pregnancy and, together with an increased volume of iodine distribution, leads to low plasma inorganic iodine concentrations and increases the thyroidal iodine clearance. Paradoxically, the absolute iodine uptake and hormone production rates remain unchanged (Glinoer 1997). While there is an increase in total T4 and T3 in the serum, this is almost all due to a concomitant increase in TBG. Free T4 and T3 are maintained, or in some women decreased below pre-pregnancy levels in the serum although the exact reasons for this remain unknown (Glinoer 1997). Hence there are three major changes in maternal thyroid physiology during pregnancy: (i) enlargement of the thyroid, (ii) alterations in iodine handling, and (iii) an increase both in thyroid hormone binding proteins and thyroid hormones. In contrast to the effects on thyroid and thyroid hormones, thyroid stimulating hormone (TSH) levels, at least in healthy women, remain within the normal range during pregnancy (Chan et al, 1975). Following parturition/birth thyroid function returns to normal levels within the first few weeks following birth (Mankar et al 2016).

8.3. Specific thyroid hormone changes in liver disease

The main transporter protein for thyroid hormones in humans, TBG, is an acute phase protein made in the liver, and in human cases of acute hepatitis, patients show elevated serum levels of total T4, due to increased production of TBG, but with normal levels of free T4. As the severity of hepatitis increases with impending liver failure, low total serum T4 levels are thought to reflect the reduced hepatocellular synthesis of TBG (Kano et al 1987). While serum T3 levels in patients with acute

hepatitis can be very variable, the free T3:T4 ratio appears to correlate negatively with the severity of the liver disease reflecting diminished type 1 deiodinase activity, resulting in a reduced conversion of T4 to T3. Some case histories have shown an association of acute hepatic failure with the development of goitre that resolved with improvement in liver function (Hegedus 1986).

Data on thyroid hormone changes in laboratory animals on routine regulatory toxicity studies, in the presence of liver damage, is not available and it is not possible to comment on whether or not thyroid hormone changes would be induced in situations of liver damage.

8.4. Effects of temperature on thyroid hormones

8.4.1. Effects in humans

Changes in the outside temperature will alter TSH secretion in humans and as a consequence the serum concentration of thyroid hormones and their metabolism. These changes are thought to be mediated centrally via the pituitary/hypothalamus and peripherally by effects on the rates of thyroid hormone degradation, through increased loss in the faeces, and by alterations in thyroid hormone receptor expression in the tissues. Such changes would be expected to decrease the metabolic rates in peripheral tissues.

In cell systems *in vitro,* changes in temperature have been shown to affect the binding affinity of T4 to its serum binding proteins and this could also function *in vivo* under conditions of extreme cold or extreme heat (Bernstein and Oppenheimer 1966). In a study of males living in Northern Finland, serum free T3 levels were shown to be lower in February than in August, and TSH levels were higher in December than at other times of the year. Serum free T3 levels correlated significantly with the mean outdoor temperature of the preceding month but serum TSH levels failed to show any correlation with the mean temperature of the month or with free T3. Low serum free T3 in winter suggests that thyroid hormone degradation was accelerated in the cold (Leppäluoto et al 1998). The interpretation of some of the human studies may have been confounded by additional variables such as altered daylight, activity levels, living conditions, and sleep deprivation accompanying prolonged residence in Arctic and Antarctic regions (Hackney et al 1995 a, b).

8.4.2. Effects in laboratory animals

The overall effects of environmental temperature have been easier to demonstrate in animals than in humans but profound species differences in thermal regulation may mean that the findings in animal models may not apply to humans (Silva and Larsson 1985). Cold exposure in animals leads to thyroid gland hyperplasia, enhanced hormonal secretion, degradation, and excretion, accompanied by an increased demand for dietary iodine (Goglia et al 1983). The prompt activation of pituitary TSH secretion after cold exposure of the rats (Panda and Turner 1975; Emerson and Utiger 1975) is thought to be due to a direct effect on the hypothalamus (Anderson 1964).

Exposure to cold has also resulted in increased secretion of TRH (Szabo and Frohman 1977), and a reduced response of pituitary TSH release to administered TRH (Hefco et al 1975). Cold exposure in laboratory studies in the rat is associated with increased rates of T4 and T3 deiodination, increased conversion of T4 to T3, enhanced hepatic binding, and increased biliary and faecal clearance of all of the iodothyronines (Balsam and Sexton 1975; Bernal and Escobar del Rey 1975; Tsukahara et al 1997; Galton and Nisula 1969; Balsam and Leppo 1974). Finally, thyroid hormone effects may be enhanced by alterations in co-activators which are able to enhance the activity of thyroid hormone receptors on gene activation (Puigserver et al 1998).

When rats were exposed to high environmental temperatures of 34°C for three weeks, there was a rapid and simultaneous decrease in hypothalamic TRH, plasma TSH, plasma T4 and thyroid activity by the 36th hour of heat exposure suggesting an effect mediated via the hypothalamus. On prolonged exposure, by day 9, there was a rebound in thyroid activity due to a peak in circulating TSH in

response to the marked decrease in plasma T4 seen at this time. From day 9 to the end of the study on day 21, all thyroid parameters returned to control levels indicating adaptation to the increased temperature regime (Rousset and Cure 1975). This result in the laboratory was mirrored by a similar effect in human subjects where a decrease in the elevated serum TSH level associated with primary hypothyroidism was induced by increases in body temperature (O'Malley et al 1980).

8.5. The relationship of fasting and obesity to thyroid hormones

In periods of limited food availability, there is central downregulation of the HPT axis, and serum T4 and T3 levels fall during fasting both in humans (Chan et al 2003) and in rodents (Ahlma et al 1996; Legradi et al 1997) to downregulate the metabolic rate in affected animals/individuals. In rats, fasting has been shown to decrease both pituitary type 2 deiodinase activity and liver type 1 deiodinase activity, and the reduction correlates with reduced peripheral T3 isolated from liver homogenates (Boelen et al 2006; 2008). Despite this reduction in pituitary and liver T3, hypothalamic type 2 deiodinase activity increases with fasting, resulting in a stimulation of release of the appetite-related proteins, neuropeptide Y (NPY) and agouti-related peptide (AgRP) from the arcuate nucleus. Thus, despite fasting-associated reductions in peripheral thyroid hormone levels, there is still a localized increase in T3 within the hypothalamus with a marked increase in orexigenic (hunger) signals, which in turn act upon the paraventricular nucleus to decrease TRH and, in consequence, TSH production. Humans who are anorexic, or who undergo severe caloric restriction, exhibit similar reductions in thyroid hormones, which are thought to be protective of vital energy stores (Langouche et al 2014; Reinehr 2010; Warren 2011). The administration of leptin (Da Veiga et al 2004), or α-MSH (Fekete et al 2000), has been shown to abolish the fasting-induced reductions in TRH and restore normal circulating TSH levels and humans and mice with mutations in the leptin receptor or in the leptin molecule itself exhibit central hypothyroidism are prone to severe obesity (Ohtake et al 1977; Clement et al 1998), which is ameliorated, at least in leptin-deficient humans, by the administration of exogenous leptin (Farooqi et al 2002).

In humans, there is a clear relationship between body weight, obesity and hypothyroidism (Amin et al 2011) and thyroid hormones are thought to directly affect appetite via their actions on the brain. Administration of TRH and TSH directly into the brain of rodents causes a reduction in food intake (Lin et al 1983; Vijayan and McCann 1977; Suzuki et al 1982) and similar effects on food intake are seen following peripheral administration of TRH (Choi et al 2002). In contrast, central and peripheral administration of T3 increases food intake and consequentially induces body weight gain (Kong et al 2004; Ishii et al 2003; 2008).

In summary therefore, any situation that significantly alters the normal intake of food by a laboratory animal will be expected to affect thyroid hormone homeostasis, not as a direct effect on the thyroid but secondarily to effects on food consumption.

9. ECPA Question: What are the non-thyroid hormone activities of the thyroid hormone transport proteins? Are there quantitative and/or qualitative species differences?

9.1. Thyroid hormone binding proteins as acute phase proteins

Since the liver is responsible for metabolism of thyroid hormones essentially any toxicity, including chemical-induced hypertrophy and enzyme induction, will affect thyroid hormone turnover, alter circulating thyroid hormone levels, and potentially feed back to the hypothalamus/pituitary to alter thyroid hormone production. Acute phase proteins are proteins that are increased or decreased in the presence of inflammatory conditions in general (Cray et al 2009). The vast majority of acute phase proteins are made in the liver and any condition that affects their hepatic production and release, irrespective of species, will alter the concentrations of these proteins (Ross et al 1983). TBG is an acute phase protein and hence in conditions such as hepatic necrosis, where total serum protein is decreased because of compromised liver function, the serum concentration of the thyroid binding

proteins will be decreased and the circulating total T4 and T3 has also been shown to decrease (Schussler et al 1978; Neto and Zantut-Wittmann 2016). While the exact identity of the thyroid binding proteins differs between different species, they are all manufactured and released from the liver and will consequently be altered, and induce alterations in circulating thyroid hormones, in the presence of compromised hepatic function (Huang and Liaw 1995).

In human cases of acute liver disease, patients show elevated serum levels of total T4 but with normal concentrations of free T4, due to increased production of TBG as an acute phase response (Jayachandran et al 2016; Neto and Zantut-Wittmann 2016; Gardner et al 1982). In severe cases of hepatitis low total T4 levels are thought to reflect the reduced hepatocellular synthesis of TBG (Kano et al 1987). While serum T3 levels in patients with acute hepatitis can be very variable, the free T3:T4 ratio appears to correlate negatively with the severity of the liver disease reflecting diminished type 1 deiodinase activity, resulting in a reduced conversion of T4 to T3. Some case histories have shown an association of acute hepatic failure with the development of goitre that resolved with improvement in liver function (Hegedus 1986).

Conditions of elevated oestrogen, including pregnancy, induce an increase in serum thyroxine binding globulin (TBG), and similarly, when exogenous oestrogen was administered to rats, elevations in TBG and total T4 were observed (Ain et al 1987). Whether this is an adaptation to impending pregnancy and the need for thyroid hormones by the developing foetus is open to speculation.

10. ECPA Question: What are the remaining uncertainties? How may they be addressed?

In order to arrive at a decision as to whether or not a given chemical is indicating thyroid disrupting properties, the draft GD suggest a two-tier approach based upon the use of the OECD Conceptual Framework levels 1 – 5 (page 9 of draft GD 2017) to categorise the various data. In terms of human health assessment, levels 2 and 3 list the specific *in vitro* and *in vivo* assays respectively that would be used to discount a generalised endocrine effect using specifically designed assays, while level 4 lists the guideline studies that would normally be routinely conducted in any submission passage for pesticides and biocides. While the majority of the assays mentioned in the various levels have OECD test guidelines already issued, there are a minority of the *in vitro* assays that are not guideline at the time of writing.

In order to adequately address the question of the remaining uncertainties, it is necessary to break it down into two separate questions that relate to whether or not we are asking basic questions of understanding, that could be used in a TIER 2 MoA type of analysis, and where the basic biology of thyroid hormone control will be questioned, or whether we are questioning the adequacy of the current testing strategies for evaluating potential thyroid disrupting chemicals which would constitute part of the TIER 1 assessment. Currently much of the debate has muddled the two parts together and has resulted in polarised views as to the adequacy of each.

10.1. The current assays and their assessment of thyroid effects

As can be seen from Table 5 the current mandated toxicity assays carry a limited assessment of thyroid effects of chemicals dependent largely upon the assessment of thyroid gland weight and thyroid gland histopathology.

Table 5: The current assays and their assessment of thyroid effects (modified from European Union 2017).

OECD TG	Study Title	Thyroid Endpoints Measured
407	Repeated dose 28-day oral toxicity study	Histopathology of Thyroid gland liver and CNS & PNS Weight of thyroid gland liver & brain
408	Repeated dose 90-day oral toxicity study in rodents	Histopathology of Thyroid gland CNS, PNS & liver. Weight of thyroid gland, liver & brain
451-3	Chronic toxicity & carcinogenicity	Histopathology of Thyroid gland, CNS, PNS and liver
414	Prenatal developmental toxicity study	No thyroid-related endpoints are included
415	One-generation reproductive toxicity study	No thyroid related endpoints are included
416	Two-generation reproductive toxicity study	Thyroid gland histopathology in P0 and F1 parents
421	Reproductive screening test	T4 from PND13 offspring and P0 adult males; T4 may also be measured in pups on PND4 and dams on PND13; optional thyroid weight and histopathology
422	Combined 28-day/reproductive screening assay	As TG421
426	Developmental neurotoxicity study	No thyroid but assessment of developing brain
443	Extended one generation reproductive toxicity study	T4/TSH in F1 offspring from cohort 1A at term (PND22); Thyroid gland weight and histopathology; when the DNT cohort is included, assessment of developing brain

While the pivotal endpoint of thyroid gland carcinogenesis is fairly well covered by the lifetime chronic toxicity/carcinogenicity test, there is concern that potential neurodevelopmental deficits, induced by low levels of circulating thyroid hormones may not be so adequately assessed in the current available test guidelines (EU 2017). While no guideline studies fully assess this issue, TG 426, the DNT study, and TG 443, the EOGRTS (with DNT cohort) should, with modifications, be able to adequately address this. Hence the inclusion of thyroid hormone measurements into TG426 would significantly improve the guidance document insofar as thyroid assessments are concerned. TG 443 has the possibility to include a DNT cohort and if so the endpoints currently included in TG 426 would strengthen any concern that the neurodevelopmental effects of hypothyroidism were being adequately assessed. Furthermore, several behavioural studies performed according to these guidelines have not been able to show any adverse effects in behaviour or on brain histopathology, even in severely hypothyroxinemic animals. This has led scientists in the field of neurotoxicology and thyroid disruption to suggest that new endpoints which are more sensitive to perinatal thyroid disruption should be

added to these guidelines, in order to better assess adverse effects on neurodevelopment (OECD 2006, Harry et al 2014).

There has also been criticism that the behavioural assays currently included into the TG are not sensitive enough to detect neurodevelopmental effects when they may be occurring (Harry et al 2014; EU 2017). This conclusion is based upon the findings that some chemicals, that clearly affect the hypothalamic/pituitary/thyroid axis, have not shown neurobehavioural effects in specifically designed studies. One example that has been used to exemplify this conclusion is the fungicide, mancozeb. This compound has shown thyroid effects in terms of hypertrophy and hyperplasia and thyroid gland weight increases in a number of toxicity studies but did not affect nervous system development in specifically conducted developmental toxicity studies in the rat (Axelstad et al 2011). The mode of action of mancozeb in affecting the thyroid gland is thought to be due to its conversion to ethylene thiourea, a known thyroid toxic chemical (Graham et al 1975) and once again, a EOGRT study with a DNT cohort carried out with ETU failed to show adverse effects on brain development or on the neurobehavioural parameters that were assessed, even though thyroid hypertrophy was present in the adult rats at both the mid and high dose levels (Marty et al 2013b, in DRAR).

There are several alternative interpretations for these data. If we accept that severe hypothyroidism can lead to serious neurodevelopmental deficits, and there is sound evidence for a limited number of chemicals to support this conclusion, then the following arguments can be used to explain the lack of behavioural effects in these studies:

1. The thyroid disrupting effects of mancozeb/ETU on the dams were not severe enough to lead to altered brain development in the offspring.
2. The offspring were not hypothyroid themselves in the postnatal period (due to limited milk transfer) and since much brain development occurs postnatally in rats, the prenatal hypothyroxinemia was not severe enough to disrupt brain development.
2. In order to induce an altered brain development, also inhibition of fetal thyroid hormone synthesis is needed. ETU was not able to enter the fetal thyroid.
3. The neurobehavioural assessment methods were not sensitive enough to detect subtle changes in brain development.
4. The decreases in circulating T4 levels lead to a compensatory upregulation of peripheral deiodinase activity, leading to an increased conversion of T4 to the active T3 sufficient to provide enough T3 for normal brain development in the pups. It is noteworthy that ETU in comparison to PTU (propylthiouracil) has only marginal inhibitory effect on 5'-deiodinase type-1.

With the exception of explanation 3, the other alternatives imply a threshold level of thyroid hormone inhibition in the dams, below which no consequences to pup neurodevelopment occur. The data also support the conclusion that chemically-induced thyroid effects in the dams occur at significantly lower chemical concentrations than do thyroid hormone induced neurodevelopmental deficits in the pups (Gilbert and Sui 2008). An alternative way of stating this is that the sensitivity of the adult thyroid gland in demonstrating the effects of chemically-induced thyroid hormone lowering is considerably greater than the induction of neurodevelopmental effects in the pups such that the degree of thyroid hormone decreases in the dams needs to be considerably lower to induce neurodevelopmental effects in the offspring. Human data from mothers living in iodine deficient parts of the world would support this conclusion where the % of hypothyroid mothers present in the population greatly exceed the incidence of neurological deficits in the children of the affected mothers (Alexander et al 2017).

Explanation point 3, namely that the neurobehavioural assays currently in use are simply not sensitive enough to detect subtle changes in brain development in the pups, is a criticism that can be levelled at almost every measured endpoint in any of the current guidance documents. The introduction of behavioural endpoints into guidance documents is always accompanied by significant efforts at validation of the introduced methods and indeed this was the case with those recommended in the

developmental neurotoxicity test, TG426, and the EOGRTS, TG443, cohorts 2A and 2B. The neurobehavioural assays are conducted in addition to extensive, and specifically targeted, neuro-histopathological and hormonal assessment and provides the most comprehensive test system currently available. Clearly the guidelines are not immutable, and when sufficient information becomes available to warrant additional assays, then these do become incorporated, albeit following prolonged and extensive validation exercises to ensure the quality of the additional assays.

10.2. The need for additional screens for chemicals that might disrupt the hypothalamus/pituitary/ thyroid (HPT) axis?

There has been much criticism that current testing strategies, for detecting thyroid disrupting chemicals, lack both specificity and sensitivity, and that the rodent assays currently employed may not necessarily predict outcomes in the human population (European Food Safety Authority/European Chemicals Agency 2016; European Union 2017).

A consequence of this debate has resulted in proposals for the greater incorporation of *in vitro* cell and protein binding assays, of basic molecular interactions of chemicals with components of the HPT axis, into the screening toolbox for detecting thyroid interfering chemicals (Murk et al 2013). There is no doubt that *in vitro* assays of various designs have advanced our basic understanding of biological processes immeasurably and they are an essential part in developing MoA and human relevance arguments, and in formulating adverse outcome pathways. However, the assays need to fill current gaps in the toolbox rather than simply adding unnecessarily to the already large list of testing assays mandated by European agrochemical regulatory requirements. The formulation of adverse outcome pathways is one way in which gaps in current understanding of thyroid hormone perturbation can be identified and novel assays naturally fit as part of the current TIER system where the primary tiers depend upon properly validated animal studies which can be supported in a TIER 2 testing strategy that uses the most appropriate assays for addressing problems raised following the TIER 1 assessment, as described in the draft GD. While there exist a plethora of various *in vitro* assays, their use as screens for chemical toxicity, in the absence of any hypothesis driven selection of the appropriate assay, could raise more questions than answers, generate unnecessary extra work to understand exactly what the *in vitro* screen is showing, and involve additional unnecessary experimentation to clarify what any single *in vitro* screen might mean in the context of the biology of the whole animal (Colnot and Dekant 2017; Hulme and Trevithick 2010).

10.3. Are there refinements that can be made to current hormone assessments?

10.3.1. When to measure hormones?

Evidence suggests that for chemicals that depress thyroid hormones, the degree of depression gets progressively less as the duration of suppression increases (Yu et al 2002). This is thought to be due to a resetting of the homeostatic level at which the thyroid hormone feedback system works. This is particularly true of elevations in TSH that tend to occur shortly following exposure to thyroid affecting chemicals but which can return to normal values within weeks of exposure. The same effect is seen with the depression of serum T4 and T3 which tend to return to normal values on continued exposure. Hence the question arises regarding the most appropriate time for measuring these hormones and what consequences the reset system might have on the long-term health of the animal. It is clear that although hormone levels return to near normal levels on prolonged dosing, the system maintains this at a higher metabolic level than does the HPT axis in untreated animals and as such would be expected to induce a sustained level of stress that long term could increase the chances of developing diseases including thyroid cancer (Vansell et al 2004). Hence a careful evaluation of the right time, or right times, for evaluating thyroid hormone levels in animal studies is needed to avoid situations where morphological thyroid changes might be seen in the apparent absence of effects on thyroid hormones (Colnot and Dekant 2017). It is not within the scope of this review to recommend sampling times but in order to generate interpretable data, a thorough knowledge of the

pharmacokinetics of the chemical under test, together with the known circadian control for some of the hormones is essential prerequisite for such studies.

10.3.2. Should thyroid hormone measures be incorporated into routine toxicity studies?

There have been suggestions that thyroid hormone measures could be incorporated into guideline toxicity studies as a routine rather than when indicated by a confirmed knowledge of a chemical class thyroid effect or some indication that the thyroid gland was a potential target. While there are clearly advantages to gaining the maximum amount of information from animal studies but there does need to be a measure of caution when advocating such additions. Thyroid hormone assays are not currently straightforward, and they require a degree of expertise not routinely present in many laboratories conducting guideline studies. Routine incorporation might well generate data that becomes uninterpretable without supporting evidence from histopathology and could throw up false positive data arising from inexperience of the conducting laboratory. Conducting laboratories need to have the necessary historical background data to understand the limits within normality that exist in laboratory animals. The timing of blood sampling for hormone measures needs to be carefully planned in the knowledge of the circadian control that some of the hormones operate under.

Lastly and perhaps most importantly, the occurrence of confounding factors, such as organ toxicity and reductions in food consumption that are frequently seen in toxicity studies, are known to affect thyroid hormone levels independent of any primary effect on the thyroid gland itself and ruling these factors out can be challenging without embarking on an extensive investigative study. There is also evidence of adaptation to thyroid inhibition where hormone depression is seen early in studies but which recover on prolonged exposures. For an unknown compound, the question would always arise as to when, in the conduct of the study, it might be appropriate to sample for potential thyroid hormone changes. Since these are likely to be different dependent upon the inhibitory MoA triggered, and the individual chemical involved, the routine incorporation of thyroid hormone measures to guideline toxicity studies presents almost insurmountable difficulties if it is to generate sensible and interpretable data that adequately assesses potential thyroid effects.

Histopathology of the thyroid gland is routinely carried out as part of acute, chronic and lifetime toxicity bioassays, and arguably is the most sensitive biomarker of thyroid hormonal disruption, at least when incorporated into a routine toxicity study (NTP 2002). Any sign of a thyroid effect by histopathology could then be best approached by undertaking specifically designed investigative *in vivo* and *in vitro* toxicity studies to investigate potential thyroid effects by targeting the hormonal endpoints relevant to the thyroid.

10.4. Is the rat the appropriate species? Should we explore different animal models?

Various alternative animal models to the rat have been proposed for testing of both the potential carcinogenic and the developmental effects of chemicals disrupting the HPT axis. The inherent advantages of the rat as a model for human risk assessment refer back to our familiarity with the vagaries of the species and our extensive understanding of its similarities, and differences in terms of biology, to humans. Clearly alternative animal species, and *in vitro* and *ex vivo* systems, exist for assessing potential thyroid hormone disrupting properties but they all have drawbacks and limitations which are discussed in the relevant sections in this and other documents (Sadamatsu et al 2006; Brent 2012b).

10.4.1. The sheep as a model for human thyroid effects

There has been some discussion as to the sheep being a more appropriate animal model to represent human health hazard with regard to disruption of thyroid hormone homeostasis (European Union 1917; page 23) especially where neurodevelopmental toxicity is concerned. The study quoted to

reinforce this statement was Leghait et al (2010) where fipronil, a compound known to produce significant thyroid hypertrophy in rats, was studied in sheep. The underlying reason for regarding the sheep as a more representative model for man, is based upon the greater similarity of thyroid binding protein in the plasma of sheep where TBG is the major serum transporter for thyroid hormones, whereas in the rat this protein is, to all intents and purposes, absent in the adult.

Leghait et al (2010) found that fipronil failed to produce thyroid toxicity in the sheep but the conclusions to the study were somewhat compromised because the formation of the sulphone active metabolite of fipronil was appreciable less in the sheep than it is in the rat. This failure highlights the problems of embarking on studies in species where the database is seriously limited, as in the case of the sheep.

The metabolism of thyroid hormones has been studied in the pregnant sheep, both in the dam and in the developing foetus (Fisher et al 1972) and the paper speculates that, on the basis of thyroid metabolism, the foetal sheep has greater similarity to human foetuses than does the rat system. While this may well be the case, the lack of detailed information on the other, not inconsiderable, variables determining chemical-induced thyroid toxicity, together with the impracticality of using such a large species for toxicity studies, compound supply alone would most likely deter such studies, would mean that at best it might be considered as a second species in place of the canine, but only after a significant period of data gathering on the current paucity of basic biochemical and biological parameters pertinent to the conduct of toxicity studies.

In spite of this drawback, a recent study has been reported that looked at the effect of exposing pregnant ewes to sewage sludge-fertilized pastures that succeeded in showing that pre-conceptual exposure of the dam increased the relative thyroid organ weights in male foetuses and decreased expression of the sodium iodide symporter in the thyroid glands (Hombach-Klonisch, et al 2013).

10.4.2 The contribution of knockout mice to understanding human relevance of thyroid hormone disruption

Several transgenic mouse models of thyroid cancer exist that contain various genes found to be overexpressed in human thyroid cancer, that are placed under the expression of the thyroglobulin (Tg) promoter, and that subsequently allow thyroid-specific over expression of the specific transgene and the subsequent development of thyroid cancer (Kim and Zhu 2009).

Of particular relevance for potential species differences in response has been the MCT8 knockout mouse which has shown little neurodegenerative effects (Dumitrescu et al 2006; Trajkovic et al 2007; Visser et al 2011) despite the human condition affecting the MCT8 transporter, where serious and profound neurological pathologies are produced in the mutation of the MCT8 gene in Allan-Herndon-Dudley Syndrome (Dumitrescu et al 2004; Friesema et al 2004; Schwartz and Stevenson 2007). In knockout mice, lacking the MCT8 transporter, they fail to show motor deficits suggesting that alternate pathways for transporting thyroid hormones into the brain exist in this species (Di Cosmo et al 2010; Roberts et al 2008). It is now known that the organic ion transporter polypeptide-14 (OATP14) is the primary thyroid hormone transporter responsible (Friesma et al 2005) and OATP14 mRNA and protein is strongly expressed in both rat and mouse cerebral microvessels, but not in human. The high expression of OATP14 in the rodent brain, as compared with the human, is one explanation for the relatively mild neurophysiological consequences of deletion of the MCT8 transporter in Mct8-null mice. The absence of an alternate transporter in the developing human brain results in serious neurodevelopmental consequences seen in individuals carrying the mutated MCT8 gene.

RET/PTC gene rearrangements have been found to be consistently involved in human thyroid cancer, and transgenic mice carrying over-expression of this gene combination have indeed been found to develop thyroid carcinogenesis (Santoro et al 1996; Jhiang et al 1996). Several other genes, such as the NTRK1, BRAF and RAS genes, are also known to be involved in the development of human cancer, and while mice transgenically designed to overexpress these have been found to reciprocate the biology, morphology and behaviour of human thyroid cancer and to help in the understand the

basic biology of the process (Knostman et al 2007; Knauf et al 2005; Vitagliano et al 2006), they have been singly unsuccessful in informing on the relative susceptibility of chemically induced thyroid cancer in either laboratory animals or in man simply because that was not what they were designed to do. Their whole purpose was to develop a model that could be used to study the development of human thyroid cancer with a view to better understand the pathophysiology and molecular biological changes leading to cancer.

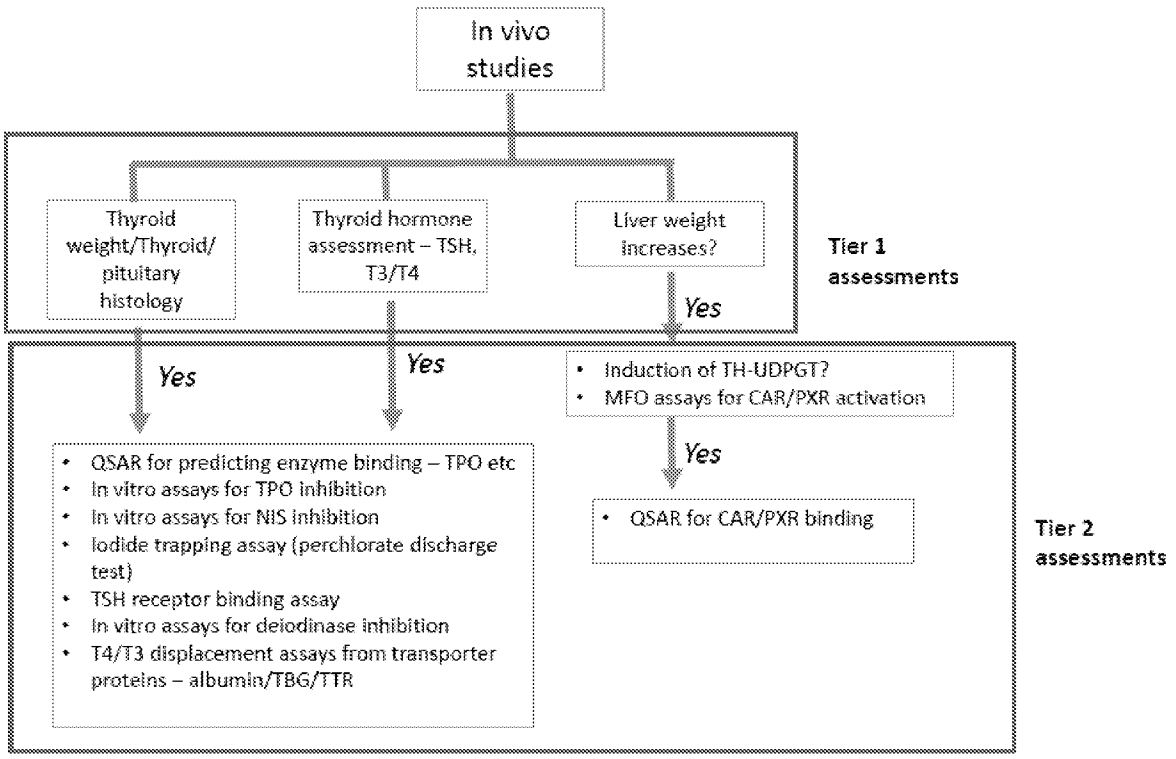
Type 2 iodothyronine deiodinase is essential for maintaining normal local concentrations of free T3 under different physiological and pathophysiological situations and has a critical role in the correct operation of the negative feedback control of TSH/TRH release from the pituitary and hypothalamus (Schneider et al 2001). In an attempt to better characterise the respective roles of the three iodothyronine deiodinase enzymes, knockout mice have been generated for each of the enzymes (Schneider et al 2001; 2006; Marsili et al 2011). Mice bearing the knocked-out gene for iodothyronine Type 2 deiodinase show incomplete development of the inner ear (Ng et al 2004), and bone and muscle malformations resulting from a deficiency in the local conversion of T3 in cochlear cells, myoblasts and osteoblasts respectively (Dentice et al 2010; Bassett et al 2010). The type 2 deiodinase KO mouse also shows impaired embryonic development of brown adipose tissue, and as a consequence the mice suffer a permanent thermogenic defect (Hall et al 2010; de Jesus et al 2001) which leaves them susceptible to hypothermia during cold challenge, and a greater susceptibility to diet-induced obesity at ambient temperatures (Castillo et al 2011).

11. A proposal for a testing strategy for assessing thyroid disrupting effects of a chemical

In the event of a chemical inducing thyroid hypertrophy/hyperplasia, or measured decreases in circulatory thyroid hormones, in rodent toxicity studies, a mode of action analysis and human relevance framework in the context of the recommended IPCS guidance should be conducted (Boobis et al. 2006) together with an adverse outcome pathway constructed to identify missing data from the proposed pathway as recommended by Vinken et al (2017).

In terms of possible thyroid effects mediated via increased clearance through the liver, the testing strategy outlined in Fig. 16 below addresses all known thyroid modes of action, and suggests a way of ruling out alternative MOAs through experimentation in appropriate assays.

Fig. 16: Suggested testing strategy to confirm a thyroid mode of action



If either of the three tier 1 effects are seen *in vivo* studies in rodents, additional assessments (new studies may have to be carried out) would then be commissioned as tier 2 assessments to specifically investigate the proposed mode of action and/or eliminate alternative modes of action. The elimination of alternative pathways is a necessary part of the IPCS process and while validated *in vitro* assays do not currently exist for all of the endpoints listed in the left-hand box, experimental *in vitro* cell assays are available for all of the endpoints listed. The proposed mode of action would need to be subject to Bradford Hill causality/association test to verify its strength, and then the human relevance framework would be applied by answering the three pivotal questions as described in Meek et al (2013):

1. Is the weight of evidence sufficient to establish a MOA in animals?
2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental qualitative differences in key events between animals and humans?
3. Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between animals and humans?

The establishment of a plausible MOA would identify some mechanisms which are known to have either qualitative species differences between rodents and humans, or quantitative species differences whereby humans have been shown to be more or less sensitive to the toxicity seen with the given chemical. An example of the latter is the activation of the CAR/PXR nuclear receptor in the rodent whereby induction of hepatic UDPGT leads to an increased rate of biliary excretion of T4 through glucuronidation, a resultant decrease in circulating T4, and a compensatory hypertrophy/hyperplasia of the thyroid follicular epithelium through TSH stimulation (Dellarco et al 2006). While human liver does contain the CAR/PXR receptor, there are significant quantitative differences that make activation considerably less sensitive in human than it is in the rodent with considerably higher dose levels need to produce the same effect.

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A LITERATURE REVIEW OF THE CURRENT STATE OF THE SCIENCE REGARDING SPECIES DIFFERENCES IN THE CONTROL OF, AND RESPONSE TO, THYROID HORMONE PERTURBATIONS. PART 1: A HUMAN HEALTH PERSPECTIVE

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1. Executive Summary

This review was commissioned by the European Crop Protection Association to address a series of questions raised following the issue of the draft guidance document (GD) from the joint ECHA/EFSA/JRC recommendations. The questions asked specifically related to the literature use by the draft guidance and whether or not it represented a state of the current science regarding chemical thyroid disruption. While the intention was that this document would present the laboratory animal data related to chemical induced disruption, while a second document would cover the human evidence, it was apparent in beginning the review that a document that ignored the human data was not going to be possible without seriously compromising the scientific content. This was because of the enormous amount of basic information, derived from human thyroid diseases and the clinical treatment of those diseases that was available and its incorporation was considered integral to understanding the laboratory animal data in order to place animal findings into the context of human relevance and risk.

Although there are numerous publications detailing the basic control of thyroid hormones it was decided that the first part of the review would require an introduction to the normal control and functioning of thyroid hormones in order for the reader to understand the subsequent sections dealing with perturbations in thyroid homeostasis. The review is structured to address the ECPA questions in order and covers species differences in the functional morphology and biology of the thyroid gland always with an eye to comparing the most common laboratory species for toxicity testing, the rat, with what we know about human thyroid responses. In terms of the effects of thyroid hormone perturbation there are two major endpoints of concern, namely neurodevelopmental effects on the foetus and neonate, and thyroid cancer. These have generally been separated where possible since the critical features of both, in terms of thyroid effects and the consequences of dysfunction in one part of the thyroid control process, are different between those acting in carcinogenesis and those determining foetal and postnatal development.

Section 4 of this review is an appraisal of the draft GDs and, on the whole, the GD provides an excellent roadmap for prospective registrants of new products and describes in detail what will be expected in terms of levels of proof, what it terms "lines of evidence". Throughout, the draft GD illustrates its points with relevant literature references to clarify where necessary. However, it is highly selective in its use of the literature and in no way does it aspire to be a "state of the science" review of the area of endocrine disruption and neither is this within the stated scope of the document. Appendix A of the document is a summary of the information regarding thyroid physiology and provides a high-level opinion on how chemicals could secondarily affect the thyroid predominantly through a primary effect on the liver. While the draft GD does mention alternative modes of action, such as the sodium-iodide symporter, as potential thyroid targets, the degree of coverage would not permit any reader from understanding these processes, let alone the other thyroid targets that are present. It is clearly not a state of the science document, as regards the thyroid gland but it does take the opportunity of repeating a fallacy of there being effects on thyroid hormones in the absence of histopathology on the gland (page 96). It would be helpful to the critical reader if a relevant mammalian literature reference could be included to substantiate this statement since it has the potential to undermine one of the most sensitive and regularly conducted endpoints for most of the in vivo assays While this is disappointing it simply displays the perils of straying off the stated scope of the document into what appears, at face value, to be an informed survey of the literature.

Section 6 of the current review document details the literature evidence for species differences between human and rodents addressing each function in thyroid hormone synthesis, metabolism and excretion separately. It then covers the respective responses to chemicals known to affect thyroid homeostasis in the rodent using classic examples of rodent thyroid disruptors to illustrate the points made. Where possible, such as is the case for the sulphonamide drugs, the responses in rodents and humans can be directly compared under similar exposure conditions. Throughout the review statements are illustrated with clear chemical examples, such as those compounds operating through induction of hepatic metabolism and excretion that show qualitative differences between human and rat thyroid effects while for others, such as those chemical inhibiting thyroperoxidase which show significant quantitative differences in responses with humans being considerably more resistant to the thyroid disruption than are rodents.

Section 6.10 discusses the underlying principles of carcinogenesis as they apply to thyroid cancer in both human and the rodent, and addresses similarities and differences (summarized in Table 2), in particular the pivotal role of thyroid stimulating hormone (TSH) in rodent thyroid cancer and its secondary role in human cancer. There are a number of additional significant differences between the rodent and human in their basic thyroid biology and in their responses to the same or similar chemicals and the differences are exemplified with particular reference to iodine deficiency which induces goitre in humans under the sustained driving influence of TSH, even though thyroid cancer under these circumstances doesn't normally occur. In contrast non-genotoxic rodent thyroid cancer appears exquisitely dependent upon sustained TSH stimulus alone without the need for additional factors. This section concludes with a discussion of the overall relevance of chemically-induced rodent thyroid cancer to humans. Section 7 discusses the presence of thresholds for thyroid hormone changes for both neurodevelopmental and carcinogenic outcomes again by reference to literature examples of situations where threshold shave been clearly demonstrated for both outcomes.

Section 8 is a discussion of the effects on extra-thyroidal changes such as concurrent disease, concurrent systemic toxicity in other organs such as the liver and kidney, starvation, and heat and cold in affecting the concentrations of circulating thyroid hormones and thyroid morphology. All of these environmental changes can profoundly affect the turnover of thyroid hormones and at the very least organ toxicity, and food and water consumption, are situations that can occur regularly in routine toxicity studies as a consequence of the maximum tolerated dose (MTD) approaches that are applied in the conduct of such studies. A consideration of these factors will be critical if thyroid hormone measurements are routinely incorporated into toxicity studies.

Throughout the review, where possible, the discussion has been undertaken with reference to the WHO/IPCS MoA/human relevance framework and the draft GD also embraces the principles outlined in the numerous publications that have arisen out the initiative. This is a welcome move to introduce more objective approaches to the regulatory process and to lay solid factual foundations to regulatory decisions.

The final part of the current review is questioning whether or not the current testing strategies for detecting thyroid disrupting chemicals are adequate for the purpose, and alternative animal models, including in vitro screens, are discussed. In light of the two-tier approaches advocated in the draft GD additional assays that specifically target potential molecular initiating events, are to be welcomed as part of the second tier for establishing MoA and determining human relevance of the particular events. The review makes use of an extensive list of references, both current and some considerably older, in making the points throughout.

2. Introduction

This document was commissioned by the European Crop Protection Association (ECPA) to appraise the Association of the current (issued 7th December 2017) ECHA/EFSA/JRC draft guidance document (GD) in terms of its fitness for purpose and its use of the current science in arriving at its conclusions. In order to carry out this remit, the current document has summarised the current state of understanding regarding the control of thyroid hormone homeostasis, in both human and animal species, and incorporates a critical review of the publicly available literature regarding the chemical perturbation of thyroid hormones particularly in those animal species that are used in the non-clinical safety evaluation studies required for the determination of human safety.

The publication of the outcome of the Thyroid European Commission-ANSES Workshop (European Union 2017) and the ECHA EFSA JRC Endocrine Disruptor Draft guidance (European Food Safety Authority/European Chemicals Agency 2016) posed a number of critical questions regarding the entirety of the information used to arrive at their conclusions, with particular emphasis on the sensitivity and specificity of the assays currently used to detect thyroid effects and the relevance of the species used in the bioassays for subsequent human risk extrapolation. This paper attempts to address those questions, by providing a comprehensive review of the available literature and to place

this into the appropriate context with regard to the value, or otherwise, of current testing strategies. It also proposes an approach by which the effects seen can be objectively used in providing appropriate assessment of the risks associated with exposure of the human population to chemical entities that could potentially perturb thyroid hormone function.

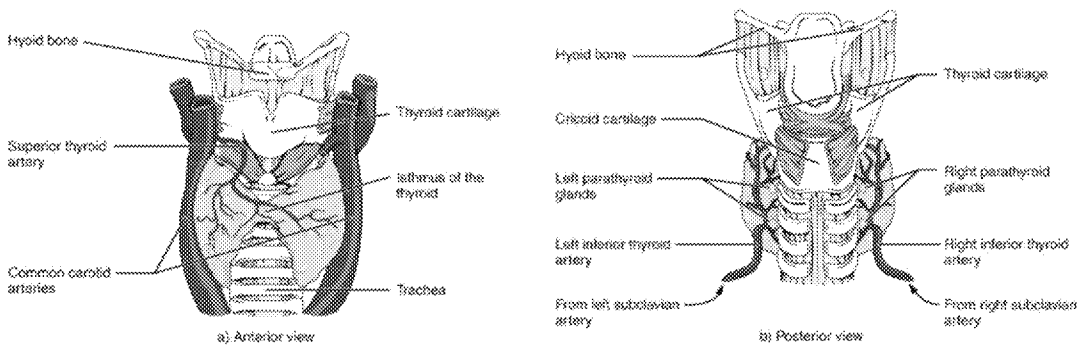
ECPA requested a focus on certain specific questions that are highlighted in italics before the respective relevant parts of the review.

3. Thyroid hormone function – the established understanding

This section summarises what is known about thyroid hormone production and secretion and is generally an amalgamation of the current knowledge derived from human and animal species. Where species differences exist, these are mentioned within the text.

The thyroid gland in mammals and birds (Fig. 1) is normally a bilobed organ with the two lobes being connected by an isthmus which lies on, and located ventro-laterally to, the trachea (McNabb and Darrass 2015). Histologically the gland consists of a number of follicles lined by low cuboidal epithelial cells and a follicular lumen containing colloid, composed of thyroglobulin, which appears pink in haematoxylin and eosin stained sections.

Fig. 1: Gross anatomy of the thyroid gland in mammals and birds - from https://courses.lumenlearning.com/ap2/chapter/the-thyroid-gland/

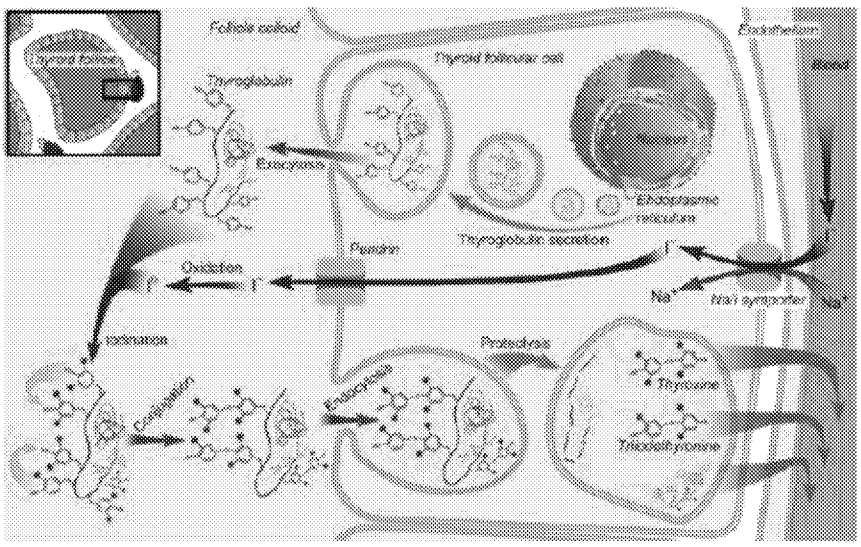


3.1. Thyroid hormone synthesis

Thyroglobulin is a glycoprotein made up of 134 tyrosine residues that is stored in the follicular lumen and which is the starting molecule for the subsequent synthesis of triiodothyronine (T3) and thyroxine (T4). Binding of thyroid stimulating hormone (TSH) to its receptor on the follicular cells triggers the up-regulation of the sodium-iodide symporter (NIS) on the basolateral membrane of follicular cells, resulting in an increase in the intracellular concentrations of iodine through a process known as iodine trapping (Rousset et al 2015). Once inside the cell the iodide is transported to the apical membrane of the follicular cell where it is acted upon by the enzyme thyroid peroxidase (TPO), an integral membrane protein present in the apical plasma membrane that catalyzes the sequential reactions needed for the formation of the respective thyroid hormones. TPO first oxidizes iodide to iodine, then iodinates tyrosine residues on thyroglobulin to produce mono- and diiodotyrosine, and finally links two tyrosine molecules together to produce T3 and T4. The peptide linkage between the thyroid hormones and thyroglobulin is then enzymatically cleaved, thyroid hormones are internalized at the apical surface of the thyroid epithelial cells by endocytosis, and hydrolytic enzymes within lysosomes

fuse with the endosomes to release the hormones which are subsequently actively exported into the circulation, through the basolateral membrane of the follicular cells via the monocarboxylate transporter 8 (Di Cosmo et al 2010). Once in the circulation the majority of the hormones reversibly complex with liver-derived binding proteins for transport to other tissues although a small proportion remains free in the plasma.

Fig. 2: Formation of hormones in the thyroid follicular cells – *from* https://courses.lumenlearning.com/boundless-ap/chapter/the-thyroid-gland/



T3 is the biologically active hormone and T4, the major thyroid hormone that is secreted from the thyroid gland, is considered a precursor or prohormone. Although all of the T4 is synthesised within the thyroid, in human beings between 40 - 80% of T3 (Bianco and Kim 2006; Bianco et al 2002; Gereben et al 2008; Gereben et al 2015) is made through deiodination of T4 in peripheral tissues, especially the liver, kidney, pituitary gland and muscle. An additional, inactive molecule, reverse T3 (rT3), is also made in peripheral tissue by the action of deiodinases on T4. T3 is produced by deiodination on the outer ring of the T4 molecule, while the inactive form, rT3 is produced through deiodination of the inner ring of the T4 molecule (Visser et al 2016). Production of rT3 is thought to be a means of controlling excess circulating T4 since it has no known biological activity. Approximately 20% of all of the bound T4 is converted to rT3 on a daily basis in the human liver and other extra-thyroidal tissues.

The deiodination reaction is catalysed by one of three enzymes called type 1, type 2 and type 3 iodothyronine deiodinases (Visser, 1988; Leonard and Visser 1986; Chanoine et al 1993). Type 1 deiodinase is the main enzyme expressed in the liver, kidney and thyroid, and the liver is considered to be the most important extra-thyroidal site for the production of T3 and for removal or rT3. Both T3 and rT3 are metabolized within the liver by 5- and 5'-deiodination respectively to produce the inactive 3,3'-di-iodothyronine (T2). In situations, such as illness, increased metabolic demand in general, or administration of drugs such as amiodarone or β-adrenergic medication, the proportions of T3 and rT3 made from T4 can change to accommodate the altered demands (Hackney et al 1995a; Narayana et al 2011; Barbesino 2014).

3.2. Tissue uptake, signal transduction, and metabolism of thyroid hormones

Tissue thyroid status depends not only on thyroid hormone uptake and secretion but also on metabolism, delivery of T3 to the nuclear receptors, and receptor expression and distribution within the target cell populations.

It had generally been assumed that thyroid hormone, due to its hydrophobicity, enters cells via passive diffusion but *in vitro* studies identified several membrane transporters belonging to the monocarboxylate, and organic anion, transporter families with the ability to actively transport thyroid hormones into cells (Janson et al 2005). A human genetic disorder, known as Allan-Herndon-Dudley Syndrome, was instructive in indicating the critical role of the monocarboxylate transporter 8 (MCT8) protein in actively sequestering hormones into target cells. (Dumitrescu et al 2004; Friesema et al 2004; Schwartz and Stevenson 2007), and severe neurologic deficits. The syndrome is due to a mutation in the MCT8 gene andit was shown that active thyroid hormone transport is required in certain tissues, especially the brain for normal functioning (Kersseboom and Visser 2011; Visser et al 2011).

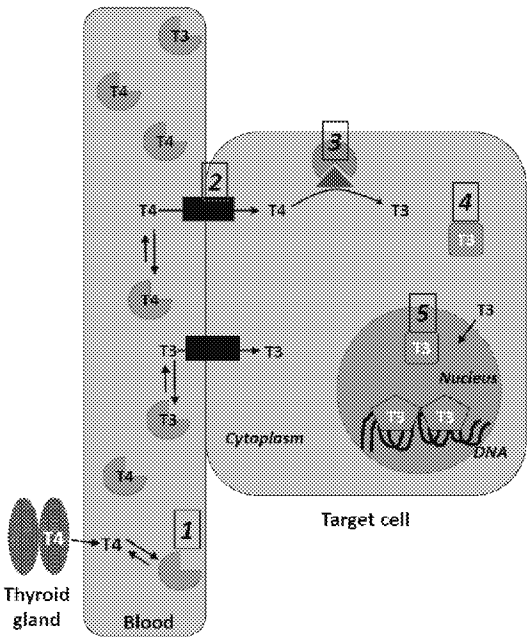
Table 1: Types of thyroid hormone transporters and their iodothyronine derivatives (Ahmed 2012)

Transporter	Iodothyronine derivatives	Specificity
MCT8	T3, T4, rT3, T2	+++
MCT10	T3. T4	++
OATP1A1	T3, T4, rT3, T2, T4S, T3S, rT3S, T2S	+
OATP1A2	T4, T3, rT3	
OATP1A3	T4, T3	
OATP1A4		
OATP1A5		
OATP1B1	T4, T3, T3S, T4S, rT3S	
OATP1B2	T3, T4	
OATP1B3	rT3, T4S, T3S, rT3S	
OATP1C1	T4, rT3, T3, T4S	++
OATP2B1	T4	+
OATP3A1 (V1/V2)		++
OATP4A1	T3, T4, rT3	+
OATP4C1	T3, T4	
OATP6B1		
OATP6C1		
LAT1	T3, T4, rT3, T2	
LAT2		
NTCP	T4, T3, T4S, T3S	++

Notes: The specificity is defined as high (+++) if the transporter only transports iodothyronine hormones, moderate (++) if it transports fewer than 5 other ligands, and low (+) if more than 5 ligands are known.

The MCT8 transporter has also been localised on the basolateral membrane of follicular epithelial cells suggesting that this protein might also act in the export of T4/T3 following its synthesis in the follicular epithelial cells (Friesma et al 2006). There are many known intracelllular thyroid hormone transporters, in addition to MCT8, and these are listed in Table 1 above (Ahmed 2012).

Fig. 3: Thyroid hormone secretion and transfer into cells (from Alshehri et al 2015)



1. = Plasma binding proteins (TBG, TTR, albumin); 2 = thyroid hormone transporters (MCT8, OCT14); 3. = iodothyronine deiodinases; 4. = cytosolic thyroid hormone binding proteins; 5. = thyroid hormone nuclear receptors.

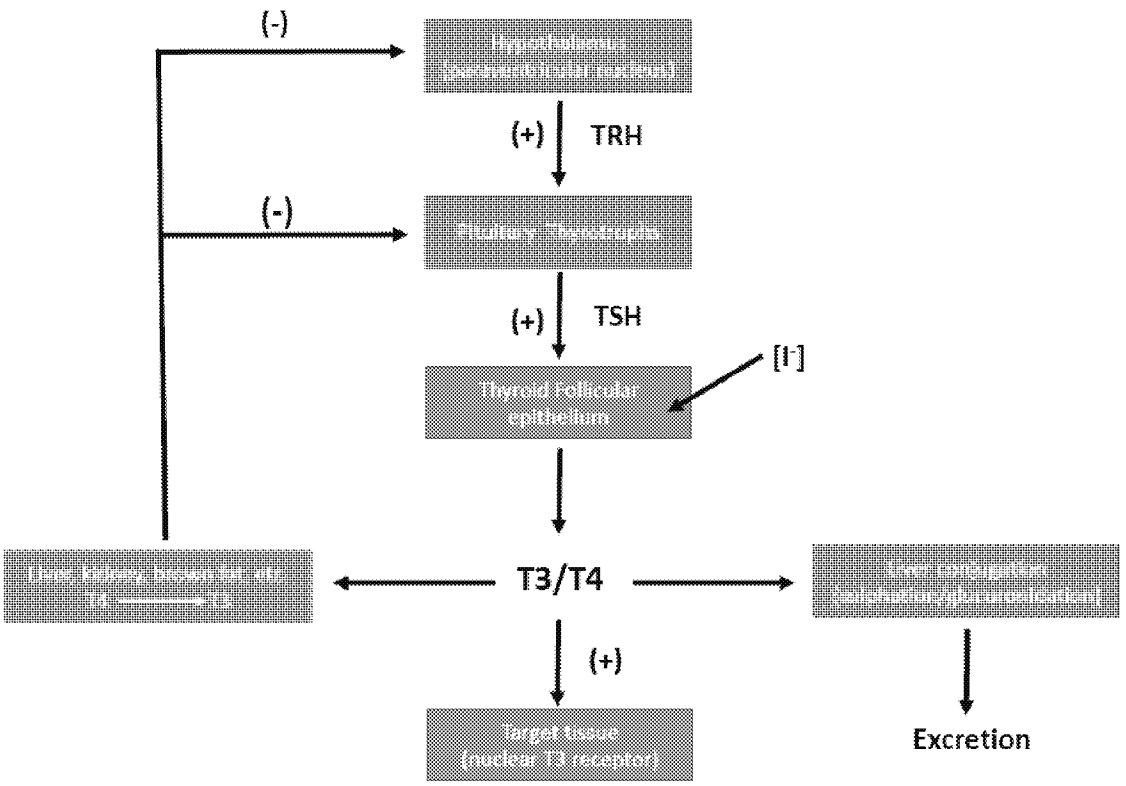
Once across the cell membrane and inside their target cells, cytosolic thyroid hormone binding proteins transfer the hormones to the nucleus where they dissociate from the carrier protein and bind to the nuclear T3 hormone receptor for subsequent cellular responses (Fig 3). Several different cytosolic proteins can act as thyroid transfer proteins including glutathione s-transferases (Ishigaki et al 1989; Kato et al 1989). It has been found that the thyroid nuclear receptor exists in several different forms, depending primarily on tissue type, some of which are able to bind T3 while others act to supress the activity of T3 (Mullur et al 2014). The different isoforms of the thyroid nuclear receptor are thought to exist to provide a pathway of tissue specific thyroid hormone action. The thyroid hormone receptor is part of the nuclear superfamily group that includes the retinoic acid receptor, retinoid X receptor, vitamin D, and the peroxisome proliferator activated receptor (Evans 1988). These receptors, on binding their respective ligand, are able to bind as monomers to thyroid response elements (TREs) on DNA, but the majority bind in the form of a heterodimer with the retinoid X receptor (RXR). Heterodimer formation is thought to enhance DNA binding affinity as well as providing target gene specificity (Kliewer et al 1992). The thyroid hormone/RXR heterodimer complex, on binding to its thyroid response element on DNA, is then able to stimulate or inhibit subsequent gene transcription within the target cell. There is considerable opportunity for crosstalk between the thyroid hormone receptor/RXR complex and other nuclear hormone receptors including the alpha and gamma forms of the peroxisome proliferator activated receptors (PPARα and PPARγ), and the liver X receptor (LXR) (Liu and Brent 2010). Since these nuclear receptors all form heterodimers with RXR, there is potential for competition for the limited amounts of RXR available (Hsu et al 1995; Liu et al 2007; Fattori et al 2015) that would result in depression of receptor activation by thyroid hormone and a possible inability or reduced ability to respond to elevated levels of thyroid hormone.

3.3. The hypothalamus/pituitary/thyroid axis (Fig. 4)

Thyroid releasing hormone, (TRH), secreted by the tanycytes within the hypothalamus, acts upon the pituitary gland, binding to G protein-coupled TRH receptors, resulting in an increase in intracellular cAMP, and subsequent thyrotropin (TSH) release from the thyrotrophs (Hershman 1974). Hormone signals that have modulatory effects on pituitary TSH secretion include dopamine, somatostatin, and leptin, and these molecules help to regulate thyroid hormone release at the level of the CNS (Scanlon et al 1979; Tanjasiri et al 1976; Seoane et al 2000; Ghamari-Langroudi et al 2010).

Thyroidectomy in rats results in a marked increase in serum TSH concentrations in response to the failure to produce T4 and T3. In experiments with thyroidectomised rats, where exogenous T4 was administered in the presence of selenium deficiency, TSH levels continued to remain high despite the maintenance of serum T4 concentrations identical to those observed in the serum of intact rats. In this experiment although serum T4 levels were normal, serum T3 levels remained low due to the diet-induced depression in deiodinase enzyme activities (selenium containing enzymes). This data confirmed the hypothesis that circulating T3, rather than T4, plays the critical role in regulating TSH secretion (Chanoine et al 1992; Abend et al 1991; Emerson et al 1989; Scanlon and Toft, 2000).

Fig. 4: Feedback control of thyroid hormone synthesis and release (adapted from Hill et al 1989).



Local tissue conversion of T4 to T3, by iodothyronine deiodinase type 2, provides negative feedback at the level of the thyrotrophs in the pituitary, and via the TRH secreting tanycytes in the hypothalamus (Fonseca et al 2013; Gereben et al 2008; Larsen and Zavacki 2012. High circulating thyroid hormone levels result in a reduction in TRH and TSH secretion, whereas low thyroid hormone

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levels stimulate TRH release from the hypothalamus and TSH release from the pituitary which act to increase thyroid production of the hormones.

On release from the pituitary gland, TSH binds to G protein-coupled TSH receptors on the thyroid follicular cells, stimulating the production and secretion of T4 and lower levels of T3, and when required to be increased, e.g. by certain chemicals and drugs, TSH stimulation will lead to hypertrophy of the follicular cells to meet the extra demand (Parmentier et al 1989; Tani et al 2004). If increased thyroid hormone production by the existing follicular cell population alone is insufficient to correct the decreased circulating hormone levels, continued TSH stimulation results in a stimulation of follicular cell proliferation, hyperplasia of the follicles, and ultimately can result in the development of follicular cell neoplasms (Smith et al 1991).

Although originally it was thought that T3 and T4 simply diffused into target cells it is now clear that there are several ion-coupled, membrane transporters that are able to actively transport the thyroid hormones into target cells (Visser 2011). The MCT8 protein is involved in transporting T3 into cells throughout the body and mutations in the gene are associated with serious neurodevelopmental abnormalities in humans. Mouse models of MCT8 gene knockout show thyroid function study changes similar to those in patients with so-called Allan-Herndon-Dudley Syndrome, a condition brought about by a mutation of the transporter, but these mice show only minor changes in brain function in comparison with the human syndrome (Dumitrescu et al 2006; Trajkovic et al 2007; Visser et al 2011). The discrepancy between the response of inactivation of the MCT8 transporter in mice, and the serious consequences that result from the same mutation of the transporter in humans, is most likely due to the former having redundant thyroid hormone transporters that are able to compensate for the loss of the MCT8 gene. MCT8 is highly expressed in the hypothalamus and certain mutations have shown impairment of central hormone regulation and an abnormal thyroid hormone feedback (Alkemade et al 2011). Without a functioning MCT8 transporter in the brain, specific brain areas are unable to absorb T3 and become hypothyroid although the liver Is still able to respond through the use of alternative thyroid uptake systems. Mutated MCT8 also means that the excess TRH/TSH is produced due to impaired negative feedback in the hypothalamus results in tissue-specific hyperthyroidism, hyper-metabolism and profound weight loss (Heuer et al 2009). Treatment with the thyroid hormone analogue, diiodothyropropionic acid (DITPA), in both animal models and humans with inactivated MCT8 gene, results in a reduction in both serum TSH and serum T3, with a consequent improvement in weight gain and decreased metabolic rates (Di Cosmo et al 2009; Verge et al 2012).

3.4. Physiological function of thyroid hormones

Thyroid hormones regulate the metabolic processes essential for normal growth and differentiation in the developing organism, as well as determining the metabolic rate in the adult (Brent 2012b; Cheng et al 2010; Oetting and Yen 2007) particularly with regard to the regulation of energy metabolism within cells (Malik and Hodgson 2002; Iwen et al 2013; Liu and Brent 2010). In healthy humans, thyroid hormone status correlates with body weight and energy expenditure (Fox et al 2008; Iwen et al 2013; Knudsen et al 2005) and hyperthyroidism promotes a hypermetabolic state characterized by increased resting energy expenditure, weight loss, reduced cholesterol levels, increased lipolysis, and gluconeogenesis (Brent 2008; Motomura and Brent 1998). In contrast, hypothyroidism induces reductions in metabolic rate that are characterized by chronic fatigue, weight gain, increased cholesterol levels, reduced lipolysis, and reduced gluconeogenesis (Brent 2012a; Oppenheimer et al 1991). The effect of thyroid hormones on metabolism is achieved through interactions with receptors in the brain, white fat, brown fat, skeletal muscle, liver, and pancreas (Mullur et al 2014).

3.5. Thyroid hormones in neurodevelopment

During the initial stages of gestation/pregnancy, the foetus relies on maternal thyroid hormones for normal brain development and growth. Deprivation of the maternal thyroid hormones, in hypothyroidism, can have devastating effects on the foetus and in humans, dysfunction of thyroid hormones in brain development is most often mediated through mutations in thyroid hormone transporters or in the deiodinases that degrade excess T3 (Ahmed 2015). Human hypothyroidism is most commonly monitored through assessment of circulating TSH levels and where needed, maternal thyroid hormone supplementation (levothyroxine) given during pregnancy corrects for any deficit (Maraka et al 2017). The recommended fixed upper threshold for TSH concentration in humans in January 2017 was 2.5 mIU/L during the first trimester and 3.0 mIU/L during the second and third trimesters (Maraka et al 2017). According to these diagnostic criteria, subclinical hypothyroidism, defined as an elevated TSH concentration with concurrent normal thyroid hormone concentrations, was estimated to affect up to 15% of pregnancies in the US and 14% in Europe. This represented a fivefold increase in apparent prevalence of hypothyroidism compared with the 2-3% prevalence of subclinical hypothyroidism before these criteria were established, raising the possibility of overdiagnosis of subclinical hypothyroidism and subsequent discussions by the American Thyroid Association (Alexander et al 2017) have revised these estimations by increasing the TSH cut-off limit to 4.0 mIU/L.

Because of increased thyroid hormone production, increased renal iodine excretion, and foetal iodine requirements, dietary iodine requirements are higher in pregnancy than they are for non-pregnant adults (Glinoer 2007).

Patients with the human genetic disorder, Allan-Herndon-Dudley Syndrome, show low levels of serum T4 and rT3, elevated T3, and normal or slightly elevated serum thyrotropin (TSH) (Dumitrescu et al 2004; Friesema et al 2004; Schwartz and Stevenson 2007), and severe neurologic developmental deficits that develop before birth. The mutated MCT8 gene translates a dysfunctional protein that is unable to adequately transport sufficient T3 into the developing brain and prevents the normal formation and growth of nerve cells and its discovery was instrumental in showing the critical requirement of thyroid hormones in the developing brain and that active thyroid hormone transport was required in the brain to ensure normal development (Kersseboom and Visser 2011; Visser et al 2011).

Studies in MCT8 knockout mice show a dramatically reduced uptake of T3 into the brain in comparison with wild type mice with an intact MCT8 system (Dumitrescu et al 2006; Trajkovic et al 2007). Because mutations in the MCT8 transporter are associated with multiple neurologic abnormalities in humans, with developmental delays, and progression to quadriplegia (Bernal 2011), the transporter is thought to have a critical role in normal brain development, and thyroid hormone transporters in general show both a specific temporal, but also spatial, pattern of expression in the developing brain (Sharlin et al 2011; Van der Deure et al 2010, Visser et al 2011).

Experimental data on thyroid hormone transport suggests significant species differences exist between humans and experimental animals, especially with regard to the uptake of thyroid hormones by the brain. In contrast to the situation in man knockout mice, lacking the MCT8 transporter, fail to show motor deficits suggesting that alternate pathways for transporting thyroid hormones into the brain exist in this species (Di Cosmo et al 2010; Roberts et al 2008). It is now known that the organic ion transporter polypeptide-14 (OATP14) is the primary thyroid hormone transporter expressed in the endothelial cells at the blood-brain barrier, whereas MCT8 mediates thyroid hormone uptake into neurons (Friesma et al 2005). OATP14 mRNA and protein is strongly expressed in both rat and mouse cerebral microvessels, but not in human, while both MCT8 and OATP14 is present in mouse and rat tanycytes, the cells in the hypothalamus that are responsible for the secretion of TRH (Roberts et al 2008). While MCT8 is primarily concerned with transporting T3 into the brain, OATP14 has been found to primarily transport T4 (Pizzagalli et al 2002; Sugiyama et al 2003; Tohyama et al 2004), and the high microvessel expression of OATP14 in the rodent, as compared with the human brain, may explain the relatively mild neurophysiological consequences of deletion of the MCT8 transporter in Mct8-null mice. In Mct8 KO mice, OATP14 is thought to be able to compensate for the loss of MCT8

in transporting thyroid hormone into the brain without loss of function, in contrast to humans lacking functional MCT8, where the absence of an alternate transporter results in serious neurodevelopmental consequences.

MCT8-mediated T3 transport itself, rather than being generated following uptake of T4 into the brain is also consistent with the finding that brain development and function is normal in type 2 deiodinase knockout mice (Schneider et al 2001). Type 2 deiodinase is responsible for converting T4 to T3 and the fact that type 2 deiodinase knockout mice are phenotypically normal indicates that direct brain uptake of T3 from the circulation can compensate for the inability of the neurones themselves to generate T3 by deiodination of T4.

Conversion of the pre-hormone T4 to the active thyroid hormone, T3, occurs mainly in peripheral tissue via the enzymic activity of a group of iodothyronine deiodinases. Deiodinases generally are present at low levels in the brain, and while the type 1 deiodinase is found at high concentrations in the liver, within the brain the type 3 deiodinase generally mediates the degradation of thyroid hormones to inactive metabolites rather than deiodinases catalysing the conversion of T4 to T3. Especially high type 3 deiodinase activity has been demonstrated in the placenta and the pregnant uterus, as well as in different foetal tissues. This is considered to be a protective mechanism to prevent exposure of foetal tissues to high T3 levels at inappropriate times in development thus allowing the normal growth of these tissues. Paradoxically, T3 is only required at the differentiation stage of tissue development, and its presence at earlier stages, in conditions of maternal hyperthyroidism, has been linked with developmental abnormalities (Batra 2013). The critical role of type 2 and 3 deiodinases, and their differential expression at different stages in the developing foetus, is exemplified in cochlear development, since mice carrying either a type 2 or a type 3 deiodinase knockout have severe hearing loss (Ng et al 2004). At immature stages of foetal development, the type 3 enzyme limits stimulation by T3 whereas postnatally, a double switch occurs with a decline in the activity of the type 3 enzyme and a concomitant increase in the activity of type 2 deiodinase, resulting in a local T3 surge which is independent of serum T3 levels and which triggers the onset of auditory function (Ng et al 2004; Visser et al 2016).

4. ECPA Question: Consider the references in the draft guidance document – is this the state of the science? If not, what is?

The GD is intended to provide guidance for applicants and risk assessors on the implementation of the draft scientific criteria for determining endocrine disrupting chemicals and as such it appears to excellently provide "point by point" guidance using relevant literature references but it clearly is not, and does not claim to be, a state of the science document. It does introduce the entire area of endocrine disruption and does a good job of introducing those assays having OECD guidelines, together with other assays not currently covered by guidelines but it clearly does not incorporate the most up to date scientific research in the area as a whole and in the thyroid gland in particular. It does address its stated scope and provides an invaluable source of information for assessors and dossier submitters that provides clear guidance on what evidence will currently constitute a chemical being classified as an endocrine disruptor of relevance to the human exposure situation.

On page 3 of the document the principles used to set out the guidance are detailed for assessing whether a substance meets the hazard based ED criteria. The strategy is based on the requirements outlined in the ED-criteria

'[…] that a substance shall be considered as having endocrine disrupting properties […] if:

(1) It shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;
(2) It has an endocrine mode of action. i.e. it alters the function(s) of the endocrine system;
(3) The adverse effect is a consequence of the endocrine mode of action.'

The link between the adverse effect and the endocrine mode of action (MoA) addressed in point (3) shall be established based on biological plausibility. The biological plausibility shall be determined in the light of current scientific knowledge and all available relevant scientific data by using a WoE approach".

The last sentence above in the GD is a critical one since its whole precept is dependent upon the scientific database being not only comprehensive, but also being critically balanced between the academic understanding of thyroid hormone disruption, obtained from non-guideline, *in vivo* and *in vitro,* laboratory studies, but also on practical experience gained through the evaluation of a broad range of chemical classes conducted following the strict guidelines laid down by the OECD TGs.

Appendix A of the draft guidance specifically addresses the problem of potential thyroid hormone disruption and is a pragmatic description of the current problems of species extrapolation between the rat and human in particular, but it also describes the evidence that would be taken to arrive at a conclusion of a relevant MoA to human risk, and how a registrant might approach disproving this human link, making excellent use of the WHO/IPCS MoA/human relevance framework approach advocated by Meek et al (2014b) and others.

Appendix A undertakes to aid the interpretation of any potential thyroid disrupting effect by setting out clearly stated decisions points on page 95 of the latest GD based upon the following "Using the current understanding of thyroid physiology and toxicology13 it is proposed that the following be applied when interpreting data from experimental animals:

1. It is presumed that substances that alter the circulating levels of T3 and/or T4 with concurrent histopathological findings in the thyroid would pose a hazard for human thyroid hormone insufficiency in adults as well as pre- and post-natal neurological development of offspring.
2. It is presumed that substances that alter the circulating levels of T3 and/or T4 without histopathological findings would still present a potential concern for neurodevelopment.
3. In the absence of substance-specific data which provide proof of the contrary, humans and rodents are presumed to be equally sensitive to thyroid-disruption (including cases where liver enzyme induction is responsible for increased TH clearance)."

Point 2 is an important statement if proven to be true. As such it warrants a literature reference to justify its inclusion as one of the three most important points in the decision-making process. It seems to suggest that only neurodevelopmental endpoints would be relevant in such circumstances and also that the control of thyroid hormones is somehow different in the thyroid carcinogenesis process than it is in the neurodevelopmental ones. There are many reasons why a histopathological investigation of thyroid glands, in the presence of decreased thyroid hormones, may not detect follicular hypertrophy/hyperplasia but I suspect the effect not being there is not one of them! This point is covered in detail in section 7.1 of this review

An objective discussion of the balance of pure and applied research is not within the stated scope of the draft GD and is clearly one of the objectives of this current review document.

5. ECPA Question: Is the way in which the references are used in the GD appropriate?

The literature references presented in the main text of the draft GD do appear to be appropriate for the intent and stated scope of the document. The literature listings are not extensive but the majority are relevant, and appear in the appropriate parts of the document to help clarify some of the more complex thinking behind the proposals. They are a pragmatic listing that does not attempt to introduce the reader to the most current scientific data being generated particularly in the human, but also in the experimental animal, field of thyroid research.

The GD is lacking particularly in its inclusion of the current state of thyroid scientific literature and, while heavily biased towards oestrogen and androgen disruption, it is still not comprehensive in its coverage of even these areas. But a state of the science review is not the intended scope of the GD!

The literature references used do appear to support the sections throughout the GD and offer clarification in those areas requiring it. They do not provide a "get-out" clause for industrial submitters to refute decisions of human relevance, but the GD does suggest appropriate actions, via their Tier 2 list of approaches, that can usefully be adopted to support MoA/human relevance cases that could possibly challenge suggestions of thyroid hormone disruption in other Tier tests.

6. ECPA Question: Comparison between human and key laboratory animal species (rodent, dog, monkey)

The physiological functioning and regulation of the pituitary-hypothalamic-thyroid system in all known mammalian species, birds and humans is qualitatively extremely similar (Choksi et al 2003; Bianco et al. 2002) and correct thyroid functioning and maturation during foetal organogenesis are essential for the development of critical organ systems, including the nervous system and reproductive tract (Jannini et al 1995; Metz et al 1996; Krassas 2000). However, the dynamics of the thyroid hormone control and turnover do differ substantially between the different mammalian species even though there are significant structural homologies between the various hormones (Imamura et al 1991; Tsykin and Schreiber 1993; Power et al 2000). Table 2 summarises the main thyroid features where differences are seen between human and rodent thyroid hormone control.

6.1. Species comparison of the functional morphology of the thyroid gland

Compared to the thyroid follicles in primates, which are large with abundant colloid and with follicular cells that are relatively flattened (low cuboidal), rodent follicles are considerably smaller, contain less thyroglobulin colloid and are most often lined by cuboidal, basophilic-staining epithelium indicative of a higher content of mRNA production, and by inference, higher rate of hormone synthesis, than the equivalent cells in those species having low eosinophilic epithelium such as primates including human. Within any single gland in both rats and mice, a small number of follicles will be large, lined by an attenuated epithelial layer and contain large amounts of thyroglobulin, in similarity with the majority of follicles in the primate and human gland, while other rodent follicles, the majority, will normally contain much smaller amounts of thyroglobulin colloid and have taller lining epithelium considered to represent more actively synthesising, and hormone secreting, cells. During times of increased hormone demand the number of active follicles, in the thyroid gland of rodents, increases while the number of resting "cold" follicles decreases. Because of this plasticity in the follicular response in

rodents, the morphological differences in the appearances of the follicles in the normal thyroid gland between control primates and rodents is supportive of the faster rates of thyroid hormone turnover in the rodent (US EPA 1998).

6.2. Species comparison of thyroid hormone binding proteins in the plasma

Once secreted into the plasma from the thyroid follicular cells, the vast majority (>90%) of the T3 and T4 are transported through the blood bound to thyroid hormone binding proteins. These liver-derived binding proteins, and the proportion of T3 and T4 that they are able to bind, varies significantly among animal species. T3 and T4, in different species, have been found to be able to reversibly bind to three different liver-derived binding proteins: thyroxine-binding globulin (TBG), transthyretin (TTR), also called thyroid-binding prealbumin, and albumin (Schussler 2000; Bartalena and Robbins 1993). Lipoproteins also bind a small fraction of the available thyroid hormones. TBG is a monomer and a member of the serine protease inhibitor (serpin) superfamily of proteins (Flink et al., 1986; Robbins, 2000) while TTR is a tetramer composed of four identical subunits each composed of 127 amino acids (Power et al., 2000). Albumin is a monomer that has substantial sequence homology with α-fetoprotein and vitamin D-binding proteins (Robbins, 2000). There is little overall amino acid sequence homology between the three major binding proteins.

In normal human plasma, there are three T4 binding proteins (Fig. 3) with respective hormone distribution being approximately 80% bound to TBG, 15% to TTR, and 5% to albumin and lipoproteins, while for T3 the respective proportions are 90% bound to TBG and the remainder to albumin and lipoproteins. The binding distribution of T4 and T3 appears to correlate exactly with the binding affinity of these hormones to their respective proteins in humans, and the affinity of both T4 and T3 for TBG is much higher than are their affinities for either albumin or TTR (Kaneko, 1989; Robbins, 2000). Although only about 0.3% or less of T3 and T4 circulates unbound, it is this free hormone fraction that is metabolically active at the tissue and cellular level although the presence of deiodinases within target tissues means that local intracellular production of T3 from T4 can occur and most probably is responsible for the majority of T3 required by these tissues.

Table 2: A comparison of thyroid function and control between humans, rats and mice (adapted from Choksi et al 2003; Jahnke et al 2004; Colnot & Dekant 2017; Lewandowski et al 2003).

Parameter	Human	Rat	Mouse
Half-life of T4	5-9 days	0.5-1 day	0.5-0.75 days
Half-life of T3	1 day	0.25 days	0.45 days
High affinity TBG	Present	Absent	Absent
Primary serum binding protein	TBG	Albumin	Albumin
Serum TSH levels (ng/ml)	0.05-0.5	0.6-6.0	unknown
Sex difference in serum TSH level?	Males = females	males>females	males>females
Sex ratio for thyroid cancer	Females>males	Males>females	Males>females
Effect of chronic TSH stimulation?	Goitre	Cancer	Cancer
Amount of T4 supplementation required in absence of functioning thyroid?	2.2 mg/kg bw/day	20 mg/kg bw/day	Unknown
Development of foetal HPT	TSH/T3 by week 20 of gestation	TH & TSH by day 17 gestation	Unknown
Effect of mutant/KO MCT8	Multiple severe neurological deficits	unknown	Normal
Morphology of the follicular epithelium	Low epithelium	Tall cuboidal epithelium	Tall cuboidal epithelium
Morphology of the follicles	Large, lots of colloid	Small, little colloid	Small, little colloid
T3 glucuronidation	Minor route	Major route	unknown
Type 2 deiodinase expression in thyroid	High	Very low/Absent	Very low/Absent
% of T4 eliminated in bile	10-15%	~50%	Unknown
Timing of thyroid nuclear receptor binding in foetus	Week 10-16 (of 39 weeks total)	Day 10-15 (of 21 days total)	Unknown

In humans, inherited or acquired variations in the concentration and/or affinity of these thyroid hormone binding proteins may produce substantial changes in serum total thyroid hormone levels but these changes do not result in hypothyroidism or hyperthyroidism because the concentration of the free thyroid hormone does not change. A deficiency in thyroid hormone binding proteins in humans is suspected when abnormally low serum total thyroid hormone concentrations are present in clinically normal (euthyroid) subjects in the presence of normal serum TSH. More specifically, low TBG is suggested to be the cause of the low total T4 levels in these circumstances as it is this protein that carries the majority of the serum hormones. Under these circumstances the assay of free T4/T3 is more diagnostic of a thyroid gland effect than measures of total hormone where decreases could occur during liver pathologies where decreased production of binding proteins could be the cause of the decreased total T4/T3 levels measured.

Compared to humans, albumin is the major thyroid hormone binding protein in adult rodents and while they do carry a TBG gene, the TBG protein is expressed at very low levels in adult animals (Vranckx et al 1990; Rouaze-Romet et al 1992; Tani et al 1994). Developmental studies in the rat have shown that TBG protein expression increases briefly postnatally in rodents, but then declines to very low levels during weaning to remain at these low levels for the remainder of the rat's life (Savu et al 1987, 1991; Vranckx et al 1990). Although the binding affinity of T3 and T4 for TBG in the adult rat is still higher than it is for TTR and albumin in the rat, the fact that serum TBG levels in the rat are so low means that both thyroid hormones, to all intents and purposes, bind only to TTR and albumin in the serum of adult rodents.

6.3. The role of transthyretin (TTR)

The protein that is currently known as transthyretin (TTR) was first described in 1942 in human serum (Kabat et al 1942) and cerebrospinal fluid (CSF) and as a result of its mobility during electrophoresis at pH 8.6, where it was the only serum protein that migrated ahead of albumin, it was named at the time as 'prealbumin' (Seibert and Nelson 1942).

TTR is a thyroid hormone binding protein that is synthesised in the liver, is secreted into the bloodstream and distributes thyroid hormones around the body (Alshehri et al 2015). A second source of TTR is the choroid plexus and at this site it is thought to be involved in the movement of thyroxine from the blood into the cerebrospinal fluid and the subsequent distribution of thyroid hormones in the brain. Adequate uptake of thyroid hormone into the developing brain is essential for normal maturation and differentiation of the nervous system in the foetus in both animals and humans. In human plasma thyroxine binding globulin has the highest affinity for binding T3 and T4 followed by transthyretin and finally albumin, and they carry respectively 75%, 15% and 10% of the thyroid hormones in human blood (Alshehri et al 2015). It has been argued that TTR is the main protein responsible for transporting T4 in rodent blood (Palha et al 1994) although this has been challenged in the light of data from TTR knockout mice where the mice are perfectly viable without phenotypic changes (see discussion below).

The vast majority of *in vivo* studies assessing the potential for chemically-induced thyroid hormone disruption are carried out in the rat and it is this species where most attention has been focussed in determining the relevance of any observed rodent thyroid effect for the human population. While humans have all three major binding proteins, healthy adult rodents have only albumin and TTR (Vranckx et al 1990a; 1990b; Savu et al 1991; Lewandowski et al., 2004). If TTR is the major binding protein in the rat then it is clear that factors other than hormone binding affinity and dissociation rates must explain the clear differences demonstrated between the hormone kinetics in the rat with T4 half-life being around 24h, versus 5-6 days in humans. It is equally true that the enhanced clearance rate in the rat is compensated for by a correspondingly higher production rate of thyroid hormones, with an equally greater basal TSH levels in the plasma, and a more active thyroid gland as a consequence (Lewandowski et al., 2004).

It is a perfectly viable hypothesis, supported by data, that the differing thyroid hormone kinetics between rats and humans make the former considerably more sensitive to the effects of many, if not the majority, of these chemical disruptors of thyroid homeostasis (McClain 1995; Colnot and Dekant 2017). A comparison of the data on the affinities and dissociation rates of thyroid hormones for the TTRs from rodents, humans and other animals have admittedly shown little variation (Chang et al 1999) but TTR is only one of the thyroid binding proteins in rodents. Albumin is the main carrier of thyroid hormones in the rat (Jahnke et al 2004) and differences in thyroid hormone binding is only one of the determining factors in the kinetics of thyroid hormone turnover between humans and rodents.

There are well described examples of both qualitative and quantitative differences in the responses of humans and rats to some of the drug induced thyroid hormone disrupters, such as with the sulphonamide antibiotics, where controlled exposures in both humans and rats have shown qualitative and significant quantitative differences in the thyroid responses (Capen 1999; McClain 1995; Colnot and Dekant 2017). Perhaps the best understood examples of drugs affecting thyroid hormone homeostasis in rodents and not in man are the rodent hepatic enzyme inducing drugs (Curran and

DeGroot 1991) where a considerable amount of controlled human exposure has been monitored for thyroid effects with drugs such as sodium phenobarbitone has failed to find such a relationship even though other anti-epileptics, such as carbamazepine and rifampin have been shown to be goitrogenic.

In an attempt to better understand the importance of TTR in rodents, TTR knockout mice have been produced and shown to be perfectly viable (Episkopou et al 1993; Richardson 2007) without neurodevelopmental abnormalities. If mice are like rats in that they make TBG in the foetus, then this thyroid hormone binding protein could, along with albumin, substitute for TTR at critical developmental stages in the knockout foetus, which are taken over exclusively by albumin postnatally. The mouse TTR knockout data therefore suggest that TTR, rather than being a key thyroid hormone transporter, as is claimed in the draft GD and in the publication by Alshehri et al (2015), has a secondary, or backup, role at most in the transport of thyroid hormones in rodents, and supports a conclusion that, in the adult rat/mouse, albumin with its relatively low affinity but high dissociation rates for carrying T4and T3, is the main thyroid binding protein in rodents (Palha et al 1994; 2000; 2002; Sousa et al 2005; Alshehri et al 2015). Considering the critical importance of thyroid hormones to brain development it is inconceivable that the absence of the major thyroid hormone transporter, as is claimed for TTR, in knockout mice would permit survival without significant neurological disorders. These results suggest that albumin is the most important thyroid hormone transporters in the mouse, and most probably also in the rat, under these circumstances.

6.4. Species differences in thyroid hormone metabolism

Thyroid hormones are metabolised in peripheral tissues by three enzyme systems, the iodothyronine deiodinases, by sulphate conjugation by sulphotransferases (SULT), and by conjugation with glucuronic acid catalysed by glucuronosyltransferases (UGT). The deiodinases and UGT enzymes are localised intracellularly within the endoplasmic reticulum while the SULTs are cytosolic enzymes (Dentice et al 2013; Radominska-Pandya et al 2005; Teubner et al 2007). Both pathways are responsible for the excretion of thyroid hormones in the rat, and the liver and kidney are major sites for this process (Vansell and Klaassen 2002; Visser et al. 1993). In the rat liver, there are a large number of UGT enzymes involved in conjugating various substrates, but with regards to the thyroid hormones, UGT1A1, UGT1A6 and UGT1A7 are reported to conjugate T4 while a number of different UGT2 enzymes conjugate T3 (Vansell and Klaassen 2002; Emi et al 2007).

T3 is not glucuronidated significantly in human liver or kidney although it is induced in certain diseases where circulating thyroid hormones increase, such as in hyperthyroidism, and where glucuronidation becomes more important (Findlay et al 2000; Visser 1996). The lack of hepatic T3 glucuronidation in humans suggests that either human liver doesn't express an enzyme homologous to rat UGT2B2, the enzyme primarily, although not solely, responsible for glucuronidating T3 in the rat, or that it does not accept T3 as a substrate. In humans therefore T3 appears to be metabolized predominantly by deiodination and sulfation. In contrast to the situation in humans, normal rat liver has been shown to have substantial T3 glucuronidation (Findlay et al 2000).

Laboratory animal studies show that there are both species-and gender-dependent variations in enzyme activity (Kelly, 2000) that can explain some of the gender specific thyroid hormone effects seen with enzyme inducing agents. The UGT enzymes have especial importance in the biology of thyroid hormones since they can be upregulated in the liver by a number of xenobiotics as part of the pleiotropic response that occurs following activation of several nuclear hormone receptors, including the constitutive androstane receptor (CAR) and PXR, and the impact of altered thyroid hormone homeostasis is considerable in terms of both thyroid cancer and neonatal and postnatal development in animal species susceptible to the induction of these enzymes (McClain et al 1988; 1989). Since the capacity for the hepatic induction of these enzymes in humans is low (Richardson et al 2014) while rodents are almost exquisitely responsive to chemically-induced activation of these nuclear hormone receptors, disruption of thyroid hormone homeostasis via induction of hepatic UDP-GT enzymes, as a MoA, is not thought to be relevant to human situation (McClean 1995).

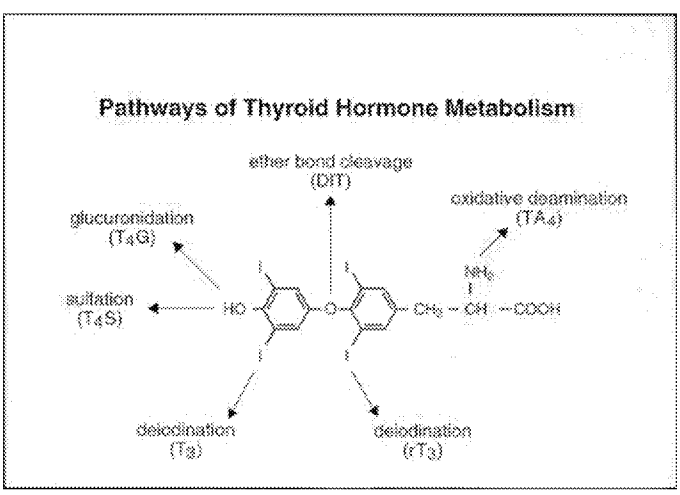
As with the UGTs, there are a number of SULT enzymes present in the liver that are involved with conjugation of thyroid hormones (Kester et al 1999). Whereas glucuronidation appears to facilitate the faecal excretion of thyroid hormones, sulfation initiates its degradation, allowing reutilization of the iodide for *de novo* thyroid hormone synthesis. In healthy humans, the sulphation pathway does not appear to contribute significantly to thyroid hormone metabolism, although its role increases in importance when Type I deiodinase activity is depressed (Visser 1994). Animal studies indicate that activation of the sulphation pathway inhibits T3 formation and increases the degradation of T4 and the inactive rT3 to additional inactive metabolites (Kelly, 2000). While minor in the adult human, T3-sulphate activity may be more important in the human foetus where, in the absence of foetal iodothyronine deiodinase to generate T3 from T4, sulphated T3 (T3S) can serve as a vital source of foetal T3 (Brucker-Davis, 1998).

Although type 2 deiodinase is normally expressed at high levels in human thyroid, and both mRNA and activity of type 2 deiodinase are induced by TSH, and agonistic TSH receptor antibodies circulating in patients with Graves' disease (Imai et al 2001; Murakami et al 2001), this enzyme is not expressed in normal rat or mouse thyroid (Wagner et al 2003). The consequences of a lack of expression of type 2 deiodinase in the rat are that intracellular conversion of T4 to T3 will not occur within the follicular cells, possibly as a result of the fact that rat follicular cells are already normally in a higher state of enzyme production than those present in humans and lack the need to increase their production rate of T3. Type 2 deiodinase can however be induced in conditions of hypothyroidism in both human and the rats in an attempt to increase the conversion of T4 to T3 (Wagner et al 2003).

6.5. Hepatic metabolism of thyroid hormones (Fig. 5).

Studies using ^{131}I labelled T4 have shown that in humans, the liver extracts between 5–10% of plasma T4 passing through the organ at any one time, a value that is considerably higher than could be accounted for by the amount of free T4 in the plasma delivered to the organ. This discrepancy shows that a substantial amount of protein bound T4, in addition to free T4, is absorbed directly into the cells (Mendel et al 1988). MCT8, a major thyroid hormone transporter has been localised to the membranes of hepatocytes and the thyroid hormone uptake occurs in a saturatable, energy and sodium-dependent, manner that enables the protein bound T4 and T3 to cross the hepatocyte membrane and to concentrate free intracellular hormones to a much higher level than those present in the plasma (Nishimura and Naito 2008). Although MCT8 is an important thyroid hormone transporter, several other proteins, such as the organic anion transporter protein 1C1 (OATP1C1), have been shown to transport thyroid hormones into cells throughout the body although they do tend to show a tissue specific pattern of expression (Abe et al 1998; Visser et al 2011).

Fig. 5: Thyroid hormone metabolism (from Visser et al 2016)



Thyroid function in a healthy individual is critically dependent upon a normally functioning liver to thyroid axis (Malik and Hodgson 2002) and while the major route for metabolism of T3 is through deiodination, significant removal of T3, rT3 and T4 occurs in the liver through conjugation reactions with sulphate and glucuronic acid (Fig. 2) catalysed respectively by iodothyronine sulfotransferase and iodothyronine glucuronyltransferase enzymes with the conjugates of the latter in particular, being excreted into the intestine through the bile (Visser 1996; Wu et al 2005).

There are some differences in the metabolism of T4 by the liver between rats and humans with approximately 50% of the T4 being eliminated via bile in rats, but only 10-15% in humans (Hill et al. 1989). However this difference does not appear to reflect a qualitative difference in metabolism, because the major metabolite in bile (glucuronide conjugate) remains the same in both species (Hard 1998). It does however suggest a kinetic difference in metabolism between the two species with the rat turning over T4 at an appreciably faster rate than in humans. This is consistent with the proposed biological differences in the transport of free and bound thyroid hormones between the two species. Iodothyronine glucuronides are rapidly excreted in large quantities in the bile with approximately 20% of human daily T4 production appearing in the faeces. This is not an end stage process as the conjugates are readily hydrolysed within the intestine by bacterial ß-glucuronidases, and some of the liberated hormone can be reabsorbed through enterohepatic cycling (Visser et al 1988).

Sulphated iodothyronine (T3S) levels are normally present at very low concentrations in plasma, bile and urine, because these conjugates are rapidly degraded by type 1 deiodinase. This has been taken to indicate that sulphate conjugation is a primary step leading to the irreversible inactivation of thyroid hormone (Visser 1994; Peeters et al 2005). Plasma concentrations, and biliary excretion, of T3S is significantly increased following inhibition of type 2 deiodinase activity with PTU or the iodine-containing radiocontrast medium, iopanoic acid, both during foetal development, and following fasting (Wu et al 2005; Visser 1994). Under these conditions, T3S may be acting as a reservoir of inactive hormone from which active T3 may be regenerated.

6.6. The importance of thyroid hormone sulphation

Serum concentrations of T3S are normally very low in healthy human subjects but are very high in the blood of the foetus and umbilical cord, and high in patients treated with the type 1 deiodinase inhibitor, triac, an acetic acid derivative of T4 (Eelkman et al 1989; Wu et al 2005). Similar high T3S/T3 ratios are also seen in some human cases of hypothyroidism, with the high sulphated T3 levels being due to a low peripheral type 1 deiodinase activity (Visser 1994; Peeters et al 2005).

An increase in T3S levels is also seen in rats when hepatic and renal type 1 deiodinase activities are decreased following exposure to enzyme inhibitors of the type 1 deiodinase enzymes, or in the presence of selenium deficiency, where marked increases in both serum and bile concentrations of iodothyronine sulphates occur (Visser 1994). These changes result from decreased clearance of the sulphated iodothyronines due to the absence of the type 1 deiodinases, but under conditions of hypothyroidism the inactivation of thyroid hormone by sulphation has been found to be reversible due to the presence of sulphatases in different tissues and in intestinal bacteria (Kester et al 2002). It is supposed therefore that the presence of relatively high concentrations of T3S in the foetus has an important function as a reservoir from which active T3 may be released in a tissue-specific, and time-dependent, manner (Santini et al 1992; Wu et al 1992; Darras et al 1999).

6.7. Species comparison of thyroid hormone and TSH half-life

The serum half-life of T4 and T3 in normal human adults is 5–9 days and 1 day, respectively (Choksi et al 2003) while in rats, the comparative values are 0.5–1 and 0.25 days, for T4 and T3 respectively. The plasma half-life of T4 and T3 in the dog has been estimated to be 8 –16 hours and 5-6 hours respectively (Kaptein et al 1993; 1994). The basis for the difference in half-lives is not completely understood, but it is proposed that the lack of the high-affinity T4 binding protein, TBG, in the adult rat plays a critical role since this leads to a higher serum level of free T4 in the rat and a greater tendency

for the bound hormones to dissociate as a consequence of the lower affinity binding that albumin and TTR has for the thyroid hormones. These increased concentrations of free hormones are thought to make the hormones far more susceptible to metabolism and excretion (U.S. EPA, 1998; Capen 1999), and to explain the very short half-lives that the thyroid hormones have in the rat and mouse in comparison to humans. The binding affinity of TBG for T4 in humans is approximately 1000 times greater than it is of TTR for T4, and it is well known that the % of free T4 is appreciable lower in species with high levels of TBG than it is in species without TBG (Capen 1999). Experiments have shown that thyroidectomised rats required 10 times more T4, equating to 20 μg/kg body weight (Frumess and Larsen 1975; Dohler et al 1979; Schlenker et al 2008), for full substitution as compared to an adult human requiring 2.2 μg/kg body weight (McAninch and Bianco 2016).

Comparative studies on the effects of perchlorate in the rat and human thyroid gland have also shown significant biochemical and physiologic differences in the relative responses. In an experiment by Yu et al. (2002) with perchlorate, inhibition of radiolabelled iodide uptake was 15%, 55%, and 65% at 1.0, 3.0, and 10 mg/kg perchlorate respectively at 1 day following perchlorate administration to the rat. However, by day 5, inhibition of iodide uptake had decreased to 0, 10%, and 30% at each respective dose level and after 14 days, inhibition of iodide uptake was only observed only at the top dose levels of 10 mg/kg. The data showed that the initial inhibition of iodide uptake by perchlorate in rats was similar to that observed in humans but that rats were able to compensate for the inhibition within 5 days of administration, most likely by increasing the expression of the sodium-iodide symporter on the follicular cells of the thyroid. A similar response was not observed in a 14-day human study with perchlorate administration (Greer et al. 2002). It was thought that compensation occurred in rats because of their smaller reserve capacity of thyroid hormones than humans and their more rapid turnover of circulating hormones.

The mitogenic hormone responsible for the normal production of thyroid hormones, and for the chemically induced thyroid hypertrophy and hyperplasia is TSH. It is this hormone that, on chronic stimulation in rodents, can lead to follicular neoplasia of the thyroid due to chemicals that interfere with thyroid hormone homeostasis (Hard 1998). The plasma levels of TSH in the male rat are approximately 6 ng/ml (Helmreich and Tylee 2011) which are about 3-fold higher than they are in the female rat (Kieffer et al 1976). This compares with values of 0.05 – 0.5 ng/ml in adult humans (Lewandowski et al 2003). In addition, production of both T4 and T3, in the rat, is appreciably higher than it is in man, due to their shorter half-life, and since production is driven by TSH levels, the higher plasma levels of TSH in rodents are thought to be responsible for the differences in follicle morphology seen between rodent and primates, including humans, with rodents having a follicular appearance consistent with a higher rate of thyroid hormone production and turnover.

6.8. Comparison of thyroid modifying chemicals in human and animal species

There is no doubt that adequate thyroid hormones are essential for the development of the foetus in both humans and laboratory animal species, as well as other vertebrate species (Williams 2008). It is also evident that thyroid hormone deficiency will result in neurodevelopmental deficits in humans and animals, the severity of which appears to be related directly to the severity of the hypothyroidism and the degree of thyroid hormone depression (Glinoer 2000; Haddow et al 1999; Klein et al 2001).

6.8.1. Thyroid modifying chemicals in humans

There are a number of environmental chemicals that have been shown to interfere with thyroid hormone homeostasis in humans that include polychlorinated biphenols (PCBs), bisphenol A, perchlorate, tetrachlorodibenzo-p-dioxin (TCDD), polychlorinated dibenzofuran (PCDF), pentachlorophenol (a breakdown product of hexachlorobenzene), triclosan, polybrominated and tetrabrominated diphenyl ethers (PBDEs) and other, naturally-occurring, chemicals such as soy isoflavones and thiocyanates in cruciferous vegetables (Miller et al 2009). Because of its ubiquitous distribution in the environment, there has been intensive study of the potential for perchlorate to cause human thyroid effects and, more importantly, neurodevelopmental deficits in the human population

and while a clear association with decreased T4 and increased TSH has described in women, no such association was found in men (Blount et al 2006).

There are a variety of drugs that have also been shown to inhibit peripheral production of T3 in humans including propylthiouracil (PTU), dexamethasone, propranolol, lithium, iodinated compounds such as the radiographic agents, iopanoic acid and ipodate, and the anti-arrhythmic drug amiodarone. PTU is a specific non-competitive inhibitor of type 1 deiodinase, while iopanoic acid and ipodate are competitive inhibitors not only of type 1 deiodinase but also of the type 2 enzyme. In addition, the radiographic agents have been shown to inhibit hepatic uptake of thyroid hormone (Dentice et al 2013). Amiodarone, and its metabolite desethylamidarone, may also interfere with peripheral thyroid hormone levels by a combination of inhibition of deiodinase activities and of tissue thyroid hormone uptake (Narayana et al 2011; Rosene et al 2010). The therapeutic use of lithium in the treatment of manic-depressive psychosis in humans has been known to be associated with the development of goitre for many years, and decreases in the rate of degradation of T4, and a decrease in serum T3 is seen in patients receiving high doses of lithium carbonate through a variety of mechanisms including enhancement of iodide induced inhibition of the NIS, inhibition of the deiodinases, and an exaggerated response of pituitary TSH release to TRH (Shopsin et al 1973; Andersen 1973; Carlson et al 1973). Little is known about the mechanisms by which propranolol and dexamethasone inhibit peripheral T3 production. In recognition of the thyroid inhibiting properties of these drugs, combinations of PTU, ipodate, dexamethasone and/or propranolol have been used to acutely decrease plasma T3 levels in patients with severe hyperthyroidism, known as a "thyrotoxic storm" (Carroll and Matfin 2010).

While a link has been established between the urinary concentrations of PCBs and human neurodevelopmental deficiencies, a study by Longnecker et al (2003) failed to establish a link between serum thyroid hormone deficits and PCB exposure even though this study was, wrongly, cited by Crofton (2008) as an example of a chemical-induced thyroid hormone effects operating in humans. Nevertheless, PCBs have been shown to alter thyroid hormone homeostasis in rats and to be associated with neurodevelopmental deficits (Gauger et al 2004).

6.8.2. Thyroid modifying chemicals in rodents

In comparison to the situation in humans, there are a far greater proportion of tested chemicals that have been shown to alter thyroid homeostasis in animals, mostly rodents, that include a number of phthalate esters, pregnenolone-16α-carbonitrile, benzodiazepines, calcium channel blockers, steroids, chlorinated hydrocarbons, such as chlordane and DDT, and polyhalogenated hydrocarbons such as PCBs and PBBs (Curran and DeGroot 1991; Capen 1999). Despite considerable variation in chemical structure and classes, for those chemicals that cause thyroid hormone effects in rodents, there are a limited number of ways (Fig. 5) in which they can affect thyroid hormone homeostasis either by direct effects on the thyroid gland itself or by effects on extra-thyroidal tissues, such as the liver, brain and kidney, responsible for the deiodination of T4 to T3 (Capen 1999).

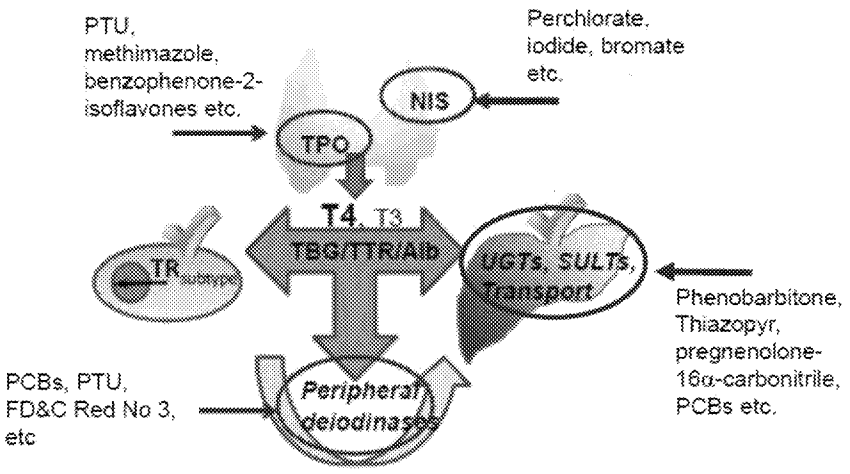
6.8.2.1. Competitive inhibitors of the sodium iodide symporter (NIS)

Chemicals such as pertechnetate, thiocyanate and perchlorate ions can directly affect the thyroid gland by acting as competitive inhibiters of the follicular cell NIS (Fig 5). This leads to a decrease in the availability of inorganic iodide in the thyroid gland and a consequential decrease in the synthesis and release of thyroid hormones (Merrell et al 2003; Capen 1999). When circulating levels of T4 and T3 decrease, compensatory release of TSH from the pituitary gland leads to hypertrophy, hyperplasia, thyroid gland growth and increases the chances of developing cancer (Fisher et al 2012).

6.8.2.2. Thyroperoxidase inhibitors

Certain chemical classes including thionamides such as 6-propylthiouracil and ethylene thiourea, some of the sulphonamide drug classes, and miscellaneous compounds such as amitrole, reversibly or irreversibly inhibit the thyroperoxidase catalysed incorporation of active iodide into thyroglobulin (Sarne 2016). Inhibition of thyroperoxidase reduces the production and release of T4 and T3 into the circulation, provoking a compensatory release of TSH from the pituitary gland and causing hypertrophy and hyperplasia in the thyroid follicular epithelium (Hard 1998).

Fig. 6: Known sites of action of chemicals in disrupting thyroid hormone homeostasis in rodents (from Paul 2014)



Notes: Abbreviations are TPO = thyroperoxidase; NIS = sodium/iodine symporter; TBG = thyroxine binding globulin; TTR = transthyretin; Alb = albumin; UGTs = UDP glucuronyltransferase; SULT = sulphotransferases; TR = thyroid hormone nuclear receptor; PCBs = polychlorinated biphenyls; PTU = propylthiourea;

6.8.2.3. Direct toxicity to follicular epithelium

Direct chemical toxicity to the thyroid follicular cells has been seen with PCBs (Collins et al 1977), which reduce the output of thyroid hormones, while lithium (Kibirge et al 2013; Bocchetta and Loviselli 2006) and excess iodide (Leung and Braverman 2014) inhibit the secretion of thyroid hormones from the follicular cells, induce retention of the hormones within the follicular lumen, seen as increased colloid, and produce a decrease in circulatory thyroid hormones (Green, 1978; Capen and Martin 1989). Both of these effects have been seen in humans. Amiodarone is an iodine-rich drug used to manage ventricular and supraventricular tachyarrhythmias, and is also associated with thyroid dysfunction, most probably through its effect as an iodide source (Minelli et al 1992).

6.8.2.4. Inhibition/induction of iodothyronine 5'-deiodinases

There are a number of chemicals that alter thyroid hormone homeostasis either by inhibiting the 5' deiodinase catalysed conversion of T4 to T3 in peripheral tissues (Capen 1999) or, in rarer cases, by enhancing the conversion of T4 to T3 through induction of the deiodinases (Morse et al 1993).

The dye erythrosine, also known as FD & C Red No 3, was shown to increase thyroid follicular adenomas in rats in lifetime bioassays (Borzelleca et al 1987) and the compound induces thyroid gland hypertrophy and hyperplasia in shorter duration studies in the rat. The thyroid morphological

changes are accompanied by decreases in serum T3 and increases in rT3, T4 and TSH, due to inhibition of 5'-deiodinase (Capen 1997; 1999; Capen and Martin 1989).

PTU has also been shown to inhibit 5'deiodination (Cavalieri and Pitt-Rivers 1981) in addition to inhibiting thyroperoxidase (TPO), and its potency in inducing thyroid hypertrophy and hyperplasia is most probably a product of a combination of effects rather than down to any single MoA.

A common component of sun-cream, octyl-methoxycinnamate, when given over a five day period, has also induced a dose-dependent decrease in serum T3, T4, TSH, and in hepatic type 1 5'-deiodinase (Klammer et al 2007). In this study TSH receptor expression in the thyroid was also increased while hypothalamic TRH expression, and the activities of TPO and NIS in the thyroid were unaffected. Oral administration of the PCB, 3,3',4,4',5,5'-hexachlorobiphenyl (HCB), or a combination of HCB with 3,3',4,4'-tetrachlorobiphenyl (TCB), to pregnant rats from day 1 to day 18 post-gestation induced significant decreases in plasma thyroid hormones in the dams, and significant increases in brain type 2 5' deiodinase activity in foetuses (Day 20 of gestation) and neonates (Days 7 and 21 postpartum), and an associated reduction in circulating total, and free, T4 levels. To complicate matters further, treatment also induced hepatic T4 glucuronidation in both 20 day gestation foetuses, and in neonates (Morse et al 1993).

In the Morse et al (1993) study as described above, the induced decreases in plasma thyroid hormones were accompanied by a highly significant induction of type 2 thyroxine 5'-deiodinase activity in brain homogenates from 20 day post-gestation foetuses, and day 7 and 21 postpartum neonates (Morse et al 1993). The increase in deiodinase activity was interpreted as indicating that local hypothyroidism had occurred in the brains of the foetal and neonatal rats exposed to HCB. Since these effects occurred during a period in which thyroid hormones play an important role in brain maturation, the data may be relevant in explaining the mechanism of developmental neurotoxicity induced by PCBs. As this group of chemicals also induce hepatic glucuronidation, that increase clearance of thyroid hormones, this group of compounds, in common with many of the well worked chemical examples of thyroid hormone disrupters, exhibits more than simply one single molecular initiating event that could operate in concert (an additive effect) to magnify the resulting thyroid hormone perturbation seen with this chemical class (Crofton 2008).

6.8.2.5. Displacement of hormones binding to thyroid transport proteins/receptors

There is some evidence from *in vitro* competitive binding studies that certain chemicals are able to displace T4 from one of the serum thyroid binding proteins, TTR (Meerts et al 2000). Although opinion differs as to the relative importance of this binding protein in the rat the findings, if substantiated *in vivo,* would have the potential to stress the thyroid system, making it more likely that dysfunction could occur (Kohrle et al., 1989; Lueprasitsakul et al., 1990; Lans et al., 1993; Cheek et al., 1999; Chauhan et al., 2000; Ishihara et al., 2003). Because of the redundancy in thyroid hormone binding proteins in humans and rodents, it is questionable whether or not such displacement of T4 from TTR alone would lead to functional consequences. *In vivo* evidence with the flavonoid, EMD 49209, which appears to displace T4 from TTR *in vitro*, shows that it reduces tissue levels of T3 *in vivo,* not through any MoA involving displacement of T4 from TTR, but as a result of a shortage of T4 as a substrate for deiodinases (Schroder-van der Elst et al 1998). Until *in vivo* evidence is produced to support this hypothetical MoA it has to be concluded that, of the time of writing, this *in vitro* phenomenon has not been reciprocated in any known *in vivo* chemical-induced thyroid disrupter.

6.8.2.6. Induction of hepatic turnover/biliary excretion of thyroid hormones

At least in rodents, certain chemicals have been shown to induce the activity of the hepatic UGT and SULT enzymes responsible for the conjugation and removal, through biliary excretion, of thyroid hormones (Oppenheimer et al 1968; McClain et al 1989; Visser et al 1993; Hood and Klaassen 2000; Liu and Klaassen 1996). This results in decreased circulating free and bound thyroid hormones, a

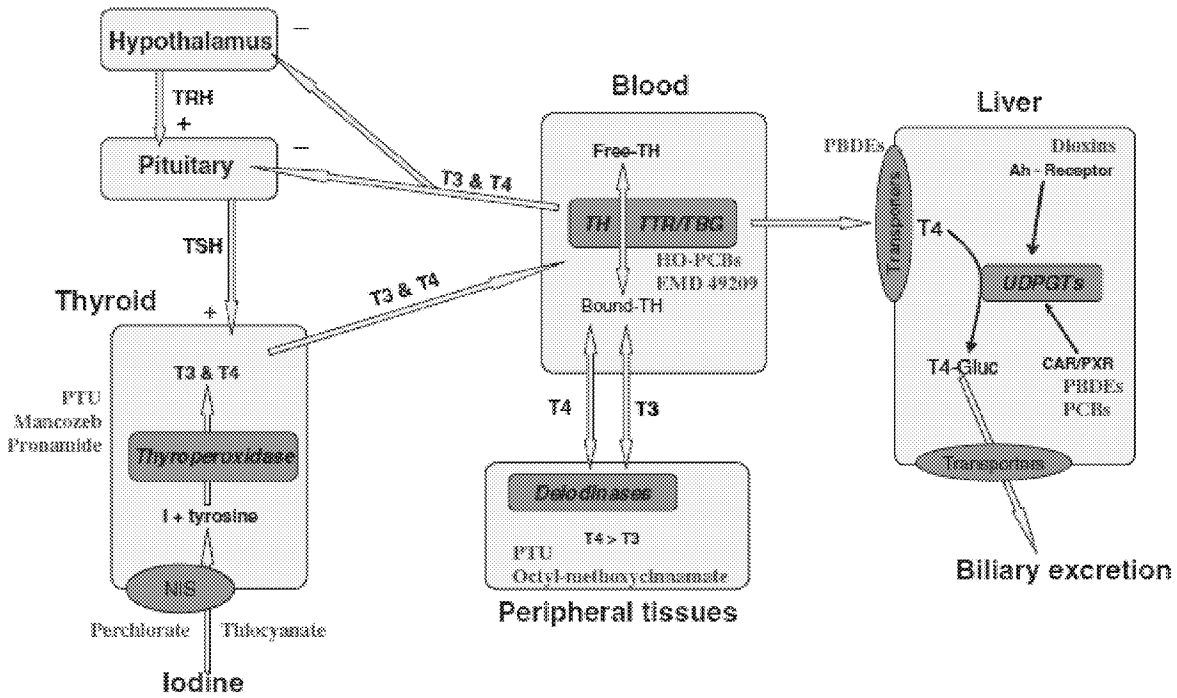
higher turnover and decreased half-life of circulating hormones, and a compensatory TRH and TSH increased release from the hypothalamus/pituitary acts on the follicular cells of the thyroid to induce hypertrophy and hyperplasia, and if sustained, thyroid follicular neoplasia (McClaln 1989; Hill et al 1989; Thomas and Williams 1991; Williams 1995). In addition to chemical induction of hepatic SULTs, a number of drugs and environmental chemicals, such as salicylic acid, clomiphene, naturally occurring chemicals such as flavonoids and phytoestrogens, and environmental chemicals such as the disinfectant, pentachlorophenol, and the antibacterial product, triclosan, have been shown to inhibit hepatic SULTs and to have the potential to increase circulating levels of thyroid hormones (Schuur et al 1998; Wang et al 2004; Wang & James 2006). There is evidence that thyroid hormone perturbations, specifically those mediated via hepatic enzyme induction and increased biliary excretion, are a rodent only phenomenon that is not able to work in humans for a number of reasons relating to the significantly greater sensitivity of hepatic enzyme induction in rodents versus humans, and the much shorter half-life, and hence greater turnover, of thyroid hormones in rodents in comparison to humans (McClean 1995; McClean et al 1989; Colnet and Dekant 2017).

The other modes of action of chemicals in affecting thyroid hormone homeostasis are not inherently species specific but pharmacokinetic and metabolism differences may render some species more sensitive to the thyroid effects than others (Zimmermann and Galetti 2015).

6.8.2.7. Miscellaneous modes of action in disrupting thyroid hormone homeostasis

There is considerable debate as to whether or not chemical disrupters are able to bind directly to thyroid receptors *in vivo* but while it is clear that certain chemicals, such as tetrabromo-bisphenol A, bisphenol A and hydroxyl PCBs, can alter thyroid hormone-responsive genes in various tissues including liver and brown fat (Moriyama et al 2002; Gauger et al 2004; Bansal et al 2005; Kitamura et al 2005), the evidence exists that the changes observed are not induced by binding to the nuclear thyroid receptors (Cheek et al 1999; Ishihara et al 2003; Gauger et al 2004). In an *in vitro* thyroid receptor binding assay, Ishihara et al (2003) showed that while several chemicals, including diethylstilboestrol, pentachlorophenol and ioxynil, were able to displace thyroid hormone from TTR, none were able to displace thyroid hormone from its receptor. Marsh et al (1998) found that two synthesised polybrominated diphenyl ethers were able to bind, *in vitro,* to both the alpha and beta forms of the thyroid hormone receptor but this has not been repeated by other groups investigating competitive displacement of thyroid hormones from the receptors. However, most *in vitro* screens for thyroid hormone receptor binding have relied upon the ligand-binding domain of the receptor, and while clearly this is the site for activation of the receptor, binding to other parts of the receptor could potentially result in allosteric hindrance that would alter the subsequent functioning of the receptor. Indeed this has been shown at least *in vitro* to be the case for PCBs (Miyazaki et al 2008).

Fig. 7: Known MoAs (in blue boxes) for disruption of thyroid hormone production and release (from Crofton 2008)



6.9. Comparison of thyroid hormone involvement in neurodevelopment in humans and rodents

6.9.1. Thyroid hormone and neurodevelopment in humans

It has been over 120 years since a committee of the UK Royal College of Physicians in London linked the pivotal role of the thyroid gland to normal human brain development (Ord 1898). Many countries have now instigated a neonatal hypothyroidism screening test for the early diagnosis of hypothyroidism, in recognition of the latter as the most common preventable cause of mental retardation in the young (Bhatara et al 2002). Thyroid hormones are known to be essential for the normal maturation and functioning of the central nervous system although they also modulate a broad range of effects on virtually all tissues (Karapanou and Papadimitriou 2011). It was originally thought that thyroid hormones were only important for human neurodevelopment post-natally. This was based on the observation that circulating foetal levels of these hormones were very low, together with the fact that the placenta was thought to present a very efficient barrier to the transfer of thyroid hormones from the mother. This prejudice was supported by the findings that post-natal thyroid support therapy could correct many of the thyroid dependent conditions that were present at birth following maternal hypothyroidism due to low iodine (Glendenning 2008). However, it is now clear that thyroid hormones are essential for both foetal and post-natal neurodevelopment, and for the regulation of neuropsychological function in children and adults (Williams 2008).

Thyroid hormones have no influence on the very early developmental events in the human foetus, such as neural induction and the establishment of cellular polarity, but they are essential to regulate later processes, including neurogenesis, myelination, dendritic proliferation and synapse formation

(Bernal et al 2003; Bernal 2007; Zoeller and Rovet 2004). There are large numbers of genes transcribed following activation of the thyroid hormone nuclear receptor and the timing of the onset of thyroid hormone action is crucial for the correct development of the brain (Zoeller and Rovet 2004; Hindmarsh 2002). A condition known as endemic neurological cretinism in humans is due to maternal iodine deficiency, and resulting maternal hypothyroxinaemia, with low maternal T4 levels causing neurological hypothyroidism in the foetus, which results in profound mental retardation, cerebral spastic diplegia, deaf-mutism and squint in the absence of general signs of hypothyroidism (Porterfield and Hendrich 1993). Although iodine dependent neurodegenerative conditions, in neonates, can be prevented by iodine supplementation, iodine deficiency remains the commonest endocrine disorder in the human population and has been estimated to be the most frequent cause of preventable mental retardation (de Escobar et al 2004).

6.9.2. Comparison of the stages of thyroid hormone dependency in the rat and human foetus

There are three stages of thyroid hormone dependent neurological development in the foetus, with the first occurring between 16-20 weeks post-conception in humans, equivalent to day E17.5-18 in the rat, and before the foetus is able to synthesise its own thyroid hormones (Fig. 8). During this first stage thyroid hormones can only be delivered to the foetus via the placenta from maternally synthesised hormone (De Escobar et al 2000; 2004; Obregon et al 2007) and its presence stimulates neuronal proliferation and migration in the cerebral cortex, hippocampus, and medial ganglionic eminence (Narayanan and Narayanan 1985; Lucio et al 1997; Cuevas et al 2005; Auso et al 2004). The second stage occupies the remainder of pregnancy after the initiation of foetal thyroid hormone synthesis when the developing brain receives a dual supply of thyroid hormones from both a foetal and maternal origin. During this stage processes dependent upon thyroid hormones include neurogenesis, neurone migration, axonal outgrowth, dendritic branching and synaptogenesis, glial cell differentiation and migration, and the onset of myelination (de Escobar et al 2000; Porterfield and Hendrich 1993).

The third stage occurs between the neonatal and post-natal period when thyroid hormone supplies to the brain are entirely derived from the child (Fig. 8). During this stage, thyroid hormone is needed for the continuing maturation of the central nervous system that involves migration of the granule cells in the dentate gyrus and cerebellum, migration of the pyramidal cells in the cerebral cortex and the Purkinje cells of the cerebellum, and the maturation of the glial system.

Fig 8: Contribution of human maternal and foetal thyroid hormones to development (from Williams 2008).

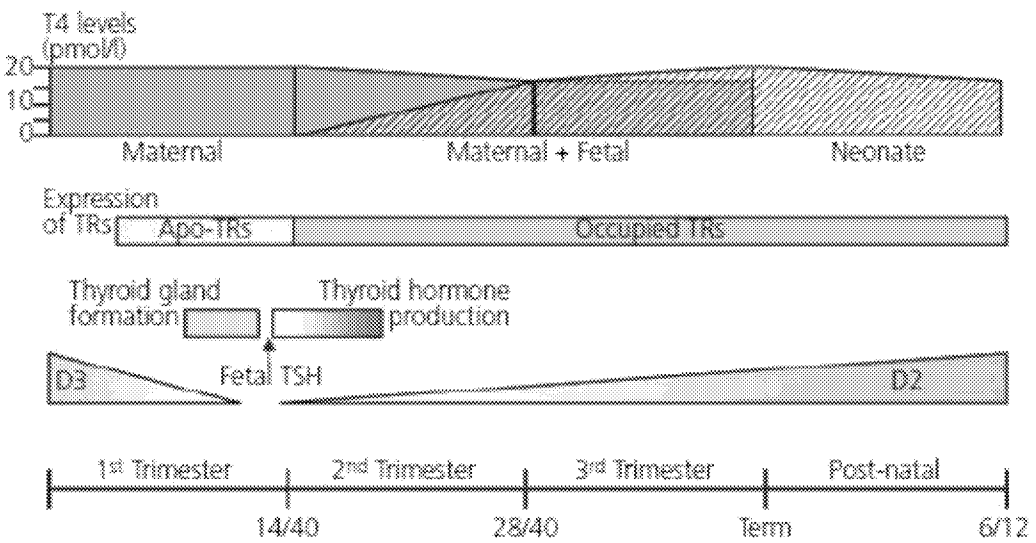


Fig. 8 above illustrates the respective contribution of foetal and maternal thyroid hormones at the respective times during gestation/pregnancy. The foetus is entirely dependent upon maternal thyroid hormone during the first trimester and entirely dependent upon its own hormone after birth. Foetal thyroid hormone production begins at the end of the first trimester peaking at term. The thyroid gland begins development midway through the first trimester and begins production of foetal hormone following a surge in foetal TSH at the end of the first trimester. D3 is an inactivating type 3 iodothyronine deiodinase which decreases during the first trimester allowing the initiation of production of the foetal hormone as concentrations of type 2 deiodinase (D2) increase after this period. TR represents thyroid hormone nuclear receptor which is able to bind thyroid hormone at the end of the first trimester. Type 3 iodothyronine deiodinase is thought to act to prevent thyroid hormone access to specific tissues at certain critical times and to prevent saturation of the thyroid hormone nuclear receptor (Bianco and Kim 2006; Bianco et al 2002). Type 3 deiodinase works by removing iodine from the inner ring of T4 or T3 and hence inactivates the hormone. It is expressed at its highest levels in the foetus whereas it is present only at low levels in adult tissue (Williams 2008).

The findings of neurodevelopmental problems following thyroid hormone deficiency in humans were subsequently confirmed and shown in animal studies (Koibuchi and Chin 2000; Thompson and Potter 2000; Morte et al 2002; Singh et al 2003). This data provided the critical evidence that thyroid hormones are essential in early (foetal) brain development, and that the associated neurological deficits depended upon the timing and severity of thyroid hormone insufficiency. Hence for chemicals that showed dose-response relationships in terms of their depression in circulating thyroid hormones, a threshold for the initiation of defects, based upon the threshold for thyroid hormone dependent effects, existed (Narayanan and Narayanan 1985). In the case of hypothyroidism occurring in adult rats, the neurological defects could be reversed by T4 supplementation (Ruiz-Marcos et al 1988) although this was not the case for perinatal and prenatal thyroid deficiency, where neurological deficits were generally permanent.

The order of development of the thyroid gland, during organogenesis, is similar for rodents, sheep, and humans, but the timing of various perinatal developmental events differs significantly between the species, with rats being born relatively immature, and hence late developmental events, that occur *in utero* in humans, only occur postnatally in rats. Because of their advanced state of development at birth, thyroid development in sheep, by comparison, occurs almost exclusively in utero (Hombach-Klonisch et al 2013).

The timing of the onset of thyroid receptor (TR) binding also differs significantly between species independent of their differing gestational times and can be first observed in the rat during mid-to late-gestation (average gestation is 21 days), in sheep during the latter two-thirds of gestation (average gestation is 20.5 weeks), and in humans between gestational weeks 10 and 16 (average gestation is 39 weeks) (Fisher and Brown, 2000).

6.10. Comparison of thyroid cancer in human and animal species

Thyroid cancer is the most common endocrine malignancy in the human population and incidence rates in most countries have been steadily increasing over the past few decades, particularly in women (Zimmermann and Galetti 2015). Known risk factors for human thyroid cancer are radiation exposure during childhood, exposure from nuclear accidents (Mazonakis et al 2007; Bounacer et al 1997), natural radiation, or medical imaging (Bard et al 1997), but suspected risk factors include obesity and the metabolic syndrome (Renehan, et al 2008; Rinaldi et al 2012), environmental pollutants (Hallgren and Darnerud 2002; Zhang et al 2008), a family history of thyroid cancer, or thyroid disorders (Franceschi et al 1999) and, for certain susceptible individuals, iodine intake (Zimmermann and Galetti 2015). While iodine deficiency together with a small number of drugs are known to induce thyroid growth (goitrogen) in humans, the only confirmed human thyroid carcinogen is ionizing radiation, and the available evidence would suggest that chemicals that induce thyroid

growth alone, in the absence of additional mutational events, will not lead to human thyroid cancer (McTiernan et al 1984; Holm et al 1988; Daminet and Ferguson 2003).

Chemically-induced rodent thyroid follicular cell cancer arises from one of two different processes that ultimately converge under the influence of continued TSH stimulation. The first process is found with chemicals that induce DNA damage/mutations specific to the thyroid, while the second is seen with chemicals that induce perturbations of hypothalamus/pituitary/thyroid axis with subsequent increased stimulation of thyroid cell growth by TSH. An example of a chemical that induces thyroid follicular cancer in rodents exclusively through a mutagenic MoA is N-Bis-(2-hydroxypropyl) nitrosamine as it is entirely devoid of effects on thyroid hormone production or activity (Hiasa et al 1991).

6.10.1 The association between iodide deficiency, goitre and thyroid cancer in the human population

The epidemiology of goitre and thyroid cancer is confusing, and confused, and the appropriate interpretation requires an in depth understanding of the molecular biology, underlying causes of thyroid cancer, and the clinical development and treatment of both goitre and cancer. One of the most scientifically enlightening causes of human thyroid disease is that resulting from iodine deficiency since it has been known for centuries that in such conditions, there will be a high incidence of thyroid hypertrophy in the local population. Humans living in areas of chronic iodide deficiency develop thyroid hypertrophy/hyperplasia manifest in the appearance of goitre (Zimmerman 2009; Kotwal et al 2007; Li and Eastman 2012), due to chronic deficiency in the production of thyroid hormones, and the continued stimulation of the thyroid gland by TSH released from the pituitary gland. Supplementation of dietary iodine is normally able to reverse the clinical symptoms of both goitre and the other physiological and neurological effects of hypothyroidism (Kotwal et al 2007; van der Haar 2007; Liesenkötter et al 1995).

6.10.1.1. The role of TSH in human thyroid cancer

The data for the role of TSH in human thyroid cancer contrasts dramatically with the situation in rats. There is a large volume of epidemiological data regarding thyroid cancer rates in areas of endemic goitre, where iodine deficiency would be expected to lead to chronic TSH stimulation of the thyroid gland. In the United States an extensive study compared regional rates of thyroid cancer mortality and endemic goitre over a 20-yr period both before, and after, the generalized introduction of iodinated salt (Pendergrast et al 1961). While the incidence of goitre significantly decreased in the areas previously affected by endemic goitre, there was no similar change in thyroid cancer mortality rates and hence no association between the two diseases. Similar results were obtained in Austria (Riccabona 1986) where large areas of the Tyrol have historically shown an abnormally high incidence of goitre due to iodine deficiency. While there are regions of the world with endemic goitre and a high incidence of thyroid cancer, there are also similar regions with a high incidence of goitre in the absence of similar high thyroid cancer rates, as well as areas without endemic goitre where the incidence of thyroid malignancies is higher than normal (Clements 1954, Riccabona 1987).

In contrast to the previous examples however, patients with goitres that arise, not from iodide deficiency but from congenital thyroid gland metabolic defects (i.e. mutations of various kinds), have been noted to have a markedly increased incidence of thyroid malignancies (Kitahara and Sosa 2016; Cooper et al 1981; McGirr et al 1959; Elman 1958). There is also an increased incidence of thyroid carcinoma in patients with thyrotoxicosis (Siegel and Lee, 1998; Verburg and Reiners 2010; Pendergrast et al 1961; Clements 1954). There are, however, no reports of an increased incidence of thyroid cancer in patients with thyroid hormone resistance where chronically high levels of circulating TSH exist, due to a non-functional negative feedback control in the hypothalamus/pituitary gland, and an inability to react to normal levels of circulating T3 (Yen 2003).

In those situations where there is an increased risk of thyroid malignancy in patients with glands showing metabolic defects, mutations, or in subjects with thyrotoxicosis, the thyroid cancers do not arise simply due to the hyperplastic stimulus of TSH alone but are complex combinations of genetic and hormonal factors. The finding that simple goitre can be fully reversed by supplementing the diet

with iodine shows that while the goitre is undoubtedly produced under a chronic TSH stimulus, the thyroid gland nevertheless retains its ability to return to normal despite many years of TSH stimulation. This is very different to the situation in rodents where the evidence points to the ability of sustained TSH stimulation alone, as can happen with chemically induced thyroid insufficiency, being able to induce neoplasia (Capen 1999). Indeed the implantation of TSH secreting pituitary tumours alone into recipient rats has been shown to be able to induce thyroid follicular neoplasms in the absence of any further treatment (Hill et al 1989).

6.10.1.2. The role of TSH in rodent cancer

Rats have higher basal levels of circulating TSH than are present in humans (Table 2; Lewandowski et al 2003) and in contrast to the situations in the human population, where plasma levels of TSH are roughly equal between men and women, male rats have higher normal levels of TSH than are present in female rats (Hill et al 1998). This finding correlates well with the observation that the height of the follicular epithelium is greater in males than in female rats, reflecting the greater TSH-induced activity in males versus females (Capen 1996). Male rats also show a higher spontaneous incidence of thyroid follicular cancer than do female rats (IARC 1999; Haseman et al 1985). In a survey of the outcome of mouse and rat carcinogenicity studies (Hurley et al 1998), twenty-four of the approximately 240 pesticides tested were found to induce thyroid follicular cell cancer, and of these twenty-two induced thyroid tumours only in rats, with the remaining two inducing thyroid cancer in both rats and mice. Additionally, chemically-induced thyroid neoplasms occurred far more frequently in male rats than in female rats, both with respect to the proportion of chemicals that induced thyroid tumours, and to final tumour incidence. In contrast to the situation in the rat, thyroid cancer incidences are greater in human females than in males, while TSH levels between men and women are the same suggesting some fundamental differences both in basal thyroid control and physiology but also in their responses to situations that cause thyroid cancer (Hill et al 1998; Parker et al 1997).

6.10.2 Comparative cancer studies in laboratory animals and humans for goitrogenic chemicals

The goitrogenic effects of sulphonamide drugs have been known for many years since the first demonstration of rat thyroid hypertrophy by the prototype sulphonamide, sulphaguanidine (MacKenzie and MacKenzie 1943; Mackenzie et al 1941; Astwood et al 1943). The sulphonamide drugs, as a class, induce thyroid changes in the rat and dog through inhibition of thyroperoxidase-catalysed binding of iodine to thyroglobulin and the resulting decrease in circulating thyroid hormones (Nishikawa 1983).The next generation of sulphonamides also turned out to be potent goitrogens in the rat, when a combination of sulfamethoxazole and trimethoprim was shown to cause dramatic decreases in T3 and T4, with compensatory increases in both circulating TSH and in the weight of the thyroid gland. While similar effects were also seen in the dog with this drug (Nishikawa 1983), monkeys and humans did not show the thyroid inhibitory effects, and when rhesus monkeys were given doses of sulfamethoxazole up to 300 mg/kg/day for 52 weeks there were no changes in either thyroid gland weight or histology (Swarm et al 1973). In contrast long-term administration of sulphonamides, at dose levels that result in prolonged stimulation of the thyroid gland by TSH, induces thyroid neoplasia in rats (Doerge and Decker 1994).

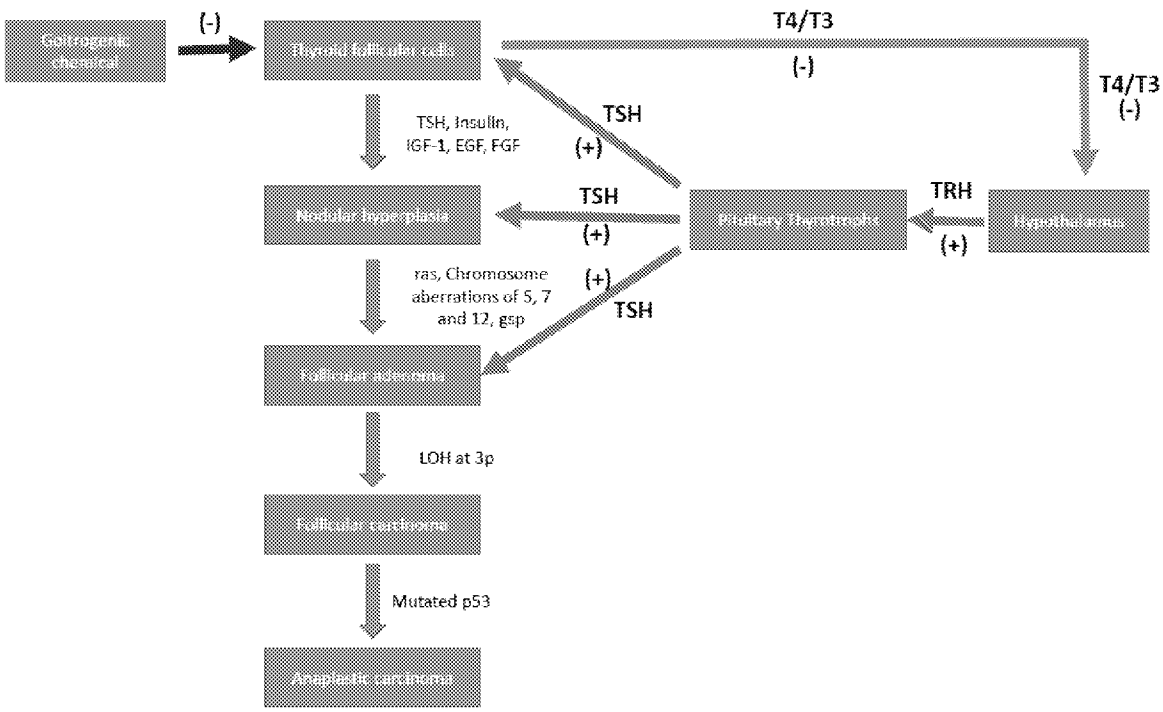
A similar species difference in sensitivity to the inhibitory effects of these drugs was found following *ex vivo* experiments in a thyroid peroxidase assay using PTU (another thyroperoxidase inhibitor) and the sulphonamide, sulphamonomethoxine. In this study, the concentration of PTU required to inhibit thyroperoxidase sourced from monkey thyroid was approximately 50x higher than that required for the same degree of inhibition in rat thyroperoxidase, while for sulphamonomethoxine the concentration required to inhibit 50% of the thyroperoxidase activity was 500 fold greater in the monkey enzyme than in rat thyroperoxidase (Takayama et al 1986). This data was strongly supportive of an intrinsic sensitivity difference in the enzyme between that derived from rat thyroid and that from monkey thyroid. These studies further demonstrated that, at least with thyroperoxidase inhibitors, significant differences in potency existed between sensitive species, such as rat, mouse and dog, and more resistant species that included non-human primates, human, guinea pig and chicken (Capen 1999). In

support of this data, only mild effects of sulfonamides have ever been observed on human thyroid function (Cohen et al 1980).

6.10.3. Thyroid hormone perturbation and thyroid cancer in rodents

In mammals in general, including humans, when demands for more thyroid hormone are small, existing thyroid follicular cells can meet any extra demand by increasing their synthesis and output, often becoming larger in size (hypertrophic). At least in rodents, inhibition of thyroid hormone synthesis and/or secretion, such as that occurring following the administration of certain chemicals, is responded to by increasing the number (hyperplasia) and size (hypertrophy) of thyroid follicular cells to enhance thyroid hormone output. This response is mediated through feedback release of TSH from the pituitary gland, and in the presence of continued thyroid hormone deficiency, and chronic TSH stimulation, there is an increase in the thyroid weight as a result of a combination of thyroid follicular hypertrophy and hyperplasia. Since the TSH-producing thyrotrophs in the pituitary gland are also producing, and secreting, at a greater rate than normal, they will also undergo hypertrophy and hyperplasia and where looked for, these are invariably seen in the adenohypophysis of the pituitary gland under conditions of moderate to severe thyroid hypertrophy (Norford et al 1993; Russfield 1967; Moriarty and Tobin 1976; Akosa et al 1982).

Fig. 9: The postulated progression of changes in rat thyroid follicular epithelium in the development of cancer from chronic exposure to a goitrogenic chemical



Notes: The red arrow indicates a thyroid disrupting chemical, the green arrows indicate a positive effect on the target cells while the blue arrow indicates the negative feedback response of the hypothalamus to low circulating T3/T4 levels.

In rodents, there is adequate evidence, with a comparatively large number of chemicals of various classes and diverse pharmacological, that continued thyroid stimulation by TSH alone eventually leads to neoplasia of the thyroid follicular cells (Hill et al 1998; Hurley et al 1998; Capen 1999). The proposed MoA for the production of rat thyroid cancers by goitrogenic chemicals is illustrated in Fig. 9. Although TSH is the main hormonal driver for follicular cell hypertrophy and hyperplasia, several other factors, including insulin, epidermal growth factor (EGF), fibroblast growth factor (FGR) and insulin-like growth factor (IGF), have been shown *in vitro* to also be mitogenic for the follicular thyroid epithelial cells (Maviel et al 1988; Logan et al 1992; Żerek-Meleń et al 1987).

The data therefore support a conclusion that intrinsic sensitivity differences occur between species, in terms of their sensitivity to thyroid hormone inhibition, and also strongly suggests that qualitative differences may also determine a cancer outcome with prolonged TSH stimulation being the main driver for rodent thyroid non-genotoxic cancer, whereas TSH is only able to promote cancer in humans in the presence of underlying thyroid problems such as inherited mutation associated metabolic disorders or thyrotoxicosis (Curran and DeGroot 1991).

6.10.4. Is chemically-induced rodent thyroid cancer relevant to man?

For regulatory purposes, and in the absence of MoA data to the contrary, chemical induced rodent thyroid tumours is presumed to be relevant to humans, and when information on differences in inter-species MoA is lacking, the default is to assume comparable carcinogenic sensitivity in rodents and humans (Hill et al 1998; Hard 1998). Where there are follicular neoplasms in the absence of evidence of follicular hyperplasia/ hypertrophy, or evidence of disruption of the thyroid-pituitary axis, such neoplasms are presumed to be relevant to humans and in terms of their dose-response relationships, the Environmental Protection Agency applies linearity when estimating the risk to thyroid cancer induced by chemical substances that either do not disrupt thyroid functioning, are mutagenic, or that lack MoA information. In terms of a neoplastic MoA for rodent thyroid tumours both IARC (1999; 2000) and the US Environmental Protection Agency (US EPA 1998) currently have established specific guidance for evaluating the human relevance of these tumours.

In order to show a thyroid-pituitary MoA for any particular chemical, the US EPA (1998) asks for evidence in the following five areas:

1. Increases in cellular growth (e.g., increased thyroid weight, hypertrophy or hyperplasia, proliferation detected by DNA labelling or mitotic indices)
2. Changes in thyroid and pituitary hormones (T3/T3, TSH)
3. Location of site of action (e.g., thyroid, liver, or peripheral, and enzyme target within the target organ);
4. Dose correlations among thyroid effects (cell proliferation/hypertrophy) and cancer;
5. Reversibility of effects when chemical dosing stops.

For those chemicals that are non-mutagenic and non-genotoxic, that reduce thyroid hormones acutely/chronically, and that increase TSH levels, in the absence of a proven MoA having no relevance to humans, the outcome is still considered to represent a human risk.

The increasing adoption of the WHO/IPCS principles of establishing MoA and human relevance for these findings have, more recently introduced a more stringent list of requirements that consider all possible alternative MoAs and that apply modified Bradford Hill criteria for estimating the strength of association of between the proposed MoA and the adverse outcome (Dellarco et al 2006; Boobis et al 2006; Meek 2008; Meek et al 2014). This has been a welcome step in applying objective scientific data to support arguments of non-adversity of effects, and non-relevance to the human population of animal toxicity results and has applied an objective approach to regulatory decision making that takes

into account non-guideline investigative study data in addition to GLP conducted guideline study data to arrive at their final conclusions.

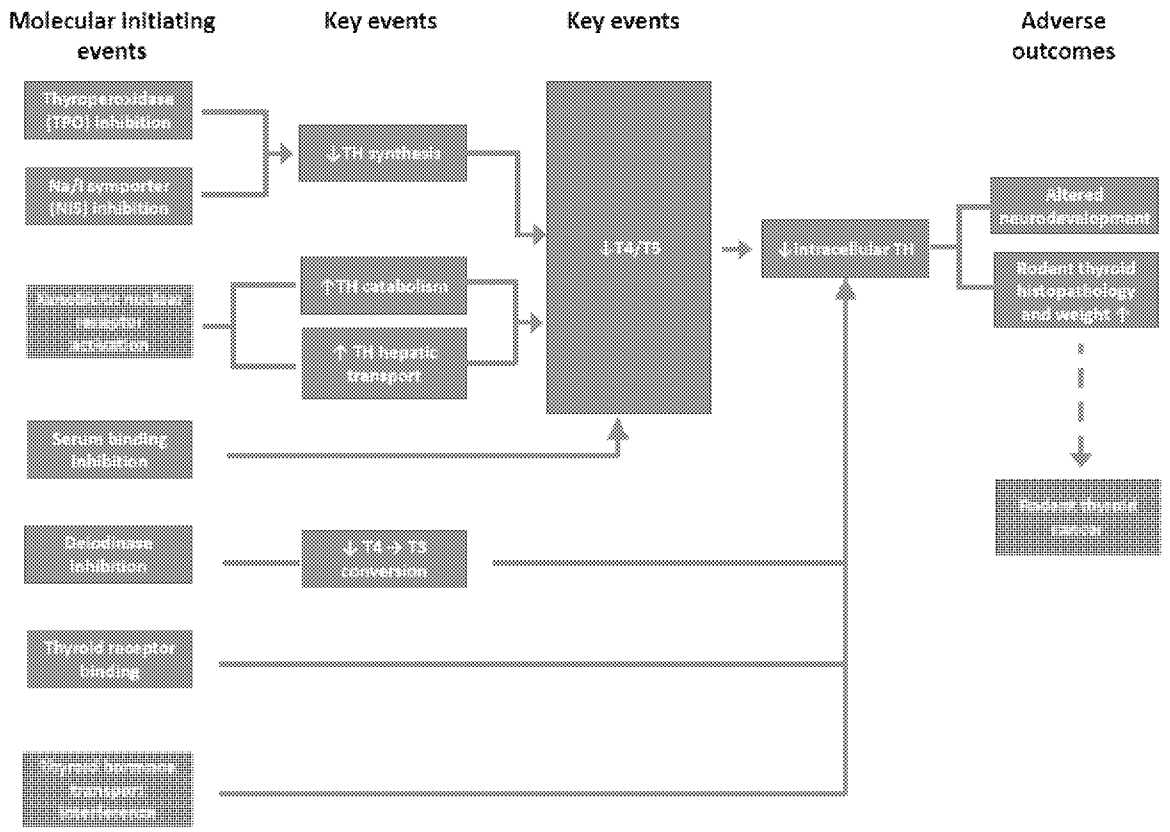
6.11. Cross-species extrapolation for thyroid effects

Inter-species extrapolation of adverse thyroid effects, normally detected in laboratory animal studies, to human exposures, is an exacting science requiring firstly a thorough demonstration of the MoA of the toxicity in the target laboratory animal species, most often the rat. Once accepted as a plausible explanation a reasoned argument would then be made, based upon sound principles, as to the human relevance of the laboratory animal MoA. Acceptable approaches to this problem have been widely publicised in the WHO/IPCS guidelines for establishing a weight of evidence conclusion to the exercise (Meek et al 2003; Boobis et al 2006) and this approach has been included in the draft GD (ECHA/EFSA/JRC 2017). There are clearly situations in which the effects of a chemical in the rat are similar to what would be predicted in humans, and perchlorate is a clear example of this principle (Wolff 1998).

For some chemicals that affect thyroid function however the situation may be very different and there may be little data to support cross-species extrapolation (Crofton 2004). Both *in vivo* and *in vitro* studies show that sodium phenobarbitone, pregnenolone-16α-carbonitrile (PCN), acetochlor and PCBs, activate nuclear receptors such as the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) either individually, or as dimers and that administration of these chemicals to rodents leads to an up-regulation of hepatic catabolic enzymes, stimulation of the biliary elimination of thyroid hormones, a decrease in circulating thyroid hormones (Schuetz et al. 1998; Liu et al 1996; Hood and Klaassen 2000), and thyroid hypertrophy and hyperplasia. Although human liver does contain both CAR and PXR (Moore et al 2000; Omiecinski et al 2011) there are significant species differences in response to those chemicals that activate these nuclear receptors both in terms of sensitivity of response (Elcombe et al 2014) and in terms of specificity with rodent PXR being activated by PCN, but not by rifampicin, whereas human PXR is activated by rifampicin but not by PCN (Kliewer et al. 2002). Additionally, while PCBs in general are agonistic to PXR in rats, *in vitro* data in human hepatocytes show that high concentrations of PCB-153 are antagonists to the human PXR (Tabb et al. 2004).

Not to make too much of a point about it, significant species differences exist between human and rodents in terms of the thyroid binding proteins in the circulation where the relative importance of TTR, TBG and albumin for transporting thyroid hormones accounts for significant differences in thyroid hormone half-life and turnover, with rodents showing appreciably higher rates of production and loss of thyroid hormones than do humans, a situation that leads to increased overall sensitivity, and in some cases species specific differences, in chemical perturbation of the HPT axis (Capen 1997; Hill et al 1998).

Fig. 10: An adverse outcome pathway analysis of chemically-induced thyroid hormone disruption (from Paul 2014).



7. ECPA Question: Is there evidence for a threshold/correlation for adversity linked to serum thyroid hormone levels (T3, T4, TSH and sulphated thyroid hormone), between species

Thyroid hormone disruption has been the subject of adverse outcome pathway (AOP) analysis (Paul 2014). Fig 10 above is taken from a presentation that clearly outlines the current understanding of the molecular initiating events (MIEs) that lead to altered hormone homeostasis (Crofton 2008; Murk et al 2013). Although the AOP above might suggest that any single chemical would act through a single molecular initiating event, many rodent thyroid-acting chemicals disrupt multiple MIEs and for several the ultimate thyroid disruption is thought to be considerably greater as a result of these multiple hits on its function. PCBs exemplify this principle where they have been shown to displace thyroid hormone from its binding protein, to inhibit deiodinase activity, and to increase thyroid hormone catabolism by inducing hepatic glucuronidation (Barter and Klaassen 1992; 1994; Brouwer 1989; Hood and Klaassen 2000).

While each of the MIEs in Fig 10 will operate essentially independent of the administered dose of a thyroid disrupting chemical, activation of subsequent key events would be expected to follow classical dose-response relationships with higher dose levels inducing greater changes in each key event, and a threshold dose level below which each respective key event will not be triggered (Dellarco et al 2006). Hence each key event would be expected to follow conventional dose-response relationships dependent upon the degree of change in the previous key event with clearly defined exposure thresholds for triggering subsequent events. In the absence of a sufficient change in the previous key

event the downstream key event would not occur. An alternative way of looking at this is in terms of chemically induced depressions in thyroid hormones where TSH release from the pituitary gland only occurs when circulating levels of T3 achieve a threshold level that stimulates the receptors in the hypothalamus to release TRH.

Comparative studies on the effects of perchlorate in the rat and human thyroid gland (Yu et al. 2002) have shown dose related inhibition of uptake of radiolabelled iodide of 15%, 55%, and 65% at 1.0, 3.0, and 10 mg/kg perchlorate respectively on day 1 following initiation of perchlorate administration to the rat but that the homeostasis was reset in animals killed after 28 days where inhibition of iodide uptake was only observed at the top dose levels of 10 mg/kg. This adaptation of the thyroid system to prolonged administration is somewhat different to the way in which other organs deal with toxicity but shows clear adaptation probably by increasing the expression of the sodium-iodide symporter on the follicular cells of the thyroid. A similar response was not observed in a 14-day study with perchlorate administration in human volunteers (Greer et al. 2002) and the species difference exhibited was thought to have occurred in rats because of their smaller reserve capacity of thyroid hormones than humans.

7.1. Thyroid hormone changes in the absence of histological changes

The negative feedback control of circulating free T3 on the hypothalamus and pituitary plays an essential role in ensuring that there is sufficient circulating thyroid hormones to satisfy the daily needs of the body. The circulating T3 concentrations are a product of the deiodination of T4 through the action of hepatic 5' deiodinases together with small amounts of T3 being released directly from the thyroid gland. Hence circulatory T3 levels are maintained by a large pool of protein bound T4 which can be deiodinised in peripheral tissues, mostly the liver, when the concentration of free circulating T3 falls below acceptable limits. In addition to TSH controlling the production and release of T4/T3 from the thyroid gland, it is also responsible for the control of the activity of peripheral tissue deiodinases to modulate the conversion of T3 from T4 (Hoermann et al 2015) and hence TSH acts to integrate the peripheral and central elements of thyroid hormone homeostasis into one overarching control system. This is the normal physiological control of thyroid hormones and it operates within defined limits of circulating hormones, but not within a narrow band of concentrations since it is clear that release of the master controller, TSH, follows a circadian rhythm of peaks and troughs in the circulation throughout the day (Fisher 1996). Indeed, the differences between peak and nadir levels of TSH in humans have been shown to differ by as much as ±50% around a mean value (Hoermann et al 2015; Fisher 1996). Despite these daily fluctuations in serum TSH levels, those of T3 and T4 remain essentially within acceptable limits underlining the critical importance of an adequate concentration of thyroid hormones in maintaining the normal physiology in the body (Bianco and Kim 2006).

It is stated in the draft GD (page 96; Appendix A) that "A decrease in T4 (total or free) in the absence of other histological changes and/or hormonal evidence of hypothyroidism is a relatively frequent observation in experimental toxicological studies, particularly in rodents." (ECHA/EFSA/JRC 2017). It is unclear exactly where this statement has come from and within what context it is meant since it is not referenced in the text. However, sporadic changes in single parameters, in the multitude of clinical chemistry endpoints measured in routine toxicity studies, is indeed a common observation and, in the absence of expected accompanying changes in linked parameters, is normally discounted (Hamada et al 1998; Lewis et al 2002). As with all parameters in such studies, the difficulty lies in linking the effects, in this case a decrease in T4, to any functional consequence since thyroid control, like most physiological systems in the body, operates within tolerances rather than being dependent upon excursions outside of narrow ranges. Considering the critical importance of thyroid hormones, there is an inbuilt redundancy in terms of high circulating T4 levels, which allows the maintenance of T3 homeostasis within the limits required for adequate physiological control. Hence a degree of decrease in T4 will still permit adequate levels of T3 to be maintained without triggering compensatory TSH release and thyroid histological changes. Therefore, in the absence of supporting evidence of histopathological effects in the thyroid gland or pituitary, or expected associated changes in TSH and T3, isolated changes in T4 need to be placed into the context of the study as a whole, and interpreted

with an eye to the duration of the study and dose-response relationships for the observed change, as it would for any other potentially adverse endpoint.

While decreases in T4 can be diagnostic of an endocrine effect, there are a number of non-physiological, and non-endocrinological, reasons why a decrease in a single parameter should not be considered biologically/toxicologically relevant (Lewis et al 2002). Despite the fact that decreases in T4 in human cases can be adverse with regard to pre- and post-natal neurological development and thyroid pathology, they are always accompanied by concomitant hormonal and/or tissue changes that may or may not be described due to the constraints placed upon clinical studies (Colnot and Dekant 2017). There are no such restrictions on laboratory animal toxicity studies and complete tissue histopathology and a broad range of hormonal and clinical chemistry assessments will accompany any appropriately OECD guide-lined test, such that expected accompanying pathophysiological endpoints will be present, alongside the change in the measured parameter, to enable that change to be placed into context.

In a situation where a chemical is suspected of affecting thyroid homeostasis then specialised studies to establish any functional consequences of the changes, including MoA and human relevance focussed experiments, might help clarify whether or not the observed change was accompanied by functional consequences. Studies such as the EOGRTS or DNT would help to clarify the biological significance of particular hormonal changes, but since hormonal disruption generally follows classical dose response relationships (Klaassen and Hood 2001; Ren et al 1988; Liu et al 1995), small changes in circulating hormones, that occur at relatively low doses of chemicals, would not be expected to result in functional consequences when the considerable redundancy in function that exists for the thyroid control system is taken into account.

A discussion of the topic of isolated thyroid hormone changes on studies has been covered in an excellent recent publication by Colnot and Dekant (2017) where they outline the considerations that would normally be taken when arriving at a decision regarding the treatment-relatedness or otherwise of a change in thyroid hormones. Hence a difference in thyroid hormone levels between controls and treated animals is unlikely to represent a treatment-related effect if it is transient and occurs in the absence of dose-response or if it occurs in one or a few animals. A difference in thyroid hormone levels is less likely related to treatment even if statistically significant if it remains within the range of hormone levels present in the serum of control animals from other studies conducted in the same laboratory (historical controls).

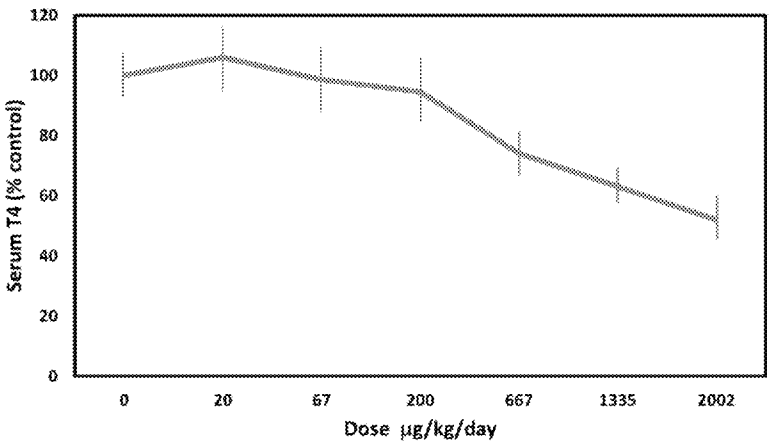
Since it is well known that circulating thyroid hormone levels are subject to a number of confounders such as generalised stress including body weight and food consumption effects, altered thyroid hormone levels are less likely to be specific and more likely to be secondary to systemic stress effects if there is no alteration in the general function of the thyroid as determined by thyroid weights and histology (DeVito et al., 1999; Choksi et al., 2003) since these endpoints are less sensitive to these secondary effects (St Germain and Galton, 1985; Cavalieri, 1991). Altered caloric intake is known to affect thyroid hormones, and reduced food and water intake is commonly observed on toxicity studies in rodents, some of the thyroid hormone changes observed, when unaccompanied by supportive changes in histology and thyroid weight may be secondary to inanition induced by the use of maximum tolerated doses or, considering the impact of disease on thyroid hormone homeostasis, be secondary to toxicity in other organs such as the kidney or liver.

7.2. Evidence for dose response/threshold relationships for thyroid hormone effects

7.2.1. Evidence in animal studies

A series of dose response studies on a range of chemical mixtures, known to disrupt thyroid hormone homeostasis, were carried out on female Long-Evans rats dosed via gavage with a number of poly-halogenated aromatic hydrocarbons for 4 consecutive days and serum total T4 was measured in samples collected 24 hr after the last dose (Crofton et al 2005). These studies clearly showed a threshold dose for the mixtures (Fig. 11), below which no effects on serum T4 were observed (Crofton et al 2005).

Fig 11: Dose dependent decrease in serum T4 concentrations following exposure to a mixture of polycyclic halogenated hydrocarbons (PHAH) – redrawn from Crofton et al 2005)



A dose response study was also carried out under the auspices of the National Toxicology Program where 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) was dosed by oral gavage to female Harlan Sprague-Dawley rats at dose levels of 10, 100, 300, 1,000, or 3,000 µg/kg 5 days per week for periods of up to 105 weeks (NTP 2006). Assessments carried out on this study included free and total thyroid hormones and potential cancer outcomes, but not thyroid organ weight.

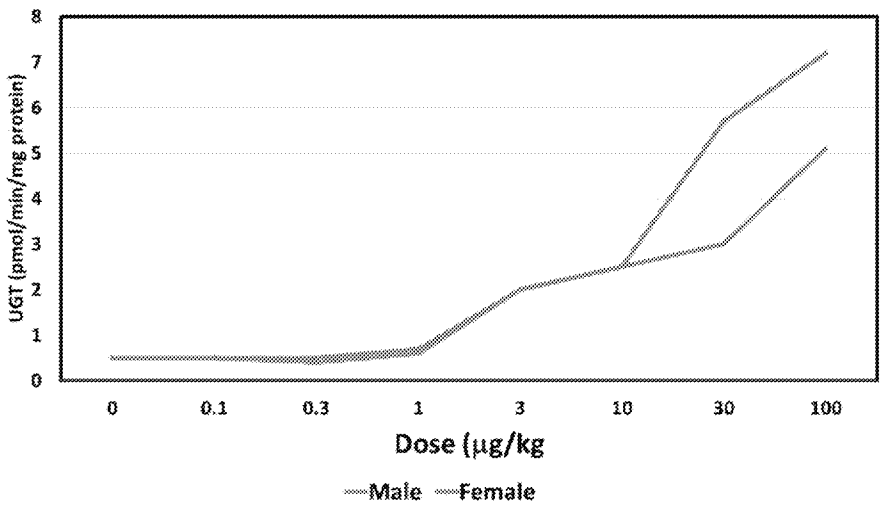
Serum total T4, free T4, and total T3 concentrations in the 3,000 µg/kg group only were significantly lower than those in the vehicle controls at the 14-week interim evaluation. At the 53-week interim evaluation, serum total T4 and free T4 concentrations in the 3,000 µg/kg group only were significantly lower than in the vehicle controls but there were no changes in total or free T3 levels. At 2 years, the incidences of minimal to mild follicular cell hypertrophy were significantly increased in the 300 µg/kg and 3,000 µg/kg (core and stop-exposure) groups although follicular hypertrophy was recorded at all dose levels including the controls (incidences of 5/51, 9/52, 9/53, 12/53, 10/53, 17/51, 12/49 at the 0, 10, 100, 300, 1000 and 3000 µg/kg).

Once again this study clearly illustrates a threshold for thyroid hormone disruption at all time points measured (NOEL at 1000 µg/kg), but not for histopathology of follicular hypertrophy at the 2 year time period. The proposed AOP for thyroid changes predicts that hormone changes would be a more sensitive endpoint than histopathology and that the latter should occur following activation of the respective MIE. The fact that neither of these was seen in this study is most probably explained as an artefact of the time points where the various endpoints were measured and emphasises the fact that bolting on thyroid hormone measure to a standard study design is most probably not going to give supportive information for establishing MoA. This data strongly supports the conclusion that specially

designed, preferably short term, studies would most likely provide the information that would give the most informative data on which to address prospective MoAs.

A similar study was conducted with another polyhalogenated hydrocarbon, PCB 126, which was dosed by oral gavage to rats daily for four days at a range of dose levels (Fig. 12) from 0.1 to 100 μg/kg/day (Craft et al 2002). The study measured effects on hepatic glucuronyltransferase and on serum T4 levels, and while showing clear thresholds for dose levels where reductions in serum T4 occurred, it also demonstrated a concomitant induction of hepatic UGT which showed a LOEL at 3 μg/kg which corresponded to a similar LOEL for reductions in serum T4 (Fig. 11).

Fig. 12: Hepatic UGT levels in rats following dosing with PCB 126 for 4 days (Craft et al 2002)



Studies in rapidly growing, prepubertal, cynomolgus monkeys, made hypothyroid by the administration of methimazole in drinking water, showed a clear dose response in the concentration of exogenous T4 required to correct the inhibition of lower leg growth rate, and to stabilise serum levels of T3, T4 and TSH. (Ren et al 1988). Hypothyroid monkeys showed a 65% decrease in lower leg growth rate, decreased T3, T4 and IGF-1, and increased TSH compared to non-hypothyroid monkeys of similar age. Following administration of exogenous T4 in this experiment, lower leg growth rate increased significantly, in a dose dependent manner, such that T4, given at dose levels of 4 and 8 μg/kg/day had 56% and 73% increases in lower leg growth rate respectively compared to controls, this change being accompanied by a return of the circulating thyroid hormone levels to normal.

7.2.2. Evidence in human studies

The evidence for thresholds in the effects of T4/T3 is also seen with TSH in human studies. In a study where PTU was administered to hypothyroid patients receiving 0.2 mg/day of T4 (Saberi et al 1974), PTU induced a fall in mean serum T3 concentrations from a mean value of 84 ng/ 100 ml to 70 ng/100 ml (a 17% decrease) after 1 day, and to 63 ng/100 ml (a 25% decrease) after 2 days of PTU. Under these conditions, and following these drops in serum T3, serum TSH concentrations did not increase. Hypothyroid patients receiving T4 at a lower dose of 0.1 mg/day showed a similar decrease in serum T3 following PTU administration which was accompanied by increases in TSH from pre-study levels of 29.6 μU/ml to a peak of 40 μU/ml on day 5-6 of administration of PTU. These results suggest that at least in hypothyroid patients, there is a tolerance before a TSH response occurs following decreases in circulating T3, and a threshold for decreasing circulatory thyroid hormones, below which no compensatory release of TSH will occur from the pituitary.

7.3. Evidence for dose responses/thresholds for neurodevelopmental disorders of thyroid homeostasis

It is clear from both human and animal studies that foetal development is critically dependent upon the correct concentration of thyroid hormones at the right phase in the foetal development. For many years human hypothyroidism during pregnancy has been known to produce severe neurological abnormalities in the offspring of affected individuals that cannot be corrected by thyroid hormone supplementation post-partum. However, the severity of the neurological deficits of neonatal hypothyroidism in humans are dependent upon the severity of the maternal hypothyroidism, and when detected are largely preventable by immediate thyroid hormone replacement (Williams 2008; Alexander et al 2017). However, untreated human neonates exhibit growth retardation and general features of hypothyroidism with mental retardation, tremor, spasticity, and speech and language deficits.

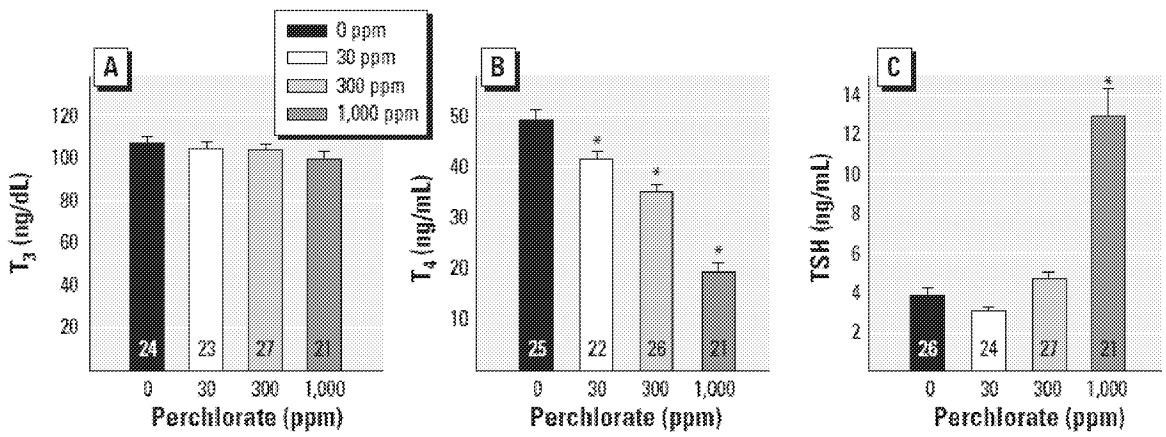
In human beings, pregnancy has a significant impact on the thyroid gland and its function. During pregnancy, the thyroid gland increases in size by 10% in countries with healthy iodine levels in the diets, but by 20% to 40% in areas of iodine deficiency. Production of T4 and T3 has been reported to increase by nearly 50% on non-pregnant women, and dietary demand for iodide increases proportionally to meet these needs (Alexander et al 2017). Subclinical thyroid disease can become clinical during pregnancy and it is for these reasons that TSH levels are monitored in women during pregnancy. Because thyroid hormone supply to the foetus during the first trimester is dependent entirely on the mother thyroid hormone deficiency at this time can have severe effects on the developing nervous system (Bath et al 2013). Results of the Avon Longitudinal Study of Parents and Children (ALSPAC) survey showed that a higher proportion of children born to women with an iodine status of less than 150 μg/g/day had sub-optimum cognitive outcomes than did those born to women in the 150 μg/g/day or more group (Bath et al 2013). Since iodine is essential for the production of the thyroid hormones and iodine deficiency is known to result in hypothyroidism, these data demonstrate a clear threshold for dietary iodine intake below which an increased risk to the developing foetus results.

Subclinical hypothyroidism in pregnant humans has been internationally defined as an upper limits of serum TSH levels of 4 mIU/L (Alexander et al 2017). Levels of TSH higher than this incur recommended therapeutic intervention, typically using the soluble thyroxine substitute, levothyroxine or LT4 (Maraka et al 2017). A typical dose of LT4 is 50 μg/day. This was shown to correct thyroid hormone deficiencies, to minimise the risk of neurodeficiencies in children, and to decrease pregnancy loss associated with low thyroid hormone levels. Adequate iodine intake is essential for the production of thyroid hormones and the World Health Organisation recommends a daily intake of 250 μg for pregnant and lactating women (Alexander et al 2017). There are many parts of the world where iodine deficiency is endemic and associated with increased pregnancy loss and cretinism in offspring and goitre in adults (de Benoist et al 2004). There have been two non-randomised trials in which neurodevelopmental outcomes were improved in children from mildly to moderately iodine-deficient areas whose mothers received iodine supplementation early in pregnancy (Berbel et al 2009; O'Donnell et al 2002)

Data in rats on chemicals that disrupt the thyroid hormone homeostasis has shown that while high doses of potent thyrotoxic chemicals will produce profound neurodevelopment abnormalities, lower doses, where decreases in circulating thyroid hormones are less, are also associated with less severe developmental defects (Auso et al. 2004; Crofton 2004; Crofton et al. 2000; Goldey et al. 1995a, 1995b; Goodman and Gilbert 2007; Morreale de Escobar 2003). These results support the concept of conventional dose response relationships of cause and effect, and although MIEs would be expected to be activated on exposure, irrespective of dose, downstream key events would be expected to follow normal dose-response relationships which would only be triggered once the previous key event reached a particular threshold.

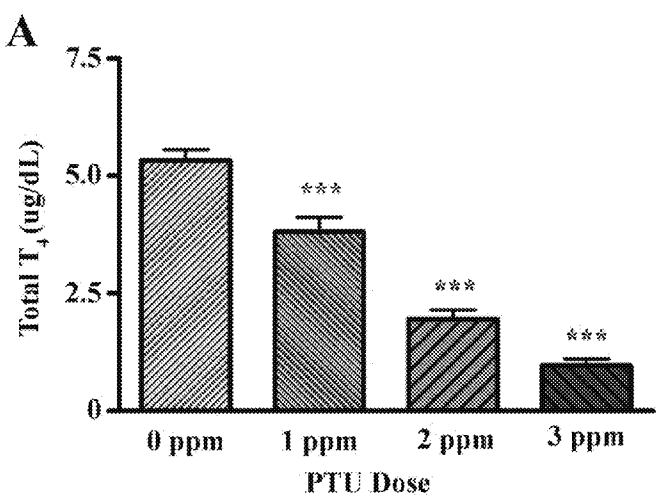
Experimental data supports the contention of thresholds for developmental abnormalities and in a study in pregnant rats, a 50% decrease in maternal T4 was shown to affect cochlear maturation in rats resulting in them being born deaf (Crofton 2004). A study in which pregnant rats were made hypothyroid by perchlorate administered in the drinking water, at dose levels up to 1000 ppm beginning on GD6 and sacrificed on PND30 (Gilbert and Sui 2008), found that T4 in the dams was reduced by 16%, 28%, and 60% in the 30, 300, and 1,000-ppm dose groups respectively accompanied by compensatory increases in TSH at the high-dose only supporting the threshold concept of preceding key events where the reductions in T4 (preceding key event), at the two lower dose levels were insufficient to trigger the subsequent key event, TSH release. Interestingly, in this study there were no changes in T3 values at any of the dose levels illustrating the degree of redundancy in the system that allows sufficient levels of T3 to be maintained despite decreases in T4. This study illustrates the expected dose-response relationships in terms of decreasing T4 and increasing TSH although the relationship was less obvious for T3 levels (Fig. 13).

Fig. 13: Changes in circulating hormone levels in rats dosed with perchlorate (Gilbert and Sui 2008)



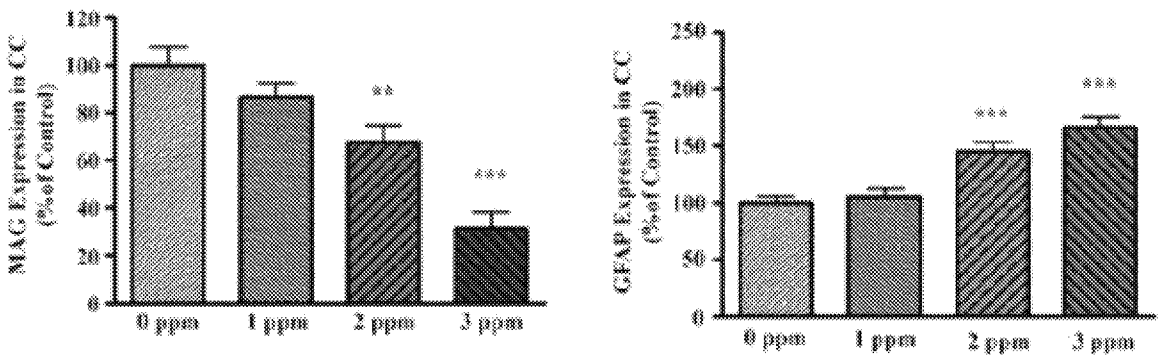
Another study where two week old rats were given PTU in the drinking water at dose levels of 0, 1, 2 and 3 ppm (Sharlin et al 2008) decreased T4 levels at all administered doses in a dose-dependent manner producing a 28%, a 63% and a 82% decrease in total T4 at the 1, 2, and 3 ppm dose levels respectively in PND 15 pups (Fig. 14).

Fig. 14: Dose dependent decrease in total T4 in the serum (from Sharlin et al 2008)



The decrease in T4 showed a direct correlation with a dose-dependent decrease in the density of oligodendrocytes, and an increase in the density of glial fibrillary acidic protein-positive astrocytes in both the corpus callosum and anterior commissure (Fig. 15). Although linear regression analyses demonstrated a strong positive correlation with each of these parameters, the 1 ppm dose level of PTU was a NOEL for both oligonucleotide and astrocytic responses even though T4 decreases were seen at all exposure levels. This again supports the concept of a threshold degree of decrease in total T4 levels below which there are no neurodevelopmental deficits presumably because of the maintenance of T3 values sufficient to satisfy the requirements of the developing foetuses.

Fig. 15: Dose dependent decrease in oligonucleocytes (identified by presence of myelin associated glycoprotein expression - MAG) and increase in GFAP astrocytes in the corpus callosum (CC) in rats exposed to PTU (from Sharlin et al 2008)



An interesting chemical example which appears to demonstrate thresholds for decreases in circulating maternal thyroid hormones not affecting the offspring is seen with the fungicide, mancozeb in two developmental toxicity studies and one EOGRTS study (Axelstad et al 2011). This compound was extensively discussed in the thyroid disruption workshop that took place in April 2017, and an account of the discussion was published in the final report of the workshop (Kortenkamp et al 2017). Mancozeb is thought to be thyrotoxic via a degradation product, ethylene thiourea, which is a known rodent goitrogen and carcinogen acting through inhibition of thyroperoxidase (Marinovich et al 1997; National Toxicology Program 1992). The maximum dose level of mancozeb permissible in the developmental neurotoxicity study was limited by maternal toxicity consisting of significant body weight loss and hind limb paralysis but the top dose levels used were still considered adequate because it depressed maternal thyroid hormone levels by ~50% and dose levels lower than those used in the study had been shown to induce thyroid weight increases, decreased thyroid hormones and thyroid gland hypertrophy and hyperplasia, in a previous ninety day rat study. None of the three studies showed effects on the developmental and behavioural parameters assessed in the pups and foetuses on the study and mancozeb is not a developmental neurotoxicant despite clear evidence of maternal thyroid effects. This data supports the concept of a threshold for depression of circulating thyroid hormones in the dam below which there are no toxicological consequences for the pups.

In summary it is apparent that both the decreases in thyroid hormones and any subsequent neurodevelopmental abnormalities arising from the altered thyroid hormone homeostasis show expected dose response relationships with clear NOELs and thresholds for responses below which no biological effect is seen.

7.4. Is there evidence for a threshold for chemicals inducing thyroid cancer through thyroid hormone disruption?

The presence or absence of a threshold for the induction of rodent thyroid cancer by chemical exposure is dependent upon the MoA of the particular chemical in inducing thyroid hormone dysfunction (McClain 1995). Clearly it would be difficult to argue any thresholds for chemicals inducing thyroid cancer through mechanisms involving mutational events but for most other non-genotoxic modes of action for producing thyroid cancer, it is the chronic effect of high TSH levels that stimulate the thyroid gland to undergo growth and sustained cell hyperplasia, the latter outcome classically increasing the chances of the hyper-proliferative follicular epithelial cells acquiring spontaneous mutations that lead eventually, through promotional and progressional changes, to cancer (Cohen and Ellwein 1991; Foster 1997).

Since the hypothalamus/pituitary sensing system for thyroid hormones is considered to be responsive only to free T3, and that this represents <10% of the total circulating T3, there is also considerable redundancy in terms of measuring any reduction in total serum thyroid hormones before a TSH response is invoked. This latter comment argues for the importance of measuring both total and free thyroid hormones when assessing potential thyroid modulating effects of a particular chemical (Kioukia et al 2000; Dayan 2001; van der Watt 2008; Li et al 2014). Furthermore, the whole postulate regarding non-genotoxic MoAs for cancer demands a sustained condition of stimulation of the target cell population (Paynter et al 1988), and the degree of thyroid dysfunction produced by a chemical over a prolonged period of time would present a significant clinical problem to affected individuals before such exposure would increase the risk for neoplasia in humans.

In an EPA series of case examples of thyroid carcinogens (EPA 2014), a thionamide chemical that inhibited both thyroperoxidase and deiodinase activity was found to cause an increase in thyroid follicular cancer in both rats (Table 3) and mice (Table 4), and a corresponding dose related increase in serum TSH. In the article the chemicals were anonymised so that their exact structure could not be obtained. The compound was stated to be non-mutagenic by standard assays and to inhibit the synthesis of T4 within the thyroid, and the subsequent conversion of T4 to the active T3 form in the peripheral tissues. The compound also induced an increase in TSH secreting pituitary adenomas in the rat oncogenicity study. The compound has also been dosed to humans and monkeys and, with regard to altered TSH levels, exposed humans showed no TSH imbalance, while monkeys showed minor TSH increases but were considerably less sensitive on a mg/kg basis than were rats (EPA 2014). Table 3 shows the outcome of a two-year carcinogenicity study with the compound and demonstrated a clear threshold for development of thyroid follicular cancer at 120 mg/kg/day for both male and female rats. A two-year study in B6C3F1 mice was also carcinogenic for the compound but the NOEL was considerably higher than was seen in the rat study (Table 4) suggesting a differential sensitivity between the two, rodent, species.

Table 3: Incidence of thyroid follicular cell tumours in F344 rats in a 2-year study of compound 1 (EPA 2014).

Dose	0		120		240		480	
Sex	M	F	M	F	M	F	M	F
Total No. rats	49	50	49	50	47	48	45	46
Follicular adenoma	1	0	1	0	10*	14*	34*	28*
Follicular carcinoma	1	0	0	0	4*	3*	8*	7*
Adenoma+carcinoma	2	0	1	0	14*	17*	42*	35*

* $p<0.05$

Table 4: Incidence of thyroid follicular neoplasms in B6C3F1 mice in a 2-year study of compound 1 (EPA 2014).

Dose	0		120		240		480	
Sex	M	F	M	F	M	F	M	F
Total No. rats	48	50	46	47	44	47	47	46
Follicular adenoma	1	2	1	1	2	0	16*	11*
Follicular carcinoma	0	0	0	0	0	0	0	1
Adenoma+carcinoma	1	2	1	1	2	1	16*	11*

* $p<0.05$

In summary, since the mitogenic stimulus for any chemical that targets the rodent thyroid gland is sustained TSH stimulation, there would be no cancer risk from non-genotoxic chemicals at exposure levels that fail to decrease circulating thyroid hormone levels sufficiently to provoke the TSH release from the pituitary gland (McClain 1995). For those rodent thyroid gland carcinogens, a threshold dose for thyroid hormone depression will exist based upon a lack of TSH induced cell proliferation in the thyroid follicular cells, below which there will be no hazard for the development of thyroid cancer in the rodent.

8. ECPA Question: Are there non-endocrine causes of the same adverse effects e.g. stress, starvation, environmental factors such as temperature?

The sensitive and tightly regulated thyroid hormone feedback control system, thyroid gland autoregulation, and the large intra- and extra-thyroidal storage pools of thyroid hormone under normal conditions are able to provide a constant supply of free thyroid hormone to peripheral tissues in the face of perturbations imposed by the external environment, chemicals and drugs, and a variety of diseases processes. However, under certain situations the homeostatic control is disturbed and compensatory regulation of synthesis and secretion can occur. This section details the secondary response of the thyroid to particular stresses unrelated to a direct effect of the particular stress on the thyroid gland itself.

It is especially important, in the context of laboratory animal studies, to understand those responses that affect thyroid hormones under stress situations since many animal safety evaluation studies are conducted at a range of dose levels that might include, at the top dose level, exposures that can compromise the normal physiology of the rat. The use of a maximum tolerated dose level can significantly influence the normal hormonal homeostasis (Carr and Kolbye 1991; Downes and Foster 2015; Apostolou 1990; Carr and Kolbye 1991; Haseman and Seilkop 1992; McConnell 1989), not as a primary effect of the chemical, but as a secondary effect of being exposed to concentrations of chemicals that inhibit food consumption, or cause disease in organs in addition to, or other than, direct toxicity to the thyroid gland. Hence the interpretation of any thyroid hormone changes under circumstances of multi-organ pathology, or significant effects on food and water consumption, will need to be very carefully evaluated to eliminate the secondary consequences of these confounding influences on circulating thyroid hormone levels.

TSH secretion by the pituitary gland thyrotrophs, and their sensitivity to TRH stimulation, has been shown in humans to be affected by renal failure, starvation, sleep deprivation, depression, and hormones, including cortisol, growth hormone, and sex steroids (Gary et al 1996; Jackson 1982).

8.1. Thyroid hormone changes in disease states

In many chronic illnesses in humans, defects occur in thyroid hormone metabolism, resulting in the "sick euthyroid syndrome" characterized by a normal total T4, normal/high free T4, low total T3, low free T3 and an elevated rT3 (Malik and Hodgson 2002). These changes reflect a reduction in type 1 deiodinase activity, an increase in type 3 deiodinase activity (Bianco et al 2002) and changes in the plasma concentration of thyroid-binding proteins and free fatty acids which displace thyroid hormones from binding proteins. There are also non-thyroidal influences on the hypothalamic-pituitary-thyroid axis such as cortisol inhibiting TSH secretion (Camacho and Dwarkanathan 1999).

Increased levels of rT3, indicating greater conversion of T4, have been observed in humans in starvation, anorexia nervosa, severe trauma and haemorrhagic shock, hepatic dysfunction, postoperative states, severe infection, and in burn patients (Chopra 1976; Boelaert and Franklyn 2005; Jabbar et al 2017). Since rT3 is an inactive form of thyroid hormone, an increased conversion of T4 suggests that certain disease states induce a lowering of the metabolic rates and recuing caloric requirements as part of the natural physiological processes involved in coping with the disease.

8.2. Specific thyroid hormone changes in liver disease

The main transporter protein for thyroid hormones in humans, TBG, is an acute phase protein made in the liver, and in human cases of acute hepatitis, patients show elevated serum levels of total T4, due to increased production of TBG, but with normal levels of free T4. As the severity of hepatitis increases with impending liver failure, low total serum T4 levels are thought to reflect the reduced hepatocellular synthesis of TBG (Kano et al 1987). While serum T3 levels in patients with acute hepatitis can be very variable, the free T3:T4 ratio appears to correlate negatively with the severity of the liver disease reflecting diminished type 1 deiodinase activity, resulting in a reduced conversion of T4 to T3. Some case histories have shown an association of acute hepatic failure with the development of goitre that resolved with improvement in liver function (Hegedus 1986).

Data on thyroid hormone changes in laboratory animals on routine regulatory toxicity studies, in the presence of liver damage, is not available and it is not possible to comment on whether or not thyroid hormone changes would be induced in situations of liver damage.

8.3. Effects of temperature on thyroid hormones

8.3.1. Effects in humans

Changes in the outside temperature will alter TSH secretion in humans and as a consequence the serum concentration of thyroid hormones and their metabolism. These changes are thought to be mediated centrally via the pituitary/hypothalamus and peripherally by effects on the rates of thyroid hormone degradation, through increased loss in the faeces, and by alterations in thyroid hormone receptor expression in the tissues. Such changes would be expected to decrease the metabolic rates in peripheral tissues.

In cell systems *in vitro,* changes in temperature have been shown to affect the binding affinity of T4 to its serum binding proteins and this could also function *in vivo* under conditions of extreme cold or extreme heat (Bernstein and Oppenheimer 1966). In a study of males living in Northern Finland, serum free T3 levels were shown to be lower in February than in August, and TSH levels were higher in December than at other times of the year. Serum free T3 levels correlated significantly with the mean outdoor temperature of the preceding month but serum TSH levels failed to show any correlation with the mean temperature of the month or with free T3. Low serum free T3 in winter suggests that thyroid hormone degradation was accelerated in the cold (Leppäluoto et al 1998). The interpretation of some of the human studies may have been confounded by additional variables such as altered daylight, activity levels, living conditions, and sleep deprivation accompanying prolonged residence in Arctic and Antarctic regions (Hackney et al 1995 a, b).

8.3.2. Effects in laboratory animals

The overall effects of environmental temperature have been easier to demonstrate in animals than in humans but profound species differences in thermal regulation may mean that the findings in animal models may not apply to humans (Silva and Larsson 1985). Cold exposure in animals leads to thyroid gland hyperplasia, enhanced hormonal secretion, degradation, and excretion, accompanied by an increased demand for dietary iodine (Goglia et al 1983). The prompt activation of pituitary TSH secretion after cold exposure of the rats (Panda and Turner 1975; Emerson and Utiger 1975) is thought to be due to a direct effect on the hypothalamus (Anderson 1964).

Exposure to cold has also resulted in increased secretion of TRH (Szabo and Frohman 1977), and a reduced response of pituitary TSH release to administered TRH (Hefco et al 1975). Cold exposure in laboratory studies in the rat is associated with increased rates of T4 and T3 deiodination, increased conversion of T4 to T3, enhanced hepatic binding, and increased biliary and faecal clearance of all of the iodothyronines (Balsam and Sexton 1975; Bernal and Escobar del Rey 1975; Tsukahara et al 1997; Galton and Nisula 1969; Balsam and Leppo 1974). Finally, thyroid hormone effects may be enhanced by alterations in co-activators which are able to enhance the activity of thyroid hormone receptors on gene activation (Puigserver et al 1998).

When rats were exposed to high environmental temperatures of 34°C for three weeks, there was a rapid and simultaneous decrease in hypothalamic TRH, plasma TSH, plasma T4 and thyroid activity by the 36th hour of heat exposure suggesting an effect mediated via the hypothalamus. On prolonged exposure, by day 9, there was a rebound in thyroid activity due to a peak in circulating TSH in response to the marked decrease in plasma T4 seen at this time. From day 9 to the end of the study on day 21, all thyroid parameters returned to control levels indicating adaptation to the increased temperature regime (Rousset and Cure 1975). This result in the laboratory was mirrored by a similar effect in human subjects where a decrease in the elevated serum TSH level associated with primary hypothyroidism was induced by increases in body temperature (O'Malley et al 1980).

8.4. The relationship of fasting and obesity to thyroid hormones

In periods of limited food availability, there is central downregulation of the HPT axis, and serum T4 and T3 levels fall during fasting both in humans (Chan et al 2003) and in rodents (Ahlma et al 1996; Legradi et al 1997) to downregulate the metabolic rate in affected animals/individuals. In rats, fasting has been shown to decrease both pituitary type 2 deiodinase activity and liver type 1 deiodinase activity, and the reduction correlates with reduced peripheral T3 isolated from liver homogenates (Boelen et al 2006; 2008). Despite this reduction in pituitary and liver T3, hypothalamic type 2 deiodinase activity increases with fasting, resulting in a stimulation of release of the appetite-related proteins, neuropeptide Y (NPY) and agouti-related peptide (AgRP) from the arcuate nucleus. Thus, despite fasting-associated reductions in peripheral thyroid hormone levels, there is still a localized increase in T3 within the hypothalamus with a marked increase in orexigenic (hunger) signals, which in turn act upon the paraventricular nucleus to decrease TRH and, in consequence, TSH production. Humans who are anorexic, or who undergo severe caloric restriction, exhibit similar reductions in thyroid hormones, which are thought to be protective of vital energy stores (Langouche et al 2014; Reinehr 2010; Warren 2011). The administration of leptin (Da Veiga et al 2004), or α-MSH (Fekete et al 2000), has been shown to abolish the fasting-induced reductions in TRH and restore normal circulating TSH levels and humans and mice with mutations in the leptin receptor or in the leptin molecule itself exhibit central hypothyroidism are prone to severe obesity (Ohtake et al 1977; Clement et al 1998), which is ameliorated, at least in leptin-deficient humans, by the administration of exogenous leptin (Farooqi et al 2002).

In humans, there is a clear relationship between body weight, obesity and hypothyroidism (Amin et al 2011) and thyroid hormones are thought to directly affect appetite via their actions on the brain. Administration of TRH and TSH directly into the brain of rodents causes a reduction in food intake (Lin et al 1983; Vijayan and McCann 1977; Suzuki et al 1982) and similar effects on food intake are seen following peripheral administration of TRH (Choi et al 2002). In contrast, central and peripheral administration of T3 increases food intake and consequentially induces body weight gain (Kong et al 2004; Ishii et al 2003; 2008).

In summary therefore, any situation that significantly alters the normal intake of food by a laboratory animal will be expected to affect thyroid hormone homeostasis, not as a direct effect on the thyroid but secondarily to effects on food consumption.

9. ECPA Question: What are the non-thyroid hormone activities of the thyroid hormone transport proteins? Are there quantitative and/or qualitative species differences?

9.1. Thyroid hormone binding proteins as acute phase proteins

Since the liver is responsible for metabolism of thyroid hormones essentially any toxicity, including chemical-induced hypertrophy and enzyme induction, will affect thyroid hormone turnover, alter circulating thyroid hormone levels, and potentially feed back to the hypothalamus/pituitary to alter thyroid hormone production. Acute phase proteins are proteins that are increased or decreased in the presence of inflammatory conditions in general (Cray et al 2009). The vast majority of acute phase proteins are made in the liver and any condition that affects their hepatic production and release, irrespective of species, will alter the concentrations of these proteins (Ross et al 1983). TBG is an acute phase protein and hence in conditions such as hepatic necrosis, where total serum protein is decreased because of compromised liver function, the serum concentration of the thyroid binding proteins will be decreased and the circulating total T4 and T3 has also been shown to decrease (Schussler et al 1978; Neto and Zantut-Wittmann 2016). While the exact identity of the thyroid binding proteins differs between different species, they are all manufactured and released from the liver and will consequently be altered, and induce alterations in circulating thyroid hormones, in the presence of compromised hepatic function (Huang and Liaw 1995).

In human cases of acute liver disease, patients show elevated serum levels of total T4 but with normal concentrations of free T4, due to increased production of TBG as an acute phase response (Jayachandran et al 2016; Neto and Zantut-Wittmann 2016; Gardner et al 1982). In severe cases of hepatitis low total T4 levels are thought to reflect the reduced hepatocellular synthesis of TBG (Kano et al 1987). While serum T3 levels in patients with acute hepatitis can be very variable, the free T3:T4 ratio appears to correlate negatively with the severity of the liver disease reflecting diminished type 1 deiodinase activity, resulting in a reduced conversion of T4 to T3. Some case histories have shown an association of acute hepatic failure with the development of goitre that resolved with improvement in liver function (Hegedus 1986).

Conditions of elevated oestrogen, including pregnancy, induce an increase in serum thyroxine binding globulin (TBG), and similarly, when exogenous oestrogen was administered to rats, elevations in TBG and total T4 were observed (Ain et al 1987). Whether this is an adaptation to impending pregnancy and the need for thyroid hormones by the developing foetus is open to speculation.

10. ECPA Question: What are the remaining uncertainties? How may they be addressed?

In order to arrive at a decision as to whether or not a given chemical is indicating thyroid disrupting properties, the draft GD suggest a two-tier approach based upon the use of the OECD Conceptual Framework levels 1 – 5 (page 9 of draft GD 2017) to categorise the various data. In terms of human

health assessment, levels 2 and 3 list the specific *in vitro* and *in vivo* assays respectively that would be used to discount a generalised endocrine effect using specifically designed assays, while level 4 lists the guideline studies that would normally be routinely conducted in any submission passage for pesticides and biocides. While the majority of the assays mentioned in the various levels have OECD test guidelines already issued, there are a minority of the *in vitro* assays that are not guideline at the time of writing.

In order to adequately address the question of the remaining uncertainties, it is necessary to break it down into two separate questions that relate to whether or not we are asking basic questions of understanding, that could be used in a TIER 2 MoA type of analysis, and where the basic biology of thyroid hormone control will be questioned, or whether we are questioning the adequacy of the current testing strategies for evaluating potential thyroid disrupting chemicals which would constitute part of the TIER 1 assessment. Currently much of the debate has muddled the two parts together and has resulted in polarised views as to the adequacy of each.

10.1. The current assays and their assessment of thyroid effects

As can be seen from Table 5 the current mandated toxicity assays carry a limited assessment of thyroid effects of chemicals dependent largely upon the assessment of thyroid gland weight and thyroid gland histopathology.

Table 5: The current assays and their assessment of thyroid effects (modified from European Union 2017).

OECD TG	Study Title	Thyroid Endpoints Measured
407	Repeated dose 28-day oral toxicity study	Histopathology of Thyroid gland liver and CNS & PNS Weight of thyroid gland liver & brain
408	Repeated dose 90-day oral toxicity study in rodents	Histopathology of Thyroid gland CNS, PNS & liver. Weight of thyroid gland, liver & brain
451-3	Chronic toxicity & carcinogenicity	Histopathology of Thyroid gland, CNS, PNS and liver
414	Prenatal developmental toxicity study	No thyroid-related endpoints are included
415	One-generation reproductive toxicity study	No thyroid related endpoints are included
416	Two-generation reproductive toxicity study	Thyroid gland histopathology in P0 and F1 parents
421	Reproductive screening test	T4 from PND13 offspring and P0 adult males; T4 may also be measured in pups on PND4 and dams on PND13; optional thyroid weight and histopathology
422	Combined 28-day/reproductive screening assay	As TG421
426	Developmental neurotoxicity study	No thyroid but assessment of developing

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		brain
443	Extended one generation reproductive toxicity study	T4/TSH in F1 offspring from cohort 1A at term (PND22); Thyroid gland weight and histopathology; when the DNT cohort is included, assessment of developing brain

Clearly the mandatory inclusion of additional thyroid endpoints to many of these guidelines would enhance theirvalue without placing extra burden on animal experimentation and would go a long way to filling in perceived shortcomings in the current test battery.

While the pivotal endpoint of thyroid gland carcinogenesis is fairly well covered by the lifetime chronic toxicity/carcinogenicity test, there is concern that potential neurodevelopmental deficits, induced by low levels of circulating thyroid hormones may not be so adequately assessed in the current available test guidelines (EU 2017). While no guideline studies fully assess this issue, TG 426, the DNT study, and TG 443, the EOGRTS (with DNT cohort) should, with modifications, be able to adequately address this. Hence the inclusion of thyroid hormone measurements into TG426 would significantly improve the guidance document insofar as thyroid assessments are concerned. TG 443 has the possibility to include a DNT cohort and if so the endpoints currently included in TG 426 would strengthen any concern that the neurodevelopmental effects of hypothyroidism were being adequately assessed. Furthermore, several behavioural studies performed according to these guidelines have not been able to show any adverse effects in behaviour or on brain histopathology, even in severely hypothyroxinemic animals. This has led scientists in the field of neurotoxicology and thyroid disruption to suggest that new endpoints which are more sensitive to perinatal thyroid disruption should be added to these guidelines, in order to better assess adverse effects on neurodevelopment (OECD 2006, Harry et al 2014).

There has also been criticism that the behavioural assays currently included into the TG are not sensitive enough to detect neurodevelopmental effects when they may be occurring (Harry et al 2014; EU 2017). This conclusion is based upon the findings that some chemicals, that clearly affect the hypothalamic/pituitary/thyroid axis, have not shown neurobehavioural effects in specifically designed studies. One example that has been used to exemplify this conclusion is the fungicide, mancozeb. This compound has shown thyroid effects in terms of hypertrophy and hyperplasia and thyroid gland weight increases in a number of toxicity studies but did not affect nervous system development in specifically conducted developmental toxicity studies in the rat (, Axelstad et al 2011). The mode of action of mancozeb in affecting the thyroid gland is thought to be due to its conversion to ethylene thiourea, a known thyroid toxic chemical (Graham et al 1975) and once again, a EOGRT study with a DNT cohort carried out with ETU failed to show adverse effects on brain development or on the neurobehavioural parameters that were assessed, even though thyroid hypertrophy was present in the adult rats at both the mid and high dose levels (Marty et al 2013b, in DRAR).

There are several alternative interpretations for these data. If we accept that severe hypothyroidism can lead to serious neurodevelopmental deficits, and there is sound evidence for a limited number of chemicals to support this conclusion, then the following arguments can be used to explain the lack of behavioural effects in these studies:

1. The thyroid disrupting effects of mancozeb/ETU on the dams were not severe enough to lead to altered brain development in the offspring.
2. The offspring were not hypothyroid themselves in the postnatal period (due to limited milk transfer) and since much brain development occurs postnatally in rats, the prenatal hypothyroxinemia was not severe enough to disrupt brain development.

3. The neurobehavioural assessment methods were not sensitive enough to detect subtle changes in brain development.
4. The decreases in circulating T4 levels lead to a compensatory upregulation of peripheral deiodinase activity, leading to an increased conversion of T4 to the active T3 sufficient to provide enough T3 for normal brain development in the pups.

With the exception of explanation 3, the other alternatives imply a threshold level of thyroid hormone inhibition in the dams, below which no consequences to pup neurodevelopment occur. The data also support the conclusion that chemically-induced thyroid effects in the dams occur at significantly lower chemical concentrations than do thyroid hormone induced neurodevelopmental deficits in the pups (Gilbert and Sui 2008). An alternative way of stating this is that the sensitivity of the adult thyroid gland in demonstrating the effects of chemically-induced thyroid hormone lowering is considerably greater than the induction of neurodevelopmental effects in the pups such that the degree of thyroid hormone decreases in the dams needs to be considerably lower to induce neurodevelopmental effects in the offspring. Human data from mothers living in iodine deficient parts of the world would support this conclusion where the % of hypothyroid mothers present in the population greatly exceed the incidence of neurological deficits in the children of the affected mothers (Alexander et al 2017).

Explanation point 3, namely that the neurobehavioural assays currently in use are simply not sensitive enough to detect subtle changes in brain development in the pups, is a criticism that can be levelled at almost every measured endpoint in any of the current guidance documents. The introduction of behavioural endpoints into guidance documents is always accompanied by significant efforts at validation of the introduced methods and indeed this was the case with those recommended in the developmental neurotoxicity test, TG426, and the EOGRTS, TG443, cohorts 2A and 2B. The neurobehavioural assays are conducted in addition to extensive, and specifically targeted, neuro-histopathological and hormonal assessment and provides the most comprehensive test system currently available. Clearly the guidelines are not immutable, and when sufficient information becomes available to warrant additional assays, then these do become incorporated, albeit following prolonged and extensive validation exercises to ensure the quality of the additional assays.

10.2. The need for additional screens for chemicals that might disrupt the hypothalamus/pituitary/ thyroid (HPT) axis?

There has been much criticism that current testing strategies, for detecting thyroid disrupting chemicals, lack both specificity and sensitivity, and that the rodent assays currently employed may not necessarily predict outcomes in the human population (European Food Safety Authority/European Chemicals Agency 2016; European Union 2017).

A consequence of this debate has resulted in proposals for the greater incorporation of *in vitro* cell and protein binding assays, of basic molecular interactions of chemicals with components of the HPT axis, into the screening toolbox for detecting thyroid interfering chemicals (Murk et al 2013). There is no doubt that *in vitro* assays of various designs have advanced our basic understanding of biological processes immeasurably and they are an essential part in developing MoA and human relevance arguments, and in formulating adverse outcome pathways. However, the assays need to fill current gaps in the toolbox rather than simply adding unnecessarily to the already large list of testing assays needed to ensure the safe use of chemicals in society. The formulation of adverse outcome pathways is one way in which gaps in current understanding of thyroid hormone perturbation can be identified and novel assays naturally fit as part of the current TIER system where the primary tiers depend upon properly validated animal studies which can be supported in a TIER 2 testing strategy that uses the most appropriate assays for addressing problems raised following the TIER 1 assessment, as described in the draft GD. While there exist a plethora of various *in vitro* assays, their use as screens for chemical toxicity, in the absence of any hypothesis driven selection of the appropriate assay, could raise more questions than answers, generate unnecessary extra work to understand exactly what the *in vitro* screen is showing, and involve additional unnecessary experimentation to clarify what any

single *in vitro* screen might mean in the context of the biology of the whole animal (Colnot and Dekant 2017; Hulme and Trevithick 2010).

10.2. Are there refinements that can be made to current hormone assessments?

10.2.1. When to measure hormones?

Evidence suggests that for chemicals that depress thyroid hormones, the degree of depression gets progressively less as the duration of suppression increases (Yu et al 2002). This is thought to be due to a resetting of the homeostatic level at which the thyroid hormone feedback system works. This is particularly true of elevations in TSH that tend to occur shortly following exposure to thyroid affecting chemicals but which can return to normal values within weeks of exposure. The same effect is seen with the depression of serum T4 and T3 which tend to return to normal values on continued exposure. Hence the question arises regarding the most appropriate time for measuring these hormones and what consequences the reset system might have on the long-term health of the animal. It is clear that although hormone levels return to near normal levels on prolonged dosing, the system maintains this at a higher metabolic level than does the HPT axis in untreated animals and as such would be expected to induce a sustained level of stress that long term could increase the chances of developing diseases including thyroid cancer (Vansell et al 2004). Hence a careful evaluation of the right time, or right times, for evaluating thyroid hormone levels in animal studies is needed to avoid situations where morphological thyroid changes might be seen in the apparent absence of effects on thyroid hormones (Colnot and Dekant 2017). It is not within the scope of this review to recommend sampling times but in order to generate interpretable data, a thorough knowledge of the pharmacokinetics of the chemical under test, together with the known circadian control for some of the hormones is essential prerequisite for such studies.

10.2.2. Should thyroid hormone measures be incorporated into routine toxicity studies?

There have been suggestions that thyroid hormone measures could be incorporated into guideline toxicity studies as a routine rather than when indicated by a confirmed knowledge of a chemical class thyroid effect or some indication that the thyroid gland was a potential target. While there are clearly advantages to gaining the maximum amount of information from animal studies but there does need to be a measure of caution when advocating such additions. Thyroid hormone assays are not currently straightforward, and they require a degree of expertise not routinely present in many laboratories conducting guideline studies. Routine incorporation might well generate data that becomes uninterpretable without supporting evidence from histopathology and could throw up false positive data arising from inexperience of the conducting laboratory. Conducting laboratories need to have the necessary historical background data to understand the limits within normality that exist in laboratory animals. The timing of blood sampling for hormone measures needs to be carefully planned in the knowledge of the circadian control that some of the hormones operate under.

Lastly and perhaps most importantly, the occurrence of confounding factors, such as organ toxicity and reductions in food consumption that are frequently seen in toxicity studies, are known to affect thyroid hormone levels independent of any primary effect on the thyroid gland itself and ruling these factors out can be challenging without embarking on an extensive investigative study. There is also evidence of adaptation to thyroid inhibition where hormone depression is seen early in studies but which recover on prolonged exposures. For an unknown compound, the question would always arise as to when, in the conduct of the study, it might be appropriate to sample for potential thyroid hormone changes. Since these are likely to be different dependent upon the inhibitory MoA triggered, and the individual chemical involved, the routine incorporation of thyroid hormone measures to guideline toxicity studies presents almost insurmountable difficulties if it is to generate sensible and interpretable data that adequately assesses potential thyroid effects.

Histopathology of the thyroid gland is routinely carried out as part of acute, chronic and lifetime toxicity bioassays, and arguably is the most sensitive biomarker of thyroid hormonal disruption, at least when incorporated into a routine toxicity study (NTP 2002). Any sign of a thyroid effect by histopathology could then be best approached by undertaking specifically designed investigative *in vivo* and *in vitro*

toxicity studies to investigate potential thyroid effects by targeting the hormonal endpoints relevant to the thyroid.

10.3. Is the rat the appropriate species? Should we explore different animal models?

Various alternative animal models to the rat have been proposed for testing of both the potential carcinogenic and the developmental effects of chemicals disrupting the HPT axis. The inherent advantages of the rat as a model for human risk assessment refer back to our familiarity with the vagaries of the species and our extensive understanding of its similarities, and differences in terms of biology, to humans. Clearly alternative animal species, and *in vitro* and *ex vivo* systems, exist for assessing potential thyroid hormone disrupting properties but they all have drawbacks and limitations which are discussed in the relevant sections in this and other documents (Sadamatsu et al 2006; Brent 2012b).

10.3.1 The sheep as a model for human thyroid effects

There has been some discussion as to the sheep being a more appropriate animal model to represent human health hazard with regard to disruption of thyroid hormone homeostasis (European Union 1917; page 23) especially where neurodevelopmental toxicity is concerned. The study quoted to reinforce this statement was Leghait et al (2010) where fipronil, a compound known to produce significant thyroid hypertrophy in rats, was studied in sheep. The underlying reason for regarding the sheep as a more representative model for man, is based upon the greater similarity of thyroid binding protein in the plasma of sheep where TBG is the major serum transporter for thyroid hormones, whereas in the rat this protein is, to all intents and purposes, absent in the adult.

Leghait et al (2010) found that fipronil failed to produce thyroid toxicity in the sheep but the conclusions to the study were somewhat compromised because the formation of the sulphone active metabolite of fipronil was appreciable less in the sheep than it is in the rat. This failure highlights the problems of embarking on studies in species where the database is seriously limited, as in the case of the sheep.

The metabolism of thyroid hormones has been studied in the pregnant sheep, both in the dam and in the developing foetus (Fisher et al 1972) and the paper speculates that, on the basis of thyroid metabolism, the foetal sheep has greater similarity to human foetuses than does the rat system. While this may well be the case, the lack of detailed information on the other, not inconsiderable, variables determining chemical-induced thyroid toxicity, together with the impracticality of using such a large species for toxicity studies, compound supply alone would most likely deter such studies, would mean that at best it might be considered as a second species in place of the canine, but only after a significant period of data gathering on the current paucity of basic biochemical and biological parameters pertinent to the conduct of toxicity studies.

In spite of this drawback, a recent study has been reported that looked at the effect of exposing pregnant ewes to sewage sludge-fertilized pastures that succeeded in showing that pre-conceptual exposure of the dam increased the relative thyroid organ weights in male foetuses and decreased expression of the sodium iodide symporter in the thyroid glands (Hombach-Klonisch, et al 2013).

10.3.2. The contribution of knockout mice to understanding human relevance of thyroid hormone disruption

Several transgenic mouse models of thyroid cancer exist that contain various genes found to be overexpressed in human thyroid cancer, that are placed under the expression of the thyroglobulin (Tg)

promoter, and that subsequently allow thyroid-specific over expression of the specific transgene and the subsequent development of thyroid cancer (Kim and Zhu 2009).

Of particular relevance for potential species differences in response has been the MCT8 knockout mouse which has shown little neurodegenerative effects () despite the human condition affecting the MCT8 transporter, where serious and profound neurological pathologies are produced in the mutation of the MCT8 gene in Allan-Herndon-Dudley Syndrome (Dumitrescu et al 2004; Friesema et al 2004; Schwartz and Stevenson 2007). In knockout mice, lacking the MCT8 transporter, they fail to show motor deficits suggesting that alternate pathways for transporting thyroid hormones into the brain exist in this species (Di Cosmo et al 2010; Roberts et al 2008). It is now known that the organic ion transporter polypeptide-14 (OATP14) is the primary thyroid hormone transporter responsible (Friesma et al 2005) and OATP14 mRNA and protein is strongly expressed in both rat and mouse cerebral microvessels, but not in human. The high expression of OATP14 in the rodent brain, as compared with the human, is one explanation for the relatively mild neurophysiological consequences of deletion of the MCT8 transporter in Mct8-null mice. The absence of an alternate transporter in the developing human brain results in serious neurodevelopmental consequences seen in individuals carrying the mutated MCT8 gene.

RET/PTC gene rearrangements have been found to be consistently involved in human thyroid cancer, and transgenic mice carrying over-expression of this gene combination have indeed been found to develop thyroid carcinogenesis (Santoro et al 1996; Jhiang et al 1996). Several other genes, such as the NTRK1, BRAF and RAS genes, are also known to be involved in the development of human cancer, and while mice transgenically designed to overexpress these have been found to reciprocate the biology, morphology and behaviour of human thyroid cancer and to help in the understand the basic biology of the process (Knostman et al 2007; Knauf et al 2005; Vitagliano et al 2006), they have been singly unsuccessful in informing on the relative susceptibility of chemically induced thyroid cancer in either laboratory animals or in man simply because that was not what they were designed to do. Their whole purpose was to develop a model that could be used to study the development of human thyroid cancer with a view to better understand the pathophysiology and molecular biological changes leading to cancer.

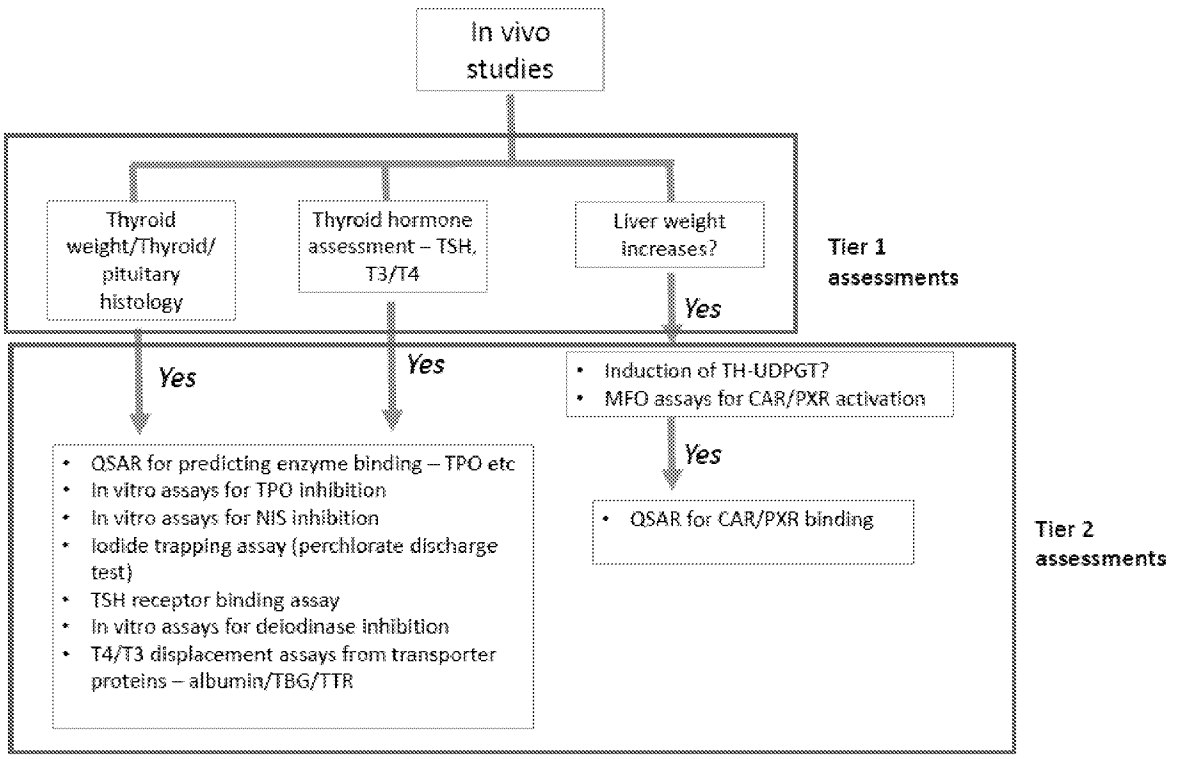
Type 2 iodothyronine deiodinase is essential for maintaining normal local concentrations of free T3 under different physiological and pathophysiological situations and has a critical role in the correct operation of the negative feedback control of TSH/TRH release from the pituitary and hypothalamus (Schneider et al 2001). In an attempt to better characterise the respective roles of the three iodothyronine deiodinase enzymes, knockout mice have been generated for each of the enzymes (Schneider et al 2001; 2006; Marsili et al 2011). Mice bearing the knocked-out gene for iodothyronine Type 2 deiodinase show incomplete development of the inner ear (Ng et al 2004), and bone and muscle malformations resulting from a deficiency in the local conversion of T3 in cochlear cells, myoblasts and osteoblasts respectively (Dentice et al 2010; Bassett et al 2010). The type 2 deiodinase KO mouse also shows impaired embryonic development of brown adipose tissue, and as a consequence the mice suffer a permanent thermogenic defect (Hall et al 2010; de Jesus et al 2001) which leaves them susceptible to hypothermia during cold challenge, and a greater susceptibility to diet-induced obesity at ambient temperatures (Castillo et al 2011).

11. A proposal for a testing strategy for assessing thyroid disrupting effects of a chemical

In the event of a chemical inducing thyroid hypertrophy/hyperplasia, or measured decreases in circulatory thyroid hormones, in rodent toxicity studies, a mode of action analysis and human relevance framework in the context of the recommended IPCS guidance should be conducted (Boobis et al. 2006) together with an adverse outcome pathway constructed to identify missing data from the proposed pathway as recommended by Vinken et al (2017).

In terms of possible thyroid effects mediated via increased clearance through the liver, the testing strategy outlined in Fig. 16 below addresses all known thyroid modes of action, and suggests a way of ruling out alternative MOAs through experimentation in appropriate assays.

Fig. 16: Suggested testing strategy to confirm a thyroid mode of action



If either of the three tier 1 effects are seen *in vivo* studies in rodents, additional assessments (new studies may have to be carried out) would then be commissioned as tier 2 assessments to specifically investigate the proposed mode of action and/or eliminate alternative modes of action. The elimination of alternative pathways is a necessary part of the IPCS process and while validated *in vitro* assays do not currently exist for all of the endpoints listed in the left-hand box, experimental *in vitro* cell assays are available for all of the endpoints listed. The proposed mode of action would need to be subject to Bradford Hill causality/association test to verify its strength, and then the human relevance framework would be applied by answering the three pivotal questions as described in Meek et al (2013):

1. Is the weight of evidence sufficient to establish a MOA in animals?
2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental qualitative differences in key events between animals and humans?
3. Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between animals and humans?

The establishment of a plausible MOA would identify some mechanisms which are known to have either qualitative species differences between rodents and humans, or quantitative species differences whereby humans have been shown to be more or less sensitive to the toxicity seen with the given chemical. An example of the latter is the activation of the CAR/PXR nuclear receptor in the rodent whereby induction of hepatic UDPGT leads to an increased rate of biliary excretion of T4 through glucuronidation, a resultant decrease in circulating T4, and a compensatory hypertrophy/hyperplasia of the thyroid follicular epithelium through TSH stimulation (Dellarco et al

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2006). While human liver does contain the CAR/PXR receptor, there are significant quantitative differences that make activation considerably less sensitive in human than it is in the rodent with considerably higher dose levels need to produce the same effect.

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Serum Half-Lives for Short- and Long-Chain Perfluoroalkyl Acids after Ceasing Exposure from Drinking Water Contaminated by Firefighting Foam

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Background: Firefighting foam–contaminated ground water, which contains high levels of perfluoroalkyl substances (PFAS), is frequently found around airports. In 2018 it was detected that employees at a municipal airport in northern Sweden had been exposed to high levels of short-chain PFAS along with legacy PFAS (i.e., PFOA, PFHxS, and PFOS) through drinking water.

Objectives: In this study, we aimed to describe the PFAS profile in drinking water and biological samples (paired serum and urine) and to estimate serum half-lives of the short-chain PFAS together with legacy PFAS.

Methods: Within 2 weeks after provision of clean water, blood sampling was performed in all 26 airport employees. Seventeen of them were then followed up monthly for 5 months. PFHxA, PFHpA, PFBS, PFPeS, and PFHpS together with legacy PFAS in water and biological samples were quantified using LC/MS/MS. Half-lives were estimated by assuming one compartment, first-order elimination kinetics.

Results: The proportions of PFHxA, PFHpA, and PFBS were higher in drinking water than in serum. The opposite was found for PFHxS and PFOS. The legacy PFAS accounted for about 50% of total PFAS in drinking water and 90% in serum. Urinary PFAS levels were very low compared with serum. PFBS showed the shortest half-life {average 44 d [95% confidence interval (CI): 37, 55 d]}, followed by PFHpA [62 d (95% CI: 51, 80 d)]. PFPeS and PFHpS showed average half-lives as 0.63 and 1.46 y, respectively. Branched PFOS isomers had average half-lives ranging from 1.05 to 1.26 y for different isomers. PFOA, PFHxS, and linear PFOS isomers showed average half-lives of 1.77, 2.87, and 2.93 y, respectively.

Discussion: A general pattern of increasing half-lives with increasing chain length was observed. Branched PFOS isomers had shorter half-lives than linear PFOS isomers. https://doi.org/10.1289/EHP6785

Introduction

Perfluoroalkyl substances (PFAS) are persistent man-made chemicals. Drinking-water sources have been contaminated around production facilities, airports, and air force bases worldwide, affecting employees drinking the polluted water as well as the general population in adjacent communities (Domingo et al. 2012; Fromme et al. 2009). Efforts have been made to reduce the emission of PFAS into the environment through the phase-out of most long-chain PFAS, especially perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) and related compounds, by voluntary and regulatory actions over the last two decades. Among the PFAS, long-chain homologs of perfluorinated carboxylic acids (PFCAs) have eight or more carbons in the carbon chain, and among perfluorinated sulfonic acids (PFSAs), a carbon chain length of six carbons or more (ITRC 2020). Due to their persistence and bioaccumulation capability, however, PFAS exposure and their associated potential toxic effects will exist long term. To address potential adverse health effects, exposure, epidemiology, and experimental studies have mainly focused on the long-chain legacy PFOA and PFOS (EFSA 2018). Less is known about short-chain PFAS and more complex structures that are now suggested as replacements by industry due to their lower bioaccumulation potential (Buck et al. 2011). There are very little data on human exposure levels and health effects of short-chain PFAS.

Serum elimination half-lives of PFOA, PFOS, and perfluorohexane sulfonic acid (PFHxS) have been estimated in a general Swedish population with high PFAS exposure through drinking water when biomonitoring started 6 months after clean water was provided (Li et al. 2018). The average half-lives were estimated to be 2.7 y for PFOA, 3.4 y for PFOS, and 5.3 y for PFHxS, with marked interindividual variation (Li et al. 2018). The estimates were in the same range as reported by others (Brede et al. 2010; Olsen et al. 2007; Worley et al. 2017). To date, studies on the serum half-lives of short-chain PFAS are very scarce. Olsen et al. (2009) reported 25.8 d as a half-life of perfluorobutane sulfonic acid (PFBS, C4) based on data from 7 occupationally exposed workers followed up to 180 d. For perfluorohexanoic acid (PFHxA, C6) and perfluoroheptanoic acid (PFHpA, C7), the apparent elimination half-lives were reported to be 14–49 d (Russell et al. 2013) and 70 d (Russell et al. 2015a) in 11 occupationally exposed subjects. A more rapid elimination of shorter-chain PFAS has been attributed to weaker affinities for renal transport proteins responsible for reabsorption and higher water solubility (Han et al. 2012).

Arvidsjaur, a small municipality in the northern part of Sweden, has one municipal waterworks for residential drinking-water supply. The regional airport, however, has its own water supply (Figure 1). In mid-August 2018, PFAS for the first time was included in the regular water quality surveillance. A first report obtained on 31 August 2018 indicated that the drinking water was contaminated with PFAS at both the municipality waterworks and at the airport. Warnings to not drink or cook with tap water were immediately issued and clean water from tanks was immediately supplied. However, confirmatory samples from 3 September 2018 showed that only the airport drinking water was contaminated and that the municipal drinking water had very low levels of PFAS (Table 1).

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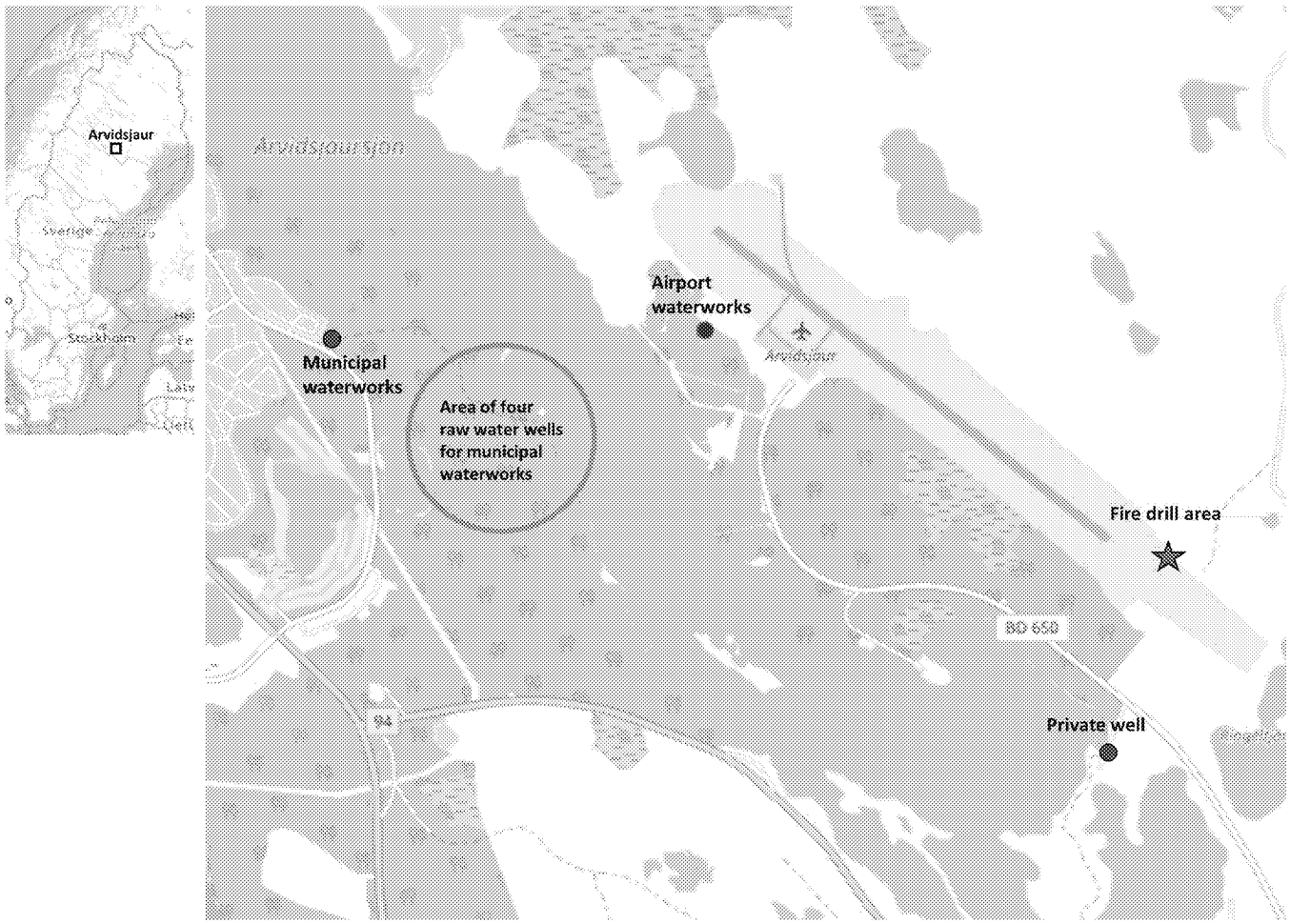

Figure 1. Map of Arvidsjaur municipality, Sweden, and the regional airport, with information of fire drill area, two waterworks, the private well, and area of four raw-water wells for municipal waterworks. Background map data: © OpenStreetMap contributors, CC BY-SA.

The source of contamination of the airport drinking water was assumed to be from firefighting foam. Notably high concentrations of short-chain PFAS, such as perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), PFHxA, PFBS, and perfluoropentane sulfonic acid (PFPeS), were found in addition to the legacy PFOA, PFHxS, and PFOS.

Our research group was contacted by the Arvidsjaur municipality. Biomonitoring of all employees at the airport was initiated 11 d after PFAS-free drinking water was supplied to the airport. This population was considered highly relevant for a study on the elimination of short-chain PFAS because the subjects were recruited almost immediately after the abrupt end of the exposure.

In the present study, we aimed to *a*) describe the PFAS profile in drinking water as well as in paired serum and urine samples, and *b*) estimate serum half-lives of short-chain PFAS including PFHxA, PFHpA, PFBS, and PFPeS and the long-chain perfluoroheptane sulfonic acid (PFHpS), along with legacy PFOA, PFHxS, and PFOS, but distinguishing between linear and branched PFOS.

Material and Methods

Study Setting

The regional airport in Arvidsjaur was established in 1990. During a few years in the early 2000s, pilots were trained at the airfield and the number of yearly landings rose to 20,000. Thereafter, the traffic was reduced markedly and has stabilized to around 1,200 landings per year. The fire drill area at the airfield is used by the airport firefighting staff and by the municipal fire brigade. No accidents with fires have occurred at the airport.

Immediately after the drinking-water contamination was revealed on 31 August 2018, clean bottled water was supplied to the employees and passengers at the airport until granular activated carbon filters were installed for tap water filtration after about 2 months. Drinking-water quality was regularly checked afterward, and no elevated levels of PFAS were further noticed.

Study Population

All 26 employees at the airport were invited for a blood sampling between 11 and 14 September 2018 (i.e., within 11 to 14 d after the termination of contaminated drinking-water exposure). Information on age, home address, employment history, working tasks, and sick leave and vacation days in August and September were collected by questionnaire. In addition, data on the number of glasses of water consumed per day, local fish consumption, and history of blood donation and medication were collected. For female employees, questions about menstruation, pregnancy, and duration of breast-feeding were asked.

Because municipal drinking water did not show elevated PFAS levels (Table 1), we are confident that there was no longer ongoing drinking-water exposure at home as long as people had no other source of drinking water. Based on the linkage of self-reported home address and municipal waterworks information,

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Table 1. Levels of measured PFAS in the airport drinking water, municipal drinking water, and from the private well.

PFAS (chain length)	Airport drinking water (ng/L)[a]	Municipal drinking water (ng/L)[a]	Drinking water from private well[a]
Perfluorinated carboxylic acids (PFCAs)			
PFBA (C4)	60	<0.6	2.2
PFPeA (C5)	180	<0.6	0.63
PFHxA (C6)	330	<0.3	0.84
PFHpA (C7)	97	<0.3	0.33
Linear PFOA (C8)	210	<0.3	0.53
Branched PFOA (C8)	88	<0.3	<0.3
Total PFOA (C8)	300	<0.3	0.53
PFNA (C9)[b]	<0.6	<0.6	<0.6
PFDA (C10)[b]	<0.6	<0.6	<0.6
PFUnDA (C11)[b]	<2	<2	<2
PFDoDA (C12)[b]	<2	<2	<2
Perfluorinated sulfonic acids (PFSAs)			
PFBS (C4)	200	<0.3	0.5
PFPeS (C5)	180	<0.3	<0.3
PFHxS (C6)	710	0.32	4.6
PFHpS (C7)	16	<0.3	<0.3
Linear PFOS (C8)	62	<0.2	1.7
Branched PFOS (C8)	64	<0.2	0.24
Total PFOS (C8)	130	<0.2	1.9
Fluorotelomer sulfonic acid			
[6:2 FTS (C6)]	6.6	<0.3	<0.3
Sum of 11 PFAS[c]	2,000	<5	12

Note: PFAS, perfluoroalkyl substances; PFBA, perfluorobutanoic acid; PFBS, perfluorobutane sulfonic acid; PFDA, perfluorodecanoic acid; PFDoDA, perfluorododecanoic acid; PFHpA, perfluoroheptanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFPeA, perfluoropentanoic acid; PFOS, perfluorooctane sulfonic acid; PFPeS, perfluoropentane sulfonic acid; PFUnDA, perfluoroundecanoic acid; 6:2 FTS, fluorotelomer sulfonic acid.

[a]The data were generated from the reports of Arvidsjaur municipality, updated, and shared with authors in December 2019. Multiple water samples were taken at different locations from 31 August to 3 September 2018. We reported results from water samples obtained on 3 September 2018.

[b]Levels of PFNA, PFDA, PFUnDA and PFDoDA were below detection levels and not further included in the statistical analysis in this paper.

[c]Sum of 11 PFAS: PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS, PFOS, 6:2 FTS.

we were able to identify private wells. One airport employee living in the vicinity of the airport had a private well (Figure 1) with low PFAS levels in the water (Table 1).

The PFAS analysis in serum from the first sampling was immediately performed and personal results were reported to the employees. Most of the employees showed elevated serum PFAS levels, especially for short-chain PFAS. Twenty-one employees with PFBS level above the limit of detection (LOD) were invited for a 4-month follow-up (from 16 October 2018 to 22 January 2019) and 17 volunteered. These 17 employees submitted four rounds of blood and first morning urine samples in parallel, at 1-month intervals. In total, there were five rounds of serum sampling (started from September) and four rounds of urine sampling (from October). For modeling of elimination, all samples were reanalyzed in a single batch. We obtained 83 serum samples with a median count of 5 (range: 4–5) samples per employee, and 59 urine samples with median of 4 (range: 1–4) samples per employee. Only one employee provided a single urine sample. The study was approved by the Regional Ethical Review Board in Lund, Sweden. All employees provided written informed consent.

For comparison with Swedish general background PFAS serum levels, we referred to the PFAS levels observed in a reference population from Karlshamn, a municipality in southern Sweden without PFAS contamination in the municipal drinking water. In 2016, 226 individuals were sampled. Fifty-nine individuals were randomly selected for analyzing other short-chain PFAS than the legacy ones, using the same modified method as for the airport employees. Fifty-eight individuals within the same age range as the airport employees (i.e., >22 years of age) were included in the comparison. For a detailed description of this population see Li et al. (2018).

Chemical Analysis

PFAS analysis in drinking water. Drinking-water sampling and analyses were performed by a commercial water analysis company, SYNLAB Analytics & Services (SYNLAB). The first water samplings of outgoing drinking water from the municipal waterworks and from the airport waterworks were performed on 15 August 2018. High levels of PFAS in water samples from both locations were reported on 31 August 2018. Resampling was immediately performed at different locations from 31 August to 3 September 2018, revealing that only the airport drinking water was contaminated while the municipal outgoing drinking water had a sum of 11 PFAS [i.e., PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS, PFOS, fluorotelomer sulfonic acid (6:2FTS)] that was <5 ng/L (Table 1). The reasons for the mistake on 15 August 2018 were never clarified. For the locations of water sampling, see Figure 1. In all, there were five separate water samples from the municipal raw and outgoing drinking water and six samples from the airport during this 2.5-week period. We here report results from the water samples obtained on 3 September 2018.

PFAS analysis in drinking water was performed according to German standard methods for determination of selected polyfluorinated compounds in water using liquid chromatography–tandem mass spectrometry (LC/MS/MS) after solid–liquid extraction (F 42) (DIN 38407-42) (German Institute for Standardization 2011). SYNLAB is accredited by SWEDAC (the national accreditation body of Sweden) according to SS-EN ISO/IEC 17025. At our request, SNYLAB expanded their report from the original 11 PFAS to 15 PFAS in December 2019. The 15 PFAS were PFBA, PFPeA, PFHxA, PFHpA, PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), PFBS, PFPeS, PFHxS, PFHpS, PFOS, and 6:2 FTS. The LODs for PFAS in water sample are listed in Table S3.

PFAS analysis in serum and urine samples. Venous whole blood samples were obtained and were centrifuged for 10 min at $1{,}500 \times g$ at room temperature to isolate the serum on site. The serum samples were collected in 6-mL BD Vacutainer® Plus plastic serum tubes without gel (BD). Urine samples were collected in 13-mL polypropylene screw cap tubes (Sarstedt). Serum and urine samples were frozen at −20°C after sampling and transported to Lund using cold chain logistics. Serum and urine samples were stored at −80°C and −20°C, respectively, before analysis.

Chemicals. An overview of the PFAS analytes and internal standards with abbreviations is given in Table S1. All native and isotopically labeled standards (PFAC-MXC, MPFAC-C-ES, P1MHpS, P5MHpS, and P6MHpS) were purchased from Wellington Laboratories as diluted reference standards. PFAC-MXC was used for the native PFC stock solution. Stable isotope–labeled PFC Standards Solution MPFAC-C-ES was used for internal standards. To quantify the branched PFOS, methanol solutions of sodium perfluoro-1-methylheptane sulfonate (P1MHpS; 1m-PFOS), sodium perfluoro-5-methylheptane sulfonate (P5MHpS; 5m-PFOS), and sodium perfluoro-6-methylheptane sulfonate (P6MHpS; 6m-PFOS) were used.

Acetonitrile (high-performance liquid chromatography grade), ammonium acetate and methanol (LC-MS grade) were purchased

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from Merck. Water was purchased from a Milli-Q Integral 5 system (Millipore).

Instrumentation. Quantitative analysis was conducted using triple quadrupole linear ion trap mass spectrometer equipped with TurboIonSpray sources (QTRAP 6500+; AB Sciex) coupled to an LC/MS/MS system (UFLCXR; Shimadzu Corporation). Nitrogen was used as the nebulizer, auxiliary, curtain, and collision gas. The MS analyses were carried out using selected reaction monitoring (SRM) in negative ion mode. The SRM parameters for the data acquisition are shown in Table S2. All data acquisition was performed using Analyst® software (version 1.6.3; AB Sciex), and data processing was performed using MultiQuant™ software (version 2.1; AB Sciex).

Sample preparation: Calibration standards, quality control samples and chemical blanks. Standard solutions were prepared by further dilution of diluted reference standards in methanol. For the calibration standards, the blank matrix, fetal bovine serum, (FBS; Gibco) was used for the serum samples. Human normal urine obtained from the colleagues at our lab was used for the urine analysis. These were prepared in the same way as the samples, except for the addition of 25 μL of the diluted standard solutions.

For serum, three quality control (QC) samples (QC1, QC2, and QC3) were prepared in-house. QC1 was prepared by pooling equal volumes of three serum samples containing different levels of PFAS. Similarly, QC2 was prepared using another three samples and pooled with equal volumes. QC3 was prepared with additional spiking with PFAS standard solutions. For urine, one QC sample was used, and the sample was prepared by pooling equal volumes of three urine samples from individuals with high levels of PFAS in serum.

Chemical blanks were prepared from Milli-Q water and were prepared in the same way as the samples. Two sets of each QC samples and three sets of chemical blanks were added to each batch, where one batch corresponded to a 96-well plate.

Sample preparation: Preparation of serum samples. The serum samples, QC samples, and chemical blanks were prepared in 96-well plates with 1.5-mL flat-bottom glass vials (Biotech solutions). Serum samples were thawed for 1–2 h and mixed on a vortex shaker for approximately 10 s two times. Once after all samples were thawed and a second time before pipetting the serum to the plate. Before pipetting, the tip was pre-wetted several times, drawing a volume into the tip and dispensing it back into the tube. Sample preparation procedures for serum samples consisted of 25 μL serum mixed with 25 μL water, 25 μL methanol, 25 μL isotopically labeled internal standards in 50% acetonitrile (see Table S1), and 100 μL acetonitrile. Thereafter, samples were mixed vigorous by shaking for 30 min and centrifuged at $2{,}600 \times g$ for 10 min at room temperature. The supernatant (0.2 mL) was transferred to 96-well plates with 0.5-mL conical glass vials (MicroLiter Analytical Supplies) and again centrifuged at $3{,}000 \times g$ for 10 min before analysis. The QC samples and chemical blanks were prepared following the same procedure as the serum samples. The calibration standards were prepared in FBS the same way as the samples, except for the addition of diluted reference standards of all analyzed compounds in 25 μL of the methanol solution.

Aliquots of 4 μL of the sample were analyzed using LC/MS/MS. For the analysis, a C18 column (4 μm, 2.1 mm i.d. × 50 mm, Genesis Lightning) was used prior to the injector to filter the mobile phases. The analytical column was an Aquity BEH (1.7 μm, 2.1 mm i.d. × 100 mm Waters). The mobile phases (A and B) were A: 5 mM ammonium acetate in water:methanol (50:50); and B: methanol. The mobile phase was kept at 0% B for 2.5 min after injection. A gradient was then applied over 11 min to 90% B, where it was then kept for 1.5 min. The column was then conditioned at 0% B for 2 min. A diverter valve was used, and the column effluent was diverted to the MS between 1.1 and 14.0 min. The flow rate was 0.32 mL/min, and the column was maintained at 60°C. The analysis was performed in negative ion mode. The ion source temperature was at 350°C.

Sample prepration: Preparation of urine samples. The urine samples, QC sample, and chemical blanks were prepared in 96-well plates with 0.5-mL conical glass vials (MicroLiter Analytical Supplies). The samples were thawed 1–2 h, then mixed by vortex shaker and manual turning. Before pipetting, the tip was pre-wetted several times, drawing a volume into the tip and dispensing it back into the tube. For the urine samples, 50 μL urine was mixed with 25 μL of methanol and 25 μL of a 50% acetonitrile solution containing isotopically labeled internal standards (see Table S1). Then samples were shaken vigorously for 30 min and centrifuged at $3{,}000 \times g$ for 10 min at room temperature. The QC sample and chemical blanks were prepared following the same procedure as the urine samples. Calibration standards were prepared in blank urine, the same way as the samples, except for the addition of diluted reference standards of all analyzed compounds in the 25 μL of methanol solution.

Aliquots of 20 μL of the sample were analyzed using LC/MS/MS. For the analysis, a C18 column (4 μm, 2.1 mm i.d. × 50 mm, Genesis Lightning) was used prior to the injector to filter the mobile phases. The analytical column was an Aquity BEH (1.7 μm, 2.1 mm i.d. × 100 mm Waters). The mobile phases were A: 5 mm ammonium acetate in water:methanol (50:50); and B: methanol. The mobile phase was kept at 0% B for 2.5 min after injection. A gradient was then applied over 11 min to 90% B, where it was then kept for 1.5 min. The column was then conditioned at 0% B for 2 min. A diverter valve was used, and the column effluent was diverted to the MS between 1.1 and 14.0 min. The flow rate was 0.32 mL/min, and the column was maintained at 60°C. The analysis was performed in negative ion mode. The ion source temperature was at 350°C.

Quantification. The concentrations of PFAS were determined by peak area ratios between analyte and internal standard. The quantifier transitions are shown in Table S2. The total, nonisomer-specific compounds for all PFAS except for PFOS are reported. For the branched PFOS, 1m-PFOS could be separated from the other isomers and evaluated using a calibration curve of 1m-PFOS. Perfluoro-2/6-methylheptanesulfonate (2/6m-PFOS) could not be separated, thus the sum of 2/6m-PFOS was evaluated using a calibration curve of 6m-PFOS. The sum of perfluoro-3/4/5-methylheptanesulfonate (3/4/5m-PFOS) was evaluated using a calibration curve of 5m-PFOS.

The limits of detection (LODs) were determined as the concentrations corresponding to the average plus three times the standard deviation of the concentrations in chemical blank samples. The LODs for each PFAS in serum and urine samples, respectively, are shown in Table S3. The results of three QC samples used in serum analysis are listed in Table S4.

The analyses of PFOS and PFOA are part of a QC program between analytical laboratories coordinated by H. Drexler, Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University of Erlangen-Nuremberg, Germany. The laboratory also participated in the European Human Biomonitoring Initiative (HBM4EU) QA/QC program, and its successful performance has resulted in its qualification as an HBM4EU laboratory for the analysis of PFPeA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, PFHxS, PFHpS, and PFOS.

In this paper, we excluded PFPeA, PFNA, PFDA, PFUnDA, and PFDoDA from further analysis. The level of PFPeA was below LOD in all serum samples. The levels for the other PFAS were below LOD in the water sample, indicating that exposure

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Table 2. Description of the 26 airport employees and PFAS levels measured in the first serum samples, together with PFAS levels measured in the reference group.

Categories	Employees participated in first blood sampling only	Employees participated in first blood sampling and repeated blood and urine sampling	Reference group[a]
Basic characteristics			
Counts (*N*)	9	17	58
Female [*N* (%)]	1 (11)	6 (35)	37 (64)
Age [median (range)]	33 (22–61)	50 (24–62)	34 (22–49)
Years of current employment [median (range)]	2 (1–27)	10 (1–28)	NA[b]
Total working days from 1 August to 11 September 2018 [median (range)]	29 (1–40)	39 (20–42)	NA
Pregnancy previously [yes (%)]	1 (100)	2 (33)	NA
Year of last pregnancy	2018	2010 and 1999	NA
Month of breast-feeding (median)	6	Both 6	NA
PFCAs in first serum sample {ng/mL [median (range)]}[c]			
PFHxA (C6)	0.38 (0.23–1.1)	0.37 (0.16–0.92)	NA
PFHpA (C7)	0.17 (0.07–1.3)	0.53 (0.20–2.2)	NA
PFOA (C8)	6.5 (2.9–11)	13 (5.0–31)	1.5 (0.26–4.6)
PFSAs in first serum sample {ng/mL [median (range)]}			
PFBS (C4)	0.10 (<LOD–1.0)[d]	0.46 (0.22–1.3)	NA
PFPeS (C5)	3.5 (1.4–11)	7.6 (3.6–17)	<LOD (<LOD–0.02)
PFHxS (C6)	60 (17–85)	133 (21–402)	0.59 (<LOD–1.2)
PFHpS (C7)	0.97 (0.28–1.9)	1.6 (0.49–6.2)	<LOD (<LOD–0.16)
L-PFOS (C8)	6.5 (5.4–13)	11 (5.5–28)	3.0 (0.30–9.8)
1m-PFOS (C8)	0.52 (0.25–2.7)	1.0 (0.32–4.1)	0.18 (<LOD–0.59)
3/4/5m-PFOS (C8)	2.9 (1.2–6.7)	4.9 (1.7–17)	0.79 (0.10–2.2)
2/6m-PFOS (C8)	1.8 (0.84–3.2)	2.6 (1.0–7.5)	0.46 (<LOD–1.4)

Note: L-PFOS, linear perfluorooctane sulfonic acid; LOD, limit of detection; PFAS, perfluoroalkyl substances; PFBS, perfluorobutane sulfonic acid; PFCA, perfluorinated carboxylic acid; PFHpA, perfluoroheptanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexane sulfonic acid; PFOA, perfluorooctanoic acid; PFPeS, perfluoropentane sulfonic acid; PFSA, perfluorinated sulfonic acid; 1m-PFOS, branched, perfluoro-1-methylheptanesulfonate; 2/6m-PFOS, branched, sum of perfluoro-2/6-methylheptanesulfonate; 3/4/5m-PFOS, branched, sum of perfluoro-3/4/5-methylheptanesulfonate.

[a]The reference group was from Karlshamn, Sweden, a municipality without PFAS contamination in drinking water (Li et al. 2018). This group was sampled in 2016. A subset of 59 individuals were randomly selected for analyzing other short-chain PFAS than the legacy ones using the same method as the airport employees, and 58 within the same age range as the airport employees (i.e., 22–62 years of age) were included in the comparison.

[b]No data available or PFAS was not measured.

[c]PFBA and PFPeA were elevated in the drinking water (Table 1) but below the LOD in all the serum samples, so they were not presented in the present paper.

[d]LODs are listed in Table S3.

through the drinking water was negligible, and therefore not relevant for half-life estimation in the present study.

Calculated Variables

The ratio of serum and water concentration for each PFAS (serum/water ratio) was calculated based on median serum level in the first serum sample obtained from all 26 airport employees divided by the drinking-water concentration. This ratio is an indicator of PFAS accumulation in body.

Urinary PFAS concentrations were specific-gravity adjusted. The ratio of urine and serum concentration for each PFAS (urine/serum ratio) at each sampling was calculated based on PFAS concentrations in paired serum and urine samples from 17 employees. Then the personal average urine/serum ratio was calculated using the mean value of repeated samplings for each employee.

Modeling of PFAS Elimination and Estimating of Half-Life

Serum PFAS concentrations were log-transformed. Time was calculated as the elapsed days between supply of clean bottled water and first blood sampling. We assumed one compartment, first-order elimination kinetics. In this kinetic model, the body is considered as one homogeneous volume in which mixing is instantaneous and from which PFAS could be absorbed, transferred, and eliminated according to the rate of elimination that is proportional to the concentration of PFAS in the body. Two methods were used to estimate serum elimination rate constant (k) and half-life for each PFAS:

Method 1: For estimation of the population average k, a linear mixed model was used as reported previously (Li et al. 2018) but without the random slope in the model. Briefly, in the linear mixed model

$$\ln C_{ij} = \alpha_i + t_{ij}\beta + X_i\beta' + \varepsilon_{ij},$$

C_{ij} is the serum concentration of PFAS for individual i at sampling round j; α_i is the subject-specific intercept (random intercept); t_{ij} is the time; β is the average slope; X_i is a vector of fixed covariates for individual i; β' stands for the fixed effect coefficients; and ε_{ij} is the random error term. We modified the model by excluding the subject-specific slope (random slope) due to a failure to converge after inclusion, which may be due to the limited number of study subjects. The negative value of the average slope ($-\beta$) is the population average k, and average half-life is calculated as $\ln 2/k$.

In the linear mixed model, we included only age and sex as covariates. We based covariate selection on factors that could conceivably confound the PFAS half-life estimation. Working task, duration of employment, number of glasses of water per day, blood donation, and pregnancy before mitigation of PFAS exposure from drinking water can influence the initial serum levels but would not be expected to influence the PFAS half-life. Medication use was checked, and no one reported usage of cholestyramine [a medication that influences PFAS elimination (Genuis et al. 2010, 2013)], therefore, medication use was not included in the model.

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A sensitivity analysis was performed using the same linear mixed model but with C_{ij} as the serum levels after subtracting general background levels (using the median levels obtained from the nonexposed reference population). If the serum PFAS levels after subtraction were lower than the half of the background levels, they were replaced with half of the background levels.

Method 2: For estimation of individual k, linear regression was used to derive the slope (β) of the logarithm of serum concentrations vs. time for each employee. The negative value of the slope ($-\beta$) for each individual was equivalent to the individual k, and individual serum elimination half-life was calculated as $\ln 2/k$ for each PFAS. If β was positive (i.e., PFAS concentrations tended to increase over the five samplings), the half-life was not calculated.

For comparison of PFAS initial levels between sexes, the Mann-Whitney U-test was used on original PFAS scales. All statistical analyses were performed in IBM SPSS (version 25.0; IBM).

Results

Basic Characteristics for the Study Population

Table 2 shows the basic characteristics and initial serum concentrations of PFAS for the employees who attended only the first sampling and those who attended repeated samplings, together with the corresponding background PFAS levels observed in the reference population (Li et al. 2018). PFHxS showed the highest serum concentration in the airport employees, with a median level of about 102–225 times higher than the level observed in the reference population. In addition, the median PFPeS concentration, although lower than PFHxS, was about 175–380 times higher than the maximum level in the reference population. As expected, the nine employees not included (five of them were not invited) in the further sampling showed much lower serum levels of short-chain PFAS (i.e., PFHpA, PFBS, PFPeS, and PFHpS), as well as of legacy PFOA, PFHxS, and PFOS.

The initial PFAS levels for males and females are listed in Table S5. There was no difference between sexes for most of the measured PFAS. Only PFHxA showed higher levels in males than females.

Comparison between PFAS Levels in Drinking Water and in First Serum Samples

The different patterns of PFAS levels in the drinking water and in serum are illustrated in Figure 2, expressed on a molar-concentration basis, and in Figure S1 on a weight-concentration basis. The corresponding summary data are listed in Table S6. The proportions of short-chain PFHxA and PFBS were higher in the water (20% and 13%, respectively) than in serum (0.4% for both). In contrast, PFHxS and PFOS (linear and branched) showed higher proportions in serum (70% and 5–7%, respectively) than in drinking water (34% and around 2.4%, respectively). In total, the legacy PFOA, PFHxS, and PFOS accounted for about 50% of total PFAS in water and 90% in serum.

Levels of PFAS measured in the drinking water at the airport and in the first serum samples taken from all 26 employees at the airport, together with the calculated serum/water ratio, are given in Table 3. For both PFCAs and PFSAs, an increasing serum/water ratio with increasing carbon chain length is suggested, indicating more bioaccumulation in the human body for the longer-chain PFAS.

Urine Levels of PFAS and Urine/Serum Ratios

Table 4 presents the descriptive analysis of PFAS concentrations in serum and urine samples for all samplings. In general, the urine concentrations of PFAS were very low compared with

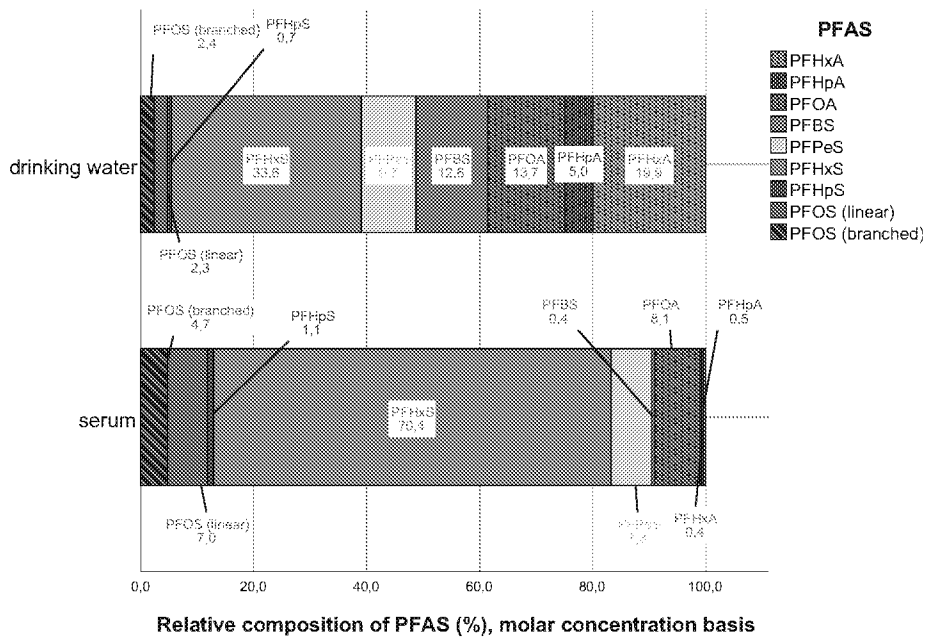

Figure 2. Composition (molar-concentration basis) of perfluorinated carboxylic acids (PFCAs, dotted bars) and perfluorinated sulfonic acids (PFSAs, open bars; branched PFOS in hatched bar) in the airport drinking water and in the first serum samples obtained from the 26 airport employees (detailed data are presented in Table S6). PFCAs and PFSAs with same number of carbons in the carbon chain (i.e., PFHxA vs. PFHxS, PFHpA vs. PFHpS, and PFOA vs. PFOS) are illustrated with the same color. The relative composition (percentage) for each PFAS is embedded in the box. Although PFBA and PFPeA were elevated in the drinking water, they were below the LOD in all the serum samples, so they were not presented. Note: PFAS, perfluoroalkyl substances; PFBS, perfluorobutane sulfonic acid; PFHpA, perfluoroheptanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexane sulfonic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFPeS, perfluoropentane sulfonic acid.

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Table 3. PFAS Levels measured in the airport drinking water and in the first serum samples from all 26 airport employees.

PFAS	Airport drinking water (ng/mL)	Serum level in the first samples {ng/mL [median (range)]}	Serum/ water ratio[a]
PFCAs[b]			
PFHxA	0.33	0.38 (0.16–1.1)	1.15
PFHpA	0.097	0.46 (0.07–2.2)	4.74
PFOA	0.30	9.1 (2.9–31)	30.3
PFSAs			
PFBS	0.20	0.33 (<LOD–1.3)	1.65
PFPeS	0.18	6.9 (1.4–17)	38.3
PFHxS	0.71	76 (17–402)	107
PFHpS	0.016	1.3 (0.28–6.2)	81.3
L-PFOS	0.062	9.5 (5.4–28)	153
B-PFOS	0.064	6.4 (2.2–28)	100

Note: B-PFOS, branched PFOS; L-PFOS, linear PFOS; LOD, limit of detection; PFAS, perfluoroalkyl substances; PFBS, perfluorobutane sulfonic acid; PFCA, perfluorinated carboxylic acid; PFHpA, perfluoroheptanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexane sulfonic acid; PFOA, perfluorooctanoic acid; PFPeS, perfluoropentane sulfonic acid; PFSA, perfluorinated sulfonic acid.
[a]Serum/water ratio was calculated as median serum level divided by drinking-water level.
[b]PFBA and PFPeA were elevated in the drinking water but below the LOD in all the serum samples, so no ratio is presented.

serum levels. The urinary PFAS levels for each sampling are presented in Table S7 and illustrated in Figure S2.

For each PFAS compound, there was a substantial variation of the urine/serum ratio within and between individuals over the follow-up time. The variation for PFPeS (representing short-chain PFAS) and L-PFOS (representing long-chain PFAS) is illustrated in Figure S3. Overall, there was no consistent pattern of variation over time. At the group level, the urine/serum ratio was highest for PFHpA, followed by PFPeS and 2/6m-PFOS, indicating that short-chained PFAS and 2/6m-PFOS are excreted more rapidly through the urine, whereas PFOA, PFHxS, and L-PFOS are excreted more slowly through the urine (Table 4). In general, for both PFCAs and PFSAs, there was a decreased urine/serum ratio together with an increased serum/water ratio with the increment of chain length.

Table 4. PFAS Levels measured in the paired serum and urine samples obtained from the second to the fifth samplings from 17 airport employees and calculated urine/serum ratio.

PFAS	Serum level {ng/mL [median (range)]}[a]	Specific gravity adjusted urine level {ng/mL [median (range)]}[a]	Personal average urine/serum ratio [median (range)][b]
PFCAs			
PFHpA	NA[c]	0.025 (<LOD–0.080)	0.086 (0.018–0.32)
PFOA	10 (4.1–28)	0.031 (0.010–0.13)	0.0032 (0.00066–0.0067)
PFSAs			
PFPeS	6.5 (1.8–14)	0.072 (0.021–0.12)	0.0089 (0.0057–0.026)
PFHxS	118 (17–399)	0.092 (0.025–0.73)	0.00093 (0.00060–0.0018)
L-PFOS	10 (4.1–24)	<LOD (<LOD–0.084)	0.00092 (0.000035–0.0056)
2/6m-PFOS	2.1 (0.57–8.1)	<LOD (<LOD–1.6)	0.0051 (0.00059–0.032)

Note: L-PFOS, linear PFOS; LOD, limit of detection; NA, not applicable; PFAS, perfluoroalkyl substances; PFCA, perfluorinated carboxylic acid; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexane sulfonic acid; PFOA, perfluorooctanoic acid; PFPeS, perfluoropentane sulfonic acid; PFSA, perfluorinated sulfonic acid; 2/6m-PFOS, branched, sum of perfluoro-2/6-methylheptanesulfonate.
[a]Median and range obtained from the second to the fifth serum or urine samplings.
[b]The urine/serum ratio was first calculated for each study subject at each sampling (i.e., each subject had four urine/serum ratios for each PFAS). Personal average urine/serum ratio was then calculated as the mean value of four ratios for each subject. Median and range were obtained from the personal average urine/serum ratio.
[c]PFAS was not measured.

Estimation of Serum Elimination Half-Lives

The population average elimination rate and half-life are shown in Table 5, and individual estimates for each employee are listed in Table 6 (for short-chain PFAS) and Table 7 (for long-chain PFAS). In general, short-chain PFAS such as PFHpA, PFBS, and PFPeS showed shorter average half-lives of about 44–230 d, whereas long-chain legacy PFAS showed much longer average half-lives of 1.5–2 y (Table 5). PFHxA (C6), however, did not significantly decrease within 5 months. The estimated average constant elimination rate of PFHxA (i.e., the slope) from the mixed model was not significantly different from zero (Table 5). Correspondingly, 53% (9 of 17) of the individuals did not show a decrement in serum concentration of PFHxA during the 5-month follow-up (Table 6). PFHxS and L-PFOS showed the longest estimated half-lives in the airport workers (all about 2.9 y). All the branched PFOS showed half-lives that, on average, were >1.5 y shorter than L-PFOS; 2/6m-PFOS showed the shortest half-life. For the PFAS with same number of carbon atoms in the molecule, PFSAs showed longer half-lives than PFCAs, for example, PFHpS had on average about a 1.3-y longer half-life than the corresponding PFHpA, and PFOS had on average about a 1.2-y longer half-life than PFOA. The pattern of half-lives of different PFAS were somewhat consistent with the serum/water ratio and urine/serum ratio, indicating that PFAS with shorter estimated half-lives were corresponding to lower serum/water ratios and larger urine/serum ratios. The median and distribution of PFAS levels in serum for each sampling are presented in Table S8. There were clear trends of decrements for short-chain PFAS over time, whereas the decrements of long-chain PFAS were less evident. The sample size is too small to detect whether there were departures from linearity.

The sensitivity analysis using serum PFAS levels after subtracting general background levels are listed in the right part of Table 5. For PFPeS and PFHxS, which were much higher in the airport workers compared with the reference population, the estimates after subtraction of background levels were quite close to estimates without subtraction of background exposure. For PFOA and PFOS, with less contrast (approximately 5-fold), the estimation of half-lives were shortened after subtraction of background levels. The reductions of half-life estimation were 8% for PFHpS, 16% for PFOA, 42% for L-PFOS, and around 28% for branched PFAS. For most of the PFAS, the individual elimination rates were not correlated with their initial serum levels ($p > 0.3$ from Spearman's correlation test). Only PFHpA showed a significant positive correlation (Spearman's $r = 0.59$, $p = 0.013$).

Discussion

In the 17 airport employees sampled between 2 weeks to 5 months after the end of exposure to PFAS-contaminated drinking water, we determined the average half-life for PFPeS as 0.63 y (i.e., 230 d) and PFHpS as 1.46 y. The shortest estimated half-life was for PFBS (0.12 y; i.e., 44 d) and the longest half-lives were observed for PFHxS and L-PFOS (2.86 and 2.91 y, respectively). The pattern of estimated half-lives was consistent with the observed PFAS serum/water ratios and urine/serum ratios of various PFAS, indicating that the lower the rate of renal clearance, the higher the serum/water ratio and the longer the half-life.

Short-chain PFCAs with chains of less than eight carbons and PFSAs with less than six carbons have been shown to be excreted faster compared with the long-chain legacy PFOS and PFOA

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Table 5. Population average constant elimination rate (yearly) and half-life (year) for each PFAS.

	Model with original serum levels[a]			Model with serum levels after subtraction of background exposure[b]		
PFAS	Constant elimination rate (95% CI)	Half-life (95% CI)	p-Value	Constant elimination rate (95% CI)	Half-life (95% CI)	p-Value
PFHxA	0.43 (−0.25, 1.1)	1.63 (NA)	0.2	NA[c]	NA	
PFHpA	4.11 (3.13, 5.09)	0.17 (0.14, 0.22)[d]	<0.001	NA	NA	
PFOA	0.39 (0.3, 0.49)	1.77 (1.43, 2.31)	0.002	0.47 (0.35, 0.58)	1.48 (1.19, 1.96)	<0.001
PFBS	5.96 (4.65, 7.27)	0.12 (0.1, 0.15)[d]	0.003	NA	NA	
PFPeS	1.1 (0.68, 1.51)	0.63 (0.46, 1.01)	0.004	1.1 (0.68, 1.52)	0.63 (0.46, 1.01)	<0.001
PFHxS	0.24 (0.16, 0.33)	2.86 (2.1, 4.47)	0.005	0.24 (0.16, 0.33)	2.84 (2.08, 4.43)	0.002
PFHpS	0.48 (0.11, 0.84)	1.46 (0.83, 6.25)	0.014	0.51 (0.12, 0.9)	1.35 (0.77, 5.71)	0.014
L-PFOS	0.24 (0.07, 0.4)	2.91 (1.71, 9.63)	0.007	0.41 (0.11, 0.71)	1.69 (0.98, 6.04)	0.010
1m-PFOS	0.55 (0.09, 1.01)	1.27 (0.69, 7.64)	0.022	0.75 (0.15, 1.36)	0.92 (0.51, 4.77)	0.018
3/4/5m-PFOS	0.64 (0.18, 1.1)	1.09 (0.63, 3.96)	0.011	0.83 (0.24, 1.42)	0.83 (0.49, 2.84)	0.011
2/6m-PFOS	0.66 (0.29, 1.04)	1.04 (0.67, 2.42)	0.001	0.95 (0.39, 1.5)	0.73 (0.46, 1.76)	0.002

Note: CI, confidence interval; L-PFOS, linear perfluorooctane sulfonic acid; NA, not applicable; PFAS, perfluoroalkyl substances; PFBS, perfluorobutane sulfonic acid; PFHpA, perfluoroheptanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexane sulfonic acid; PFOA, perfluorooctanoic acid; PFPeS, perfluoropentane sulfonic acid; 1m-PFOS, branched, perfluoro-1-methylheptanesulfonate; 2/6m-PFOS, branched, sum of perfluoro-2/6-methylheptanesulfonate; 3/4/5m-PFOS, branched, sum of perfluoro-3/4/5-methylheptanesulfonate.

[a]Linear mixed model with age and sex as covariates. Serum PFAS levels were log-transformed. The constant elimination rate denotes the negative value of the slope ($-\beta$) from the model, and half-life is calculated as $\ln 2/k$.

[b]Sensitivity analysis using same linear mixed model. Serum PFAS levels were first subtracted with the median levels from the reference population (background exposure levels, presented in Table 2). If the levels after subtraction were lower than half of the corresponding background levels, they were replaced with half of the background levels.

[c]No background serum PFAS levels available.

[d]Half-life and 95% CI in days: PFHpA: 62 d (95% CI: 51–80 d); PFBS: 44 d (95% CI: 37–55 d).

(Conder et al. 2008). The rapid excretion and lower bioaccumulation of short-chain PFAS has made it difficult to find an exposed population that can be used to study human half-lives. The major strength of the present study is that first serum samples were taken within only 2 weeks after the exposure to the highly contaminated water was terminated. This provided us with an opportunity to address half-lives of some short-chain PFAS in human blood. We were confident that all individuals in the study no longer had ongoing drinking-water exposure at home after thoroughly checking water supply information at each individual's home address. Moreover, we used two methods to estimate PFAS elimination rate at the population level and individual level, respectively. The mixed model is preferable for a population average estimation and provides more robust estimates when random error of sampling exists. The linear regression for each individual estimates served as an alternative method to show interindividual variations. Because up to only five measurements were used in each regression, the statistical power was limited and, therefore, not considered as main results.

Our study also has some limitations. Principally, that the number of study subjects was relatively small, although we succeeded in obtaining five blood samples and four urine samples from most subjects during 5 months. For long-chain PFAS with long half-lives, only 5 months is relatively short for estimating the half-life. However, the study was sufficient to estimate the half-life for several PFAS with reasonable precision. The statistical power for individual estimates would still benefit from further follow-up with more sampling points.

For short-chain PFAS, limited human studies about half-lives are available. In the present study, the individual serum half-life of PFBS ranged from 21.9 to 87.6 d, with an average estimation of 43.8 d. The estimation of PFBS by Olsen et al. (2009) was 25.8 d (geometric mean) with a range of 13.1–45.7 d. Although the average estimation was about half compared with our study,

Table 6. Individual constant elimination rate (yearly) and half-life (year) in the 17 airport employees for short-chain PFAS.

		PFHxA			PFHpA			PFBS			PFPeS		
Subject	Sample counts	Elimination rate[a]	HL[a]	p-Value[a]	Elimination rate	HL	p-Value	Elimination rate	HL	p-Value	Elimination rate	HL	p-Value
1	5	0.2	4.1	0.78	2.1	0.3	0.02	3.2	0.2	0.03	0.7	1.0	0.02
2	5	−0.1	NA[b]	0.87	3.7	0.2	0.01	4.0	0.2	0.02	0.6	1.2	0.04
3	5	1.6	0.4	0.04	1.7	0.4	0.03	5.1	0.1	0.00	0.7	0.9	0.03
4	5	−0.6	NA	0.57	3.2	0.2	0.14	3.1	0.2	0.44	0.8	0.8	0.05
5	5	−1.7	NA	0.24	1.3	0.5	0.02	3.0	0.2	0.00	0.4	1.7	0.11
6	4	−1.6	NA	0.04	4.1	0.2	0.03	2.9	0.2	0.43	0.9	0.7	0.08
7	5	1.5	0.5	0.27	4.6	0.1	0.00	6.2	0.1	0.02	0.7	0.9	0.01
8	5	−0.7	NA	0.44	5.4	0.1	0.02	4.4	0.2	0.14	0.5	1.4	0.07
9	5	−0.2	NA	0.88	7.8	0.1	0.01	11.7	0.1	0.00	0.7	1.0	0.00
10	5	0.4	1.9	0.75	2.2	0.3	0.04	3.8	0.2	0.03	0.6	1.2	0.08
11	5	2.8	0.2	0.03	2.6	0.3	0.01	7.1	0.1	0.02	2.0	0.3	0.13
12	5	−0.3	NA	0.64	6.7	0.1	0.00	5.4	0.1	0.01	1.1	0.6	0.00
13	5	−0.7	NA	0.25	5.4	0.1	0.00	6.2	0.1	0.02	0.8	0.9	0.06
14	4	−0.5	NA	0.87	6.6	0.1	0.02	10.7	0.1	0.01	1.2	0.6	0.08
15	5	2.7	0.3	0.09	3.9	0.2	0.02	5.7	0.1	0.01	2.5	0.3	0.07
16	5	1.1	0.6	0.08	0.9	0.7	0.17	3.6	0.2	0.01	0.4	1.6	0.07
17	5	2.5	0.3	0.06	5.2	0.1	0.04	10.4	0.1	0.02	2.4	0.3	0.06

Note: HL, half-life; PFBS, perfluorobutane sulfonic acid; PFHpA, perfluoroheptanoic acid; PFHxA, perfluorohexanoic acid; PFPeS, perfluoropentane sulfonic acid.

[a]The elimination rate and p-value were obtained from linear regression. Serum PFAS levels were log-transformed. The elimination rate denotes the negative value of the slope ($-\beta$) from the model, and half-life is calculated as $\ln 2/k$.

[b]Not applicable because the elimination rate was negative, indicating PFAS levels did not decrease during the 5-month follow-up.

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Table 7. Individual constant elimination rate (yearly) and half-life (year) in the 17 airport employees for long-chain PFAS.

Subject	Sample counts	PFOA Elimination rate[a]	PFOA HL[a]	PFOA p-Value[a]	PFHxS Elimination rate	PFHxS HL	PFHxS p-Value	PFHpS Elimination rate	PFHpS HL	PFHpS p-Value	L-PFOS Elimination rate	L-PFOS HL	L-PFOS p-Value	1m-PFOS Elimination rate	1m-PFOS HL	1m-PFOS p-Value	3/4/5m-PFOS Elimination rate	3/4/5m-PFOS HL	3/4/5m-PFOS p-Value	2/6m-PFOS Elimination rate	2/6m-PFOS HL	2/6m-PFOS p-Value
1	5	0.3	2.4	0.17	0.2	3.5	0.19	0.5	1.3	0.31	−0.2	NA	0.30	0.3	2.0	0.10	0.4	1.9	0.13	−0.2	NA	0.76
2	5	0.2	2.9	0.27	0.3	2.0	0.09	0.2	3.4	0.64	0.4	1.7	0.07	0.1	5.4	0.83	0.3	2.1	0.11	0.5	1.3	0.20
3	5	0.6	1.2	0.04	0.5	1.5	0.09	0.2	3.0	0.34	0.5	1.3	0.21	0.8	0.9	0.09	0.4	1.7	0.15	0.7	1.0	0.12
4	5	0.3	2.3	0.15	0.1	9.6	0.75	0.001	526	1.00	−0.3	NA	0.24	−0.1	NA	0.78	0.1	11	0.87	0.0	51	0.97
5	5	0.3	2.2	0.15	0.1	4.7	0.20	−0.3	NA	0.19	0.6	1.2	0.13	0.1	5.9	0.75	0.2	3.1	0.16	0.4	1.6	0.56
6	4	0.2	2.9	0.39	−0.1	NA[b]	0.42	0.1	6.1	0.43	0.04	17	0.87	−0.1	NA	0.51	0.04	19	0.49	0.4	1.7	0.04
7	5	0.4	1.6	0.03	0.3	2.3	0.02	−0.04	NA	0.88	0.006	108	0.92	−0.3	NA	0.03	0.1	4.8	0.02	1.1	0.6	0.29
8	5	0.007	100	0.98	−0.01	NA	0.94	−0.6	NA	0.42	−0.4	−1.6	0.23	−0.5	NA	0.13	−0.2	NA	0.24	−0.2	NA	0.44
9	5	0.4	1.9	0.05	−0.04	NA	0.81	0.05	15	0.69	0.4	1.8	0.21	−0.3	NA	0.10	0.1	10	0.70	0.2	4.2	0.50
10	5	0.3	2.1	0.25	0.4	1.9	0.11	0.4	1.8	0.46	−0.2	NA	0.32	0.6	1.2	0.10	0.2	4.0	0.52	−0.007	NA	0.97
11	5	0.5	1.5	0.12	0.2	3.0	0.39	1.7	0.4	0.20	0.1	4.7	0.56	1.9	0.4	0.15	2.0	0.4	0.15	1.7	0.4	0.10
12	5	0.7	1.0	0.00	0.4	1.6	0.04	0.6	1.1	0.08	0.8	0.9	0.01	0.03	26	0.95	0.5	1.3	0.09	1.7	0.4	0.03
13	5	0.4	1.6	0.02	0.5	1.4	0.01	−0.2	NA	0.70	0.2	3.7	0.34	1.1	0.6	0.08	0.5	1.5	0.04	1.0	0.7	0.20
14	4	0.4	1.6	0.40	0.6	1.2	0.31	1.0	0.7	0.21	0.3	2.0	0.67	1.7	0.4	0.08	0.3	2.0	0.55	1.5	0.5	0.44
15	5	0.6	1.1	0.04	0.3	2.2	0.06	1.6	0.4	0.09	0.5	1.3	0.01	1.3	0.6	0.18	2.2	0.3	0.08	1.2	0.6	0.18
16	5	0.3	2.7	0.19	0.04	19	0.89	0.1	12	0.61	0.6	1.2	0.05	−0.3	NA	0.02	0.3	2.0	0.12	−0.1	NA	0.81
17	5	0.6	1.2	0.09	0.4	1.6	0.07	1.7	0.4	0.13	0.3	2.1	0.19	2.2	0.3	0.12	1.9	0.4	0.13	1.9	0.4	0.10

Note: HL, half-life; L-PFOS, linear perfluorooctane sulfonic acid; PFAS, perfluoroalkyl substances; PFHpS, perfluoroheptane sulfonic acid; PFHxS, perfluorohexane sulfonic acid; PFOA, perfluorooctanoic acid; 1m-PFOS, branched, perfluoro-1-methylheptanesulfonate; 2/6m-PFOS, branched, sum of perfluoro-2/6-methylheptanesulfonate; 3/4/5m-PFOS, branched, sum of perfluoro-3/4/5-methylheptanesulfonate.

[a]The elimination rate and p value were obtained from linear regression. Serum PFAS levels were log-transformed. The elimination rate denotes the negative value of the slope ($-\beta$) from the model, and half-life is calculated as $\ln 2/k$.

[b]Not applicable because the elimination rate was negative, indicating PFAS levels did not decrease during the 5-month follow-up.

the ranges overlapped; four to six subjects in the study by Olsen et al. (2009) had a half-life estimation within the range observed in the present study, with one more subject showing 21.2 d as the PFBS half-life. Their follow-up of 180 d was a little longer. Further, PFBS serum levels reported by Olsen et al. (2009) (average level: 397 ng/mL) were much higher than in the present study (median of initial level: 0.46 ng/mL). Given the large variation in exposure levels between the two studies, a dose-dependency of PFBS serum elimination half-life cannot be ruled out.

For PFHpA, the present study showed a similar half-life (0.17 y, corresponding to 62 d) to that reported by Russell et al. (2015a), who calculated the first-order apparent elimination half-life of 70 d (geometric mean) in whole blood based on repeated samples from five ski wax technicians after occupational exposure during the skiing season. Another study performed in a Chinese population reported estimated half-lives of 0.82 and 1.0 y (geometric mean for young females and for older females plus males, respectively) for PFHpA (Zhang et al. 2013). The latter study was based only on single time-paired serum (or whole blood) and urine samples and assumed renal clearance as total clearance in their calculation, so it is not strictly comparable. The underestimation of total clearance would lead to an overestimation of half-life.

For PFHxA, about half of the airport employees did not show a clear decrease of serum concentration (i.e., 53% had no individual estimated half-life). A reason could be that we measured PFHxA in serum, which only presents a very small fraction of PFHxA in blood (although all serum levels were >LOD). Unlike the other PFAS we present, PFHxA is more bound to blood cells and whole blood would therefore be a more suitable blood matrix to determine PFHxA (Poothong et al. 2017). Several other studies also failed to detect PFHxA in serum/plasma (Christensen et al. 2016; Eriksson et al. 2017; Salihovic et al. 2015). Russell et al. (2013) reported an apparent elimination half-life of PFHxA in whole blood from seven ski wax technicians as 32 d with a range of 14–49 d. Due to the fact that PFHxA was measured in serum in the present study, which did not adequately reflect the real body burden level, our estimation of the half-life is, therefore, unreliable.

For the short-chain PFPeS with a five-carbon chain length, our half-life estimation (0.63 y) was slightly shorter than the observation from the highly exposed general population from Ronneby, Sweden, which showed an average half-life of 1.0 y [95% confidence interval (CI): 0.9, 1.0 y] (Li et al. 2019). However, for the long-chain PFHpS with a seven-carbon chain length, the estimated half-life from this study (1.46 y) was much shorter than the Ronneby population [4.7 y (95% CI: 4.3, 5.3 y)] (Li et al. 2019). No other study was available for further comparison.

A shorter half-life estimation in the present study than the corresponding half-lives derived over 2 y in the Ronneby population was also observed for other PFAS compounds. The half-lives of PFOA (1.77 y), PFHxS (2.86 y), and L-PFOS (2.91 y) were each shorter compared with findings of Li et al. (2018) (2.7, 5.3, and 3.4 y for total PFOS, respectively). The difference in study population (e.g., age range, sex composition) may be one explanation. In addition, the follow-up period was different between two studies. The first serum samples in the Ronneby population were obtained 6 months after the exposure stopped. Therefore, the elimination rate in the first 6 months could not be directly compared between these populations. There is some evidence of a possible nonlinear clearance process of PFAS from the literature, mainly about PFOA, showing that the estimated half-life appeared to decrease with increased follow-up time. For instance, based on a 5-y follow-up, Olsen et al. (2007) estimated an

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Table 8. Summary of PFAS half-lives in the present study and in other studies referred to in the discussion.

PFAS	Half-life in the present study	Half-life in other study	Study population	Year of study	Follow-up time	Reference
PFHpA	0.17 y (62 d)	70 d	5 ski wax technicians	2007–2011	7 months	Russell et al. 2015a
PFOA	1.77 y	2.7 y	106 Swedes	2014–2016	2 y	Li et al. 2018
		3.8 y	26 retired fluorochemical production workers	1999–2004	5 y	Olsen et al. 2007
		3.3 y	138 Germans	2006–2008	2 y	Brede et al. 2010
		2.3 y	200 Americans	2007–2008	1 y	Bartell et al. 2010
PFBS	0.12 y (44 d)	25.8 d	6 3M employees	2004	3 months	Olsen et al. 2009
PFPeS	0.63 y	1.0 y	108 Swedes	2014–2019	5 y	Li et al. 2019
PFHxS	2.86 y	5.3 y	106 Swedes	2014–2016	2 y	Li et al. 2018
L-PFOS	2.91 y	3.4 y	106 Swedes	2014–2016	2 y	Li et al. 2018

Note: L-PFOS, linear perfluorooctane sulfonic acid; PFAS, perfluoroalkyl substances; PFBS, perfluorobutane sulfonic acid; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexane sulfonic acid; PFOA, perfluorooctanoic acid; PFPeS, perfluoropentane sulfonic acid.

average half-life of PFOA of 3.8 y in retired fluorochemical production workers, whereas Brede et al. (2010) reported a half-life of PFOA of 3.3 y based on a 2-y follow-up and Bartell et al. (2010) reported of 2.3 y with a 1-y follow-up. Our PFOA half-life of 1.77 y is consistent with this pattern that elimination is faster in the early time window after exposure stopped and becomes slower after 1 or 2 y.

Half-life estimation can also be influenced by ongoing exposure, which could contribute to explaining the different half-lives reported in different studies. Our sensitivity analysis of estimations with and without subtraction of general background level also clearly showed that for PFPeS and PFHxS, much higher initial serum levels compared with the background levels, the estimates did not differ much. For PFOA and PFOS, however, the estimated half-lives were shortened after subtracting background levels. The result is in line with the findings of Russell et al. (2015b) that if the background exposure compared to the contaminated level is not small, then ignoring the background exposure will lead to an overestimated of half-life. The half-life comparison between studies is summarized in Table 8.

The average serum/water ratio of PFOA observed in our study (30.3) was lower than in other studies, which ranged from 100 to 231 (Post et al. 2009; Hoffman et al. 2011; Zhang et al. 2019). In those studies, PFOA was measured in the tap water supplied by public water systems (Post et al. 2009; Zhang et al. 2019) or in private wells (Hoffman et al. 2011). It is reasonable to assume that their population was exposed to PFOA-contaminated drinking water at home. In our study, however, the individuals were only exposed at work and had a PFAS-free water supply at home. Therefore, the contribution from drinking water to serum level may be less compared with those who are constantly exposed at home.

Although PFAS are eliminated through urine to different degrees, urinary levels of PFAS are generally low in adults. In our study, despite the fact that 11 PFAS were successfully measured in serum, only 6 PFAS (PFHpA, PFOA, PFPeS, PFHxS, L-PFOS, and 2/6m-PFOS) could be detected and quantified in urine samples. Moreover, the urine/serum ratios were observed to be low (all <0.010) among the airport employees. Our observed urine/serum ratios of PFOS (0.00092) and PFOA (0.0032) were similar to the findings of a Chinese study (Zhang et al. 2015), which reported urine/serum ratios of PFOS of 0.0004 for pregnant women and 0.013 for nonpregnant women and urine/serum ratios of PFOA of 0.0011 and 0.0028 for pregnant and nonpregnant women, respectively. Our results showed that the urine/serum ratio for PFOA was larger than linear PFOS, suggesting that PFOA is excreted faster through urine than PFOS. Our results also showed a lower urine/serum ratio of L-PFOS than 2/6m-PFOS, indicating that 2/6m-PFOS is preferentially excreted through urine compared with L-PFOS, which is consistent with other studies that reported a smaller renal clearance of L-PFOS than branched PFOS except 1m-PFOS (Zhang et al. 2013; Zhou et al. 2014).

In general, for both PFCAs and PFSAs, there was a decreased serum/water ratio and increased urine/serum ratio with decreasing chain length, indicating a faster excretion of PFAS with shorter chain length and a corresponding shorter half-life. For instance, short-chain PFPeS had a 10 times higher urine/serum ratio than long-chain L-PFOS. Correspondingly, PFPeS had a half-life 4 times shorter than L-PFOS. The shorter carbon chain length, the less accumulation, and shorter half-lives may be attributed to the difference in potential binding affinities to different organic anion transporter proteins (Han et al. 2012; Weaver et al. 2010). In animal models, it has been found that one organic anion transport protein (OATP 1a1) expressed in the kidney had a stronger interaction with long-chain PFAS and led to higher reabsorption and lower elimination levels (Yang et al. 2009). To some extent, higher water solubility of short-chain PFAS compared with longer ones may also be associated with faster excretion (Bhhatarai and Gramatica 2011). One exception to the above conclusion that PFAS elimination and half-lives is dependent on chain length was C6 PFHxS, which had a longer half-life than C7 PFHpS and a half-life similar to that of C8 PFOS. The reason is unclear, but such findings have been observed in other studies as well (Li et al. 2018; Olsen et al. 2007).

It should be noticed that even though short-chain PFAS showed a lower serum/water ratio than the legacy long-chain PFAS, they were clearly detected in the sera of the airport employees 2 weeks and longer after the cessation of exposure from the contaminated drinking water. For example, PFPeS showed much higher serum levels among the exposed employees even 5 months after exposure ceased than the levels observed in general population without PFAS exposure through drinking water (median 6.22 ng/mL vs. <LOD; i.e., <0.02 ng/mL). Consequently, populations with high daily exposure to short-chain PFAS from highly contaminated drinking water will have clearly elevated serum levels of these PFAS above background as long as exposure continues. Therefore, high short-chain PFAS contamination of drinking water may be a serious environmental health problem that should be taken into account in future epidemiological studies.

Conclusion

In the present study of 17 airport employees from Arvidsjaur, Sweden, who had been exposed to PFAS through drinking water at work, the estimated average half-lives after abrupt cessation of drinking-water exposure, ranged from 44 d for short-chain PFBS to 2.9 y for PFHxS and PFOS. A general pattern of increasing half-lives with chain length was observed. Branched PFOS had shorter half-lives than linear PFOS. Both PFOA and PFOS half-lives in the present study were shorter than published estimates, suggesting a possible time-dependent PFAS elimination process, with more rapid elimination in the first few months after the end of exposure.

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CHAPTER 21

TOXIC RESPONSES OF THE ENDOCRINE SYSTEM

Charles C. Capen

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INTRODUCTION

Endocrine glands are collections of specialized cells that synthesize, store, and release their secretions directly into the bloodstream. They are sensing and signaling devices located in the extracellular fluid compartment and are capable of responding to changes in the internal and external environments to coordinate a multiplicity of activities that maintain homeostasis.

Endocrine cells that produce polypeptide hormones have a well-developed rough endoplasmic reticulum that assembles hormone and a prominent Golgi apparatus for packaging hormone into granules for intracellular storage and transport. Secretory granules are unique to polypeptide hormone- and catecholamine-secreting endocrine cells and provide a mechanism for intracellular storage of substantial amounts of preformed active hormone. When the cell receives a signal for hormone secretion, secretory granules are directed to the periphery of the endocrine cell, probably by the contraction of microfilaments.

Steroid hormone-secreting cells are characterized by prominent cytoplasmic lipid bodies that contain cholesterol and other precursor molecules. The lipid bodies are in close proximity to an extensive tubular network of smooth endoplasmic reticulum and large mitochondria which contain hydroxylase and dehydrogenase enzyme systems. These enzyme systems function to attach various side chains to the basic steroid nucleus. Steroid hormone-producing cells lack secretory granules and do not store significant amounts of preformed hormone. They are dependent on continued biosynthesis to maintain the normal secretory rate for a particular hormone.

Many diseases of the endocrine system are characterized by dramatic functional disturbances and characteristic clinicopathological alterations affecting one or several body systems. The affected animal or human patient may have clinical signs that primarily involve the skin (hair loss caused by hypothyroidism), nervous system (seizures caused by hyperinsulinism), urinary system (polyuria caused by diabetes mellitus, diabetes insipidus, and hyperadrenocorticism), or skeletal system (fractures induced by hyperparathyroidism) (Capen, 2006b).

The literature suggests that chemically induced lesions of the endocrine organs are most commonly encountered in the adrenal glands, followed in descending order by the thyroid, pancreas, pituitary, and parathyroid glands. In the adrenal glands, chemically induced lesions are most frequently found in the zona fasciculata/zona reticularis and to a lesser extent in either the zona glomerulosa or medulla. In a survey, conducted by the Pharmaceutical Manufacturers Association, of tumor types developing in carcinogenicity studies, endocrine tumors developed frequently in rats, with the thyroid gland third in frequency (behind the liver and mammary gland), followed by the pituitary gland (fourth), and adrenal gland (fifth). Selected examples of commonly encountered toxic endpoints involving endocrine organs in laboratory animals are discussed in this chapter. Mechanistic data is included whenever possible to aid in the interpretation of findings in animal toxicology studies and to determine their significance in risk assessment (Alison *et al.*, 1994).

PITUITARY GLAND

Normal Structure and Function

The pituitary gland (hypophysis) is divided into two major compartments: (1) the adenohypophysis (anterior lobe) of the human pituitary composed of the pars distalis, pars tuberalis, and pars intermedia; and (2) the neurohypophyseal system which includes the pars nervosa (posterior lobe), infundibular stalk, and nuclei (supraoptic and paraventricular) in hypothalamus that contain the neurosecretory neurons that synthesize and package into secretory granules the neurohypophyseal hormones. The pars intermedia forms the thin cellular zone between the adenohypophysis and neurohypophysis. The pituitary gland lies within the sella turcica of the sphenoid bone. The gland receives its blood supply via the posterior and anterior hypophyseal arteries which originate from the internal carotid arteries. Arteriolar branches penetrate the pituitary stalk near the median eminence, lose their muscular coat, and form a capillary plexus. These vessels drain into the hypophyseal portal veins which supply the adenohypophysis. The hypothalamic–hypophyseal portal system functionally is important as it transports the hypothalamic releasing- and release-inhibiting hormones directly to the adenohypophysis where they interact with their specific populations of trophic hormone-producing cells.

The adenohypophysis in many animal species completely surrounds the pars nervosa of the neurohypophyseal system in contrast to human beings where it is situated on the anterior surface. The pars distalis is the largest portion and is composed of the multiple populations of endocrine cells that secrete the pituitary trophic hormones. The secretory cells are surrounded by abundant capillaries derived from the hypothalamic–hypophyseal portal system (Capen, 1996a). The pars tuberalis consists of dorsal projections of supportive cells along the infundibular stalk. It functions primarily as a scaffold for

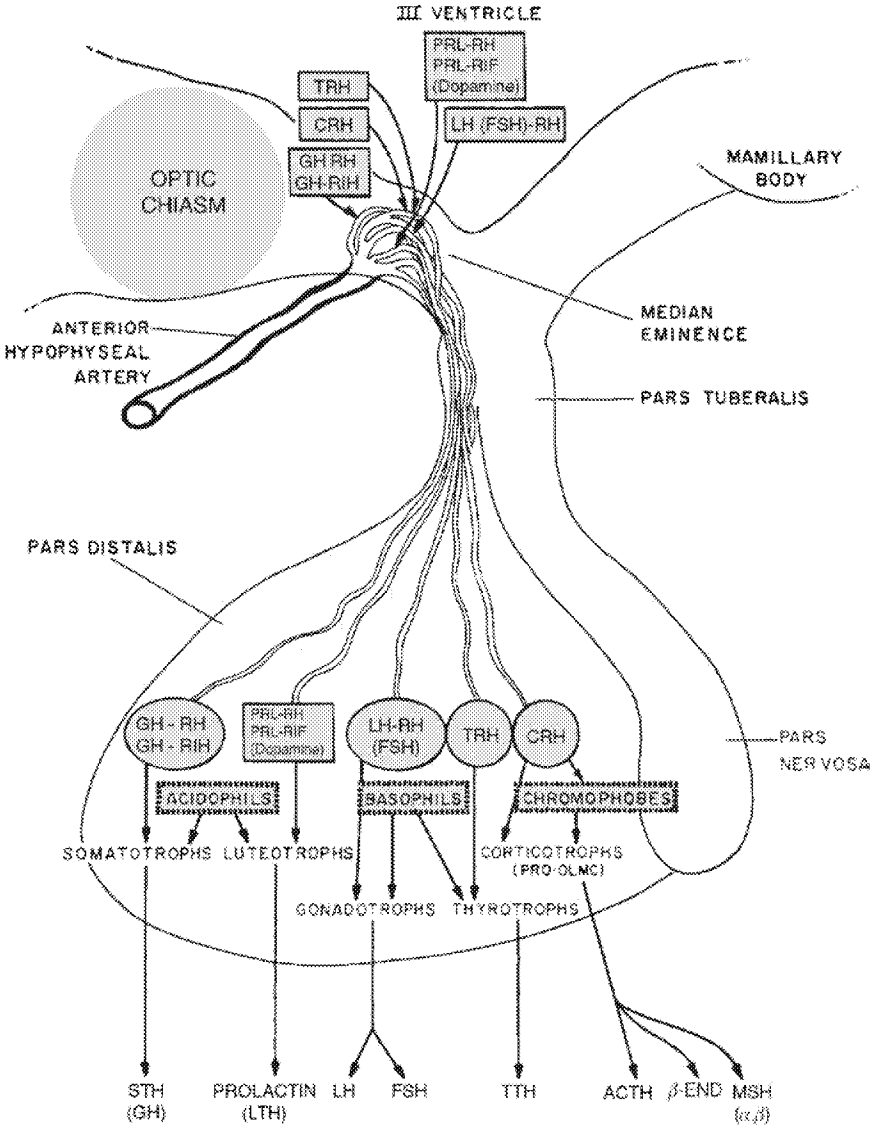

Figure 21-1. Control of trophic hormone secretion from the adenohypophysis by hypothalamic releasing hormones (RH) and release-inhibiting hormones (RIH).

The releasing and release-inhibiting hormones are synthesized by neurons in the hypothalamus, transported by axonal processes, and released into capillary plexus in the median eminence. They are transported to the adenohypophysis by the hypothalamic–hypophyseal portal system where they interact with specific populations of trophic hormone-secreting cells to govern the rate of release of preformed hormones, such as somatotropin (GH, STH), prolactin (LTH), prolactin (LTH), lutenizing hormone (LH), follicle-stimulating hormone (FSH), thyrotropic hormone (TTH), adrenocorticotropic hormone (ACTH), and melanocyte-stimulating hormone (MSH). There are RIH for those trophic hormones (e.g., prolactin and growth hormone) that do not directly influence the activity of target cells and result in production of a final endocrine product (hormone) that could exert negative feedback control.

the capillary network of the hypophyseal portal system during its course from the median eminence to the pars distalis. The pars intermedia is located between the pars distalis and pars nervosa and lines the residual lumen of Rathke's pouch. It contains two populations of endocrine cells in certain species. One of these cell types (B-cell) in the dog synthesizes and secretes adrenocorticotropic hormone (ACTH).

A specific population of endocrine cells is present in the pars distalis (and in the pars intermedia of dogs for ACTH) that synthesizes, processes, and secretes each of the pituitary trophic hormones. Secretory cells in the adenohypophysis formerly were classified either as acidophils, basophils, or chromophobes based on the reactions of their secretory granules with pH-dependent histochemical stains. Based upon contemporary specific immunocytochemical procedures, acidophils can be further subclassified functionally into somatotrophs that secrete growth hormone (GH; somatotrophin) and luteotrophs that secrete luteotropic hormone (LTH; prolactin). Their granules contain simple protein hormones. Basophils include both gonadotrophs that secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and thyrotrophs that secrete thyrotropic

hormone (thyroid-stimulating hormone [TSH]). Chromophobes are pituitary cells that by light microscopy do not have stainable cytoplasmic secretory granules. They include the pituitary cells involved with the synthesis of ACTH and melanocyte-stimulating hormone (MSH) in some species, nonsecretory follicular (stellate) cells, degranulated chromophils (acidophils and basophils) in the actively synthesizing phase of the secretory cycle, and undifferentiated stem cells of the adenohypophysis.

Each type of endocrine cell in the adenohypophysis is under the control of a specific releasing hormone from the hypothalamus (Fig. 21-1). These releasing hormones are small peptides synthesized and secreted by neurons of the hypothalamus. They are transported by short axonal processes to the median eminence where they are released into capillaries and are conveyed by the hypophyseal portal system to specific trophic hormone-secreting cells in the adenohypophysis. Each hormone stimulates the rapid release of preformed secretory granules containing a specific trophic hormone. Specific releasing hormones have been identified for TSH, FSH and LH, ACTH, and GH. Prolactin (PRL) secretion is stimulated by a number of factors, the most important of which appears to be thyrotropin-releasing hormone (TRH). Dopamine serves as the major prolactin-inhibitory factor and suppresses prolactin secretion and also inhibits cell division and DNA synthesis of luteotrophs (Parkening *et al.*, 1980). Dopamine also suppresses ACTH production by corticotrophs in the pars intermedia of some species. Another hypothalamic release-inhibiting hormone is somatostatin (somatotropin-release inhibiting hormone, SRIH). This tetradecapeptide inhibits the secretion of both growth hormone and TSH. Control of pituitary trophic hormone secretion also is affected by negative feedback by the circulating concentration of target organ (thyroid, adrenal cortex, and gonad) hormones.

The neurohypophysis is subdivided into three anatomic parts. The pars nervosa (posterior lobe of the human pituitary) represents the distal component of the neurohypophyseal system. The infundibular stalk joins the pars nervosa to the overlying hypothalamus and is composed of long axonal processes from neurosecretory neurons in the hypothalamus. It is composed of numerous capillaries, supported by modified glial cells (pituicytes), which are termination sites for the nonmyelinated axonal processes of neurosecretory neurons. The neurohypophyseal hormones (i.e., oxytocin and antidiuretic hormone) are synthesized in the cell body of hypothalamic neurons, packaged into secretory granules, transported by long axonal processes, and released into the bloodstream in the pars nervosa.

Antidiuretic hormone (ADH or vasopressin) and oxytocin are nonapeptides synthesized by neurons situated either in the supraoptic (primarily ADH) or paraventricular (primarily oxytocin) nuclei of the hypothalamus. ADH and its corresponding neurophysin are synthesized as part of a common larger biosynthetic precursor molecule, termed propressophysin. The hormones are packaged with a corresponding binding protein (i.e., neurophysin) into membrane-limited neurosecretory granules and transported by axons to the pars nervosa for release into the circulation. As the biosynthetic precursor molecules travel along the axons in secretion granules from the neurosecretory neurons, the precursors are cleaved into the active hormones and their respective neurophysins. These secretory products can be detected immunocytochemically. In Brattleboro rats with hereditary hypothalamic diabetes insipidus, nerve cells in the hypothalamus that normally produce ADH and neurophysin-I are negative immunocytochemically for both proteins whereas neurosecretory stain positive for cells that produce vasopressin and neurophysin-II are positive.

In addition to the specific trophic hormone-secreting cells, a population of supporting cells is also present in the adenohypophysis. These cells are referred to as stellate (follicular) cells and can be stained selectively with antibodies to S-100 protein. The stellate cells typically have elongate processes and prominent cytoplasmic filaments. These cells appear to provide a phagocytic or supportive function in addition to producing a colloid-like material that accumulates in follicles.

Mechanisms of Pituitary Toxicity

Pituitary tumors can be induced readily by sustained uncompensated hormonal derangements leading to increased synthesis and secretion of pituitary hormones. The absence of negative feedback inhibition of pituitary cells leads to unrestrained proliferation (hyperplasia initially, neoplasia later). This effect can be potentiated by the concurrent administration of ionizing radiation or chemical carcinogens.

In the pituitary–thyroid axis, thyroxine (T_4) and triiodothyronine (T_3) normally regulate the pituitary secretion of TSH by a classical negative feedback control system. Surgical removal or radiation-induced ablation of the thyroid or interference with the production of thyroid hormones by the use of specific chemical inhibitors of thyroid hormone synthesis leads to a stimulation of TSH synthesis and secretion with elevated blood levels. The thyrotrophic cells in the adenohypophysis undergo prominent hypertrophy. Subsequently, hyperplasia of the thyrotrophs occurs concurrently with hypertrophy as a consequence of the lack of normal negative feedback control. In rodents foci of hyperplasia may progress to the formation of adenomas in the pituitary gland. The role of gonadectomy in pituitary tumor induction has been studied most intensively in mice. The pituitary tumors induced by gonadectomy in mice are markedly strain-dependent and may contain FSH, LH, or both.

The administration of estrogens is a reproducible method for inducing pituitary tumors in certain experimental animals. The effect of exogenous estrogen on the rat pituitary includes stimulation of prolactin secretion and the induction of prolactin-secreting tumors (Hart, 1990). The administration of estrogens in susceptible strains results in elevated serum prolactin levels, increased numbers of prolactin cells within the pituitary, enhanced incorporation of tritiated thymidine within the gland, and increased mitotic activity (Osamura *et al.*, 1982). The pituitary of the ovariectomized F344 female rat is more responsive to the tumorigenic effect of diethylstilbestrol than the intact female; however, there is considerable variation in the induction of pituitary tumors by estrogens in different rat strains. For example, F344 and Holtzman rats respond to an initial estrogen stimulus by increasing the rate of DNA synthesis in the pituitary within 2–4 days. The rate of DNA synthesis declines after 7–10 days of treatment to unstimulated levels in the Holtzman strain but remains elevated in F344 rats.

Sarkar *et al.* (1982) have reported that estrogen-induced pituitary adenomas derived from prolactin-secreting cells are associated with loss of hypothalamic dopaminergic neurons, which normally inhibit the function of prolactin-secreting cells. Prolactin-producing tumors when transplanted subcutaneously were also associated with degenerative changes in hypothalamic dopaminergic neurons. The tumorigenic action of estrogen may not be due exclusively to its effect on the hypothalamus because estrogen can produce prolactinomas in pituitaries grafted beneath the renal capsule. The effects of estrogens on prolactin cells have been studied in hypophysectomized rats bearing transplanted pituitaries beneath the kidney capsule. The studies of El Etreby *et al.* (1988) using this model have shown

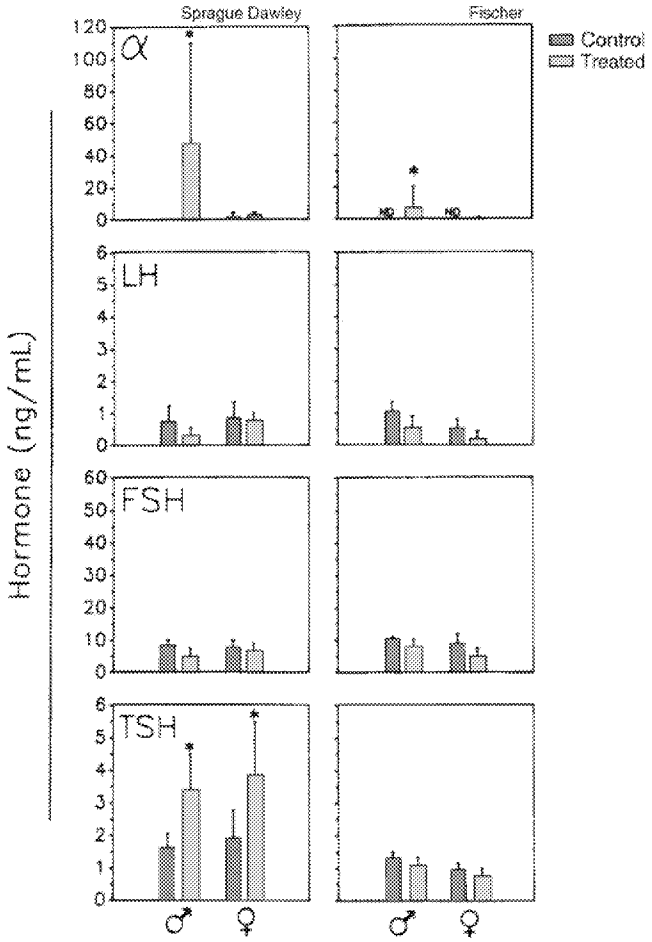

Figure 21-2. Serum glycoprotein hormone levels in rats treated chronically with salmon calcitonin.

Sprague–Dawley (left panels) or Fischer rats (right panels) were treated for 52 weeks with vehicle (open bars) or calcitonin (80 IU/kg/d) (black bars). Results are the mean ± SD; $^*P < 0.05$. (From Jameson *et al.*, 1992).

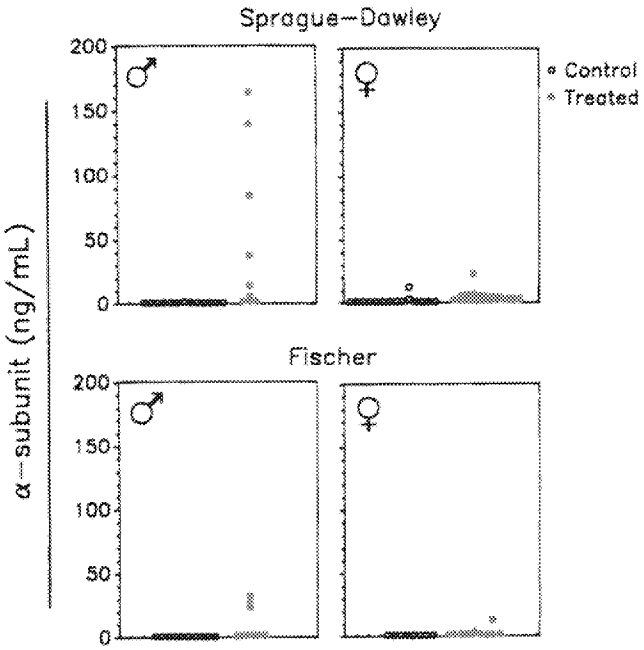

Figure 21-3. Serum α-subunit levels in individual male rats treated chronically with calcitonin.

The serum levels for individual animals are denoted as 0 = vehicle; • = calcitonin-treated. (From Jameson *et al.*, 1992).

that dopamine agonists, including lisuride and bromocriptine, antagonize the direct stimulatory effects of estrogens on the prolactin cells. These dopamine agonists may act directly on dopaminergic receptors within the transplanted pituitaries.

Other chemicals, including caffeine, have been implicated in the development of pituitary adenomas in rats (Yamagami *et al.*, 1983). The administration of *N*-methylnitrosourea also is associated with the development of pituitary adenomas in Wistar rats. The neuroleptic agent sulpiride has been reported to cause the release of prolactin from the anterior pituitary in the rat and to stimulate DNA replication. The administration of clomiphene prevents the stimulation of DNA synthesis produced by sulpiride, but does not affect prolactin release from the gland. These findings suggest that the intracellular prolactin content of the pituitary plays a role in the regulation of DNA synthesis through a mechanism mediated by estrogens (Gunnison *et al.*, 1997).

Morphologic Alterations and Proliferative Lesions of Pituitary Cells

Jameson *et al.* (1992) reported that the administration of salmon calcitonin for one year to Sprague–Dawley and Fisher 344 rats was associated with an increased incidence of focal hyperplasia and adenomas in the pituitary. The association of calcitonin treatment and pituitary tumors was dose-dependent and was more pronounced with salmon calcitonin than with porcine calcitonin (Brown *et al.*, 1993). Using both immunohistochemical analysis and measurements of serum hormone levels, they provided evidence that prolonged administration of calcitonin resulted in pituitary tumors that produced the common α-subunit of the glycoprotein hormones (leutinizing hormone [LH], follicle-stimulating hormone [FSH] and thyroid-stimulating hormones [TSH]), a type of tumor that has been reported to comprise a significant fraction of pituitary tumors in humans. Immunohistochemistry and in situ hybridization demonstrated that most pituitary tumors associated with the chronic administration of high doses of calcitonin expressed a glycoprotein hormone α-subunit, whereas expression of the α-subunit was identified infrequently in hyperplastic lesions of control rats.

Serum levels of each of the major pituitary hormones were measured in both sexes of Sprague–Dawley and Fisher rats administered calcitonin. There were no significant alterations in the circulating levels of growth hormone, prolactin, or ACTH and the tumors were negative by immunohistochemical and in situ hybridization assessment for these hormones. Serum LH and FSH levels were unaffected by the treatment with calcitonin; however, TSH levels were elevated 2.1-fold after calcitonin treatment in Sprague–Dawley but not Fischer rats of both sexes (Fig. 21-2). Interestingly, thyroid weights were decreased by 43% in calcitonin-treated male rats and there was atrophy of thyroid follicular cells in some treated rats, suggesting that the immunoreactivity detected by the TSH assay was not biologically active. After treatment with calcitonin, serum α-subunit levels were increased at least 20-fold in Sprague–Dawley males and fourfold in male Fischer rats (Figs. 21-3 and 21-4). There was a good correlation between histopathologic evidence of α-subunit-producing pituitary tumors and elevated serum levels. In each of the calcitonin-treated rats that had adenomas, the tumors were positive

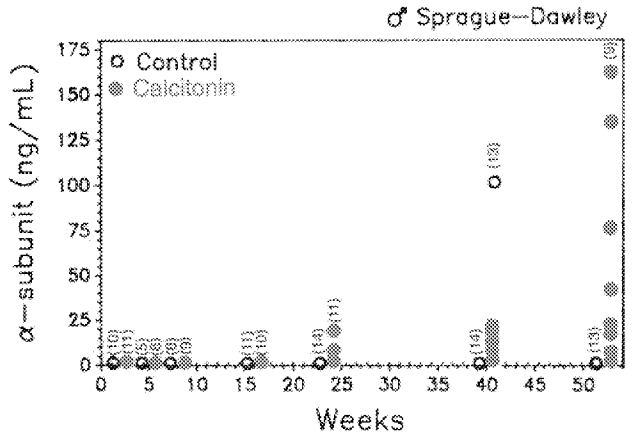

Figure 21-4. Time course for the increase in serum α-subunit levels in male Sprague–Dawley rats.

Symbols in the undetectable range represent values for more than one animal. The number of animals in each group is shown in parenthesis. 0 = control; ● = calcitonin-treated. (From Jameson *et al.*, 1992).

for α-subunit by immunohistochemistry and in situ hybridization for expression of α-subunit mRNA.

Serum levels of α-subunit were elevated in male Sprague–Dawley rats after 2, 5, 8, 16, 24, 40, and 52 weeks to determine the time course for hormone elevation. Elevated levels of α-subunit were detected as early as 24 weeks in rats treated with calcitonin and the majority of animals had increased α-subunit levels by 40 weeks of treatment (Fig. 21-4), suggesting that pituitary tumors developed only after several months of exposure to calcitonin. Levels of α-subunit in vehicle-treated rats were below the detection limits of the assay at each time point.

The studies reported by Jameson *et al.* (1992) did not determine whether the effects of calcitonin on the pituitary were direct or indirect. Calcitonin is known to be produced in large amounts in the posterior hypothalamus and median eminence where it may normally exert an effect on the hypothalamus–pituitary axis. Calcitonin receptors have been identified in the hypothalamus and lower numbers of receptors are found in the pituitary gland. A striking feature of the calcitonin-induced pituitary tumors and elevated serum α-subunit levels was the predilection for male compared with female rats. The basis for the sex- and species-specific effects of calcitonin was not determined. The relevance of the effects of calcitonin in the rat pituitary gland to human pathophysiology is uncertain at present. However, neither the treatment of patients with calcitonin nor patients with the multiple endocrine neoplasia syndrome II with medullary thyroid cancer and elevated serum calcitonin levels have resulted in the development of pituitary tumors. The doses of calcitonin used in rats were from 25- to 50-fold greater on a per-weight basis than doses administered to patients. In addition, several strains of rats are known to be highly predisposed to develop pituitary tumors compared to humans.

The high frequency of spontaneous pituitary adenomas in laboratory rats is a well-recognized phenomenon which must be considered in any long-term toxicological study (Attia, 1985). The incidence of pituitary tumors is determined by many factors including strain, age, sex, reproductive status, and diet (Berry, 1986). Studies from the National Toxicology Program (NTP) historical database of 2-year-old F344 rats have shown that the incidence of pituitary adenomas was 21.7% and 44% for males and females, respectively. Corresponding figures for carcinomas arising in the adenohypophysis were 2.4% and 3.5% for males and females, respectively. Numerous hypotheses have been invoked to explain the high incidence of pituitary adenomas in certain inbred rat strains. Both hereditary factors and the levels of circulating sex steroids have been suggested as important etiological mechanisms. The hypothalamus has also been incriminated in the development of these tumors (Azad *et al.*, 1989). Age-related hypothalamic changes may result in diminished activity of dopamine, the major prolactin-inhibitory factor.

Numerous studies have demonstrated the striking degree of strain variation in the incidence of pituitary tumors in rats, which has been reported to range from 10% to more than 90% (van Putten *et al.*, 1988; van Putten and van Zwieten, 1988; Sher *et al.*, 1982; Haseman *et al.*, 1998; Pickering and Pickering, 1984). Particularly high incidences of pituitary adenomas have been reported in Wistar, WAG/Rij, Osborne–Mendel, Long–Evans, Amsterdam, and Columbia-Sherman rats. In the BN/Bi strain, pituitary adenomas have been found in 26% of females and 14% of males. Adenomas were identified in 95% of females and 96% of males in the WAG/Rij strain whereas (WAG/Rij 3 BN) F_1 rats had incidences of 83% for females and 64% for males. Rapid body growth rates and high levels of conversion of feed to body mass in early life or high protein intake in early adult life predispose any strain of rat to the development of pituitary adenomas (Keenan *et al.*, 1994, 1995). In rats fed a low protein diet (less than 12.7% crude protein), the overall tumor incidence, the numbers of multifocal tumors, and the degree of cellular atypia within tumors are significantly lower than in rats fed a standard diet.

Cystoid Degeneration Cystoid degeneration has been used to describe foci of parenchymal cell loss in the adenohypophysis. Foci of cystoid degeneration (50–150 mm in diameter) have margins composed of normal secretory cells of the pars distalis. Cystoid degeneration also may occur in hyperplastic foci and in neoplasms of the pituitary. The frequency of cystoid degeneration is increased by feeding diets containing diethylstilbestrol to female C3H HeN (MTV+) mice.

Pituitary Hyperplasia and Neoplasia The separation between focal hyperplasia, adenoma, and carcinoma utilizing histopathological techniques is difficult in the pituitary gland. However, criteria for their separation have been established and should be applied in a consistent manner in the evaluation of proliferative lesions of the pituitary gland (Barsoum *et al.*, 1985; Berkvens *et al.*, 1980). For the specific trophic hormone-secreting cells of the adenohypophysis there appears to be a continuous spectrum of proliferative lesions between diffuse or focal hyperplasia and adenomas derived from a specific population of secretory cells. It appears to be a common feature of endocrine glands that prolonged stimulation of a population of secretory cells predisposes to the subsequent development of a higher than expected incidence of focal hyperplasia and tumors. Long-continued stimulation may lead to the development of clones of cells within the hyperplastic foci that grow more rapidly than the rest and are more susceptible to neoplastic transformation when exposed to the right combination of promoting carcinogens.

Focal ("nodular") hyperplasia in the adenohypophysis appears as multiple small areas that are well demarcated but not encapsulated from adjacent normal cells. Cells in areas of focal hyperplasia closely resemble the cells of origin; however, the cytoplasmic area may be slightly enlarged and the nucleus more hyperchromatic than in the normal cells. Adenomas usually are solitary nodules that are larger than the often multiple areas of focal hyperplasia. They are

sharply demarcated from the adjacent normal pituitary glandular parenchyma and there often is a thin, partial to complete, fibrous capsule. The adjacent parenchyma is compressed to varying degrees depending on the size of the adenoma. Cells composing an adenoma closely resemble the cells of origin morphologically and in their architectural pattern of arrangement.

Carcinomas usually are larger than adenomas in the pituitary and usually result in a macroscopically detectable enlargement. Histopathological features that are suggestive of malignancy include extensive intraglandular invasion, invasion into adjacent structures (e.g., dura mater, sphenoid bone), formation of tumor cell thrombi within vessels, and particularly the establishment of metastases at distant sites. The growth of neoplastic cells subendothelially in highly vascular benign tumors of the pituitary should not be mistaken for vascular invasion. Malignant cells often are more pleomorphic than normal, but nuclear and cellular pleomorphism are not a consistent criterion to distinguish adenoma from carcinoma in the adenohypophysis of rodents.

The vast majority of pituitary adenomas in humans and rodents have been described as chromophobic in type by light microscopy; however, many of these tumors have been found to stain for prolactin by immunohistochemistry. Of the prolactin-producing tumors, most are sparsely granulated with low levels of prolactin immunoreactivity by immunohistochemistry and having small numbers of secretory granules by electron microscopy (McComb *et al.*, 1984,1985; Sandusky *et al.*, 1988). Diffuse hyperplasia of the prolactin cells has been observed adjacent to some adenomas. The development of tumors and hyperplasia of prolactin-secreting cells often is accompanied by increasing serum levels of prolactin. Occasionally, prolactin cells within adenomas may be admixed with FSH/LH-, TSH-, or ACTH-positive cells in the rat pituitary.

ADRENAL CORTEX

Normal Structure and Function

The adrenal cortex is composed of three distinct zones. The outer zone is the zona glomerulosa and is composed of a thin region of columnar cells arranged in an arched or arcuate pattern. This zone is also called the zona multiformis in animals because of its different patterns of arrangement of secretory cells. The zona glomerulosa produces the steroid hormone, aldosterone, which is responsible for increasing sodium reabsorption and stimulating potassium excretion by the kidneys and thereby indirectly regulating extracellular fluid volume. Loss of this zone or the inability to secrete aldosterone may result in death due to retention of high levels of potassium with excess loss of sodium, chloride, and water. The zona fasciculata is the thickest zone (>70% of the cortex) and is composed of columns of secretory cells separated by prominent capillaries. The cells are polyhedral and have many intracellular lipid droplets. This zone produces glucocorticoids. The zona reticularis is also composed of polyhedral cells, whose arrangement is less linear and more as round nests or clumps of cells. The zona reticular produces glucocorticoids and in some species small amounts of sex steroids, namely, androgens, estrogens, and progestins. This zone is more distinct in rats compared to mice.

These are unique anatomical features of the adrenal gland blood supply that are important for its function and development of lesions. The gland is supplied by arterioles that penetrate the capsule, lose their muscular wall, and form a capillary bed that supplies the adrenal cortex. The arterioles that penetrate the capsule have a rapid reduction in lumen diameter and are a common site of embolization of bacteria or tumor cells. The capillaries of the cortex supply blood to the medulla via the cortical–medullary portal blood system. This results in a high concentration of glucocorticoids in the blood supplied to the medulla. Some of the arterioles directly supply the medulla with blood, so the medulla has two sources of blood, namely, cortical capillaries and primary arterioles. Blood leaves the adrenal gland via medullary veins.

Ultrastructural features of the cortical cells can be very useful for their identification. All three zones have prominent mitochondria that can be distinguished by the shape of their cristae. Cells of the zona glomerulosa are characterized by prominent mitochondria and Golgi apparatus, whereas cells of the zona fasciculata have many cytoplasmic lipid droplets, mitochondria with vesicular cristae, and abundant smooth endoplasmic reticulum. The zona reticularis is distinguished by the presence of prominent lysosomes in the cytoplasm.

The adrenal cortex of animals is prone to develop degenerative and proliferative lesions, the etiology of which may be either spontaneous in nature or experimentally induced (Ribelin, 1984). Therefore, testing of xenobiotic chemicals using various laboratory animal species is a valid means of assessing the toxic potential for humans exposed to various xenobiotic chemicals. The choice of test animal species also is critical as a number of studies have demonstrated that there often is a variable species susceptibility to chemical toxicity. This suggests that interspecies differences in metabolism play a role in the development of adrenal cortical toxicity and in the inhibition of steroidogenesis. The age of the test animal, to a lesser degree, can be a factor in the development of chemically induced adrenal cortical lesions.

The adrenal (suprarenal) glands in mammals are flattened bilobed organs located in close proximity to the kidneys. The adrenal glands receive arterial blood from branches of the aorta or from the phrenic, renal, and lumbar arteries resulting in a subcapsular sinusoidal vascular plexus that drains through the cortex into the medulla. The ratio of cortex:medulla is approximately 2:1 in healthy laboratory-reared animals.

The cortex is histologically characterized by defined regions or zones. The cortical zones consist of the zona glomerulosa (multiformis), zona fasciculata, and zona reticularis. The zones are not always clearly delineated, as in the normal rat adrenal cortex. The mineralocorticoid-producing zona glomerulosa (multiformis) (approximately 15% of the cortex) contains cells aligned in a sigmoid pattern in relationship to the capsule. Degeneration of this zone or an interference in the ability to produce mineralocorticoids (namely, aldosterone) results in a life-threatening retention of potassium and hypovolemic shock associated with the excessive urinary loss of sodium, chloride, and water. The largest part of the cortex is the zona fasciculata comprising >70% of the cortical width. Cells in this zone are arranged in long anastomosing columns separated by vascular sinusoids and are responsible for the secretion of glucocorticoid hormones (e.g., corticosterone or cortisol). The innermost portion of the cortex is the zona reticularis (>15% of the cortex), which secretes minute quantities of adrenal sex hormones.

The adrenal cortical cells contain large cytoplasmic lipid droplets which consist of cholesterol and other steroid hormone precursors. The lipid droplets are in close proximity to the smooth endoplasmic reticulum and large mitochondria which contain the specific hydroxylase and dehydrogenase enzyme systems required to synthesize the different steroid hormones. Unlike polypeptide-hormone-secreting cells, there are no secretory granules in the cytoplasm because there is direct secretion without significant storage of preformed steroid hormones.

Steroid-hormone-producing cells of the adrenal cortex synthesize a major parent steroid with one to four additional carbon atoms added to the basic 17-carbon steroid nucleus. Because steroid hormones are not stored in any significant amount, a continued rate of synthesis is required to maintain a normal secretory rate. Once in the circulation, cortisol or corticosterone are bound reversibly to plasma proteins (such as transcortin, albumin). Under normal conditions 10% of the glucocorticoids are in a free unbound state.

Adrenal steroids are synthesized from cholesterol by specific enzyme-catalyzed reactions and involve a complex shuttling of steroid intermediates between mitochondria and endoplasmic reticulum. The specificity of mitochondrial hydroxylation reactions in terms of precursor acted upon and the position of the substrate which is hydroxylated is confined to a specific cytochrome P-450. The common biosynthetic pathway from cholesterol is the formation of pregnenolone, the basic precursor for the three major classes of adrenal steroids. Pregnenolone is formed after two hydroxylation reactions at the carbon 20 and 22 positions of cholesterol and a subsequent cleavage between these two carbon atoms. In the zona fasciculata, pregnenolone is first converted to progesterone by two microsomal enzymes. Three subsequent hydroxylation reactions occur involving, in order, carbon atoms at the 17, 21, and 11 positions. The resulting steroid is cortisol, which is the major glucocorticoid in teleosts, hamsters, dogs, nonhuman primates, and humans. Corticosterone is the major glucocorticoid produced in amphibians, reptiles, birds, rats, mice, and rabbits. It is produced in a manner similar to the production of cortisol, except that progesterone does not undergo 17α-hydroxylation and proceeds directly to 21-hydroxylation and 11β-hydroxylation.

In the zona glomerulosa, pregnenolone is converted to aldosterone by a series of enzyme-catalyzed reactions similar to those involved in cortisol formation; however, the cells of this zone lack the 17α-hydroxylase and thus cannot produce 17α-hydroxyprogesterone which is required to produce cortisol. Therefore, the initial hydroxylation product is corticosterone. Some of the corticosterone is acted on by 18-hydroxylase to form 18-hydroxycorticosterone, which in turn interacts with 18-hydroxysteroid dehydrogenase to form aldosterone. Since 18-hydroxysteroid dehydrogenase is found only in the zona glomerulosa, it is not surprising that only this zone has the capacity to produce aldosterone. In addition to the aforementioned steroid hormones, cells in the zona reticularis also produce small amounts of sex steroids including progesterone, estrogens, and androgens.

The mineralocorticoids (e.g., aldosterone) are the major steroids secreted from the zona glomerulosa under the control of the rennin-angiotensin II system. The mineralocorticoids have their effects on ion transport by epithelial cells, particularly renal cells, resulting in conservation of sodium (chloride and water) and loss of potassium. In the distal convoluted tubule of the mammalian nephron, a cation exchange exists which promotes the resorption of sodium from the glomerular filtrate and the secretion of potassium into the lumen.

Under conditions of decreased blood flow or volume, the enzyme renin is released into the circulation at an increased rate by cells of the juxtaglomerular apparatus of the kidney. Renin release has also been associated with potassium loading or sodium depletion. Renin in the peripheral circulation acts to cleave a plasma globulin precursor (angiotensinogen produced by the liver) to angiotensin-I. An angiotensin-converting enzyme (ACE) subsequently hydrolyzes angiotensin-I to angiotensin-II, which acts as a trophic hormone to stimulate the synthesis and secretion of aldosterone. Under normal conditions negative feedback control to inhibit further renin release is exerted by the elevated levels of angiotensin (principally angiotensin-II) as well as the expanded extracellular fluid volume resulting from the increased electrolyte (sodium and chloride) and water reabsorption by the kidney.

The principal control for the production of glucocorticoids by the zona fasciculata and zona reticularis is exerted by adrenocorticotropin (ACTH), a polypeptide hormone produced by corticotrophs in the adenohypophysis of the pituitary gland. ACTH release is largely controlled by the hypothalamus through the secretion of corticotropin-releasing hormone (CRH). An increase in ACTH production results in an increase in circulating levels of glucocorticoids and under certain conditions also can result in weak stimulation of aldosterone secretion. Negative feedback control normally occurs when the elevated blood levels of cortisol act either on the hypothalamus, anterior pituitary, or both to cause a suppression of ACTH secretion (Rothuizen *et al.*, 1991).

Fetal Adrenal Cortex A specialized fetal adrenal cortex exists in primates during late gestation (Mesiano and Jaffe, 1997). The cortex is composed of large polyhedral cells that produce abundant cortisol and estrogen precursors. The hormones secreted by the cortex are important for normal development of the fetus, and the steroid precursor dihydroepiandrosterone is converted to estrogen by the placenta. The cells of the fetal cortex are produced in the outer cortex and migrate medially, where they undergo hypertrophy and eventually apoptosis. After birth, there is a rapid regression, apoptosis, and lysis of the fetal cortex with dilatation of cortical capillaries and replacement by the typical three cortical zones. It is important not to misinterpret this as a lesion in neonatal primates since it represents physiological replacement of the fetal cortex with the definitive postnatal adrenal cortex.

X-Zone of Adrenal Gland The X-zone in the mouse adrenal cortex is a similar unique physiologic phenomenon as the fetal cortex in primates. In contrast to the fetal cortex of primates, the X-zone develops postnatally in the inner cortex of mice and is fully formed at weaning. Its function is unknown, but it may be similar to the fetal zone in primates. After weaning, the X-zone degenerates at variable rates, depending on the sex of the mouse. In male mice, the X-zone undergoes degeneration at puberty with accumulation of intracellular fat globules. In unbred females, the zone undergoes slow regression and degeneration during the first pregnancy. As with the fetal zone in primates, it is important not to misinterpret the degeneration associated with regression of the X-zone in mice as a lesion.

Mechanisms of Adrenal Cortical Toxicity

The reason the adrenal cortex is predisposed to the toxic effects of xenobiotic chemicals appears to be related to at least two factors. First, adrenal cortical cells of most animal species contain large stores of lipids used primarily as substrate for steroidogenesis. Many adrenal cortical toxic compounds are lipophilic and therefore can accumulate in these lipid-rich cells. Second, adrenal cortical cells have enzymes capable of metabolizing xenobiotic chemicals, including enzymes of the cytochrome P450 family. Many of these enzymes function in the biosynthesis of endogenous steroids and are localized in membranes of the endoplasmic reticulum or mitochondria. A number of toxic xenobiotic chemicals serve as

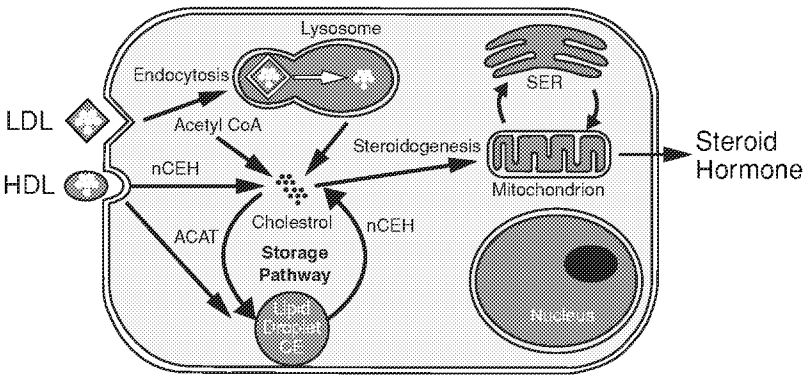

Figure 21-5. Cholesterol metabolism and steroid biosynthesis in adrenocortical and ovarian interstitial cells.

Cholesterol is the substrate for steroid biosynthesis. Conversion of cholesterol to pregnenolone occurs in the mitochondria and oxidative reactions catalyzed by P450 enzymes occur in the smooth endoplasmic reticulum and mitochondria. Sources of cholesterol include lipoprotein uptake from serum (LDL and HDL), de novo synthesis from acetate via the acetyl coenzyme A pathway, and hydrolysis of cholesteryl ester (CE) by neutral CE hydrolase (nCEH). The storage pool in the form of lipid droplets is derived principally from the conversion of free cholesterol to CE catalyzed by acyl coenzyme A:cholesterol acyltransferase (ACAT). Direct uptake of CE from serum to the storage pool is minimal in the rat. (From Latendresse *et al.*, 1993).

pseudosubstrates for these enzymes and can be metabolized to reactive toxic compounds. These reactive compounds result in direct toxic effects by covalent interactions with cellular macromolecules or through oxygen activation with the generation of free radicals (Colby, 1988; Hinson and Raven, 2006).

Impaired Steroidogenesis Impaired steroidogenesis is an important mechanism of toxicity in the adrenal cortex. It can occur by inhibition of cholesterol biosynthesis or metabolism and by disruption of cytochrome P-450 enzymes. Both these mechanisms will lead to the accumulation of increased cytoplasmic lipid in the form of discrete droplets.

Toxin Activation by CYP-450 Enzymes Toxins may be activated by many of the cytochrome P-450 enzymes in the cortical cells. Activation of toxins can result in the generation of reactive oxygen metabolites, membrane damage, and produce phospholipidosis in the cells.

Exogenous Steroids Exogenous steroids can disrupt normal function and structure of the adrenal cortex. Exogenous agonists will induce negative feedback inhibition of ACTH secretion by the pituitary and will result in atrophy of the zona fasciculata and reticularis. Some steroids, such as the sex steroids, can induce proliferative lesions in the adrenal cortex. Exogenous steroid antagonists will block steroid hormone action, lead to increased ACTH secretion, and diffuse hyperplasia of the cortex.

There is considerable species variation in the response of the adrenal cortex to exogenous chemicals. This is due to both inherent differences in the sensitivity to certain drugs and differences in the metabolic pathways of steroidogenesis. An interesting example is *o,p′*-DDD (Mitotane) which was originally developed to treat metastatic adrenal cortical cancer in humans; however, humans are relatively insensitive to the effects of *o,p′*-DDD, and the drug was not useful in the treatment of adrenal cancer. In contrast, dogs are more sensitive to the effects of *o,p′*-DDD, and it has been used effectively to treat pituitary-dependent hyperadrenocorticism due to autonomous secretion of ACTH by pituitary (corticotroph) tumors in a dose-dependent manner. *o,p′*-DDD is a selective toxin for the zona fasciculata and reticularis, thereby sparing the important functions of the zona glomerulosa (Vilar and Tullner, 1959).

The zonae reticularis and fasciculata appear to be the principal targets of xenobiotic chemicals in the adrenal cortex. Classes of chemicals known to be toxic for the adrenal cortex include short chain (3 or 4 carbon) aliphatic compounds, lipidosis-inducers, and amphiphilic compounds (Yarrington *et al.*, 1981, 1983, 1985). A variety of other compounds also may affect the medulla. The most potent aliphatic compounds are of 3-carbon length with electronegative groups at both ends. These compounds frequently produce necrosis, particularly in the zonae fasciculata and reticularis. Examples include acrylonitrile, 3-aminopropionitrile, 3-bromopropionitrile, 1-butanethiol, and 1,4-butanedithiol (Szabo *et al.*, 1980). By comparison, lipidosis inducers can cause the accumulations, often coalescing, of neutral fats which may be of sufficient quantity to cause a reduction or loss of organellar function and eventual cell destruction.

Lipidosis-Producing Compounds Cholesterol is the precursor substrate required to synthesize steroid hormones. Steroidogenic cells obtain cholesterol exogenously from serum lipoproteins and endogenously from *de novo* synthesis via the acetyl coenzyme A pathway (Fig. 21-5). The adrenal cortical cells and OI cells in the rat preferentially utilize serum high-density lipoproteins (HDLs) for their primary source of cholesterol and resort to *de novo* synthesis if HDL does not meet the demand of steroidogenesis. This is in contrast to Leydig cells of the testis, which preferentially utilizes *de novo* synthesis of cholesterol and uses an exogenous source only when intracellular synthesis does not meet the demand and the cholesterol pool has been depleted (Payne *et al.*, 1985). Examples of the compounds causing lipidosis include aminoglutethimide, amphenone, and anilines.

Tricresyl phosphate (TCP) and other triaryl phosphates cause a defect in cholesterol metabolism by blocking both the uptake from

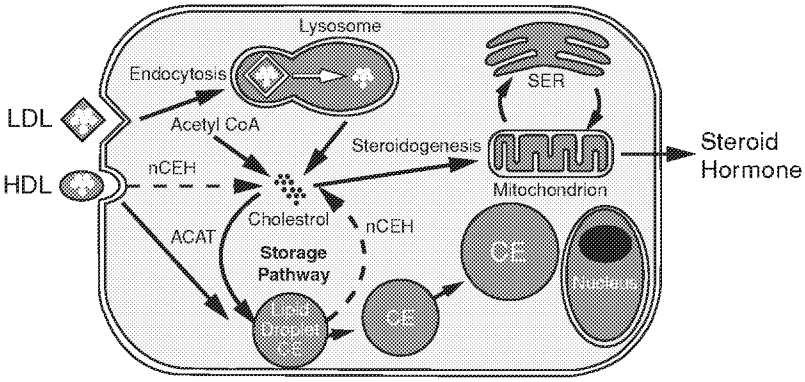

Figure 21-6. Pathogenesis of cholesteryl lipidosis in adrenocortical cells and ovarian interstitial cells.

The defect in cholesterol metabolism occurs in the uptake from serum and storage pathways. An inhibition of neutral cholesteryl ester hydrolase (nCEH) by a xenobiotic chemical results in the accumulation of CE in the form of lipid droplets in the cytoplasm. Acyl coenzyme A:cholesterol acyltransferase (ACAT) (catalyzes the formation of CE from cholesterol) activity remains near normal levels. (From Latendresse *et al.*, 1993).

serum and storage pathways. An inhibition of cytosolic neutral cholesteryl ester hydroxylase (nCEH) by triaryl phosphate (97% inhibition compared to controls) results in the progressive accumulation of cholesteryl ester in the form of lipid droplets in the cytoplasm of adrenal cortical and ovarian interstitial (OI) cells (Fig. 21-6) but not in testicular Leydig cells of rats (Latendresse *et al.*, 1993). Acyl coenzyme A: cholesterol acyl transferase (ACAT), an enzyme that esterifies cholesterol to make cholesteryl ester, was depressed only 27% (compared to controls) resulting in elevated intracellular storage of cholesterol in the form of lipid droplets (Fig. 21-7) (Latendresse *et al.*, 1994a,b, 1995).

Disruption of Membrane Organellar Membrane Turnover Biologically active cationic amphiphilic compounds produce a generalized phospholipidosis that involves primarily the zonae reticularis and fasciculata and produce microscopic phospholipid-rich inclusions. These compounds affect the functional integrity of lysosomes, which appear ultrastructurally to be enlarged and filled with membranous lamellae or myelin figures. Examples of compounds known to induce phospholipidosis include chloroquine, triparanol, and chlorphentermine.

Selective Destruction of Mitochondria and Smooth Endoplasmic Reticulum In addition, there is a miscellaneous group of chemicals that affect hydroxylation and other functions of mitochondrial and microsomal fractions (e.g., smooth endoplasmic reticulum) in the adrenal cortex. Examples of these compounds include *o,p'*-DDD and α-(1,4-dioxido-3-methylquinoxalin-2-yl)-*N*-methylnitrone (DMNM). Other compounds in this miscellaneous category cause their effects by means of cytochrome P-450 metabolism and the production of toxic metabolites. A classic example is the activation of carbon tetrachloride, resulting in lipid peroxidation and covalent binding to cellular macromolecules of the adrenal cortex.

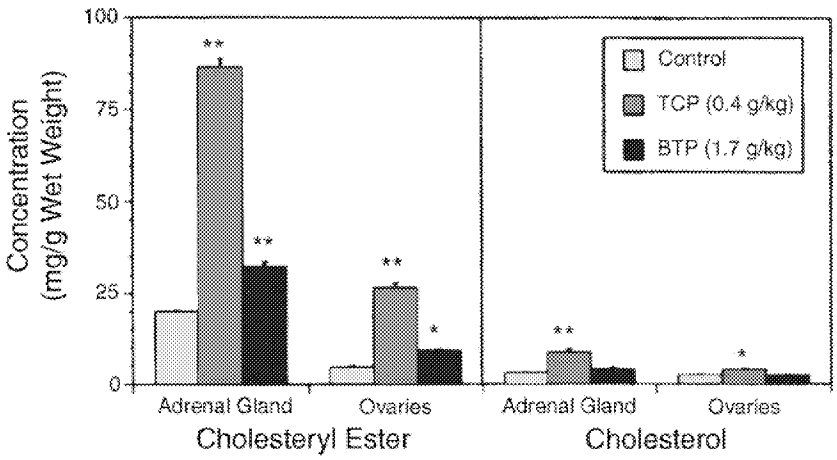

Figure 21-7. The effects of tricresyl phosphate (TCP) and butylated triphenyl phosphate (BTH) on the concentration of cholesteryl ester (CE) and cholesterol in adrenal gland and ovary of rats.

Mean ± SEM ($n = 8$–9) mg/g wet weight of adrenal gland and ovary. ***Different ($p \leq 0.01$, $p \leq 0.05$, respectively) from control. (From Latendresse *et al.*, 1993).

Studies with *o,p'*-DDD have shown a dose-dependent mitochondrial degeneration with vacuolization in the zonae fasciculata and reticularis (cortisol- and sex hormone-producing zone) with sparing of the aldosterone-producing zona glomerulosa (multiformis) (Hart *et al.*, 1971). The canine adrenal cortex is unusually sensitive to the cytotoxic effects of *o,p'*-DDD, which has led to the development of an effective drug (Lysodren or Mitotane) for the medical management of pituitary-dependent hypercortisolism (Cushing's disease). Cessation of weekly maintenance treatment with *o,p'*-DDD after regression of clinical signs often results in a recrudescence of functional disturbances of cortisol-excess due to chronic ACTH over-stimulation of the adrenal cortex leading to hypertrophy and hyperplasia of the cortisol-producing zones. The canine adrenal cortex is considerably more sensitive to the effects of *o,p'*-DDD than the adrenal cortices of humans.

Selective Inhibition of Angiotensin Converting Enzyme (ACE) Drugs such as Captopril, used in the medical management of hypertension, selectively inhibit ACE and result in atrophy of the outer zona glomerulosa (multiformis) in the adrenal cortex. Inhibition of ACE decreases the synthesis of angiotensin II from angiotensin I leading to trophic atrophy of the zona glomerulosa. The decreased production of aldosterone results in decreased renal tubular reabsorption of sodium and chloride, increased serum potassium due to decreased tubular excretion, and decreased tubular reabsorption of water resulting in lower blood volume and pressure.

Selective Inhibition of ACYL-CoenzymeA: Cholesterol ACYL Transferase (ACAT) Compounds that inhibit ACAT often result in degeneration of the zonae fasciculata and reticularis in the adrenal cortex. There is a distinct species sensitivity with dogs > guinea pigs > rabbits > monkeys > rats. It is uncertain whether the mechanism of toxicity is due to a direct cytotoxic effect of the compound or the result of the selective ACAT inhibition (Dominick *et al.*, 1993a,b; Reindel *et al.*; 1994; Junquero *et al.*, 2001; Robertson *et al.*, 2001).

Many of the chemicals that cause morphological changes in the adrenal glands also affect cortical function. Chemically induced changes in adrenal function result either from blockage of the action of adrenocorticoids at peripheral sites or by inhibition of synthesis and/or secretion of hormone. In the first mechanism, many antisteroidal compounds (antagonists) act by competing with or binding to steroid hormone-receptor sites; thereby, either reducing the number of available receptor sites or by altering their binding affinity. Cortexolone (11α-deoxycortisol) an antiglucocorticoid and spironolactone, an antimineralocorticoid, are two examples of peripherally acting adrenal cortical hormone antagonists.

Xenobiotic chemicals affecting adrenal function often do so by altering steroidogenesis and result in histologic and ultrastructural changes in adrenal cortical cells. For example, chemicals causing increased lipid droplets often inhibit the utilization of steroid precursors, including the conversion of cholesterol to pregnenolone. Chemicals that affect the fine structure of mitochondria and smooth endoplasmic reticulum often impair the activity of 11α-, 17α-, and 21α-hydroxylases, respectively, and are associated with lesions primarily in the zonae reticularis and fasciculata. Atrophy of the zona glomerulosa may reflect specific inhibition of aldosterone synthesis or secretion, either directly (e.g., inhibition of 18α- hydroxylation) or indirectly (e.g., suppression of the rennin-angiotensin system II) by chemicals such as spironolactone and captopril.

It is well documented that synthetic and naturally occurring corticosteroids are potent teratogens in laboratory animals. The principal induced defect is cleft lip or palate; however, there is a paucity of information on the direct effect of xenobiotic chemicals on the development of the adrenal cortex. For example, adrenal aplasia occurred in 7.6 of 9.8% of white Danish rabbits when thalidomide was given to their dams.

Pathologic Alterations and Proliferative Lesions in Cortical Cells

Macroscopic lesions of chemically affected adrenal glands are characterized either by enlargement or reduction in size that often is bilateral. Cortical hypertrophy due to impaired steroidogenesis or hyperplasia due to long-term stimulation often is present when the adrenal cortex is increased in size. Small adrenal glands often are indicative of degenerative changes or trophic atrophy of the adrenal cortex. Midsagittal longitudinal sections of adrenal glands under the above conditions will reveal either a disproportionately wider cortex relative to the medulla or vice versa, resulting in an abnormal cortical:medullary ratio. Nodular lesions that distort and enlarge one or both adrenal glands suggest that a neoplasm is present. A single well-demarcated nodular lesion suggests a cortical adenoma whereas widespread incorporation of the entire adrenal gland by a proliferative mass is suggestive of cortical carcinoma, especially if there is evidence of local invasion into periadrenal connective tissues or into adjacent blood vessels and the kidney.

Non-neoplastic lesions of the adrenal cortex induced by xenobiotic chemicals are characterized by changes ranging from acute progressive degeneration to reparative processes such as multifocal hyperplasia. Early degenerative lesions characterized by enlarged cortical cells filled with cytoplasmic vacuoles (often lipid) may result in a diffuse hypertrophy of the cortex. A lesion of this type has been observed in rats treated with an antibacterial compound α-(1,4-dioxido-3-methylquinoxalin-2-yl)-*N*-methylnitrone (DMNM). This type of vacuolar degeneration is a reflection of impaired steroidogenesis resulting in an accumulation of steroid precursors. More destructive lesions such as hemorrhage and/or necrosis are associated with an inflammatory response in the cortex. If the zona glomerulosa remains functional there will be no life-threatening electrolyte disturbances and no signs of hypoadrenocorticism (Addison's disease). While many chemical agents that affect the adrenal cortex initially involve the zona reticularis and inner zona fasciculata, certain chemicals such as DMNM can cause a progressive degeneration of the entire adrenal cortex. Occasionally, a chemical's effect is limited to a specific zone of the adrenal cortex and may be species specific (Yarrington and Johnston, 1994).

Ultrastructural alterations of adrenal cortical cells associated with chemical injury are quite diverse in nature. The zonae reticularis and fasciculata typically are most severely affected, although eventually the lesions involve the zona glomerulosa. These lesions may be classified as follows: endothelial damage (e.g., acrylonitrile), mitochondrial damage (e.g., DMNM, *o, p'*-DDD, amphenone), endoplasmic reticulum disruption (e.g., triparanol), lipid aggregation (e.g., aniline), lysosomal phospholipid aggregation (e.g., chlorophentermine), and secondary effects due to embolization by medullary cells (e.g., acrylonitrile). Mitochondrial damage with vacuolization and accompanying changes in the endoplasmic reticulum and autophagocytic responses appear to be among the most common ultrastructural changes observed following chemical injury in the adrenal cortex. Because mitochondria and smooth endoplasmic

reticulum form an intimate subcellular organellar network in cortical cells with important hydroxylases and dehydrogenase enzymes, it is not surprising that many chemical agents altering the ultrastructural morphology of cortical cells inhibit steroidogenesis.

Chemically induced proliferative lesions of the adrenal cortex are less frequently reported and include hyperplasia, adenoma, and carcinoma. Unlike the diffuse cortical hyperplasia/hypertrophy associated with excess ACTH stimulation, chemically induced hyperplasia usually is nodular in type, often multiple in distribution, and composed of increased numbers of normal or vacuolated cortical cells.

A variety of different chemicals are associated with an increased incidence of adrenal cortical neoplasia. Most of the reported tumors tend to be benign (adenomas) although an occasional tumor may be malignant (carcinomas). The zonae reticularis and fasciculata are more prone to develop tumors following chemical injury whereas the zona glomerulosa is spared unless invaded by an expanding tumor in the adjacent zones of the cortex. The tumorigenic agents of the adrenal cortex have a diverse chemical nature and use.

Spontaneous proliferative lesions may be found in all zones of the adrenal cortex but in adult rats are found most frequently in the zona fasciculata. Spontaneous nodular hyperplasia of the adrenal cortex is common in the rabbit, golden hamster, rat, mouse, dog, cat, horse, and baboon. Naturally occurring adrenal cortical tumors are found infrequently in domestic animals, except adult dogs and castrated male goats. However, cortical adenomas and (to a lesser extent) cortical carcinomas have been reported in moderately high incidence in certain strains of laboratory hamsters (e.g., BIO 4.24 and BIO 45.5 strains) and rat (e.g., Osborne–Mendel, WAG/Rij, BUF, and BN/Bi strains). The incidence often increases markedly in rats over 18 months of age. Adrenal cortical neoplasms in mice are uncommon but the incidence may be increased by gonadectomy.

Assessment of Function of the Adrenal Cortex

It may be useful to measure the function of the adrenal cortex. This can be accomplished by measuring glucocorticoid hormone concentrations in the blood or urine (expressed as a ratio to creatinine). It is important to remember the diurnal variations in secretion. Provocative testing is a useful tool to evaluate the functional capacity of the zonae fasciculata and reticularis by measuring the increase in secretion of glucocorticoids in response to exogenous ACTH. Light and electron microscopy and histomorphometry (cortico:medullary ratio and width of different cortical zones) are useful to characterize lesions that may disrupt the function of the cortical cells.

ADRENAL MEDULLA

Normal Structure and Function

The medulla constitutes approximately 10% of the volume of the adrenal gland. In the normal rodent adrenal gland and in most other laboratory animal species the medulla is sharply demarcated from the surrounding cortex. The bulk of the medulla is composed of chromaffin cells, which are the sites of synthesis and storage of the catecholamine hormones (Tischler *et al.*, 1988a). In the rat and mouse, norepinephrine and epinephrine are stored in separate cell types that can be distinguished ultrastructurally after fixation in glutaraldehyde and postfixation in osmium tetroxide. The norepinephrine-containing core of the secretory granules appears electron-dense and is surrounded by a wide submembranous space whereas epinephrine-containing granules are less dense, have a finely granular core, and a narrow space beneath the limiting membrane. Granules of varying densities may be found in the same cell types in the adrenal medulla of immature rats (Tischler and DeLellis, 1988a). Human adrenal medullary cells may contain both norepinephrine and epinephrine within a single chromaffin cell.

The adrenal medulla contains variable numbers of ganglion cells in addition to chromaffin cells (Reznik *et al.*, 1980). A third cell type has been described in the medulla and designated the small granule-containing (SGC) cell or small intensely fluorescent (SIF) cell. These cells morphologically appear intermediate between chromaffin cells and ganglion cells, and may function as inter-neurons. The adrenal medullary cells also contain serotonin and histamine, but it has not been determined if these products are synthesized in situ or taken up from the circulation. A number of neuropeptides also are present in rat chromaffin cells including enkephalins, neurotensin, and neuropeptide Y.

In the catecholamine biosynthetic pathway, tyrosine is acted on by tyrosine hydroxylase to produce dopa, which is converted to dopamine by dopa decarboxylase. Dopamine in turn, is acted on by dopamine beta-hydroxylase to form norepinephrine, which is converted to epinephrine by phenylethanolamine-*N*-methyltransferase (PNMT). Tyrosine hydroxylase and PNMT are the principal rate limiting steps in catecholamine synthesis. The conversion of tyrosine into dopa and dopamine occurs within the cytosol of chromaffin cells. Dopamine then enters the chromaffin granule where it is converted to norepinephrine. Norepinephrine leaves the granule and is converted into epinephrine in the cytosol and epinephrine re-enters and is stored in the chromaffin granule. In contrast to the synthesis of catecholamines, which occurs in the cytosol, neuropeptides and chromogranin-A proteins are synthesized in the granular endoplasmic reticulum and are packaged into granules in the Golgi apparatus.

Innervation plays an important role in regulating the functions of chromaffin cells. During adult life, stresses such as insulin-induced hypoglycemia or reserpine-induced depletion of catecholamines produces a reflex increase in splanchnic nerve discharge, resulting both in catecholamine secretion and transsynaptic induction of catecholamine biosynthetic enzymes, including tyrosine hydroxylase. These effects become apparent during the first week of life, following an increase in the number of nerve terminals in the adrenal medulla. Other environmental influences including growth factors, extracellular matrix, and a variety of hormonal signals that generate cyclic AMP, may also regulate the function of chromaffin cells.

Mechanisms of Adrenal Medullary Toxicity

Proliferative lesions of the medulla, particularly in the rat, have been reported to develop as a result of a variety of different mechanisms (Rosol *et al.*, 2001; Tischler *et al.*, 1985, 1988b, 1991). Warren *et al.* (1966) studied over 700 pairs of rats with parabiosis and found that more than 50% of male irradiated rats developed adrenal medullary tumors. A relationship exists between adenohypophyseal (anterior pituitary) hormones and the development of adrenal medullary proliferative lesions (Manger *et al.*, 1982). For example, the long-term administration of growth hormone is associated with an increased incidence of pheochromocytomas as well as the development of tumors at other sites. Prolactin-secreting pituitary tumors, which occur commonly in many rat strains, also play a role in the development of proliferative medullary lesions. In addition,

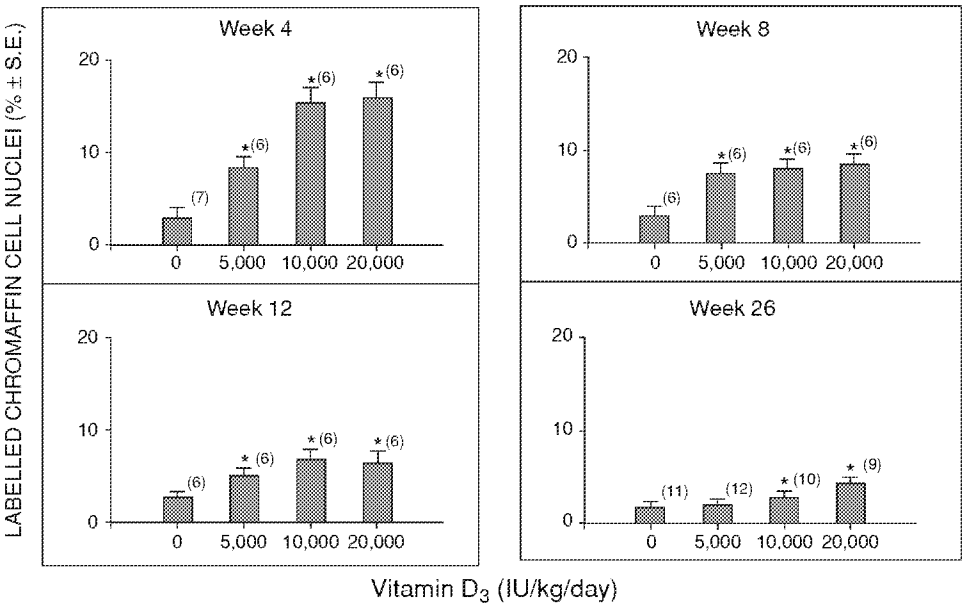

Figure 21-8. Effects of vitamin D_3 on percent of chromaffin cells labeled with BrdU during weeks 4, 8, 12, and 26 of dietary supplementation.

Asterisks indicate statistically significant increases over corn oil controls. Numbers in parenthesis indicate numbers of rats scored for each point. Premature deaths or euthanasia caused loss of three animals from the 20,000 IU/kg/day group, two from the 10,000 IU/kg/day group and one control. Histological examination of these animals' adrenal glands showed no detectable abnormalities. One extra animal at the start of the experiment was assigned to the control group at week 1. At least 2500 chromaffin cells were scored for each rat. (From Tischler *et al.*, 1999).

several neuroleptic compounds that increase prolactin secretion by inhibiting dopamine production have been associated with an increased incidence of proliferative lesions of medullary cells in chronic toxicity studies in rats.

Both nicotine and reserpine have been implicated in the development of adrenal medullary proliferative lesions in rats. Both chemicals act by a shared mechanism since nicotine directly stimulates nicotinic acetylcholine receptors whereas reserpine causes a reflex increase in the activity of cholinergic nerve endings in the adrenal (Tischler *et al.*, 1995). A short dosing regimen of reserpine administration in vivo stimulates proliferation of chromaffin cells in the adult rat and the mechanism may involve a reflex increase in neurogenic stimulation via the splanchnic nerve. Several other drugs have been reported to increase the incidence of adrenal medullary proliferative lesions. These include zomepirac sodium (a nonsteroidal inflammatory drug) (Mosher and Kircher, 1988), isoretinoin (a retinoid), and nafarelin (LHRH analog), atenolol (β-adrenergic blocker), terazosin (α-adrenergic blocker), ribavirin (antiviral), and pamidronate (bisphosphonate) (Davies and Monro, 1995).

Lynch *et al.* (1996) have reported that nutritional factors have an important modulating effect on the spontaneous incidence of adrenal medullary proliferative lesions in rats. Several sugars and sugar alcohols have produced adrenal medullary tumors at high dosages (concentrations of 10–20% in the diet), including xylitol, sorbitol, lacitol, and lactose (Baer, 1988). Although the exact mechanism involved is not completely understood, an important role for calcium has been suggested. High doses of slowly absorbed sugars and starches increase the intestinal absorption and urinary excretion of calcium. Hypercalcemia is known to increase catecholamine synthesis in response to stress, and low-calcium diets will reduce the incidence of adrenal medullary tumors in xylitol-treated rats. Other compounds that may act by a similar mechanism of altered calcium homeostasis include the retinoids (which produce hypercalcemia) and conditions such as progressive nephrocalcinosis in aging male rats treated with nonsteroidal anti-inflammatory agents.

Roe and Bar (1985) have suggested that environmental and dietary factors may be more important than genetic factors as determinants of the incidence of adrenal medullary proliferative lesions in rats. The incidence of adrenal medullary lesions can be reduced by lowering the carbohydrate content of the diet. Several of the chemicals that increase the incidence of adrenal medullary lesions, including sugar alcohols, increase absorption of calcium from the gut. Calcium ions as well as cyclic nucleotides and prostaglandins may act as mediators capable of stimulating both hormonal secretion and cellular proliferation.

Tischler *et al.* (1996, 1999) presented data that vitamin D is the most potent in vivo stimulus yet identified for chromaffin cell proliferation in the adrenal medulla. Vitamin D_3 (5000, 10,000, or 20,000 IU/kg/day in corn oil) resulted in a four- to fivefold increase in bromodeoxyuridine (BrdU) labeling at week 4 that diminished to a twofold increase by week 26 (Fig. 21-8). An initial preponderance of epinephrine-labeled (PNMT-positive) cells subsequently gave way to norepinephrine-labeled cells. By week 26, 89% of rats receiving the two highest doses of vitamin D_3 had focal medullary proliferative lesions (BrdU-labeled focal hyperplasia, "hot spots") and pheochromocytomas in contrast to absence of proliferative lesions in controls. This increase in medullary cell proliferation was associated with a significant increase in circulating levels of both calcium and phosphorus after vitamin D administration (Fig. 21-9). The nuclei of hyperplastic chromaffin cells were labeled by BrdU but were phenylethanolamine-*N*-methyl transferase-negative indicating that they most likely were norepinephrine-producing cells

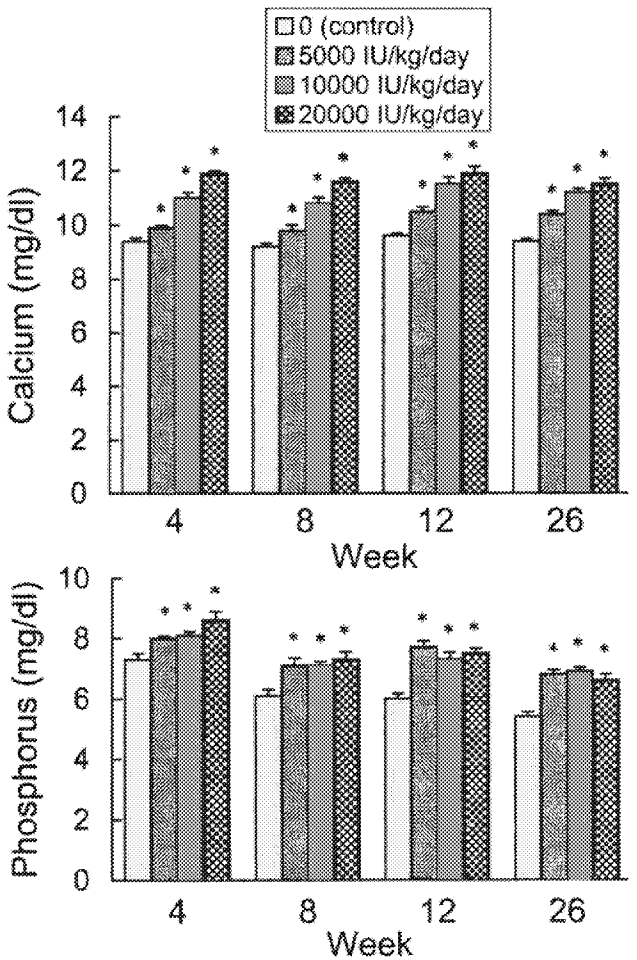

Figure 21-9. Effects of vitamin D_3 on serum calcium and phosphorus levels at weeks 4, 8, 12, and 26. Sustained perturbation of both Ca^{++} and PO_4 concentrations persisted throughout the time course. (From Tischler et al., 1999).

of the rat medulla (Fig. 21-10). The proliferative lesions usually were multicentric, bilateral, peripheral in location in the medulla, nearly all were PNMT negative, and appeared to represent morphologic continuum rather than separate entities. Earlier studies reported by the same research group had demonstrated that the vitamin D_3 (20,000, 40,000 IU/kg/day) increase in chromaffin cell proliferation was observed as early as 1 week and had declined by 4 weeks. These findings support the hypothesis that altered calcium homeostasis is involved in the pathogenesis of pheochromocytomas in rodents, most likely through effects on increasing chromaffin cell proliferation (Fig. 21-11). Vitamin D_3, calcitrol (active metabolite of D_3), lactose, and xylitol all failed to stimulate directly the proliferation of rat chromaffin cells in vitro.

In summary, three dietary factors have been suggested to lead to an increased incidence of adrenal medullary proliferative lesions in chronic toxicity studies in rats (Roe and Bar, 1985). These are (1) excessive intake of food associated with feeding ad libitum; (2) excessive intake of calcium and phosphorus, since commercial diets contain 2–3 times more calcium and phosphorus than needed by young rats; and (3) excessive intake of other food components (e.g., vitamin D and poorly absorbable carbohydrates) which increase calcium absorption.

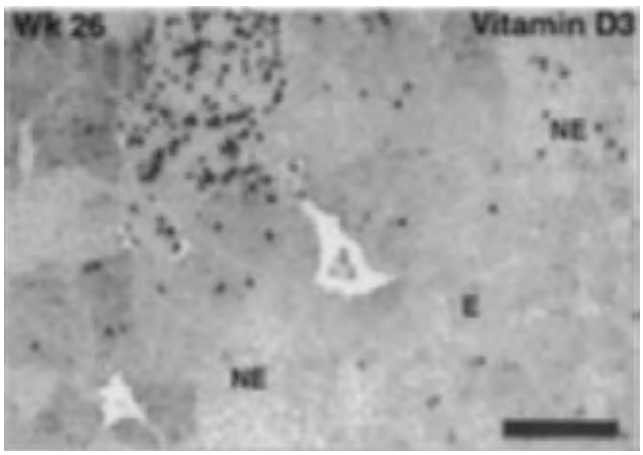

Figure 21-10. Photomicrographs of adrenal medullary sections stained for BrdU (dark nuclei) and PNMT (dark cytoplasm) to compare BrdU labeling of epinephrine (E) and norepinephrine (NE) cells.

Vitamin D_3 (20,000 IU/kg/day) caused a dramatic increase in BrdU labeling of predominantly E-cells at week 4. The response was greatly reduced at week 26 and there was no longer an E-cell predominance. A representative hyperplasic nodule in the vitamin D_3-stimulated adrenal medulla at week 26 (top left) is densely labeled with BrdU and is PNMT-negative (bar = 100 μm). (From Tischler *et al.*, 1999).

Pathologic Alterations and Proliferative Lesions of Adrenal Medullary Cells

Adrenal Medullary Hyperplasia and Neoplasia The adrenal medulla undergoes a series of proliferative changes ranging from diffuse hyperplasia to benign and malignant neoplasia. The latter neoplasms have the capacity to invade locally and to metastasize to distant sites. Diffuse hyperplasia is characterized by symmetrical expansion of the medulla with maintenance of the usual sharp demarcation between the cortex and the medulla. The medullary cell cords often are widened, but the ratio of norepinephrine to epinephrine cells is similar to that of normal glands. Focal hyperplastic lesions are often juxtacortical but may occur within any area of the medulla. The small nodules of hyperplasia in general are not associated with compression of the adjacent medulla; however, the larger foci may be associated with limited medullary compression. The foci of adrenal medullary hyperplasia are typically composed of small cells with round to ovoid nuclei and scanty cytoplasm. At the ultrastructural level, the cells composing these focal areas of hyperplasia contain small numbers of dense core secretory granules resembling the granules of SIF or SGC cells (Tischler and DeLellis, 1988b).

Larger benign adrenal medullary proliferative lesions are designated as pheochromocytomas. These lesions may be composed of relatively small cells similar to those found in smaller hyperplastic foci or larger chromaffin cells or a mixture of small and large cells. According to some authors, the lack of a positive chromaffin reaction in these focal proliferative lesions precludes the diagnosis of pheochromocytoma; however, the chromaffin reaction is quite insensitive and catecholamines (particularly norepinephrine) can be demonstrated in these proliferative lesions by biochemical extraction studies and by the formaldehyde- or glyoxylic-acid-induced fluorescence methods. Even in some of the larger medullary lesions, the chromaffin reaction is equivocal but catecholamines can be demonstrated both biochemically and histochemically.

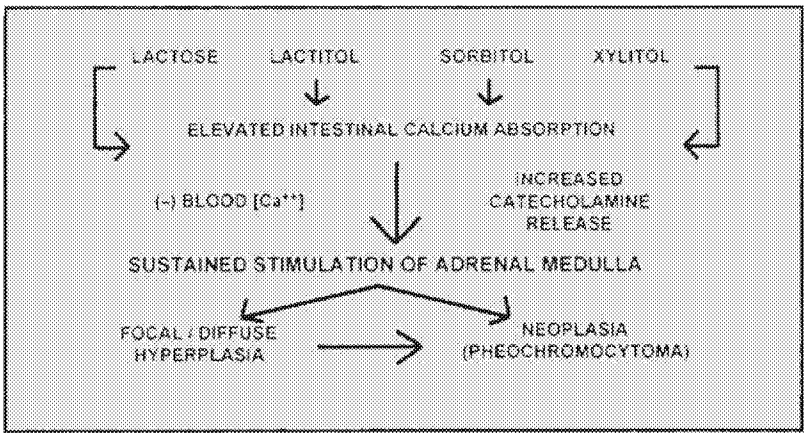

Figure 21-11. Pathogenesis of adrenal medullary proliferative lesions associated with ingestion of polyols resulting in elevation of the blood calcium concentration. (Modified from Lynch et al., 1996).

Malignant pheochromocytomas invade the adrenal capsule and often grow in the periadrenal connective tissues with or without distant metastases.

Proliferative lesions occur with high frequency in many strains of laboratory rats. The incidence of these lesions varies with strain, age, sex, diet, and exposure to drugs, and a variety of environmental agents (Nyska *et al.*, 1999; Roe *et al.*, 1995). Studies from the NTP historical database of two-year-old F344 rats have reported that the incidence of pheochromocytomas was 17.0% and 3.1% for males and females, respectively. Malignant pheochromocytomas were detected in 1% of males and 0.5% of females. In addition to F344 rats, other strains with a high incidence of pheochromocytomas include Wistar, NEDH (New England Deaconess Hospital), Long–Evans, and Sprague–Dawley. Pheochromocytomas are considerably less common in Osborne–Mendel, Charles River, Holtzman, and WAG/Rij rats. Most studies have revealed a higher incidence in males than in females (Figs. 21-12 and 21-13). Crossbreeding of animals with high and low frequencies of adrenal medullary proliferative lesions results in F_1 animals with an intermediate tumor frequency. Pheochromocytomas are less common in mice than in most strains of rats.

There is a conspicuous relationship between increasing age and the frequency, size, and bilateral occurrence of adrenal medullary proliferative lesions in the rat. In the Long–Evans strain, medullary nodules have been found in less than 1% of animals under 12 months of age. The frequency increases to almost 20% in 2-year-old animals and to 40% in animals between 2 and 3 years of age. The mean tumor size increases progressively with age as does the frequency of bilateral and multicentric occurrence.

A variety of techniques may be used for the demonstration of catecholamines in tissue sections. The chromaffin reaction is the oxidation of catecholamines by potassium dichromate solutions and results in the formation of a brown-to-yellow pigment in medullary tissue. The chromaffin reaction as traditionally performed possesses a low level of sensitivity and should not be used for the definitive demonstration of the presence of catecholamines in tissues. Similarly, both the argentaffin and argyrophil reactions, which have been used extensively in the past for the demonstration of chromaffin cells, also possess low sensitivity and specificity. Fluorescence techniques using formaldehyde or glyoxylic acid represent the methods of choice for the demonstration of catecholamines at the cellular level. These aldehydesform highly fluorescent derivatives

RAT STRAIN	INCIDENCE (%) MALES (%)	FEMALES (%)	REFERENCE
CHARLES RIVER-CD	109/1211 (9.0)	24/1204 (2.0)	SHER *et al.* (1982)
F344 (NTP)	156/1794 (8.8)	55/1754 (3.1)	GOODMAN *et al* (1979)
HOLTZMAN	4/806 (0.5)	3/618 (0.5)	SCHARDIEN *et al* (1968)
F344	76/448 (17.0)	14/450 (3.1)	SHER *et al.* (1982)

Figure 21-12. Rat strains with a high incidence of pheochromocytomas. (Modified from Lynch et al., 1996).

RAT STRAIN	INCIDENCE (%) MALES (%)	FEMALES (%)	REFERENCE
OSBORNE-MENDEL (NTP)	21/975 (2.2)	9/970 (0.9)	GOODMAN et al. (1980)
SPRAGUE-DAWLEY (CIBA-GEIGY)	121/578 (20.9)	36/585 (6.2)	McMARTIN et al. (1992)
WISTAR (BOR:WISW [SPF Cpb])	131/445 (29.4)	16/447 (3.6)	BOMHARD (1992) (30 mo.)
WISTAR (BOR:WISW [SPF Cpb])	133/1230 (10.6)	27/1242 (2.1)	BOMHARD & RINKE (1994) (24 mo.)
WISTAR (LOBUND)	46/66 (69.2)	NO DATA	POLLARD & LUCKERT (1989)

Figure 21-13. Rat strains with a low incidence of pheochromocytomas. (Modified from Lynch et al., 1996).

with catecholamines, which can be visualized by ultraviolet microscopy. Immunohistochemistry provides an alternative approach for the localization of catecholamines in chromaffin cells and other cell types. Antibodies are available that permit epinephrine- and norepinephrine-containing cells to be distinguished in routinely fixed and embedded tissue samples. Several of the important enzymes involved in the biosynthesis of catecholamine hormones also may be demonstrated by immunohistochemical procedures. Antibodies to chromogranin-A can be used for the demonstration of this unique protein in chromaffin cells (Puchacz *et al.*, 1996; Tischer *et al.*, 1990, 1994).

Pheochromocytomas in rats and human beings are both composed of chromaffin cells with variable numbers of hormone-containing secretory granules (Fig. 21-14). The incidence is high in many strains of rats by comparison to human patients where pheochromocytomas are uncommon except in patients with inherited clinical syndromes of multiple endocrine neoplasia (MEN). These tumors in rats usually do not secrete excess amounts of catecholamines whereas human pheochromocytomas episodically secrete increased amounts of catecholamines leading to hypertension and other clinical disturbances. There appears to be a striking species difference in the response of medullary chromaffin cells to mitogenic stimuli with rats being very sensitive compared to humans (Fig. 21-14).

Tischler and Riseberg (1993) reported that adult rat chromaffin cells had a marked (10–40%) increase in bromodeoxyuridine (BrdU) labeled nuclei in vitro following the addition of the following mitogens: nerve growth factor (NGF), fibroblast growth factor (FGF), forskolin, and phorbol myristate (PMA) whereas human chromaffin cells had a minimal (<0.1%) response to the same mitogens (Fig. 21-15). This striking difference in sensitivity to mitogenic stimuli may explain the lower frequency of adrenal medullary proliferative lesions in humans compared to many rat strains (Tischler and Riseberg, 1993). The mouse adrenal medulla, which like humans has a low spontaneous incidence of proliferative lesions of chromaffin cells, also failed to respond to a variety of mitogenic stimuli (Fig. 21-15). These findings and others suggest that chromaffin cells of the rat represent an inappropriate model to assess the potential effects of xenobiotic chemicals on chromaffin cells of the human adrenal medulla (Lynch *et al.*, 1996).

CHARACTERISTICS	RATS	HUMANS
TUMOR CELLS	SMALLER THAN NORMAL	SMALL OR LARGE
SECRETORY GRANULES	OFTEN SMALL	VARIABLE (E + NOR E)
HORMONE CONTENT	NOR E	E + NOR E
FUNCTIONAL STATUS	INACTIVE	CATECHOLAMINE EXCESS
INCIDENCE	HIGH (STRAIN DIFFERENCE)	LOW (MEN SYNDROME)
RESPONSE OF AM TO MITOGENIC STIMULI	SENSITIVE	RESISTANT

Figure 21-14. Characteristics of pheochromocytomas in rats compared to humans. (Modified from Lynch et al., 1996).

BrdU INCORPORATION *IN VITRO*

	CONTROL	NGF	FGF	FORSKOLIN	PMA
RAT	<1%	UP TO 40%	UP TO 40%	UP TO 40%	UP TO 10%
HUMAN	<0.1%	<0.1%	<0.1%	<0.1%	<0.1%
BOVINE	0	0	0	0	0
MOUSE	<0.1%	0	UP TO 5%	UP TO 1 %	UP TO 1%

PM = PHORBOL MYRISTATE; NGF = NERVE GROWTH FACTOR; FGF = FIBROBLAST GROWTH FACTOR; BrdU = BROMODEOXYURIDINE

[1] RAT (3- TO 5-MONTH-OLD MALE AND FEMALE F344 OR CHARLES RIVER SPRAGUE-DAWLEY) BOVINE (ADULT FEMALE); [2] MOUSE (129/SVJ x C57 L/6J; 3-MONTH-OLD FEMALES)

FROM: TISCHLER AND RISEBERG, ENDOCRINE PATH 1993, 15-19

Figure 21-15. Species differences in mitogenic responses of chromaffin cells in vitro from adult rat, human, bovine, and mouse adrenals. (From Tischler and Riseberg (1993).

Chemically Induced Medullary Toxicity

Medullary chromaffin cells in rats are susceptible to acute necrosis and cytolysis by salinomycin, which occurs in less than 10 hours (Chen-Pan *et al.*, 1999). However, the adrenal medulla is capable of replenishing the chromaffin cells in as little as 24 hours by an unknown mechanism. It appears that the adrenal medulla recruits undifferentiated chromaffin cells to repopulate the medulla by a rapid and orderly process of differentiation. Repopulation is not dependent on mitosis. Therefore, chemically induced acute necrosis of the adrenal medulla may be easily overlooked if short experimental time points (<24 hours) were not examined.

Chen-Pan *et al.* (2002), in a subsequent study with salinonycin, found the rapid recovery to be based on differentiation of preexisting primitive undifferentiated chromaffin cells, rather than by mitosis, due to a lack of 5-bromo-2-deoxyuridine incorporation in the differentiating and/or differentiated chromaffin cells. Schwann cells actively functioned as phagocytes and appeared to indirectly stimulate the differentiation of undifferentiated chromaffin cells. Inflammatory cells were rare with only a few lymphocytes evident inside the clusters of necrotic medullary cells.

THYROID GLAND (FOLLICULAR CELLS)

Normal Structure and Function

The thyroid gland is the largest of the endocrine organs that functions exclusively as an endocrine gland (Capen 2000). The basic histologic structure of the thyroid is unique for endocrine glands, consisting of follicles of varying size (20–250 μm) that contain colloid produced by the follicular cells (thyrocytes). The follicular cells are cuboidal to low columnar (under conditions of normal iodine intake) and their secretory polarity is directed toward the lumen of the follicles. An extensive network of interfollicular capillaries provides the follicular cells with an abundant blood supply. Follicular cells have extensive profiles of rough endoplasmic reticulum and a large Golgi apparatus in their cytoplasm for synthesis and packaging substantial amounts of protein (e.g., thyroglobulin) that are then transported into the follicular lumen. The interface between the luminal side of follicular cells and the colloid is modified by numerous microvillous projections.

The synthesis of thyroid hormones is unique among endocrine glands because the final assembly of hormone occurs extracellularly within the follicular lumen. Essential raw materials, such as iodide ion (I^-), are trapped by follicular cells from plasma, transported rapidly against a concentration gradient to the lumen of the follicle, and oxidized by a peroxidase enzyme in the microvillar membranes to iodine (I_2).

Biosynthesis of Thyroid Hormones The assembly of thyroid hormones within the follicular lumen is made possible by a unique protein, thyroglobulin, synthesized by follicular cells (Fig. 21-16). Thyroglobulin is a high molecular-weight glycoprotein synthesized in successive subunits on the ribosomes of the endoplasmic reticulum in follicular cells. The constituent amino acids (tyrosine and others) and carbohydrates (mannose, fructose, galactose) are derived from the circulation. Recently synthesized thyroglobulin leaving the Golgi apparatus is packaged into apical vesicles which are extruded into the follicular lumen. The amino acid tyrosine, an essential component of thyroid hormones, is incorporated within the molecular structure of thyroglobulin. Iodine is bound to the tyrosyl residues in thyroglobulin at the apical surface of follicular cells to form successively monoiodotyrosine (MIT) and diiodotyrosine (DIT). These biologically inactive iodothyronines subsequently are coupled together under the influence of the thyroperoxidase to form the two biologically active iodothyronines (thyroxine-T4, triiodothyronine-T3) secreted by the thyroid gland.

The functionally important thyroperoxidase enzyme in the thyroid hormone synthetic pathway is present in the apical plasma membrane and microvilli as well as in other membranous structures of the follicular cells (Tice and Wollman, 1972, 1974). Thyroperoxidase is a membrane-bound, heme-containing glycoprotein composed of 933 amino acids with a transmembrane domain. This important enzyme oxidizes (in the presence of hydrogen peroxidase) iodide ion (I^-) taken up by follicular cells through the function of the sodium-iodide symporter into reactive iodine (I_2), which binds to the tyrosine residues in the thyroglobulin. Iodine is incorporated not

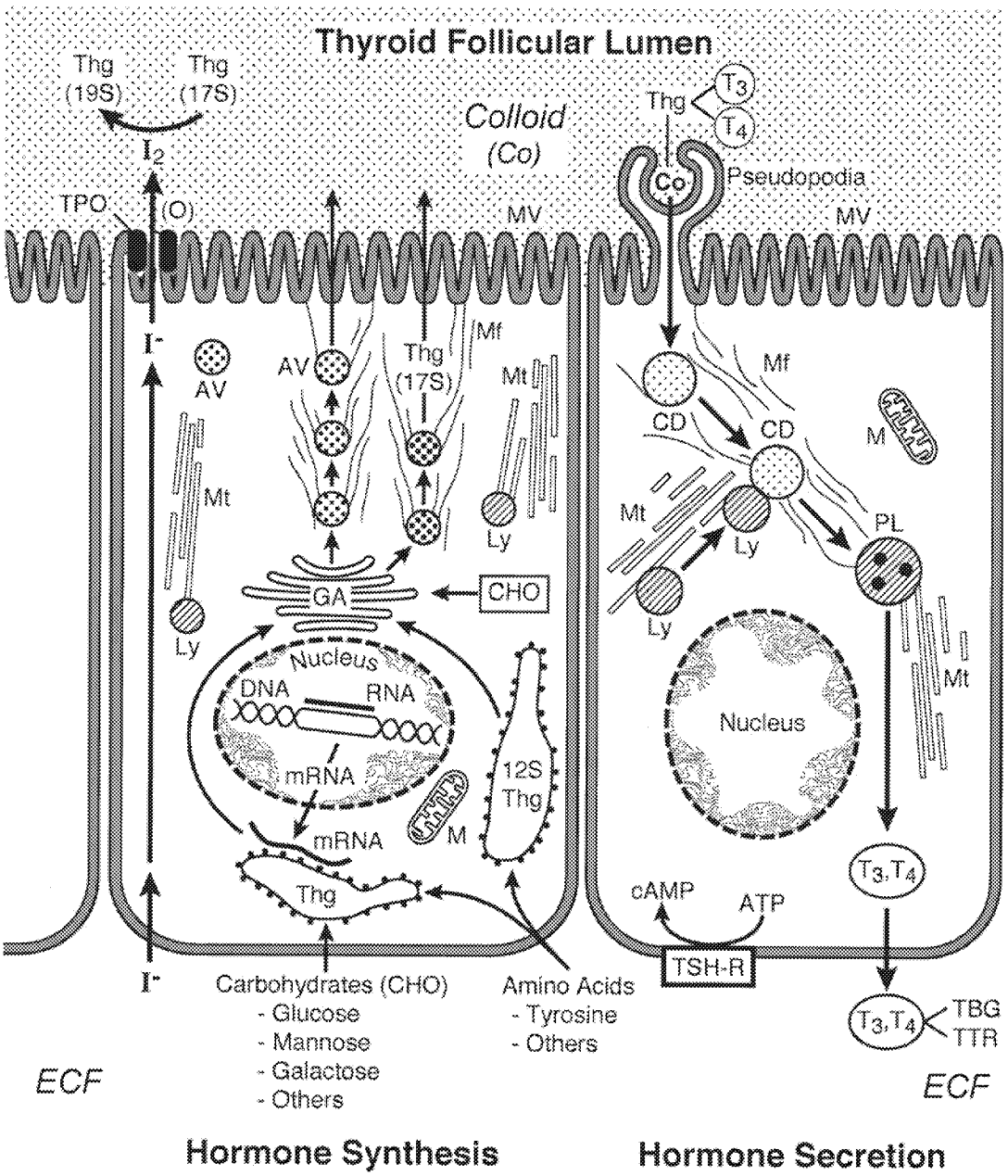

Figure 21-16. Normal thyroid follicular cells illustrating two-way traffic of materials from capillaries into the follicular lumen and from lumen back into interfollicular capillaries.

Raw materials, such as iodine, are concentrated by follicular cells and rapidly transported into the lumen (left). Amino acids (tyrosine and others) and sugars are assembled by follicular cells into thyroglobulin (thg), packaged into apical vesicles (AV), and released into the lumen. The iodination of tyrosyl residues occurs within the thyroglobulin molecule to form thyroid hormones in the follicular lumen. Elongation of microvilli and endocytosis of colloid by follicular cells occurs in response to thyroid-stimulating hormone (TSH) stimulation (right). The intracellular colloid droplets (Co) fuse with lysosomal bodies (Ly), active thyroid hormone is enzymatically cleaved from thyroglobulin, and free tetraiodothyronine (T4) and triiodothyronine (T3) are released into circulation. M, mitochondria; N, nucleus; CHO, carbohydrates; GA, Golgi apparatus; R, receptor; TgB, thyroid-binding globulin; PL, phagolysosome; TTR, transthyretin; ECF, extra cellular fluids; Mf, microfilaments; and Mt, microtubules. (From Capen, 2004).

only into newly synthesized thyroglobulin recently delivered to the follicular lumen but also into molecules already stored in the lumen. Thyroperoxidase also functions as a coupling enzyme to combine MIT and DIT to form triiodothyronine (T_3) or two DITs to form thyroxine (T_4).

The mechanism of active transport of iodide has been shown to be associated with a sodium-iodide (Na^+-I^-) symporter (NIS) present in the basolateral membrane of thyroid follicular cells (Fig. 21-17) (LaPerle and Jhiang, 2003). Transport of iodide ion across the thyroid cell membrane is linked to the transport of Na^+.

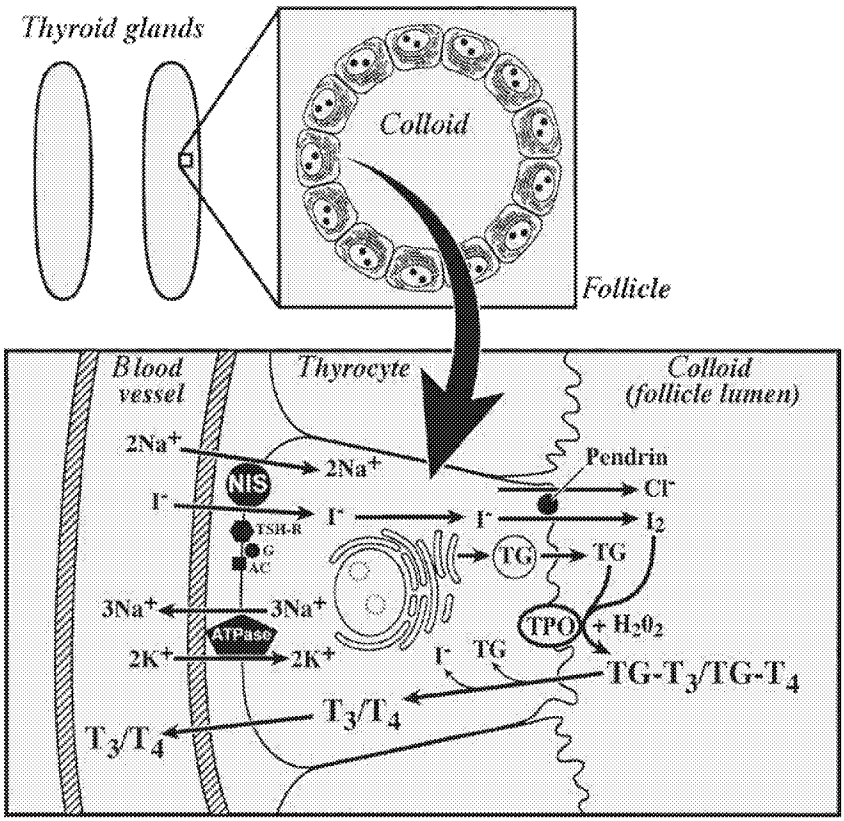

Figure 21-17. The sodium-iodide (NA+-I⁻) symporter (NIS) and thyroid hormone biosynthesis.

The NIS located in the basolateral membrane of thyroid follicular cells is responsible for the rapid uptake and concentration of iodide ion (I^-) from the circulation. The enzyme thyroperoxidase (TPO) in microvilli on the lumenal surface oxidizes I^- in the presence of hydrogen peroxide (H_2O_2) to reactivate iodine (I_2) that subsequently binds to tyrosyl residues in the thyroglobulin (TG) molecule of the colloid. Pendrin is a Cl^-/I^- transport protein on the apical surface of thyrocytes. AC, adenylate cyclase; CD, colloid droplet; Cl-, chlorine ion; G, G protein; I^-, iodide ion; R, receptor; T3, triiodothyronine; T4, tetraiodothyronine (thyroxine); and TSH, thyroid-stimulating hormone. (From La Perle *et al.*, 2003.)

The ion gradient generated by the Na^+–K^+ ATPase appears to be the driving force for the active co-transport of iodide. The transporter protein is present in the basolateral membrane of thyroid follicular cells and is a large protein containing 643 amino acids with 13 transmembrane domains.

Other tissues such as the salivary gland, gastric mucosa, choroid plexus, ciliary body of the eye, and lactating mammary gland also have the capacity to actively transport iodide, albeit at a much lower level than the thyroid (Lacroix *et al.*, 2001). In the salivary glands the NIS protein has been detected in ductal cells but not in acinar cells. Only the thyroid follicular cells accumulate iodide in a TSH-dependent manner.

The NIS gene is complex (15 exons, 14 introns) and its expression in the thyroid is upregulated by TSH. The functionally active iodine transport system in the thyroid gland has important pathophysiologic applications in the evaluation, diagnosis, and treatment of several thyroid disorders, including cancer. The NIS and active transport of iodide can be selectively inhibited by competitive anion inhibitors (e.g., perchlorate, thiocyanate); thereby, effectively blocking the ability of the gland to iodinate tyrosine residues in thyroglobulin and synthesize thyroid hormones.

The ability of NIS to concentrate iodide holds clinical relevance, in addition to its physiological importance, by providing the basis for using radioactive iodine in the treatment and management of various thyroid diseases. Radioactive iodine is commonly used to destroy hyperactive thyrocytes in patients with thyrotoxicosis, to ablate normal and malignant thyroid tissues in patients who have undergone total thyroidectomy for thyroid carcinoma, and to perform whole-body scans for the detection of recurrent and metastatic thyroid cancer.

Iodide ion once within the thyrocyte is passively transported via pendrin into the follicular lumen (Royaux *et al.* 2000; Kohn *et al.* 2001). Pendrin is a ~86 kDa chloride/iodide transport protein, is located at the apical membrane of thyrocytes, and is responsible for the transport of iodide ion into the follicular lumen (Fig. 21-17) (Scott *et al.*, 1999). It is the product of the gene responsible for Pendred syndrome, a genetic disorder in human patients characterized clinically by sensorineural hearing loss and goiter (Everett *et al.*, 1999, 2001; Kopp, 1999; Bidart *et al.*, 2000). Pendrin is characterized by 11 or 12 putative-transmembrane domains and is closely related to a family of sulfate transport proteins; however, pendrin does not transport sulfate and unlike NIS does not require sodium for its transport of iodide.

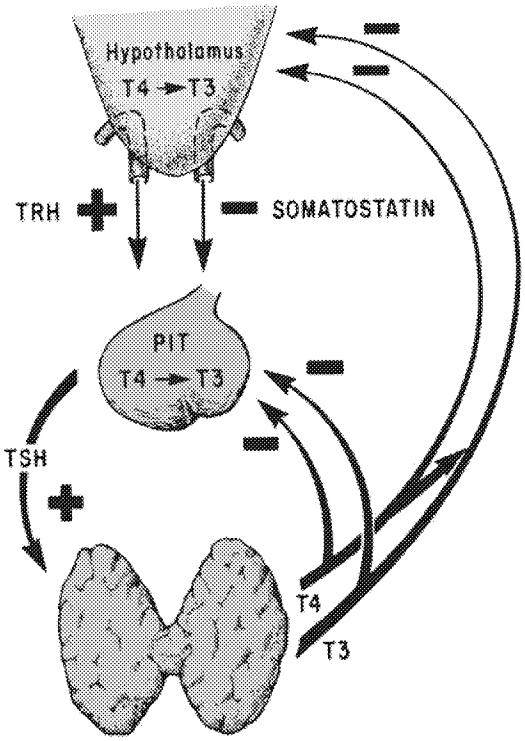

Figure 21-18. Hypothalamic–pituitary–thyroid axis.

Thyroid-stimulating hormone (TSH) from the pituitary stimulates the secretion of both the tetraiodothyronine (T_4) and triiodothyronine (T_3). These act at the pituitary (PIT) level to control secretion of TSH by a negative-feedback mechanism. In addition, T_4 is converted to T_3 within the pituitary by a monodeiodinase. TSH secretion is stimulated by thryotropin-releasing hormone (TRH) from the hypothalamus and inhibited by somatostatin and, to a lesser extent, by dopamine. Thyroid hormones act at the hypothalamus to stimulate the secretion of somatostatin. T_4 also is deiodinated to T_3 within the hypothalamus, and this conversion may play a role in feedback regulation. (From Reichlin, 1998.)

Thyroid Hormone Secretion The secretion of thyroid hormones from stores within luminal colloid is initiated by elongation of microvilli on follicular cells and formation of pseudopodia. These elongated cytoplasmic projections are increased by pituitary TSH, extend into the follicular lumen, and indiscriminatingly phagocytize a portion of adjacent colloid (Fig. 21-16). Colloid droplets within follicular cells subsequently fuse with numerous lysosomal bodies that contain proteolytic enzymes (Wollman *et al.*, 1964; Sagartz *et al.*, 1995; Ozaki *et al.*, 1995; Nilsson *et al.*, 1986). Triiodothyronine (T_3) and thyroxine (T_4) are released from the thyroglobulin molecule and because of their hydrophobic nature diffuse out of follicular cells into the adjacent capillaries. The biologically inactive iodotyrosines (MIT, DIT) simultaneously released from the colloid droplets are deiodinated enzymatically and the iodide generated is either recycled to the follicular lumen to iodinate new tyrosyl residues or released into the circulation under normal conditions. Thyroxine is rapidly bound in plasma to albumin and several globulin fractions (especially high-affinity thyroxine-binding globulin [TBG]) produced by the liver, and triiodothyronine is bound to albumin (transthyretin) and one globulin fraction in dogs.

Negative feedback control of thyroid hormone secretion is accomplished by the coordinated response of the adenohypophysis and specific hypothalamic nuclei to circulating levels of thyroid hormones (especially T_3). A decrease in thyroid hormone concentration in plasma is sensed by groups of neurosecretory neurones in the hypothalamus that synthesize and secrete a small peptide (3 amino acids), thyrotropin-releasing hormone (TRH), into the hypophyseal portal circulation. Thyroid-stimulating hormone (TSH) or thyrotropin (TTH) is conveyed to thyroid follicular cells where it binds to the basilar aspect of the cell, activates adenyl cyclase, and increases the rate of biochemical reactions concerned with the synthesis and secretion of thyroid hormones (Wynford-Thomas *et al.*, 1987) (Fig. 21-18).

Biologic Effects of Thyroid Hormones Thyroxine (T_4) and triiodothyronine (T_3) once released into the circulation act on many different target cells in the body. The overall functions of the hormones are similar though much of the biologic activity is the result of monodeiodination by 5′-deiodinase to 3,5,3′-triiodothyronine (active T_3) prior to interacting with target cells. Under certain conditions (protein starvation, neonatal animals, liver and kidney disease, febrile illness, etc.) or exposure to xenobiotic chemicals, thyroxine is preferentially monodeiodinated by 5′-deiodinase to 3,3′,5′-triiodothyronine ("reverse T3"). Because this form of T_3 is biologically inactive, monodeiodination to form reverse T3 provides a mechanism to attenuate the metabolic effects of thyroid hormones in peripheral tissues.

The overall physiologic effects of thyroid hormones are to: increase the basal metabolic rate; make more glucose available to meet the elevated metabolic demands by increasing glycolysis, gluconeogenesis, and glucose absorption from the intestine; stimulate new protein synthesis; increase lipid metabolism and conversion of cholesterol into bile acids and other substances; activate lipoprotein lipase and increase sensitivity of adipose tissue to lipolysis by other hormones; stimulate the heart rate, cardiac output, and blood flow; increase neural transmission, cerebration, and neuronal development in young animals (Lima *et al.*, 2001).

The subcellular mechanism of action of thyroid hormones resembles that for steroid hormones in that free hormone enters target cells and binds loosely to cytosol-binding proteins (Fig. 21-19). Free triiodothyronine initially binds to receptors on the inner mitochondrial membrane to activate mitochondrial energy metabolism, and subsequently binds to high-affinity nuclear receptors and increases transcription of the genetic message (mRNA) to facilitate new protein synthesis (e.g., structural, enzymatic, and binding proteins).

Thyroid hormone functions in target cells are mediated by three nuclear receptors: T_3R alpha$_1$, T_3R beta$_1$, and T_3R beta$_2$ that are encoded by two genes, namely T_3R alpha and T_3R beta. Thyroid hormones are essential for numerous postnatal developmental processes including growth and neurogenesis. The T_3R alpha gene is widely expressed from early development whereas the T_3R beta gene is highly restricted and expressed later in development (Fraichard *et al.*, 1997). Mice with targeted disruption of the T_3R alpha gene have markedly reduced production of both T_4 and T_3 associated with growth arrest and delayed development of bones and small intestine.

Species Differences in Thyroid Hormone Economy

Long-term perturbations of the pituitary–thyroid axis by various xenobiotics or physiological alterations (e.g., iodine deficiency, partial thyroidectomy, and natural goitrogens in food) are more likely to predispose the laboratory rat to a higher incidence of proliferative lesions (e.g., hyperplasia and benign tumors [adenomas] of follicular

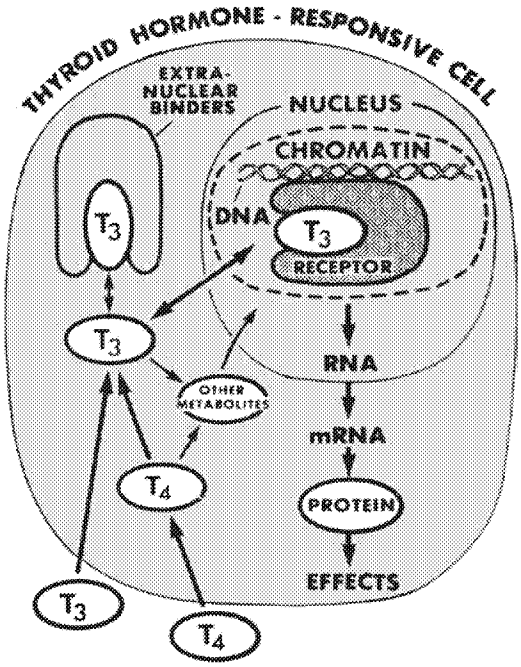

Figure 21-19. Subcellular mechanism of action of thyroid hormones in target cells.

Free triiodothyronine (T_3) primarily enters target cells because most of the tetraiodothyronine (T_4) undergoes monodeiodination in the liver or elsewhere in the periphery to form T_3. In the cell T_3 binds either to cytosolic-binding proteins (CBPs) or to high-affinity receptors on the inner mitochondrial (MT) membrane and activates oxidative phosphorylation in target cells. Binding to nuclear receptors by T_3 increases transcription of messenger RNA, which returns to the cytoplasm to direct the synthesis of new proteins. The increased synthesis of new proteins (structural or enzyme) carries out the multiple biological effects of the thyroid hormones.

cells) in response to chronic TSH stimulation than in the human thyroid (Capen and Martin, 1989; Curran and DeGroot, 1991; Capen, 1997; Zbinden *et al.*, 1988). This is particularly true in the male rat which has higher circulating levels of TSH than in females. The greater sensitivity of the rodent thyroid to derangement by drugs, chemicals, and physiological perturbations also is related to the shorter plasma half-life of thyroxine T_4 in rats than in humans due to the considerable differences in the transport proteins for thyroid hormones between these species (Döhler *et al.*, 1979).

The plasma T_4 half-life in rats is considerably shorter (12–24 hours) than in humans (5–9 days). In human beings and monkeys circulating T_4 is bound primarily to thyroxine-binding globulin (TBG), but this high-affinity binding protein is not present in rodents, birds, amphibians, or fish (Table 21-1).

The binding affinity of TBG for T_4 is approximately one thousand times higher than for prealbumin. The percent of unbound active T_4 is lower in species with high levels of TBG than in animals in which T_4 binding is limited to albumin and prealbumin. Therefore, a rat without a functional thyroid requires about 10 times more T_4 (20 μg/kg body weight) for full substitution than an adult human (2.2 μg/kg body weight). Triiodothyronine (T_3) is transported bound to TBG and albumin in human beings, monkey, and dog but only to albumin in mouse, rat, and chicken (Table 21-2). In general, T_3 is bound less avidly to transport proteins than T_4, resulting in a faster turnover and shorter plasma half-life in most species. These differences in plasma half-life of thyroid hormones and binding to

Table 21-1
Thyroxine (T_4) Binding to Serum Proteins in Selected Vertebrate Species

SPECIES	T_4-BINDING GLOBULIN	POSTALBUMIN	ALBUMIN	PREALBUMIN
Human being	++*	—	++	+
Monkey	++	—	++	+
Dog	++*	—	++	—
Mouse	—*	++	++	—
Rat	—	+	++	+
Chicken	—	—	++	—

+, ++, Degree of T_4 binding to serum proteins; —, absence of binding of T_4 to serum proteins. (From Döhler *et al.*, 1979).

Table 21-2
Triiodothyronine (T_3) Binding to Serum Proteins in Selected Vertebrate Species

SPECIES	T_4-BINDING GLOBULIN	POSTALBUMIN	ALBUMIN	PREALBUMIN
Human being	+*	—	+	—
Monkey	+	—	+	—
Dog	+	—	+	—
Mouse	—*	+	+	—
Rat	—	—	+	—
Chicken	—	—	+	—

+, ++, Degree of T_3 binding to serum proteins; —, absence of binding of T_3 to serum proteins. (From Döhler *et al.*, 1979).

transport proteins between rats and human beings may be one factor in the greater sensitivity of the rat thyroid to developing hyperplastic and/or neoplastic nodules in response to chronic TSH stimulation.

Thyroid-stimulating hormone levels are higher in male than female rats and castration decreases both the baseline serum TSH and response to thyrotropin-releasing hormone (TRH) injection. Follicular cell height often is higher in male than in female rats in response to the greater circulating TSH levels. The administration of exogenous testosterone to castrated male rats restores the TSH level to that of intact rats. Malignant thyroid tumors (carcinomas or "cancer") develop at a higher incidence following irradiation in males than females (2:1) and castration of irradiated male rats decreases the incidence to that of intact irradiated female rats. Testosterone replacement to castrated male rats restores the incidence of irradiation-induced thyroid carcinomas in proportion to the dose of testosterone and, similarly, serum TSH levels increase proportionally to the dose of replacement hormone. Likewise, higher incidence of follicular cell hyperplasia and neoplasia has been reported in males compared to female rats following the administration of a wide variety of drugs and chemicals in chronic toxicity/carcinogenicity testing.

There are also marked species differences in the sensitivity of the functionally important peroxidase enzyme to inhibition by xenobiotics. Thioamides (e.g., sulfonamides) and other chemicals can selectively inhibit the thyroperoxidase and significantly interfere with the iodination of tyrosyl residues incorporated in the thyroglobulin molecule, thereby, disrupting the orderly synthesis of T_4 and T_3. A number of studies have shown that the long-term administration of sulfonamides results in the development of thyroid nodules frequently in the sensitive species (such as the rat, dog, and mouse)

PROMOTING EFFECT OF IODINE DEFICIENCY (ID) ON THYROID CARCINOGENESIS INDUCED BY N-NITROSOMETHYLUREA (NMU) AT 33 WEEKS

GROUP	THYROID WEIGHT ($\bar{x}$ mg + SD)	DIFFUSE FC HYPERPLASIA (%)	FOLLICULAR CELL (FC) ADENOMA (%)	FOLLICULAR CELL (FC) CARCINOMA (%)	TOTAL FOCAL FC LESIONS (no./cm²)
NMU + ID DIET	632 ± 208*	100x	100xx	100xxxx	11 ± 6
NMU + IODINE ADEQUATE (IA) DIET	46 ± 13**	0	70xxx	10	14 ± 14
NMU + CONTROL DIET	29 ± 4***	0	50	0	17 ± 20
ID DIET	109 ± 12****	100x	0	0	0.4 ± 0.8
IODINE ADEQUATE DIET	40 ± 16	0	0	0	0
CONTROL DIET	36 ± 4	0	0	0	0

* P<0.01 vs. GROUPS 2, 3, 4, 5, or 6
** P<0.05 vs. GROUPS 3 or 4
*** P<0.01 vs. GROUP 4
**** P<0.01 vs. GROUPS 5 or 6

x P<0.001 vs. GROUPS 2, 3, 5 or 6
xx P<0.001 vs. GROUPS 4, 5, or 6
xxx P<0.01 vs. GROUPS 4, 5, or 6
xxxx P<0.001 vs. GROUPS 2, 3, 4, 5, or 6

CONTROL DIET = STERILIZABLE WAYNE BLOX DIET; IODINE ADEQUATE = ID (REMINGTON) DIET + 0.01 gm/kg K IODATE.
NMU 41 mg/kg IV AT 6 WK OF AGE (2 WK BEFORE DIETS STARTED).

Figure 21-20. The potent promoting effects of increased TSH secretion caused by iodine deficiency demonstrated in rats administered a single intravenous dose of a known initiator (N-nitrosomethylurea) of thyroid neoplasms. (Redrawn from Ohshima and Ward, 1984).

but not in species resistant (e.g., monkey, guinea pig, chicken, and human beings) to the inhibition of peroxidase in follicular cells.

Mechanisms of Thyroid Follicular Cell Toxicity

Numerous studies have reported that chronic treatment of rodents with goitrogenic compounds results in the development of follicular cell adenomas (Capen, 1996b, 1998, 1999, 2006a; Hard, 1988). Thiouracil and its derivatives have this effect in rats (Napalkov, 1976) and mice (Morris, 1955). This phenomenon has also been observed in rats that consumed brassica seeds (Kennedy and Purves, 1941), erythrosine (FD&C Red No. 3) (Borzelleca *et al.*, 1987; Capen and Martin, 1989), sulfonamides (Swarm *et al.*, 1973), and many other compounds (Paynter *et al.*, 1988; Hill *et al.*, 1989). The pathogenetic mechanism of this phenomenon has been understood for some time (Furth, 1954) and is widely accepted by the scientific community. These goitrogenic agents either directly interfere with thyroid hormone synthesis or secretion in the thyroid gland, increase thyroid hormone catabolism and subsequent excretion into the bile, or disrupt the peripheral conversion of thyroxine (T_4) to triiodothyronine (T_3). The ensuing decrease in circulating thyroid hormone levels results in a compensatory increased secretion of pituitary thyroid-stimulating hormone (TSH) (Hotz *et al.*, 1997). The receptor-mediated TSH stimulation of the thyroid gland leads to proliferative changes of follicular cells that include hypertrophy, hyperplasia, and ultimately, neoplasia in rodents (Hill *et al.*, 1998, 1999; Hurley *et al.*, 1998).

Excessive secretion of TSH alone (i.e., in the absence of any chemical exposure) has also been reported to produce a high incidence of thyroid tumors in rodents (Ohshima and Ward, 1984, 1986). This has been observed in rats fed an iodine-deficient diet (Axelrod and Leblond, 1955) and in mice that received TSH-secreting pituitary tumor transplants (Furth, 1954). The pathogenetic mechanism of thyroid follicular cell tumor development in rodents involves a sustained excessive stimulation of the thyroid gland by TSH. In addition, iodine deficiency is a potent promoter of the development of thyroid tumors in rodents induced by intravenous injection of *N*-methyl-*N*-nitrosourea (Fig. 21-20) (Ohshima and Ward, 1984). The subsequent parts of thyroid section discuss specific mechanisms by which xenobiotic chemicals disrupt thyroid hormone synthesis and secretion, induce hepatic microsomal enzymes that enhance thyroid hormone catabolism or inhibit enzymes involved in monodeiodination in peripheral tissues that result in perturbations of thyroid hormone economy, which in rodents predisposes to the development of follicular cell tumors in chronic studies.

Xenobiotic Chemicals that Directly Inhibit Thyroid Hormone Synthesis

Blockage of Iodine Uptake The biosynthesis of thyroid hormones is unique among endocrine glands because the final assembly of the hormones occurs extracellularly within the follicular lumen. Essential raw materials, such as iodide, are trapped efficiently at the basilar aspect of follicular cells from interfollicular capillaries, transported rapidly against a concentration gradient to the lumen, and oxidized by a thyroid peroxidase in microvillar membranes to reactive iodine (I_2) (Fig. 21-21). The mechanism of active transport of iodide has been shown to be associated with a sodium-iodide (Na^+-I^-) symporter (NIS) present in the basolateral membrane of thyroid follicular cells. Transport of iodide ion across the thyroid cell membrane is linked to the transport of Na^+. The ion gradient generated by the Na^+-K^+ ATPase appears to be the driving force for the active co-transport of iodide. The transporter protein is present in the basolateral membrane of thyroid follicular cells (thyrocytes) and is a large protein containing 643 amino acids with 13 transmembrane domains.

Immunohistochemical staining using a polyclonal antibody against the human NIS fusion protein (hNIS) revealed that expression of the protein is heterogenous in the normal human thyroid and detected only in occasional thyrocytes of a follicle (Jhiang *et al.*, 1998b). The hNIS-positive thyrocytes usually were detected in small

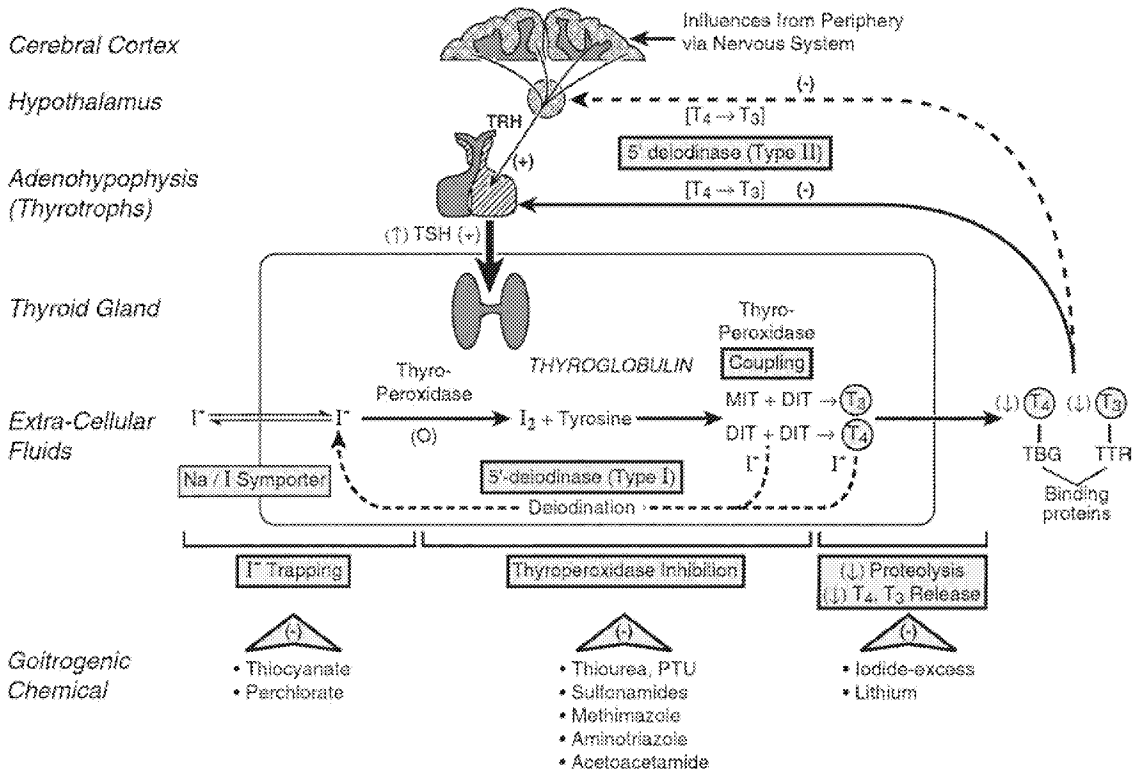

Figure 21-21. Mechanism of action of goitrogenic chemicals on thyroid hormone synthesis and secretion.

Chemicals can interfere with thyroid function either by (a) blocking iodide ion trapping, (b) inhibiting organic binding–coupling, or (c) disrupting proteolysis and release of T_4 and T_3 from colloid. DIT, diiodotyrosine; I_2, reactive iodine; MIT, monoiodotyrosine; PTU, propylthiouracil; T_3, triiodothyronine; T_4, tetraiodothyronine; TBG, thyroxine-binding globulin; TRH, thyrotropin-releasing hormone; and TSH, thyroid-stimulating hormone. (From Capen, 2004a).

follicles composed of cuboidal to columnar cells but rarely were detected in large follicles composed of flattened thyrocytes. The heterogeneity of hNIS expression among thyroid follicles is consistent with the finding that iodide concentrating ability also varies between follicles in the thyroid gland (Spitzweg *et al.*, 2000).

Other tissues such as the salivary gland, gastric mucosa, choroid plexus, ciliary body of the eye, and lactating mammary gland also have the capacity to actively transport iodide, albeit at a much lower level than the thyroid. In the salivary glands the hNIS protein was detected in ductal cells but not in acinar cells. Only the thyroid follicular cells accumulate iodide in a TSH-dependent manner. The NIS gene is complex (15 exons, 14 introns) and its expression in the thyroid is upregulated by TSH. The functionally active iodine transport system in the thyroid gland has important clinical applications in the evaluation, diagnosis, and treatment of several thyroid disorders, including cancer. The NIS and active transport of iodide can be selectively inhibited by competitive anion inhibitors; thereby, effectively blocking the ability of the gland to iodinate tyrosine residues in thyroglobulin and synthesize thyroid hormones.

The initial step in the biosynthesis of thyroid hormones is the uptake of iodide from the circulation and transport against a gradient across follicular cells to the lumen of the follicle. A number of anions act as competitive inhibitors of iodide transport in the thyroid, including perchlorate (ClO_4^-), thiocyanate (SCN^-), and pertechnetate (Fig. 21-21) (Atterwill *et al.*, 1987). Thiocyanate is a potent inhibitor of iodide transport and is a competitive substrate for the thyroid peroxidase but it does not appear to be concentrated in the thyroid. Blockage of the iodide trapping mechanism has a disruptive effect on the thyroid–pituitary axis, similar to iodine deficiency. The blood levels of T_4 and T_3 decrease, resulting in a compensatory increase in the secretion of TSH by the pituitary gland. The hypertrophy and hyperplasia of follicular cells following sustained exposure results in increased thyroid weights and the development of goiter.

Perchlorate from combustion of solid-propelled rocket engines and missiles, and other environmental sources (e.g., explosives, fireworks, road flares, commercial fertilizers, and airbag inflation systems) has contaminated water sources nationwide and more than 11 million people have perchlorate in their public drinking water at concentrations of 4 ppb (4 μg/L). Perchlorate is an ion that can affect thyroid function because it competitively inhibits the transport of iodine into thyroid follicular cells by inhibiting the action of the sodium-iodide symporter (Soldin *et al.*, 2001; Clewell *et al.*, 2004). Exposure to perchlorate in drinking water is a concern due to potential disruption of thyroid function with the development of hypothyroidism at various stages of life and subsequent effects on neurointellectual development (Li *et al.*, 2000; Lamm *et al.*, 1999; Lamm and Doemland, 1999). A normal secretion of thyroid hormones are required for the development and growth of neurones and supporting glial cells, the formation of synapses between neurones, the formation of myelin sheaths surrounding neuronal processes, and the development of neurotransmitters (National Research Council, 2005).

Review of the extensive scientific literature following perchlorate exposure or administration to humans and animals by a National Academy of Sciences committee concluded that the most

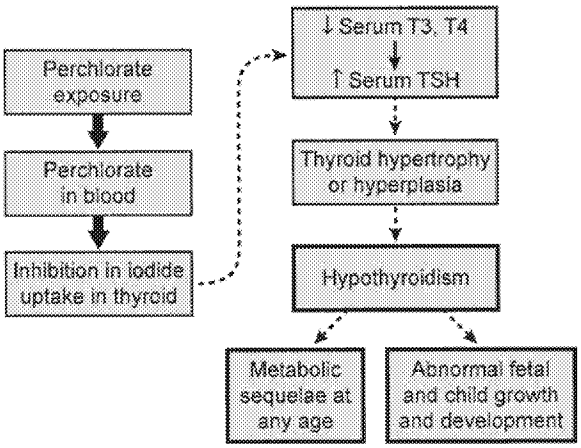

Figure 21-22. Suggested mode-of-action model for perchlorate toxicity in humans.

Solid arrows represent outcomes observed in humans during perchlorate exposure. Dashed arrows represent outcomes not clearly demonstrated in humans exposed to perchlorate but biologically plausible in absence of adequate compensation. The thyroid response to increased serum TSH concentrations and the independent increase in thyroid iodide uptake would act to raise T_3 and T_4 production to normal and thus prevent later steps of the model from occurring. (From Health Implications of Perchlorate Ingestion. National Research Council, The National Academic Press, Washington, DC, 2005).

reasonable mode of action pathway of events after perchlorate exposure would be changes in thyroid hormones and TSH secretion followed by thyroid follicular cell hypertrophy and hyperplasia. Hypothyroidism would develop only in people unable to compensate with an increased thyroid iodide uptake (National Research Council, 2005) (Fig. 21-22). Mechanisms exist that permit the body to compensate for decreases in T_4 and T_3 production. The compensatory increase in TSH secretion and thyroidal iodide uptake returns T_4 and T_3 production to normal without causing adverse affects (National Research Council, 2005). Any effects that follow and result from hypothyroidism (e.g., metabolic sequelae at any age such as decreased basal metabolic rate and slowing of function of many organ systems or abnormal growth and development of fetuses and children) clearly would be adverse. The Committee that investigated the health implications of perchlorate ingestion concluded that transient changes in serum thyroid hormones or TSH concentrations are not adverse effects but simply biochemical changes that might precede adverse effects. Based upon clinical studies, occupational and environmental epidemiologic studies, and investigations following the long-term administration of perchlorate to patients with hyperthyroidism, the committee recommended that a perchlorate reference dose (RfD) of 0.0007 μg/kg/day should protect the health of even the most sensitive populations (National Research Council, 2005). This is equivalent to 24.5 ppb concentration of perchlorate in drinking water assuming a body weight of 70 kg, daily water consumption of 2 L and drinking water as the only dietary source of perchlorate (DeGroef *et al.*, 2006). Recent studies in healthy volunteers have reported that 6 months of exposure to perchlorate at doses up to 3 mg/day had no effect on thyroid function including thyroidal iodide uptake as well as serum levels of thyroid hormones, TSH, and thyroglobulin (Braverman *et al.*, 2006).

Sodium chlorate ($NaClO_3$) is another xenobiotic chemical that results in perturbations of thyroid hormone economy, most likely by an inhibition of sodium-iodide symporter, and in chronic studies increased the incidence of thyroid follicular cell tumors when administered in drinking water to rats (Hooth *et al.*, 2001). The dose-dependent decrease in serum T4 was greater after 4 than 21 days but on day 90 T4 levels were similar to controls. The significant compensatory increases in serum TSH were greater after 4 and 21 days than at 90 days when only the high dose rats had a very modest increase in circulating levels. This increase in TSH is associated with the administration of $NaClO_3$ resulted in a 13% incidence of thyroid follicular cell adenoma plus carcinoma in males and a 9% incidence in females compared to 2% in controls of both sexes (Hooth *et al.*, 2001).

- THIOUREA
- PROPYLTHIOUREA (PTU)
- ANILINE DERIVATIVES: SULFONAMINDES
  - SULFAMETHAZINE
  - MANY OTHERS, NOT ALL
- METHIMAZOLE, CARBIMAZOLE
- AMINOTRIAZOLE
- ACETOACETAMIDE

Figure 21-23. Chemicals disrupting thyroid function (decreased synthesis of thyroid hormones) by inhibiting thyroperoxidase.

Inhibition of Thyroid Peroxidase Resulting in an Organification Defect A wide variety of chemicals, drugs, and other xenobiotics affect the second step in thyroid hormone biosynthesis (Fig. 21-21). The stepwise binding of iodide to the tyrosyl residues in thyroglobulin requires oxidation of inorganic iodide (I^2) to molecular (reactive) iodine (I_2) by the thyroid peroxidase present in the luminal aspect (microvillar membranes) of follicular cells and adjacent colloid. Classes of chemicals that inhibit the organification of thyroglobulin include (1) the thionamides (such as thiourea, thiouracil, propylthiouracil, methimazole, carbimazole, and goitrin); (2) aniline derivatives and related compounds (e.g., sulfonamides, paraaminobenzoic acid, paraaminosalicylic acid, and amphenone); (3) substituted phenols (such as resorcinol, phloroglucinol, and 2,4-dihydroxybenzoic acid); and (4) miscellaneous inhibitors (e.g., aminotriazole, tricyanoaminopropene, antipyrine, and its iodinated derivative [iodopyrine]) (Fig. 21-23).

Many of these chemicals exert their action by inhibiting the thyroid peroxidase which results in a disruption both of the iodination of tyrosyl residues in thyroglobulin and also the coupling reaction of iodotyrosines [(e.g., monoiodothyronine (MIT) and diiodothyronine (DIT)] to form iodothyronines (T_3 and T_4) (Fig. 21-24). Propylthiouracil (PTU) has been shown to affect each step in thyroid hormone synthesis beyond iodide transport in rats. The order of susceptibility to the inhibition by PTU is the coupling reaction (most susceptible), iodination of MIT to form DIT, and iodination of tyrosyl residues to form MIT (least susceptible). Thiourea differs from PTU and other thioamides in that it neither inhibits guaiacol oxidation (the standard assay for peroxidase) nor inactivates the thyroid peroxidase in the absence of iodine. Its ability to inhibit organic iodinations is due primarily to the reversible reduction of active I_2 to $2I^-$.

The goitrogenic effects of sulfonamides have been known for more than 50 years, since the reports of the action of sulfaguanidine on the rat thyroid. Sulfamethoxazole and trimethroprim exert a potent goitrogenic effect in rats, resulting in marked decreases in circulating T_3 and T_4, a substantial compensatory increase in TSH,

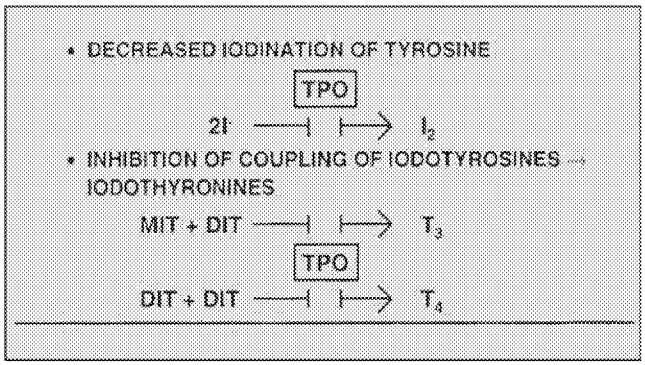

Figure 21-24. Mechanisms by which xenobiotic chemicals decrease thyroid hormone synthesis by inhibiting thyroperoxidase in follicular cells.

SENSITIVE SPECIES	RESISTANT SPECIES
• RAT	• HUMAN BEINGS
• MOUSE	• NONHUMAN PRIMATES
• DOG	• GUINEA PIG
• PIG	• CHICKEN

Figure 21-25. Variable species sensitivity of thyroperoxidase inhibition by sulfonamides. (From Takayama et al., 1986).

and increased thyroid weights due to follicular cell hyperplasia. The dog also is a species sensitive to the effects of sulfonamides, resulting in markedly decreased serum T_4 and T_3 levels, hyperplasia of thyrotrophic basophils in the pituitary gland, and increased thyroid weights.

By comparison, the thyroids of monkeys and human beings are resistant to the development of changes that sulfonamides produce in rodents (rats and mice) and the dog. Rhesus monkeys treated for 52 weeks with sulfamethoxazole (doses up to 300 mg/kg/day) with and without trimethroprim had no changes in thyroid weights and the thyroid histology was normal. Takayama *et al.* (1986) compared the effects of PTU and a goitrogenic sulfonamide (sulfamonomethoxine) on the activity of thyroid peroxidase in the rat and monkey using the guaiacol peroxidation assay. The concentration required for a 50% inhibition of the peroxidase enzyme was designated as the inhibition constant$_{50}$ (IC_{50}). When the IC_{50} for PTU was set at 1 for rats it took 50 times the concentration of PTU to produce a comparable inhibition in the monkey. Sulfamonomethoxine was almost as potent as PTU in inhibiting the peroxidase in rats. However, it required about 500 times the concentration of sulfonamide to inhibit the peroxidase in the monkey compared to the rat. Studies such as these with sulfonamides demonstrate distinct species differences between rodents and primates in the response of the thyroid to chemical inhibition of hormone synthesis. It is not surprising that the sensitive species (e.g., rats, mice, and dogs) are much more likely to develop follicular cell hyperplasia and thyroid nodules after long-term exposure to sulfonamides than the resistant species (e.g., subhuman primates, human beings, guinea pigs, and chickens) (Fig. 21-25).

Recent evidence suggests that propylthiouracil (PTU) or feeding a low iodine diet markedly increase thyroid follicular cell proliferation in rats by disrupting the movement of small molecular weight ions and molecules through gap junctions (Kolaja *et al.*, 2000). Inhibition of gap-junction intercellular communication (GJIC) prior to induction of cell proliferation has been reported with several tumor promoters and in proliferative diseases. After 14 days of either PTU or a low iodine diet (plus 1% $KClO_4$ in water) serum T_3 and T_4 were decreased to undetectable levels, serum TSH was increased significantly and thyroid follicular cell proliferation was increased nearly threefold. This was accompanied by a 30–35% decrease in GJIC (determined by an ex vivo method with Lucifer-yellow—a small molecular weight [457] fluorescent dye) and a twofold increase in apoptosis in both treated groups. Therefore, inhibition of GJIC by PTU or a low iodine diet may result in increased thyroid follicular cell proliferation, similar to other tissues, possibly by disrupting the passage of regulatory substance(s) through these highly permeable intercellular channels.

A contemporary example of a chemical acting as a thyroperoxidase inhibitor is sulfamethazine (Poirier *et al.*, 1999). This is a widely used antibacterial compound in food-producing animals with a current permissible tissue residue level of 100 ppb. Carcinogenicity studies at NCTR reported a significant increase of thyroid tumors in male Fischer 344 rats administered the high dose (2400 ppm) of sulfamethazine (McClain, 1995). The incidence of thyroid tumors was increased in both male and female $B_6C_3F_1$ mice after two years in the high-dose (4800 ppm) group but not in the lower dose groups. Quantitative risk assessment based upon these carcinogenicity findings, using low-dose linear extrapolation, yielded a 1×10^6 lifetime risk of 90 ppb in female rats and 40 ppb in male rats. A consideration of the ratio of intact drug to metabolites further reduced the tissue residue level to 0.4 ppb, which would be unachievable in practice (McClain, 1995).

A number of mechanistic studies have been performed in collaboration with Dr. McClain and others with the objective of developing a database that would support the hypothesis that the thyroid tumors observed in rats and mice from chronic studies were secondary to hormonal imbalances following the administration of high doses of sulfamethazine. In a 4-week mechanistic study, the effects of 10 dose levels (0–12,000 ppm) of sulfamethazine, spanning the range that induced thyroid tumors in rodents, were evaluated on thyroid hormone economy in Sprague–Dawley rats. There was a characteristic log–dose response relationship in all parameters of thyroid function evaluated. There were no significant changes at the six lower doses (20–800 ppm) of sulfamethazine, followed by sharp relatively linear changes at the four higher dose levels (1600–12,000 ppm) in percent decrease of serum T_3 and T_4, increase in serum TSH, and increase in thyroid weight. A similar, nonlinear dose response was present in the morphologic changes of thyroid follicular cells following the feeding of varying levels of sulfamethazine. Follicular cell hypertrophy was observed at lower doses of sulfamethazine than hyperplasia, which was increased only at dose levels of 3300 ppm and above (Fig. 21-26).

Other mode-of-action studies have demonstrated sulfamethazine to be a potent inhibitor of thyroperoxidase in rodents with an IC_{50} of 1.2×10^{-6} M. The morphologic effects on the thyroid were reversible after withdrawal of compound, and supplemental T_4 in the diet inhibited the development of the functional and morphologic changes in thyroid follicular cells (McClain, 1995). Hypophysectomized rats (with no TSH) administered sulfamethazine did not develop morphologic changes in the thyroid. Sulfamethazine

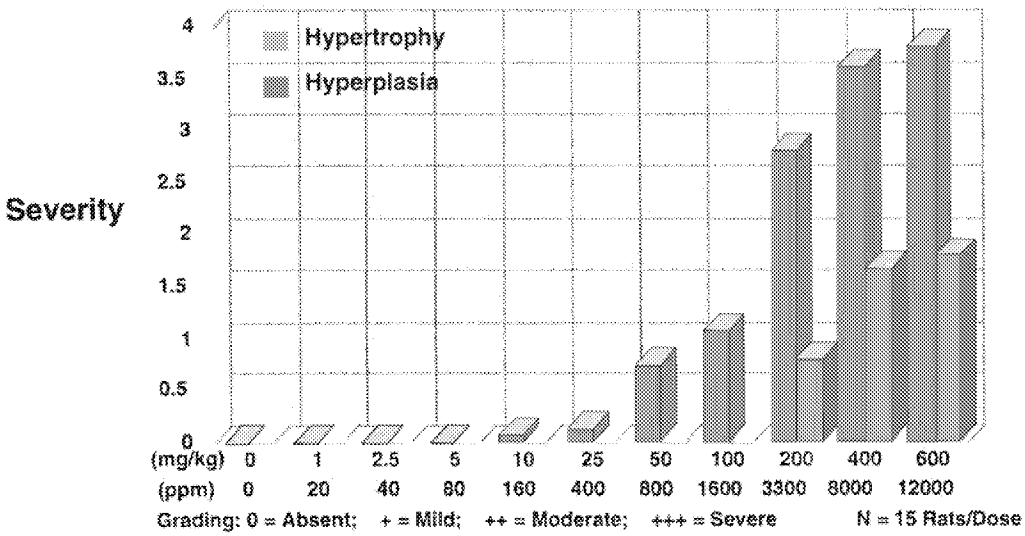

Figure 21-26. Nonlinear dose–response in morphologic changes in thyroid follicular cells in response to 10 dose levels of sulfamethazine administered in the feed to male Sprague–Dawley rats. (From Capen, 1997).

did not increase thyroid cell proliferation in vitro in the absence of TSH and there was no effect on thyroid structure/function in cynomologous monkeys administered sulfamethazine. Nonhuman primates and human beings are known to be more resistant than rodents to the inhibition of thyroperoxidase.

Xenobiotic Chemicals that Disrupt Thyroid Hormone Secretion

Blockage of Thyroid Hormone Secretion Relatively few chemicals selectively inhibit the secretion of thyroid hormone from the thyroid gland (Fig. 21-21). An excess of iodine has been known for years to inhibit secretion of thyroid hormone and occasionally can result in goiter and subnormal function (hypothyroidism) in animals and human patients. High doses of iodide have been used therapeutically in the treatment of patients with Grave's disease and hyperthyroidism to lower circulating levels of thyroid hormones. Several mechanisms have been suggested for this effect of high iodide levels on thyroid hormone secretion, including a decrease in lysosomal protease activity (in human glands), inhibition of colloid droplet formation (in mice and rats), and inhibition of TSH-mediated increase in cAMP (in dog thyroid slices). Rats fed an iodide-excessive diet had a hypertrophy of the cytoplasmic area of follicular cells with an accumulation of numerous colloid droplets and lysosomal bodies (Collins and Capen, 1980a; Kanno *et al.*, 1994; Many *et al.*, 1985). However, there was limited evidence ultrastructurally of the fusion of the membranes of these organelles and of the degradation of the colloid necessary for the release of active thyroid hormones (T_4 and T_3) from the thyroglobulin. Circulating levels of T_4, T_3, and rT_3 all would be decreased by an iodide-excess in rats.

Lithium has also been reported to have a striking inhibitory effect on thyroid hormone release (Fig. 21-21). The widespread use of lithium carbonate in the treatment of manic states occasionally results in the development of goiter with either euthyroidism or occasionally hypothyroidism in human patients. Lithium inhibits colloid droplet formation stimulated by cAMP in vitro and inhibits the release of thyroid hormones.

Xenobiotic-Induced Thyroid Pigmentation or Alterations in Colloid

The antibiotic minocycline produces a striking black discoloration of the thyroid lobes in laboratory animals and humans with the formation of brown pigment granules within follicular cells (Ambrogio *et al.*, 1999; Eisen and Hakim, 1998; Tajima *et al.*, 1985). The pigment granules stain similarly to melanin and are best visualized on thyroid sections stained with the Fontana–Masson procedure. Electron-dense material first accumulates in lysosome-like granules and in the rough endoplasmic reticulum. The pigment appears to be a metabolic derivative of minocycline and the administration of the antibiotic at high dose to rats for extended periods may result in a disruption of thyroid function and the development of goiter. The release of T_4 from perfused thyroids of minocycline-treated rats was significantly decreased but the follicular cells retained the ability to phagocytose colloid in response to TSH and had numerous colloid droplets in their cytoplasm.

Other xenobiotics [or metabolite(s)] selectively localize in the thyroid colloid of rodents resulting in abnormal clumping and increased basophilia to the colloid. Brown to black pigment granules may be present in follicular cells, colloid, and macrophages in the interthyroidal tissues resulting in a macroscopic darkening of both thyroid lobes. The physiochemically altered colloid in the lumen of thyroid follicles appears to be less able than normal colloid either of reacting with organic iodine in a step-wise manner to result in the orderly synthesis of iodothyronines or being phagocytized by follicular cells and enzymatically processed to release active thyroid hormones into the circulation. Serum T_4 and T_3 are decreased, serum TSH levels are increased by an expanded population of pituitary thyrotrophs, and thyroid follicular cells undergo hypertrophy and hyperplasia. As would be expected, the incidence of thyroid follicular cell tumors in 2-year carcinogenicity studies is increased at the higher dose levels usually with a greater effect in males than females. Autoradiographic studies often demonstrate tritiated material to be preferentially localized in the colloid and not within follicular cells. Tissue distribution studies with ^{14}C-labelled compound may reveal preferential uptake and persistence in the thyroid gland compared to other tissues. However, thyroperoxidase activity is normal

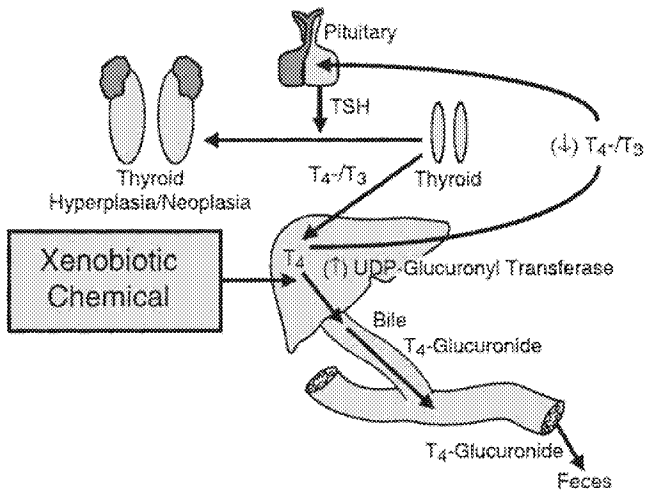

Figure 21-27. Hepatic microsomal enzyme induction by the chronic administration of xenobiotic chemicals leading to thyroid follicular cell hyperplasia and neoplasia.

and the thyroid's ability to take up radioactive iodine often is increased compared to controls in response to the greater circulating levels of TSH. Similar thyroid changes and/or functional alterations usually do not occur in dogs, monkeys, or humans.

Hepatic Microsomal Enzyme Induction

Hepatic microsomal enzymes play an important role in thyroid hormone economy because glucuronidation is the rate-limiting step in the biliary excretion of T_4 and sulfation primarily by phenol sulfotransferase for the excretion of T_3 (Vansell and Klaassen, 2001, 2002a; Shelby *et al.*, 2003; Dunn and Klaassen, 2000). Long-term exposure of rats to a wide variety of different chemicals may induce these enzyme pathways and result in chronic stimulation of the thyroid by disrupting the hypothalamic–pituitary–thyroid axis (Curran and DeGroot, 1991; Vansell and Klaassen, 2002b). The resulting chronic stimulation of the thyroid by increased circulating levels of TSH often results in a greater risk of developing tumors derived from follicular cells in 2-year or lifetime chronic toxicity/carcinogenicity studies with these compounds in rats (Fig. 21-27). Recent studies have suggested that glucuronidation and enhanced biliary excretion of T_3 may be the reason why serum TSH is increased in short-term (7 days) studies with some microsomal enzyme-inducing chemicals (e.g., phenobarbital, pregnenolone-16α-carbonitrile) but is less affected with others (3-methylcholanthrene, PCB). However, microsomal enzyme inducers are more effective in reducing serum T_4 than serum T_3 (Hood and Klaassen, 2000a). Outer-ring deiodinase (ORD) activity, an enzyme involved in the peripheral conversion of T_4 (major secretory product of the thyroid) to T_3, was reduced (not increased as would be expected if this was the mechanism) following the administration of four well-characterized enzyme inducers in rats. Type I ORD was measured in thyroid, kidney, and liver whereas type II ODR was quantified in brown adipose tissue, pituitary gland, and brain (Hood and Klaassen, 2000b).

Xenobiotics that induce liver microsomal enzymes and disrupt thyroid function in rats include CNS-acting drugs (e.g., phenobarbital, benzodiazepines); calcium channel blockers (e.g., nicardipine, bepridil); steroids (spironolactone); retinoids; chlorinated hydrocarbons (e.g., chlordane, DDT, TCDD), polyhalogenated biphenyls (PCB, PBB), among others (Waritz *et al.*, 1996). Most of the hepatic microsomal enzyme inducers have no apparent intrinsic carcinogenic activity and produce little or no mutagenicity or DNA damage. Their promoting effect on thyroid tumors usually is greater in rats than in mice, with males more often developing a higher incidence of tumors than females. In certain strains of mice these compounds alter liver cell turnover and promote the development of hepatic tumors from spontaneously initiated hepatocytes.

Phenobarbital has been studied extensively as the prototype for hepatic microsomal inducers that increase a spectrum of cytochrome P-450 isoenzymes (McClain *et al.*, 1988). McClain *et al.* (1989) reported that the activity of uridine diphosphate glucuronyltransferase (UDP-GT), the rate-limiting enzyme in T_4 metabolism, is increased in purified hepatic microsomes of male rats when expressed as picomoles/min/mg microsomal protein (1.3-fold) or as total hepatic activity (3-fold). This resulted in a significantly higher cumulative (4-hours) biliary excretion of ^{125}I-T_4 and bile flow than in controls.

Phenobarbital-treated rats develop a characteristic pattern of changes in circulating thyroid hormone levels (McClain *et al.*, 1988, 1989). Plasma T_3 and T_4 are markedly decreased after 1 week and remain decreased for 4 weeks. By 8 weeks T_3 levels return to near normal due to compensation by the hypothalamic–pituitary–thyroid axis. Serum TSH values are elevated significantly throughout the first month but often decline after a new steady state is attained. Thyroid weights increase significantly after 2–4 weeks of phenobarbital, reach a maximum increase of 40–50% by 8 weeks, and remain elevated throughout the period of treatment.

McClain *et al.* (1988) in a series of experiments have shown that supplemental administration of thyroxine (at doses that returned the plasma level of TSH to the normal range) blocked the thyroid tumor-promoting effects of phenobarbital and that the promoting effects were directly proportional to the level of plasma TSH in rats. The sustained increase in circulating TSH levels results initially in hypertrophy of follicular cells, followed by hyperplasia, and ultimately places the rat thyroid at greater risk to develop an increased incidence of benign tumors.

Phenobarbital has been reported to be a thyroid gland tumor promoter in a rat initiation–promotion model. Treatment with a nitrosamine followed by phenobarbital has been shown to increase serum TSH concentrations, thyroid gland weights, and the incidence of follicular cell tumors in the thyroid gland (McClain *et al.*, 1988, 1989). These effects could be decreased in a dose-related manner by simultaneous treatment with increasing doses of exogenous thyroxine. McClain *et al.* (1989) have demonstrated that rats treated with phenobarbital have a significantly higher cumulative biliary excretion of ^{125}I-thyroxine than controls (Fig. 21-28). Most of the increase in biliary excretion was accounted for by an increase in T_4-glucuronide due to an increased metabolism of thyroxine in phenobarbital-treated rats. This is consistent with enzymatic activity measurements which result in increased hepatic T_4-UDP-glucuronyl transferase activity in phenobarbital-treated rats (Fig. 21-29). Results from these experiments are consistent with the hypothesis that the promotion of thyroid tumors in rats was not a direct effect of phenobarbital on the thyroid gland but rather an indirect effect mediated by TSH secretion from the pituitary secondary to the hepatic microsomal enzyme-induced increase of T_4 excretion in the bile.

The activation of the thyroid gland during the treatment of rodents with substances that stimulate thyroxine catabolism is a well-known phenomenon and has been investigated extensively with phenobarbital and many other compounds (Curran and DeGroot, 1991). It occurs particularly with rodents, first because UDP-glucuronyl transferase can easily be induced in rodent species, and second

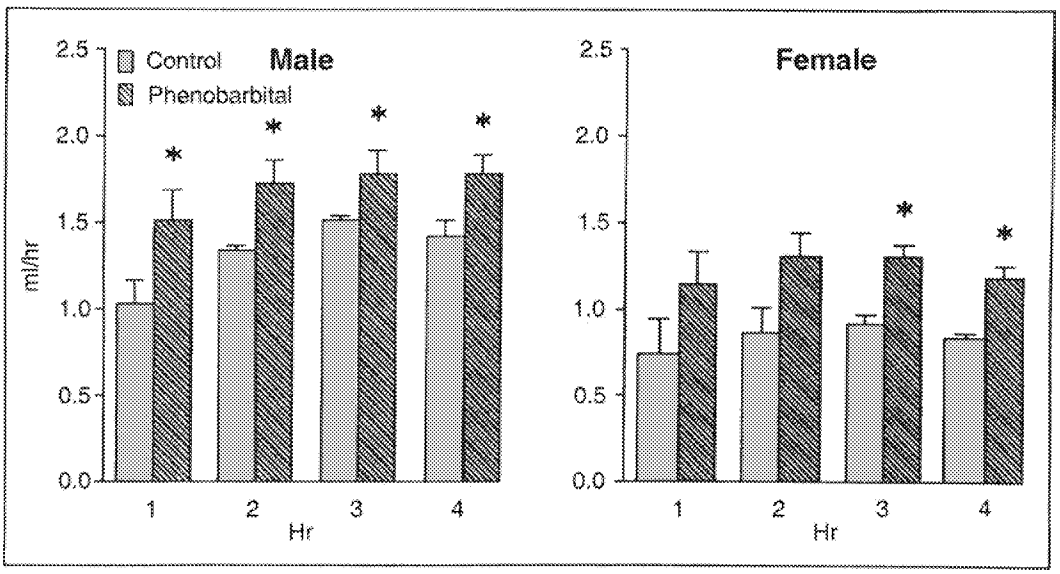

Figure 21-28. Cumulative biliary excretion of ^{125}I-thyroxine (percentage of administered dose) in control and phenobarbital treated rats (100 mg/kg/day in the diet for 4–6 weeks).

Phenobarbital treatment resulted in an increase in the cumulative excretion of thyroxine over a 4-hour period. Thin layer chromatography of bile samples indicated that most of the increase in biliary excretion was accounted for by an increase in the fraction corresponding to thyroxine-glucuronide. (From McClain, 1998).

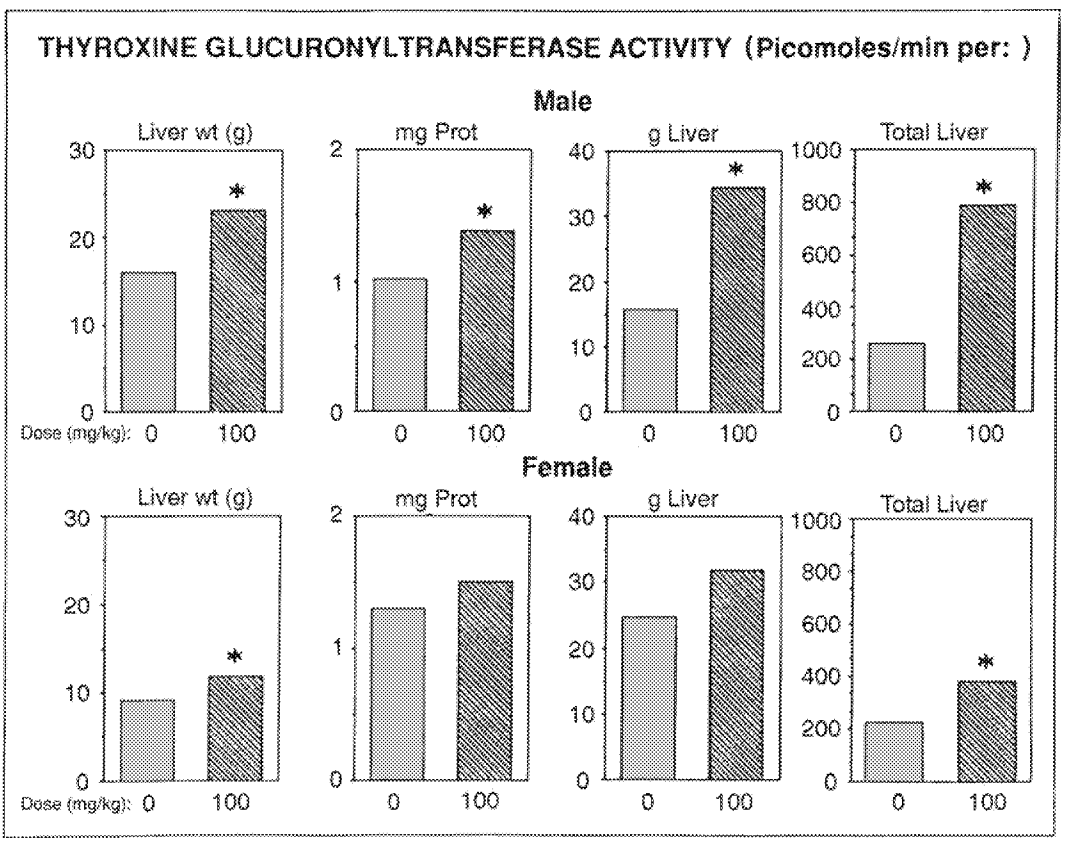

Figure 21-29. Hepatic thyroxine glucuronyltransferase activity in control and phenobarbital-treated rats (100 mg/kg/day in the diet for 4 weeks).

Glucuronyltransferase activity was measured in hepatic microsomes using thyroxine as a substrate. Phenobarbital treatment induced thyroxine-glucuronyltransferase in male and female rats; however, the effect in male rats was quantitatively larger. (From McClain, 1998).

because thyroxine metabolism takes place very rapidly in rats in the absence of thyroxine-binding globulin. In humans a lowering of the circulating T_4 level but no change in TSH and T_3 concentrations has been observed only with high doses of very powerful enzyme-inducing compounds, such as rifampicin with and without antipyrine.

Although phenobarbital is the only UDP-glucuronyl transferase (UDP-GT) inducer that has been investigated in detail to act as a thyroid tumor promoter, the effects of other well-known UDP-GT inducers on the disruption of serum T_4 TSH and thyroid gland have been investigated. For example, pregnenolone-16α-carbonitrile (PCN), 3-methylcholanthrene (3MC), and aroclor 1254 (PCB) induce hepatic microsomal UDP-GT activity toward T_4 (Barter and Klaassen, 1992a). These UDP-GT inducers reduce serum T_4 levels in both control as well as in thyroidectomized rats that are infused with T_4, indicating that reductions in serum T_4 levels are not due to a direct effect of the inducers on the thyroid gland (Barter and Klaassen, 1992b, 1994). However, serum TSH levels and the thyroid response to reductions in serum T_4 levels by UDP-GT inducers are not always predictable. While PCN increased serum TSH and resulted in thyroid follicular cell hyperplasia, similar to that observed with phenobarbital, 3MC and PCB in these short-duration experiments and at the dose levels used did not increase serum TSH levels or produce thyroid follicular cell hyperplasia (Hood *et al.*, 1995; Liu *et al.*, 1995). These findings support the overall hypothesis that UDP-GT inducers can adversely affect the thyroid gland by a secondary mechanism, but this applies only to those UDP-GT inducers that increase serum TSH in addition to reducing serum T_4 (Kolaja and Klaassen, 1998).

Additional investigations by this research group demonstrated that hepatic microsomal enzyme-inducing xenobiotic chemicals [e.g., phenobarbital and pregnenolone-16α-carbonitrile (PCN)] increased serum TSH (0–75%) much less than the thyroperoxidase inhibitor propylthiouracil (PTU) (830%) (Hood *et al.*, 1999a,b). Phenobarbital and PCN administration increased thyroid weight approximately 80% compared to a 500% increase in PTU-treated rats. Thyroid follicular cell proliferation (determined by BrdU labeling) was increased 260, 330, and 850% in rats treated with phenobarbital, PCN, and PTU, respectively, for 7 days but the labeling index had returned to control levels by the 45th day of treatment. These findings demonstrate that certain hepatic microsomal enzyme-inducing chemicals that result in mild to modest elevations in serum TSH lead to dramatic increases in thyroid follicular cell proliferation which peaked after 7 days of treatment and then rapidly returned to control values (Klaassen and Hood, 2001). These findings are similar to those of Wynford-Thomas *et al.* (1982), who reported a maximal proliferative response (evaluated by mitotic index) after 7 days of treatment with aminotriazole (a thyroperoxidase inhibitor). The decline in thyroid follicular cell proliferation was suggested to be due to desensitization of the cells to the mitogenic actions of TSH (Wynford-Thomas *et al.*, 1982a).

Hood *et al.* (1999) reported that moderate increases in serum TSH of between 10 and 20 ng/mL increased the number of proliferating thyroid follicular cells but had no effect on thyroid weight, emphasizing that small increases in serum TSH can be sufficient to stimulate proliferation. These important findings suggest that quantitation of follicular cell proliferation may be more useful than thyroid weights for assessing alterations in thyroid growth in rats administered xenobiotic chemicals that produce only small to moderate increases in serum TSH.

Another classic example of a chemical that induces hepatic microsomal enzymes and disrupts thyroid function are the polychlorinated biphenyls (Vansell *et al.*, 2004). Polychlorinated biphenyls are commonly used industrial compounds that have been released into the environment and have caused widespread contamination. The disease-producing capability of these compounds includes alterations in reproduction, growth, and development. PCBs cause a significant reduction in serum levels of thyroid hormones due to alterations in thyroid structure, in addition to the well-known induction of hepatic UDP-glucuronyl transferase and increased secretion of thyroxine-glucuronide in the bile (Collins and Capen, 1980b).

Feeding of PCB produced a dose-dependent significant reduction in serum T_4 levels in rats. Following withdrawal of PCB from the diet, blood T_4 levels return to the normal range at 35 weeks but not at 12 weeks. These changes in circulating levels of T_4 were accompanied by a striking hypertrophy and hyperplasia of thyroid follicular cells compared with controls. The most consistent lesions in follicular cells following the feeding of PCB were the accumulation of numerous large colloid droplets and irregularly shaped lysosomal bodies in the expanded cytoplasmic area. Microvilli on the lumenal surface were shortened with abnormal branchings. The chronic administration (12-week) of PCB resulted in a striking distention of many follicular cells with large lysosomal bodies that were strongly acid phosphatase positive and with colloid droplets, blunt and abnormally branched microvilli, and mitochondrial vacuolation. The principal lesion produced by PCB in follicular cells that contributed to the altered thyroid function appeared to be an interference in the interaction between the numerous colloid droplets and lysosomal bodies that is necessary for the enzymatic release of thyroid hormones.

Another recently-investigated example of an inducer of hepatic microsomal enzymes that increase the incidence of thyroid follicular cell tumors in rats is pyrethrins. Pyrethrins comprise a mixture of natural products isolated from Pyrethrum flowers, which have been used for many years as an insecticide for household and other applications. Pyrethrins exhibit a low order of oral toxicity in mammals and are rapidly metabolized. Short-term tests for genotoxic potential have demonstrated that pyrethrins are not genotoxic agents. Pyrethrins were not carcinogenic to male and female CD-1 mice in an 18-month study when administered at dietary levels of 0, 100, 2500, and 5000 ppm (Schoenig, 1995). The treatment of male and female Sprague–Dawley (CD) rats for 2 years with 100 ppm pyrethrins had no effect on tumor incidence. However, higher doses of pyrethrins produced effects in the liver of female rats and in the thyroid glands of both sexes. A small increase in the incidence of hepatocellular adenoma was observed in female rats given 3000 ppm pyrethrins. Although treatment with 1000 ppm pyrethrins had no significant effect on the incidence of thyroid gland follicular cell tumors in female rats, the combined incidence of follicular cell adenomas and/or carcinomas was significantly increased in male rats given 1000 and 3000 ppm pyrethrins and in female rats fed 3000 ppm pyrethrins.

In a subsequent mechanistic study, male Sprague–Dawley (CD) rats were fed diets containing 0 and 8000 ppm pyrethrins and female rats diets containing 0, 100, 3000, and 8000 ppm pyrethrins for periods of 7, 14, and 42 days followed by 42 days reversal (Finch *et al.*, 2006). Rats also were fed diets containing 1200–1558 ppm sodium phenobarbital (NaPb) for 7 and 14 days as a positive control. Treatment of male rats with 8000 ppm pyrethrins, female rats with 3000 and 8000 ppm pyrethrins, and both sexes with NaPb resulted in increased thyroid gland weights

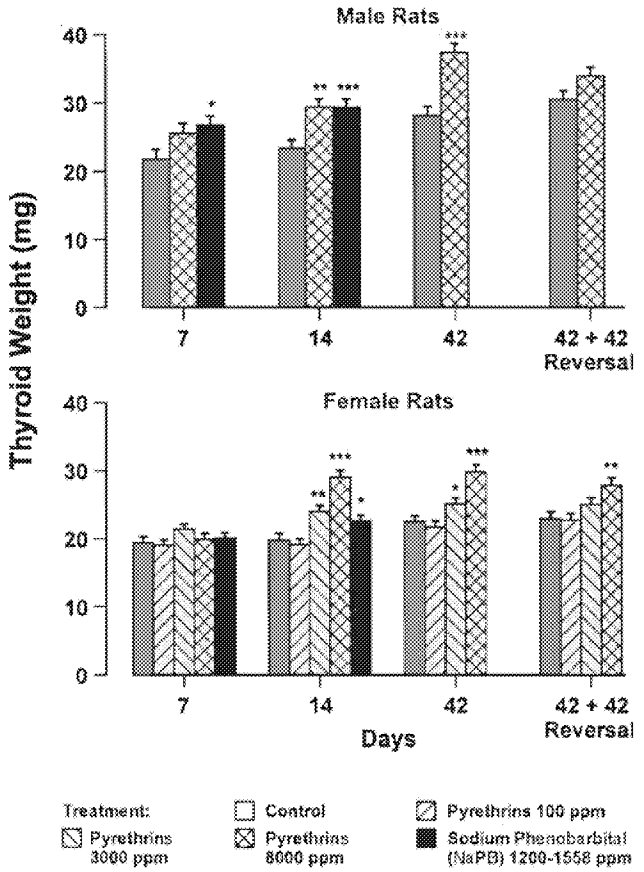

Figure 21-30. Effect of pyrethrins and NaPB on thyroid weights of Sprague–Dawley rats. (From Finch et al., 2006).

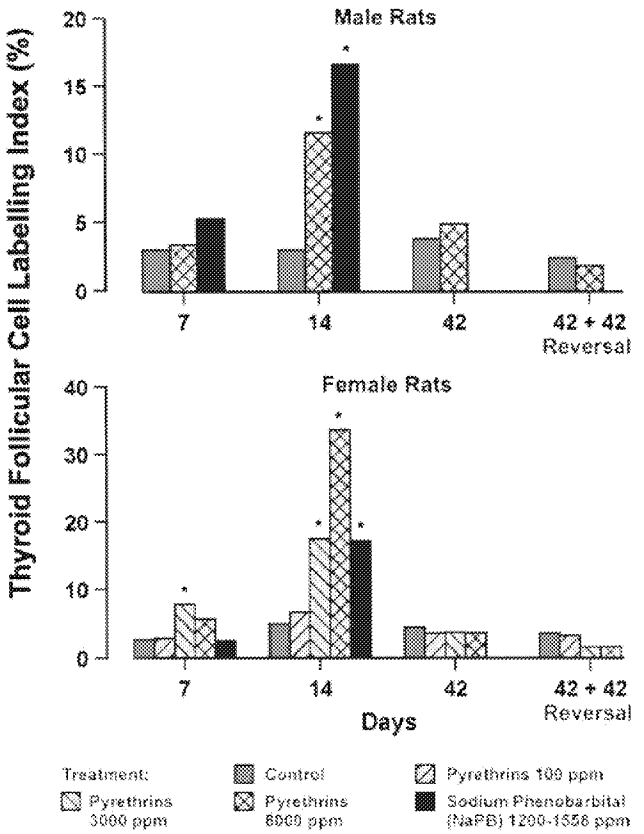

Figure 21-31. Effect of pyrethrins and NaPB on thyroid follicular cell labelling index of Sprague–Dawley rats. (From Finch et al., 2006).

(Fig. 21-30) associated with follicular cell hypertrophy. Thyroid follicular cell replicative DNA synthesis was increased by treatment of both males and females with pyrethrins and NaPb for 14 days (Fig. 21-31). Treatment with pyrethrins and NaPb increased serum TSH (Fig. 21-32) and reduced serum levels of T_4 (Fig. 21-33) (Finch *et al.*, 2006).

The treatment of male and female rats with 8000 ppm pyrethrins for 7, 14, and 42 days and 1200–1558 ppm NaPb for 7 and 14 days produced increases in hepatic microsomal cytochrome P450 (CYP) content and a marked induction of CYP2B-dependent 7-pentoxyresorufin *O*-depentylase and testosterone 16β-hydroxylase activities (Price *et al.*, 2007). Significant increases also were observed in CYP3A-dependent testosterone β-hydroxylase and thyroxine UDP-glucuronosyltransferase activities (Fig. 21-34). Relative potency calculations indicated that NaPb was 8.8 and 3.8X more potent than pyrethrins in inducing CYP2B and CYP3A enzymes in male and female rats, respectively. Hepatic effects of pyrethrins were dose-dependent in female rats (100 ppm being a no effect level) and after the cessation of treatment were reversible in both sexes. The mode of action of pyrethrins-induced rat liver and thyroid tumors appears to be similar to that of other nongenotoxic inducers of hepatic xenobiotic metabolism (Price *et al.*, 2007).

In addition to disturbances in thyroid hormone economy associated with induction of hepatic microsomal enzymes and increased biliary excretion of conjugated thyroid hormones, recent studies have reported xenobiotic chemical-induced rapid hepatobiliary clearance of unconjugated T_4 (Wong *et al.*, 2005). Serum T_4 levels were significantly lowered after 12 hours and were undetectable after 72 hours of administration of a selective corticotrophin-releasing factor (CRF) receptor-1 antagonist to male Sprague–Dawley rats. By comparison propylthiouracil (PTU) (used as a positive control) did not lower serum T4 levels until 72 hours. A significant compensatory increase in serum TSH also was detected after 12 hours with the CRF-1 receptor-1 antagonist compared to after 48 hours with PTU. A decreased mean ^{125}I–T_4 plasma concentration was detected after 1 hour when the first sample was collected and continued to rapidly decrease over a 24-hours period. Phenobarbital (used as a positive control) resulted only in a slight decrease of ^{125}I–T_4 in plasma compared to controls over a similar 24-hours experimental period (Wong *et al.*, 2005). A single oral dose of the compound resulted in a rapid (at 3 hours) significant increase in mRNA for hepatic organic anion transporting protein (Oatp2) that persisted over the 24 hours of the experiment. Other studies have reported that microsomal enzyme inducers can increase the expression of these transporter proteins that mediate the uptake of xenobiotics from blood into the liver (Klaassen and Slitt, 2005). In addition, the CRF receptor-1 antagonist also induced the mRNA's of the multidrug resistance protein-2, a canalicular transporter, that mediates the efflux of compounds from liver into bile (Johnson and Klaassen, 2002a,b; Klaassen, 2002; Wong *et al.*, 2005; Chen and Klaassen, 2004; Chen *et al.*, 2003, 2005; Cheng *et al.*, 2005; Cherrington *et al.*, 2002, 2003; Guo *et al.*, 2002; Maher *et al.*, 2005; Slitt *et al.*, 2006).

Xenobiotic chemical-induced perturbations in thyroid hormone economy are encountered more frequently in rats than in mice. Viollon-Badie *et al.* (1999) administered four classic hepatic microsomal enzyme-inducing chemicals (e.g., clofibrate [300 mg/kg],

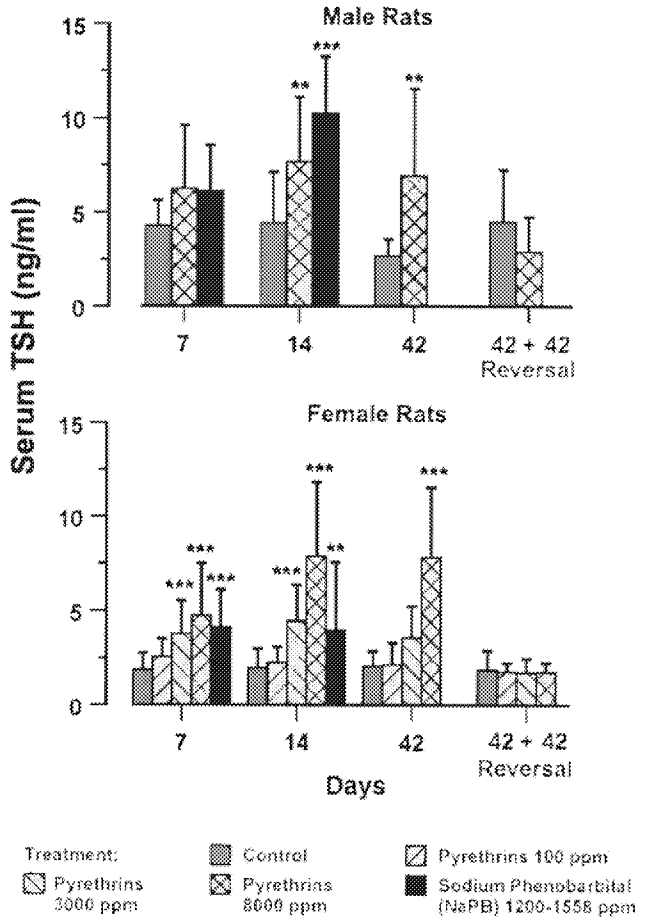

Figure 21-32. Effect of pyrethins and NaPB on serum TSH in Sprague–Dawley rats. (From Finch et al., 2006).

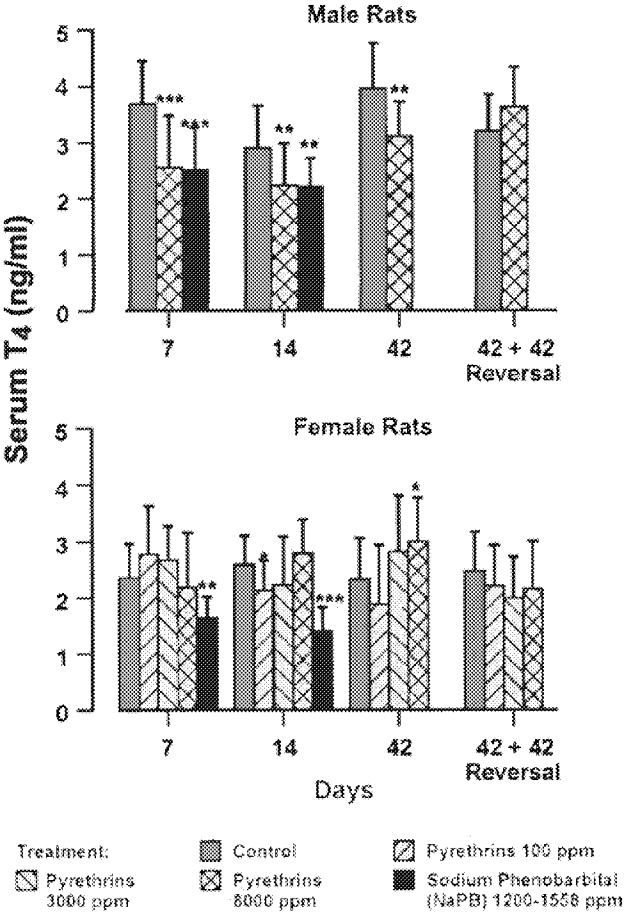

Figure 21-33. Effect of pyrethrins and NaPB on serum thyroxine (T_4) in Sprague–Dawley rats. (From Finch et al., 2006).

phenobarbital (PB) [80 mg/kg], pregnenolone-16α carbonitrile [PCN] [100 mg/kg], and β-naphthoflavone [80 mg/kg]) to rats and mice and compared the effects on hepatic T_4-UDP-glucuronyl transferase. Rats had a consistent and significant increase in enzyme activity whereas the modest increase in mice was not significant compared to controls. However, Hood *et al.* (2003) clearly demonstrated that B6C3F male mice respond to classic hepatic enzyme inducers (e.g., PB, PCB, PCN, and 3-methylcholanthrene (3-MC) by increasing liver weight, increased hepatic T4-UDP-glucuronyl transferase activity, decreased serum T_4 (only with PB and PCB), and increased serum TSH (markedly by PCN and 3-MC, only slight by PB and PCB). All four microsomal enzyme inducers dramatically increased thyroid follicular cell proliferation in mice as quantitated by the BrdU-labeling index.

There is no convincing evidence that humans treated with drugs or exposed to chemicals that induce hepatic microsomal enzymes are at increased risk for the development of thyroid cancer (Curran and DeGroot, 1991). In a study on the effects of microsomal enzyme-inducing compounds on thyroid hormone metabolism in normal healthy adults, phenobarbital (100 mg daily for 14 days) did not affect the serum T_4, T_3, or TSH levels (Ohnhaus *et al.*, 1981). A decrease in serum T_4 levels was observed after treatment with either a combination of phenobarbital plus rifampicin or a combination of phenobarbital plus antipyrine; however, these treatments had no effect on serum T_3 or TSH levels (Ohnhaus and Studer, 1983). Epidemiological studies of patients treated with therapeutic doses of phenobarbital have reported no increase in risk for the development of thyroid neoplasia (Clemmesen *et al.*, 1974; Clemmesen and Hjalgrim-Jensen, 1977, 1978, 1981; White *et al.*, 1979; Friedman, 1981; Shirts *et al.*, 1986; Olsen *et al.*, 1989). Highly sensitive assays for thyroid and pituitary hormones are readily available in a clinical setting to monitor circulating hormone levels in patients exposed to chemicals potentially disruptive of pituitary–thyroid axis homeostasis.

Likewise, there is no substantive evidence that humans treated with drugs or exposed to chemicals that induce hepatic microsomal enzymes are at increased risk for the development of liver cancer. This is best exemplified by the extensive epidemiological information on the clinical use of phenobarbital. Phenobarbital has been used clinically as an anticonvulsant for more than 80 years. Relatively high microsomal enzyme-inducing doses have been used chronically, sometimes for lifetime exposures, to control seizure activity in human beings. A study of over 8000 patients admitted to a Danish epilepsy center from 1933 to 1962 revealed no evidence for an increased incidence of hepatic tumors in phenobarbital-treated humans when patients receiving thorotrast, a known human liver carcinogen, were excluded (Clemmesen and Hjalgrim-Jensen, 1978). A follow-up report on this patient population confirmed and extended this observation (Clemmesen and Hjalgrim-Jensen, 1981; Olsen *et al.*, 1989). The results of two other smaller studies (2099 epileptics and 959 epileptics) also revealed no hepatic tumors in patients treated with phenobarbital (White *et al.*, 1979).

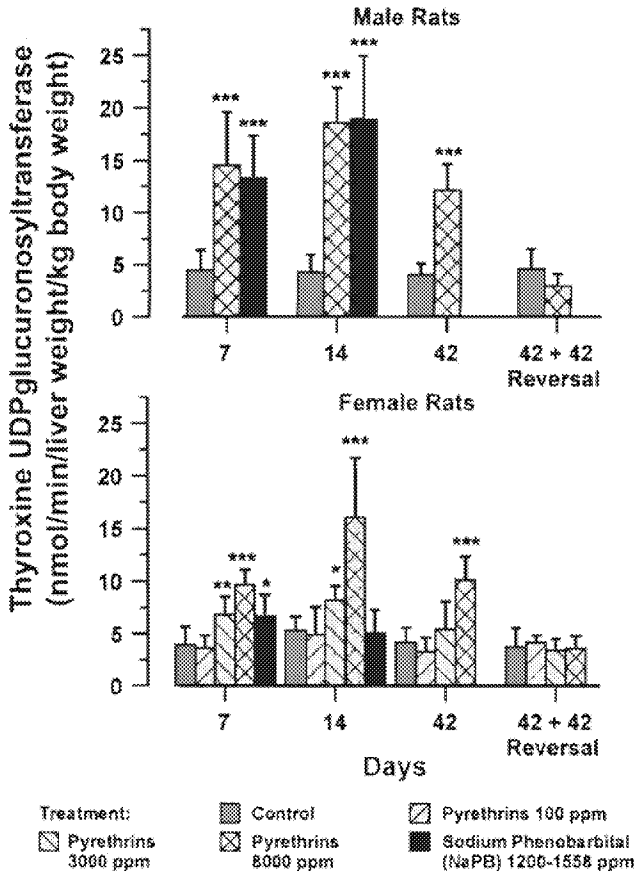

Figure 21-34. Effect of pyrethrins and NaPB on hepatic T_4-UDP-GT (nmol/min/liver wt./body wt.) in Sprague–Dawley rats. (From Finch et al., 2006).

Chemical Inhibition of 5′-Monodeiodinase

FD&C Red No. 3 (erythrosine) is an example of a well-characterized xenobiotic that results in perturbations of thyroid function in rodents and in long-term studies is associated with an increased incidence of benign thyroid tumors. Red No. 3 is a widely used color additive in foods, cosmetics, and pharmaceuticals. A chronic toxicity/carcinogenicity study revealed that male Sprague–Dawley rats fed a 4% dietary concentration of Red No. 3 beginning in utero and extending over their lifetime (30 months) developed a 22% incidence of thyroid adenomas derived from follicular cells compared to 1.5% in control rats and a historical incidence of 1.8% for this strain (Borzelleca *et al.*, 1987; Capen, 1989) (Fig. 21-35). There was no significant increase in follicular cell adenomas in the lower dose groups of male rats or an increase in malignant thyroid follicular cell tumors. Female rats fed similar amounts of the color did not develop a significant increase in either benign or malignant thyroid tumors. Feeding of the color at the high dose (4%) level provided male rats with 2464 mg/kg of Red No. 3 daily; by comparison human consumption in the United States is estimated to be 0.023 mg/kg/day.

The results of mechanistic studies with FD&C Red No. 3 have suggested that a primary (direct) action on the thyroid is unlikely to result from: (1) failure of the color (^{14}C-labeled) to accumulate in the gland, (2) negative genotoxicity and mutagenicity assays, (3) lack of an oncogenic response in mice and gerbils, (4) a failure to promote thyroid tumor development at dietary concentrations of 1.0% or less in male and female rats (Capen, 1989), and (5) no increased tumor development in other organs. Investigations with radiolabeled compound have demonstrated that the color does not accumulate in the thyroid glands of rats following the feeding of either 0.5% or 4.0% FD&C Red No. 3 for 1 week prior to the oral dose of ^{14}C-labeled material.

Mechanistic investigations with FD&C Red No. 3 included, among others, a 60-day study of male Sprague–Dawley rats fed either 4% (high dose) or 0.25% (low dose) Red No. 3 compared to controls, whose food was without the color, in order to determine the effects of the color on thyroid hormone economy. The experimental design of the study was to sacrifice groups of rats ($n = 20$ rats/interval and dose) fed Red No. 3 and their control groups after 0, 3, 7, 10, 14, 21, 30, and 60 days.

A consistent effect of Red No. 3 on thyroid hormone economy was the striking increase in serum reverse T_3 (Fig. 21-36). In the rats fed high doses, reverse T_3 was increased at all intervals compared to controls and this also held for rats killed at 10, 14, and 21 days in the low-dose group. The mechanisms responsible for the increased serum reverse T_3 appear to be, first, substrate (T_4) accumulation due to 5′-monodeiodinase inhibition with subsequent conversion to reverse T_3 rather than active T_3; and, second, reverse T_3 accumulation due to 5′-monodeiodinase inhibition resulting in an inability to degrade reverse T_3 further to diiodothyronine (T_2).

Groups	Original Study					High-Dose Study	
	I_A	I_B	II	III	IV	I_C	V
Red No. 3 (%)	0	0	0.1	0.5	1.0	0	4.0
(mg/kg/day)	—	—	(49)	(251)	(507)	—	(2.464)
F.C. Adenoma (%)	0	0	0	2.9	1.5	1.5	21.8
F.C. Carcinoma (%)	0	0	4.5	1.5	4.4	2.9	4.4
Cystic follicular hyperplasia (%)	2.9	1.5	12	16.2	7.3	0	23.2
Diffuse or focal F.C. hyperplasia (%)	1.4	0	7.	7.4	26.1	5.8	67.0
Follicular cysts (%)	10	14.5	9.0	11.8	11.6	2.9	14.5

Figure 21-35. Thyroid lesions in male Sprague–Dawley rats fed varying doses of FD&C Red No. 3 beginning in utero and for a lifetime of 30 months. (From Borzelleca et al., 1987).

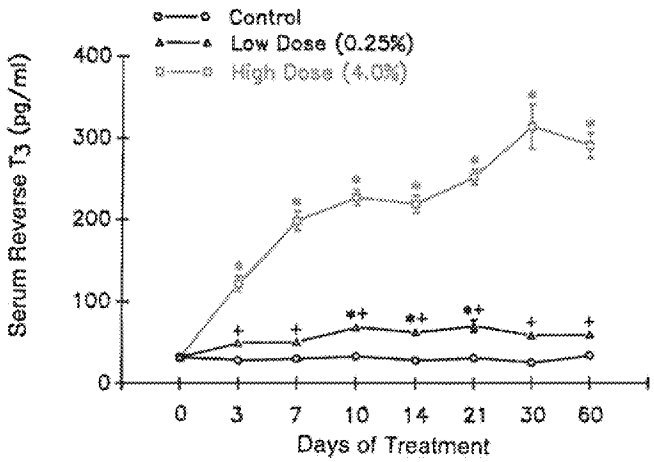

Figure 21-36. Rapid and significant increase in serum reverse triiodothyronine (rT_3) levels in Sprague–Dawley rats ($N = 20$ per group and interval) administered a high (4%) and low (0.25%) dose of FD&C Red No. 3.

The significant increase in rT_3 was detected at the initial interval of 3 days and persisted during the 60-day experiment in the high-dose group. (Courtesy of the Certified Color Manufacturers Association, Inc., and Dr. L.E. Braverman and Dr. W.J. DeVito, University of Massachusetts Medical School.)

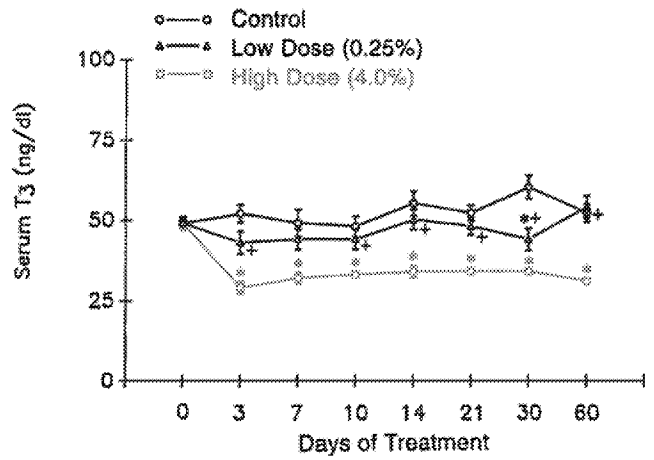

Figure 21-37. Changes in serum triiodothyronine (T_3) following administration of a high (4%) and low (0.25%) dose of FD&C Red No. 3 in the diet to Sprague–Dawley rats. (Courtesy of the Certified Color Manufacturers Association, Inc., and Dr. L.E. Braverman and Dr. W.J. DeVito, University of Massachusetts Medical School.)

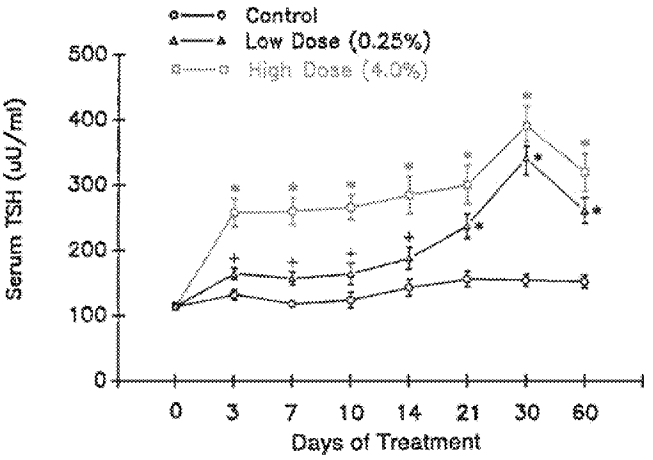

Figure 21-38. Changes in serum thyroid-stimulating hormone (TSH) following administration of a high (4%) and low (0.25%) dose of FD&C Red No. 3 in the diet to Sprague–Dawley rats. (Courtesy of the Certified Color Manufacturers Association, Inc. and Dr. L.E. Braverman and Dr. W.J. DeVito, University of Massachusetts Medical School.)

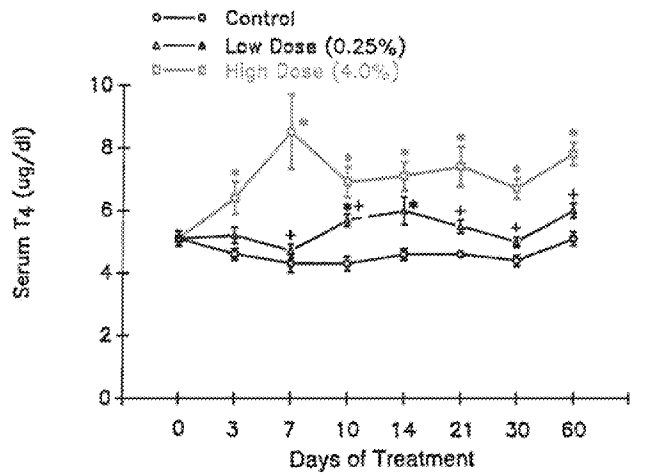

Figure 21-39. Changes in serum thyroxine (T_4) following administration of a high (4%) and low (0.25%) dose of FD&C Red No. 3 in the diet to Sprague–Dawley rats. (Courtesy of the Certified Color Manufacturers Association, Inc., and Dr. L.E. Braverman and Dr. W.J. DeVito, University of Massachusetts Medical School.)

Serum triiodothyronine (T_3) was decreased significantly at all intervals in rats of the high-dose group compared to interval controls (Fig. 21-37). The mechanism responsible for the reduced serum T_3 following feeding of Red No. 3 was decreased monodeiodination of T_4 due to an inhibition of the 5′-monodeiodinase by the color.

Serum TSH was increased significantly at all intervals in rats of the high-dose (4%) group compared to controls. Rats fed 0.25% Red No. 3 had increased serum TSH only at days 21, 30, and 60 (Fig. 21-38). The mechanism responsible for the increased serum TSH following ingestion of Red No. 3 was a compensatory response by the pituitary gland to the low circulating levels of T_3 that resulted from an inhibition of the 5′-monodeiodinase. Serum T_4 also was increased at all intervals in rats fed 4% Red No. 3 compared to controls (Fig. 21-39). The mechanism responsible for the increased serum T_4 was, first, accumulation due to an inability to monodeiodinate T_4 to T_3 in the liver and kidney from the inhibition of 5′-monodeiodinase by the color; and, second, TSH stimulation of increased T_4 production by the thyroid gland.

^{125}I-labeled T_4 metabolism was altered in liver homogenates prepared from rats fed 4% FD&C Red No. 3. Degradation of labeled T_4 was decreased to approximately 40% of the values in control homogenates (Fig. 21-40). This was associated with a 75% decrease in percent generation of ^{125}I and an approximately 80% decrease in percent generation of ^{125}I-labeled T_3 from radiolabeled T_4 substrate. These mechanistic investigations suggested that the color results in a perturbation of thyroid hormone economy in rodents by inhibiting the 5′-monodeiodinase in the liver, resulting in long-term stimulation of follicular cells by TSH, which over their lifetime predisposed to an increased incidence of thyroid tumors (Borzelleca *et al.*, 1987; Capen and Martin, 1989). The color tested negative in

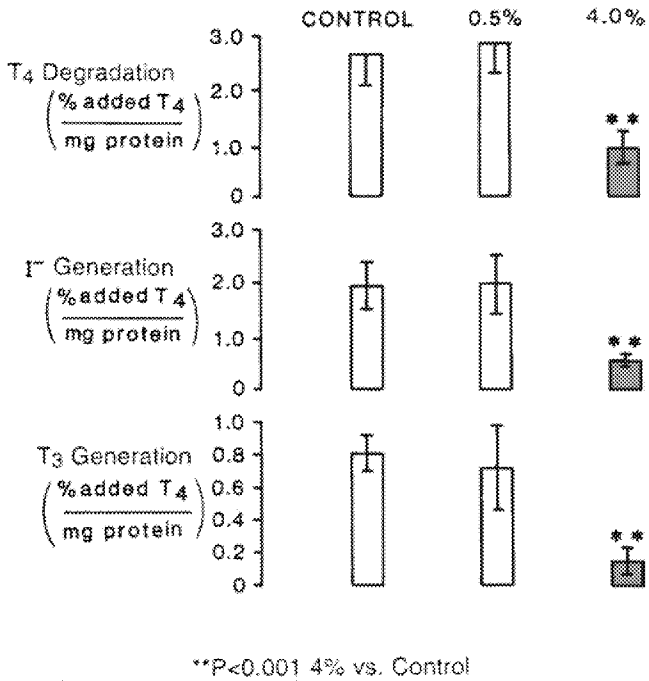

Figure 21-40. Effects of dietary FD&C Red No. 3 on the hepatic metabolism of ^{125}I-labeled thyroxine in male Sprague–Dawley rats fed diets containing 0.5 and 4.0% color compared to controls. (Courtesy of the Certified Color Manufacturers Association, Inc. and the late Sidney H. Ingbar, M.D.)

standard genotoxic and mutagenic assays, and it did not increase the incidence of tumors in other organs.

Morphometric evaluation was performed on thyroid glands from all rats at each interval during the 60-day study. Four levels of exposure of rat thyroid to Red No. 3 were evaluated, with 25 measurements from each rat. The direct measurements included the diameter of thyroid follicles, area of follicular colloid, and height of follicular cells. Thyroid follicular diameter was decreased significantly in both low- and high-dose groups at 3, 7, 10, and 14 days compared to interval controls. The area of follicular colloid generally reflected the decrease in thyroid follicular diameter and was decreased significantly at days 3 and 10 in high-dose rats and days 7 and 10 in the low-dose group compared to interval controls. These reductions in thyroid follicular diameter and colloid area were consistent with morphological changes expected in response to an increased serum TSH concentration.

Thyroid follicular height was increased significantly only after feeding FD&C Red No. 3 for 60 days in both the high- and low-dose groups compared to interval controls. The absence of morphometric evidence of follicular cell hypertrophy at the shorter intervals was consistent with the modest increase (15.8%) in thyroid gland:body weight ratio after this relatively short exposure to the color. The lack of follicular cell hypertrophy at the shorter intervals of feeding Red No. 3 in rats with severalfold elevations in serum TSH levels may be related, in part, to the high iodine content (58% of molecular weight) interfering with the receptor-mediated response of thyroid follicular cells to TSH. The thyroid responsiveness to TSH is known to vary inversely with iodine content (Ingbar, 1972; Lamas and Ingbar, 1978). Thyroid glands of rats fed FD&C Red No. 3 would be exposed to an increased iodine concentration primarily from sodium iodide contamination of the color and, to a lesser extent, from metabolism of the compound and release of iodide.

Secondary Mechanisms of Thyroid Tumorigenesis and Risk Assessment

Understanding the mechanism of action of xenobiotics on the thyroid gland provides a rational basis for extrapolation findings from long-term rodent studies to the assessment of a particular compound's safety for humans (McClain, 1994). Many chemicals and drugs disrupt one or more steps in the synthesis and secretion of thyroid hormones, resulting in subnormal levels of T_4 and T_3, associated with a compensatory increased secretion of pituitary TSH (Fig. 21-41). When tested in highly sensitive species, such as rats and mice, early on these compounds resulted in follicular cell hypertrophy/hyperplasia and increased thyroid weights, and in long-term studies they produced an increased incidence of thyroid tumors by a secondary (indirect) mechanism associated with hormonal inbalances.

Review of the U.S. *Physicians' Desk Reference* (PDR) reveals a number of marketed drugs that result in a thyroid tumorigenic response when tested at high concentrations in rodents, primarily in rats. A broad spectrum of product classes are represented including antibiotics, calcium-channel blockers, antidepressants, hypolipidemic agents, amongst others (Fig. 21-42). Amiodarone (an antiarrhythmic drug) and iodinated glycerol (an expectorant) are highly iodinated molecules that disrupt thyroid hormone economy by mechanisms similar to the food color, FD&C Red No. 3 (Fig. 21-43).

In the secondary mechanism of thyroid oncogenesis in rodents, the specific xenobiotic chemical or physiological perturbation evokes another stimulus (e.g., chronic hypersecretion of TSH) that promotes the development of nodular proliferative lesions (initially hypertrophy, followed by hyperplasia, subsequently adenomas, infrequently carcinomas) derived from follicular cells (Hiasa *et al.*, 1987). Thresholds for "no effect" on the thyroid gland can be established by determining the dose of xenobiotic that fails to elicit an elevation in the circulating level of TSH. Compounds acting by this indirect (secondary) mechanism with hormonal imbalances usually show little or no evidence for mutagenicity or for producing DNA damage.

In human patients who have marked changes in thyroid function and elevated TSH levels, as is common in areas with a high incidence of endemic goiter due to iodine deficiency, there is little if any increase in the incidence of thyroid cancer (Doniach, 1970; Curran and DeGroot, 1991). The relative resistance to the development of thyroid cancer in humans with elevated plasma TSH levels is in marked contrast to the response of the thyroid gland to chronic TSH stimulation in rats and mice. The human thyroid is much less sensitive to this pathogenetic phenomenon than rodents (McClain *et al.*, 1989).

Human patients with congenital defects in thyroid hormone synthesis (dyshormonogenetic goiter) and markedly increased circulating TSH levels have been reported to have an increased incidence of thyroid carcinomas (McGirr *et al.*, 1959; Cooper *et al.*, 1981). Likewise, thyrotoxic patients with Grave's disease, in which follicular cells are autonomously stimulated by an immunoglobulin (long-acting thyroid stimulator, or LATS) also appear to be at greater risk of developing thyroid tumors (Clements, 1954; Pendergrast *et al.*, 1961). Therefore, the literature suggests that prolonged stimulation of the human thyroid by TSH will induce neoplasia only in exceptional circumstances, possibly by acting together with some other metabolic or immunologic abnormality (Curran and DeGroot, 1991).

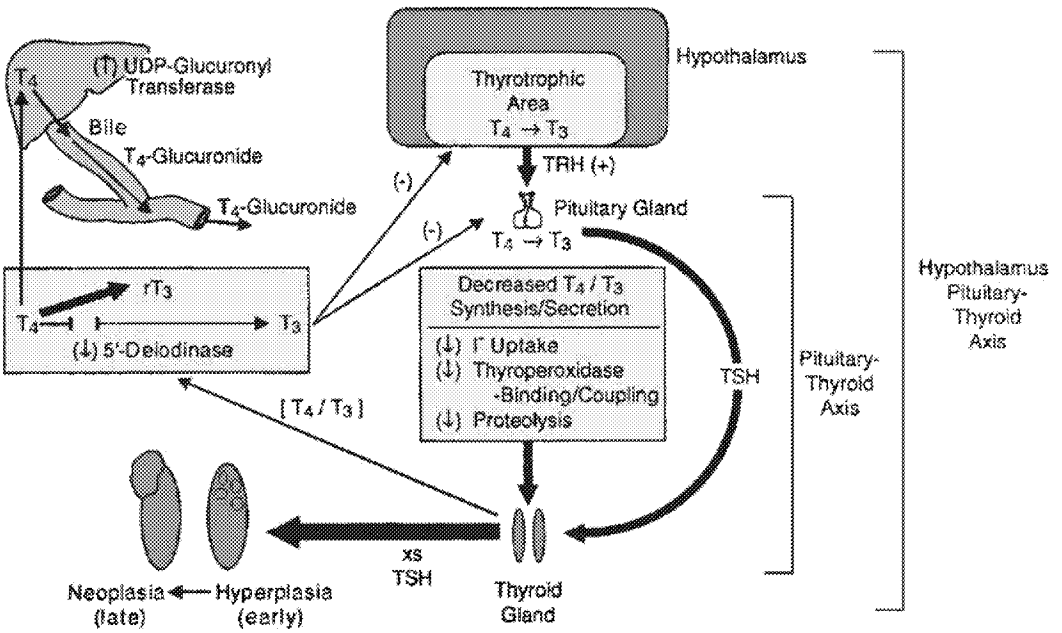

Figure 21-41. Multiple sites of disruption of the hypothalamic–pituitary–thyroid axis by xenobiotic chemicals.

Chemicals can exert direct effects by disrupting thyroid hormone synthesis or secretion and indirectly influence the thyroid through an inhibition of 5′-deiodinase or by inducing hepatic microsomal enzymes (e.g., T_4-UDP glucuronyltransferase). All of these mechanisms can lower circulating levels of thyroid hormones (T_4 and/or T_3), resulting in a release from negative-feedback inhibition and increased secretion of thyroid-stimulating hormone (TSH) by the pituitary gland. The chronic hypersecretion of TSH predisposes the sensitive rodent thyroid gland to develop an increased incidence of focal hyperplastic and neoplastic lesions (adenomas) by a secondary (epigenetic) mechanism.

DRUG	PRODUCT CLASS	SPECIES
• MINOCYCLINE	ANTIBIOTIC	R
• OXAZEPAM	ANTIANXIETY	R
• NICARDIPINE	Ca-CHANNEL BLOCKER	R
• SERTRALINE	ANTIDEPRESSANT	R
• SIMVASTATIN	HYPOLIPIDEMIC	R
• SPIRONOLACTONE	DIURETIC	R
• VIDARABINE	ANTIVIRAL	R

Figure 21-42. Examples of marketed drugs with a tumorigenic response in the thyroid gland of rats. (Modified from Davis and Monro, 1995).

DRUG	PRODUCT CLASS	SPECIES
• AMIODARONE	ANTIARRHYTHMIC	R
• ATENOLOL	β-ADRENERGIC BLOCKER	R
• BEPRIDIL	Ca-CHANNEL BLOCKER	R
• DAPSONE	ANTINEOPLASTIC	R
• GRISEOFULVIN	ANTIBIOTIC	R
• IODINATED GLYCEROL	EXPECTORANT	R
• METHINAZOLE	ANTI-THYROID	R
• MIDAZOLAM	SEDATIVE	R

Figure 21-43. Marketed drugs with a thyroid tumorigenic response. (Modified from Davis and Monro, 1995).

Initiating Chemicals in Thyroid Carcinogenesis

In contrast to the previous categories of indirect-acting thyrotoxic compounds, certain chemicals and irradiation appear to have a direct effect on the thyroid gland resulting in genetic damage that leads to cell transformation and tumor formation in animals (Doniach, 1963; Lee *et al.*, 1982; Capen *et al.*, 1999). Examples of thyroid initiators include 2-acetylaminofluorine (2-AAF), *N*-methyl-*N*-nitrosourea [MNU], *N*-bis(2-hydroxypropyl) nitrosamine [DHPN], methylcholanthrene, dichlorobenzidine, and polycyclic hydrocarbons. Chemicals in this group often increase the incidence of both benign and malignant thyroid tumors. Iodine deficiency is a strong promoter of MNU-initiated thyroid tumors in rats.

Thyroid Tumors in Humans

Thyroid carcinomas are the most common endocrine neoplasms in humans, affecting approximately 1% of the population (Sherman, 2003). Roughly 95% of all thyroid tumors are of thyroid follicular epithelial cell origin, including papillary, follicular, and anaplastic thyroid carcinomas. The remaining 5% are medullary thyroid carcinomas of C-cell origin (Cho *et al.*, 1999; Sherman, 2003). The subclassification of thyroid cancers into these four categories is clinically significant. Papillary thyroid carcinomas metastasize via lymphatics to local lymph nodes in an estimated 50% of cases but have the most favorable prognosis, with a 98% 10-year survival rate. Follicular carcinomas are associated with poorer prognoses. Follicular thyroid carcinomas are more prevalent in areas of dietary iodine deficiency, metastasize hematogenously, and are less likely than papillary thyroid carcinomas to take up radioactive iodide for imaging and therapeutic ablation. However, the 10-year survival rate for follicular thyroid carcinomas is still high at 92%. Anaplastic thyroid carcinomas are almost invariably fatal due to rapid invasion of critical structures in the neck, distant metastases, and a failure to take up radioactive iodide.

Medullary thyroid carcinomas, also commonly known as C-cell carcinomas, arise from calcitonin-secreting C-cells and are associated with inherited syndromes, such as multiple endocrine neoplasia (MEN), in approximately 20–25% of cases (Cho *et al.*, 1999; Sherman, 2003). Medullary carcinomas frequently metastasize via the bloodstream, in addition to lymphatic spread, and are treated with surgical resection and/or external beam radiation. In contrast to follicular origin thyroid tumors, C-cells and tumors arising from them do not have the ability to take up radioactive iodide. The five-year survival rate for medullary thyroid carcinomas is approximately 50%.

Genetic Events in Thyroid Tumors of Follicular Cell Origin

Thyroid follicular cells are responsible for iodide uptake and thyroid hormone synthesis and can undergo neoplastic transformation to carcinomas of three histotypes: papillary, follicular, and anaplastic. It is well known that papillary thyroid carcinomas can occur secondary to ionizing radiation exposure, particularly in children (Sherman, 2003; Boice, 2005). After the Chernobyl nuclear reactor accident in 1986, the incidence of thyroid carcinomas in children in affected areas of Belarus increased from less than one per million to more than 90 per million (Cardis *et al.*, 2005). The primary known molecular mechanism of radiation-induced papillary carcinoma development is through the Ret/PTC group of oncogenes. *Ret* proto-oncogene is a receptor tyrosine kinase normally involved in the glial-derived neurotropic factor signaling pathway in neuroendocrine and neural cells. In thyroid follicular cells, the alignment of chromosomes during interphase places the *ret* proto-oncogene in close proximity to several other constitutively expressed genes with which it can recombine during repair of ionizing radiation-induced double-stranded DNA breaks (Nikiforov, 2002, 2000). These Ret/PTC rearrangements allow for unregulated expression of chimeric oncoproteins with constitutive tyrosine kinase activity. Ret/PTC expression is found in roughly one-third of all papillary carcinomas, but in the majority of all radiation-induced papillary carcinomas (Klugbauer *et al.*, 1995). Whereas Ret/PTC1-expressing papillary carcinomas are often well differentiated, Ret/PTC3 expression is associated with a solid phenotype and a more aggressive clinical course (Nikiforov, 2002; Williams *et al.*, 2004).

Sporadic papillary thyroid carcinomas unrelated to radiation exposure make up more than two-thirds of all cases, and several genetic events have been identified as important in their tumorigenesis (Nikiforov, 2004). A form of the B-type Raf kinase, or BRAF, with a point mutation resulting in V600E has been identified in approximately 45% of sporadic papillary carcinomas, particularly the tall-cell variant (Xing, 2005). BRAF expression has been associated with dedifferentiation and disease progression. NTRK1 is a receptor tyrosine kinase normally involved in nerve growth factor signaling (Tallini, 2002). Like Ret/PTC rearrangements, NTRK1 can recombine with the 5′ end of other heterologous genes and form a constitutively active oncogene, such as TRK-T1, leading to papillary carcinomas. However, NTRK1 rearrangements are less frequent than Ret/PTC rearrangements and are not associated with radiation exposure.

Genetically Engineered Mouse Models of Thyroid Cancer

Genetically engineered mouse models of thyroid cancer facilitate analysis of the roles of specific genetic mutations in thyroid tumorigenesis (Jhiang *et al.*, 1996, 1998b; Sagartz *et al.*, 1997; Capen and Sagartz, 1998). Because thyroid cancer truly encompasses several diseases with different etiologies and relevant genetic mutations, no single transgenic mouse model of thyroid cancer can fully recapitulate the full spectrum of disease. However, several models have successfully reproduced various aspects and have offered insight into genetic mutations underlying thyroid tumorigenesis. These mouse models of thyroid cancer offer examples of positive genotype–phenotype correlation (Knostman *et al.*, 2007) (Table 21-3).

Over the past decade, several genetically engineered mouse models of thyroid cancer have been utilized to replicate variants of the human disease (Table 21-3). In most cases, transgenic mice have been produced using the highly active bovine thyroglobulin (Tg) promoter to specifically target transgene expression to thyroid follicular cells or using the human or rat calcitonin/calcitonin gene-related peptide (CGRP) promoter to target transgene expression to C-cells. In some cases, tumor suppressor gene knockout mice have been created and cross-bred with transgenic mice expressing thyroid-specific oncogenes, resulting in increased tumorigenesis and/or an aggressive phenotype. Transgenic mice expressing Ret/PTC1, Ret/PTC3 Trk-T1, BRAF or ras offer a reasonable approximation of the features of papillary thyroid carcinomas in humans. However, even with a p53 knock-out, no model has been able to demonstrate significant tumor dedifferentiation or metastasis (LaPerle *et al.*, 2000). One limitation has been the fact that p53 knockout mice develop extrathyroidal neoplasms at a high rate,

Table 21-3

Comparison of Human Thyroid Tumors and Genetically Engineered Mouse Models of Thyroid Cancer with Similar Genetic Mutations

GENETIC ALTERATION	TUMOR MORPHOLOGY		BIOLOGIC BEHAVIOR		HORMONAL DERANGEMENT	
	HUMAN	MOUSE	HUMAN	MOUSE	HUMAN	MOUSE
Ret/PTC1	Typical PTC	Typical PTC	Frequent local invasion, regional lymph node and less common distant metastases	Local invasion without any metastasis	None typical	Hypothyroidism
Ret/PTC3	Typical to solid PTC	Typical to solid PTC	Frequent local invasion, regional lymph node and less common distant metastases	Local invasion with rare lymph node metastases	None typical	NR
$BRAF^{V600E}$	Tall-cell variant or typical PTC with necrosis and anaplasia	Tall-cell variant or typical PTC with foci of anaplasia	Very frequent local invasion and anaplasia; loss of differentiation markers	Local invasion and anaplasia; maintenance of thyroglobulin expression	None typical	High TSH euthyroid
TRK	Typical PTC	PTC with absence of characteristic nuclear abnormalities	Local invasion, regional lymph node and less common distant metastases	Invasion not reported; absence of metastases	None typical	None
ras	FTC or follicular variant PTC	Typical PTC	Local invasion, regional lymph node, and/or hematogenous metastases	Local invasion with rare lung metastases	None typical	None
p53 inactivation/loss	ATC	Increased anaplasia, invasion and metastases in Ret/PTC models of PTC (p53 not examined alone)	Rapid local invasion and metastases with loss of differentiation markers	Local invasion with rare metastases and early death	None typical	Hypothyroidism (in combination with Ret/PTC transgene expression)
Ret	MTC	MTC +/− PTC	C-cell hyperplasia progressing to MTC*; distant spread common in advanced disease; tumors produce calcitonin	C-cell hyperplasia progressing to MTC; very rare metastases; tumors produce calcitonin	Hypercalcitonism	Hypercalcitonism

PTC, papillary thyroid carcinoma; FTC, follicular thyroid carcinoma; ATC, anaplastic thyroid carcinoma; MTC, medullary thyroid carcinoma; NR, not reported; *hereditary cases (e.g., MEN 2A) From Knostman *et al.*, 2007.

CHAPTER

58

Endocrine System

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1. INTRODUCTION

A review of the literature of chemically induced lesions of the endocrine organs indicates that the adrenal glands are the most commonly affected, followed in descending order by the thyroid, pancreas, pituitary, and parathyroid glands. In the adrenal glands, chemically induced lesions are found most frequently in the zona fasciculata, zona reticularis and, to a lesser extent, in either the zona glomerulosa or the medulla. In a survey of tumor types developing in carcinogenicity studies, conducted by the Pharmaceutical Manufacturers Association, endocrine tumors developed frequently in rats. The thyroid gland was third in frequency (behind liver and mammary gland), followed by the pituitary gland (fourth) and adrenal gland (fifth).

For purposes of the following discussion, pharmacological as well as toxicologic effects will be reviewed, with emphasis on the latter. Pharmacological effects are defined as beneficial and desired drug-related changes with minimal side effects and morphological alterations (which are often reversible), whereas toxicologic effects are more severe adverse effects that often are irreversible.

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PART I: ADRENAL CORTEX

2. STRUCTURE AND FUNCTION

2.1. Gross and Microscopic Anatomy

In mammals, the adrenal or suprarenal glands are flattened bilobed organs located in close proximity to the kidneys. They receive arterial blood from branches of the aorta or from the phrenic, renal, and lumbar arteries, resulting in a vascular plexus; perfusion occurs by separate sinusoids to the capsule and to the entire gland, including both the cortex and the medulla. Venous blood flow is derived from a sinusoidal network originating around the cells of the adrenal cortex with eventual flow into the medulla at its periphery. A venous tree is present within the medulla that ultimately flows into the adrenal vein by way of its larger branches.

A midsagittal section of the adrenal glands reveals a clear separation between the cortex and the medulla. The cortex is firm and yellow, and occupies approximately two-thirds of the entire cross-sectional diameter of the organ. In contrast, the medulla is soft and has a prominent gray-tan coloration. The ratio of cortex : medulla is approximately 2 : 1 in healthy laboratory-reared animals.

Defined regions or zones histologically characterize the cortex. Cortical zones consist of the zona glomerulosa (multiformis), zona fasciculata, and zona reticularis. The zones are not always clearly delineated, as illustrated in the normal rat adrenal cortex (Figure 58.1). The mineralocorticoid-producing zona glomerulosa (multiformis) (15% of the cortex) contains cells aligned in a sigmoid pattern in relationship to the capsule. Loss of this zone or the inability to secrete mineralocorticoids (e.g. aldosterone) may result in death of the animal due to the retention of inappropriately high levels of potassium in association with an excessive loss of sodium chloride and water. The largest zone is the zona fasciculata (> 70% of the cortex). Cells in this zone are arranged in long anastomosing cords or columns, separated by small capillaries/sinusoids. They are responsible for the secretion of glucocorticoid hormones (e.g., corticosterone in rat,

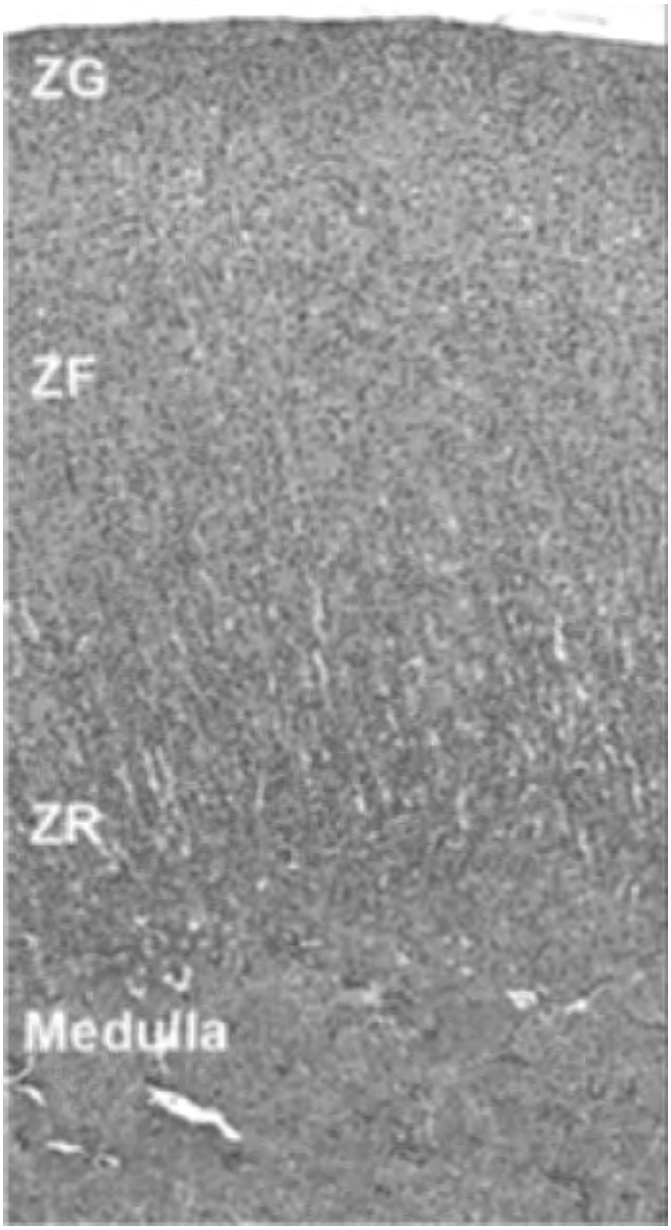

FIGURE 58.1 Zones of the adrenal gland in a normal rat. A thin fibrous capsule surrounds the adrenal cortex. The adrenal cortex consists of three zones, the zona glomerulosa (ZG), zona fasiculata (ZF), and zona reticularis (ZR). The ZG is the thinnest zone of the cortex. The ZF is the thickest zone of the cortex and is composed of columns or fascicles of cortical cells separated by sinusoids. The ZF : ZR boundary is not distinct. The cells of the ZR are smaller and less vacuolated compared to the ZF. The central region is the adrenal medulla composed primarily of chromaffin cells. H&E stain.

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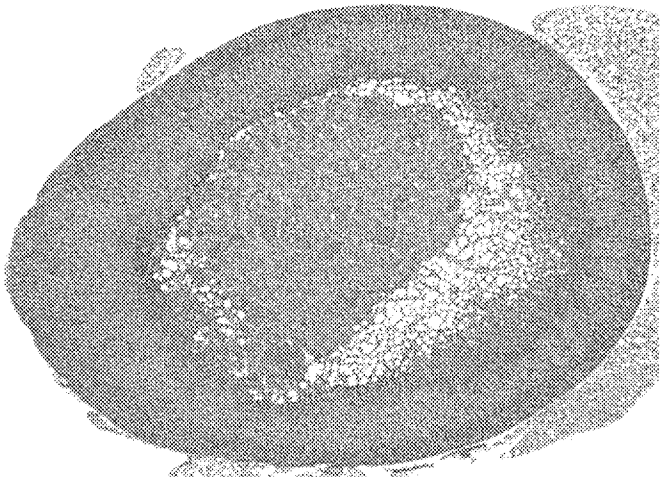

FIGURE 58.2 Corticomedullary vacuolation (X-zone) in female CD-1 mouse. H&E stain.

mouse, and rabbit, or cortisol in dog, pig, monkey, and human), which promote the elevation of blood glucose in addition to many other effects. The innermost portion of the cortex is the zona reticularis (15% of the cortex), which normally secretes minute quantities of adrenal sex hormones in some species.

There are structural and functional differences between species and sexes. Accessory cortical tissue is often seen in mice and cynomolgus monkeys, which is not to be mistaken for proliferative lesions. The marmoset has no obvious zona reticularis, and in the dog the zona fasciculata and zona reticularis are less well demarcated than in other species. The mouse develops the x-zone, a prominent fourth cortical layer immediately adjacent to the medulla, which is especially prominent in females (Figure 58.2); this zone degenerates, leaving clumps of brown-yellow lipofuscin at the corticomedullary junction. Primates develop an area of cortical involution in late fetal life that regenerates after birth. The zona reticularis is three times thicker in male Syrian hamsters than in females. Male mice are more sensitive to the effects of prednisone than females at the same doses.

2.2. Ultrastructural Anatomy

The adrenal cortical cells contain large cytoplasmic lipid droplets, which consist of cholesterol and other steroid precursors. The lipid droplets are in close proximity to the smooth endoplasmic reticulum and large mitochondria, which contain the specific hydroxylase and dehydrogenase enzyme systems required to synthesize the different steroid hormones. Unlike polypeptide hormone-secreting cells, there are no secretory granules in the cytoplasm because there is direct secretion without significant storage of preformed steroid hormones.

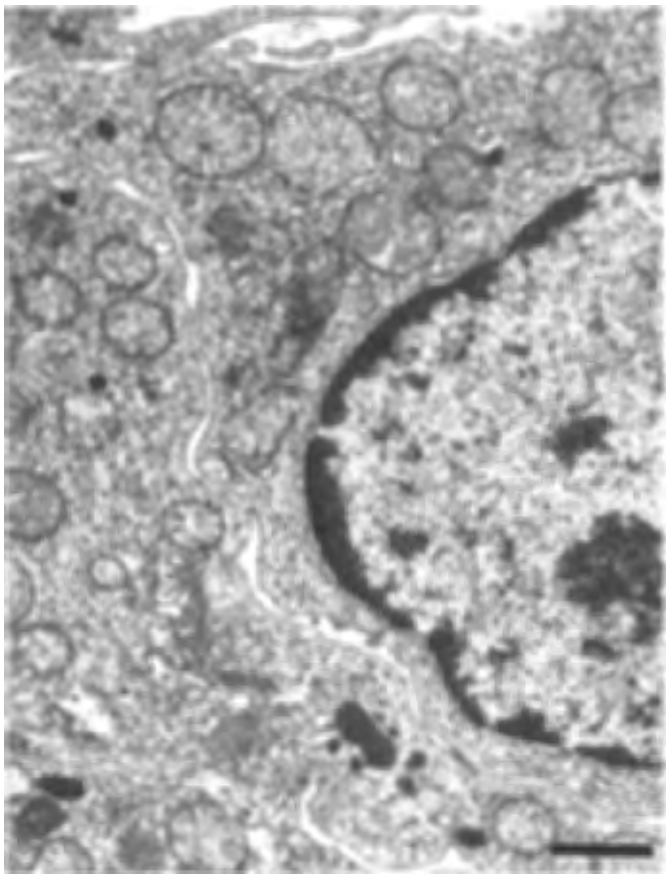

FIGURE 58.3 Adrenal cortical cell from the zona glomerulosa of rat adrenal cortex with mitochondria that have distinct leaf-like cristae. Bar = 1 μm. *Figure reproduced from Yarrington* et al. *(1985) Degeneration of the rat and canine adrenal cortex caused by α-(1,4-dioxido-3-methylquinoxalin-2-yl)-*N*-methylnitrone (DMNM),* Fundam. Appl. Toxicol. *5, 370–381, with permission.*

The three cortical zones have unique ultrastructural characteristics in the rat adrenal cortex. Zona glomerulosa cells (Figure 58.3) contain moderate numbers of neutral lipid

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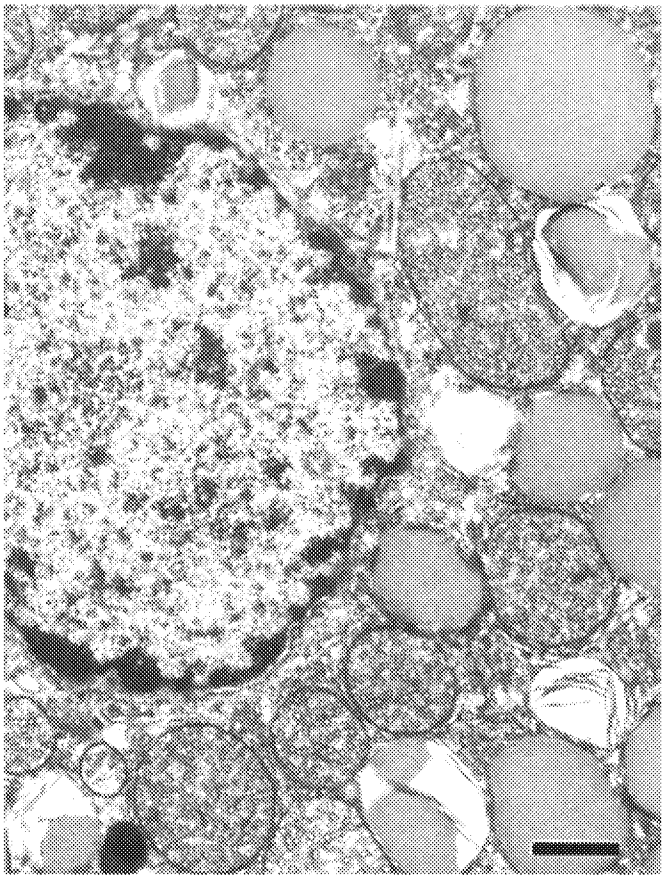

FIGURE 58.4 Cell from the zona fasciculata of rat adrenal cortex with prominent lipid droplets. Mitochondria have vesicular cristae. Bar = 1 μm. *Figure reproduced from Yarrington* et al. *(1985) Degeneration of the rat and canine adrenal cortex caused by α-(1,4-dioxido-3-methylquinoxalin-2-yl)-*N*-methylnitrone (DMNM),* Fundam. Appl. Toxicol. *5, 370–381, with permission.*

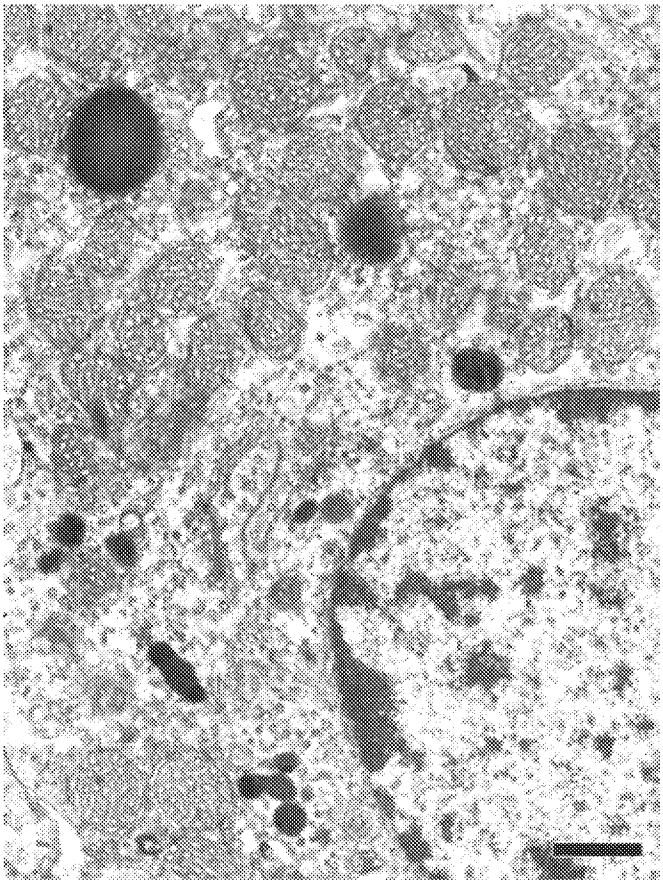

FIGURE 58.5 Cell from the zona reticularis of rat adrenal cortex with mitochondria that have typical tubulovesicular cristae. Bar = 1 μm. *Figure reproduced from Yarrington* et al. *(1985) Degeneration of the rat and canine adrenal cortex caused by α-(1,4-dioxido-3-methylquinoxalin-2-yl)-*N*-methylnitrone (DMNM),* Fundam. Appl. Toxicol. *5, 370–381, with permission.*

droplets, mitochondria with leaf-like cristae, extensive Golgi regions, minimal smooth and rough endoplasmic reticulum, and a few polyribosomes or free ribosomes. In comparison, the larger cells of the zona fasciculata have abundant smooth endoplasmic reticulum, occasional lysosomes, many lipid droplets of various sizes, and round mitochondria with vesicular cristae (Figure 58.4). Linear densities, which often have a crystalline structure, are occasionally found in the mitochondria. In contrast, the typical cell of the zona reticularis in the rat has abundant mitochondria with tubulovesicular cristae, few lipid droplets, numerous lysosomes, and distinct endoplasmic reticulum (Figure 58.5).

2.3. Physiological and Functional Considerations

All hormones produced by the adrenal cortex are steroids. Steroid-producing endocrine organs such as the adrenal cortex synthesize a major parent steroid compound with 1–4 additional carbon atoms added to the basic 17 carbon-containing steroid nucleus. Because steroid hormones are not stored in any significant amount, a continued rate of synthesis is required to maintain a normal secretory rate. This in turn requires continued stimulation of the adrenal cortex by pituitary derived adrenocorticotropic hormone (ACTH; see later) to a greater or lesser degree according to physiological requirements. Once in the circulation, the steroid hormones

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(e.g., cortisol or corticosterone) are bound to plasma proteins (e.g., transcortin [CBG, cortisol/corticosterone binding globulin], albumin). Depending on the nature of the plasma proteins the binding affinity may be high or low, but nonetheless reversible to allow the steroid to be in a free unbound state when interacting with target cells. Under normal conditions, 10% of the glucocorticoids are in a free unbound state. The unbound steroid is free to interact with target cells either to exert metabolic effects or to be transformed into an inactive metabolite. In conditions of elevated secretion of adrenal glucocorticoid above basal rates, the free steroid fraction in the blood is increased and available to evoke the physiological response in target cells and tissues.

Once entry into the cell occurs through the cell membrane, the steroid hormones are attached to soluble cytoplasmic proteins. The hormone-receptor complex is subsequently transported to the nucleus of the target cells, where the hormone is bound to DNA binding sites. The subsequent interaction results in the increased transcription of messenger ribonucleic acid (mRNA), which directs new protein synthesis by the target cells (e.g., enzymes, binding, or structural proteins) to effect the physiological function of the hormone.

Adrenal steroids are synthesized from cholesterol, which in turn is derived from acetate. A complex shuttling of steroid intermediates between mitochondria and endoplasmic reticulum characterizes specific synthetic processes. The specificity of mitochondrial hydroxylation reactions in terms of the steroid attacked and the position of the substrate that is hydroxylated is confined to a specific cytochrome P450 (CYP). The common biosynthetic pathway from cholesterol is the formation of pregnenolone, the basic precursor for the three major groups of adrenal steroids (Figure 58.6). Pregnenolone is formed after two hydroxylation reactions at the carbon 20 and 22 positions of cholesterol and a subsequent cleavage between these two carbon atoms.

In the zona fasciculata, pregnenolone is first converted to progesterone by two microsomal enzymes. Three subsequent hydroxylation reactions occur involving, in order, carbon atoms at the 17, 21, and 11 positions. The first two hydroxylation reactions occur in microsomes, while the 11β-hydroxylation occurs in the mitochondria. The resulting steroid is cortisol, which is the major glucocorticoid in teleosts, hamsters, dogs, non-human primates, and humans. Corticosterone is the major glucocorticoid produced in amphibians, reptiles, birds, rats, mice, and rabbits. It is produced in a manner similar to the production of cortisol, except that progesterone escapes 17α-hydroxylation and proceeds directly to 21-hydroxylation and 11β-hydroxylation. Essentially, rodents lack CYP17 and this is an important consideration for toxicology, as compounds that inhibit this enzyme may not be fully detected in rodent species. Species with CYP17 produce cortisol and those lacking CYP17 produce corticosterone as the major glucocorticoid. This may also account for species differences in adrenocortical toxicity between rodent and non-rodent species. Spironolactone and ketoconazole inhibit CYP17 (but also other enzymes in the steroidogenic pathway), and a full review of chemical compounds and their various targets in the steroidogenic pathway is given in Harvey *et al.* (2007, 2009).

In the zona glomerulosa, pregnenolone is converted to aldosterone by a series of enzymatic reactions similar to those in cortisol formation; however, the cells of this zone lack 17α-hydroxylase and thus cannot produce 17α-hydroxyprogesterone, which is required to produce cortisol. Therefore, the initial hydroxylation product is corticosterone. Some of the corticosterone is acted on by 18-hydroxylase to form 18-hydroxycorticosterone, which in turn interacts with 18-hydroxysteroid dehydrogenase to form aldosterone. Because 18-hydroxysteroid dehydrogenase is found only in the zona glomerulosa, it is not surprising that only this zone has the capacity to produce aldosterone. In addition to the aforementioned steroid hormones, the adrenal cortex also produces small amounts of sex steroids, including progesterone, estrogens, and androgens. Thus, the adrenal cortex as a whole has all the necessary enzymes to synthesize the full range of steroids, differentially located across the various zones, and this property has been useful in developing human derived adrenocortical cell lines (e.g., H295R) to examine steroidogenesis. Estrogens and testosterone are not produced in rodents as they lack CYP17.

After their synthesis, secretion, and interaction with target cells, the adrenal steroid hormones are ultimately metabolized in peripheral tissues. Inactivation occurs in the liver by two main steps

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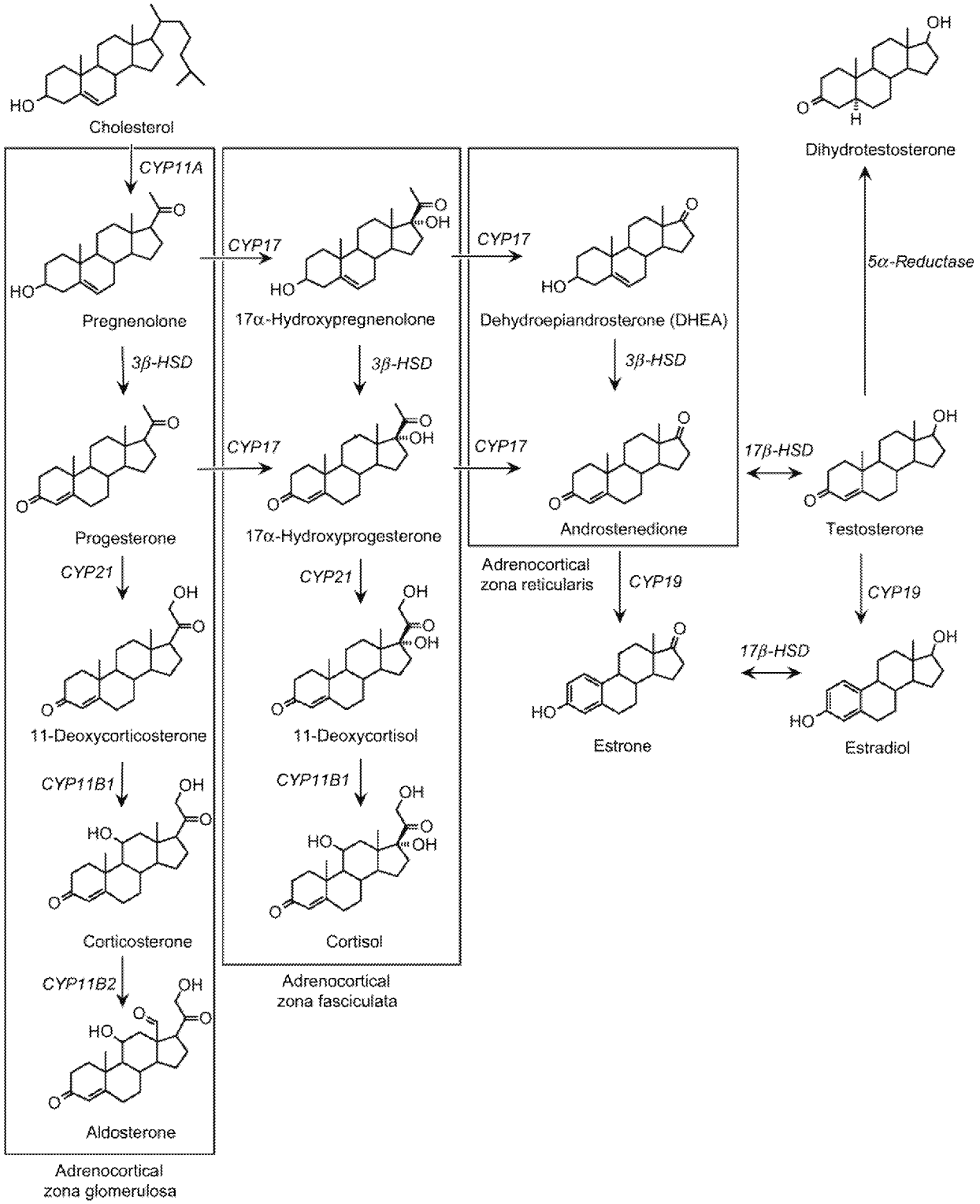

FIGURE 58.6 Adrenocortical steroidogenesis. This diagram shows enzyme expression and steroid production in the zona glomerulosa, zona fasiculata, and zona reticularis of the **human adrenal cortex**. The ability of the cells to produce a specific enzyme conveys the ability to produce a specific steroid and only cells of the zona glomerulosa express CYP11B2 capable of producing aldosterone. CYP17 is required for the production of cortisol and is not expressed in the rat and mouse, which secrete corticosterone as the dominant glucocorticoid. HSD: hydroxysteroid dehydrogenase. *Figure reproduced from Sanderson (2009) Adrenocortical toxicology* in vitro*: assessment of steroidogenic enzyme expression and steroid production in H295R cells. In:* Adrenal Toxicology, *(Harvey, P. W., Everett, D. J., and Springall, C. J., eds), Informa Healthcare, pp. 175–182.*

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that include reduction or side chain removal, and conjugation to glucuronic acid or sulfate. In the presence of liver disease, the turnover of steroid hormones, particularly cortisol, may be decreased and can result in abnormal adrenal function tests in patients or test animals without adrenal cortical lesions. Occasionally, peripheral tissues may activate steroid hormones (e.g., testosterone to dihydrotestosterone) or, as in the case of cortisol, convert the steroid to other less active forms of the hormone. Mineralocorticoids (e.g., aldosterone) are the major steroids secreted from the zona glomerulosa under the control of the renin–angiotensin II system. Mineralocorticoids have their effects on ion transport by epithelial cells, particularly renal cells, resulting in the conservation of sodium (chloride and water) and loss of potassium. In the distal convoluted tubule of the mammalian nephron, a cation exchange exists that promotes the resorption of sodium from the glomerular filtrate and the secretion of potassium into the lumen.

Under conditions of decreased blood flow or volume, the enzyme renin is released into the circulation at an increased rate by cells of the juxtaglomerular apparatus of the kidney. Renin release has also been associated with potassium loading or sodium depletion. Renin in the peripheral circulation acts to cleave a plasma globulin precursor (angiotensinogen that is produced by the liver) to angiotensin. By means of angiotensin-converting enzyme (ACE), angiotensin I is subsequently hydrolyzed to angiotensin II, which acts as a trophic hormone to stimulate the synthesis and secretion of aldosterone. Some of the angiotensin II undergoes further enzymatic modification by removal of the terminal aspartic acid to form angiotensin III. Angiotensin III appears to be nearly as active as angiotensin II in stimulating aldosterone secretion. Under normal circumstances the elevated levels of angiotensins (principally angiotensin II) exert negative feedback control to inhibit further renin release as well as the expanded extracellular fluid volume resulting from the electrolyte and water reabsorption by the kidney. Thus the adrenal cortex is fundamentally involved in the regulation of blood volume, water and electrolyte balance, and blood pressure, and disturbance of these mechanisms can lead to rapid life-threatening conditions (see Holt and Hanley [2009] for review).

Glucocorticoid hormones increase glucose production with a concomitant breakdown of proteins for purposes of gluconeogenesis and, in some tissues, promote a decreased utilization. Glucocorticoids also suppress inflammation in association with the attenuation of fibroplasia and immunological responses. The suppression of the immunological responses is largely related to the stabilization of lysosomal membranes of phagocytic cells, inhibition of a number of lymphoid cell functions, and lysis of lymphocytes. The increase in blood glucose is an important physiological response in adverse situations, but the most important physiological effect of the glucocorticoids in stressful circumstances is to quench the inflammatory response to prevent it developing to the point where it overwhelms the animal (the role of the adrenal cortex in the stress response and the physiological, pharmacological, and molecular actions of the glucocorticoids is reviewed in Buckingham, 2009 and Everds et al., 2013).

The principal control for the production of steroids by the zona fasciculata and zona reticularis is mediated by adrenocorticotropic hormone (adrenocorticotropin; ACTH), a polypeptide hormone produced by corticotrophs in the pituitary adenohypophysis. ACTH release is largely controlled by the hypothalamus through the secretion of corticotropin-releasing hormone (CRH). An increase in ACTH production normally results in an increase in circulating levels of glucocorticoids, although it can cause weak stimulation of aldosterone secretion as well. Negative feedback control normally occurs when the elevated blood levels of cortisol act on the hypothalamus, anterior pituitary, or both to cause a suppression of ACTH secretion. It is this negative feedback mechanism that can also be involved in the expression of adrenocortical toxicity in that if a compound inhibits a critical enzyme on the glucocorticoid synthesis pathway, the loss of glucocorticoid production results in less or no feedback inhibition of ACTH, and the persistent overstimulation of the adrenal cortex by ACTH can produce marked adrenal hypertrophy (see Harvey and Sutcliffe, 2010).

In contrast to the normal negative feedback mechanism, abnormally high corticosteroid levels in the plasma above physiological levels (due to exogenous administration of steroids or cortisol-producing adrenal lesions) will cause marked ACTH suppression. If the suppression is

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prolonged, secretory cells in the zonae fasciculata and reticularis will undergo atrophy, with a corresponding decrease in their future capability to synthesize and secrete corticosteroid hormones. However, when corticosteroid levels are subnormal, ACTH release from the pituitary gland is increased markedly due to the loss of feedback inhibition to enhance hormonal output from the adrenal cortex. In a normally functioning adrenal cortex, the increased ACTH will restore/stimulate glucocorticoid secretion.

3. MECHANISMS OF TOXICITY

As mentioned previously, the adrenal is the most common target organ for toxicity in the endocrine system and factors contributing to this vulnerability have been recognized (see Harvey [2010] and Hinson and Raven [2006]). First, there is the dependence of the adrenal cortex on the trophic support of hormones from the pituitary and hypothalamus, and also hormones from other endocrine tissues such as adrenomedullary neuropeptides where, for example, adrenomedullin has a role in aldosterone and cortisol secretion. Toxicity in these other sites therefore could ultimately influence the adrenal cortex. Additionally, the adrenal cortex has both anatomic and molecular characteristics that convey vulnerability to toxic insult, and the following factors predispose the adrenal cortex to toxic insult *in vivo*.

1. Functional dependence on the hypothalamus and pituitary and peripheral hormone-carrier molecules (e.g., CBG)
2. The large number of potential toxicological targets such as enzymes, receptors, and biochemical functional mediators (e.g., adrenomedullin)
   - The sequentially dependent steroidogenic steps in cortisol/corticosterone or aldosterone production and secretion are at the end of the pathway with highest probability of effect from upstream toxicity.
3. High vascularity and disproportionately large blood volume received per unit mass of adrenal tissue ensuring exposures to toxicants
   - However, the adrenal lacks a significant direct arterial blood supply to the deep cortex and the blood delivered to deep zones has already perfused the outer layers: the deep zones may therefore receive blood containing reduced oxygen and previously secreted steroids, potentially influencing CYP induction profiles.
4. The high content of unsaturated fatty acids in adrenocortical cell membranes that are susceptible to lipid peroxidation both directly and indirectly (see below)
5. Lipophilicity due to rich cholesterol and steroid content favoring deposition of lipophilic compounds
6. The high content of CYP enzymes present in the adrenal cortex that can produce:
   - reactive metabolites of toxicants that then mediate toxicity and
   - hydroxylation reactions that may generate free radicals which then damage adrenocortical cells and membrane (as above).

Classes of chemicals known to be toxic for the adrenal cortex include short chain (three or four carbon) aliphatic compounds, lipidosis inducers, and amphiphilic compounds. It would also appear that hormones, especially exogenous steroids, have a direct effect on the adrenal cortex. A variety of other compounds may also affect the cortex. The most potent aliphatic compounds are of three-carbon length, with electronegative groups at both ends. These compounds frequently produce necrosis, particularly in the zonae fasciculata and reticularis. Examples include acrylonitrile, 3-aminopropionitrile, 3-bromopropionitrile, 1-butanethiol, and 1,4-butanedithiol. By comparison, lipidosis inducers can cause the accumulations (often coalescing) of neutral fats, which may be of sufficient quantity to cause a loss of organellar function and cellular destruction. The zonae reticularis and fasciculata appear to be the principal targets of xenobiotic chemicals. Examples of the compounds causing lipidosis include aminoglutethimide, amphenone, anilines, and imidazole antimycotic drugs. Biologically active cationic amphiphilic compounds tend to produce a generalized phospholipidosis that involves primarily the zonae reticularis and fasciculata. They cause microscopic and subcellular phospholipid-rich inclusions. These compounds affect the functional integrity of lysosomes, which appear ultrastructurally to

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be enlarged and filled with membranous lamellae or myelin figures. Examples of compounds known to induce these types of effect include chloroquine, triparanol, and chlorphentermine.

Another class of compounds that affects the adrenal cortex is certain hormones, particularly natural and synthetic steroids. Some of these steroid hormones (corticosteroids) may cause functional inactivity and morphological atrophy during prolonged exogenous use (Figure 58.7). Other steroid hormones (natural and synthetic estrogens and androgens) have been reported to cause proliferative lesions in the adrenal cortex of laboratory animals.

The final class of compounds represents a miscellaneous group of chemicals that affect hydroxylation and other functions of mitochondrial and microsomal fractions (smooth endoplasmic reticulum). Examples of these compounds include *o,p'-DDD* and α1-(l,4-dioxido-3-methylquinoxalin-2-yl)-N-methylnitrone (DMNM). Additional compounds in this category cause their effects by means of cytochrome P450 metabolism and the activation of toxic metabolites. An example is the activation of carbon tetrachloride, resulting in lipid peroxidation and covalent binding to cellular macromolecules of the adrenal cortex. Harvey *et al.* (2007, 2009) review over 70 compounds and their targets in adrenocortical cells, including downregulation of the ACTH receptor or Steroid Acute Regulatory (StAR) protein, through to inhibition of steroidogenic enzymes. Of note is that compounds may have multiple targets; for example, aminoglutethimide downregulates the ACTH receptor, and inhibits CYP11A1 (cholesterol side chain cleavage) and CYP11B1 (CYP11β/18), which is the terminal enzyme in cortisol synthesis.

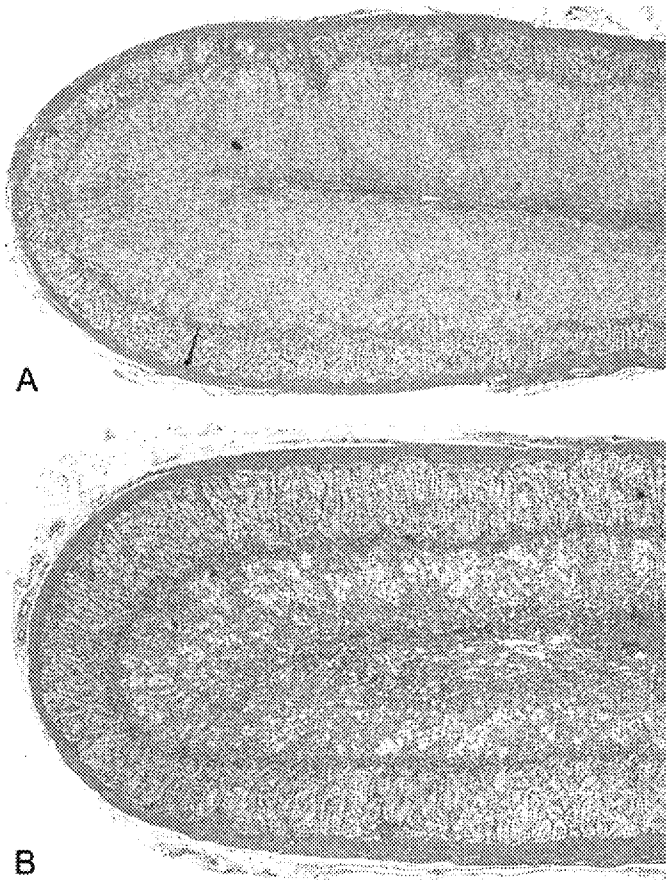

FIGURE 58.7 Adrenal of control dog (A) and adrenal of dog with cortical atrophy (B) following treatment with excessive topical corticosteroids. H&E stain.

Many of the chemicals that cause morphological changes in the adrenal glands can also affect adrenal cortical function (Table 58.1). Chemically

TABLE 58.1 Examples of Pharmacological Inhibition of Adrenal Steroid Biosynthesis, Secretion, or Function

Compound	Steroid or conversion site inhibited	Mechanism of action
Aminoglutethimide	Cholesterol to pregnenolone	Competitive inhibition of 20α-hydroxylase
o,p'-DDD	Cholesterol to pregnenolone; 11-deoxycortisol to cortisol	Partial 11β-hydroxylase inhibition
DMNM	Cholesterol to pregnenolone?	Unknown
Triparanol	Desmosterol (24-dehydrocholesterol) to cholesterol	Inhibited reduction of 24, 25 bond

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TABLE 58.1 Examples of Pharmacological Inhibition of Adrenal Steroid Biosynthesis, Secretion, or Function—cont'd

Compound	Steroid or conversion site inhibited	Mechanism of action
Cyanoketone	δ^5-3β-ol steroids to δ^4-3-oxo steroids	3β-Hydroxysteroid dehydrogenase Inhibition
Trilostane	Δ^5-3β-ol steroids to Δ^4-3-oxo steroids	3β-Hydroxysteroid dehydrogenase Inhibition
Su-9055	Cortisol; aldosterone	Inhibition of 17α-hydroxylase; interference of oxidation at C18
Su-8000	Cortisol; aldosterone	Inhibition of 17α-hydroxylase; interference of oxidation at C18
Metapyrone	11-Deoxycortisol to cortisol	Inhibition of 11β-hydroxylase; inhibition of other hydroxylation reactions depending on species
SKF 12185	11-Deoxycortisol to cortisol	Inhibition of 11β-hydroxylase
Carbon tetrachloride	Non-specific inhibition	Inhibition of cytochrome P450 portion of microsomal enzymes 17α- and 21-hydroxylases
Cadmium	Non-specific inhibition	Inhibition of NADPH-cytochrome P450 reductase portion of 21-hydroxylase; other microsomal as well as mitochondrial hydroxylases may also be affected
Amphenone	Non-specific inhibition	Inhibition of 20α-, 11β-, 17α-, and 21-hydroxylases?
Cortexolone (11-deoxycortisol)	Competitive binding to glucocorticoid receptors	Diminished translocation of glucocorticoid—receptor complex to nucleus of target cell
ROl-8307/ heparinoids	Aldosterone	Inhibition of 18-oxidation
Spironolactone	Aldosterone	Competitive inhibition of peripheral receptor sites, resulting in sodium diuresis; possible direct effects on synthesis and secretion
Captopril	Aldosterone; inactivation of renin—angiotensin system	Inhibition of angiotensin-converting enzyme
Triaryl phosphate	Cholesterol ester to cholesterol	Neutral cholesterol ester hydrolase inhibitor
PD 132301-2	Esterification of cholesterol	Inhibition of acyl-CoA; cholesterol acyltransferase
2,3,7,8-Tetrachlorodibenzo-p-dioxin	Cholesterol side chain cleavage	Cytochrome P450s

Table modified from Handbook of Toxicologic Pathology, *2nd Ed. W. M. Haschek, C. G. Rousseaux, and M. A. Wallig, eds. (2002) Academic Press, Vol. 2, Table I, p. 688.*

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induced changes in adrenal gland function result either from blockage of the effects of the adrenocorticoids at peripheral sites or from inhibition of synthesis and/or secretion (steroidogenesis). In the former case, many antisteroidal compounds (antagonists) act by competing with or binding to steroidal receptor sites, thereby either reducing the available receptor sites or altering their functional activity. Cortexolone (11α-deoxycortisol, an anti-glucocorticoid) and spironolactone (an anti-mineralocorticoid) are two examples of peripherally acting hormone antagonists. Pharmacologically, many of these antagonists are beneficial for either diagnostic or therapeutic purposes.

Most chemicals affecting adrenal function appear to do so by altering steroidogenesis. A study of the effects of these chemicals on the histology and ultrastructure of adrenal cortical cells often can give insight into possible selection sites of inhibition of steroidogenesis. For example, chemicals causing increased lipid droplets may be involved in inhibiting the utilization of steroid precursors, including the conversion of cholesterol to pregnenolone. Chemicals that affect the fine structure of mitochondria and smooth endoplasmic reticulum would be expected to impair the activity of 11α-hydroxylase and 17α- and 2l-hydroxylases, respectively. The previously mentioned examples of impaired steroidogenesis would result in lesions found primarily in the zonae reticularis and fasciculata. However, atrophy of the zona glomerulosa may reflect specific inhibition of aldosterone synthesis or secretion, either directly (inhibition of 18Q1-hydroxylation) or indirectly (inactivation of the renin–angiotensin system II), by chemicals such as spironolactone and captopril, respectively. The inhibition of steroidogenesis in some situations is non-specific, as many hydroxylation reactions are affected, such as with carbon tetrachloride and cadmium intoxications.

4. RESPONSE TO INJURY

4.1. Disorders of Hyperfunction and Hypofunction

A number of spontaneous pathological conditions can give rise to changes in the functional status of the adrenal cortex. For example, ACTH-secreting pituitary tumors or glucocorticoid hormone-producing adrenal cortical tumors have been associated with hypercortisolism, especially in dogs. Primary hypoadrenocorticism (Addison's disease) has several causes, including idiopathic (most likely immune-mediated) bilateral cortical adrenalitis and eventual atrophy, and inflammatory disease processes, which destroy all three zones of cortical parenchyma of both adrenals. Secondary hypoadrenocorticism with atrophy of only the zonae fasciculata and reticularis is caused by destructive lesions or neoplasms in the pituitary, resulting in a loss of ACTH production.

Chemicals can also produce functional alterations of the adrenal cortex. Prolonged use of exogenous glucocorticoids can mimic a syndrome of excess adrenal cortical function. Abrupt cessation of steroid use may cause a patient to develop secondary adrenal cortical insufficiency because of the prolonged suppression of ACTH production and the trophic atrophy of cells in the adrenal cortex. More significant from a toxicologic point of view are the degenerative effects of chemicals on the adrenal cortex that result in primary adrenal cortical hypofunction.

Of the experimental laboratory animal species, disturbances in adrenal cortical function have been best characterized in the dog. Because of this, and the fact that canine hyper- and hypoadrenocorticism are similar to the corresponding clinical syndromes in humans, the remaining discussion in this section will be principally confined to the dog.

Exogenous glucocorticoid hormone therapy (daily at high doses) often mimics naturally occurring cases of hypercortisolism (e.g., Cushing's syndrome). Clinical observations include polyuria, polydipsia, an enlarged pendulous abdomen, muscular wasting, alopecia, thinning of the skin with cutaneous pigmentation and mineralization, and hepatomegaly. Osteoporosis is an important finding in human patients with cortisol excess, but is not a common finding in the dog. Significant laboratory findings include an increase in alkaline phosphatase (steroid-induced isoenzyme), an eosinopenia with marked lymphopenia, and leukocytosis due to the increased

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formation of neutrophils. Dogs with hypercortisolism infrequently develop significant alterations in serum concentrations of sodium, potassium, or chloride, in contrast to those electrolyte imbalances seen in humans with Cushing's syndrome.

Urinary and plasma levels of 17-hydroxycorticosteroids or metabolites often are increased modestly in resting non-stimulated patients. The use of stimulation and suppression tests, including evaluation of the cortisol response to exogenous ACTH and dexamethasone (low and high dose) administration, is helpful in establishing a diagnosis and in determining whether the primary lesion is in the pituitary/hypothalamus (pituitary dependent) region or in the adrenal cortex (adrenal dependent). During the prolonged use of exogenous glucocorticoids, ACTH administration results in an inadequate release and blunted increase in blood levels of cortisol. In spontaneous cases of functional adrenal cortical hyperplasia that remains partially under the control of pituitary ACTH, a marked response to the exogenous ACTH challenge occurs, resulting in an exaggerated increase in blood cortisol levels.

In hypoadrenocorticism, caused by natural disease or experimentally by the administration of xenobiotic chemicals, the clinical signs often are not pathognomonic. Depression, anorexia, weakness, non-specific gastrointestinal disorders, hypotension, hemoconcentration, and elevated blood urea nitrogen (BUN) may be found. Additional findings include an abnormal electrocardiogram with spiked T waves and flattening of the P wave. These electrocardiographic alterations appear to be due to the prominent increase in serum potassium in dogs with hypoadrenocorticism, and subnormal secretion of aldosterone.

Plasma and urinary 17-hydroxycorticosteroids often are at low levels in the resting state. Stimulation tests, including the response to exogenous ACTH challenge, usually reveal a subnormal ("blunted") increase in plasma cortisol due to the reduced number of cortical cells that can respond to the challenge.

4.2. Effects during Embryogenesis

It is well documented that synthetic and naturally occurring corticosteroids are potent teratogens in laboratory animals. The principal induced defect is cleft lip or palate; however, there is a paucity of information regarding the direct effect of chemicals on the development of the adrenal cortex. Adrenal aplasia occurred in a subset of white Danish rabbits when thalidomide was given to their dams.

Indirect effects of chemicals on the development and function of the fetal adrenal cortex are illustrated by the syndrome of prolonged gestation in sheep due to the consumption of the plants *Veratrum californicum* or *Salsola tuberculata.* Both plants cause a syndrome of prolonged gestation. *V. californicum* may also produce giant fetuses that have cyclopian deformities. Common to both plant toxicities is the presence of hypoplastic adrenal cortices in the affected fetuses.

In *V. californicum* intoxication, the zonae fasciculata and reticularis may both be poorly differentiated while the width of the entire cortex may be less than half the total diameter of the adrenal gland. The pituitary, although developed, lacks the normal neural and vascular connections with the hypothalamus due to extensive central nervous system (CNS) malformations. The hypoplastic fetal adrenal cortices synthesize little cortisol and thus fail to induce the 17α-hydroxylase in the placenta that is necessary to synthesize increased estrogens near term. Subnormal estrogen levels and maintenance of midgestational levels of progesterone in the dam at term result in prolongation of the gestation period.

In S. *tuberculata* intoxication, the pituitary is small with a lack of normal granulation of the trophic hormone-secreting cells of the adenohypophysis. In both plant toxicities there is a loss of functional activity of the hypothalmic–pituitary–adrenal cortical axis that is necessary for the normal induction of parturition in the ewe. It has been suggested that the plant S. *tuberculata* contains chemicals that inhibit fetal hypothalamic-releasing hormones.

4.3. Morphologic Alterations

Macroscopic lesions of chemically affected adrenal glands are characterized by either enlargement or reduction in size that often is

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bilateral. Initially, cortical hypertrophy or swelling due to impaired steroidogenesis or hyperplasia due to long-term stimulation often is seen when the adrenal is increased in size. Similar gross findings may be the result of medullary hyperplasia or pheochromocytoma. In contrast, small adrenal glands often are indicative of degenerative changes, resulting in atrophy. Midsagittal longitudinal sections of the glands will reveal a disproportionately wider cortex relative to the medulla, or vice versa, resulting in an abnormal cortical : medullary ratio.

Non-Neoplastic Lesions

Histologically, non-neoplastic lesions of the adrenal cortex induced by chemical agents are characterized by changes ranging from acute progressive degenerative to reparative in nature. These may be exacerbations of normal spontaneous findings or directly compound-induced lesions. There is delayed involution of the x-zone in mice after treatment with luteinizing hormone, and in B6C3F1 mice after 13 weeks treatment with acetonitrile and the antifungal, ketoconazole (Figure 58.2). There can be a treatment-related increase in the pigment lipofuscin (formed from lipid products resulting from altered fat metabolism) in the zona reticularis in rats, which increases normally with age, but can be increased after dosing with estrogens, antithyroid agents, the neurotoxin MPTP, and adrenocorticosteroids. It can also be increased after feeding diets high in saturated fats and with Vitamin E deficiency. This ceroid pigment can be demonstrated with fuschinophilic stains (Ziehl-Neelson and Periodic acid-Schiff) which can help to differentiate it from from hemosiderin (a brown blood-breakdown product) which can, in turn, be demonstrated using Perl's stain (for iron) (Figure 58.8).

Early degenerative lesions characterized by enlarged cortical cells filled with cytoplasmic vacuoles (often lipid) may result in a diffuse hypertrophy of the cortex. Lipid vacuolation is commonly seen in the zona fasciculata in normal unstressed animals, with numerous lipid droplets containing esterified cholesterol ~0.5 μm diameter. This increases with age, and in response to reproductive and physiological changes (Figure 58.9).

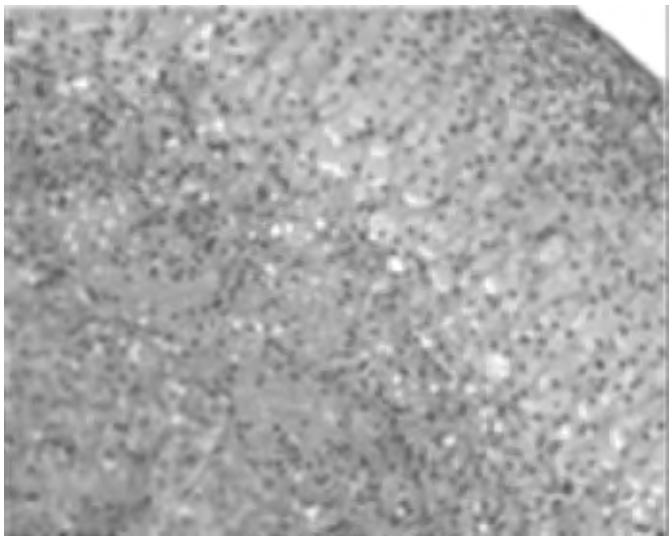

FIGURE 58.8 Lipofuscin pigment at the cortico-medullary junction (rat). H&E stain.

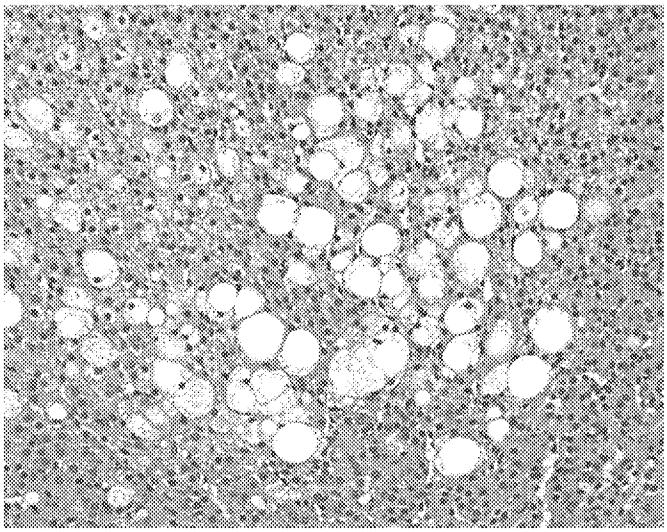

FIGURE 58.9 Lipid droplets in the zona fasciculata of a dog. H&E stain.

However, a treatment-related change is detected by the presence of excessive coalescing lipid droplets. A lesion of this type has been observed in rats treated with 100 mg/kg/day of the antibacterial compound DMNM for 35 days (Figure 58.10). This vacuolar type of degeneration is a reflection of impaired steroidogenesis, resulting in excess storage of unmetabolized steroid precursors. More destructive lesions may be observed in the form of hemorrhage and/or necrosis

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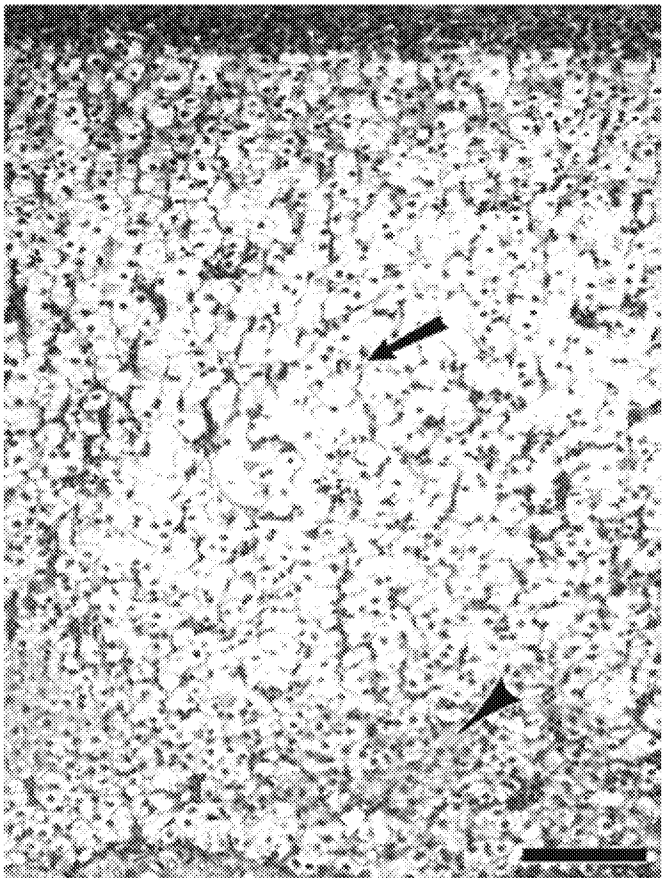

FIGURE 58.10 Vacuolar (arrow) and granular (arrowhead) degeneration of the adrenal cortex of a rat treated with 100 mg/kg/day of DMNM for 31 days. Bar = 100 μm. *Figure reproduced from Yarrington* et al. *(1985) Degeneration of the rat and canine adrenal cortex caused by α-(1,4-dioxido-3-methylquinoxalin-2-yl)-*N*-methylnitrone (DMNM),* Fundam. Appl. Toxicol. *5, 370–381, with permission.*

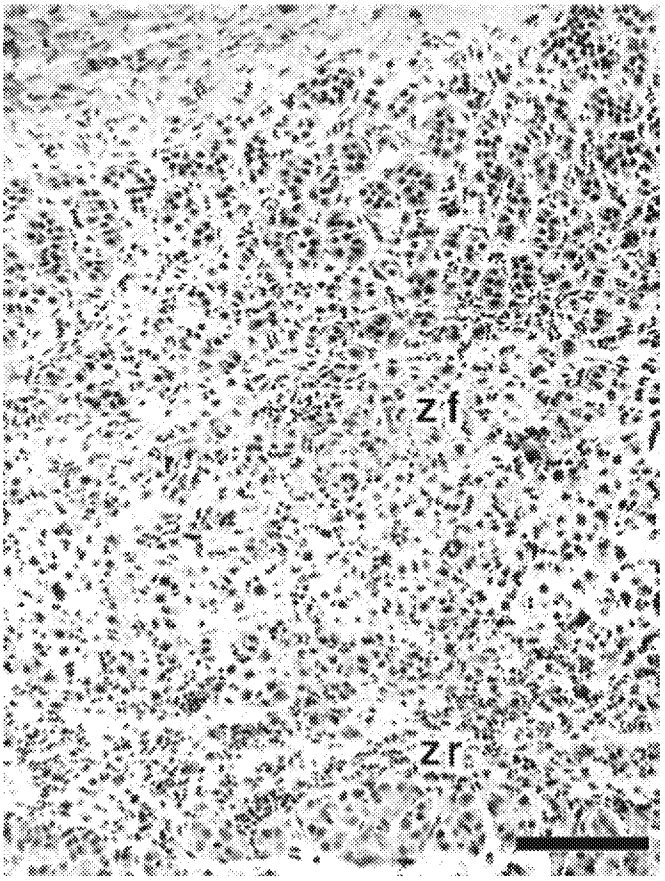

FIGURE 58.11 Necrosis of the zona fasciculata (zf) and the zona reticularis (zr) in the adrenal cortex of a monkey given 75 mg/kg/day of a hematinic compound (MDL 80,478). Bar = 1 μm. *Figure reproduced from Yarrington* et al. *(1983) Comparative toxicity of the hematinic MDL 80,478: Effects on the liver and adrenal cortex of the dog, rat and monkey,* Fundam. Appl. Toxicol. *3, 86–94, with permission.*

(Figure 58.11), often in association with an inflammatory response. At the same time, one area of the cortex (e.g., the zona glomerulosa) may undergo hypertrophy while another area has degenerative lesions (e.g., vacuolar degeneration) of the zona fasciculata (Figure 58.12). The zona glomerulosa remains functional and there are no clinically significant signs of hypoadrenocorticism, and chronic regenerative changes may develop subsequently. Usually, the adrenal cortex will be shrunken or atrophic with fibrosis and areas of multinodular hyperplasia. In the case of chronic DMNM toxicity in rats, fibrosis and occasionally hemosiderin pigment may be found in the zona reticularis. Cortical nodules of presumably functional remnants of the zona fasciculata and discernible areas of the zona glomerulosa were found in rats that survived treatment for 90 days (Figure 58.13).

While many adrenocorticolytic agents affect the adrenal cortex initially at the zona reticularis and inner zona fasciculata, some chemicals such as DMNM can cause a progressive degeneration of the adrenal cortex. Occasionally, the effect of a chemical is limited to a specific zone of the adrenal cortex and may be species specific (Figure 58.14). For example, the compound PD 132301-2, when administered to monkeys, induces a narrow

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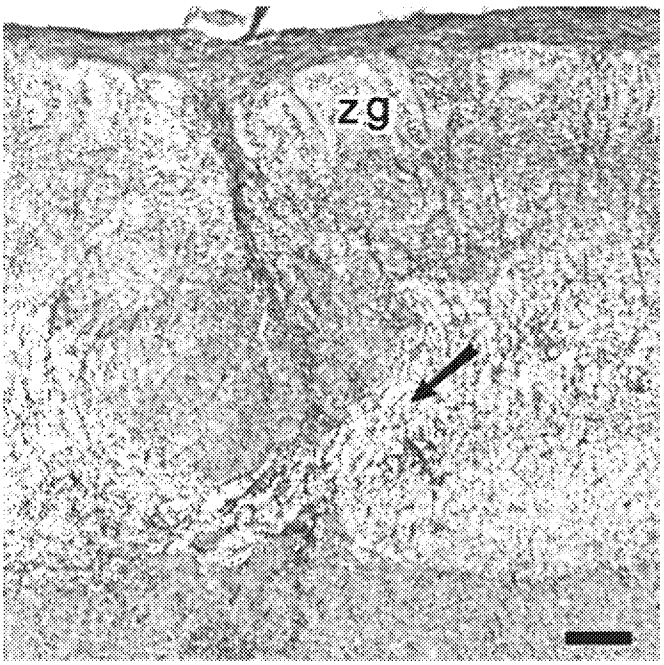

FIGURE 58.12 Hypertrophy of the zona glomerulosa (zg) and vacuolar degeneration of the remainder of the cortex (arrow) in a dog given 75 mg/kg/day of MDL 80,478. Bar = 100 μm. *Figure reproduced from Yarrington* et al. *(1983) Comparative toxicity of the hematinic MDL 80,478: Effects on the liver and adrenal cortex of the dog, rat and monkey,* Fundam. Appl. Toxicol. *3, 86–94, with permission.*

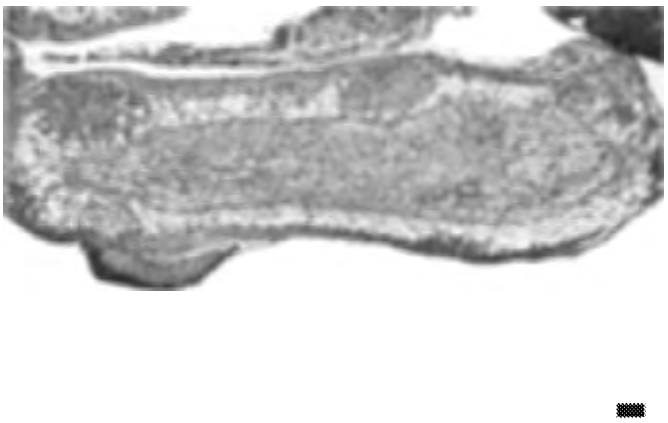

FIGURE 58.13 Atrophy, vacuolar degeneration, and nodular hyperplasia (arrows) of the adrenal cortex from a rat treated with 50 mg/kg/day of DMNM for 90 days. Bar = 100 μm. *Figure reproduced from Yarrington* et al. *(1985) Degeneration of the rat and canine adrenal cortex caused by α-(1,4-dioxido-3-methylquinoxalin-2-yl)-*N*-methylnitrone (DMNM),* Fundam. Appl. Toxicol. *5, 370–381, with permission.*

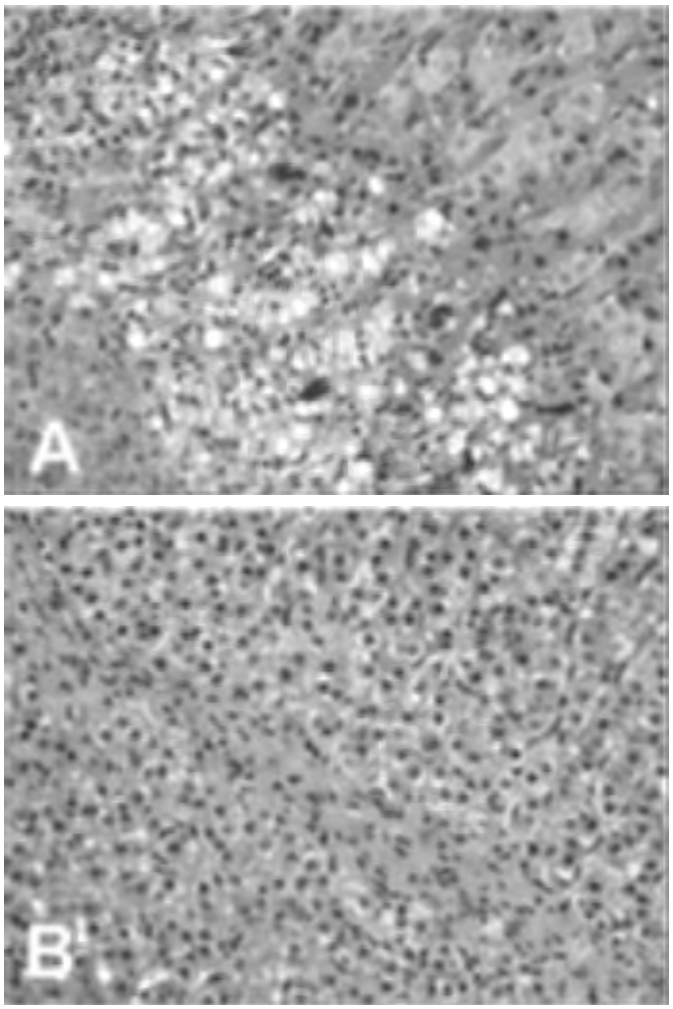

FIGURE 58.14 (A) Adrenocortical cell necrosis and vacuolation in the zona fasciculata of a cynomolgus monkey (treated with PD-132301-2). (B) Adrenocortical fibrosis in a cynomolgus monkey following a 4-week treatment-free period. H&E stain.

band of degeneration or necrosis of cortical cells in the mid to outer zona fasciculata. In contrast, all three cortical zones are affected in the adrenal glands of dogs treated with this compound. Xenobiotic chemicals that cause degeneration of the adrenal cortex are summarized in Table 58.2, together with the predilection site of their effects and the most significant lesions. Ultrastructural alterations of adrenal cortical cells associated with chemical injury are quite diverse in nature (Table 58.2). The zonae reticularis and fasciculata typically are most severely affected, although eventually the lesions involve the zona glomerulosa. These alterations may be classified as

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follows: endothelial damage (e.g., acrylonitrile), mitochondrial damage (e.g., DMNM, *o,p'-DDD*, amphenone), endoplasmic reticulum changes (e.g., triparanol), lipid aggregation (e.g., aniline), lysosomal phospholipid aggregation (e.g., chlorophentermine), and possible secondary effects due to embolization by medullary cells (e.g., acrylonitrile).

Mitochondrial damage with vacuolization (Figure 58.15) and accompanying changes in the endoplasmic reticulum and autophagocytic responses are among the most common ultrastructural changes observed following chemical injury in the adrenal cortex. Because mitochondria and smooth endoplasmic reticulum form an intimate network in cortical cells and important hydroxylases and dehydrogenase enzymes are found in these organelles, it is not surprising that many agents altering the ultrastructural morphology inhibit steroidogenesis. Similarly, increased lipid droplets (Figure 58.16) and lysosomal phospholipidosis (Figure 58.17) are compatible with altered steroid biosynthesis as a result of chemical inhibition of steroid precursors (e.g., cholesterol). The increased accumulation of lipid and severe mitochondrial vacuolization correspond to light microscopic findings of marked cytoplasmic vacuolar and granular degeneration. More severe ultrastructural injury may result and some parenchymal cells of the cortex have an electron-dense cytoplasm, chromatolysis, and disruption of the plasma membranes (Figure 58.18).Frequently, macrophages containing cholesterol clefts, numerous lipid droplets, and membranous debris can be observed among the necrotic cells.

Proliferative Lesions

Spontaneous hyperplasia of the adrenal cortex is common in the rat, rabbit, golden hamster, mouse, dog, cat, horse, and baboon. Spontaneous proliferative lesions may be found in all zones of the adrenal cortex, but are present most frequently in the zona fasciculata of adult rats. These proliferative foci or larger, capsule-distorting nodules are recorded when there is no change in the architectural arrangement of cells, and no obvious compression of adjacent cells, but the cellular aggregates are cytologically different from those cells surrounding them in terms of size and tinctorial properties. Large eosinophilic nodules in the rat often show vacuolated/cystic degeneration and hemorrhage which can replace many of the large eosinophilic adrenocortical cells (Figure 58.19). Cortical adenomas and, to a lesser extent, cortical carcinomas have been reported in moderately high incidence in certain strains of rat (e.g., Osborne Mendel, *WAG/Rij*, BUF, and BN/Bi strains). The incidence often increases markedly in rats over 18 months of age. A high incidence of cortical adenomas and fewer carcinomas have also been reported in the laboratory hamster (e.g., BIO 4.24 and BIO 45.5 strains). Subcapsular cell proliferation and adenoma are common in laboratory mice; however, adrenal cortical neoplasms are rare but may be induced by gonadectomy. Naturally occurring adrenal cortical tumors are found infrequently in domestic animals, except adult dogs and castrated male goats.

Less frequently reported are chemically induced proliferative lesions of the adrenal cortex. Unlike the diffuse hyperplasia and hypertrophy associated with the adrenal cortical response to excess ACTH stimulation, chemically induced hyperplasia usually is nodular in type and often multiple in distribution. Each focus or larger nodule is an oval-to-spherical lesion of variable size consisting of enlarged normal or vacuolated cells.

A summary of chemicals causing adrenocortical neoplasia is present in Table 58.3. Most of the reported tumors tend to be benign (adenoma), although an occasional one may be malignant (carcinoma). The zonae reticularis and fasciculata are more prone to develop tumors, whereas the zona glomerulosa is spared unless invaded by an expanding tumor in the adjacent zones of the cortex. Tumorigenic agents of the adrenal cortex have a diverse chemical nature and use (Table 58.3). Several of the compounds have a steroid nucleus and are natural or synthetic estrogens or androgens.

An adrenal cortical adenoma has characteristics similar to nodular hyperplasia, with the exception that adjacent parenchymal cells are compressed and often atrophic due to pressure. Cortical adenomas show loss of the cellular radial architecture, but are well differentiated and circumscibed and may be partially

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TABLE 58.2 Examples of Chemically-Induced Microscopic and Ultrastructural Changes of the Adrenal Cortex

Compound	Initial predilection site	Histology	Ultrastructure
Nafenopin	Zona fasciculata	Hypertrophy	SER[a] and peroxisome proliferation
Acrylonitrile	Zona reticularis	Hemorrhage	Damage to vascular endothelium; embolization of medullary cells and cell fragments of capillaries
Aminoglutethimide	All zones; more marked in outer zona fasciculata	Vacuolar degeneration; increased lipid	Mitochondrial hypertrophy and cavitation
o,p′-DDD	Zona reticularis and fasciculata	Vacuolar degeneration; cytotoxic cellular atrophy	Mitochondrial vacuolization; SER dilation
α-(1,4-Dioxido-3-methylquinoxalin-2-yl)-N-methylnitrone	Zona reticularis and fasciculata	Granular and vacuolar degeneration; cytotoxic cellular atrophy	Mitochondrial vacuolization; SER dilation
Triparanol	All zones; most marked in zona fasciculata	Increased eosinophilia and inclusions	Decreased lipid droplets; mitochondrial alterations; SER hypertrophy; lysosomal formation
Cysteamine (1-mercaptoethylamine)	Zona reticularis and fasciculata	Hemorrhage and necrosis	Retrograde emboli of medullary cells
Amphenone	Zona reticularis and fasciculata	Fatty degeneration	Mitochondrial alterations
7,12-Dimethylbenzanthracene	Zona reticularis and fasciculata	Necrosis; hemorrhage; calcification	Mitochondrial alterations, including variation in size
Corticosteroids, (e.g., prednisolone, dexamethasone)	Zona reticularis and fasciculata	Atrophy	Increased lipid droplets surrounded by membranous "whorls"; increased myelin figures and lysosomes
Propylthiouracil	Zona reticularis	Ceroid degeneration	Lipid and mitochondrial degeneration
Carbon tetrachloride	Zona reticularis and fasciculata	Necrosis	Swelling of SER

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TABLE 58.2 Examples of Chemically-Induced Microscopic and Ultrastructural Changes of the Adrenal Cortex—cont'd

Compound	Initial predilection site	Histology	Ultrastructure
Tamoxifen	Zona reticularis and fasciculata	Degeneration and necrosis; lipid droplets	Necrosis; lipid droplets in macrophages; few lysosomal inclusions
Spironolactone	Zona glomerulosa	Hypertrophy and inclusions	Lipid droplets surrounded by whorls of SER ("spironolactone bodies"); mitochondrial alterations
Hexadimethrine bromide (polybene)	Zona glomerulosa	Necrosis and infarction	Protein-containing vacuoles and (polybrene) hyalin bodies; microthrombi
RO1-8307, a sulfated mucopolysaccharide	Zona glomerulosa	Condensation	
Captopril	Zona glomerulosa	Atrophy	Decreased mitochondria and SER
1,1′-Thio-diethylidene-ferrocene (MDL 80, 478)	All zones	Granular and vacuolar degeneration; hyperplastic zona glomerulosa	Mitochondrial vacuolation; increased lipid droplets
Aniline	All zones	Hypertrophic cortical cells laden with lipid droplets	Increased lipid droplets; hypertrophic SER; mitochondrial degeneration
Chlorophentermine	All zones	Nothing remarkable	Increased lysosomal alterations in the form of lamellated cytoplasmic inclusions
Triaryl phosphate	All zones	Cytoplasmic lipid droplet	Increased number/size cytoplasmic droplets
PD 132301-2	Zona fasciculata	Coarse vacuolation	SER aggregation; changes of autophagosomes

[a] *Smooth endoplasmic reticulum.*

Table modified from Handbook of Toxicologic Pathology, *2nd Ed. W. M. Haschek, C. G. Rousseaux, and M. A. Wallig, eds. (2002) Academic Press, Vol. 2, Table II, pp. 693–694.*

encapsulated by a thin band of fibrous connective tissue (Figure 58.20).

Adrenal cortical carcinomas are composed of large polyhedral or pleomorphic cells with an eosinophilic or vacuolated cytoplasm. Tumor cells have prominent nucleoli and variable numbers of mitotic figures, and form different histologic patterns, includ-

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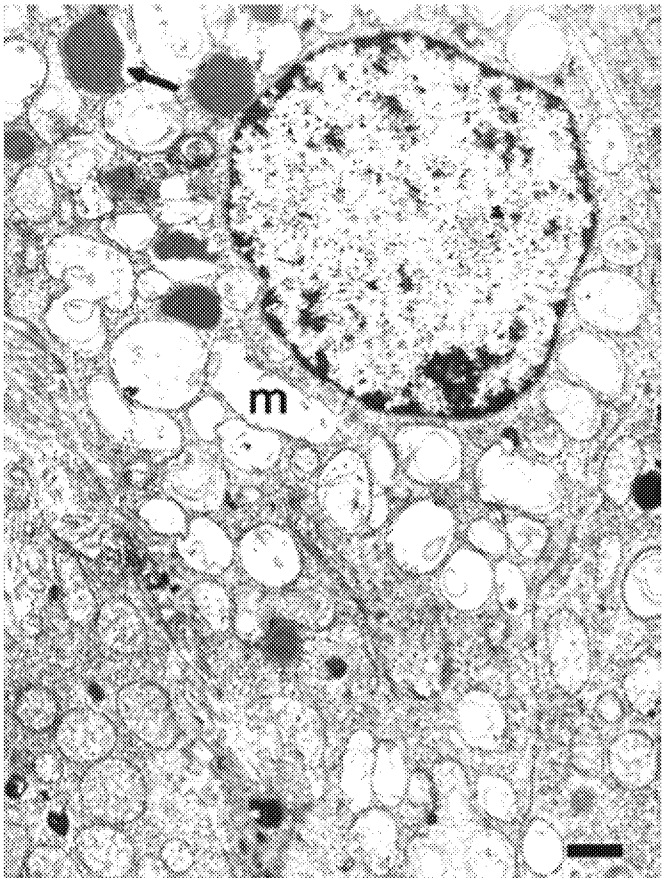

FIGURE 58.15 Cell of the zona fasciculata of the adrenal cortex of a rat treated with 100 mg/kg/day of DMNM for 21 days. Note vacuolated mitochondria (m) and some lipid droplets undergoing lipolysis (arrow). Bar = 1 μm. *Figure reproduced from Yarrington* et al. *(1985) Degeneration of the rat and canine adrenal cortex caused by α-(1,4-dioxido-3-methylquinoxalin-2-yl)-N-methylnitrone (DMNM),* Fundam. Appl. Toxicol. *5, 370–381, with permission.*

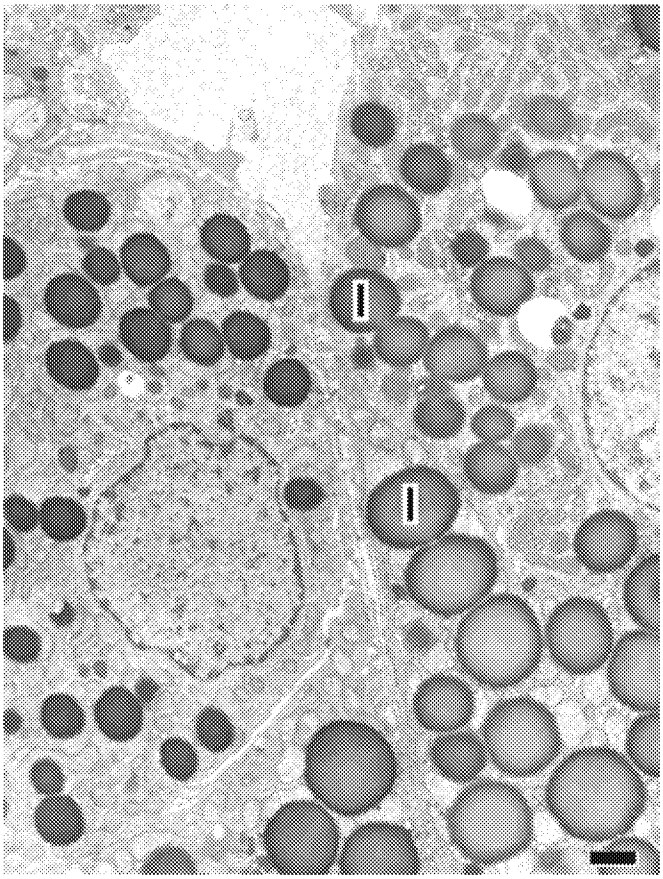

FIGURE 58.16 Numerous lipid droplets (l) in the cytoplasm of cells of the zona fasciculata and glomerulosa from a rat treated with 500 mg/kg/day of MDL 83,478. Bar = 1 μm. *Figure reproduced from Yarrington* et al. *(1983) Comparative toxicity of the hematinic MDL 80,478: Effects on the liver and adrenal cortex of the dog, rat and monkey,* Fundam. Appl. Toxicol. *3, 86–94, with permission.*

ing sheets, lobules, and cords. The invasive nature of the malignant cells is also apparent by penetration through the capsule, obliteration of the normal architecture of the affected gland, and metastasis to distant sites. Blood-filled spaces and localized areas of necrosis are common in cortical carcinomas (Figure 58.21).

Adrenal tumors are being recognized with greater frequency in ferrets as they are being kept as household pets and living to a more advanced age. The adrenal enlargements are either bilateral (approximately 45%) due to diffuse or nodular hyperplasia, or unilateral (approximately 55%) due to adrenal cortical carcinoma or adenoma. Clinical signs in ferrets with adrenal cortical tumors include vulvar enlargement, bilaterally symmetrical alopecia (especially on the ventral abdomen and medial aspects of the rear legs), polyuria, polydipsia, and the presence of a palpable mass at the cranial pole of the kidney (left side greater frequency than right). Adrenal tumors develop in adult ferrets (mean age 5 years), with females affected more frequently than males (sex ratio of 2 : 1 or greater). The history frequently indicates that the ferrets were gonadectomized at an early age (5 or 6 weeks). Other functional disturbances, including anemia, thrombocytopenia,

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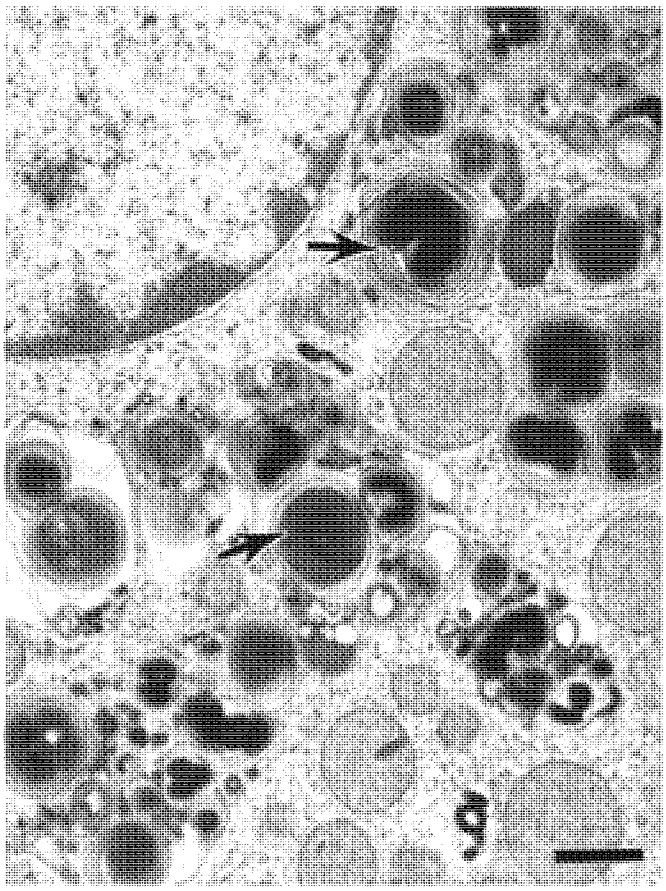

FIGURE 58.17 Large myelinoid bodies (arrows) compatible with lysosomal phospholipidosis in an adrenal cortical cell of a rat treated with a cationic amphiphilic compound. Bar = 1 μm. *Figure reproduced from* Handbook of Toxicologic Pathology, *2nd Ed. W. M. Haschek, C. G. Rousseaux and M. A. Wallig, eds. (2002) Academic Press, Vol. 2, Fig. 13, p. 696, with permission.*

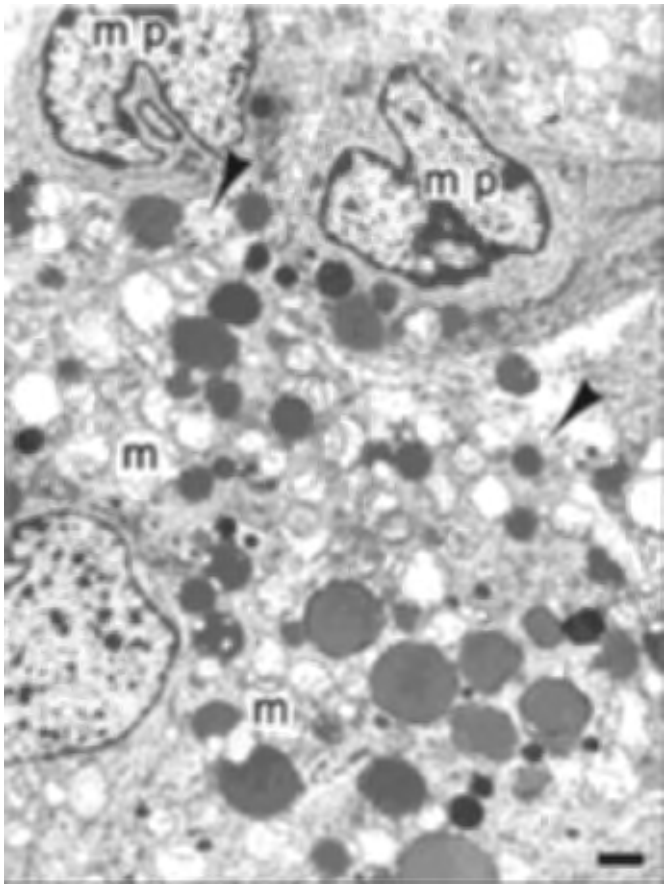

FIGURE 58.18 Necrotic cell of the zona reticularis in a dog treated with 22.5 mg/kg/bid of DMNM for 21 days. Many degenerative mitochondria (m) and numerous lipid droplets are present in cytoplasm with disruption of the plasma membrane (arrowheads). Two macrophages (mp) are present. Bar = 1 μm. *Figure reproduced from Yarrington* et al. *(1985) Degeneration of the rat and canine adrenal cortex caused by α-(1,4-dioxido-3-methylquinoxalin-2-yl)-*N*-methylnitrone (DMNM),* Fundam. Appl. Toxicol. *5, 370–381, with permission.*

pyometra, and endometrial hyperplasia, are consistent with an overproduction of estrogenic steroids by the adrenal tumors. Some of the functional disturbances resemble intact females with persistent or prolonged estrus, as ferrets are seasonally polyestrous and must undergo induced ovulation. About one-third of ferrets with adrenal cortical tumors also have neoplasms derived from the insulin-producing β cells of the pancreatic islets, which can be associated with hypoglycemia and elevated levels of serum insulin, resulting in seizures, episodic lethargy, ptyalism, ataxia, and hind-leg weakness.

The most consistent endocrinologic change in ferrets with adrenal tumors is an elevation in plasma levels of estradiol-17β. It is presumed that the estradiol-17β is produced by the tumor directly, but an alternative possibility would be that the adrenal tumors secrete androgenic steroids that are aromatized in the skin and possibly elsewhere to estrogenic steroids. There is no increase in circulating estradiol-17β levels in response to exogenous ACTH, but plasma levels decrease following adrenalectomy. Plasma cortisol and corticosterone levels in ferrets with adrenal tumors either are in the

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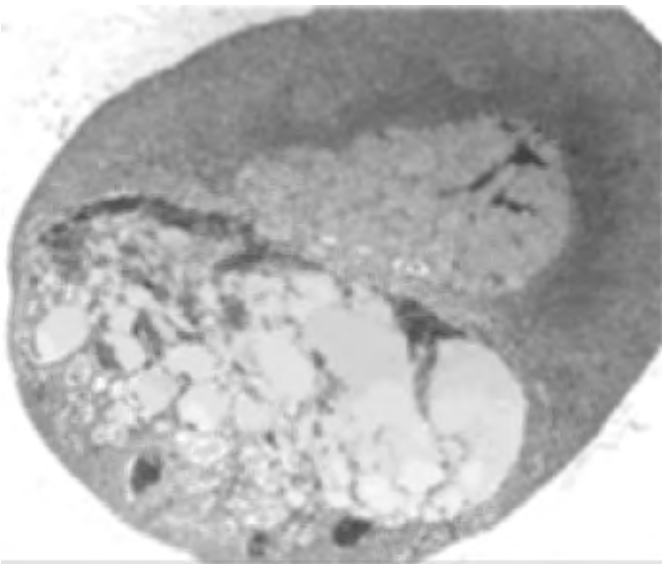

FIGURE 58.19 Eosinophilic nodule in a rat, with cystic degeneration, hemangiectasis, hemorrhage and large eosinophilic adrenocortical cells. H&E stain.

range of normal or below, and do not show an exaggerated increase in response to exogenous ACTH. There is not a decrease in plasma cortisol following unilateral adrenalectomy, and the contralateral adrenal cortex is not atrophic as would be expected if the adrenal tumor was secreting excess cortisol. However, urinary cortisol : creatinine has been found to be elevated in ferrets with adrenal cortical tumors (5.98×10^{-6}) compared to controls (0.34×10^{-6}). Although sensitive for detecting elevated urinary cortisol excretion, this test is not specific for the syndrome of cortisol excess. Clinical signs in ferrets with adrenal cortical tumors can be effectively reversed by adrenalectomy (especially of the left side if there is no macroscopic enlargement) but not by chemotherapy with *o,p′-DDD*. Complete regrowth of hair usually occurs by 2–3 months post-adrenalectomy.

TABLE 58.3 Examples of Compounds Producing Tumors of the Adrenal Cortex[a]

Compound	Chemical properties	Type of lesion	Animal affected
Aflatoxin/stilbesterol	Fungal metabolite/ steroid	Adenoma and hyperplasia	Rat
Cholesterol	Steroid	Microadenomas	Rabbit
Dibromochloropropane	Soil fumigant	Adenoma	Rat
7,12-Dimethylbenzanthracene	Organic solvent	Adenoma; eosinophilic and basophilic foci	Rat
Estrone, estriol, diethylstilbesterol	Natural and synthetic estrogens	Adenocarcinoma	Rat
Formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide	Antimicrobial	Adenoma	Hamster
Parathion	Insecticide	Adenoma; carcinoma	Rat
Tetrachlorovinphos	Insecticide	Adenoma	Rat
Testosterone	Hormone	Adenoma	Hamster
Urethane	Organic solvent; intermediate in organic synthesis	Adenoma	Rat
Linoleic acid	Unsaturated fatty acid	Carcinoma	Rat

[a] *Predilection sites are the zona reticularis and fasciculata.*
Table modified from Handbook of Toxicologic Pathology, *2nd Ed. W. M. Haschek, C. G. Rousseaux, and M. A. Wallig, eds. (2002) Academic Press, Vol. 2, Table III, p. 697.*

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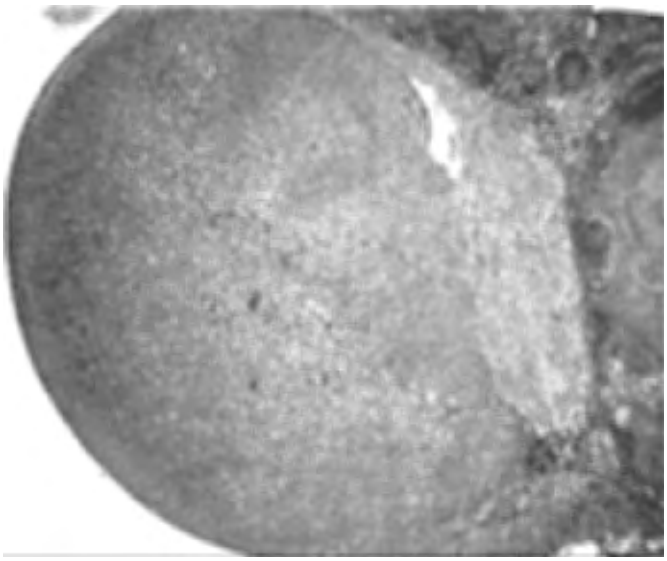

FIGURE 58.20 Adrenal cortical adenoma in a rat showing compression of adjacent parenchyma and loss of radial architecture, but well differentiated and circumscribed.

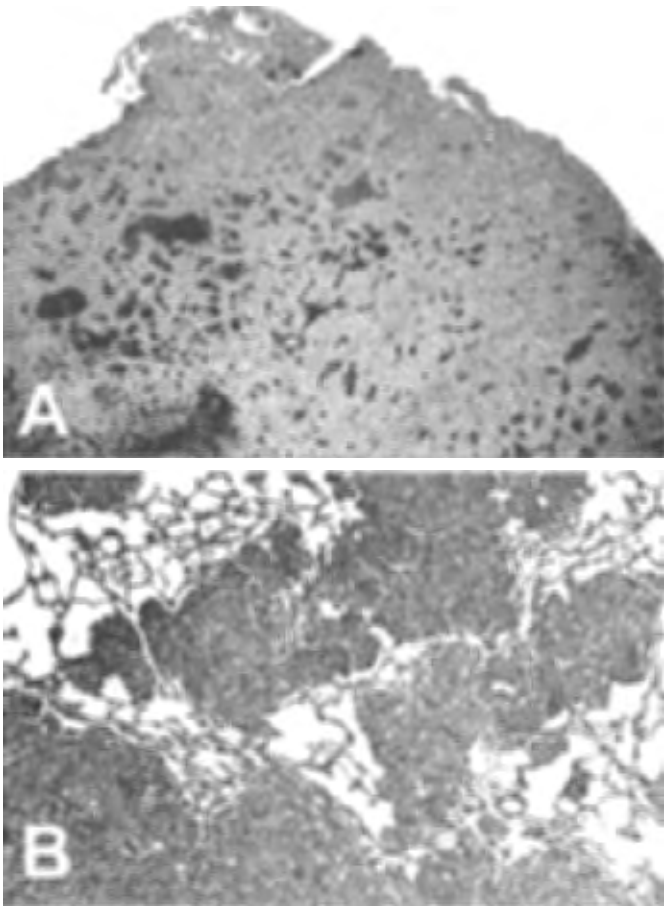

FIGURE 58.21 (A) Adrenocortical carcinoma in a rat with hemorrhage, dilated vascular spaces, and extracapsular invasion. (B) Lung metastases from an adrenocortical carcinoma in a rat.

5. TESTING FOR TOXICITY

Although a compound affects steroidogenesis, it is important to define the extent to which it impairs adrenal cortical functional reserve capacity. In some instances, clinical signs of hypoadrenocorticism may be observed in association with lower urinary and plasma corticosteroid levels. When such findings are not obvious, provocative testing for the evaluation of adrenal cortical functional reserves is essential to determine the extent of cortical damage. The most commonly used provocative test is the administration of ACTH to human patients or test animals (the dog is the ideal test animal for evaluation). ACTH has been shown to be a potent stimulus for the synthesis and secretion of naturally occurring glucocorticoids in non-compromised cells of the zonae reticularis and fasciculata.

The following experiment illustrates the potential effects of chemicals on adrenal cortical reserves. Male and female Beagle dogs were treated with 22.5 mg/kg of DMNM given twice a day for 21 days. During the course of treatment the dogs had no significant clinicopathological abnormalities, although at the end of the study the adrenal cortices were found to have diffuse granular and vacuolar degeneration in all three zones. In order to evaluate the adrenal cortical reserves, an intravenous ACTH infusion was administered to each dog at baseline 7 days before treatment with DMNM and again after 14 days of treatment for comparison.

A comparison of plasma cortisol and aldosterone levels following ACTH infusion in dogs pretest and after 14 days of treatment with DMNM, respectively, is shown in Figure 58.22. Plasma cortisol was decreased slightly to moderately in dogs given DMNM at 0.5–1.0 hour following ACTH infusion. One dog did not have diminished adrenal cortical reserves, but it did have a delayed cortisol response, suggesting that treatment with DMNM for 14 days did not affect the synthesis of cortisol but possibly impaired the secretion of the hormone. Similarly, plasma aldosterone values for all treated dogs did not reach pretest levels.

In vitro studies are extremely useful in determining the specific cellular consequences of xenobiotic exposure on steroidogenesis. In many instances, the results of these *in vitro* assessments are helpful in correlating the

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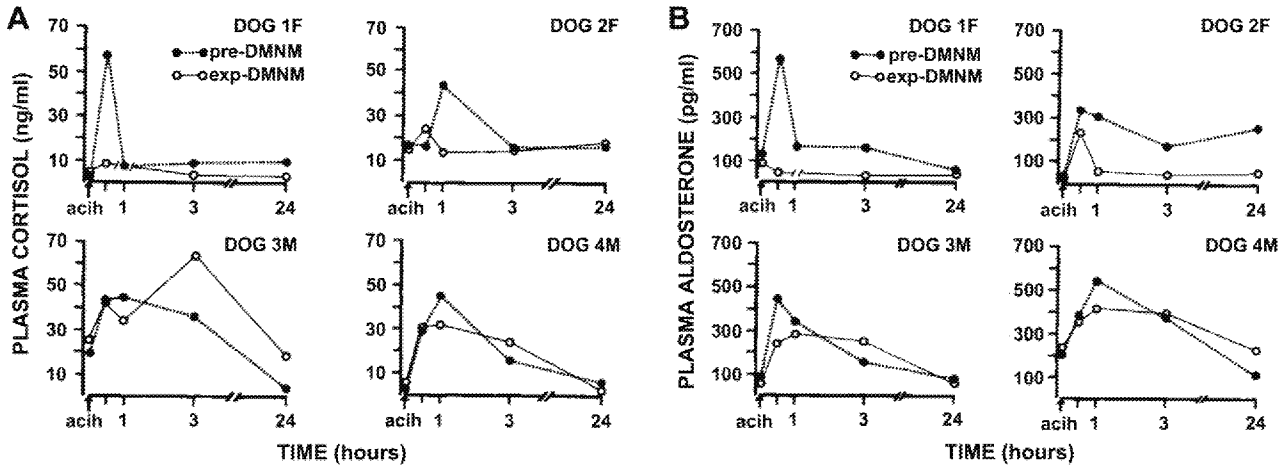

FIGURE 58.22 (A) Plasma cortisol response to intravenous infusion of exogenous ACTH to dogs treated with 22.5 mg/kg/day of DMNM for 14 days. (B) Corresponding plasma aldosterone response to the same ACTH infusion. *Figure reproduced from Yarrington* et al. *(1985) Degeneration of the rat and canine adrenal cortex caused by α-(1,4-dioxido-3-methylquinoxalin-2-yl)-*N*-methylnitrone (DMNM),* Fundam. Appl. Toxicol. *5, 370–381, with permission.*

development of adrenocortical degeneration to an inhibited pathway of steroidogenesis. By far the best available *in vitro* model is the H295R cell line. This is a human adrenocortical carcinoma cell line that retains full adrenocortical steroidogenic enzyme capability and can secrete aldosterone, cortisol, and androgens, estrogens and progestogens and their precursors in response to appropriate challenge. Although it has ACTH receptors, their concentrations are lower than normal adrenal cells, and challenge is usually induced by forskolin administration which acts downstream of ACTH via cAMP. A typical protocol is to expose cells to a range of concentrations of the test compound, administer the challenge, and compare steroid secretion (e.g., cortisol) relative to the untreated but challenged controls (angiotensin II is used to evaluate aldosterone secretion in these cells).

By looking at steroid profiles it is possible to distinguish where in the pathway a drug inhibits an enzyme, but molecular techniques also permit direct analysis of steroidogenic enzymes and gene expression studies. The majority of chemicals reported to induce adrenocortical functional toxicity over the past decade have used this system, particularly to identify molecular sites of toxicity, and H295R has also been used to assess changes in ACTH receptor regulation and Steroid Acute Regulatory (StAR) protein (the first-step transporter of cholesterol into mitochondria) activity. Reviews of the utility of the use of this cell line in toxicology are given in Parmar and Rainey (2009) and Sanderson (2009). It should be noted that rodent cell lines do exist but are deficient in at least CYP17, and the Y1 mouse cell line is only capable of producing progesterone because of deficiencies in numerous other steroidogenic enzymes. Rodent cell lines therefore have little use in establishing mechanisms of toxicity, at least in terms of human relevance.

Diagnostic imaging has now become a more practical technology to demonstrate-morphological alterations of the adrenal cortex caused by xenobiotics in a non-invasive way. Imaging procedures such as ultrasonography and computer tomography are being superseded by high resolution magnetic resonance imaging (MRI) and single-photon emission computed tomography (SPECT).

Following the death of a test animal, morphological evaluation commences with macroscopic observation of the adrenal glands to detect changes in size, color, and/or appearance (e.g., nodularity). The subsequent histological examination of adrenal tissue (including both cortex and medulla) on midsagittal sections stained with hematoxylin and eosin is performed routinely. Microscopic study may be supplemented by the use of special stains and techniques to confirm or identify a particular pigment, cell type, or enzyme. Examples of useful stains are Congo red

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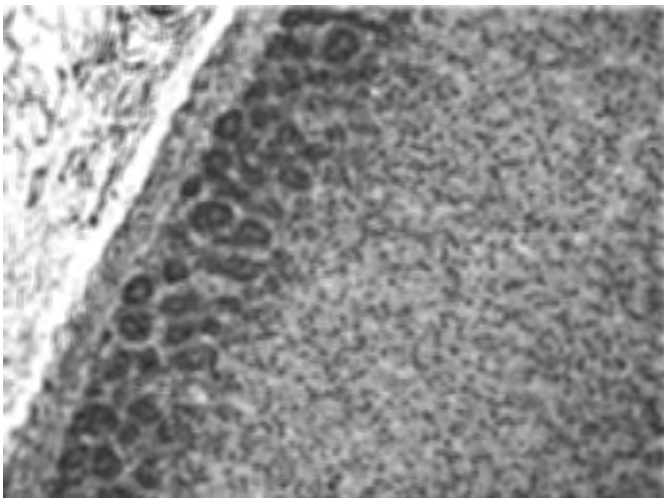

FIGURE 58.23 Cytoplasm of the cells in the zona glomerulosa has strong positivity for aldosterone synthase using immunohistochemistry with K16 antibody in the cortex of a rat.

(amyloid), acid fast stains (ceroid pigment), Sudan black or Oil red O (lipid), Perls stain (iron) and immunohistochemistry for enzymes (e.g., aldosterone synthase in the zona glomerulosa; Figures 58.23). Histomorphometric analysis can be used to assess subtle differences in cell size, width of the different cortical zones, and cortical : medullary ratio. Transmission electron microscopy is an important tool for detecting changes in the internal architecture of adrenal cortical cells, especially when this is performed in association with morphometric analysis, cytochemistry, and immunohistochemistry procedures.

5.1. Use of Animals as Models

The adrenal cortex of animals is prone to develop proliferative and degenerative lesions, the etiology of which may be either spontaneous in nature or induced experimentally. Therefore, chemical testing using various domestic or laboratory animals is a valid means of assessing the toxic potential for humans exposed to various xenobiotic chemicals. In order to evaluate chemically induced lesions of the adrenal cortex in laboratory animals, a complete understanding of naturally occurring spontaneous lesions is essential.

Species, Age, and Sex Variables

The choice of species of the test animals may also be critical. A number of studies have demonstrated that there often is a variable species susceptibility to toxicity. This observation suggests that differences in metabolism play a role in the development of adrenal cortical toxicity and in the inhibition of steroidogenesis. As mentioned previously, the rodent is deficient in CYP17, and compounds inhibiting steroidogenesis and particularly cortisol production via this enzyme would not be adequately evaluated using the rodent only, but dog and monkey could be used to detect such effects. For discussion of mechanisms of hypothalamo-pituitary–adrenocortical mechanisms of toxicity specific to the dog, see Colagiovanni and Meyer (2009); for discussion of adrenal pathological findings specific to non-human primates, see Kaspareit (2009).

Likewise, the age of the test animal, to a lesser degree, may be a factor in the development of chemically induced adrenocortical lesions. For example, adrenal cortical necrosis was induced in rats at 50 days of age but not at 25 days by the administration of 7,12-dimethylbenz[*a*]anthracene. Age- and sex-related differences in hypothalamo-pituitary–adrenal axis function, which ultimately could modulate the response to toxicity, are reviewed in Buckingham (2009), and species, age, sex, circadian rhythm, and other environmental factors specifically influencing adrenal toxicity expression are discussed in Harvey (2010).

Stress as a Variable in Animal Models: Distinguishing Stress and Adrenocortical Toxicity as a Cause of Adrenocortical Hypertrophy

Although the rat is not the best model to fully evaluate adrenal toxicity in terms of human relevance, because of differences in steroidogenesis, it often shows adrenal changes in toxicity studies. One of the most common findings in the rat is adrenocortical hypertrophy, which is indicative of ACTH stimulation (Figure 58.24). Adrenal hypertrophy may be a result of stress or indeed may result from functional impairment of the adrenal cortex and reduced capacity to secrete glucocorticoids (in turn resulting in diminished negative feedback inhibition of ACTH and overstimulation of the gland). It is therefore important to establish, in all cases of adrenocortical hypertrophy induced by excess ACTH

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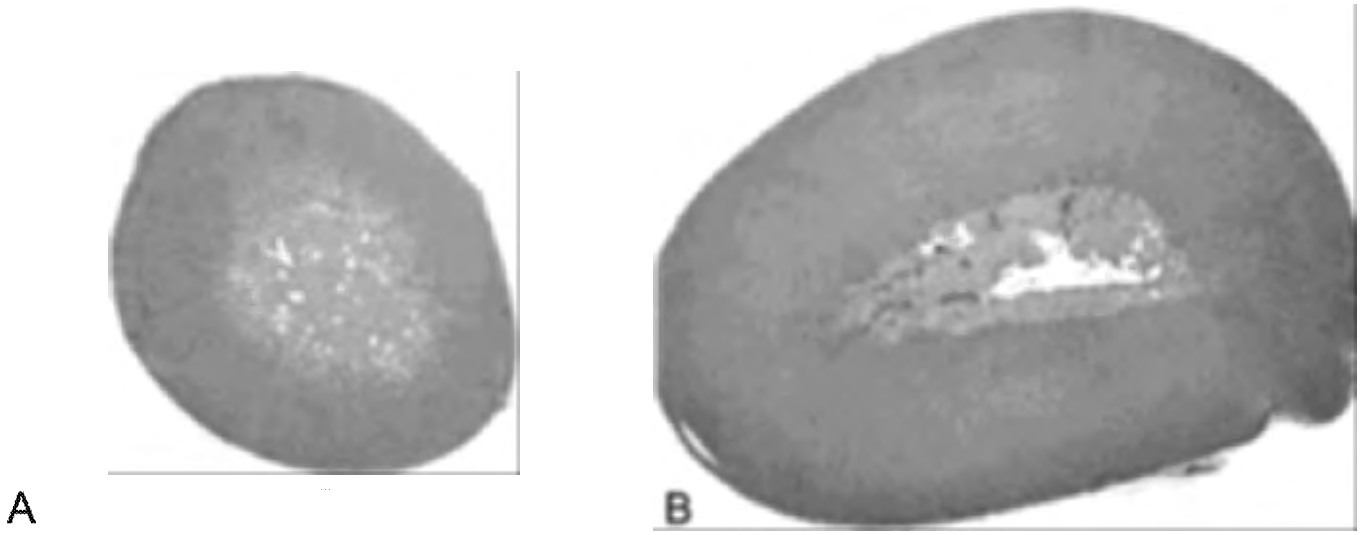

FIGURE 58.24 Adrenal of control rat (A) and rat with grade 3 cortical hypertrophy (B). H&E stain.

stimulation, the mechanism of ACTH elevation (i.e., stress versus adrenal toxicity/insufficiency), and this invariably requires additional evidence and work (see Harvey and Sutcliffe 2010).

If the only finding is adrenocortical hypertrophy, this is insufficient evidence to attribute to either a stress mechanism or a toxicity mechanism. Further supportive evidence is required to prove functionality of the adrenal cortex if stress (of little toxicological relevance) is to be suggested. Thymic and other lymphoid atrophy is a useful surrogate marker for adrenocortical competence, as it is induced by excess glucocorticoid secretion; similarly, the stress leukogram is useful evidence. The point is that stress is a natural adaptive response designed to better equip the animal to survive, and it should result in both elevated ACTH and, critically, glucocorticoids, and elevation of the latter is diagnostic and proof of adrenocortical functional competence. Stress should not produce irreversible histopathologic lesions in the adrenal cortex. By contrast, toxicity to the adrenal cortex may be obvious due to marked and often irreversible histopathological lesions (degeneration, necrosis, fibrosis), but in cases of pharmacotoxicological inhibition of steroidogenic enzymes there may be no histopathological lesions although grossly impaired glucocorticoid production could result, which is of clear toxicological concern.

Importantly, if findings are restricted to adrenocortical hypertrophy (i.e., no thymic atrophy, stress leukogram or other supportive stress-related changes) then stress cannot be confirmed as the cause, and further work to test adrenocortical functionality should be undertaken. This can take the form of an ACTH challenge test *in vivo* where glucocorticoid secretion in blood is monitored (it may also be useful to conduct *in vitro* work in H295R cells at this stage). Typical findings, diagnostic features, and mechanistic protocols for distinguishing stress from toxicity, and particularly pharmacological steroidogenesis inhibition, are given in Harvey and Sutcliffe (2010).

In this context, it is worth mentioning that acute, reversible pharmacological inhibition of adrenocortical steroidogenesis has resulted in patient deaths. The case of etomidate (a single-use human anesthetic induction agent) graphically illustrates that any form of functional impairment, even of short duration, and even if "pharmacological" and reversible (typically used as justification of lack of toxicological significance), can have catastrophic consequences in some patients, producing Addisonian crises, cardiovascular collapse, and death (see Harvey *et al.*, 2009). Etomidate was eventually discovered to be a potent and selective CYP11B1 inhibitor that blocked cortisol production.

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PART II: ADRENAL MEDULLA

6. STRUCTURE AND FUNCTION

6.1. Anatomy

The medulla constitutes approximately 10% of the volume of the adrenal gland. In the normal rodent gland and in most other species the medulla is sharply demarcated from the surrounding cortex. The bulk of the medulla is composed of chromaffin cells, which are the sites of synthesis and storage of catecholamines. The normal adult male Wistar rat adrenal contains an average of 29 nmol norepinephrine and 71 nmol epinephrine. In the rat and mouse, norepinephrine and epinephrine are stored in separate cell types that can be distinguished ultrastructurally after fixation in glutaraldehyde and post-fixation in osmium tetroxide. Norepinephrine-containing granules appear highly electron-dense, whereas epinephrine granules are less dense with finely granular matrices. In immature rat adrenals, granules of varying densities may be found in the same cell types. Human adrenal medullary cells contain both norepinephrine and epinephrine within a single cell.

In addition to chromaffin cells, the adrenal medulla contains variable numbers of ganglion cells. A third cell type has also been described, and has been designated the small granule containing (SGC) cell or small intensely fluorescent (SIF) cell. These cells appear morphologically intermediate between chromaffin cells and ganglion cells. They possibly may function as interneurons.

Adrenal medullary cells also contain serotonin and histamine, but it has not been determined if these products are synthesized *in situ* or taken up from the circulation. A number of neuropeptides, including enkephalins, neurotensin, and neuropeptide Y, are also present in rat chromaffin cells. Another peptide, adrenomedullin, has been shown to modulate function of the adrenal cortex: this peptide has hypotensive actions, and as such can influence the renin–angiotensin–aldosterone axis functionally. Pharmacotoxicological effects of a compound on the adrenal medulla can therefore have indirect effects on adrenocortical function.

6.2. Biochemistry and Physiology

The catecholamine biosynthetic pathways are in general well known. Tyrosine is acted on by tyrosine hydroxylase to produce L-DOPA, which is converted to dopamine by aromatic L-amino acid (DOPA) decarboxylase. In turn, dopamine is acted on by dopamine β-hydroxylase to form norepinephrine, which is converted to epinephrine by phenylethanolamine-N-methyltransferase (PNMT). Tyrosine hydroxylase is the rate-limiting step. The conversion of tyrosine into L-DOPA and dopamine occurs within the cytosol. Dopamine then enters the chromaffin granule, where it is converted to norepinephrine. Norepinephrine leaves the granule to be converted into epinephrine in the cytosol, and epinephrine then re-enters the granule. In contrast to the synthesis of catecholamines, which occurs in the cytosol, neuropeptides and chromogranin A proteins are synthesized in the granular endoplasmic reticulum and are packaged into granules in the Golgi apparatus.

The role of innervation in regulating the functions of chromaffin cells has been studied extensively. During adult life, stresses such as insulin-induced hypoglycemia or reserpine-induced depletion of catecholamines produce a reflex increase in splanchnic nerve discharge, resulting in both catecholamine secretion and transsynaptic induction of catecholamine biosynthetic enzymes, including tyrosine hydroxylase. These effects become apparent during the first week of life, following an increase in the number of nerve terminals in the adrenal medulla. Other environmental influences, including growth factors, extracellular matrix, and a variety of hormonal signals that generate cyclic AMP, may also regulate the function of chromaffin cells.

Physiological effects of adrenaline include an increase in systolic blood pressure and heart rate, diversion of blood to limb muscle beds/away from gut, a decrease in gut motility, bronchodilation, reduced mucus secretion, piloerection, and mydriasis. Noradrenaline only stimulates α and β1 receptors so does not cause bronchodilation, which is a β2 response, and although it also increases systolic (and diastolic) blood pressure leading to an increased mean arterial pressure, it antagonizes the effects of adrenaline on heart rate and decreases heart

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rate. Many of these physiological responses become pronounced in pathological conditions affecting the medulla, particularly pheochromocytoma, where excess catecholamine release typically results in hypertension and other distinctive symptoms.

7. MECHANISMS OF TOXICITY

Proliferative lesions of the medulla, particularly in the rat, have been reported to develop as a result of a variety of different mechanisms. Warren and co-workers (1966) studied over 700 pairs of rats with parabiosis, and found that more than 50% of male irradiated rats developed adrenal medullary tumors. However, other authors have suggested that the tumorigenic effect of ionizing radiation is equivocal for the adrenal medulla.

Some data are available on the relationship of anterior pituitary hormones to the development of adrenal medullary lesions. For example, the long-term administration of growth hormone is associated with the development of pheochromocytomas as well as tumors in other sites. Some authors have suggested that prolactin-secreting pituitary tumors, which occur commonly in many rat strains, may play a role in the development of proliferative medullary lesions. Growth hormone and prolactin effects may be related because the two hormones are approximately 40% homologous; growth hormone, when present in high concentrations in the circulation, can bind to prolactin receptors. Estrogens and thyrotropin-releasing hormone also would be expected to stimulate prolactin release. Evidence for a role of pituitary hormones in the development of medullary lesions is provided by data suggesting that hypophysectomy eliminates the development of these lesions in a susceptible strain. Although not all animals with adrenal medullary lesions have concurrent pituitary abnormalities, at least some with histologically normal pituitaries might have hypothalamic defects resulting in the decreased dopaminergic inhibition of prolactin release. It is also possible that certain neuroleptics increase prolactin by decreasing dopamine release.

Both nicotine and reserpine have been implicated in the development of adrenal medullary proliferative lesions. Both agents may act by a shared mechanism, as nicotine directly stimulates nicotinic acetylcholine receptors, whereas reserpine causes a reflex increase in the activity of cholinergic nerve endings in the adrenal. A short dosing regimen of reserpine administration *in vivo* stimulates the proliferation of chromaffin cells in the adult rat, and the mechanism may involve a reflex increase in neurogenic stimulation via the splanchnic nerve. Treatment with antithyroid drugs such as propylthiouracil also may affect chromaffin cells by a similar mechanism, as hypothyroid rats have increased sympathetic activity. An additional component of the action of reserpine occurs through the depletion of hypothalamic dopamine stores.

Several other drugs have been reported to increase the incidence of adrenal medullary proliferative lesions. These include zomepirac sodium (a non-steroidal anti-inflammatory drug), isoretinoin (a retinoid), and gemfibrozil (a hypolipidemic drug). However, the mechanisms responsible for the stimulation of adrenal medullary proliferation by these drugs are unknown. Elmore *et al.* (2009) provide a recent review of the adrenal medulla as a toxicological target organ; an up-to-date list of chemicals producing treatment-related pheochromocytoma (with chlorinated compounds comprising almost half the positive NTP list, such as chlorinated paraffins, P-chloroaniline hydrochloride, 4-chloro-M-phenylenediamine, P-dichlorobenzene, hexachloroethane, 4,4′-methylenedianiline dihydrochloride, pentochlorophenol and 1,1,2-trichloroethane); and recent background control incidence of adrenomedullary tumors in B6C3F1 mice and F344 rats, showing striking species and sex variation. For example, the incidence of benign pheochromocytoma in male F344 rats was 208/1443 but in females was only 32/1339, and the incidence in male B6C3F1 mice was 4/1335 and in females was 14/1239. Therefore, male F344 male rats have a high propensity to develop these tumors spontaneously where as F344 females and mice (B6C3F1) do not. There is an association with severe nephropathy and lung pathology (specific to inhalation studies where pulmonary fibrosis and inflammation results in hypoxemia) with pheochromocytoma.

Nutritional factors have an important modulating effect on the spontaneous incidence of adrenal medullary proliferative lesions in rats.

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Several sugars and sugar alcohols have produced adrenal medullary tumors at high dosages, including xylitol, sorbitol, lacitol, and lactose at concentrations of 10–20% in the diet. Although the mechanism involved is not completely understood, a role for calcium (Ca^{2+}) has been postulated. High doses of slowly absorbed sugars and starches increase the absorption and urinary excretion of Ca^{2+}. Hypercalcemia is known to increase catecholamine synthesis in response to stress, and low-calcium diets will reduce the incidence of adrenal medullary tumors in xylitol-treated rats. Other compounds that might act via altered Ca^{2+} homeostasis include the retinoids (which will produce hypercalcemia) and conditions such as progressive nephrocalcinosis in aging male rats treated with non-steroidal anti-inflammatory agents.

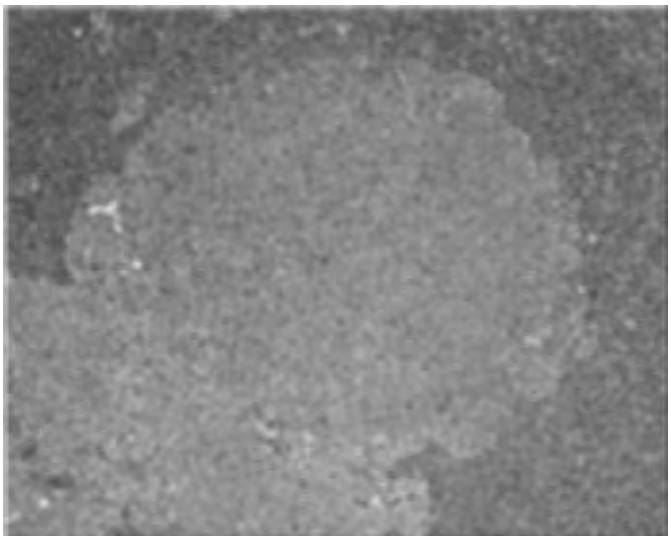

FIGURE 58.25 Focal medullary hyperplasia in Sprague-Dawley rat. H&E stain.

8. RESPONSE TO INJURY

8.1. Characteristics of Proliferative Lesions

The adrenal medulla undergoes a series of proliferative changes ranging from diffuse hyperplasia to benign and malignant neoplasia. The latter neoplasms have the capacity to invade locally and to metastasize to distant sites. Diffuse hyperplasia is characterized by symmetric expansion of the medulla with maintenance of the usual sharp demarcation of the cortex and the medulla. The medullary cell cords often are widened, but the ratio of norepinephrine to epinephrine cells is similar to that of normal glands.

Focal hyperplastic lesions are often juxtacortical, but may occur within any area of the medulla. The small nodules of hyperplasia in general are not associated with compression of the adjacent medulla (Figure 58.25); however, the larger foci often are associated with medullary compression. Foci of adrenal medullary hyperplasia are typically composed of small cells with round to ovoid nuclei and scant cytoplasm. The classification of such lesions has engendered considerable controversy in the literature. According to some authors, the lack of a chromaffin reaction in such nodules has been said to preclude the diagnosis of pheochromocytoma. However, the chromaffin reaction is quite insensitive, and catecholamines, particularly norepinephrine, can be demonstrated by biochemical extraction studies and by formaldehyde- or glyoxylic acid-induced fluorescence methods. At the ultrastructural level, the cells composing these focal areas of hyperplasia contain small numbers of dense core secretory granules resembling the granules of SIF or SGC cells (Figure 58.26).

Larger adrenal medullary proliferative lesions are accepted generally as pheochromocytomas. These lesions may be composed of relatively small cells similar to those found in smaller proliferative foci. Alternatively, they may be composed of larger cells or a mixture of small and large cells (Figure 58.27). Even in the larger medullary lesions, the chromaffin reaction often is equivocal but catecholamines maybe demonstrated both biochemically and histochemically (Figure 58.28). Invasion of the capsule of the adrenal with or without distant metastases occurs in malignant pheochromocytomas. The criteria for proliferative lesions of the rodent adrenal medulla, including distinction between benign and malignant pheochromocytoma, are as follows.

Focal hyperplasia:

1. Circumscribed focus of medullary cells that blends with surrounding normal parenchyma;
2. No or minimal compression;

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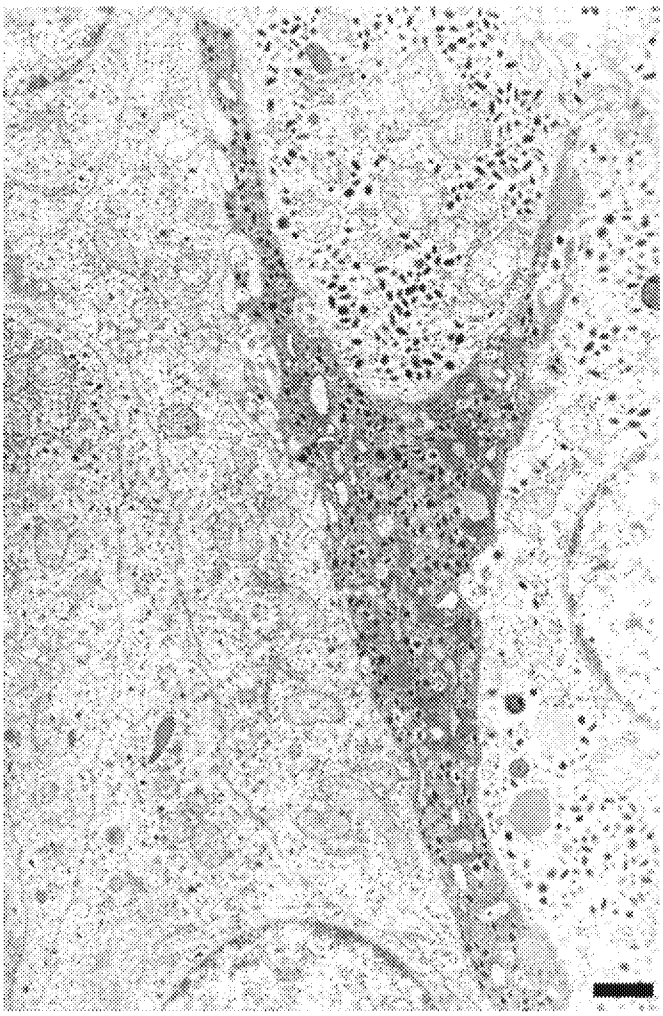

FIGURE 58.26 Focal hyperplasia ("nodule") of adrenal medulla from an old male Long-Evans rat. Cells comprising the nodule (left) have few small secretory granules compared to the larger and more pleomorphic granules of the adjacent medullary cells. Bar = 1 μm. *Figure reproduced from Tischler and DeLellis (1988b) The rat adrenal medulla. II. Proliferative lesions,* J. Am. Coll. Toxicol. *7, 23–44, with permission.*

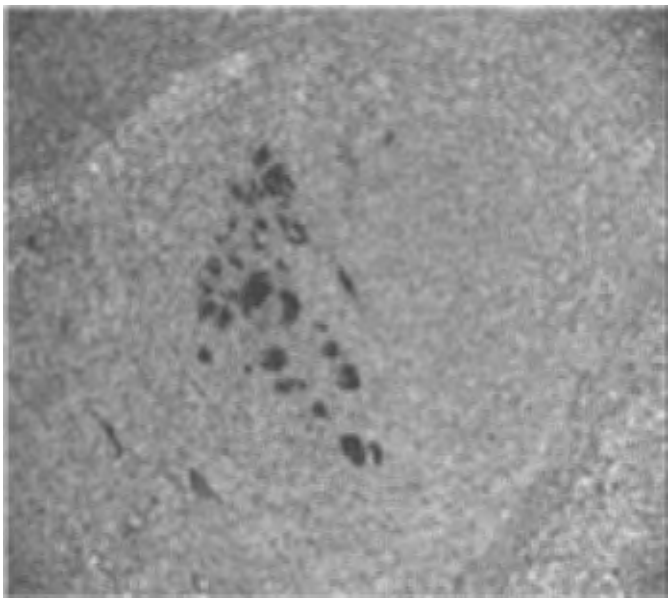

FIGURE 58.27 Pheochromocytoma in a Wistar rat. Note the dilated vascular spaces and compression of the adjacent adrenal cortical and medullary tissue. H&E stain.

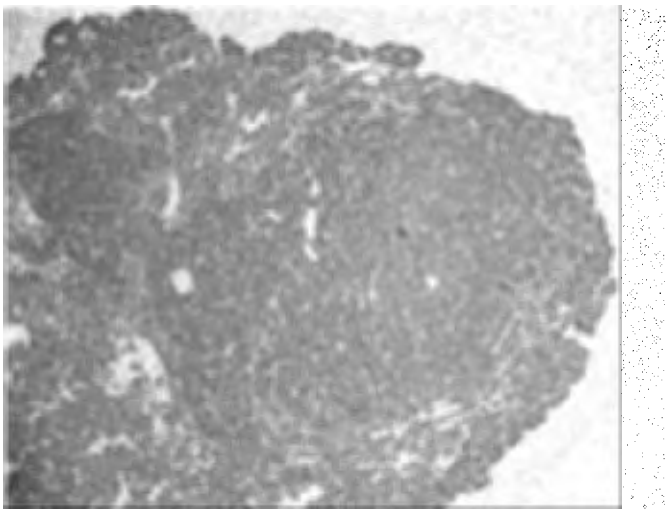

FIGURE 58.28 Pheochromocytoma in a Wistar rat. Synaptophysin immunohistochemistry.

3. Minimal alteration in architecture with cells arranged in packets or solid clusters slightly larger than normal; and
4. Minimal to mild alteration in size, shape, and staining qualities of affected cells (nuclei and cytoplasm).

Pheochromocytoma, benign:

1. Well-delineated mass of medullary cells;
2. Minimal to marked compression of surrounding parenchyma;
3. Altered architecture with cells arranged in large solid clusters or thick trabeculae; growth pattern may be variable; and
4. Mild to marked alteration in size, shape, and staining qualities of affected cells; cellular atypia and pleomorphism may be marked.

Pheochromocytoma, malignant:

1. Invasion of capsule and periadrenal soft tissue; and
2. Metastasis.

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In contrast to the high frequency of pheochromocytomas, neuroblastomas occur infrequently in the adrenal medulla in rodents. Neuroblastomas develop as a centrally located expansive mass that compresses the surrounding cortex, and are composed of small cells with round to ovoid hyperchromatic nuclei and scanty cytoplasm. Cells comprising neuroblastomas resemble lymphocytes and tend to form pseudorosettes. Neurofibrils or unmyelinated nerve fibers usually can be demonstrated in neuroblastomas.

Ganglioneuromas usually are small benign tumors arising in the medulla and compressing the surrounding cortex. They are composed of multipolar sympathetic ganglion cells and neurofibrils with a prominent fibrous connective tissue stroma (Figure 58.29). Neoplastic cells in medullary tumors occasionally differentiate along two lines, resulting in adjacent pheochromocytomas and ganglioneuromas ("collision tumors") in the same adrenal gland. The two adjacent masses in the same adrenal gland have typical microscopic characteristics of a ganglioneuroma and pheochromocytoma.

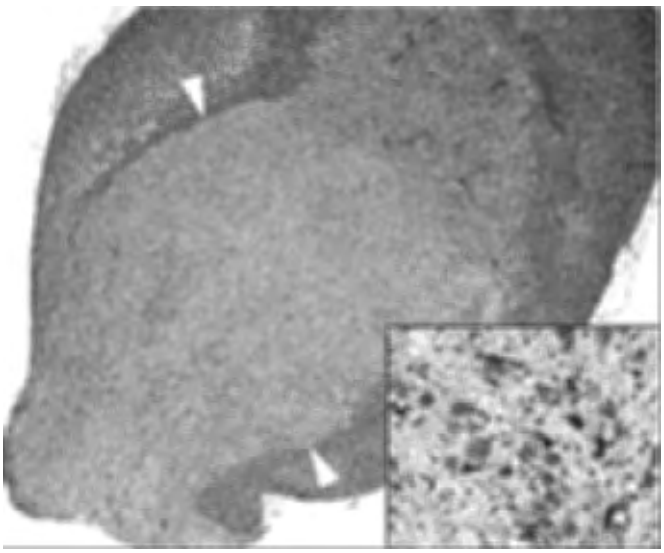

FIGURE 58.29 Ganglioneuroma in a Wistar rat arising from the adrenal medulla with penetration through the adrenal cortex and capsule (arrowheads). The inset demonstrates a higher magnification of ganglion cells (basophilic cells) and Schwann cells (eosinophilic stroma). H&E stain.

8.2. Occurrence of Proliferative Lesions

Proliferative lesions occur with high frequency in many strains of laboratory rats. The incidence of these lesions varies with strain, age, sex, diet, and exposure to drugs, and a variety of environmental agents. Studies from the NTP historical data base of 2-year-old F344 rats have reported that the incidence of pheochromocytomas was 17% and 3.5% for males and females, respectively. Malignant pheochromocytomas were detected in 1% of males and 0.5% of females. In addition to F344 rats, other strains with high incidences of pheochromocytoma include Wistar, NEDH (New England Deaconess Hospital), Long-Evans, and Sprague-Dawley.

Pheochromocytomas are considerably less common in Osborne-Mendel, Charles River, Holtzman, and WAG/Rij rats. Most studies have revealed a higher incidence in males than in females. Cross-breeding of animals with high and low frequencies of adrenal medullary proliferative lesions results in first generation (F_1) animals with an intermediate tumor frequency. Pheochromocytomas are less common in the mouse than in most strains of rats.

There is a striking relationship between age and the frequency, size, and bilateral occurrence of adrenal medullary nodules in the rat. In the Long-Evans strain, medullary nodules have been found in less than 1% of animals under 12 months of age. The frequency increases to almost 20% in 2-year-old animals, and to 40% in animals between 2 and 3 years of age. The mean tumor size increases progressively with age, as does the frequency of bilateral and multicentric occurrence.

Roe and Bar (1985) have called attention to the fact that environmental and dietary factors may be more important than genetic factors as determinants of the incidence of adrenal medullary proliferative disorders in rats. The incidence of adrenal medullary lesions can be reduced by lowering the carbohydrate content of the diet.

Sugar alcohols, including mannitol, sorbitol, xylitol, and lacitol, have been reported to increase the incidence of all types of proliferative lesions of the adrenal medulla. In view of the fact that sugar alcohols are not

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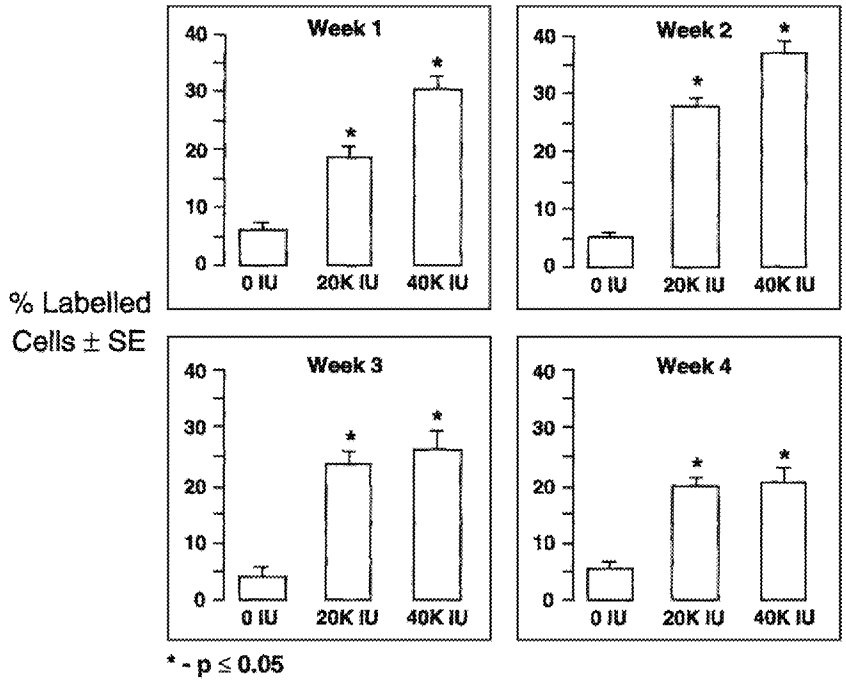

FIGURE 58.30 Effects of Vitamin D_3 on percentage of chromaffin cells labeled with BrdU during weeks 1, 2, 3, or 4 of dietary supplementation. An asterisk indicates a statistically significant increase over corn oil controls. Bars represent means ± SE for six rats per group. At least 500 chromaffin cells were scored for each rat. *Figure reproduced from Tischler* et al. *(1996). Vitamin D_3, lactose, and xylitol stimulate chromaffin cell proliferation in the rat adrenal medulla,* Toxicol. Appl. Pharmacol. *140, 115–123, with permission.*

mutagenic, it is likely that the increased incidence of adrenal medullary lesions represents a response to metabolic change. Several of the agents that increase the incidence of adrenal medullary lesions, including sugar alcohols, increase absorption of Ca^{2+} from the gut. A link between hypercalcemia and the development of adrenal medullary lesions has been suggested, and it has been reported that hypercalcemia in rats leads to catecholamine release and hypertension. Ca^{2+}, as well as cyclic nucleotides and prostaglandins, may act as mediators capable of stimulating both hormonal secretion and cellular proliferation. The likelihood of medullary lesions also appears greater in rats with metastatic calcification and nephrocalcinosis.

Vitamin D is the most potent *in vivo* stimulus yet identified for chromaffin cell proliferation in the adrenal medulla. Vitamin D_3 (5000, 10 000, or 20 000 IU/kg/day in corn oil) resulted in a four- to five-fold increase in bromodeoxyuridine (BrdU) labeling at week 4 that diminished to a two-fold increase by week 26. An initial preponderance of epinephrine-labeled cells (phenylethanolamine-N-methyl transferase [PNMT] positive cells] subsequently gave way to norepinephrine-labeled cells. By week 26, 89% of rats receiving the two highest doses of Vitamin D_3 had focal medullary proliferative lesions (BrdU-labeled focal hyperplasias ["hot spots"]) and pheochromocytomas in contrast to the absence of lesions in controls.

The proliferative lesions usually were multicentric, bilateral, peripheral in location in the medulla, nearly all were PNMT negative, and appeared to represent a morphologic continuum rather than separate entities. Earlier studies reported by the same research group had demonstrated that the vitamin D_3 (20 000, 40 000 IU/kg/day) increased chromaffin cell proliferation as early as 1 week and had declined by 4 weeks (Figure 58.30). These findings support the hypothesis that altered Ca^{2+} homeostasis is indirectly involved in the pathogenesis of pheochromocytomas in rodents, most likely via effects on increasing chromaffin cell proliferation. Vitamin D_3, calcitrol (active metabolite of D_3), lactose, and xylitol all failed to stimulate directly the proliferation of rat chromaffin cells *in vitro*.

It has been suggested that at least three dietary factors may lead to hypercalcemia and increased incidence of adrenal medullary proliferative lesions. These are excessive intake of food associated with feeding *ad libitum*; excessive intake

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of Ca^{2+} and phosphorus, as commercial diets contain two to three times more Ca^{2+} and phosphorus than needed by young rats; and excessive intake of other food components (e.g., Vitamin D and poorly absorbable carbohydrates) which predispose to increased Ca^{2+} absorption.

9. TESTING FOR TOXICITY

9.1. Morphological Evaluation

A variety of techniques may be used for the demonstration of catecholamines in tissue sections. The chromaffin reaction is the oxidation of catecholamines by potassium dichromate solution, and results in the formation of a brown-to-yellow pigment that may be seen both grossly and microscopically. The chromaffin reaction as performed traditionally possesses a very low level of sensitivity, and should not be used for the routine demonstration of catecholamines. Similarly, both argentaffin and argyrophil reactions, which have been used extensively in the past for the demonstration of chromaffin cells, also possess low sensitivity and specificity. Fluorescence techniques using formaldehyde or glyoxylic acid can be used to demonstrate catecholamines at the cellular level. These aldehydes form highly fluorescent derivatives with catecholamines, which can be visualized by ultraviolet microscopy.

Immunohistochemistry provides an alternative approach for the localization of catecholamines in chromaffin cells and other cell types. Antibodies are now available that permit epinephrine- and norepinephrine-containing cells to be distinguished even in routinely fixed and embedded tissue samples. Tumor cells show positivity for general neuroendocrine markers, including Chromogranin A, synaptophysin, and CD56. Such antibodies are useful for differentiating pheochromocytoma from an adrenal cortical tumor. Catecholamine biosynthetic enzymes can also be demonstrated by immunohistochemical procedures (e.g., tyrosine hydroxylase), and can differentiate a paraganglioma from other neuroendocrine tumors.

PART III: PITUITARY GLAND

10. STRUCTURE AND FUNCTION

10.1. Anatomy

The pituitary gland (hypophysis) may be divided into two major compartments: (1) the adenohypophysis (anterior lobe), composed of the pars distalis, pars tuberalis, and pars intermedia; and (2) the neurohypophysis (posterior lobe), which includes the pars nervosa or infundibular process, infundibulum, infundibular stem, and tuber cinereum. In most animals and in human fetuses, the thin cellular zone between the adenohypophysis and neurohypophysis is referred to as the pars intermedia or intermediate lobe.

The pituitary lies within the sella turcica of the sphenoid bone. The gland receives its blood supply via the posterior and anterior hypophyseal arteries, which originate from the internal carotid arteries. Arteriolar branches penetrate the pituitary stalk, lose their muscular coat, and form a capillary plexus near the median eminence. These vessels drain into the hypophyseal portal veins, which supply the adenohypophysis. This hypothalamic–hypophyseal portal system transports hypothalamic-releasing and release-inhibiting hormones directly to the adenohypophysis for interactions with their specific target cells.

10.2. Functional Cytology

The neurohypophysis is joined to the hypothalamus via the infundibular stalk, and is composed of densely packed bundles of nonmyelinated axons and capillaries that are supported by modified glial cells or pituicytes. The capillaries in the pars nervosa are termination sites for unmyelinated axons, which originate from the hypothalamic neurosecretory neurons. Axons that arise from neurons in the infundibular and tuberal regions (tuberohypophyseal tract) terminate predominantly in the median eminence. Axons arising from supraoptic and paraventricular nuclei (supraopticohypophyseal tract) terminate in the pars nervosa. Both oxytocin and vasopressin (antidiuretic hormone [ADH]) are synthesized in supraoptic and paraventricular nuclei as large

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precursor molecules, which contain both active hormones and their associated neurophysins. As the biosynthetic precursor molecules travel along the axons in secretion granules from the neurosecretory neurons, the precursors are cleaved into the active hormones and their respective neurophysins. These secretory products can be detected immunocytochemically. In Brattleboro rats with hereditary hypothalamic diabetes insipidus, nerve cells in the hypothalamus that normally produce ADH and neurophysin-I are negative immunocytochemically for both proteins. Neurosecretory cells that produce vasopressin and neurophysin-II are positive.

The adenohypophysis represents the largest portion of the pituitary gland. The cells within this lobe are responsible for the synthesis of at least six major hormones: growth hormone (GH), prolactin (PRL), and adrenocorticotropin (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH) or thyrotropin. With hematoxylin and eosin (H&E) staining, cells of the anterior lobe have been divided into acidophils, basophils, and chromophobes, constituting approximately 40%, 10%, and 50% of the total cell population, respectively.

The growth hormone-secreting acidophils (somatotrophs) constitute approximately 50% of the total cell population in the pars distalis (Figure 58.31). These cells are distributed throughout the adenohypophysis and are generally round to ovoid in shape. The growth hormone-producing cells stain positively with orange C and eosin, and these cells typically have numerous secretory granules, which have an average diameter of 300 nm. Occasional growth hormone-containing secretory granules may measure up to 500 nm in diameter. Although most of the growth hormone-synthesizing cells correspond to acidophils, some may be chromophobic, as they are in an actively synthesizing phase of the secretory cycle. At the ultrastructural level, actively synthesizing cells have few secretory granules but abundant granular endoplasmic reticulum and Golgi complexes.

Prolactin-secreting acidophils (luteotrophs) compose 15–25% of the total cell population. They tend to be concentrated in the dorsocephalic region of the adenohypophysis.

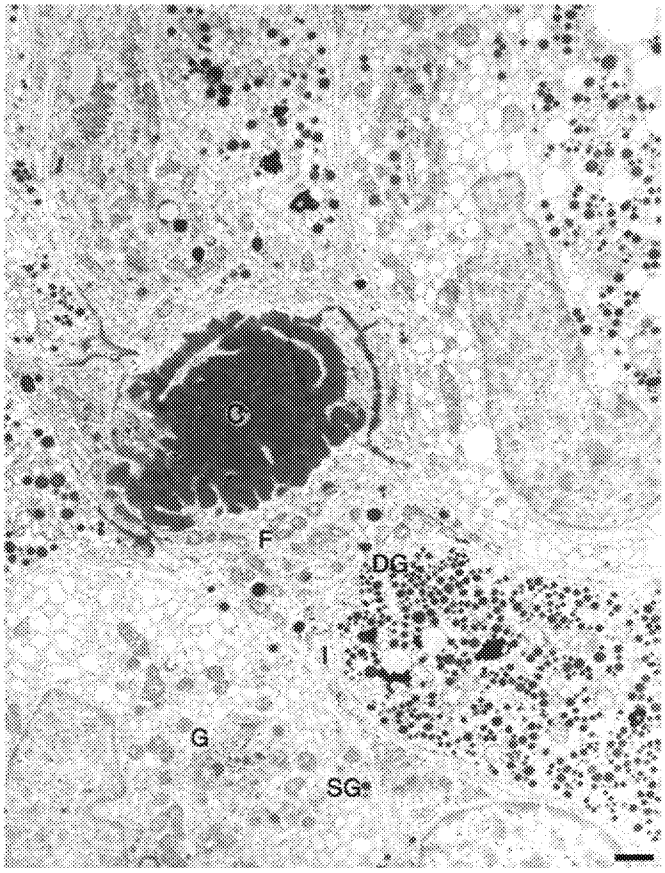

FIGURE 58.31 Adenohypophysis from a dog. Densely granulated acidophils (DG) are in the storage phase and contain numerous secretory granules. Sparsely granulated acidophils (SG) have few mature secretory granules but many dilated cisternae of granular endoplasmic reticulum and well-developed Golgi regions (G). Follicular (stellate) cell, F; colloid, C. Bar = 1 μm. *Figure reproduced from Capen and Koestner (1967) Functional chromophobe adenomas of the canine adenohypophysis: An ultrastructural evaluation of a neoplasm of pituitary corticotrophs,* Vet. Pathol. *4, 326–347, with permission.*

Ultrastructurally, two types of prolactin cells have been recognized. One type contains secretory granules, which measure 200–300 nm in diameter, whereas the second type has secretory granules, which measure 600–900 nm. Immunoperoxidase stains reveal that prolactin cells have angular cell processes that may be closely applied to the cytoplasm of gonadotrophs. Prolactin cells, particularly those with larger granules, stain positively with erythrosine and carmoisine. However, the sparsely granulated cells with smaller granules often fail to stain

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with these dyes, and appear chromophobic. Pregnancy and lactation are associated with hyperplasia of prolactin-secreting cells. These so called "pregnancy cells" are chromophobic by routine staining techniques, but do contain prominent arrays of granular endoplasmic reticulum, well-developed Golgi complexes, and few secretory granules.

ACTH-producing cells (corticotrophs) are round to ovoid, may be chromophobic or lightly basophilic (in humans), and can stain weakly positive with PAS. These cells are widely distributed within the pars distalis and form the bulk of the pars intermedia. The granules have an average diameter of 250 nm and are frequently aligned along the plasma membranes. Occasional larger granules may also be present within corticotrophs. Crooke's hyaline change refers to the intracellular accumulation of an eosinophilic homogeneous material within the cytoplasm of these cells. Immunohistochemical staining reveals that this material represents a keratin-like moiety, which accumulates in conditions associated with glucocorticoid hormone excess.

Thyrotropin-producing (TSH) basophils or thyrotrophs account for about 5% of the cells of the adenohypophysis. These cells tend to occur in small clusters within the pars distalis, and have a stellate to polygonal shape. They may be basophilic or chromophobic, and are also PAS positive. Secretory granules have an average diameter of 150 nm. "Thyroidectomy cells" are enlarged and vacuolated TSH cells that contain prominent, often dilated granular endoplasmic reticulum, conspicuous Golgi complexes, and few secretory granules. Large PAS-positive lipofuscin granules often are found within these cells. Any condition that leads to hypofunction of the thyroid, as a result of either thyroidectomy or the administration of goitrogenic drugs or chemicals that inhibit the biosynthesis of triiodothyronine (T_3) and thyroxine (T_4) or increase the hepatic degradation of thyroid hormones, results in the development of thyroidectomy cells in the pituitary gland. Hypertrophy and hyperplasia of TSH cells under these conditions are due to a lack of negative feedback by T_3 and, to a lesser extent, T_4 on the hypothalamus and thyrotrophs.

Gonadotropin-producing (GTH) basophils or gonadotrophs are relatively large round to oval cells, which account for approximately 10% of the cells in the pars distalis. These cells are responsible for the production of FSH and LH. They tend to be concentrated in the dorsocephalic region of the pars distalis. Ultrastructurally, gonadotrophs have granules that vary from 200 to 300 nm in diameter. Immunoperoxidase stains have revealed that some cells contain both FSH and LH, whereas others contain only LH or FSH.

Gonadotroph cells undergo a series of changes following castration, resulting in the formation of "gonadectomy cells." As a result of the lack of negative feedback by gonadal steroids, gonadotrophs are actively stimulated to synthesize and secrete FSH and LH. Gonadotrophs undergo hypertrophy, and the cytoplasm becomes vacuolated due to distention of endoplasmic reticulum with finely granular material in response to long-term stimulation by gonadotropin-releasing hormone (GnRH) from the hypothalamus. In some cells, a single large vacuole occupies most of the cytoplasm, producing cells with a "signet ring" appearance. Ultrastructurally, gonadectomy cells contain abundant dilated cisternae of endoplasmic reticulum, well-developed Golgi complexes, and few secretory granules.

In addition to specific hormone-secreting cells, a population of supporting cells is also present in the adenohypophysis. These cells have been referred to as stellate (follicular) cells, and can be stained selectively with antibodies to S-100 protein. Stellate cells typically have elongate processes and prominent cytoplasmic filaments. These cells appear to provide a phagocytic or supportive function in addition to producing a colloid-like material (Figure 58.31).

The hypothalamus serves as the major regulator of the adenohypophysis. Each cell type within the adenohypophysis is under the control of a corresponding releasing hormone that is synthesized within nerve cell bodies of the hypothalamus. The releasing hormones are transported via axonal processes to the median eminence, where they are released into capillaries and are carried by the hypophyseal portal system to trophic hormone-producing cells in the adenohypophysis. Specific releasing factors have been identified for TSH, FSH and LH, ACTH, and GH. Prolactin (PRL) secretion is stimulated by a number of factors, the most

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important of which appears to be thyrotropin-releasing hormone (TRH). TRH stimulates the release of prolactin with many of the same dose–response characteristics as the stimulation of TSH release.

Multiple influences contribute to the control of adenohypophyseal hormone secretion. Dopamine serves as the major prolactin inhibitory factor. This amine suppresses virtually all aspects of prolactin secretion, and also inhibits cell division and DNA synthesis of this cell type. Dopamine also suppresses ACTH production by corticotrophs in the pars intermedia of some species. A second hypothalamic release-inhibiting hormone is somatostatin (somatotropin release-inhibiting hormone, SRIH). This tetradecapeptide inhibits the secretion of both growth hormone and TSH. In some situations, SRIH also inhibits the secretion of PRL and ACTH. The control of pituitary hormone secretion is also affected by negative feedback loops resulting from the interaction of end-organ hormones, adenohypophyseal hormones, and corresponding hypothalamic-releasing and release-inhibiting hormones.

11. MECHANISMS OF TOXICITY

11.1. Induction of Pituitary Tumors

Pituitary tumors can be induced readily by sustained uncompensated hormonal derangements leading to increased synthesis and secretion of pituitary hormones. In such a situation, the absence of feedback inhibition of the pituitary cell may lead to its unrestrained proliferation. This effect can be potentiated by the concurrent administration of ionizing radiation or chemical carcinogens.

For example, in the case of the pituitary–thyroid axis, T_4 and T_3 normally control the pituitary secretion of TSH in a classical negative feedback loop. Surgical removal or radiation-induced ablation of the thyroid or interference with the production of thyroid hormones by the use of specific inhibitors leads to the prolonged stimulation of TSH synthesis and secretion. As an initial response, TSH synthesis is enhanced markedly and is reflected by an increase in polyribosomes and granular endoplasmic reticulum within the cells. Typically, cisternae of the granular endoplasmic reticulum become dilated and filled with a flocculent proteinaceous material. There is a concurrent increase in the number of saccules and vesicles of the Golgi apparatus. These cells appear hypertrophic at the light-microscopic level.

Hyperplasia of the thyrotrophs occurs concurrently with cellular hypertrophy as a consequence of the absence of normal inhibitory feedback mechanisms. Foci of hyperplasia may progress to the formation of adenomas. The role of gonadectomy in pituitary tumor induction has been studied most intensively in mice. Pituitary tumors induced by gonadectomy in this species are markedly strain-dependent, and may contain FSH, LH, or both.

The administration of estrogens is a reproducible method for inducing pituitary tumors in some experimental animals. The effect of exogenous estrogen on the rat pituitary includes stimulation of prolactin secretion and the induction of prolactin-secreting tumors. The administration of estrogens in susceptible strains results in increased serum prolactin levels, increased numbers of prolactin cells within the pituitary, and increased mitotic activity. There is considerable variation in the inducibility of pituitary tumors by estrogens in different rat strains. For example, F344 rats are more sensitive than Holtzman rats.

The precise mechanisms of estrogen-induced prolactin tumors are unknown. Such tumors have been associated with a loss of hypothalamic dopaminergic neurons, which normally inhibit the function of prolactin-secreting cells. When transplanted subcutaneously, prolactin-producing tumors were also associated with degenerative changes in hypothalamic dopaminergic neurons, suggesting that prolactin itself could be neurotoxic.

The tumorigenic action of estrogen may not be due exclusively to its effect on the hypothalamus because estrogen can produce prolactinomas in pituitaries grafted beneath the renal capsule. Dopamine agonists, including lisuride and bromocriptine, antagonize the direct stimulatory effects of estrogens on the prolactin cells. Dopamine agonists may act directly on dopaminergic receptors within the transplanted pituitaries. Prophylactic treatment of rats with lisuride decreases the incidence of pituitary adenomas in old animals. The mean levels of prolactin in

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the serum are considerably lower in lisuride-treated animals than in controls.

Other agents, including caffeine, have also been implicated in the development of pituitary adenomas. Additionally, the administration of N-methylnitrosourea is also associated with the development of pituitary adenomas in Wistar rats. The neuroleptic agent sulpiride has been reported to cause the release of prolactin from the anterior pituitary in the rat and to stimulate DNA replication. The administration of clomiphene prevents the stimulation of DNA synthesis produced by sulpiride, but does not affect prolactin release from the gland. These findings have suggested that the intracellular prolactin content of the anterior pituitary plays a role in the regulation of DNA synthesis through a mechanism mediated by estrogens.

Administration of salmon calcitonin for 1 year to Sprague-Dawley and Fisher 344 rats was associated with an increased incidence of pituitary gland hyperplasia and adenomas. The pituitary tumors produced the common α subunit of the glycoprotein hormones (LH, FSH, and TSH), a type of tumor that has been shown to comprise a significant fraction of pituitary tumors in humans. After treatment with calcitonin, serum α-subunit levels were increased by at least 20-fold in Sprague-Dawley males (Figure 58.32) and 4-fold in male Fischer rats. There was a good correlation between histopathologic evidence of α-subunit-producing pituitary tumors and elevated serum levels.

Studies have not determined whether the effects of calcitonin are direct or indirect. Calcitonin is produced in large amounts in the posterior hypothalamus and median eminence, where it may normally exert an effect on the hypothalamus–pituitary axis. Calcitonin receptors have been identified in the hypothalamus, and lower numbers of receptors are found in the pituitary gland. A striking feature of calcitonin-induced pituitary tumors and elevated serum α-subunit levels was the predilection for male compared with female rats. The basis for sex- and species-specific effects of calcitonin has not been determined. The relevance of the effects of calcitonin in the rat pituitary gland to human pathophysiology is uncertain. Neither the treatment of patients with calcitonin nor the treatment of patients with the multiple endocrine neoplasia syndrome II with medullary thyroid cancer and elevated calcitonin levels have resulted in the development of pituitary tumors. The doses of calcitonin used were from 25- to 50-fold greater on a per weight basis than doses administered to patients. In addition, rats of several strains are known to be highly predisposed to develop pituitary tumors compared to humans.

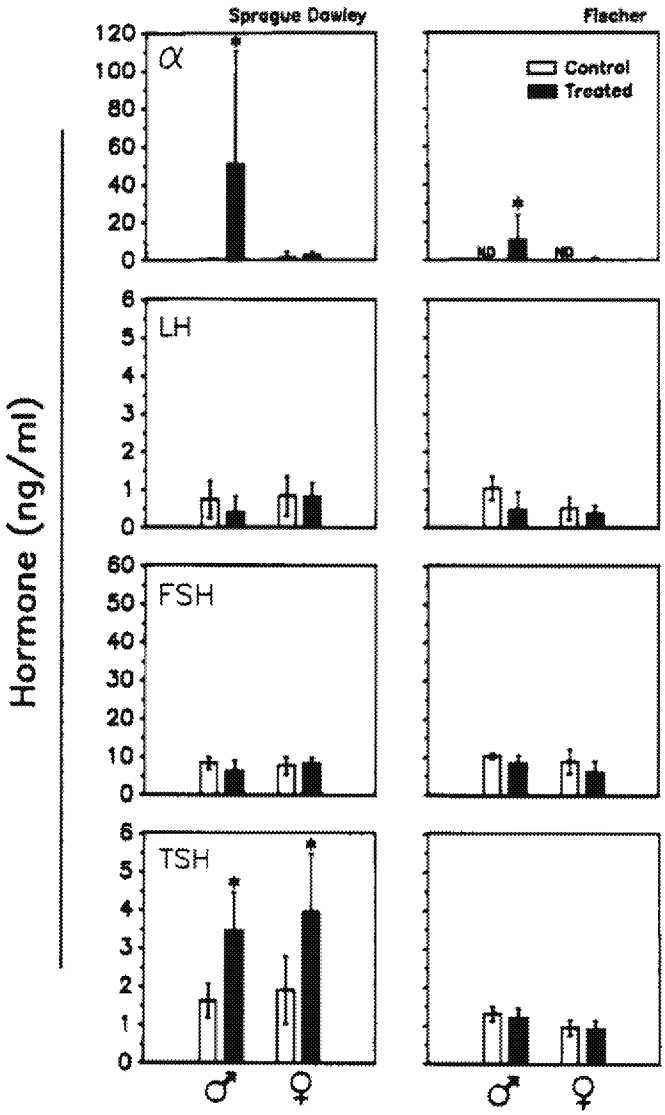

FIGURE 58.32 Serum glycoprotein hormone levels in rats treated chronically with salmon calcitonin. Sprague-Dawley or Fischer rats were treated for 52 weeks with vehicle (open bars) or calcitonin (80 IU/kg/day) (black bars). Results are the mean ± SD; $^*P < 0.05$. *Figure reproduced from Jameson* et al. *(1992) Glycoprotein hormone alpha-subunit-producing pituitary adenomas in rats treated for one year with calcitonin,* Am. J. Pathol. *140, 75–84, with permission.*

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11.2. Etiology and Pathogenesis of Spontaneous Pituitary Adenomas

Numerous hypotheses have been invoked to explain the high incidence of pituitary adenomas in certain inbred rat strains. Both hereditary factors and the levels of circulating sex steroids have been suggested as important etiological mechanisms. The hypothalamus has also been incriminated in the development of these tumors. Age-related hypothalamic changes may result in the diminished activity of dopamine, the major prolactin inhibitory factor. In this regard, studies of aging male rats have demonstrated abnormal concentrations and metabolism of hypothalamic dopamine, norepinephrine, and serotonin, and decreased hypothalamic prolactin inhibitory activities.

12. RESPONSE TO INJURY

12.1. Non-Neoplastic Lesions

The pituitary gland develops from a dorsal evagination of the oropharyngeal ectoderm (Rathke's pouch or craniopharyngeal duct) for the adenohypophysis, and a ventral downgrowth of diencephalic neuroectoderm for the neurohypophysis. Pituitary cysts developing spontaneously from remnants of Rathke's pouch in rodents are usually of microscopic size, but occasional larger cysts may develop from these remnants. The cysts either are confined to the adenohypophysis or may be joined to the lumen of the craniopharyngeal duct. The cysts are typically lined by a ciliated cuboidal to columnar epithelium, and may be uni- or multilocular. Squamous epithelium may also line the cysts. The cysts are often filled with a proteinaceous eosinophilic material. Cysts may contain PAS-positive acini with apical, basal, and myoepithelial cells that resemble parotid glands. Rathke's pouch (craniopharyngeal duct) cysts typically occur in the pars distalis and pars intermedia. Cysts may occur rarely in the neurohypophysis.

Pituitary dwarfism in dogs usually is associated with a failure of the oropharyngeal ectoderm of Rathke's pouch to differentiate into trophic hormone-secreting cells of the pars distalis, and occurs most frequently in German Shepherd dogs.

The term "cystoid degeneration" has been used to describe foci of cell loss in the adenohypophysis. Foci of cystoid degeneration usually measure 50–150 mm in diameter. The edges of these foci are composed of cell types of the pars distalis. Occasional cell debris may be found within areas of cystoid degeneration, together with granular eosinophilic material. Cystoid degeneration may also occur in hyperplastic foci and in neoplasms of the pituitary. The frequency of cystoid degeneration is increased by feeding diets containing diethylstilbestrol to female C3H HeN (MMTV+ mice).

12.2. Neoplastic Lesions

Craniopharyngioma

The craniopharyngioma is an uncommon, naturally occurring neoplasm in rodents. The tumor may originate from the craniopharyngeal duct epithelium of Rathke's pouch, and is composed of nests and cords of squamous cells with areas of cyst formation. As these tumors enlarge, they may extend into the adjacent brain; however, the tumors rarely metastasize.

In dogs, craniopharyngioma is a benign neoplasm that is derived from epithelial remnants of the oropharyngeal ectoderm of the craniopharyngeal duct (Rathke's pouch). It often occurs in young dogs, and is present in either a suprasellar or an infrasellar location. Craniopharyngiomas often are large and grow along the ventral aspect of the brain, where they can surround several cranial nerves. In addition, they extend dorsally into the hypothalamus and thalamus. Microscopically, craniopharyngiomas have alternating solid and cystic areas. The solid areas are composed of nests of epithelial cells (cuboidal, columnar, or squamous) with focal areas of mineralization. The cystic spaces are lined by either columnar or squamous cells, and contain keratin debris and colloid. It has been proposed that some pleomorphic neoplasms in the suprasellar region of younger dogs be classified as primary germ cell tumors rather than craniopharyngiomas. The diagnosis of germ cell tumors was based on three criteria: (1) midline suprasellar location; (2) presence of several distinct cell types (resembling a seminoma, dysgerminoma, teratoma, and secretory

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glandular and squamous elements); and (3) positive staining for α-fetoprotein.

Pituicytoma

Primary neoplasms of the pars nervosa are extremely uncommon. The tumors that have been reported to occur at this site have been designated as pituicytomas. These naturally occurring tumors are composed of small, closely packed spindle cells arranged in cords and bundles. Most pituicytomas are quite small, but larger tumors may extend into the adenohypophysis and into the adjacent brain tissue. Metastases have not been reported. Occasionally, the larger tumors may be impossible to distinguish from gliomas or meningiomas of the brain. Pituicytomas that stain positive for glial fibrillary acid protein are consistent with astrocytomas.

Proliferative Lesions of the Adenohypophysis

The histopathological separation among nodular hyperplasia, adenoma, and carcinoma often is more difficult in endocrine glands, such as the pituitary, than in most organs of the body. However, criteria for their separation should be established and applied in a uniform manner. For trophic hormone-secreting cells of the adenohypophysis, there appears to be a continuous spectrum of proliferative lesions between diffuse or focal hyperplasia and adenomas. Prolonged stimulation of pituitary secretory cells predisposes to the subsequent development of a higher than expected incidence of tumors.

Focal ("nodular") hyperplasia usually appears as multiple small areas that are well demarcated but not encapsulated from adjacent normal cells. Cells making up an area of focal hyperplasia in the adenohypophysis closely resemble the cells of origin; however, the cytoplasmic area may be slightly enlarged and the nucleus more hyperchromatic than in normal cells.

Adenomas usually are solitary nodules that are larger than the multiple areas of focal hyperplasia. They are sharply demarcated from the adjacent normal pituitary glandular parenchyma, and there often is a thin, partial to complete, fibrous capsule. The adjacent parenchyma is compressed to varying degrees depending on the size of the adenoma. Cells composing an adenoma may closely resemble the cells of origin morphologically and in their architectural pattern of arrangement; however, there often are histological differences such as multiple layers of cells lining follicles and vascular trabeculae or solid clusters of secretory cells subdivided into packets by a fine fibrovascular stroma.

Carcinomas usually are larger than adenomas in the pituitary, and generally result in a macroscopically detectable enlargement. The separation between adenoma and carcinoma of an endocrine gland often is difficult using only morphological criteria. Histopathological features that are suggestive of malignancy include extensive intraglandular invasion, invasion into adjacent structures (e.g., dura mater, sphenoid bone), formation of tumor cell thrombi within vessels, and, particularly, the establishment of metastases at distant sites. The growth of neoplastic cells subendothelially in highly vascular benign tumors should not be mistaken for vascular invasion. Malignant endocrine cells often are more pleomorphic (including oval or spindle shaped) than normal, but nuclear and cellular pleomorphism is not a consistent criterion to distinguish adenoma from carcinoma in the adenohypophysis of rodents. Mitotic figures may be frequent in malignant cells, but the significance of this criterion can vary considerably with the degree of background stimulation of the endocrine gland.

Many neoplasms derived from parenchymal cells of the pituitary are functionally active, secrete an excessive amount of hormone either continuously or episodically, and result in clinical syndromes of hormone excess. Quantitation of hormone levels in the serum of plasma in the basal or stimulated state and/or the measurement of the hormone or metabolites in the urine over a 24-hour period of excretion is essential to confirm that an endocrine tumor is functional and releasing hormone at an abnormally elevated rate. Morphologically, an endocrine tumor often can be interpreted as endocrinologically active if the rim of normal tissue around the tumor undergoes trophic atrophy due to negative feedback inhibition by the elevated hormone levels or an altered blood constituent. In response to the autonomous secretion of hormone by the tumor, these non-neoplastic secretory cells, especially the cytoplasmic area, become smaller than normal, and eventually the number of cells is decreased. Functional

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pituitary neoplasms secreting an excess of a particular trophic hormone (e.g., ACTH or prolactin) will be associated with striking hypertrophy and hyperplasia of target cells in the adrenal cortex (e.g., zonae fasciculata and reticularis) or mammary gland (ductal hyperplasia, galactocele formation, and increased incidence of mammary tumors) in chronic rodent studies.

Adenoma of Pars Intermedia

Adenomas of the pars intermedia are uncommon in the rat. These lesions may vary considerably in size. Larger adenomas may compress the adenohypophysis and neurohypophysis, whereas smaller lesions may be of microscopic dimensions. These tumors are nonencapsulated but do compress the adjacent normal tissue. The cells often have faintly basophilic cytoplasm with round to ovoid nuclei. Tumor cells in pigmented rats (e.g., Long-Evans) often contain melanin pigment similar to that found in the normal intermediate lobe. Tumors of the pars intermedia have not been produced experimentally in the rat, but some data indicate that estrogen administration to the Syrian hamster is related to the development of hyperplasia and adenomas in the intermediate lobe. In contrast to the relative rarity of adenomas derived from cells of the pars intermedia in the rat, these tumors are common neoplasms in the horse and dog.

Adenomas derived from cells of the pars intermedia are the most common type of pituitary tumor in horses and the second most common type in dogs, infrequent in non-human primates, and rare in other species. Two cell populations have been identified in the pars intermedia of normal dogs by immunocytochemistry. The predominant cell type (A cell) stains strongly for α-MSH. A second cell type (B cell) stains intensely for ACTH but not for α-MSH. This second cell population may account for the high bioactive ACTH concentration found in the pars intermedia of dogs and may give rise to ACTH-secreting adenomas of the pars intermedia in dogs with the syndrome of cortisol excess.

Pituitary Carcinoma

Pituitary carcinomas are uncommon neoplasms compared with adenomas. They usually are endocrinologically inactive, but can cause significant functional disturbances by destruction of the pars distalis and neurohypophyseal system, leading to adult-onset panhypopituitarism and diabetes insipidus. Carcinomas are large and invade extensively into the overlying brain, along the ventral aspect of the cranial cavity incorporating cranial nerves (II, III, IV), and locally into the adjacent sphenoid bone of the sella turcica, where they may cause osteolysis. Metastases occur infrequently to regional lymph nodes or to distant sites such as the spleen or liver. Carcinomas are highly cellular and often have large areas of hemorrhage and necrosis. Giant cells, nuclear pleomorphism, and mitotic figures are encountered more frequently than in adenomas.

12.3. Functional Characteristics of Pituitary Adenomas

The vast majority of pituitary adenomas in humans and rodents have been described as chromophobic in type; however, many of these tumors have been found to stain for prolactin by immunohistochemistry. Of the prolactin-producing tumors, most are sparsely granulated with low levels of prolactin immunoreactivity by immunohistochemistry and small numbers of secretory granules measuring 100–200 μm in diameter by electron microscopy. Diffuse hyperplasia of the prolactin cells has been noted adjacent to some adenomas.

The development of tumors and hyperplasia of prolactin-secreting cells is accompanied by increasing serum levels of prolactin. Occasionally, prolactin cells within adenomas may be admixed with FSH/LH-, TSH-, or ACTH-positive cells. Infrequent adenomas composed of populations of ACTH or FSH/LH cells have also been reported (Figure 58.33). Pituitary nodules (focal hyperplasia and adenomas) composed primarily of gonadotrophs occur commonly in male rats of the Lobund-Wistar strain.

Functional (endocrinologically active) neoplasms arising in the pituitary gland of dogs most likely are derived from corticotroph (ACTH-secreting) cells in either the pars distalis or the pars intermedia. These neoplasms cause a clinical syndrome of cortisol excess (Cushing's disease). The pituitary gland is consistently

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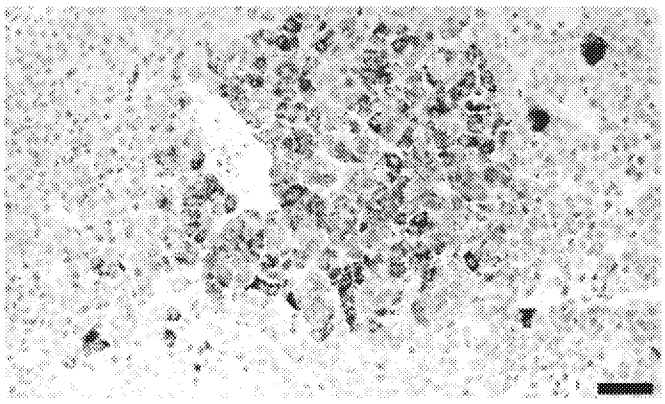

FIGURE 58.33 Pituitary adenoma in an old male Long-Evans rat. The section was stained for the β chain of luteinizing hormone. Occasional cells have intense immunoreactivity whereas others have only moderate to weak immunoreactivity. Bar = 100 μm. *Figure reproduced from Lee* et al. *(1982) Pituitary proliferative lesions in aging male Long-Evans rats: A model of mixed multiple endocrine neoplasia syndrome,* Lab. Invest. *47, 595–602, with permission.*

enlarged. Because the diaphragma sella is incomplete in the dog, the line of least resistance favors dorsal expansion of the gradually enlarging pituitary mass with extension into the overlying brain leading to eventual compression or replacement of the hypothalamus, and possible extension into the thalamus. Pituitary corticotroph adenomas are composed of well-differentiated, large or small, chromophobic cells supported by fine connective tissue septa. The cytoplasm of the neoplastic cells stains immunocytochemically for ACTH and MSH. Hormone-containing secretory granules can be demonstrated by electron microscopy. Bilateral enlargement of the adrenal glands occurs in dogs with functional corticotroph adenomas due to cortical hypertrophy/hyperplasia, primarily of the zonae fasciculata and reticularis.

12.4. Spontaneous Pituitary Adenomas

The high frequency of spontaneous pituitary adenomas in laboratory rats is a well-recognized phenomenon that must be considered in any long-term toxicological study. The incidence of these tumors is determined by many factors, including strain, age, sex, reproductive status, and diet. Studies from the National Toxicology Program (NTP) historical data base of 2-year-old F344 rats have shown that the incidence of pituitary adenomas was 21.7% and 44% for males and females, respectively. Corresponding figures for carcinomas arising in the adenohypophysis were 2.4% and 3.5% for males and females, respectively. Studies from 56 NTP rat studies completed between 1983 and 1987 revealed that the incidence of adenomas was 28.6% for males and 43.3% for females, while a diagnosis of pituitary hyperplasia was made in 10.4% of males and 10.3% of females.

Numerous studies have called attention to the fact that there is a striking degree of strain variation in the incidence of pituitary tumors in rats, which has been reported to range from 10% to more than 90%. Particularly high incidences have been reported in Wistar, WAG/Rij, Osborne-Mendel, Long-Evans, Amsterdam, and Columbia-Sherman rats. In the BN/Bi strain, pituitary adenomas have been noted in 26% of females and 14% of males. Adenomas were identified in 95% of females and 96% of males in the WAG/Rij strain, whereas (WAG/Rij 3 BN) F_1 rats had incidences of 83% for females and 64% for males.

There is a striking relationship between age and the development of pituitary adenomas. These lesions are uncommon in animals less than 1 year of age. When present in this age group, they are generally small and are present as single lesions. Pituitary adenomas are most common in rats at 2 years or more of age, and tend to be larger and more frequently multicentric than in younger animals.

Rapid body growth rates and high levels of conversion of feed to body mass in early life, or high protein intake in early adult life, predispose any strain of rat to the development of pituitary adenomas. In rats fed a low-protein diet (less than 12.7% crude protein), the overall tumor incidence, incidence of fatal tumors, number of multifocal tumors, and degree of cellular atypia within tumors are significantly lower than in rats fed a standard diet.

Dopamine suppresses prolactin secretion. The dopamine agonist, bromocriptine, has been reported to decrease serum prolactin levels in female Long-Evans rats with spontaneously developing prolactinomas when compared to untreated age-matched controls, and the mean pituitary weight was reduced in rats treated for 44 days with bromocriptine compared to controls.

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13. TESTING FOR TOXICITY

13.1. Morphologic Evaluation

Immunohistochemical evaluation is an important approach for the functional and morphological analysis of normal and abnormal pituitary glands. Hormones of the various types of adenohypophyseal cells in the rodent gland have cross-reactions with antibodies to corresponding human pituitary hormones. However, higher levels of specificity may be achieved with the use of antibodies to the rat pituitary hormones. For most routine toxicological studies, formalin fixation provides adequate preservation of the pituitary hormones for immunohistochemical studies; however, some authors have recommended Bouin's fixation for immunohistochemical staining of the pituitary because this fixative produces more precise localization of the hormones at the light microscopic level. In addition, *in situ* hybridization techniques have been employed for the identification of messenger RNAs encoding the hormonal peptides.

PART IV: THYROID C CELLS

14. STRUCTURE AND FUNCTION

C cells or parafollicular cells of the thyroid gland, named after their major secretory product (calcitonin), are located within thyroid follicles between the basal aspects of the follicular cells and the basement membrane of the follicle or are present in a parafollicular position. In addition to calcitonin, C cells contain a variety of other peptides, including somatostatin and bombesin, and they are also positive for a wide variety of generic neuroendocrine markers, including chromogranin proteins and synaptophysin. In most mammalian species, C cells are concentrated within the central regions of the lobes, being most prominent at the levels of the parathyroid glands. By light microscopy C cells often have a clear or light appearance, but silver-positive cytoplasmic granules can be demonstrated by argyrophilic staining. At the ultrastructural level, the cells contain variable numbers of membrane-bound secretory granules in which calcitonin is stored prior to its release into the circulation. Although the secretory granules have considerable heterogeneity in terms of size and density, all of the granules contain immunoreactive calcitonin.

Calcitonin is a 32-amino acid peptide that is derived from a 141-amino acid precursor (procalcitonin). The gene that encodes calcitonin consists of 6 exons that are alternatively spliced in a tissue-specific manner to yield the mRNAs encoding calcitonin and the calcitonin gene-related peptide (CGRP). The mRNA encoding calcitonin results from splicing of the first 4 exons and represents more than 95% of the mature transcripts in the thyroid. Splicing of the first 3 exons to exons 5 and 6 results in a mRNA that encodes alpha-CGRP. Alpha-CGRP is expressed in many tissues and is the only mature transcript of the calcitonin gene detected in neural tissues. A second CGRP gene encodes the closely related beta-CGRP. The tissue distributions of alpha- and beta-CGRP are similar.

The levels of ionized calcium in plasma and extracellular fluids are the major physiologic stimulus for the secretion of calcitonin. The cell membrane calcium-sensing receptor, cloned from parathyroid cells, is also expressed in C cells and contributes to the regulation of calcitonin secretion. Calcitonin interacts with specific receptors in target cells, principally in bone and kidney. Calcitonin inhibits osteoclast activity in bone, renal tubular resorption of Ca^{2+}, and Ca^{2+} absorption by the intestines. Calcitonin secretion is increased in response to a high Ca^{2+} meal often before a significant rise in plasma Ca^{2+} levels can be detected. An oral Ca^{2+} load stimulates the secretion of gastrin, cholecystokinin, and glucagon, and these hormones also serve as calcitonin secretagogues. Calcitonin also protects against Ca^{2+} loss from the skeleton during periods of Ca^{2+} mobilization, such as growth, pregnancy, and lactation.

15. MECHANISMS OF TOXICITY

Radiation administered to newborn rats is followed by a striking reduction in the numbers of C cells. The numbers of C cells increase after 1 year, but their distribution within the gland is often patchy, suggesting a clonal regrowth. Further studies have demonstrated that the incidence of C-cell tumors is increased after

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irradiation, but high levels of Ca^{2+} in drinking water do not appear to potentiate the carcinogenic effects of irradiation in C cells. However, it has been noted that a significant increase in C-cell tumor incidence has been associated with excess Vitamin D_3 in the diet. These findings suggest that Vitamin D or one of its metabolites may affect C-cell growth directly. It is known that certain Vitamin D metabolites can stimulate calcitonin secretion in Vitamin D-deficient pigs.

The administration of the glucagon-like peptide (GLP-1) analogs, such as liraglutide, results in calcitonin release, upregulation of calcitonin gene expression, and C-cell proliferative lesions in rats and, to a lesser extent, in mice. In long-term rodent studies (104 weeks), C-cell tumors increased in a dose-dependent manner. Liraglutide-treated rats had significantly more adenomas and carcinomas than control rats, while treated mice had only significantly more adenomas than controls. In contrast to rodents, humans and/or cynomolgus monkeys had low GLP-1 receptor expression in thyroid C cells, and GLP-1 receptor agonists did not activate adenylyl cyclase or generate calcitonin release in primates. Moreover, prolonged treatment with liraglutide did not lead to C-cell hyperplasia in monkeys. Other pharmaceutical agents, such as the parathyroid hormone analog teriparatide, have been associated with the development of C-cell tumors in rodents.

16. RESPONSE TO INJURY

16.1. Proliferative Lesions of C Cells

Hyperplasia of C cells is not usually associated with any macroscopic abnormality in the thyroid gland. Two types of hyperplasia are evident microscopically: diffuse (Figure 58.34) and focal (nodular) (Figure 58.35). In diffuse hyperplasia, the numbers of C cells are increased throughout the thyroid lobes, to a point where they may be more numerous than follicular cells. The relationship of C cells to follicular cells in cases of diffuse hyperplasia is similar to that noted in juvenile thyroid glands. Focal (nodular) hyperplasia of C cells often occurs concurrently with diffuse hyperplasia in the thyroid glands of rats. The follicles become progressively enlarged with increasing numbers of C cells. While normal and hyperplastic C cells are uniformly positive for calcitonin, relatively few also express somatostatin.

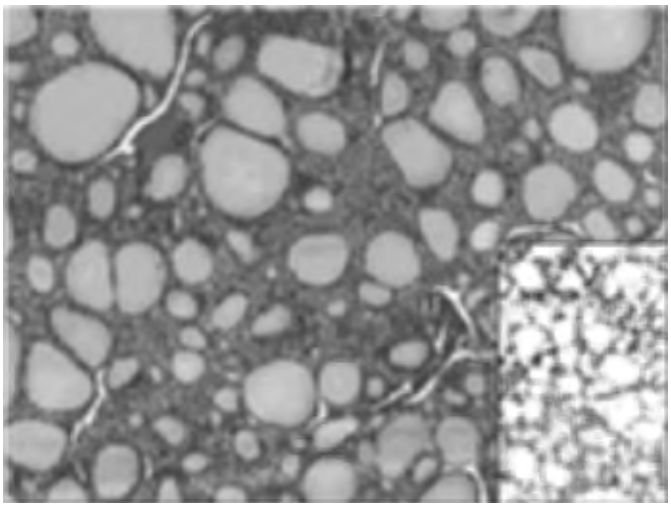

FIGURE 58.34 Diffuse C-cell hyperplasia (rat). There is a diffuse increase in C cells throughout the thyroid lobe (H&E). Inset: Immunostaining for calcitonin.

C cells are more numerous in rats (compared to mice) and increase in number with age. C cells in rats can be identified in routine H&E-stained tissue sections. Normal and diffuse hyperplasia of C cells in mice typically requires immunohistochemistry for calcitonin to identify the C cells.

At later stages of development, hyperplastic C-cell-filled follicles often assume irregular, twisted and elongate configurations. Occasional colloid-filled follicles are entrapped among the proliferating C cells. The histological distinction between focal hyperplasia and adenoma of C cells is often difficult to define. Typically, focal (nodular) hyperplasia is characterized by lesions measuring less than five average colloid-containing follicles without compression of the adjacent follicular cells. Cell boundaries are often indistinct.

C-cell adenomas are discrete expansive masses, or nodules of C cells larger than five average colloid-containing follicles (Figure 58.36). Adenomas are either well circumscribed, or partially encapsulated with varying degrees of compression of adjacent thyroid follicles. C cells have relatively abundant cytoplasm that is faintly eosinophilic with a round to ovoid nucleus with finely stippled chromatin. In some cases, individual tumor cells may be spindle-shaped.

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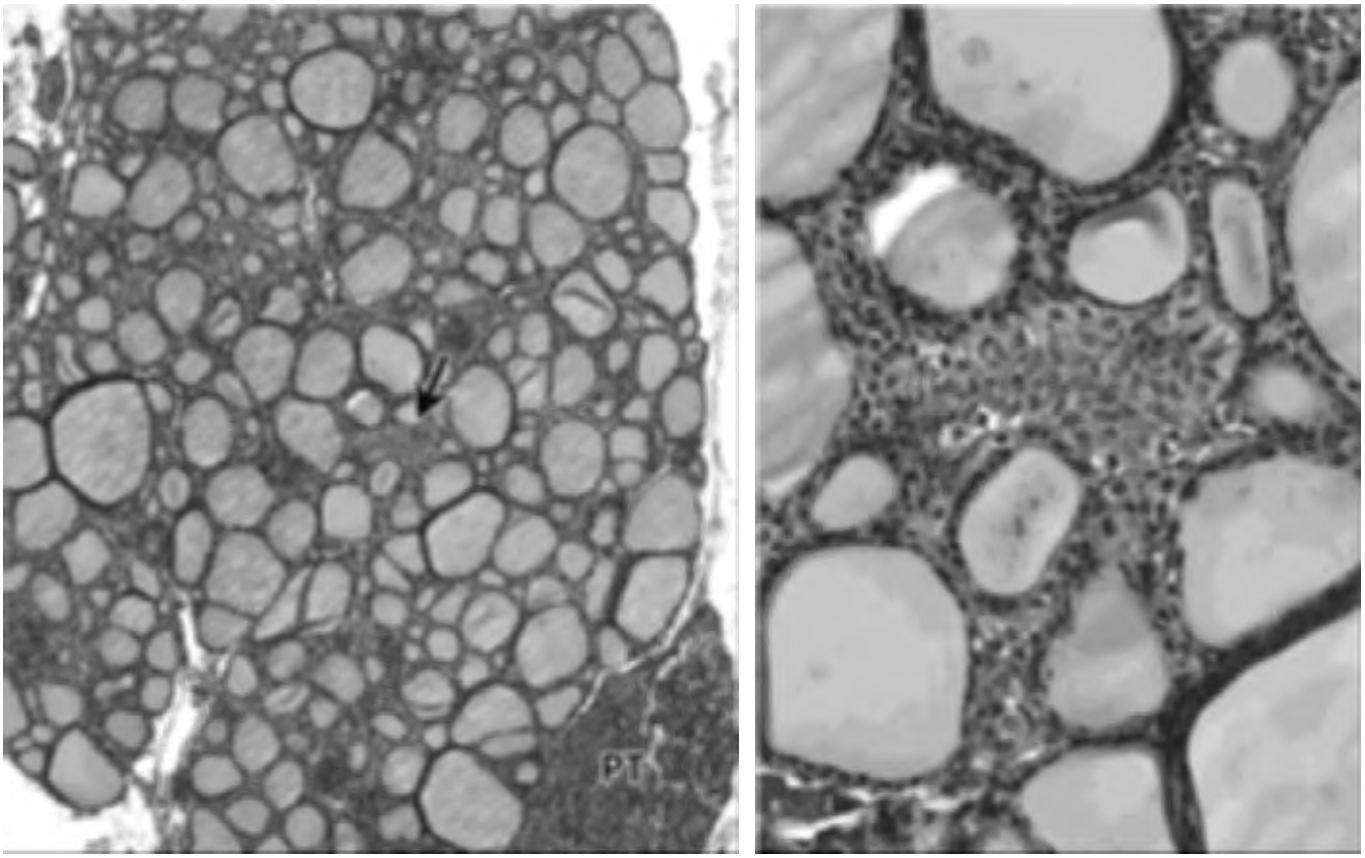

FIGURE 58.35 Diffuse and focal (nodular) C-cell hyperplasia (rat). Low-power view (left) of diffuse and focal (arrow) hyperplasia in the thyroid lobe; PT, parathyroid gland. Higher magnification (right) of focal hyperplasia. A single follicle in this field is expanded by proliferating C cells. H&E stain.

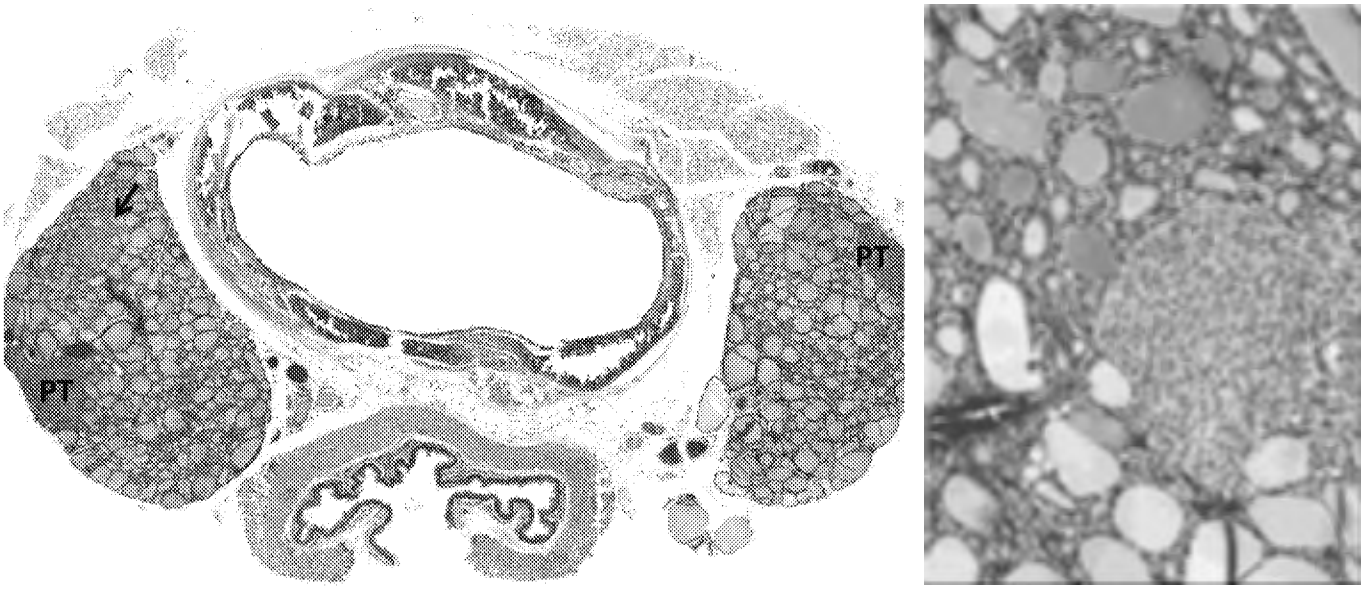

FIGURE 58.36 C-cell adenoma (rat). Low magnification of small C-cell adenoma (arrow) (left); PT, parathyroid gland. Higher magnification of C-cell adenoma (right) with a few follicles entrapped at its periphery. H&E stain.

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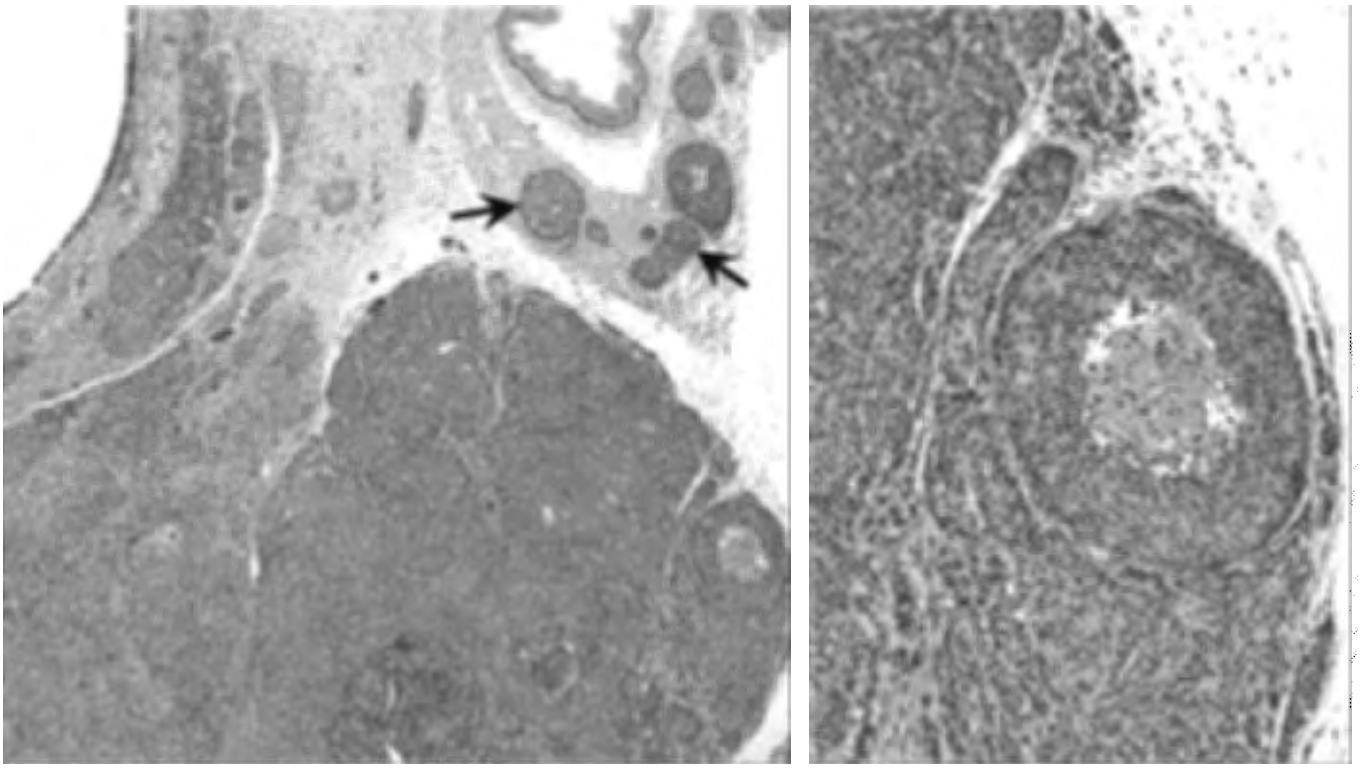

FIGURE 58.37 C-cell carcinoma (rat). Low-magnification view of C-cell carcinoma (left). This tumor involved the entire lobe with foci of lymphovascular invasion of the adjacent tissues (arrows). High-magnification view of tumor (right) with focal central necrosis. H&E stain.

Adenomas may be subdivided into small cell groups by fine connective tissue septae and capillaries, some of which may be distended with red blood cells. Amyloid deposits may be present in focal hyperplasia and adenomas.

C-cell (medullary) carcinomas are characterized by infiltration of C cells into the adjacent thyroid stroma, extracapsular tissues, and lymphovascular channels (Figure 58.37). Areas of hemorrhage and necrosis may be particularly prominent in larger tumors. The component C cells are often more pleomorphic (cuboidal, oval, spindle-shaped) than those comprising focal hyperplasia or C-cell adenomas. Mitotic activity may be prominent in some cases, and foci of necrosis may be evident (Figure 58.37). Occasional tumors may be composed of large cells with amphophilic cytoplasm, and large vesicular nuclei containing coarse chromatin and prominent nucleoli. The latter cells may bear a striking resemblance to ganglion cells. C-cell carcinomas often exhibit considerable heterogeneity in calcitonin immunoreactivity, in contrast to adenomas which generally have more uniform staining. Most C-cell carcinoma cells and some C-cell adenoma cells express somatostatin in addition to calcitonin. Amyloid deposits may be present between tumor cells, around blood vessels, and in the interstitium.

16.2. Spontaneous C-Cell Lesions

C-cell proliferative lesions, including adenomas and carcinomas/ultimobranchial body tumors, occur in a wide variety of domestic animals, including bulls, horses, sheep, and dogs. Up to 30% of aged bulls have been reported to develop C-cell tumors (predominantly adenomas) and 15–20% have evidence of C-cell hyperplasia. The tumors develop most commonly in bulls fed a high Ca^{2+} diet, while they occur rarely in cows. Affected bulls develop a spectrum of skeletal abnormalities, including vertebral osteosclerosis with ankylosing spondylosis deformans, osteophytes, vertebral fractures, and degenerative osteoarthrosis. Both lymph node and pulmonary metastases may occur.

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Pheochromocytomas may develop in association with C-cell tumors, and in some bovine breeds (Guernsey) an autosomal dominant mode of inheritance has been proposed.

C-cell tumors occur spontaneously in many rat strains, including the Long-Evans, Osborne Mendel, Buffalo, Harlan Sprague-Dawley, Fischer 344/N, WAG/Rij, and Wistar strains. There is a striking correlation between the age of the animals and the extent of C-cell abnormalities. For example, approximately 80% of Long-Evans rats between 12 and 24 months of age have extensive diffuse and/or nodular C-cell hyperplasia, with C-cell adenomas or carcinomas occurring in 14%. By the age of 24–36 months, approximately 25% of the rats have evidence of C-cell adenomas or carcinomas. Rats with C-cell tumors also commonly have adrenal medullary hyperplasia, pheochromocytomas, and pituitary adenomas. In contrast to the high frequency of spontaneous C-cell adenomas and carcinomas in the rat, these tumors are rare in the mouse.

Spontaneous C-cell tumors in humans are uncommon, accounting for less than 10% of all thyroid malignancies. Autosomal dominant forms of these tumors occur in association with the multiple endocrine neoplasia (MEN) 2 syndromes. MEN2A is characterized by the presence of C-cell and adrenal medullary proliferative lesions in association with parathyroid hyperplasia. MEN2B is characterized by C-cell and adrenal medullary lesions, often in association with a Marfanoid habitus and gastrointestinal ganglioneuromatosis. Isolated medullary thyroid carcinoma occurs in patients with familial medullary thyroid carcinoma (FMTC).

16.3. Molecular Genetics

The heritable forms of medullary thyroid carcinoma in humans develop as a consequence of germline mutations in the RET proto-oncogene. MEN2A most commonly develops as a result of mutations affecting codons 609, 611, 618, 620, and 634, while MEN2B most commonly results from mutations in codons 918 and 813. FMTC shares many of the same mutations as MEN2A. A significant number of sporadic human C-cell tumors have somatic mutations affecting codon 918. Mutations similar to those occurring in human C-cell tumors have been sought in spontaneously occurring C-cell tumors in the WAG/Rij strain, but none has been found.

Several transgenic mouse models expressing the various MEN2A- and 2B-associated mutations have been developed. Animals of three independent transgenic mouse lines bearing a MEN2A mutation under the control of the CGRP/calcitonin promoter developed C-cell hyperplasia as early as 3 weeks of age and subsequently developed bilateral and multifocal C-cell tumors. Some transgenic mice also developed follicular cell tumors resembling human papillary thyroid carcinomas. C-cell tumors have also been observed in transgenic mice bearing MEN2B mutations.

PART V: THYROID FOLLICULAR CELLS

17. STRUCTURE AND FUNCTION

17.1. Organogenesis

The thyroid gland originates as a thickened plate of epithelium in the floor of the pharynx. It is intimately related to the aortic sac in its development, and this association leads to the frequent occurrence of accessory thyroid parenchyma in the mediastinum. This accessory thyroid tissue may undergo neoplastic transformation as, for example, in the adult dog. The ultimobranchial bodies fuse with the lateral extensions and deliver C cells (neural crest origin) to each thyroid lobe. A portion of the thyroglossal duct may persist postnatally and form a cyst, which develops in the ventral aspect of the anterior cervical region. Their lining epithelium may undergo neoplastic transformation and give rise to papillary carcinomas in dogs.

Accessory thyroid tissue is common in the dog, and may be located anywhere from the larynx to the diaphragm. About 50% of adult dogs have accessory thyroids embedded in the fat on the intrapericardial aorta. These nodules are usually 1–2 mm in size and may number from one to five. They are completely lacking in C cells, but their follicular structure and function are the same as that of the main thyroid lobes.

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17.2. Thyroid Histology

The thyroid gland is the largest of the organs that function exclusively as endocrine glands. The basic structure of the thyroid is unique for endocrine glands, consisting of follicles of varying size (20–250 μm) that contain colloid produced by the follicular cells. Follicular cells are cuboidal to columnar, and their secretory polarity is directed toward the lumen of the follicles. An extensive network of inter-and intrafollicular capillaries provides the follicular cells with an abundant blood supply. Follicular cells have long profiles of rough endoplasmic reticulum and a large Golgi apparatus in their cytoplasm for the synthesis and packaging of substantial amounts of protein that are transported into the follicular lumen. The interface between the lumenal side of follicular cells and the colloid is modified by numerous microvillar projections.

17.3. Thyroid Hormone Synthesis

The biosynthesis of thyroid hormones is also unique among endocrine glands because the final assembly of the hormones occurs extracellularly within the follicular lumen. Essential raw materials, such as iodide, are trapped efficiently by follicular cells from plasma, transported rapidly against a concentration gradient to the lumen, and oxidized by a thyroid peroxidase in microvillar membranes and colloid to iodine (I_2) (Figure 58.38). The assembly of thyroid hormones within the follicular lumen is made possible by a unique protein (thyroglobulin) synthesized by follicular cells.

Thyroglobulin is a high molecular weight (600 000–750 000) glycoprotein synthesized in successive subunits on ribosomes of the endoplasmic reticulum in follicular cells. The constituent amino acids (tyrosine and others) and carbohydrates (i.e., mannose, fructose, galactose) come from the circulation. Recently synthesized thyroglobulin (17S) leaving the Golgi apparatus is packaged into apical vesicles and extruded into the follicular lumen. The amino acid tyrosine, an essential component of thyroid hormones, is incorporated in the molecular structure of thyroglobulin. Iodine is bound to tyrosyl residues in thyroglobulin at the apical surface of follicular cells to form, successively, monoiodotyrosine (MIT) and diiodotyrosine (DIT) (Figure 58.39). The resulting MIT and DIT combine to form the two biologically active iodothyronines – thyroxine, T_4, and triiodothyronine, T_3 – secreted by the thyroid gland.

The mechanism of active transport of iodide is associated with a sodium iodide (Na^+I^-) symporter (NIS). The transport of iodide ion across the thyroid cell membrane is linked to the transport of Na^+. The ion gradient generated by the Na^+/K^+-ATPase appears to be the driving force for the active cotransport of iodide. The transporter protein is present in the basolateral membrane of thyroid follicular cells (thyrocytes) and is a large protein containing 643 amino acids with 12 transmembrane domains. Other tissues, such as the salivary gland, gastric mucosa, and lactating mammary gland, also have the capacity to actively transport iodide, albeit at a much lower level than the thyroid. The NIS gene is complex (16 exons, 14 introns) and its expression in the thyroid is upregulated by TSH. The functionally active iodine transport system in the thyroid gland has important clinical applications in the evaluation, diagnosis, and treatment of several thyroid disorders, including cancer. Radioiodine is used to ablate residual tumor tissues as well as recurrent and metastatic thyroid cancer. Chemicals such as perchlorate and thiocyanate can selectively inhibit the NIS and active transport of iodide, thereby effectively blocking the ability of the gland to synthesize thyroid hormones.

17.4. Species Differences in Thyroid Hormone Economy

Long-term perturbations of the pituitary–thyroid axis by various xenobiotics or physiologic alterations (e.g., iodine deficiency, partial thyroidectomy, and natural goitrogens in food) are more likely to predispose the laboratory rat to a higher incidence of proliferative lesions (e.g., hyperplasia and adenomas of follicular cells) in response to chronic TSH stimulation than in the human thyroid. This is particularly true in the male rat, which has higher circulating levels of TSH than females. The greater sensitivity of the rodent thyroid to derangement by drugs, chemicals, and physiologic perturbations is also related to the shorter plasma half-life of T_4 than in man due to the considerable

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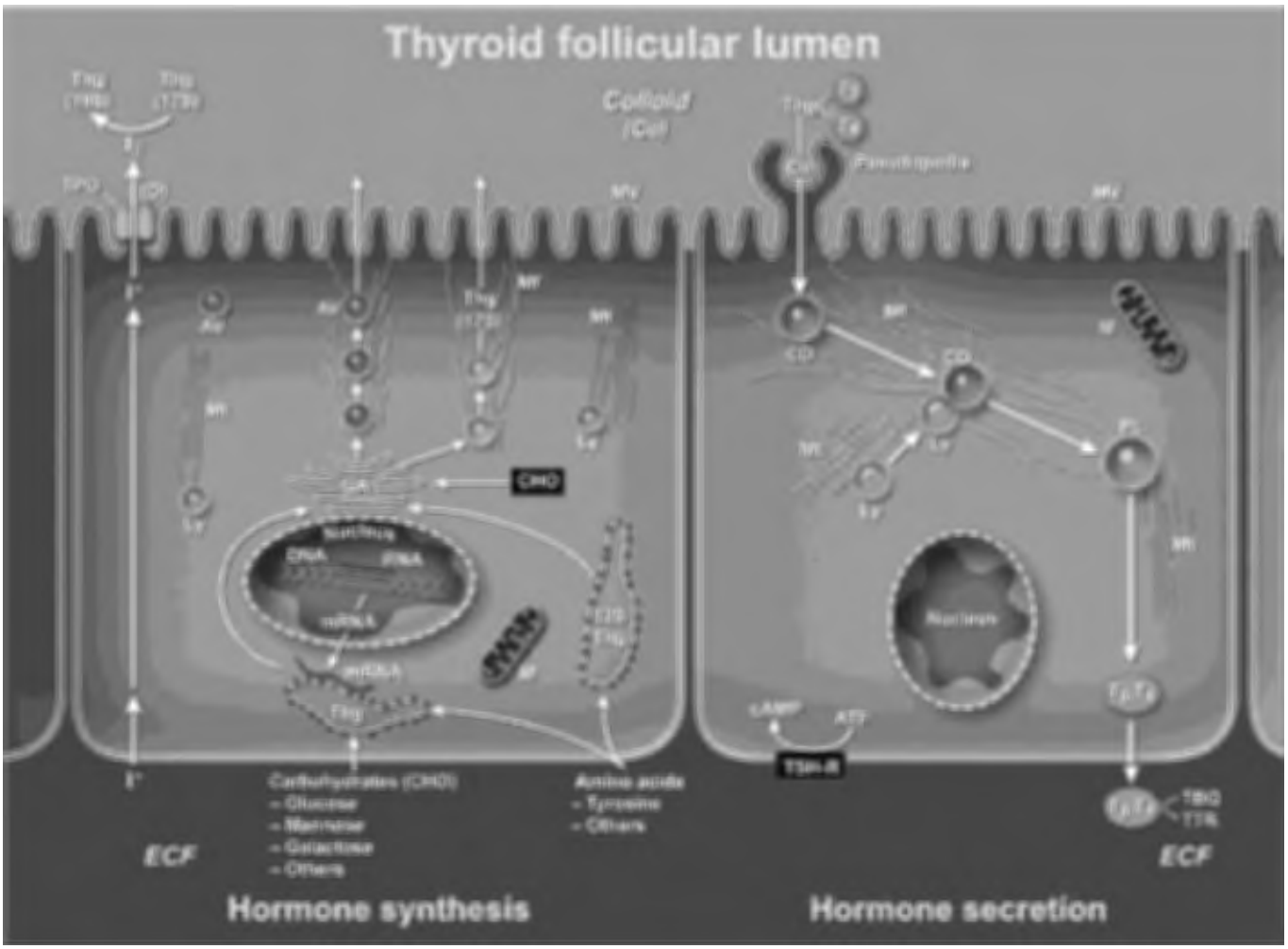

FIGURE 58.38 Normal thyroid follicular cells illustrating two-way traffic of materials from capillaries into the follicular lumen. Raw materials, such as iodide (I^-), are concentrated by follicular cells and are transported rapidly into the lumen (left). Amino acids (tyrosine and others) and sugars (CHO) are assembled by follicular cells into thyroglobulin (Thg), packaged into apical vesicles (AV), and released into the lumen. Iodination of tyrosyl residues occurs within the thyroglobulin molecule to form thyroid hormones in the follicular lumen. Elongation of microvilli and endocytosis of colloid by follicular cells occur in response to TSH stimulation (right). Intracellular colloid droplets (Co) fuse with lysosomal bodies (Ly) and the active thyroid hormone is cleaved enzymatically from thyroglobulin, and free T_4 and T_3 are released into circulation. Mf, microfilaments; M, mitochondria; Mt, microtubules; TBG, thyroxine binding globulin; TTR, transthyretin.

differences between species in the transport proteins for thyroid hormones.

The plasma T_4 half-life in rats is considerably shorter (12–24 hours) than in man (5–9 days). In human beings and monkeys, circulating T_4 is bound primarily to thyroxine-binding globulin (TBG), but this high-affinity binding protein is not present in rodents, birds, amphibians, or fish (Table 58.4).

The binding affinity of TBG for T_4 is approximately 1000 times higher than for transthyretin (prealbumin). The percentage of unbound active T_4 is lower in species with high levels of TBG than in animals in which T_4 binding is limited to albumin, transthyretin, and postalbumin. Therefore, a rat without a functional thyroid requires about 10 times more T_4 (20 μg/kg body weight) for full substitution than an adult human (2.2 μg/kg body weight). Triiodothyronine is transported bound to TBG and albumin in human beings, monkey, and dog, but only to albumin in mouse, rat, and chicken (Table 58.5).

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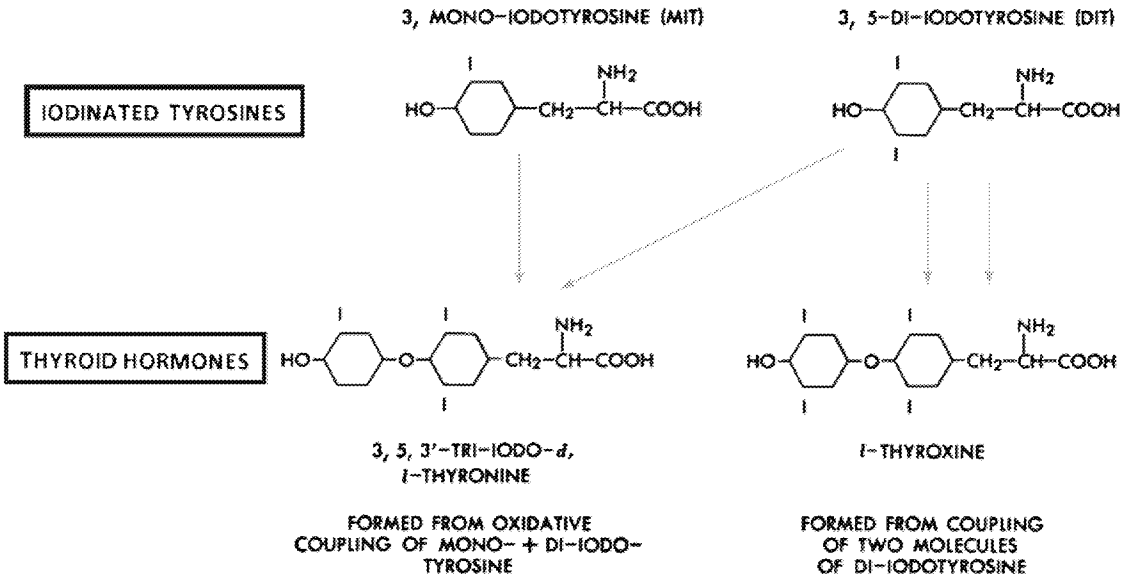

FIGURE 58.39 Formation of thyroid hormones (3,5,3′-triiodothyronine, l-thyroxine) from iodinated tyrosines (MIT and DIT) within the follicular lumen of the thyroid gland. *Figure reproduced from Capen and Martin (1989), The effects of xenobiotics on the structure and function of thyroid follicular cells and C-cells,* Toxicol. Pathol. ***17****, 266–293, with permission.*

TABLE 58.4 Thyroxine (T_4) Binding to Serum Proteins in Selected Vertebrate Species[a]

Species	T_4-binding globulin	Postalbumin	Albumin	Prealbumin (Transthyretin)
Human	++	–	++	+
Monkey	++	–	++	+
Dog	+	–	++	–
Mouse	–	++	++	–
Rat	–	+	++	+
Chicken	–	–	++	–

[a] *Degree of T_4 binding to serum proteins: + or ++. Absence of binding of T_4 to serum protein: –.*
Table modified from Döhler et al. *(1979) The rat as model for the study of drug effects on thyroid function: Consideration of methodological problems,* Pharmacol. Ther. *5: 305–318.*

In general, T_3 is bound less avidly to transport proteins than T_4, resulting in a faster turnover and shorter plasma half-life in most species (Table 58.5). These differences in plasma half-life of thyroid hormones and binding to transport proteins between rats and human beings may be one factor in the greater sensitivity of the rat thyroid to develop hyperplastic and/or neoplastic nodules in response to chronic TSH stimulation.

17.5. Thyroid Hormone Secretion

The elongation of microvilli and the formation of pseudopods on follicular cells initiate the secretion of thyroid hormones from stores within lumenal colloid. The pseudopods are increased by pituitary TSH and phagocytize a portion of adjacent colloid by endocytosis (Figure 58.40). Colloid droplets within follicular cells fuse

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TABLE 58.5 Triiodothyronine (T_3) Binding to Serum Proteins in Selected Vertebrate Species[a]

Species	T_3-binding globulin	Postalbumin	Albumin	Prealbumin (Transthyretin)
Human	+	–	+	–
Monkey	+	–	+	–
Dog	+	–	+	–
Mouse	–	+	+	–
Rat	–	–	+	–
Chicken	–	–	+	–

[a] *Degree of T_3 binding to serum proteins: + or ++. Absence of binding of T_3 to serum protein: –. Table modified from Döhler* et al. *(1979) The rat as model for the study of drug effects on thyroid function: Consideration of methodological problems,* Pharmacol. Ther. *5: 305–318.*

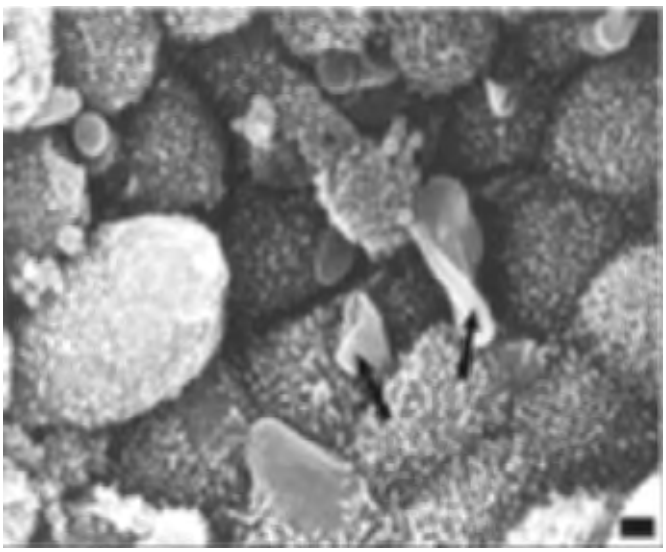

FIGURE 58.40 Scanning electron micrograph of the lumenal surface of thyroid follicular cells following TSH stimulation. Note the numerous expanded cytoplasmic processes (arrows) extending into the follicular+ lumen. Bar = 1 μm. *Figure reproduced from Capen and Martin (1989), The effects of xenobiotics on the structure and function of thyroid follicular cells and C-cells,* Toxicol. Pathol. ***17****, 266–293, with permission.*

with numerous lysosomal bodies that contain proteolytic enzymes and release thyroxine and triiodothyronine, which diffuse out of the follicular cells and enter interfollicular capillaries. Iodinated tyrosines (monoiodotyrosine [MIT] and diiodotyrosine [DIT]) released from the colloid droplets are deiodinated enzymatically, and the iodide generated under normal conditions either is recycled to the lumen to iodinate new tyrosyl residues in thyroglobulin or is released into the circulation.

Negative feedback control of thyroid hormone secretion is accomplished by the coordinated response of the adenohypophysis and certain hypothalamic nuclei to circulating and local tissue levels of T_3. In the rat, 50% or more of the pituitary content of T_3 is generated locally from circulating T_4 by a 5′-deiodinase (type II). A decrease in thyroid hormone concentration in plasma is sensed by groups of neurosecretory neurons in the hypothalamus that synthesize and secrete thyrotropin-releasing hormone (TRH; 361 Da) into the hypophyseal portal circulation. TRH binds to receptors on the plasma membrane of thyrotrophic basophils in the adenohypophysis and activates adenylyl cyclase. The intracellular accumulation of cyclic AMP in thyrotrophic basophils results in an inflow of Ca^{2+} that leads to release of TSH-containing secretory granules into pituitary capillaries.

Thyroid-stimulating hormone is conveyed to thyroid follicular cells, where it binds to the basilar aspect of the cell, activates adenylyl cyclase, and increases the rate of the biochemical reactions involved in the synthesis and secretion of thyroid hormones. One of the initial responses by follicular cells to TSH is the formation of cytoplasmic pseudopodia resulting in the increased endocytosis of colloid and the release of preformed hormone stored within the follicular lumen (Figure 58.40). If the secretion of TSH is sustained (hours or days), thyroid follicular cells become more columnar and follicular lumens

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become smaller due to the increased endocytosis of colloid. Numerous PAS-positive colloid droplets are present in the lumenal aspect of the hypertrophied follicular cells.

Conversely, in response to an increase in circulating levels of thyroid hormones (T_4 and T_3), there is a corresponding decrease in circulating pituitary TSH. Thyroid follicles become enlarged and distended with colloid due to the decreased TSH-mediated endocytosis of colloid. Follicular cells lining the involuted follicles are low cuboidal and have few endocytic vacuoles at the interface with the colloid.

17.6. Degradation and Metabolism

Thyroid hormones are degraded primarily by conjugation in the liver. Thyroxine is conjugated on the outer phenolic ring with glucuronic acid in a reaction catalyzed by thyroxine UDP glucuronosyltransferase, and conjugated T_4 is excreted in the bile. Triiodothyronine is conjugated, also on the outer ring, with sulfate in a reaction catalyzed by phenol sulfotransferase. Deamination and decarboxylation of the side chain at the other end of the thyroid hormone molecule and cleavage of the ether linkage are relatively minor pathways of degradation. A wide variety of drugs and chemicals can influence thyroid hormone metabolism by inducing one or more classes of hepatic microsomal enzymes that increase the degradation of thyroid hormones.

The stepwise monodeiodination of thyroxine in the liver, kidney, and elsewhere is also important in the metabolism of thyroid hormones. The removal of an iodine molecule from the 5 position on the outer phenolic ring by 5′-deiodinase results in the formation of biologically active T_3 (3,5,3′-triiodothyronine; Figure 58.41). However, if a molecule of iodine is removed from the 5 position of the inner phenolic ring of T_4 by another enzyme, 5-deiodinase, reverse T_3 (3,3′,5′-triiodothyronine) is produced, which is biologically inactive. Reverse T_3 subsequently is degraded further by 5′-deiodinase and T_3 by 5-deiodinase; both form diiodotyrosine (T_2). Chemicals such as FD&C Red No. 3 (erythrosine) and amiodarone disrupt thyroid hormone metabolism by inhibiting the 5′-deiodinase in liver (type I enzyme), thereby inhibiting the conversion of T_4 to T_3. In long-term studies in rats, the lowered serum T_3 results in increased circulating levels of TSH that leads to the development of follicular cell hyperplasia (goiter) and predisposes to a greater incidence of thyroid tumors in 2-year or lifetime studies.

In dog plasma, T_4 is bound to albumin and three globulin fractions, and T_3 is bound to albumin and one globulin fraction. The overall binding affinity of the plasma proteins for T_4 is lower in dogs than in humans. Most importantly, the affinity of the canine inter-α-globulin fraction for T_4 is much less than that of the human thyroxine-binding globulin (TBG). As a result of this weaker binding, as well as an

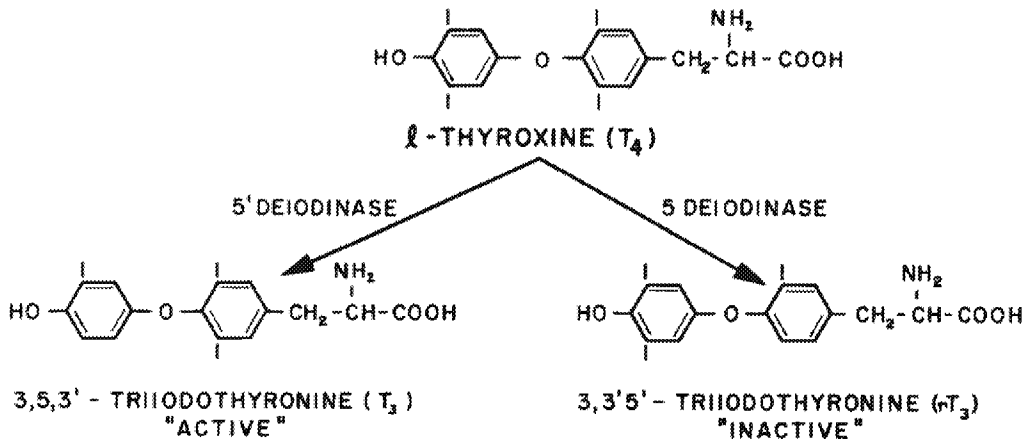

FIGURE 58.41 Monodeiodination of thyroxine to form either active T_3 (left) or inactive (reverse) rT_3 (right) depending on the need for the metabolic actions of thyroid hormone. *Figure reproduced from Capen and Martin (1989), The effects of xenobiotics on the structure and function of thyroid follicular cells and C-cells,* Toxicol. Pathol. ***17****, 266–293, with permission.*

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efficient enterohepatic excretory mechanism, the total T_4 concentration is lower and the rate of thyroid hormone turnover is more rapid in dogs than in humans.

The ratio of T_4 to T_3 in canine plasma is about 20 : 1, even though they are produced by the thyroid at a ratio of about 2 : 1. The average plasma T_4 concentration is about 1.8 μg/100 ml, and the average plasma T_3 level is 84 ng/100 ml, in the dog. These values represent measurements made between 11 am and noon. The time of measurement is important because there are diurnal oscillations in plasma T_3 and T_4. The peak concentration usually occurs at about midday and the minimum at about midnight. Both the overall rates of turnover and the loss of hormone in the feces are much higher in dogs than in humans. Fecal wastage substantially reduces the efficiency of hormone utilization, but also explains the remarkable tolerance of the dog for excess thyroid hormone.

17.7. Biological Action

Thyroxine and triiodothyronine, once released into the circulation, act on many different target cells in the body. The overall functions of T_4 and T_3 are similar, although much of the biological activity is the result of monodeiodination of T_4 to T_3 (3,5,3′-triiodothyronine) prior to interaction with target cells or by the target cells. Under certain conditions (protein starvation, neonatal animals, liver and kidney disease, febrile illness) thyroxine is preferentially monodeiodinated to 3,3′,5′-triiodothyronine (reverse T_3) (Figure 58.41). Because this form of T_3 is biologically inactive, monodeiodination to reverse T_3 provides a mechanism to attenuate the metabolic effects of thyroid hormones. Certain xenobiotics (e.g., FD&C Red No. 3, amiodarone, iopanoic acid) selectively block 5′-deiodinase, resulting in the conversion of most of T_4 to reverse T_3, leading to subnormal T_3 and a compensatory increase in TSH secretion by the pituitary gland.

Thyroxine stimulates oxygen utilization and heat production by many different populations of body cells. It causes increased utilization of carbohydrates, increased protein catabolism, as indicated by a greater excretion of nitrogen, and greater oxidation of fats as suggested by loss in body weight. The administration of thyroxine will increase the heart rate by a direct effect on heart muscle cells.

Normal function of the CNS is dependent on thyroxine. During periods of deficient thyroxine levels, the CNS fails to function normally and the animal is lethargic, dull, and mentally deficient. Myelin in the fiber tracts is decreased, cortical neurons are smaller and fewer, and vascularity of the CNS is reduced. The neurons are permanently damaged by a deficiency of thyroid hormones in a young growing animal fed a severely deficient iodine diet. However, an excess of thyroid hormone production, as occurs in a hyperthyroid animal or human patient, stimulates CNS activity to the extent that the animal is nervous, irritable, and hyperactive.

The subcellular mechanism of action of thyroid hormones resembles that of steroid hormones because free hormone enters into target cells and binds to a cytosol-binding protein. Free T_3 either binds to receptors on the inner mitochondrial membrane to activate mitochondrial energy metabolism, or binds to nuclear receptors and increases transcription of mRNA to facilitate new protein synthesis. The overall effects of thyroid hormones are to (1) increase the basal metabolic rate; (2) increase glycolysis, gluconeogenesis, and glucose absorption from the intestine; (3) stimulate new protein synthesis; (4) increase lipid metabolism and conversion of cholesterol into bile acids and other substances, activate lipoprotein lipase, and increase the sensitivity of adipose tissue to lipolysis by other hormones; (5) stimulate the heart rate, cardiac output, and blood flow; and (6) increase neural transmission, cerebration, and neuronal development in young animals.

18. MECHANISMS OF TOXICITY

18.1. Direct Thyroid Effect

Inhibitors of thyroid hormone synthesis and secretion have a direct thyroid effect.

Inhibitors of Thyroid Hormone Synthesis

BLOCKAGE OF IODINE UPTAKE

The initial step in the biosynthesis of thyroid hormones is the uptake of iodide from the

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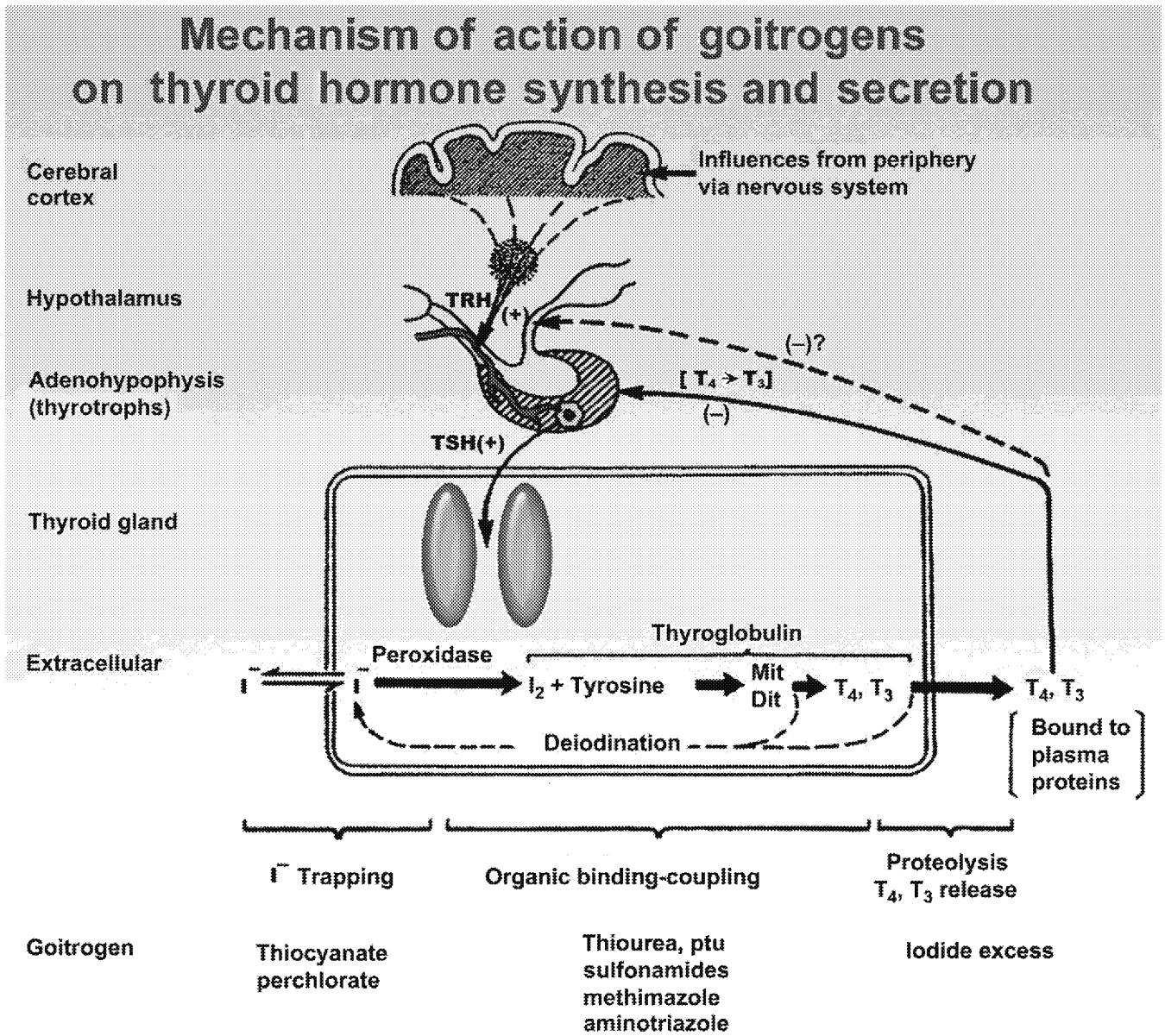

FIGURE 58.42 Mechanism of action of goitrogenic chemicals on thyroid hormone synthesis and secretion. Goitrogens affect iodide trapping, organic binding-coupling, or proteolysis of colloid and release of T_4 and T_3. *Figure reproduced from Capen and Martin (1989), The effects of xenobiotics on the structure and function of thyroid follicular cells and C-cells,* Toxicol. Pathol. ***17****, 266–293, with permission.*

circulation and its subsequent transport against a gradient across follicular cells by a sodium-iodide symporter to the lumen of the follicle. A number of anions act as competitive inhibitors of iodide transport in the thyroid, e.g., perchlorate (ClO_4^-), thiocyanate (SCN^-), and pertechnetate (Figure 58.42). Thiocyanate is a potent inhibitor of iodide transport and is a competitive substrate for thyroid peroxidase, but it does not appear to be concentrated in the thyroid. Blockage of the iodide-trapping mechanism has a disruptive effect on the thyroid–pituitary axis similar to the effect of iodine deficiency. The blood levels of T_4 and T_3 decrease, resulting in a compensatory increase in the secretion of TSH by the pituitary gland. Hypertrophy and hyperplasia of follicular cells following sustained exposure result

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in an increased thyroid weight and the development of goiter.

ORGANIFICATION DEFECT: INHIBITION OF THYROID PEROXIDASE (THYROPEROXIDASE)

A wide variety of chemicals, drugs, and other xenobiotics affect the second step in thyroid hormone biosynthesis (Figure 58.42). The stepwise binding of iodide to the tyrosyl residues in thyroglobulin requires oxidation of inorganic iodide (I^-) to molecular (reactive) iodine (I_2) by the thyroid peroxidase present in the lumenal aspect (microvillar membranes) of follicular cells and adjacent colloid. Classes of chemicals that inhibit the organification of thyroglobulin include: (1) thionamides such as thiourea, thiouracil, propylthiouracil, methimazole, carbimazole, and goitrin; (2) aniline derivatives and related compounds such as sulfonamides, p-aminobenzoic acid, p-aminosalicylic acid, and amphenone; (3) substituted phenols such as resorcinol, phloroglucinol, and 2,4-dihydroxybenzoic acid; and (4) miscellaneous inhibitors such as aminotriazole, tricyanoaminopropene, and antipyrine and its iodinated derivative, iodopyrine.

Many of these chemicals exert their action by inhibiting thyroid peroxidase, which results in a disruption both of the iodination of tyrosyl residues in thyroglobulin and of the coupling reaction of iodotyrosines (i.e., MIT and DIT) to form iodothyronines (T_3 and T_4). In rats, propylthiouracil (PTU) has been shown to affect each step in thyroid hormone synthesis beyond iodide transport. The order of susceptibility to the inhibition by PTU is the coupling reaction (most susceptible), iodination of MIT to form DIT (moderately susceptible), and iodination of tyrosyl residues to form MIT (least susceptible). Thiourea differs from PTU and other thioamides because it does not inhibit guaiacol oxidation (the standard assay for peroxidase) and does not inactivate thyroid peroxidase in the absence of iodine. Its ability to inhibit organic iodinations is due primarily to the reversible reduction of active I_2 to 2 I^-.

The goitrogenic effects of sulfonamides have been known since the reports of the action of sulfaguanidine on the rat thyroid in the early 1950s. Sulfamethoxazole and trimethroprim exert a potent goitrogenic effect in rats, resulting in marked decreases in circulating T_3 and T_4, a substantial compensatory increase in TSH, and increased thyroid weights due to follicular cell hyperplasia. The dog is also a sensitive species to the effects of sulfonamides, showing markedly decreased serum T_4 and T_3 levels, hyperplasia of thyrotrophic basophils, and increased thyroid weights. By comparison, the thyroids of monkeys and humans are resistant to the development of changes that sulfonamides produce in rodents (rats and mice) and dogs. Using the guaiacol peroxidation assay, comparison of the effects of PTU and a goitrogenic sulfonamide (sulfamonomethoxine) on the activity of thyroid peroxidase in the rat and monkey has been studied. The concentration of sulfonamide required to inhibit thyroid peroxidase in the monkey was about 500 times that required in the rat. Studies such as these with sulfonamides demonstrate distinct species differences between rodents and primates in the response of the thyroid to chemical inhibition of hormone synthesis. The sensitive species (e.g., rat, mouse, and dog) are much more likely to develop follicular cell hyperplasia and thyroid tumors after long-term exposure to sulfonamides than are resistant species (e.g., non-human primate, human, guinea pig, and chicken).

Inhibitors of Thyroid Hormone Secretion

BLOCKAGE OF THYROID HORMONE RELEASE BY EXCESS IODIDE AND LITHIUM

Relatively few chemicals selectively inhibit the secretion of thyroid hormone from the thyroid gland (Figure 58.42). An excess of iodine has been known for years to inhibit the secretion of the thyroid hormone, and occasionally can result in goiter and hypothyroidism in animals and human patients (see iodide-excess goiter, in Section 19.2). High doses of iodide have been used therapeutically in the treatment of patients with Grave's disease and hyperthyroidism to lower circulating levels of thyroid hormones. Several mechanisms have been suggested for this effect of high iodide levels on thyroid hormone secretion, including a decrease in lysosomal protease activity (human glands), inhibition of colloid droplet formation (mice and rats), and inhibition of TSH-mediated increase in cAMP (dog thyroid slices). Rats fed an iodide excess diet had hypertrophy of the cytoplasmic area of follicular cells with an accumulation of

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numerous colloid droplets and lysosomal bodies. However, there was limited evidence ultrastructurally of fusion of the membranes of these organelles and degradation of the colloid necessary for the release of T_4 and T_3 from the thyroglobulin. Circulating levels of T_4, T_3, and rT_3 would all be decreased by an iodide excess.

Lithium has also been reported to have a striking inhibitory effect on thyroid hormone release (Figure 58.42). The widespread use of lithium carbonate in the treatment of manic states occasionally results in the development of goiter with either euthyroidism or occasional hypothyroidism in human patients. Lithium inhibits colloid droplet formation stimulated by cAMP *in vitro*, and inhibits the release of thyroid hormones.

XENOBIOTIC- (OR METABOLITE-) INDUCED THYROID PIGMENTATION OR COLLOID ALTERATION

The antibiotic minocycline produces a striking black discoloration of thyroid lobes in laboratory animals and humans with the formation of brown pigment granules within follicular cells. The pigment granules stain similarly to melanin, and are best visualized on thyroid sections stained with the Fontana-Masson procedure. Electron-dense material first accumulates in lysosome-like granules and in the rough endoplasmic reticulum. The pigment appears to be a metabolic derivative of minocycline, and administration of the antibiotic at a high dose to rats for extended periods may result in a disruption of thyroid function and the development of thyroid enlargement ("goiter"). The release of T_4 from perfused thyroids of minocycline-treated rats was decreased significantly, but follicular cells retained the ability to phagocytize colloid in response to TSH and had numerous colloid droplets in their cytoplasm.

Other xenobiotics (or metabolite[s]) selectively localize in the thyroid colloid of rodents, resulting in abnormal clumping and increased basophilia in the colloid. Brown to black pigment granules may be present in follicular cells, colloid, and macrophages in the interthyroidal tissues, resulting in a macroscopic darkening of both thyroid lobes. The physiochemically altered colloid in the lumen of thyroid follicles appears to be less able than normal colloid either to react with organic iodine in a stepwise manner to result in the orderly synthesis of iodothyronines or to be phagocytized by follicular cells and processed enzymatically to release active thyroid hormones into the circulation. Serum T_4 and T_3 are decreased, serum TSH levels are increased by an expanded population of pituitary thyrotrophs, and thyroid follicular cells undergo hypertrophy and hyperplasia. As would be expected, the incidence of thyroid follicular cell tumors in 2-year carcinogenicity studies in rodents is increased significantly at the higher dose levels, usually with a greater effect in male than in female rats. The compound is localized preferentially in the colloid and not within follicular cells. There is preferential uptake and persistence in the thyroid gland compared to other tissues. However, thyroid peroxidase activity is normal, and the ability of the thyroid to take up radioactive iodine often is increased in response to the greater circulating levels of TSH. Similar thyroid changes or functional alterations usually do not occur in dogs, monkeys, or humans.

18.2. Effect on Peripheral Metabolism of Thyroid Hormones

Inhibitors of 5′-Deiodinase

FD&C RED NO. 3 (ERYTHROSINE)

FD&C Red No. 3 is one of the best characterized xenobiotic chemicals that acts as a 5′-deiodinase inhibitor and results in perturbations of thyroid function in rodents. FD&C Red No. 3 (erythrosine) is a red dye widely used as a color additive in foods, cosmetics, and pharmaceuticals. The FDA peer review panel on Red No. 3 (1987) estimated the average quantity of the dye ingested in the United States to be 1.4 mg per person daily (0.023 mg/kg/day for a 60-kg person). A number of studies have examined the potential thyroid toxicity of FD&C Red No. 3 because it is a tetraiodinated derivative of fluorescein and iodine accounts for approximately 58% of the molecular weight of the dye.

A chronic toxicity/carcinogenicity study that was required by the FDA and sponsored by the Certified Color Manufacturers' Association (CCMA) revealed that male Sprague-Dawley rats fed a 4% dietary concentration of Red No. 3 beginning *in utero* and extending over their lifetime (30 months) developed a 21.8%

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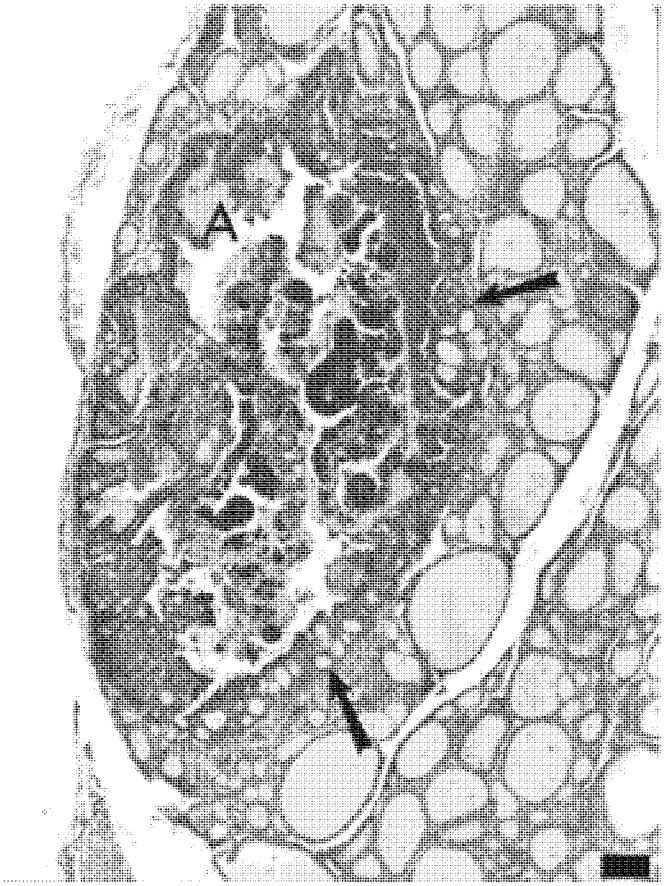

FIGURE 58.43 Thyroid follicular cell adenoma (A) in a male Sprague-Dawley rat exposed to FD&C Red No. 3 *in utero* and fed at the 4% level for the lifetime of 30 months. The adenoma is well circumscribed (arrows) and adjacent follicles are slightly compressed. Hyperchromatic neoplastic cells line a cystic space and form papillary projections into the lumen. Bar = 100 μm. *Figure reproduced from Capen (1983) Chemical Injury of the Thyroid: Pathologic and Mechanistic Considerations. In: The Toxicology Forum. Proceedings of the 1983 Annual Winter Meeting. Washington, DC, 21–23 February, 1983, pp. 260–268, with permission.*

incidence of thyroid adenomas derived from follicular cells compared with 1.5% in control rats and a historical incidence of 1.8% for this strain (Figure 58.43). There was not a significant increase in follicular cell adenomas in the lower dose groups of male rats or an increase in malignant thyroid follicular cell tumors. Female rats fed similar amounts of the color additive did not develop a significant increase in either benign or malignant thyroid tumors. Feeding of the color additive at the high-dose (4%) level provided male rats with 2464 mg/kg of Red No. 3 daily.

Studies on the absorption, distribution, metabolism, and excretion of FD&C Red No. 3 confirmed earlier reports that the dye is absorbed poorly from the gastrointestinal tract. The dye does not accumulate in the thyroid glands of rats. A primary (direct) mechanism of action of FD&C Red No. 3 on the thyroid is unlikely because of (1) the failure of the dye to accumulate in the gland, (2) consistently negative genotoxicity data, (3) lack of an oncogenic response in mice and gerbils, and (4) a failure to result in thyroid tumor development at dietary concentrations of 1.0% or less in male and female rats.

Subsequent studies have investigated the mechanisms responsible for the proliferation of follicular cells and increased development of benign thyroid tumors in male Sprague-Dawley rats fed Red No. 3. Serum T_4 concentrations in rats receiving 4% of the dye were increased from 1 through 6 months and serum T_3 was reduced significantly compared with controls. Circulating levels of reverse T_3 increased approximately seven-fold after 1 month and TSH levels were approximately three times baseline. Serum TSH levels in rats fed Red No. 3 and receiving daily T_3 injections were decreased to undetectable levels.

T_4 metabolism was altered significantly in liver homogenates prepared from rats fed 4% Red No. 3 (Figure 58.44). The degradation of ^{125}I-thyroxine was decreased to approximately 40% of values in control homogenates. This was associated with an approximately 80% decrease in ^{125}I-T_3 from the radiolabeled T_4 substrate. These mechanistic investigations suggest that the dye results in a perturbation of thyroid hormone economy in rodents, resulting in a long-term stimulation of follicular cells by TSH that over their lifetime predisposes to an increased incidence of thyroid tumors.

In this example of xenobiotic-induced thyroid toxicity, the changes in circulating levels of thyroid hormones and morphologic evidence of follicular cell stimulation in rats are the result of significant alterations in the peripheral metabolism of T_4. An inhibition of 5′-deiodinase in the liver and kidney by Red No. 3 would explain the lower circulating T_3 levels. The monodeiodination of T_4 by another enzyme (5-deiodinase) to reverse T_3 and

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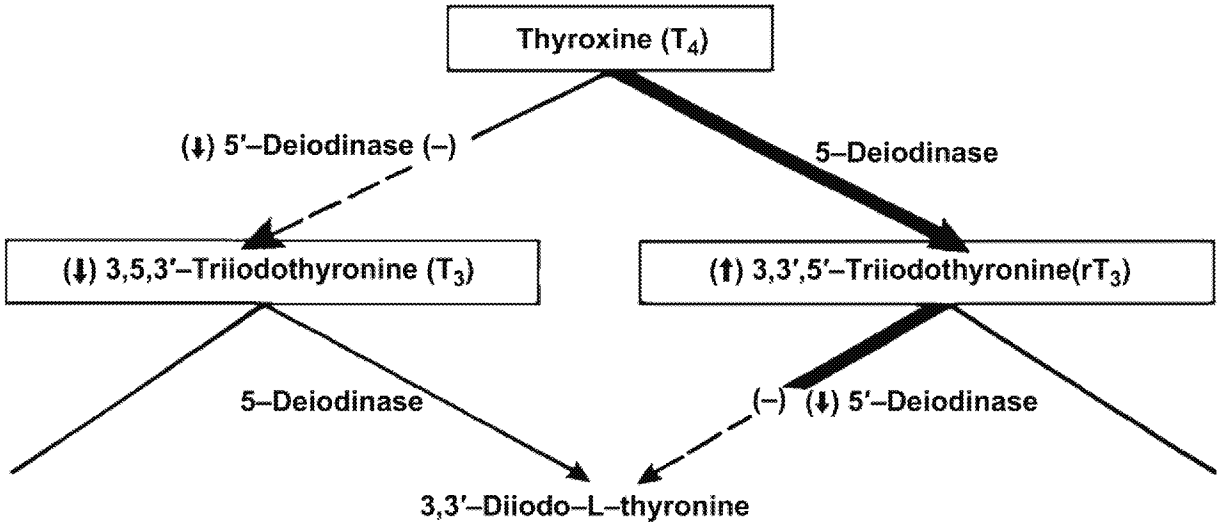

FIGURE 58.44 Effects of FD&C Red No. 3 (Erythrosine) on *in vivo* metabolism of thyroxine by inhibiting the 5′-deiodinase that normally converts T_4 to T_3. *Figure reproduced from Capen and Martin (1989), The effects of xenobiotics on the structure and function of thyroid follicular cells and C-cells,* Toxicol. Pathol. ***17****, 266–293, with permission.*

inhibition of the 5′-deiodinase (which is necessary to further degrade this inactive iodothyronine to 3,3′-diiodothyronine [T_2]) explains the striking accumulation of serum reverse T_3. The pituitary, sensing the lowered circulating levels of T_3, compensates by increasing the secretion of TSH, which results in the morphological evidence of follicular cell stimulation.

A no-effect level could be established at which the compound did not increase circulating levels of TSH and did not result in morphological changes in thyroid follicular cells. The compound increased the incidence of benign tumors in one species (rat) only at the high-dose level, and tumors developed in thyroids with TSH-mediated hypertrophy and hyperplasia of follicular cells secondary to alterations in the peripheral metabolism of thyroid hormones. The dye was negative in standard genotoxic and mutagenic assays, and did not increase the incidence of tumors in other organs.

Other compounds that are known to inhibit 5′-monodeiodinase in peripheral tissues and disrupt thyroid hormone economy by inhibiting the conversion of T_4 to T_3 include amiodarone and iopanoic acid. These xenobiotics (as well as FD&C Red No. 3) are iodinated compounds, but it is unlikely that their effects are due to the iodide component because even large doses of iodide (5 mg/day) do not affect T_4 metabolism in rats.

AMIODARONE

Amiodarone, an iodinated drug used to treat tachyarrhythmias, inhibits the *in vivo* 5′-monodeiodination of T_4. It blocks the 5′-monodeiodinase (type I enzyme) in rat liver homogenates but not the 5′-deiodinase in the rat pituitary gland (type II enzyme). Male and female Sprague-Dawley rats administered amiodarone by gavage in a 2-year carcinogenicity study had an increased incidence of thyroid follicular cell hyperplasia and adenomas. There was a numerical increase in carcinomas in each treatment group, but there was no dose response. Male and female B6C3F1 mice that received amiodarone orally for 2 years did not develop an increased incidence of thyroid follicular cell hyperplasia or tumors. In a shorter (14-week) study, male Sprague-Dawley rats that received oral amiodarone (50 mg/kg/day) had significant increases in serum TSH, T_4, and T_3, and a significant decrease in serum rT_3. These changes in thyroid hormone and TSH were similar to those observed following the administration of Red No. 3. It is likely that amiodarone, like FD&C Red No. 3, produces thyroid follicular cell adenomas in rats through the TSH-mediated secondary mechanism.

The effects of amiodarone (a readily absorbed substance) on human thyroid economy are well documented, and include decreased serum T_3 concentrations, increased serum T_4 and rT_3

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concentrations, and initial increases in serum TSH concentrations at therapeutic doses. By comparison, FD&C Red No. 3 (a poorly absorbed substance) had no effect on thyroid hormone economy in humans at doses up to 200 mg/day for 14 days, except for a minimal increase in serum TSH that was within the normal range. This TSH increase does not appear to be caused by FD&C Red No. 3 *per se* but rather by the high iodide intake associated with the large dose (200 mg/day) of the dye.

IOPANOIC ACID

Iopanoic acid is an iodinated radiographic contrast agent that inhibits both type I and type II 5′-monodeiodinase in rats. Iopanoic acid and FD&C Red No. 3 share the ability to inhibit the T_4 to T_3 conversion and increase serum TSH concentrations in rats. However, iopanoic acid inhibits both type I and type II 5′-monodeiodinase, whereas Red No. 3 inhibits only the type I enzyme in liver and kidney and not the type II enzyme in brain, pituitary, and brown adipose tissue. Iopanoic acid and FD&C Red No. 3 differ in their ability to affect human thyroid hormone economy. Iopanoic acid in doses used commonly in clinical radiographic studies results in increased serum concentrations of T_4 and rT_3, decreased serum T_3 with a compensatory increase in basal serum TSH concentrations, and increased TSH response to TRH after 5–7 days of treatment.

Inducers of Hepatic Microsomal Enzymes

Hepatic microsomal enzymes play an important role in thyroid hormone economy because glucuronidation is the rate-limiting step in the biliary excretion of T_4 and sulfation is the rate-limiting step in the excretion of T_3 by phenol sulfotransferase. Long-term exposure of rats to a wide variety of different chemicals induces these enzyme pathways and results in chronic stimulation of the thyroid by disrupting the hypothalamic–pituitary–thyroid axis. The resulting chronic stimulation of the thyroid by increased circulating levels of TSH often results in a greater risk of developing tumors derived from follicular cells in 2-year or lifetime studies with these compounds in rats. Xenobiotics that induce liver microsomal enzymes and disrupt thyroid function in rats include CNS drugs (e.g., phenobarbital, benzodiazepines), calcium channel blockers (e.g., nicardipine, bepridil), steroids (spironolactone), retinoids, chlorinated hydrocarbons (e.g., chlordane, DDT, TCDD), and polyhalogenated biphenyls (e.g., PCB, PBB). Most of the hepatic microsomal enzyme inducers have no apparent intrinsic carcinogenic activity and produce little or no mutagenicity or DNA damage. Their promoting effect on thyroid tumors usually is greater in rats than in mice, with males developing a higher incidence of tumors more often than females. In certain strains of mice, these compounds alter liver cell turnover and promote the development of hepatic tumors.

Many of the liver microsomal enzyme inducers in rats (such as phenobarbital [PB], pregnenolone-16a-carbonitrile [PCN], 3-methylcholanthrene [3MC], and Arochlor 1254 [PCB]) that affect thyroid hormone economy typically induce the activity of T_4-UDP glucuronosyltransferase (UGT), which is associated with increased excretion of T_4-glucuronide and decreased serum T_4 concentration. Klaassen and Hood (2001) report that activation of T_3-UGT is more predictive of increased TSH in rats compared to T_4-UGT. For example, administration of PB, PCN, 3MC, and Arochlor 1254 to rats induced T_4-UGT in the liver, but only PB and PCN also induced T_3-UGT and were associated with increased serum TSH concentrations and thyroid follicular cell proliferation. All four compounds were associated with relatively normal serum concentrations of T_3, which suggests that new steady states were attained by counter-regulatory mechanisms involved in thyroid hormone economy.

PHENOBARBITAL

Phenobarbital has been studied extensively as the prototype for hepatic microsomal inducers that increase a similar spectrum of cytochrome P450 isoenzymes. The activity of thyroxine-UDP glucuronosyltransferase (T_4-UGT), the rate-limiting enzyme in T_4 metabolism, is increased in purified hepatic microsomes of male rats (three-fold). This resulted in a significantly higher cumulative biliary excretion of [^{125}I]-thyroxine. Most of the increase in biliary excretion was T_4 glucuronide due to an increased metabolism of thyroxine in phenobarbital-treated rats. Phenobarbital-treated rats develop a characteristic pattern of changes in

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circulating thyroid hormone levels. Plasma T_3 and T_4 are decreased markedly after 1 week and remain decreased for 4 weeks. By 8 weeks, T_3 levels return to near normal due to compensation by the hypothalamic–pituitary–thyroid axis. Serum TSH values are elevated significantly throughout the first month but often decline somewhat after a new steady state is attained. Thyroid weights increase significantly after 2–4 weeks of phenobarbital, reach a maximum increase of 40–50% by 8 weeks, and remain elevated throughout the period of treatment. Results from these studies are consistent with the hypothesis that the promotion of thyroid tumors in rats was not a direct effect of phenobarbital on the thyroid gland but rather an indirect effect mediated by TSH secretion from the pituitary secondary to the hepatic microsomal enzyme-induced increase of T_4 excretion in the bile.

The activation of the thyroid gland during the treatment of rodents with substances that stimulate thyroxine catabolism is a well-known phenomenon, and has been investigated extensively with phenobarbital and many other compounds. It occurs particularly with rodents because (1) UDP-glucuronosyltransferase can be induced easily in rodent species, and (2) thyroxine metabolism takes place very rapidly in rats in the absence of thyroxine-binding globulin. In man, a lowering of the circulating thyroxine level but no change in TSH and T_3 concentrations has been observed only with high doses of very powerful enzyme-inducing compounds such as rifampicin with and without antipyrine.

There is no convincing evidence that humans treated with drugs or exposed to chemicals that induce hepatic microsomal enzymes are at increased risk for the development of thyroid cancer. In a study of the effects of microsomal enzyme-inducing compounds on thyroid hormone metabolism in normal healthy adults, phenobarbital (100 mg daily for 14 days) did not affect serum T_4, T_3, or TSH levels. A decrease in serum T_4 levels was observed after treatment with either a combination of phenobarbital plus rifampicin or a combination of phenobarbital plus antipyrine; however, these treatments had no effect on serum T_3 or TSH levels. Epidemiologic studies of patients treated with therapeutic doses of phenobarbital have reported no increase in risk for the development of thyroid neoplasia. Highly sensitive assays for thyroid and pituitary hormones are readily available clinically to monitor circulating hormone levels in patients who are exposed to chemicals that could potentially disrupt homeostasis of the pituitary–thyroid axis. Likewise, there is no substantive evidence that humans treated with drugs or exposed to chemicals that induce hepatic microsomal enzymes are at increased risk for the development of liver cancer.

Studies on the mode of action of phenobarbital on the promotion of thyroid tumors have been conducted using an initiation-promotion model. Rats are treated for 4–6 weeks with N-bis(2-hydroxypropyl) nitrosamine (DHPN), followed by a 15-week or longer interval of phenobarbital (500 ppm) in the diet. There is a marked increase in incidence of follicular cell tumors in phenobarbital-treated rats over rats receiving only DHPN. The incidence of tumors increases with the duration of phenobarbital treatment, and the promoting effect appears to be greater in male than in female rats. The supplemental administration of thyroxine (at doses that returned the plasma level of TSH to the normal range) blocked the thyroid tumor promoting effects of phenobarbital, and the promoting effects were directly proportional to the level of plasma TSH in rats. The sustained increase in circulating TSH levels results initially in the hypertrophy of follicular cells, followed by hyperplasia, and ultimately places the rat thyroid at greater risk to develop an increased incidence of benign tumors.

POLYCHLORINATED BIPHENYLS (PCBS)

Another classic example of a chemical that induces hepatic microsomal enzymes and disrupts thyroid function is polychlorinated biphenyl (PCB). Polychlorinated biphenyls are commonly used industrial compounds that have been released into the environment and have caused widespread contamination. The disease-producing capability of these compounds includes alterations in reproduction, growth, and development. PCBs cause a significant reduction in serum levels of thyroid hormones due to alterations in thyroid structure, in addition to the well-known induction of hepatic UDP-glucuronosyltransferase and increased secretion of thyroxine-glucuronide in the bile.

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Feeding of PCB (50–500 ppm) produced a dose-dependent significant reduction in serum T_4 levels in rats. Following withdrawal of PCB from the diet, blood T_4 levels returned to the normal range at 35 weeks but not at 12 weeks. The changes in circulating levels of T_4 were accompanied by a striking hypertrophy and hyperplasia of thyroid follicular cells compared with controls. The most consistent lesions in follicular cells following the feeding of PCB were the accumulation of numerous large colloid droplets and irregularly shaped lysosomal bodies in the expanded cytoplasmic area. Microvilli on the lumenal surface were shortened with abnormal branchings. The chronic administration (12-week) of PCB resulted in a striking distension of many follicular cells with large lysosomal bodies (that were strongly acid phosphatase-positive) and colloid droplets, blunt and abnormally branched microvilli, and mitochondrial vacuolation. The principal lesion produced by PCB in follicular cells that contributed to the altered thyroid function appeared to be an interference in the interaction between the numerous colloid droplets and lysosomal bodies that is necessary for the enzymatic release of thyroid hormones.

Subsequent studies have investigated whether this direct effect on follicular cells interfering with hormone secretion contributes to the lowering of thyroid hormone levels produced by PCB. These investigations have utilized Gunn rats, which have an impaired ability to conjugate T_4 with glucuronic acid. The serum T_4 concentration was significantly reduced to a similar degree in both homozygous and heterozygous Gunn rats fed 500 ppm PCB daily for 6 weeks. The bile-to-plasma ratio of $[^{125}I]$-T_4 was increased more than five-fold in heterozygous Gunn rats. This was a reflection of the ability of heterozygous Gunn rats to respond to PCB with the induction of hepatic T_4-UDP-glucuronosyltransferase and increased conjugation and excretion of T_4-glucuronide into the bile. The bile-to-plasma ratio of homozygous control Gunn rats was only one-half that of heterozygous Gunn rats, reflecting their reduced biliary clearance of conjugated T_4. Biliary clearance of exogenous radiolabeled T_4 following the feeding of PCB was elevated only to that of control heterozygous Gunn rats.

18.3. Secondary Mechanisms of Thyroid Oncogenesis

Understanding the mechanism of action of xenobiotics on the thyroid gland provides a more rational basis to extrapolate findings from long-term rodent studies to safety assessment of a particular compound for humans. Many chemicals and drugs disrupt one or more steps in the synthesis, secretion, and peripheral metabolism of thyroid hormones, resulting in subnormal levels of T_4 and T_3, associated with a compensatory increased secretion of pituitary TSH (Figure 58.45). When tested in a highly sensitive species, such as rats, these compounds result early in follicular cell hypertrophy/hyperplasia and increased thyroid weights, and in long-term studies in an increased incidence of thyroid tumors by a secondary (indirect) mechanism.

In the secondary mechanism of thyroid oncogenesis in rodents, the specific xenobiotic chemical or physiologic perturbation evokes another stimulus (e.g., chronic hypersecretion of TSH) that promotes initially hypertrophy and hyperplasia and subsequently the development of proliferative lesions (initially focal hyperplasia, subsequently adenomas, infrequently carcinomas) derived from follicular cells. Thresholds for a no effect on the thyroid gland can be established by determining the dose of xenobiotic that fails to elicit an elevation in the circulating level of TSH. Compounds acting by this indirect (secondary) mechanism with hormonal imbalance usually have little or no evidence for mutagenicity or for producing DNA damage.

In human patients with markedly altered changes in thyroid function and chronically elevated TSH levels, as in areas with a high incidence of endemic goiter due to iodine deficiency, there is little if any increase in the incidence of thyroid cancer. The relative resistance to the development of thyroid cancer in humans with elevated plasma TSH levels is in marked contrast to the response of the thyroid gland to chronic TSH stimulation in rats and mice. The human thyroid is much less sensitive to this pathogenetic phenomenon than is the rodent thyroid.

Human patients with congenital defects in thyroid hormone synthesis (dyshormonogenetic goiter) and markedly increased circulating TSH

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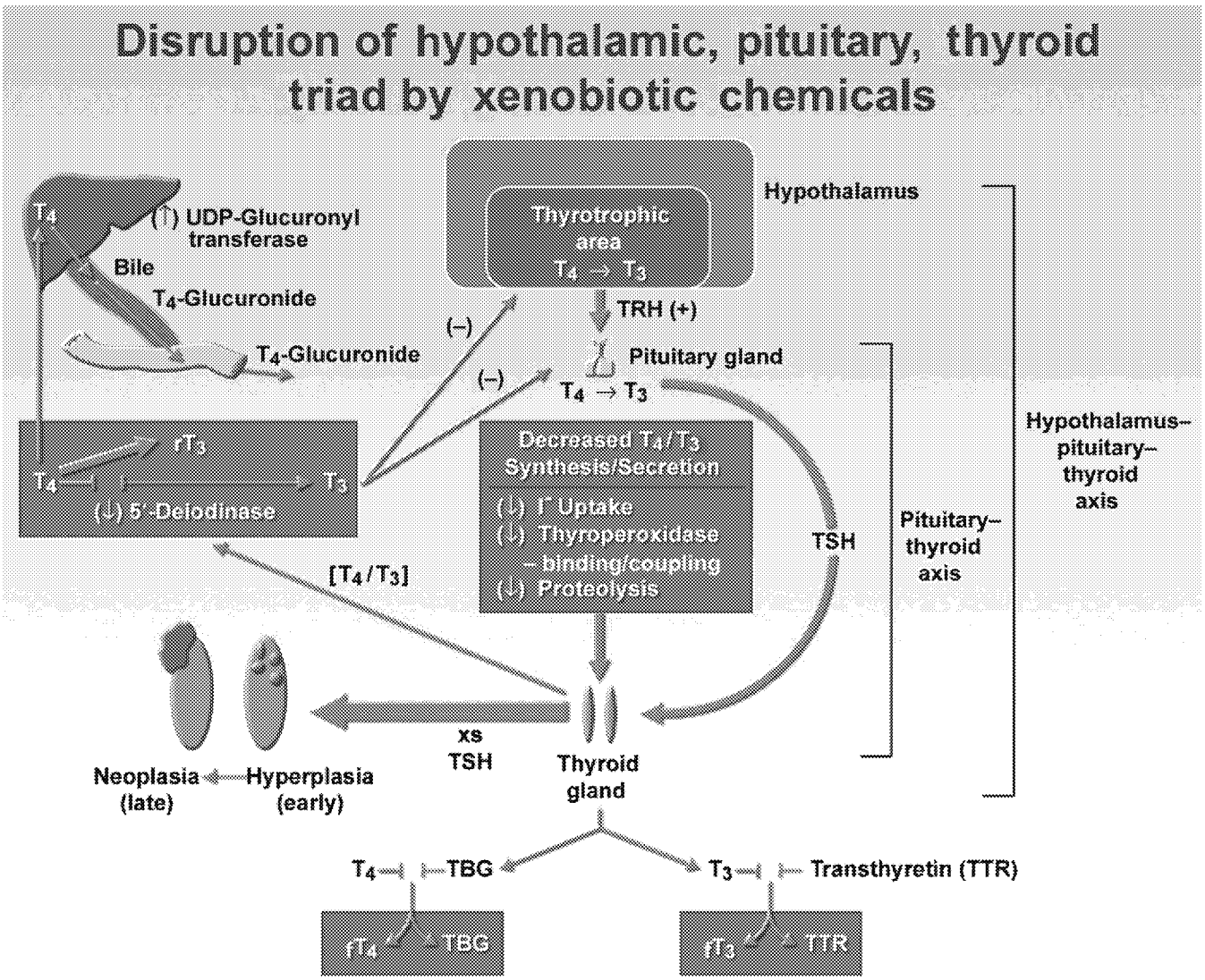

FIGURE 58.45 Multiple sites of disruption of the hypothalamic–pituitary–thyroid triad by xenobiotic chemicals. Chemicals can exert direct effects by disrupting thyroid hormone synthesis or secretion and indirectly influence the thyroid through an inhibition of 5′-deiodinase or by inducing hepatic microsomal enzymes (e.g., T_4-UDP-glucuronosyltransferase). All of these mechanisms can lower the circulating levels of thyroid hormones (T_4 and T_3), resulting in a release from negative feedback inhibition and a compensatory increased secretion of TSH by the pituitary gland. The chronic hypersecretion of excess (xs) TSH predisposes the sensitive rodent thyroid gland to develop an increased incidence of focal hyperplastic and neoplastic (adenomas) lesions by a secondary (epigenetic) mechanism.

levels have been reported to have an increased incidence of thyroid carcinomas. Likewise, thyrotoxic patients with Grave's disease where follicular cells are stimulated autonomously by an immunoglobulin (long-acting thyroid stimulator [LATS]) also appear to be at a greater risk to develop thyroid tumors. In summary, the literature suggests that prolonged stimulation of the human thyroid by TSH will induce neoplasia only in exceptional circumstances and possibly acting together with some other metabolic or immunologic abnormality.

18.4. Initiators of Thyroid Carcinogenesis

Specific chemicals and irradiation appear to have a direct effect on the thyroid gland, resulting in genetic damage that leads to cell transformation and tumor formation. Examples of thyroid initiators include 2-acetylaminofluorene

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(2-AAF), N-methyl-N-nitrosourea (MNU), DHPN, methylcholanthrene, dichlorobenzidine, and polycyclic hydrocarbons. Chemicals in this group often increase the incidence of both benign and malignant thyroid tumors over controls. Iodine deficiency is a strong promoter of MNU-initiated thyroid tumors in rats. MNU-treated rats on an iodine-deficient diet for 52 weeks had significantly increased thyroid weights and a 90% incidence of follicular cell carcinomas compared with a 32% and 20% incidence of carcinomas in rats fed iodide-supplemented and control diets, respectively. The majority of the thyroid carcinomas were transplantable and invasive into the mammary fat pad of weanling F344/NCr rats. No other tumors induced by MNU were affected by the iodine-deficient diet.

A common component of permanent hair dye preparations, 2,4-diaminoanisole sulfate (2,4-DAAS), when fed at high doses (0.5%) to F344 rats for 107 weeks, resulted in a 58% incidence of thyroid neoplasms in male rats and 42% in females compared with 7–8% in controls. Follicular cell carcinomas are the principal type of neoplasm induced by 2,4-DAAS in thyroids without a background of diffuse hyperplasia of follicular cells. The carcinomas (papillary, cystic, and solid) are invasive but did not metastasize. 2,4-DAAS has been found to be mutagenic for *Salmonella typhimurium* but is not teratogenic. In the thyroid it results in a dose-dependent accumulation of brown granules in follicular cells that are basic fuchsin positive; PAS, acid fast, and iron negative; and ultrastructurally different than lipofuscin. The thyroid pigment may represent a reaction product of 2,4-DAAS and iodine in the cytoplasm of follicular cells.

19. RESPONSE TO INJURY

Thyroid response to injury can be divided into two sections: histopathological criteria of proliferative lesions, and disorders of thyroid function.

19.1. Histopathological Criteria of Proliferative Lesions

Follicular Cystic Hyperplasia

Follicular cystic hyperplasia is a nodule (single or multiple) in the thyroid formed by the coalescence of adjacent colloid-distended follicles. The follicular wall (or remnants) is lined by one or two layers of low cuboidal epithelium with hyperchromatic nuclei that occasionally form papillary projections (Figure 58.46). Adjacent thyroid follicles are normal, with minimal evidence of compression and no encapsulation by fibrous connective tissue.

FIGURE 58.46 Follicular cystic hyperplasia in a male Sprague-Dawley rat exposed to FD&C Red No. 3 *in utero* and fed at the 4% level for a lifetime of 30 months. There is a coalescence of adjacent colloid-distended follicles that are lined by cuboidal epithelium with hyperchromatic nuclei with occasional papillary projections into the cyst (arrow). Adjacent thyroid follicles are only minimally compressed and there is no evidence of encapsulation. Bar = 100 μm. *Figure reproduced from Capen (1983) Chemical Injury of the Thyroid: Pathologic and Mechanistic Considerations. In: The Toxicology Forum. Proceedings of the 1983 Annual Winter Meeting. Washington, DC, 21–23 February, 1983, pp. 260–268, with permission.*

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Focal or Multifocal Hyperplasia of Follicular Cells

Focal ("nodular") or multifocal areas of hyperplasia of thyroid follicular cells usually only enlarge the affected lobe(s) slightly. The areas of focal hyperplasia are not encapsulated and the adjacent thyroid parenchyma is not compressed. Hyperplastic nodules are composed of irregularly shaped, usually colloid-filled follicles lined by more basophilic cuboidal follicular cells. The multiple nodules of follicular cell hyperplasia may coalesce to form macroscopically observable thyroid adenomas. Focal hyperplasia is different than the diffuse (TSH-mediated) type of follicular cell hyperplasia observed with iodine deficiency and a wide variety of xenobiotic drugs and chemicals that disrupt thyroid hormone economy to result in an increased secretion of TSH.

Follicular Cell Adenoma

An adenoma is a well-circumscribed, usually solitary, benign tumor formed by the autonomous proliferation of follicular cells within the thyroid lobe (Figure 58.43). Adjacent thyroid follicles are compressed to varying degrees, depending on the size of the adenoma. Adenomas usually do not have a complete fibrous capsule, but larger adenomas may be partially encapsulated by a thin layer of fibrous connective tissue. Neoplastic cells often are more hyperchromatic than the surrounding normal follicular cells and form variably sized colloid-containing follicles, line large cystic spaces, or solid sheets. Neoplastic cells may form papillary structures extending into larger cysts. Neoplastic cells vary from cuboidal to low columnar, and the nucleus : cytoplasm ratio often is high. There is no evidence of vascular invasion. Endocrinologically active thyroid adenomas result in the colloid involution of follicles in the rim of the surrounding thyroid due to the inhibition of TSH secretion by elevated blood T_4 and T_3 levels.

Follicular Cell Carcinoma

A carcinoma is an extensive proliferation of neoplastic follicular cells with evidence of intrathyroidal, capsular, or vascular invasion that often enlarges the affected thyroid lobe considerably (Figure 58.47). Malignant follicular cells form colloid-containing follicles of varying size, papillary projections extending into cystic spaces, or solid sheets of cells. The cells are pleomorphic, and nuclei are hyperchromatic with occasional mitotic figures. A desmoplastic reaction often accompanies the infiltration of neoplastic cells into the thyroid capsule. The perithyroidal connective tissue, as well as adjacent trachea and cervical muscles, may be invaded by larger carcinomas.

19.2. Disorders of Thyroid Function

Hypothyroidism

Hypothyroidism is a well-recognized clinical entity in dogs, and occurs in many purebred and mixed breed dogs. Certain breeds, including Doberman Pinschers, Golden Retrievers, Beagles, and Irish Setters, appear to be more commonly affected. Subnormal function of the thyroid is recognized less frequently as a naturally occurring entity in other animal species. However, a large number of drugs, chemicals, dietary deficiencies, or excesses can disrupt one or more steps of thyroid hormone biosynthesis, secretion, metabolism, or degradation and result in subnormal blood levels of thyroid hormones (Figure 58.42). Hypothyroidism in dogs often is the result of primary diseases of the thyroid gland, especially idiopathic follicular atrophy ("follicular collapse") and immune-mediated

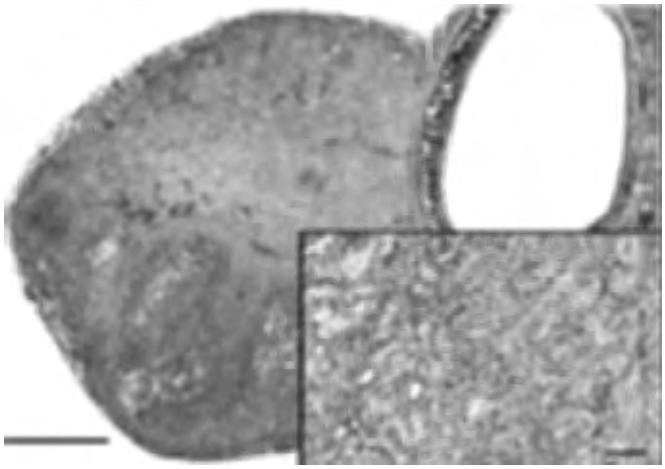

FIGURE 58.47 Follicular cell carcinoma (rat) with enlargement of the thyroid gland, capsular invasion and growth of neoplastic cells in the perithyroidal connective tissues (Bar = 1 μm). Tumor cells form follicles of varying size and colloid content plus solid areas (Inset: bar = 50 μm). H&E stain.

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lymphocytic thyroiditis. Hypertrophy and hyperplasia of TSH-secreting basophils occur in the pars distalis of dogs with hypothyroidism.

Lymphocytic thyroiditis in dogs, Buffalo rats, OS strain of chickens, and marmoset monkeys resembles Hashimoto's disease in humans, and appears to be genetically conditioned, at least in certain breeds. The immunological basis for the development of chronic lymphocytic thyroiditis in both humans and dogs appears to be through the production of autoantibodies directed against thyroglobulin, a microsomal (thyroid peroxidase) antigen, TSH receptor protein, nuclear antigen, and a second colloid antigen. Histopathological alterations in the thyroid glands consist of either a diffuse or a nodular infiltration of lymphocytes, plasma cells, and macrophages. Many of the remaining thyroid follicles are small and lined by tall columnar follicular cells, reflecting the long-standing stimulation by TSH in an attempt to compensate for the low blood levels of thyroid hormones.

Hypothyroidism may also develop secondary to longstanding pituitary or hypothalamic lesions that prevent the release of either TSH or thyrotropin-releasing hormone. The thyroid gland is moderately reduced in size and is composed of colloid-distended follicles lined by flattened follicular cells due to a lack of TSH-induced endocytosis of colloid and secretion of thyroid hormones. Somatostatin inhibits both growth hormone and TSH secretion in the pars distalis of the pituitary gland. Long-acting somatostatin analogs, such as octreotide, have the potential to reduce TSH and serum thyroid hormone concentrations.

Many functional disturbances associated with hypothyroidism are due to a reduction in the basal metabolic rate. A gain in body weight without an associated change in appetite occurs frequently. The rate of lipid metabolism decreases in longstanding cases of hypothyroidism, and blood lipid levels often are elevated because of a decreased rate of intestinal cholesterol excretion and a decreased rate of hepatic conversion into bile acids and other compounds. The elevated blood cholesterol level predisposes to the development of atherosclerosis of small vessels (especially coronary arteries) and deposition of lipid in the liver. The cardiovascular system is further affected by the reduced level of thyroid hormones due to a slowing of the heart rate. The moderate slowing in the heart rate may be difficult to detect unless a normal resting heart rate has been measured previously.

The accumulation of excessive lipid in the liver often results in varying degrees of hepatomegaly with abdominal distension in animals with long-term hypothyroidism. Renal glomeruli may become plugged with lipid in hypothyroid animals with markedly elevated plasma cholesterol, resulting in progressive renal failure.

Abnormalities in reproduction are common when a breeding animal develops hypothyroidism. Lack of libido and reduction in sperm count may occur in males, whereas abnormal or absent estrus cycles with reduced conception rates often result in females. Obesity and changes in behavior resulting from hypothyroidism may have detrimental effects on reproduction.

Hyperthyroidism

Disturbances of growth in the thyroid gland, which secretes excess thyroid hormones, resulting in hyperthyroidism, are uncommon in animals except in adult to aged cats. Follicular cell adenomas, often developing in a thyroid with multinodular hyperplasia, are encountered more commonly than malignant thyroid tumors. Prior to 1977, most proliferative lesions encountered in the feline thyroid gland were found incidentally at necropsy and were not recognized as being associated with obvious clinical disturbances. A syndrome of hyperthyroidism has been recognized with increasing frequency since the early 1980s in aged cats that is associated with multinodular hyperplasia, adenomas, or adenocarcinomas derived from follicular cells of the thyroid. Factors of possible importance in the increasing incidence of functional thyroid lesions in cats include exposure to lawn herbicides, fertilizers, or herbicides; regular treatment with flea sprays or powders; non-Siamese breeds; and a canned food diet.

Neoplastic cells release both T_4 and T_3 at an uncontrolled rate, resulting in markedly elevated blood levels of both hormones. Follicles in the rim of the thyroid around a functional adenoma are enlarged markedly and distended by the accumulation of colloid. The follicular cells are low cuboidal and atrophic with little endocytotic activity in response to the elevated levels of thyroid hormones. Thyroid tissue

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from cats with hyperthyroidism is transplantable into athymic ("nude") mice and continues to secrete excess thyroid hormone.

The most common disturbance of function in hyperthyroidism is weight loss, despite a normal or increased appetite. Other clinical signs include polydipsia and polyuria, increased frequency of defecation, increased volume of stools, restlessness, and increased physical activity.

Thyroid tumors in the dog only occasionally secrete sufficient thyroid hormone to produce clinical signs of hyperthyroidism. It is surprising that hyperthyroidism occurs even with functional tumors, as experimental induction of hyperthyroidism in the dog requires daily administration of about 25 times the normal replacement dose of thyroid hormone. Dogs have a very efficient enterohepatic excretory mechanism for thyroid hormones that it is difficult to overload, either from endogenous production by a tumor or exogenous administration of hormone.

Therefore, the likelihood of developing clinical hyperthyroidism associated with thyroid neoplasms in animals depends on the capability of tumor cells to synthesize T_4 and T_3 (e.g., well-differentiated thyroid tumors that form follicles and produce colloid are more likely to synthesize thyroid hormones than poorly differentiated solid neoplasms), and the degree of elevation of circulating levels of T_4 and T_3, which depends on a balance between the rate of secretion of thyroid hormones by the tumor and the rate of degradation of thyroid hormones. Cats are very sensitive to phenol and phenol derivatives (such as T_4). They have a poor ability to conjugate phenolic compounds with glucuronic acid and hence to excrete T_4-glucuronide into the bile. The capacity to conjugate T_3 to sulfate, an alternative pathway, is limited in all species and easily overloaded.

Hyperplasia of Follicular Cells ("Goiter")

MECHANISMS OF GOITROGENESIS

Non-neoplastic and non-inflammatory enlargement of the thyroid ("goiter") develops in all laboratory animals, domestic mammals, birds, and submammalian vertebrates. Certain forms of thyroid hyperplasia, especially nodular, may be difficult to differentiate from benign tumors (adenomas). The major pathogenic mechanisms responsible for the development of thyroid hyperplasia include iodine-deficient diets, goitrogenic compounds that interfere with thyroid hormone synthesis, dietary iodide excess, and genetic enzyme defects in the biosynthesis of thyroid hormones (Figure 58.48). All of these seemingly divergent factors result in inadequate T_4 and T_3 synthesis and decreased blood levels of thyroid hormones. The lowered blood levels are detected by the hypothalamus and pituitary to increase the secretion of TSH, which results in hypertrophy and hyperplasia of follicular cells in the thyroid gland.

DIFFUSE HYPERPLASTIC (IODINE-DEFICIENT) GOITER

Iodine deficiency in the diet resulting in diffuse thyroid hyperplasia was common in many goitrogenic areas throughout the world before the widespread addition of iodized salt to animal diets. Presently, marginal iodine-deficient diets containing certain goitrogenic compounds result in thyroid follicular cell hyperplasia and clinical evidence of goiter with hypothyroidism. These goitrogenic substances include thiouracil, sulfonamides, anions of the Hofmeister series, and a number of plants from the genus *Brassica* (Figure 58.42).

Both lateral lobes of the thyroid are enlarged uniformly in animals with diffuse hyperplastic goiter. The enlargements may be extensive and result in palpable swelling in the cranial cervical area. The affected lobes are firm and dark red because an extensive interfollicular capillary network develops under the influence of long-term TSH stimulation. The thyroid enlargements are the result of intense hypertrophy and hyperplasia of follicular cells, often with the formation of papillary projections into the lumens of follicles or multiple layers of cells lining follicles. Endocytosis of colloid usually proceeds at a rate greater than synthesis, resulting in the progressive depletion of colloid.

Under longstanding TSH stimulation, thyroid follicles become smaller than normal and there may be a partial collapse of follicles due to the lack of colloid. Hypertrophic-lining follicular cells are columnar with a deeply eosinophilic cytoplasm and small hyperchromatic nuclei that often are situated in the basilar part of the cell. The follicles are lined by either single or multiple layers of hyperplastic follicular cells that, in some follicles, form papillary projections

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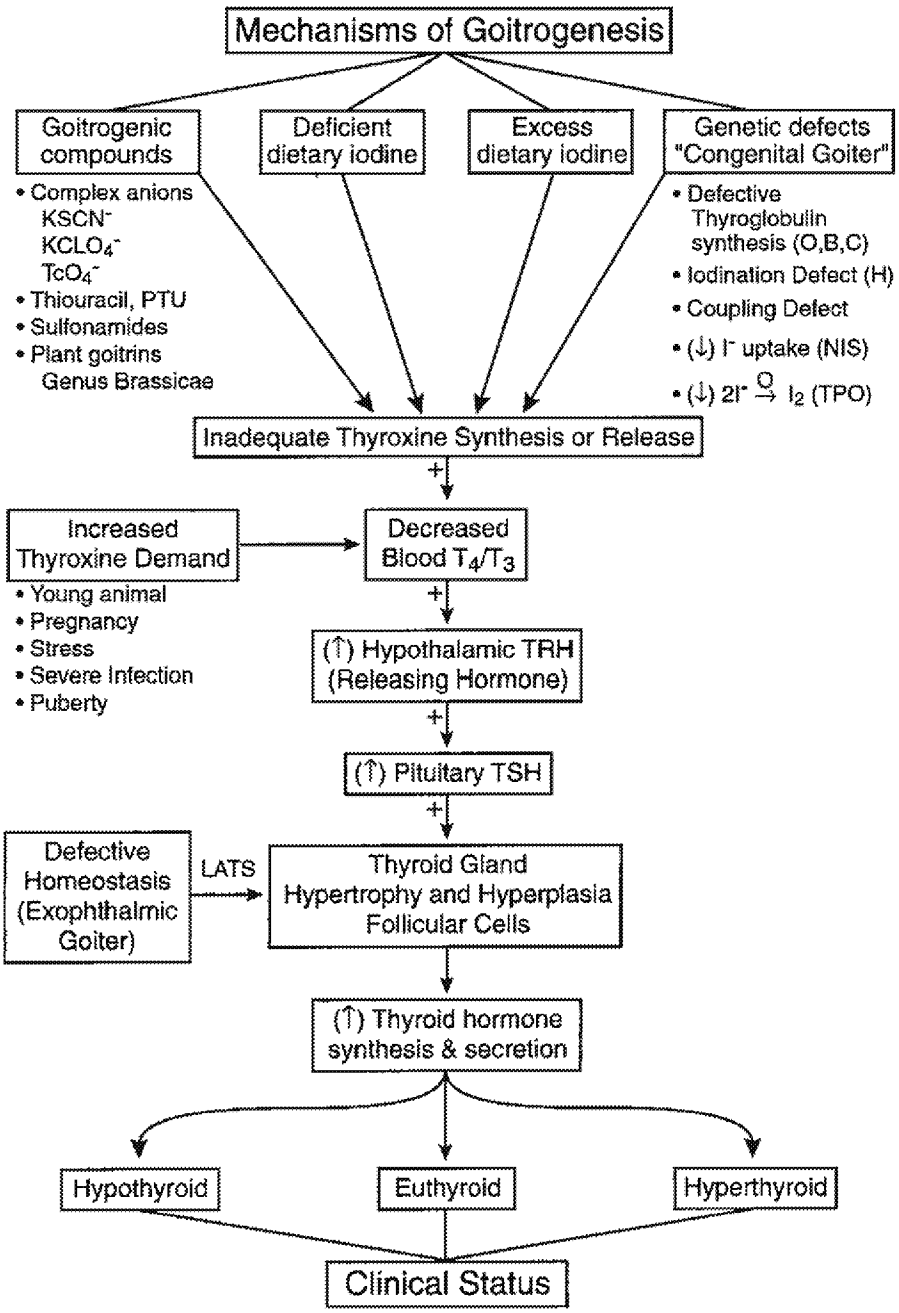

FIGURE 58.48 Mechanisms of goitrogenesis. Multiple pathogenic factors (goitrogenic compounds, deficient and excess dietary iodine intake, and genetic enzyme defects) result in inadequate thyroxine (T_4) and triiodothyronine (T_3) synthesis and lead to the long-term stimulation of thyroid follicular cells by an increased secretion of pituitary TSH. LATS (long-acting thyroid stimulator) is an autoantibody that directly stimulates follicular cells by binding to the TSH receptor on follicular cells of the human thyroid gland in patients with Graves' Disease. PTU, phenylthiouracil; NIS, NaI symporter; TPO, thyroid peroxidase; O, ovines; B, bovines; C, caprines. *Figure reproduced from* Handbook of Toxicologic Pathology, *2nd Ed. W. M. Haschek, C. G. Rousseaux and M. A. Wallig, eds. (2002) Academic Press, Vol. 2, Fig. 47, p. 748, with permission.*

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into the lumen. Similar proliferative changes are present in ectopic thyroid parenchyma in the neck and mediastinum.

COLLOID GOITER

Colloid goiter represents the involutional phase of diffuse hyperplastic goiter in young adults. The markedly hyperplastic follicular cells continue to produce colloid, but the endocytosis of colloid is decreased due to diminished pituitary TSH secretion in response to the return of blood T_4 and T_3 to the normal range. Colloid goiter may develop either after sufficient amounts of iodide have been added to the diet or after the requirements for T_4 have diminished in an older animal. Both thyroid lobes are enlarged diffusely but are more translucent and lighter in color than with hyperplastic goiter. The differences in macroscopic appearances are the result of less vascularity in colloid goiter and the development of macrofollicles distended with colloid. Follicular cells lining the macrofollicles are flattened and atrophic, and the interface between the colloid and the lumenal surface of follicular cells is smooth.

IODIDE-EXCESS GOITER

Although seemingly paradoxical, an excess of iodide in the diet also can result in thyroid hyperplasia in animals and humans. The thyroid glands of the young are exposed to higher blood iodide levels than those of the dam because of the concentration of iodide: *first* by the placenta and *subsequently* by the mammary gland. High blood iodide interferes with one or more steps of thyroxinogenesis, leading to lowered blood thyroid hormone levels and a compensatory increase in pituitary TSH secretion. Excess iodide appears primarily to block the release of T_3 and T_4 from thyroglobulin by interfering with the proteolysis of colloid by lysosomes, but also interferes with the peroxidation of $2\ I^-$ to I_2 and disrupts the conversion of MIT to DIT.

NODULAR GOITER

Multinodular hyperplasia ("goiter") in thyroid glands of old animals appears as white-to-tan nodules of varying size in one or both lobes. The affected lobes are moderately enlarged and irregular in contour. Nodular goiter in most animals (except cats) is endocrinologically inactive and encountered as an incidental lesion at necropsy. However, thyroid hormone-secreting adenomas in old cats with hyperthyroidism often develop in a gland with multinodular hyperplasia. Certain cats with a clinical syndrome of thyroid hormone excess only have multinodular hyperplasia in their thyroids.

Nodular goiter consists of multiple foci of hyperplastic follicular cells that are sharply demarcated from the adjacent thyroid parenchyma, but are not encapsulated, and result in minimal compression of adjacent parenchyma. Some hyperplastic cells form small follicles with little or no colloid. Other nodules are formed by larger irregularly shaped follicles lined by one or more layers of columnar cells that form papillary projections into the lumen. These changes appear to be the result of alternating periods of hyperplasia and colloid involution in the thyroid glands of old animals. The areas of nodular hyperplasia may be microscopic or grossly visible, causing modest enlargement of the thyroid.

CONGENITAL (DYSHORMONOGENETIC) GOITER

An inability to synthesize and secrete adequate amounts of thyroid hormones beginning before or at birth has been documented in several animal species and in human infants. The more prevalent forms of inherited goiter in humans include defects in the iodination of tyrosines, deiodination of iodotyrosines, synthesis and proteolysis of thyroglobulin, coupling of iodotyrosines to form iodothyronines, and transport of iodide from the blood to the follicular lumen.

Congenital dyshormonogenetic goiter in animals is inherited by an autosomal recessive gene in sheep (Corriedale, Dorset Horn, Merino, and Romney breeds), Afrikander cattle, and Saanen dwarf goats. Thyroid glands are symmetrically enlarged at birth due to an intense diffuse hyperplasia of follicular cells. Thyroid follicles are lined by tall columnar cells but they often have collapsed because of lack of colloid resulting from the marked endocytotic activity.

Although thyroidal uptake and turnover of $^{131}I^-$ are increased greatly compared with euthyroid controls, circulating T_4 and T_3 levels are consistently low. The lack of thyroglobulin in the most common form of congenital goiter in animals appears to be due to a defect in thyroglobulin mRNA, leading to aberrant processing

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of primary transcripts or aberrant transport of the mRNA from the nucleus to the endoplasmic reticulum.

20. EVALUATION OF TOXICITY

20.1. Thyroid Function Tests

Thyroid Hormones

The most sensitive and accurate method for measuring blood T_4 and T_3 levels is radioimmunoassay (RIA). The normal blood level of T_4 in the dog ranges between 1.5 and 3.6 μg/dL (mean: 2.5 μg/dL) and T_3 ranges between 48 and 154 ng/dL (mean: 95 ng/dL). In dogs with hypothyroidism, the T_4 level is usually below 1.0 μg/dL and T_3 is below 50 ng/dL. When the levels are border-line, clearer separation of animals with hypothyroidism from those that are euthyroid can be made by injecting exogenous TSH. In the euthyroid dog, the T_4 level will at least double 8 hours after iv or im administration of TSH. In animals with hypothyroidism, the T_4 and T_3 levels do not change significantly after the injection of TSH. Free thyroxine can be measured in dogs by equilibrium dialysis with tracer. Other methods of measuring free T_4 are technically less difficult but much less accurate. Serum free T_4 in normal dogs ranges from 0.2 to 1.0 ng/dL. The advantages of measuring free T_4 over total T_4 include (1) higher correlation with thyroid secretory function, (2) less overlap of hypothyroid and euthyroid levels, and (3) slower decrease with non-thyroidal illness.

Serum T_4 in the rat is higher than in the dog with a mean of approximately 4.5 ± 0.2 μg/dL. Serum T_3 in rats is more variable than circulating T_4 levels with a range of 50–100 ng/dL, with T_3 values tending to decrease with advancing age. The serum reverse T_3 level in the normal rat usually ranges between 55 and 75 pg/mL, but will increase dramatically and remain elevated following the administration of xenobiotics that inhibit the 5′-deiodinase. The range of values given for serum T_4, T_3, and reverse T_3 should be considered only as general guidelines. A specific range of values for rats of different ages, strains, and sexes should be established in conjunction with the endocrine laboratory performing the assays on serum from a particular experiment. Whenever possible, samples at different intervals from an experiment should be frozen and all assays performed at a single run at the termination of the experiment to minimize interassay variations. Serum collection should be performed during the morning hours, if possible, and rats from control and experimental groups alternated during the collection interval. The review by Döhler *et al.* (1979) is a valuable summary of the variations in circulating TSH and thyroid hormone levels that occur in rats with respect to sex, age, time of day, estrus cycle, strain, environmental temperature, method of blood collection, cage transport, handling, and other conditions. They document the important fact that an elevation or reduction of a particular hormone (TSH or thyroid hormone) value may not necessarily be an indication of thyrotoxic effects of a particular test substance.

Thyroid-Stimulating Hormone

Thyroid-stimulating hormone (TSH or thyrotropin) is a glycoprotein synthesized by thyrotrophic basophils in the adenohypophysis and is composed of α and β subunits. The α subunit is similar to that of several pituitary hormones of the same family, but the β subunit is specific for TSH. The hormone circulates primarily in the free (unbound) form and has a short (approximately 15-min) plasma half-life. TSH is the major regulator of the synthetic and secretory processes of thyroid follicular cells.

In the evaluation of potential thyroid toxicity of various xenobiotics in rodents, an accurate quantitation of circulating levels of TSH is essential in order to determine whether proliferative lesions of follicular cells are mediated by a chronic hypersecretion of TSH. The immunoassay for TSH is highly species-specific, with considerable interanimal and interassay variation.

Xenobiotics that disrupt thyroid hormone synthesis, secretion, or peripheral metabolism often result in prompt increases in circulating TSH levels. It is important in the design of experiments to evaluate the effect of a xenobiotic on thyroid function to have several early collection intervals. For example, FD&C Red No. 3 (a relatively mild goitrogen in rats) at a high dose (4%) resulted in a significant elevation in serum TSH at 3 days, which persisted throughout

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a 60-day experiment. Previous experiments with the same compound at the same dose (4%) using fewer rats and sequential periorbital bleeding demonstrated an upward trend in TSH, but the differences were not significant due to considerable animal-to-animal variations.

Thyroid-stimulating hormone levels are higher in male than in female rats, and castration decreases both the baseline serum TSH and the response to thyrotropin-releasing hormone injection. Follicular cell height often is higher in male than in female rats in response to the greater circulating TSH levels. The administration of exogenous testosterone to castrated male rats restores the TSH level to that of intact rats. Thyroid carcinomas develop at a higher incidence following irradiation in males than in females (2 : 1), and castration of irradiated male rats decreases the incidence to that of intact irradiated female rats. Testosterone replacement in castrated male rats restores the incidence of radiation-induced thyroid carcinomas in proportion to the dose of testosterone; similarly, serum TSH levels increase proportionally to the dose of replacement hormone. A similar higher incidence of follicular cell hyperplasia and neoplasia has been reported in male rats than in female rats following the administration of a wide variety of drugs and chemicals in toxicity and carcinogenicity testing.

Commercial TSH assays are available to measure canine TSH, and include immunoradiometric, luminescence, or ELISA formats. The β subunit of canine TSH has an 89% homology with the human subunit, and circulating TSH levels in dogs are 10- to 20-fold lower than those in humans. The canine TSH assays have a low diagnostic sensitivity since some hypothyroid dogs do not have increased serum TSH concentrations.

Recommended Design Features for Measurement of Thyroid Hormones

There are critical design features that are recommended for mechanistic toxicity studies involving the measurement of thyroid hormones in animals. These include the following.

1. The animals should be sexually mature.
2. Stress must be kept to a minimum during the study. Stress is known to both disturb diurnal rhythms and alter hormone secretion. The rats can be conditioned to human handling daily to reduce the effects of stress. Intravenous catheters can be used to mimimize stress and permit serial sample collection after dosing.
3. Blood samples should be collected to minimize hemolysis and sample contamination. Blood for hormone assays at sacrifice should be collected from the abdominal aorta to provide a larger sample with minimal contamination by tissue fluids.
4. Blood sampling should be conducted by an experienced technician who can perform the technique quickly.
5. Blood samples should be collected in the morning at the same time to avoid variation due to diurnal rhythms.
6. Blood collection schedules should be consistent across dose groups within narrow time ranges (e.g., within 1 hour).
7. Rats should be housed three rats per cage; ventilated caging is preferable.
8. Group sizes should be a minimum of 10 rats and preferably 15–20 rats per group to identify mild changes in serum hormone concentrations. This is especially important when measuring thyroid hormones (particularly TSH), since serum concentrations of thyroid hormones are known to be variable.
9. Rats should be sacrificed between 8 am and noon to minimize the effects of diurnal variations on hormone levels, and sacrifice of rats should be rotated between all experimental groups.
10. Aliquots of serum or plasma should be frozen so that endocrine assays can be conducted on common days with the same kits or methods. All samples from one study should be performed at the same time.
11. Assays for hepatic enzyme induction (e.g., UGT and 5′-deiodinase) should use sensitive methods with T_4 and/or T_3 as substrates.

20.2. Morphological and Morphometric Evaluation of Thyroid Follicles

Morphological and morphometric evaluations of the follicular cells are sensitive

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indicators of potential toxic effects of xenobiotics on the thyroid gland. Fixation of the thyroid lobes attached to the adjacent trachea in buffered formalin is the best procedure for routine studies. After 24 hours of fixation, each thyroid lobe is dissected carefully from the perithyroidal connective tissue (preferably by one person), blotted gently with filter paper, and weighed individually. The combined weight of the right and left thyroid lobes can be expressed as a ratio to total body or brain weight. Changes in thyroid weight are a sensitive overall indicator of the effects of relatively mild goitrogenic chemicals on thyroid structure. Immunohistochemical procedures are particularly helpful with selected proliferative lesions to determine whether they are of follicular cell (thyroglobulin-positive) or C-cell (calcitonin-positive) origin.

Morphometric evaluation of thyroids is a valuable adjunct to histopathological evaluation and determination of thyroid weights to detect and quantitate subtle changes in follicular cells. This is particularly true for rats, where the histology of the normal thyroid (tall columnar follicular cells lining mainly small follicles containing small amounts of lightly eosinophilic colloid) reflects the high baseline TSH levels. Follicle diameter, colloid area, and follicular cell height can be measured from representative follicles in H&E-stained sections using image analysis software. Large, colloid-distended, inactive follicles at the periphery of the lobes are avoided.

Ultrastructural evaluation of thyroids of selected animals from control and experimental groups often is helpful in documenting early or subtle changes induced by xenobiotics. Rapid fixation of thyroid tissue is essential due to the high concentration of lysosomal enzymes in follicular cells. Perfusion with a glutaraldehyde-based fixative is preferred (especially in rodents), and consistently gives the best preservation of fine structural detail in thyroid follicular cells.

Histological examination of a biopsy of the thyroid is a useful and reliable aid in the diagnosis of thyroid disease in larger animals when either the results of serum assays for T_4 and T_3 are equivocal or a nodule is palpated in the thyroid area. Removal of the caudal quarter of either lobe of the thyroid for histological examination is a simple surgical procedure without significant risk.

20.3. Hematology

A moderate normocytic normochromic anemia often is associated with subnormal function of the thyroid in dogs. This anemia has also been observed in humans and in experimental animals, and is known to be of a non-regenerative type. The hemogram is characteristic of anemia associated with a variety of chronic diseases, including neoplasia and chronic infection.

20.4. *In Vitro* Systems

In vitro systems using non-transformed, TSH-dependent rat thyroid follicular cells (FRTL-5) are valuable additions to *in vivo* studies to further investigate the molecular mechanisms that control thyroid proliferative activity. Thyroid-stimulating hormone interaction with its surface receptor on quiescent thyroid cells in culture results in the activation of adenylyl cyclase and a striking intracellular increase of mRNAs for the cellular protooncogenes *c-fos* (within 15–30 minutes) and *c-myc* (peak 1–2 hours). These effects of TSH on thyroid cells *in vitro* can be mimicked by the direct addition of cAMP analogs or other factors that increase intracellular cAMP. The protein products of the *c-fos* and *c-myc* protooncogenes are largely restricted to the cell nucleus, and play a role in the replication of cells. Interestingly, adenomas and carcinomas of the human thyroid are characterized by *c-myc* expression but this is not found in the surrounding normal tissue. The enhanced expression of oncogenes in thyroid cells by long-term TSH stimulation, such as could result in the toxicity testing of xenobiotics, may result in interactions with other factors (certain point or structural mutations, growth factors, among others) that bring about cell transformation which ultimately results in neoplasia of the gland.

Although numerous *in vitro* thyroid follicular cell culture systems have been described (such as the Fisher rat thyroid line, FRTL-5), secretion of the thyroid hormones T_3 and T_4 is seldom measured *in vitro* due to the reduced ability of follicular cells growing in monolayer culture (i.e., lacking follicular organization) to concentrate and efficiently iodinate thyroglobulin. Thyroid function *in vitro* often is assessed by

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evaluating specific phases of thyroid hormone synthesis, including iodide trapping; thyroglobulin expression, secretion, and iodination; thyroid peroxidase synthesis and activity; and TSH receptor expression. These functions are TSH-dependent and can be altered in response to various growth factors, hormones, second messengers, and xenobiotics.

Thyroid follicular cell phagocytic activity can be quantitated by a sensitive non-radioactive assay using a normal rat thyroid follicular cell line (FRTL-5) with fluoresceinated latex beads and flow cytometry. This assay *in vitro* permits discrimination of the number of functionally active cells as well as the ability to estimate the level of activity of these cells. Phagocytosis can be stimulated by forskolin, cholera toxin, 8-BrcAMP, calcitriol, and TGF-β. In contrast, phagocytosis was inhibited by insulin, NaI, $CaCl_2$, and aminotriazole. The phagocytosis of latex beads was regulated in a manner similar to iodide trapping and could be altered by the addition of numerous compounds. Phagocytic activity was stimulated by both cAMP-dependent and cAMP-independent pathways. Flow cytometric evaluation of phagocytosis of fluorescent latex beads represents a simple, rapid, non-radioactive index of thyroid function *in vitro* following exposure to a variety of xenobiotic chemicals.

20.5. Animals as Models

Much of the significant physiological and pathological data in the literature on thyroid function and structure has come from studies in animals. Although the basic hypothalamic–pituitary–thyroid axis functions in a similar manner in animals and humans, there are differences between species that are important when extrapolating animal data from toxicity and carcinogenicity studies for human risk assessment. Several of these differences have been summarized previously in this section on thyroid follicular cells.

Long-term perturbations of the pituitary–thyroid axis by various xenobiotics or physiological alterations (e.g., iodine deficiency, partial thyroidectomy) are more likely to predispose the laboratory rat to a higher incidence of proliferative lesions in the thyroid (e.g., hyperplasia and adenomas of follicular cells) than is seen in the human thyroid. This appears to be particularly true in the male rat, in which there usually are higher circulating levels of TSH than in the female. The greater sensitivity of the rodent thyroid to derangement by drugs, chemicals, and physiological perturbations is also related to the shorter plasma half-life of thyroxine (T_4) in the rat (12–24 hours) than in humans (5–9 days) due, in part, to the considerable differences between species in the transport proteins for T_4.

There also are marked species differences in the sensitivity of the functionally important peroxidase enzyme to inhibition by xenobiotics. Thioamides (e.g., sulfonamides) and other chemicals can selectively inhibit the thyroperoxidase and significantly interfere with the iodination of tyrosyl residues incorporated in the thyroglobulin molecule, thereby disrupting the orderly synthesis of T_4 and T_3. A number of studies have shown that the long-term administration of sulfonamides results in the development of thyroid nodules, frequently in species (rat, dog, and mice) sensitive to but not in species (monkey, guinea pig, chicken, and human) resistant to the inhibition of peroxidase in follicular cells.

PART VI: PARATHYROID GLAND

21. INTRODUCTION

Parathyroid glands are present in all air-breathing vertebrates. Phylogenetically, parathyroids first appear in amphibians, coincident with the transition from an aquatic to a terrestrial life. It has been suggested that the appearance of parathyroid glands in tetrapods may have arisen from the need to protect against the development of hypocalcemia and the necessity to maintain skeletal integrity in terrestrial animals, which often are in a relatively low calcium-high phosphorus environment.

Calcium ion plays a key role in many fundamental biological processes, including muscle contraction, blood coagulation, enzyme activity, neural excitability, hormone release, and membrane permeability, in addition to being an essential structural component of the skeleton. Therefore, the precise control of Ca^{2+} in extracellular fluids is vital to the health of humans and animals. To maintain a constant concentration

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of Ca^{2+}, despite variations in intake and excretion, endocrine control mechanisms have evolved that primarily consist of the interaction of parathyroid hormone (PTH), calcitonin, and the active form of Vitamin D (1,25-dihydroxyvitamin D or calcitriol).

22. STRUCTURE AND FUNCTION

22.1. Embryology and Macroscopic Anatomy

Parathyroid glands in most animal species consist of two pairs of glands situated in the anterior cervical region. Rats are the exception because they have a single pair of parathyroid glands that are located close to the thyroid. Embryologically, parathyroids are of entodermal origin, derived from the third and fourth pharyngeal pouches in close association with the primordia of the thymus (Figure 58.49). The entodermal bud that forms the thyroid gland arises on the midline at the level of the first pharyngeal pouch. This gives rise to the thyroglossal duct that migrates caudally. In the dog and cat, both external and internal parathyroids are close to the thyroid gland. The external parathyroid (III) in the dog is 2–5 mm in length and is found in the loose connective tissue cranial and slightly lateral to the anterior pole of the thyroid. The internal parathyroid (IV) is smaller, flatter, and situated on the medial surface of the thyroid beneath the fibrous capsule.

22.2. Functional Cytology

Chief Cells

Parathyroids contain a single type of secretory cell concerned with the elaboration of a single hormone. Parathyroids of animals and humans are composed of chief cells in different stages of secretory activity and in transition to oxyphil cells in certain species (Figure 58.50). Chief cells interpreted to be in an inactive (resting or involuted) stage of their secretory cycle predominate in the parathyroid glands under normal

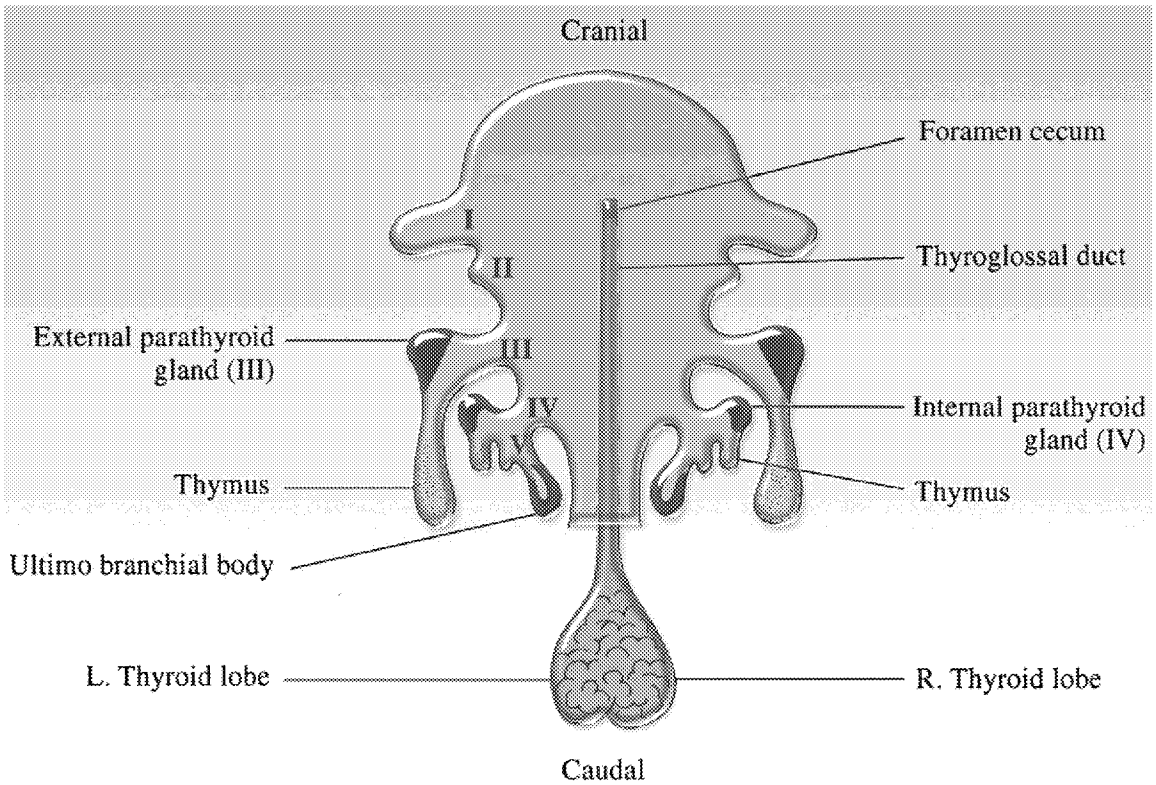

FIGURE 58.49 Embryology of the thyroid and parathyroid glands. The thyroid gland develops from a midline primordia (thyroglossal duct), whereas parathyroid glands develop from the cranial portions of the third and fourth pharyngeal pouch in association with the thymus.

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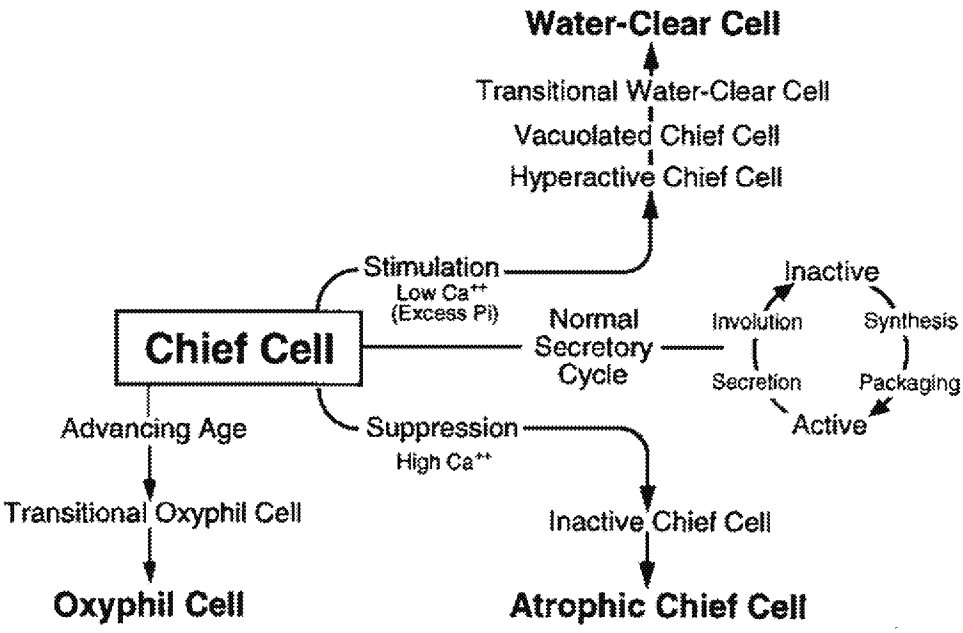

FIGURE 58.50 Functional cytology of parathyroid glands under normal and pathologic conditions. *Figure reproduced from Capen and Rosol (1993) Pathobiology of parathyroid hormone and parathyroid hormone-related protein: Introduction and evolving concepts. In:* Pathology of the Thyroid and Parathyroid Gland: An Update, *(LiVolsi, V. A. and DeLellis, R. A., eds), Williams & Wilkins, pp. 1–33, with permission.*

conditions. Inactive chief cells are cuboidal and have uncomplicated interdigitations between contiguous cells. The relatively electron-transparent cytoplasm contains poorly developed organelles and infrequent secretory granules. The cytoplasm often has either numerous lipid bodies and lipofuscin granules, or aggregations of glycogen particles. Chief cells in the active stage of the secretory cycle occur less frequently in the parathyroid glands of most species. The cytoplasm of active chief cells has an increased electron density due to the closer proximity of organelles and secretory granules, increased density of the cytoplasmic matrix, and loss of glycogen particles and lipid bodies.

Oxyphil Cells

The second cell type in the parathyroid glands of certain animal species and humans is the oxyphil cell (Figure 58.50). These cells are absent in parathyroids of the rat, chicken, and many species of lower animals. Oxyphil cells are observed either singly or in small groups interspersed between chief cells. They are larger than chief cells, and their abundant cytoplasmic area is filled with numerous large, often bizarre-shaped, mitochondria. Glycogen particles and free ribosomes are interspersed between the mitochondria. Granular endoplasmic reticulum, Golgi apparatuses, and secretory granules are poorly developed in oxyphil cells of normal parathyroid glands, suggesting that oxyphil cells do not have an active function in the biosynthesis of parathyroid hormone. Associated with the marked increase in mitochondria, oxyphil cells have been shown histochemically to have higher oxidative and hydrolytic enzyme activity than chief cells.

Cells are observed with cytoplasmic characteristics intermediate between those of chief and oxyphil cells. These transitional oxyphil cells have numerous mitochondria but other organelles are present, including rough endoplasmic reticulum, Golgi apparatuses, and secretory granules. The significance of oxyphil cells in the pathophysiology of the parathyroid glands has not been elucidated completely. They are not altered in response to either short-term hypocalcemia or hypercalcemia in animals, but both oxyphil cells and transitional forms may be increased in response to the long-term stimulation of human parathyroid glands. Therefore, oxyphil cells do not appear to be degenerate chief cells, as previously thought, but are metabolically active cells, derived from chief cells as the result of aging or some other metabolic derangement.

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22.3. Parathyroid Hormone

Biosynthesis and Chemistry

A larger biosynthetic precursor of parathyroid hormone is first synthesized on ribosomes of the rough endoplasmic reticulum in chief cells. Preproparathyroid hormone (pre-proPTH) is the initial translation product synthesized on ribosomes (Figure 58.51). It is composed of 115 amino acids and contains a hydrophobic signal sequence of 25 amino acid residues that facilitates the penetration and subsequent vectorial discharge of the nascent peptide into the cisternal space of the rough endoplasmic reticulum. Pre-proPTH is converted rapidly (within 1 minute or less) to proparathyroid hormone (proPTH) by the proteolytic cleavage of 25 amino acids from the NH_2 terminus of the molecule. The intermediate precursor, proPTH, is composed of 90 amino acids and moves within the membranous channels of the rough endoplasmic reticulum to the Golgi apparatus (Figure 58.51). Enzymes within membranes of the Golgi apparatus cleave a hexapeptide from the NH_2-terminal (biologically

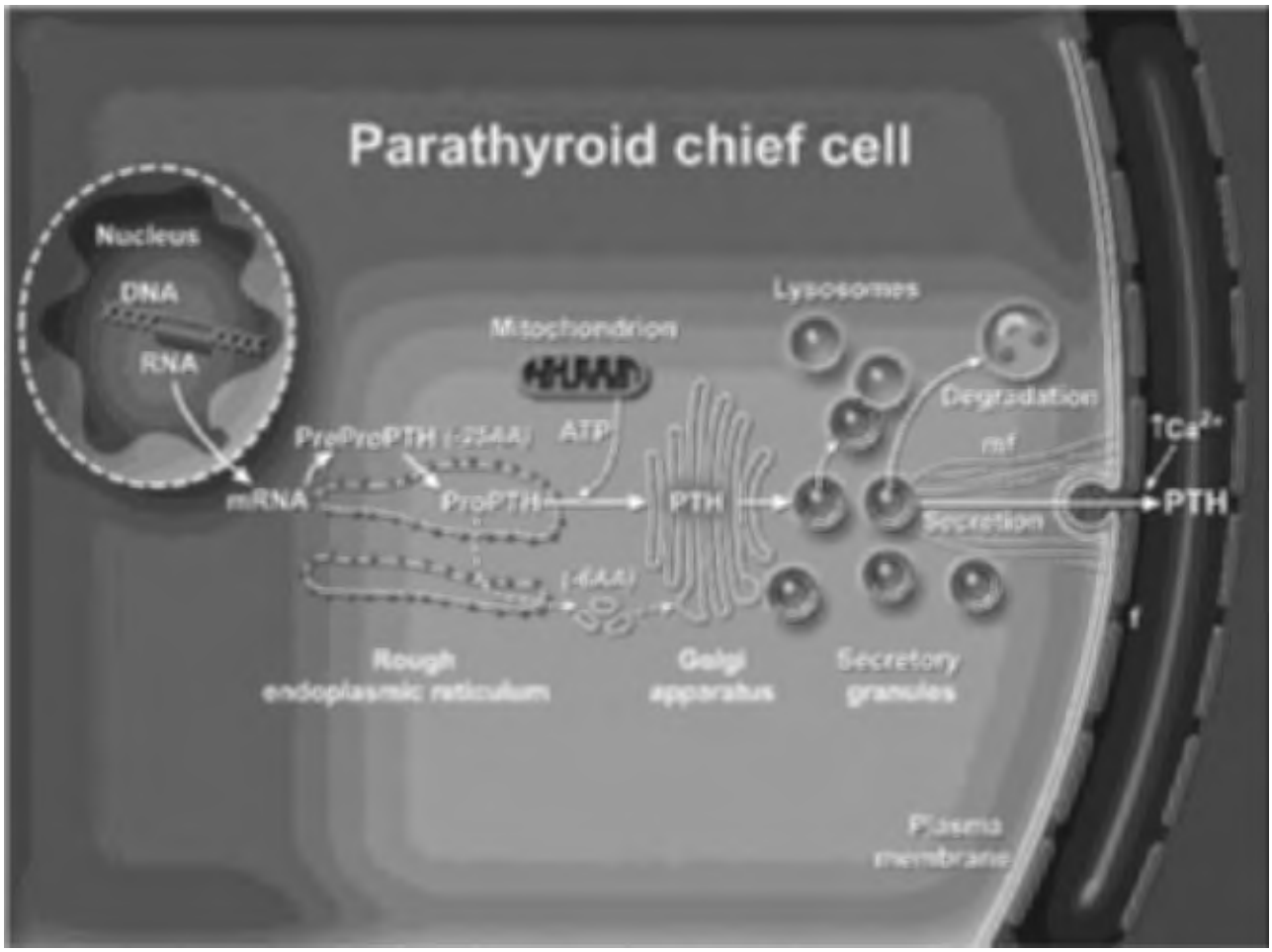

FIGURE 58.51 Subcellular compartmentalization, transport, and cleavage of precursors of parathyroid hormone (PTH). Preproparathyroid hormone (PreProPTH) is the initial translation product from ribosomes of the rough endoplasmic reticulum, which is converted rapidly to proparathyroid hormone (ProPTH). The hydrophobic sequence on the amino-terminal end of the PreProPTH facilitates penetration of the leading portion of the nascent peptide into the lumen of the endoplasmic reticulum. ProPTH is transported to the Golgi apparatus where it is converted enzymatically by a carboxypeptidase to biologically active PTH with a loss of six amino acids (AA). A major portion of the biosynthetic precursors and active PTH is degraded by lysosomal enzymes and is not secreted by chief cells under normal conditions. Extracellular Ca^{2+} binds to a membrane calcium receptor (not shown) that induces secretion of mature secretory granules with the participation of microfilaments (mf). PTH enters the blood through capillary fenestrae (f).

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active) end of the molecule, forming active parathyroid hormone (Figure 58.51). Active PTH is packaged into membrane-limited secretory granules in the Golgi apparatus for subsequent storage in chief cells. Under certain conditions of increased demand, PTH may be released directly from chief cells without being packaged into secretory granules.

The biologically active parathyroid hormone secreted by chief cells is a straight chain polypeptide consisting of 84 amino acid residues with a molecular weight of 9500. Molecular fragments of PTH are formed in the peripheral circulation and in the liver. The immunoheterogeneity created by the multiple circulating fragments of PTH has caused significant problems in the development and application of highly specific radioimmunoassays to diagnostic problems in human patients and animals.

Storage and Secretion

Secretory (storage) granules have been demonstrated ultrastructurally within chief cells of the parathyroid glands in humans and all animal species examined. The paucity of secretory granules in certain species (e.g., rat) suggests that chief cells, in general, do not store a large concentration of preformed hormone. PTH in the cytoplasm and secretory granules can be stained by immunofluorescent or immunohistochemical techniques. Oxyphil cells do not stain for PTH.

Chief cells synthesize and secrete another major protein, chromogranin A (parathyroid secretory protein I). It is a 70-kDa protein composed of 430–448 amino acids that is costored and secreted with PTH. A similar molecule has been found in secretory granules of a wide variety of peptide hormone-secreting cells and in neurotransmitter secretory vesicles. An internal region of chromogranin A is identical in sequence to pancreastatin, a C-terminal amidated peptide that inhibits glucose-stimulated insulin secretion. This 49 amino-acid proteolytic cleavage product of the parathyroid secretory protein has been reported to inhibit a low calcium-stimulated secretion of parathyroid hormone and chromogranin A from parathyroid cells. These findings suggest that chromogranin A-derived peptides may act locally in an autocrine manner to inhibit the secretion of active hormone by endocrine cells, such as those of the parathyroid gland.

Chief cells in closely related species may vary considerably in the number of storage granules in their cytoplasm. For example, granules are infrequent in rats but numerous in mouse parathyroids. Secretory granules in chief cells usually are small, ranging from 100 to 300 μm in greatest diameter, and are composed of fine, dense particles. They are electron-dense, surrounded by a closely applied limiting membrane, and are round to oval in shape. Secretion granules consistently are smaller and more regular in shape than the acid phosphatase-positive lysosomal bodies (450–800 μm) and lipofuscin granules present in chief cells.

In response to the appropriate stimulus, secretory granules migrate peripherally in chief cells and their limiting membrane fuses with the plasma membrane of the cell. An internal cytoskeleton composed of microtubules and contractile microfilaments is important in the control of peripheral movement of secretory granules and liberation of secretory products. The administration of colchicine, an inhibitor of intracellular transport and release of secretory products in a number of endocrine organs, also blocks the secretion of PTH from chief cells in rats.

Secretory cells in the parathyroid gland store small amounts of preformed hormone but are capable of responding to minor fluctuations in Ca^{2+} concentration rapidly by altering the rate of hormonal secretion, and more slowly by altering the rate of hormonal synthesis. In contrast to most endocrine organs that are under complex controls involving both long and short feedback loops, parathyroids have a unique feedback controlled by the concentration of Ca^{2+} (and, to a lesser extent, magnesium) ion in the serum. If the blood Ca^{2+} is elevated by the intravenous infusion of Ca^{2+}, there is a rapid and pronounced reduction in circulating levels of immunoreactive parathyroid hormone (iPTH). Conversely, if the blood Ca^{2+} is lowered by ethylenediaminetetraacetic acid (EDTA), there is a brisk and substantial increase in iPTH levels. The concentration of blood phosphorus has no direct regulatory influence on the synthesis and secretion of PTH; however, certain disease conditions with hyperphosphatemia in both animals and humans are associated clinically with secondary hyperparathyroidism. An elevated blood phosphorus level may lead indirectly to parathyroid stimulation by virtue of its ability to lower blood Ca^{2+}. If

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the blood phosphorus is elevated significantly by an infusion and Ca^{2+} is administered simultaneously in amounts to prevent the accompanying reduction of blood Ca^{2+}, plasma iPTH levels remain within the normal range. Magnesium ion has an effect on parathyroid secretion rate similar to that of Ca^{2+}, but its effect is not equipotent to that of Ca^{2+}.

Recently synthesized and processed active PTH may be released directly in response to increased demand and bypass the storage pool of mature secretory granules in the cytoplasm of chief cells. Bypass secretion of parathyroid hormone can be stimulated only by a low circulating concentration of Ca^{2+}.

Serum Ca^{2+} binds to the cell membrane Ca^{2+} receptor on the chief cell, which permits the serum Ca^{2+} to regulate chief cell function. The receptor is present on the plasma membranes of parathyroid chief cells, renal epithelial cells, C cells of the thyroid gland, and other cells that respond to extracellular Ca^{2+}. Mutations in one or both of the Ca^{2+} sensing receptor genes in humans result in familial hypocalciuric hypercalcemia or neonatal severe hypercalcemia, respectively. Interaction of serum Ca^{2+} with its receptor on chief cells results in the formation of an inverse sigmoidal relationship between serum Ca^{2+} and PTH concentrations. The serum Ca^{2+} that results in half maximal PTH secretion is defined as the serum Ca^{2+} "set point," and is stable for an individual animal. The sigmoidal relationship between serum [Ca^{2+}] and PTH secretion permits the chief cells to respond rapidly to a reduction in serum Ca^{2+}. The major inhibitors of PTH synthesis and secretion are increased serum [Ca^{2+}] and 1,25-dihydroxyvitamin D. Inhibition of PTH synthesis by 1,25-dihydroxyvitamin D completes an important endocrine feedback loop between the parathyroid chief cells and the renal epithelial cells because PTH stimulates renal production of 1,25-dihydroxyvitamin D.

Biological Actions

Parathyroid hormone is the principal hormone involved in the minute-to-minute fine regulation of blood Ca^{2+} in mammals. It exerts its biological actions by directly influencing the function of target cells, primarily in bone and kidney, and indirectly influencing cells in the intestine to maintain plasma Ca^{2+} at a level sufficient to ensure the optimal functioning of a wide variety of body cells. The action of PTH on bone is the mobilization of Ca^{2+} from skeletal reserves into extracellular fluids. The increase in blood Ca^{2+} results from an interaction of PTH with osteoblasts and osteoclasts in bone along with increased tubular reabsorption of Ca^{2+} in the kidney.

BONE

Osteoclasts are primarily responsible for the catabolic action of PTH on bone by increasing resorption. PTH stimulates increased numbers and activity of osteoclasts; however, osteoclasts do not have receptors for PTH. Receptors for PTH are present on osteoblasts. Isolated osteoclasts respond to PTH only with the concurrent presence of osteoblasts. PTH stimulates osteoblasts to produce RANKL (receptor activator of NFκB ligand), which binds to its receptor, RANK) on osteoclast precursers and osteoclasts to increase the number and function of osteoclasts, respectively. PTH inhibits the production of osteoprotegerin (OPG) by osteoblasts. OPG is a secreted decoy receptor for RANKL, which blocks its action in bone. Therefore the ratio of RANKL : OPG in the bone microenvironment regulates the level of osteoclastic bone resorption. OPG and antibodies to RANKL have been used experimentally or clinically to inhibit bone resorption *in vivo*.

KIDNEY

Parathyroid hormone has a rapid (5- to 10-minute) and direct effect on renal tubular function, leading to decreased reabsorption of phosphorus and phosphaturia in the proximal tubule of the nephron. PTH binds to a receptor on the basolateral aspect of renal epithelial cells. The hormone stimulates adenylyl cyclase, increases intracellular cAMP, and inhibits phosphorus reabsorption across the brush border through the actions of protein kinases.

PTH also increases the reabsorption of Ca^{2+}, which is of considerable importance in the maintenance of Ca^{2+} homeostasis. The effect of PTH on tubular reabsorption of Ca^{2+} appears to be due to a direct action on the distal convoluted tubule, and is coupled to increases in intracellular cAMP. Transcellular transport of Ca^{2+} from the renal tubule lumen to the blood by the kidney involves the Ca^{2+}-selective channels (TRPV5 and 6) on the luminal (apical) side of the renal epithelial cells, intracellular calbindins

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to buffer Ca^{2+}, and the Ca^{2+}–Na^{+} exchanger (NCX) and Ca^{2+}-ATPase (PMCA) on the basilar side of the epithelial cells. In addition to PTH, the cell membrane Ca^{2+} receptor, calcitriol, and calcitonin regulate renal Ca^{2+} reabsorption.

Subcellular Mechanism of Action

The receptor for the N-terminal portion of PTH, composed of 34 amino acids, is considered the most important region in terms of Ca^{2+} regulation. The receptor for N-terminal PTH is a seven transmembrane domain receptor that is expressed in renal epithelial cells, osteoblasts, and dermal fibroblasts, and is also found on cells that are not associated with the actions of PTH. Binding of PTH to the receptor results in increased levels of cytoplasmic cAMP and Ca^{2+} by stimulation of the adenylyl cyclase and phosphatidyl inositol pathways.

Degradation

Parathyroid hormone is secreted continuously from chief cells primarily as the intact (1–84 amino acid sequence) molecule under normal conditions. In the liver (Küpffer cells) PTH is cleaved into the smaller (approximately one-third of the molecule) NH_2-terminal (biologically active) portion and a larger (approximately two-thirds of the molecule) C-terminal (biologically inactive) portion. The kidney is also a major organ for the degradation and excretion of PTH. Biologically active PTH from the peritubular capillaries is degraded by specific proteases on the surface of renal tubular cells. In addition, both biologically active and inactive fragments are degraded intracellularly by lysosomal enzymes in renal tubular cells. C-terminal PTH has a longer circulating half-life compared to full-length and NH_2-terminal PTH.

23. MECHANISMS OF TOXICITY

23.1. Agents Influencing the Development of Proliferative Lesions

Age

There are relatively few chemicals or experimental manipulations reported in the literature that significantly increase the incidence of parathyroid tumors. Longstanding renal failure with intense diffuse hyperplasia does not appear to increase the development of chief cell tumors in animals. The historical incidence of parathyroid adenomas in untreated control male F344 rats in studies conducted by the National Toxicology Program was 4 in 1315 (0.3%); the incidence in female F344 rats was 2 in 1330 (0.15%). Parathyroid adenomas in F344 rats are an example of a neoplasm whose incidence increases dramatically when comparing 2-year studies to lifetime data, where the incidence of parathyroid adenomas increased in males from 0.1% at 2 years to 3.1% in lifetime studies. Corresponding data for female F344 rats was 0.1% at 2 years and 0.6% in lifetime studies.

Irradiation and Vitamin D

Irradiation significantly increases the incidence of parathyroid adenomas in inbred Wistar albino rats, and the incidence can be modified by feeding diets with variable amounts of Vitamin D. Neonatal Wistar rats were given either 5 or 10 μCi radioiodine (^{131}I) within 24 hours of birth. In rats 12 months of age and older, parathyroid adenomas were found in 33–37% of those administered ^{131}I compared to 0% in unirradiated controls. The incidence of parathyroid adenomas was highest (55%) in normocalcemic rats fed a low Vitamin D diet and lowest (20%) in irradiated rats fed a high Vitamin D diet (40 000 IU/kg) and having a significant elevation in plasma Ca^{2+}.

Gonadectomy

Oslapas and colleagues (1982) reported an increased incidence of parathyroid adenomas in female (34%) and male (27%) rats of the Long-Evans strain administered 40 μCi sodium ^{131}I and saline at 8 weeks of age. There were no significant changes in serum Ca^{2+}, phosphorus, and parathyroid hormone compared with controls. Gonadectomy performed at 7 weeks of age decreased the incidence of parathyroid adenomas in irradiated rats (7.4% in gonadectomy as opposed to 27% in intact controls), but there was little change in irradiated females. X-irradiation of the thyroid–parathyroid region also increased the incidence of parathyroid adenomas. When female Sprague-Dawley rats received a single absorbed dose of X-rays at 4 weeks of age, they subsequently developed

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a 24% incidence of parathyroid adenomas after 14 months.

Xenobiotics

Parathyroid adenomas have been encountered infrequently following the administration of a variety of chemicals in 2-year bioassay studies in Fischer rats. In a study of the pesticide rotenone in F344 rats, there appeared to be an increased incidence of parathyroid adenomas in high-dose (75 ppm) males compared with low-dose (38 ppm) males, control males, or NTP historical controls. It was uncertain whether the increased incidence of this uncommon tumor was a direct effect of rotenone feeding or the increased survival time in high-dose males. Chief cell hyperplasia was not present in parathyroids that developed adenomas.

23.2. Modification of Parathyroid Function Associated with Metabolic Disorders

Renal Hyperparathyroidism

Secondary hyperparathyroidism as a complication of chronic renal failure is a metabolic state characterized by an excessive, but not autonomous, rate of PTH secretion. The secretion of hormone by the hyperplastic parathyroid gland usually remains responsive to fluctuations in blood Ca^{2+}. The primary etiologic mechanism in this disorder is longstanding progressive renal disease. When the renal disease progresses to the point at which there is a significant reduction in the glomerular filtration rate, phosphorus is retained and progressive hyperphosphatemia develops (Figure 58.52). Hyperphosphatemia contributes to parathyroid stimulation by virtue of its ability to lower blood Ca^{2+} levels.

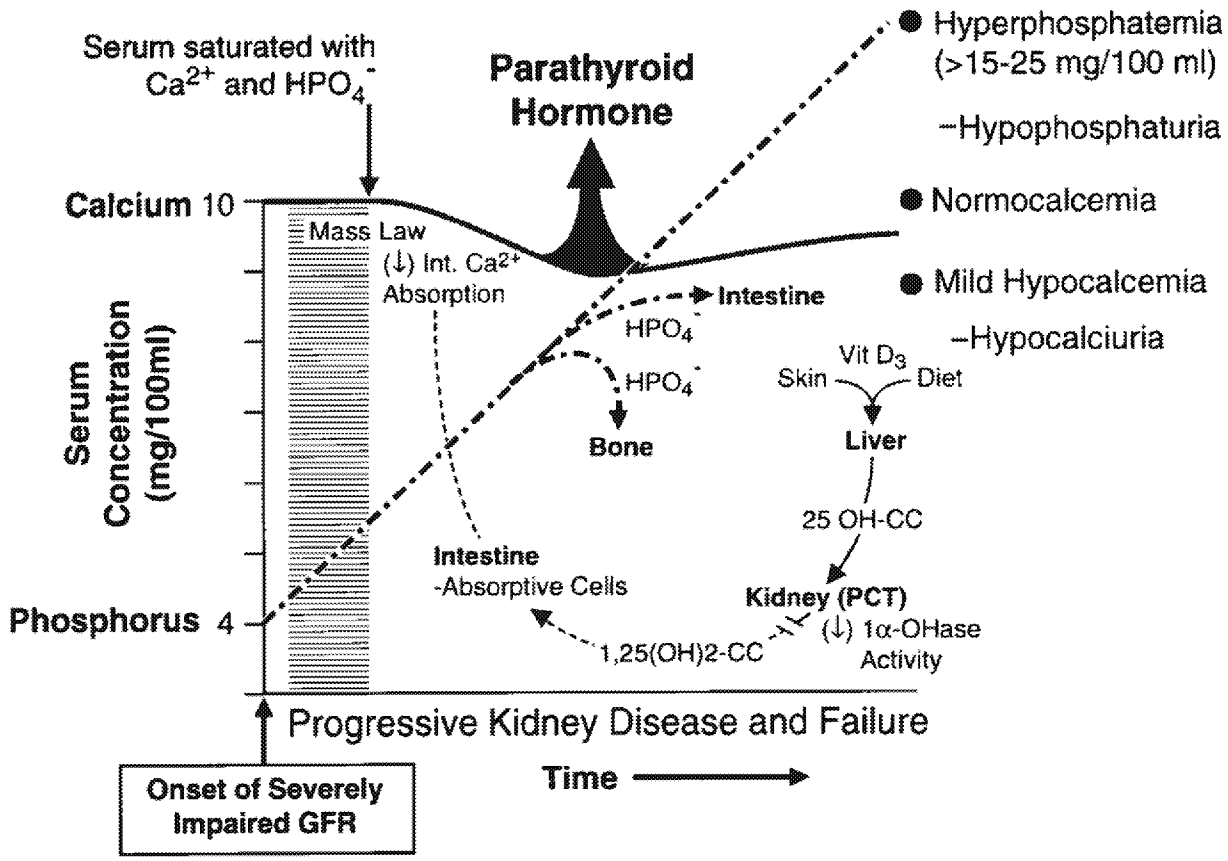

FIGURE 58.52 Alterations in levels of serum calcium and phosphorus during the pathogenesis of secondary hyperparathyroidism associated with chronic progressive renal failure. *Figure reproduced from* Handbook of Toxicologic Pathology, *2nd Ed. W. M. Haschek, C. G. Rousseaux and M. A. Wallig, eds. (2002) Academic Press, Vol. 2, Fig. 13, p. 696, with permission.*

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Parathyroid stimulation associated with chronic renal disease also can be attributed to hypocalcemia. An impaired intestinal absorption of Ca^{2+} due to an acquired defect in Vitamin D metabolism plays a role in the development of hypocalcemia associated with renal insufficiency. Chronic renal disease interferes with the production of 1,25-dihydroxyvitamin D_3 by the kidney, thereby diminishing intestinal Ca^{2+} transport. 1,25-Dihydroxyvitamin D_3 also completes an important negative feedback loop with the parathyroid gland since it inhibits the synthesis and secretion of PTH by the chief cells. Decreased 1,25-dihydroxyvitamin D_3 will lead to the release of chief cells from negative feedback and secondary hyperparathyroidism. All parathyroids are considerably enlarged as a result initially of hypertrophy of chief cells, and subsequently due to hyperplasia as a compensatory mechanism.

Nutritional Hyperparathyroidism

The increased secretion of PTH in nutritional hyperparathyroidism is a compensatory mechanism directed against a disturbance in mineral homeostasis induced by nutritional imbalances. The disease occurs in dogs, cats, monkeys, laboratory rodents, and others fed improper diets. Dietary mineral imbalances of etiologic importance in the pathogenesis are a low content of Ca^{2+}, excessive phosphorus with normal or low Ca^{2+}, and inadequate amounts of cholecalciferol (Vitamin D_3) in New World non-human primates housed indoors without exposure to sunlight. The significant end result is hypocalcemia, which results in parathyroid stimulation (Figure 58.53). A diet low in Ca^{2+} fails to supply the daily requirement, even though a greater proportion of ingested Ca^{2+} is absorbed, and hypocalcemia develops. Ingestion of excessive phosphorus results in increased

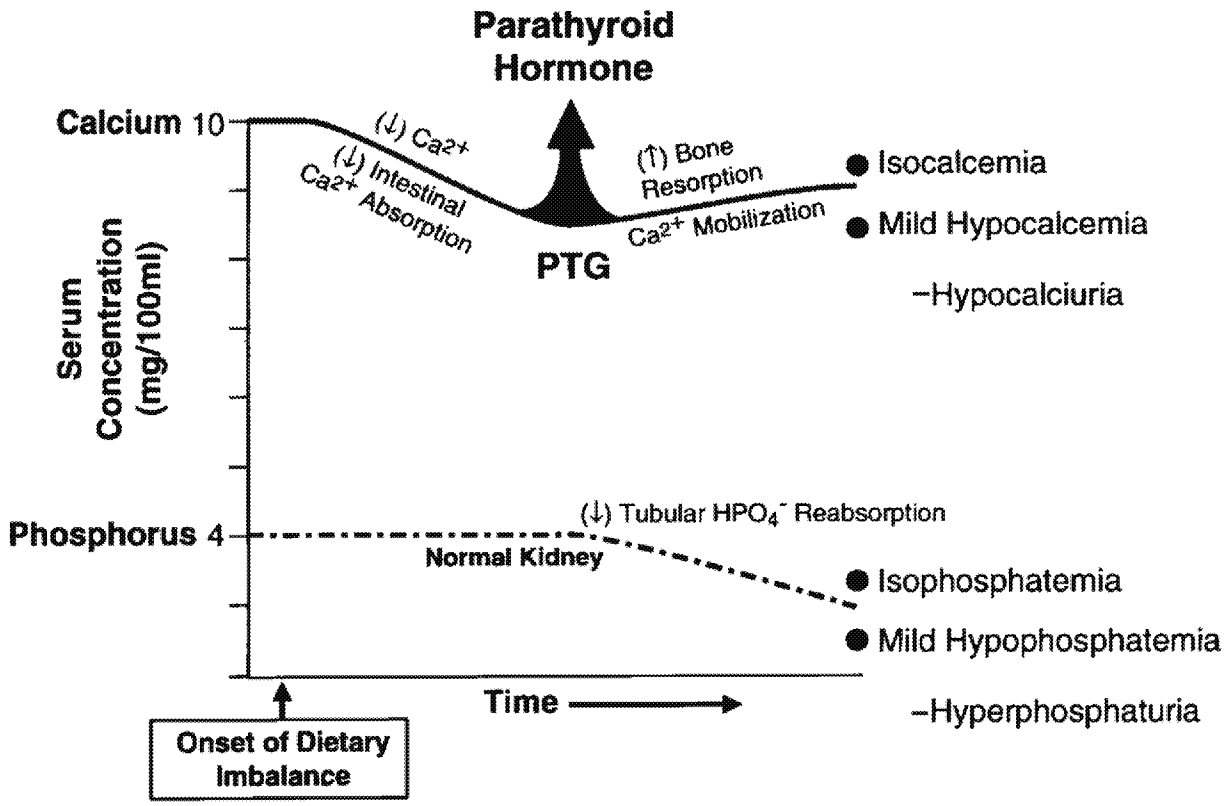

FIGURE 58.53 Alterations in serum calcium and phosphorus in the pathogenesis of nutritional secondary hyperparathyroidism caused by feeding a diet low in calcium or deficient in cholecalciferol but with normal amounts of phosphorus. *Figure reproduced from* Handbook of Toxicologic Pathology, *2nd Ed. W. M. Haschek, C. G. Rousseaux and M. A. Wallig, eds. (2002) Academic Press, Vol. 2, Fig. 54, p. 763, with permission.*

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intestinal absorption and elevation in blood phosphorus levels. Hyperphosphatemia does not stimulate the parathyroid gland directly but does so indirectly by virtue of its ability to lower blood Ca^{2+} levels and suppress the synthesis of 1,25-dihydroxycholecalciferol by the kidney. In response to nutritionally induced hypocalcemia, all parathyroid glands undergo cellular hypertrophy and hyperplasia.

Hypoparathyroidism

Hypoparathyroidism is a metabolic disorder in which either subnormal amounts of PTH are secreted by pathologic parathyroids, or the hormone secreted is unable to interact normally with target cells. Hypoparathyroidism often is associated with diffuse lymphocytic parathyroiditis, resulting in the extensive degeneration of chief cells and replacement by fibrous connective tissue. In the early stages of lymphocytic parathyroiditis there is infiltration of the gland with lymphocytes and plasma cells, and nodular regenerative hyperplasia of the remaining chief cells. Lymphocytic parathyroiditis appears to develop by immune-mediated mechanisms, as a similar destruction of secretory parenchyma and lymphocytic infiltration has been produced experimentally by repeated injections of parathyroid tissue emulsions. The functional disturbances of hypoparathyroidism primarily are the result of increased neuromuscular excitability and tetany. Bone resorption is decreased because of a lack of PTH, and blood Ca^{2+} levels diminish and serum phosphate levels increase progressively.

Primary Hyperparathyroidism

In primary hyperparathyroidism, PTH is produced in excess by a functional tumor in the gland. The normal control of PTH secretion by the concentration of blood Ca^{2+} is lost in primary hyperparathyroidism. Hormone secretion is autonomous, and the parathyroid produces excessive hormone despite the increased blood Ca^{2+}. PTH acts initially on cells in the renal tubules to promote the excretion of phosphorus and the retention of Ca^{2+}. A prolonged increased secretion of PTH results in accelerated bone resorption and increased renal production of 1,25-dihydroxyvitamin D_3. The lesion in the parathyroid gland responsible for the excessive secretion of PTH usually is an adenoma composed of active chief cells.

Cancer-Associated Hypercalcemia

There are three mechanisms by which tumors can induce hypercalcemia: hematologic cancers present in the bone marrow induce hypercalcemia by causing the local destruction of bone; solid tumors can widely metastasize to bone and result in local bone loss associated with the tumor metastases; and humoral hypercalcemia of malignancy (HHM), in which solid tumors that have no or few metastases to bone induce their effects distant from the site of the tumor by the elaboration of one or more factors.

Humoral hypercalcemia of malignancy is a syndrome associated with diverse malignant neoplasms in dogs, humans, and cats, and rarely in other animals, but it has been reported in rats and mice. Characteristic clinical findings with HHM include hypercalcemia, hypophosphatemia, hypercalciuria (often with decreased fractional Ca^{2+} excretion), increased fractional excretion of phosphorus, increased nephrogenous cAMP, and increased osteoclastic bone resorption. Hypercalcemia is induced by humoral effects on bone, kidneys, and possibly the intestine.

Malignant neoplasms that are commonly associated with HHM in animals include the adenocarcinoma derived from apocrine glands of the anal sac in dogs, some T-cell lymphomas of dogs, and miscellaneous carcinomas that induce HHM sporadically. Excessive secretion of biologically active parathyroid hormone-related protein (PTHrP) by cancer cells plays a central role in the pathogenesis of hypercalcemia in most forms of HHM.

PTHrP is produced by a number of normal tissues in neonates and adults, where it functions primarily as a paracrine factor. For example, PTHrP expression by chondrocytes regulates the growth and differention of chondrocytes in the growth plate and, indirectly, the length of the long bones. In the fetus, PTHrP regulates Ca^{2+} balance and placental Ca^{2+} transport. Genetic disruption of PTHrP leads to death after birth.

In HHM, PTHrP binds to the common N-terminal PTH/PTHrP (PTH1) receptor in bone and kidney, but does not cross-react immunologically with native PTH. PTHrP stimulates adenylyl cyclase and increases intracellular Ca^{2+} in bone and kidney cells. Increased osteoclastic bone resorption is a consistent finding in HHM with increased Ca^{2+} release from bone.

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The kidneys play a critical role in the pathogenesis of hypercalcemia, as renal Ca^{2+} reabsorption is stimulated by PTHrP. In some forms of HHM there are increased serum 1,25-dihydroxyvitamin D_3 levels, which may increase Ca^{2+} absorption from the intestine. Other tumor-related factors, such as interleukin-1, transforming growth factors α and β, and tumor necrosis factor, stimulate bone resorption *in vivo* and *in vitro* and function synergistically with PTHrP in animals with HHM.

24. RESPONSE OF PARATHYROID CHIEF CELLS TO INJURY

24.1. Parathyroid Cysts

Small cysts are observed frequently as naturally occurring lesions within the parenchyma of the parathyroid or in the immediate vicinity of the glands in rats and dogs, and occasionally in other animal species. Parathyroid cysts usually are multiloculated, lined by a cuboidal to columnar (often partially ciliated) epithelium, and contain a densely eosinophilic proteinic material. Parathyroid (Kürsteiner's) cysts develop from a persistence and dilatation of remnants of the duct that connects the parathyroid and thymic primordia during embryonic development (Figure 58.49). Cyst fluid has been reported to contain higher levels of immunoreactive PTH (1–84 intact molecule and 39–84 carboxyl fragments) than serum. The lining cells stain for PTH by immunohistochemistry. Similar cysts may be present in the anterior mediastinum when remnants of the embryonic duct are displaced with the caudal migration of the thymus. Parathyroid cysts are distinct from midline cysts derived from remnants of the thyroglossal duct. The latter are lined by multilayered thyroidogenic epithelium that often has colloid-containing follicles. They usually are located near the midline from the base of the tongue caudally into the mediastinum.

24.2. Proliferative Lesions of Parathyroid Chief Cells

Incidence

Proliferative lesions of the parathyroid gland include hyperplasia (diffuse and focal), adenomas, and carcinomas. Neoplasms of the parathyroid glands are uncommon in all species of laboratory and domestic animals, but occur at low incidence in rats, Syrian hamsters, dogs, and, rarely, mice. Parathyroid hyperplasia may be primary or secondary. Primary parathyroid hyperplasia or tumors may be functional (endocrinologically active) or non-functional, depending on their ability to secrete PTH. Primary hyperparathyroidism is the disorder that results from the excessive and autonomous secretion of PTH from adenomas or chief cell hyperplasia. Atrophy of the remaining normal parathyroid tissue is present with functional tumors and multinodular parathyroid hyperplasia.

Hyperplasia

FOCAL ("NODULAR") HYPERPLASIA

Chief cell hyperplasia may affect the parathyroid in a distinctly focal or multifocal distribution. In focal parathyroid hyperplasia there are single or multiple nodules in one or both glands where there is an increased number of closely packed chief cells often with an expanded cytoplasmic area (Figure 58.54). The focal area(s) of chief cell hyperplasia is poorly demarcated and not encapsulated from adjacent parenchyma. Chief cells within the nodules have a relatively uniform composition with a high cytoplasm : nucleus ratio and a slightly more

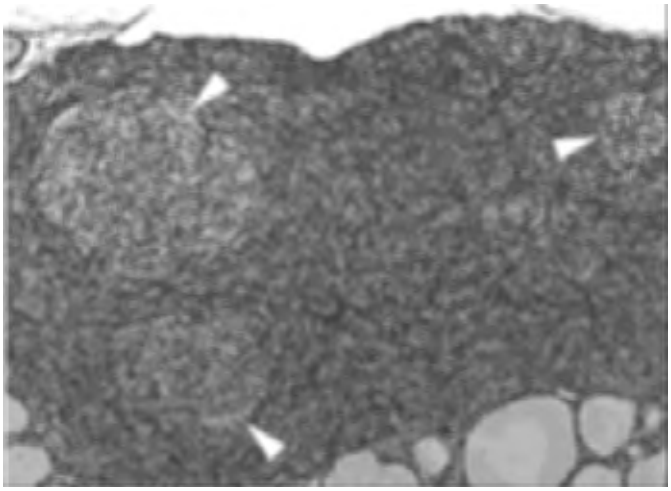

FIGURE 58.54 Multifocal (nodular) hyperplasia (arrowheads) of chief cells in parathyroid glands of a Wistar Han rat. The focal areas of increased cellularity are poorly demarcated and not encapsulated from adjacent parathyroid parenchyma. H&E stain.

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hyperchromatic nucleus than adjacent normal chief cells. There may be slight compression of adjacent chief cells around larger focal areas of hyperplasia. Focal chief cell hyperplasia often is difficult to separate from a chief cell adenoma using only morphological criteria. The presence of multiple nodules of varying sizes and uniform cellularity in one or both parathyroids with minimal compression and no encapsulation is more compatible with an interpretation of focal hyperplasia than chief cell adenoma.

DIFFUSE HYPERPLASIA

Parathyroid hyperplasia, as is seen with chronic renal failure and long-term dietary imbalances, results in a uniform enlargement of all parathyroid glands. In rats with chronic renal failure, diffusely hyperplastic parathyroid glands may be detected macroscopically as 1- to 2-mm nodules projecting from the surface of each thyroid lobe. The uniform enlargement of parathyroid glands in diffuse hyperplasia is due to both hypertrophy and hyperplasia of chief cells. There is no peripheral rim of compressed atrophic parathyroid parenchyma as seen around a functional adenoma, but there is a uniform population of hyperplastic chief cells extending to the capsule of the gland (Figure 58.55). Chief cells are packed together closely, often with indistinct cell boundaries. The expanded cytoplasmic area of chronically stimulated chief cells is lightly eosinophilic, with occasional distinct vacuoles. A more prominent fibrovascular stroma in some diffusely hyperplastic parathyroids may result in a lobulated appearance. In other hyperplastic parathyroids, chief cells form distinct acinus-like structures in the gland.

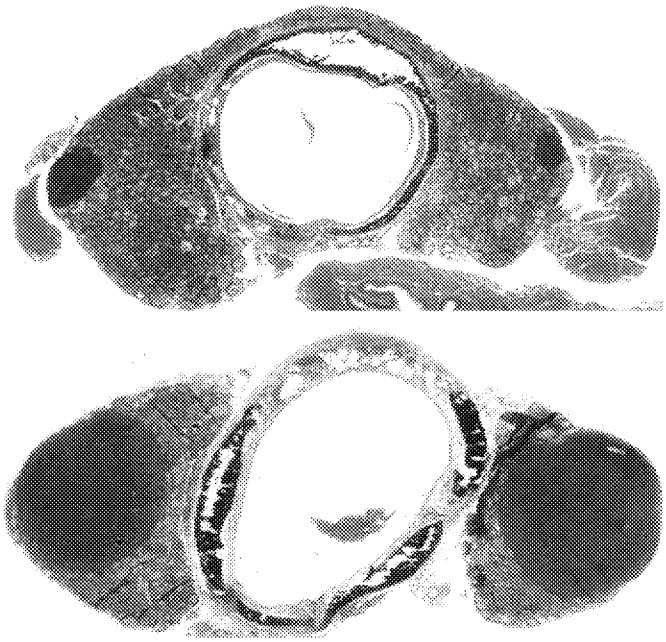

FIGURE 58.55 Bilateral diffuse hyperplasia of parathyroid chief cells with overall enlargement of parathyroid glands of a rat with chronic renal disease (lower image) compared to a normal rat (upper image). There is a uniform increase in cellularity due to hypertrophy and hyperplasia of chief cells. Increased cytoplasm renders the hypertrophic chief cells more eosinophilic compared to normal chief cells with a greater nuclear : cytoplasmic ratio in the control rat. H&E stain.

ADENOMA

Parathyroid adenomas in rats vary from microscopic in size to unilateral nodules several millimeters in diameter, located in the cervical region by the thyroids or, infrequently, in the thoracic cavity near the base of the heart. Parathyroid neoplasms in the precardiac mediastinum are derived from ectopic parathyroid tissue displaced into the thorax with the expanding thymus during embryonic development. Tumors of parathyroid chief cells do not appear to be sequelae of longstanding secondary hyperparathyroidism of either renal or nutritional origin. The unaffected parathyroid glands may be atrophic if the adenoma is functional, normal if the adenoma is nonfunctional, or enlarged if there is concomitant hyperplasia. In functional adenomas, the normal mechanism by which PTH secretion is controlled by the concentration of blood Ca^{2+} is lost and hormone secretion is excessive, despite an increased level of blood Ca^{2+}. However, PTH secretion is not completely autonomous because secretion can be reduced by extracellular Ca^{2+} *in vitro*.

Adenomas are solitary nodules that are sharply demarcated from adjacent parathyroid parenchyma (Figure 58.56). Because the adenoma compresses the rim of surrounding parathyroid to varying degrees, depending on its size, there may be a partial fibrous capsule, either from compression of existing stroma or from proliferation of fibrous connective tissue (Figure 58.56). Adenomas are usually nonfunctional in rats but may be functional in

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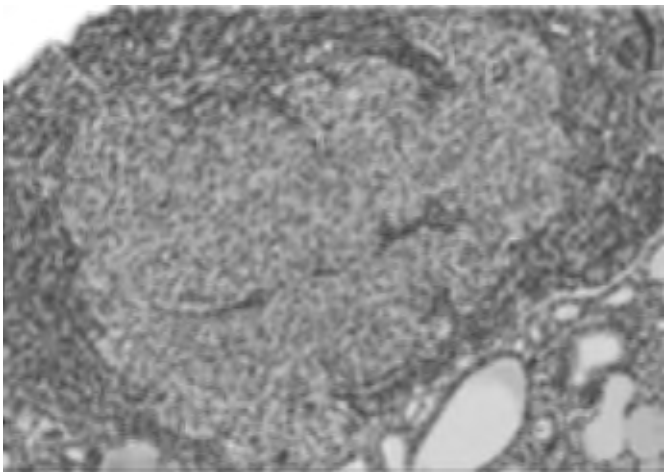

FIGURE 58.56 Chief cell adenoma that is irregular in shape and well demarcated from adjacent parathyroid parenchyma in a Fischer rat. Chief cells in the adenoma have a larger cytoplasmic area than those in the compressed rim of the parathyroid gland. H&E stain.

dogs, cats, and humans. Chief cells in nonfunctional adenomas are cuboidal or polyhedral, and arranged in a diffuse sheet, lobules, or acini with or without lumens. Nuclei are round to oval and often vesicular, and mitotic figures may be present; however, they usually are infrequent. Chief cells from functional adenomas often are closely packed into small groups by fine connective tissue septae. Chief cells are cuboidal and the cytoplasm stains lightly eosinophilic. The cytoplasmic area varies from normal size to an expanded area. There is a much lower density of cells in a functional parathyroid adenoma than in the adjacent rim with atrophic chief cells. Occasional oxyphil cells, water-clear cells, and transitional forms may be distributed throughout the adenoma.

Some parathyroid adenomas in the rat become cystic. The cystadenomas contain solid areas of tumor cells and large cystic areas lined by neoplastic chief cells. The cysts contain a densely eosinophilic proteinaceous fluid. As the cystic spaces enlarge, the tumor cells lining the cysts become flattened. Interspersed between the cysts are solid islands of tumor growth. Chief cells comprising functional parathyroid adenomas ultrastructurally are in the actively synthesizing stage of the secretory cycle. Few mature secretory granules are present in autonomous chief cells, suggesting that the rate of PTH secretion is faster than synthesis and storage.

Larger parathyroid adenomas, such as those that are detected macroscopically, often nearly incorporate the entire affected gland. A narrow rim of compressed parenchyma may be detected at one side of the gland, or the affected parathyroid may be completely incorporated by the adenoma. Chief cells in this rim often are compressed and atrophic due to pressure and persistent hypercalcemia. Peripherally situated follicles in the adjacent thyroid lobe may be compressed to a limited extent by larger parathyroid adenomas. Parathyroid glands that do not contain a functional adenoma also undergo trophic atrophy in response to the hypercalcemia, and become smaller. Atrophic parathyroids, particularly in certain species, have an interstitial infiltration of fat that replaces the normal parenchyma. Multiple white foci may be seen macroscopically in the thyroids of animals with functional parathyroid tumors. These represent areas of C-cell hyperplasia in response to long-term hypercalcemia.

CARCINOMA

Chief cell carcinomas are encountered rarely in laboratory rats and domestic animals. Carcinomas result in a macroscopically detectable enlargement of one gland. Parathyroid carcinomas often are more fixed in position than chief cell adenomas due to invasion of either the adjacent thyroid lobe or adjacent cervical skeletal muscle. Some of the enlargement may be due to central necrosis and hemorrhage in the carcinoma.

Malignant chief cells are arranged in solid sheets subdivided into lobules by a fibrovascular stroma, palisade along blood sinusoids, or form acinar structures. There is usually complete incorporation of the affected gland and evidence of invasion through the parathyroid capsule. Evidence of vascular invasion and formation of tumor cell emboli are observed infrequently. Malignant chief cells may be more pleomorphic than those that constitute adenomas, but mitotic figures are infrequent. The cytoplasmic area stains lightly eosinophilic, and boundaries of adjacent chief cells are indistinct.

24.3. Multinucleated Syncytial Cells

Parathyroid glands of dogs, rats, and other animals occasionally develop a unique

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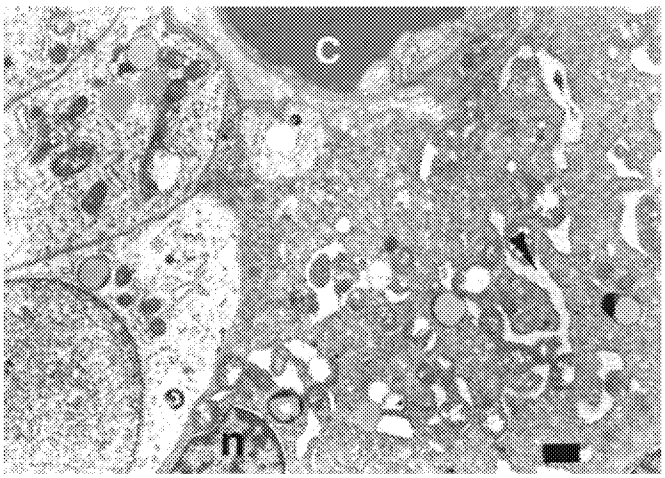

FIGURE 58.57 Syncytial ("giant") cell (right) and adjacent inactive chief cells (left) in the parathyroid gland from a dog. The large cytoplasmic area of the syncytial cell is formed by the fusion of adjacent chief cells following the disruption of plasma membranes. The multiple nuclei in the syncytial cell are smaller and more electron-dense than in the normal chief cells (n) (left) and have condensed chromatin. Some mitochondria are swollen with disruption of cristae, and profiles of endoplasmic reticulum are distended in syncytial cells (arrowhead). C, capillary. Bar = 100 μm. *Figure reproduced from Capen and Rosol (1989) Recent advances in the structure and function of the parathyroid gland in animals and the effects of xenobiotics,* Toxicol. Pathol. *17, pp. 233–345, with permission.*

multinucleated syncytial giant cell. Syncytial cells often are more numerous near the periphery of the parathyroid gland, but this is variable between animals, and considerable numbers can be present in the more central portions of the gland. The number of syncytial cells often varies considerably between parathyroids in the same animal, but may account for up to one-half of the parenchyma of the gland. These cells appear to form by the fusion of the cytoplasmic area of adjacent chief cells. The cytoplasm of syncytial cells is densely eosinophilic and homogeneous, and the plasma membranes between adjacent cells often are indistinct (Figure 58.57). Nuclei are smaller, more hyperchromatic, and more oval than those in adjacent chief cells. Membrane-limited secretory granules are observed infrequently in the cytoplasm. The mechanism by which syncytial cells form is uncertain, but it has been suggested that syncytial cells may be an artifact of immersion fixation with aldehydes due to membrane disintegration.

25. EVALUATION OF TOXICITY

25.1. Morphologic Evaluation

The parathyroid gland is infrequently injured directly by the acute or chronic administration of xenobiotics. However, parathyroid function may be altered by a wide variety of chemicals that either elevate or lower the blood Ca^{2+} concentration. In response to hypocalcemia, chief cells undergo hypertrophy and eventually hyperplasia. On formalin- or Bouin's-fixed sections, the expanded cytoplasmic area is lightly eosinophilic and vacuolated compared with chief cells in normal animals. Perivascular spaces are narrow in a hyperplastic parathyroid and there are few fat cells in the interstitium. In response to hypercalcemia, the cytoplasmic area of chief cells is decreased and more densely eosinophilic, often with a widening of intercellular and pericapillary spaces. If the hypercalcemia is prolonged, there is an overall reduction of glandular parenchyma with increased fibrous or adipose connective tissue in the interstitium. Subtle differences between treated and control groups can be best evaluated by the morphometric evaluation of parenchyma : interstitium and cytoplasmic : nuclear area of chief cells.

Ultrastructural evaluation of chief cells is a sensitive means of assessing morphologically whether a particular drug or chemical affects the parathyroid gland. Perfusion of the thyroid–parathyroid area with glutaraldehyde-based fixatives followed by post-fixation with osmium tetroxide results in the best retention of structural detail in parathyroids of animals. Morphometric studies at the ultrastructural level can be used to quantitate total cytoplasmic area, and area occupied by a particular organelle (e.g., secretory granule).

In response to an acute lowering of blood Ca^{2+}, a larger percentage of chief cells ultrastructurally will be in the active stage of synthesis and secretion than under steady-state conditions. This is indicated by a peripheral migration of secretory granules and alignment along the plasma membrane, aggregation of the endoplasmic reticulum into lamellar arrays, and enlargement of the Golgi apparatus associated with many small dense granules in the process of formation. Conversely, chief cells in

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response to hypercalcemia are predominantly in the inactive stage of the secretory cycle as evaluated by electron microscopy, with straight plasma membranes, dispersed profiles of endoplasmic reticulum, small Golgi complexes with few granules, and often accumulations of either glycogen or lipid (depending on the species) in the cytoplasm. Secretory granules accumulate initially in response to an elevation in blood Ca^{2+}, but subsequently decrease due to degradation by lysosomal enzymes. Atrophic chief cells develop in response to sustained or more severe hypercalcemia. Their cytoplasm is more electron-dense and irregularly shrunken with widened intercellular spaces. Cytoplasmic organelles are poorly developed and may have early degenerative changes suggested by mitochondrial vacuolation with disruption of cristae, and distension of endoplasmic reticulum, with loss of ribosomes.

25.2. Assay of Circulating Parathyroid Hormone

The metabolism of PTH and the formation of multiple circulating forms of PTH have made the development of clinically useful immunoassays challenging. The principal circulating forms of PTH include intact PTH (1–84) and C-terminal peptides (e.g., PTH 35–84). Parathyroid hormone has two functional domains. N-terminal PTH (PTH 1–34) is the region of major biological activity in relation to Ca^{2+} regulation. N-terminal PTH does not exist in the circulation in a biologically relevant concentration, but administration of exogenous PTH (1–34) will induce the typical actions of PTH on bone and kidney cells. The function of C-terminal PTH peptides is less well understood.

PTH is secreted in two forms: intact PTH (1–84) and C-terminal peptides. PTH (1–84) is the circulating form of biologically active PTH. PTH (1–84) is removed rapidly from the circulation by endopeptidases on Kupffer cells or glomerular filtration, and its half-life is less than 5 minutes. Some of the C-terminal peptides are released from the Kupffer cells back into the circulation and are excreted by glomerular filtration. Therefore, C-terminal PTH has a greater serum half-life than PTH (1–84) and is present in the serum at greater concentrations (50–90% of total PTH) than PTH (1–84), especially in states of hyperparathyroidism. End organs (bone and kidney) also are responsible for degradation of a small fraction of serum PTH (1–84). This involves internalization of PTH–receptor complexes and intracellular degradation by lysosomal enzymes. Rapid removal of PTH (1–84) from the circulation allows the *in vivo* actions of PTH to be controlled by the rate of PTH secretion from the parathyroid chief cells.

Early immunoassays for PTH were single-site radioimmunoassays for C-terminal peptides. These assays were suboptimal, as the biologically active form of PTH was not measured. However, they were clinically useful in states of hyperparathyroidism in patients with normal renal function. C-terminal RIAs measure both intact PTH and C-terminal PTH, which makes them less clinically relevant. In addition, conditions that reduce glomerular filtration rate, such as renal failure, will result in a large increase in the serum concentration of C-terminal PTH.

Intact serum PTH concentrations are best measured by two-site immunoradiometric assay (IRMA) or N-terminal RIA. Mouse- and rat-specific PTH assays are commercially available. Serum-intact PTH can be measured in dogs and cats with assays developed for human PTH due to the cross-reactivity of the antisera used in the assays, but internal validation should be performed for preclinical toxicity studies. Canine PTH consists of 84 amino acids and has a high degree of homology to bovine, porcine, human, and rat PTH. There are only two substitutions in the first 40 amino acids of canine PTH compared to human PTH at positions 7 and 16. These substitutions are not unique to canine PTH because they also occur in bovine (positions 7 and 16) and porcine (position 16) PTH.

PART VII: ENDOCRINE PANCREAS

26. INTRODUCTION

The endocrine pancreas consists of a distributed system of endocrine cells organized into the islets of Langerhans (islets) and small clusters of cells or individual cells in or adjacent to the exocrine pancreatic ductules, exocrine acini, or connective tissue. The islets are well defined clusters of cells that range in size (100–200 μm)

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and are easily discerned from the exocrine pancreas. The islets are not randomly distributed throughout the pancreas, but are concentrated in different lobes of the pancreas depending on the species. Each islet consists of multiple different endocrine cells that have separate functions, yet interact in a paracrine manner and by electrical gap-junction coupling. The most numerous endocrine cell (70–80%) is the β cell, which is responsible for secretion of insulin and regulation of glucose and fat metabolism. Electrically coupled β cells secrete insulin more effectively than single cells. Other cell types include the α cell (secretes glucagon), δ cell (secretes somatostatin), the PP or F cell (secretes pancreatic polypeptide), and the ε cell (secretes ghrelin). Isolated or small clusters of endocrine cells in the pancreas are usually β cells.

27. STRUCTURE AND FUNCTION

27.1. Functional Cytology

Immunohistochemistry and immunofluorescence are effective techniques to identify the endocrine cell types in the pancreas. In addition, ultrastructural examination of the endocrine cells can be used to identify cell types and evaluate cellular pathology. The secretory granules, including their dense cores and submembranous spaces, have distinct sizes and shapes depending on the cell type and species. For example, in rats, insulin-containing secretory granules have variably sized round dense cores and wide submembranous spaces. In dogs and other species, some of the β-cell dense cores of secretory granules are crystalline and elongate.

The endocrine cells of the islets are organized so that β cells can have homologous contacts with other β cells and heterologous contacts with α cells. In rats and mice, the types of islet endocrine cells are organized in a specific manner. The β cells are the most numerous endocrine cell type, and are located in the central region of the islet (Figure 58.58). The α cells are located in the periphery or mantle of the islet (Figure 58.58). Small numbers of δ cells and PP cells are also scattered in the periphery of the islets. This pattern is different in humans and non-human primates. The α cells are located in the mantle of the islets, but they are also located in the more central regions along blood vessels, especially in the larger islets. Beta cells are most prevalent in the central region of the islets. Pigs have variably sized islets. The smaller islets are composed of β cells. The large islets have β cells concentrated in the central regions; however, a small percentage of the large islets may have β cells concentrated in the periphery of the islets. In the dog, the left lobe

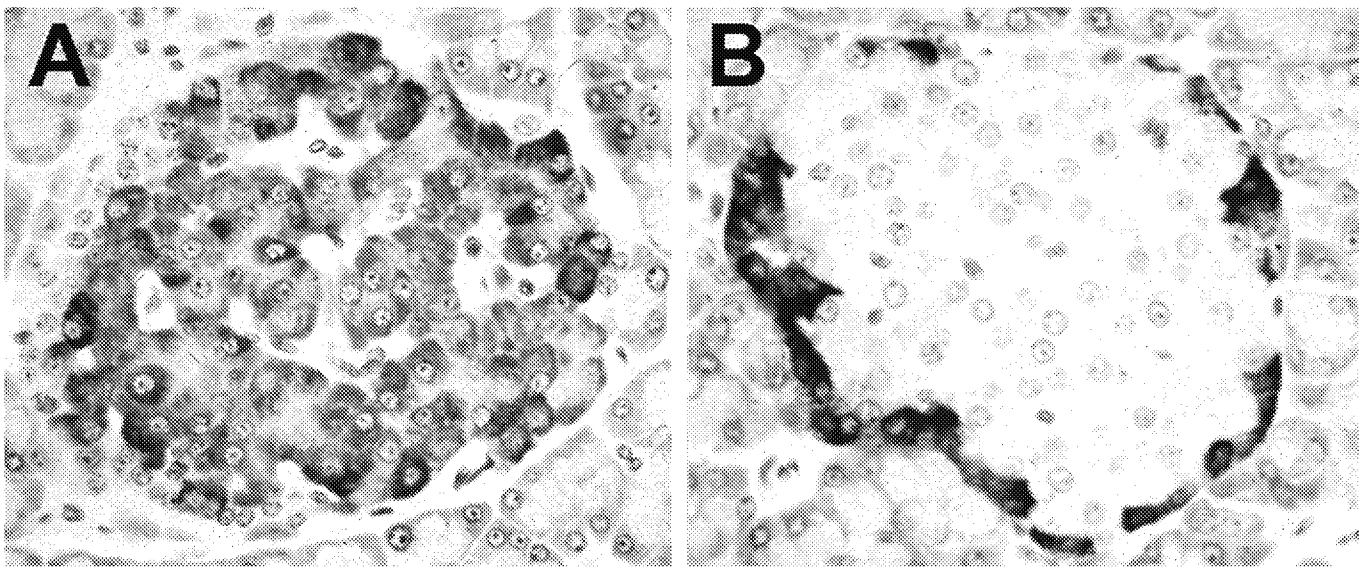

FIGURE 58.58 Immunohistochemistry of a rat pancreatic islet. (A) Insulin-positive cells in the central region of the islet. (B) Glucagon-positive cells in the periphery of the islet.

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of the pancreas contains well-defined islets, whereas the right lobe contains more single cells or small clusters of endocrine cells. Beta cells are concentrated either in the central or peripheral regions of the islets. Alpha cells are usually concentrated in the central region of the islets, but may also be found in the periphery. In cats, the β cells are peripheral and the α cells central in the islets.

27.2. Physiological and Functional Considerations

A major role for the endocrine pancreas is to maintain normal concentrations of blood glucose during different physiological states. Insulin decreases blood glucose by increasing the uptake of glucose by muscle, fat, and other cells, and promoting the synthesis of glycogen. The liver and brain are not dependent on insulin for glucose uptake. Insulin secretion is regulated by blood glucose, glucagon-like peptide-1 (GLP-1), gastric inhibitory peptide (GIP), growth hormone, glucocorticoids, and the autonomic nervous system. In contrast, glucagon increases blood glucose by stimulating glycogenolysis in the liver and kidney. Glucagon secretion is stimulated by decreased blood glucose, catecholamines, insulin, and the sympathetic nervous system.

Diabetes mellitus (DM) is the clinical condition where there is failure of the control of blood glucose with the development of hyperglycemia and hyperglucosuria. There are many serious sequelae to DM, including increased predisposition to infections, cataract formation, hepatic lipidosis and cirrhosis, atherosclerosis, microangiopathy (such as in the retina, glomerulus, and skin), peripheral neuropathy and glomerulosclerosis and renal failure. Type 1 DM (T1DM) is a chronic autoimmune disease that results in a primary lack of β cells and decreased insulin production and secretion. T1DM is usually seen in young animals or children, and is due to a genetic lack or inflammatory destruction of β cells. The Non-Obese Diabetic (NOD) mouse is a strain that partially mimics the pathogenesis of T1DM in humans with similar genetics and autoimmune inflammatory response. Type 2 DM (T2DM) is the most common form of DM in humans. T2DM is a heterogeneous group of disorders, and is associated with obesity, physical inactivity, and genetic predisposition. It is initially due to impaired insulin action from reduced sensitivity of insulin receptors to insulin. Insulin levels may be initially elevated, but then are reduced when there is failure of the β cells with prolonged T2DM. In animals, T2DM occurs in obese mice, rats, cats, rabbits, horses, and pigs. Interestingly, obese dogs are resistant to development of T2DM. Some strains of mice and rats are predisposed to developing T2DM with obesity. Islet amyloidosis may be associated with T2DM in humans and cats. Animal models of T2DM include the ob/ob mouse, db/db mouse, Zucker diabetic fatty (ZDF) rat, obese Rhesus monkeys, Sand rat, streptozotocin-induced adult diabetic animals, complete or partial pancreatectomy, insulin receptor knockout mouse, and mice with knockout of the insulin receptor in β cells.

27.3. Islet Cell Regeneration

Islet β cells have a remarkable plasticity and capability to regenerate after injury. New β cells can develop from proliferation of differentiated β cells, neogenesis from uncommitted stem cells in the islets or pancreatic ducts, or by transdifferentiation from other islet cell types, such as α cells. β-Cell regeneration from the three potential mechanisms has been demonstrated in rodents and rodent models of β-cell injury, but the mechanisms and relevance are still controversial in humans. In humans, α cells appear to be more capable of proliferation than β cells in response to disease or injury of the islets. Some of the regenerative α cells may be positive immunohistochemically for both insulin and glucagon. Proliferation of islet cells can be demonstrated using proliferation markers, such as Ki-67. It has been estimated that β cells survive for about 30 days in growing rats, after which they undergo apoptosis and are replaced by proliferation. Proliferation may decrease with age, but islet cell proliferation can still occur in aged rodents.

Investigations from different laboratories using rodent models of islet cell injury report variable degrees of β-cell regeneration with similar or dissimilar mechanisms. Injury models include partial pancreatectomy, pancreatic duct ligation, cellophane wrapping, chemical injury

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(streptozotocin or alloxan), or genetically modified mice. In general, the chemically induced models of islet cell injury are not associated with β-cell proliferation/regeneration; however, transdifferentation and neogenesis of islet cells are often reported. Treatment of rodents after chemical injury of the pancreatic islets with GLP-1, betacellulin, or activin A may stimulate β-cells proliferation and partial regeneration.

28. MECHANISMS OF TOXICITY

28.1. Streptozotocin (Streptozocin) and Alloxan

Streptozotocin is a glucosamine-nitrosourea compound and alkylating agent that damages DNA and is particularly toxic to islet β cells. Streptozotocin spontaneously degrades to carbonium ions (CH_3^+), which forms DNA adducts by alkylating purine and pyrimidine bases. It was discovered from a soil bacterium (*Streptomyces achromogenes*) as a chemical with antibacterial properties. It is approved by the FDA for use in patients with metastatic β-cell cancer. Streptozotocin is widely used to induce T1DM in animal models, such as rats and mice. A single dose of 50 mg/kg body weight in a rat will cause necrosis of β cells followed by β-cell loss and atrophy of the islets. Alloxan is a cyclic urea analog that is chemically similar to streptozotocin. It is selectively toxic to β cells and can induce T1DM. Alloxan is reduced to dialuric acid in the body and then auto-oxidized to H_2O_2 and hydroxyl free radicals, which can form hydroxyl adducts with DNA.

The DNA damage and strand breaks induced by streptozotocin or alloxan can be repaired by enzymes (such as poly[ADP]-ribose synthetase) that require NAD. Depletion of NAD also leads to β-cell damage and death. DNA damage eventually leads to development of β-cell neoplasms. Nicotinamide can be used to maintain NAD levels in β cells and reduce the toxicity of streptozotocin and alloxan.

28.2. Zinc

Zinc is an essential micronutrient, and is associated with insulin production and metabolism and the normal function of β cells. Zinc is present in the secretory granules of β and α cells. Zinc deficiency predisposes to T1DM and T2DM, and zinc supplementation has a protective effect likely through antioxidant mechanisms and induction of metallothionein.

29. RESPONSE TO INJURY

29.1. Islet Inflammation (Insulitis)

Inflammation is important in the pathogenesis of islet cell loss in both Types 1 and 2 DM. Type 1 DM is due to autoimmune inflammation of the pancreatic islets. In the occult (early) phase there is a mixed population of leukocytes (lymphocytes and macrophages) in the islets that induce β-cell death. Microvascular leakage occurs in the early phases of the inflammatory response. In the overt phase, when DM is present, there are few β cells and little inflammation. Biomarkers of autoimmune insulitis are serum autoantibodies to a set of β-cell antigens (such as insulin, glutamic acid decarboxylase (GAD), and protein tyrosine phosphatase-related molecules). In T2DM there are increased macrophages and lymphocytes in the islets associated with increased levels of cytokines (such as interleukin-1β) and chemokines. Fibrosis results from the chronic inflammation. Amyloidosis may be seen in humans or cats. Hyperglycemia contributes to β-cell apoptosis possibly by induction of the Fas receptor by IL-1β (which is induced by high glucose concentrations). Fatty acids and oxidative stress are also involved in the pathogenesis of inflammation-induced damage to the pancreatic islets.

29.2. Islet Cell Amyloidosis

Some species are prone to developing amyloidosis of the islets, which can be exacerbated by inflammation and T2DM (Figure 58.59). The species affected include humans, cats, raccoons, Celebese apes, and the South American degu. The amyloid is formed from the peptide amylin (islet amyloid peptide, IAPP), which has 37 amino acids and shares sequence homology with calcitonin gene-related peptide. Amylin is secreted along with insulin and is present in the submembranous clear zone region of the secretory granules. The

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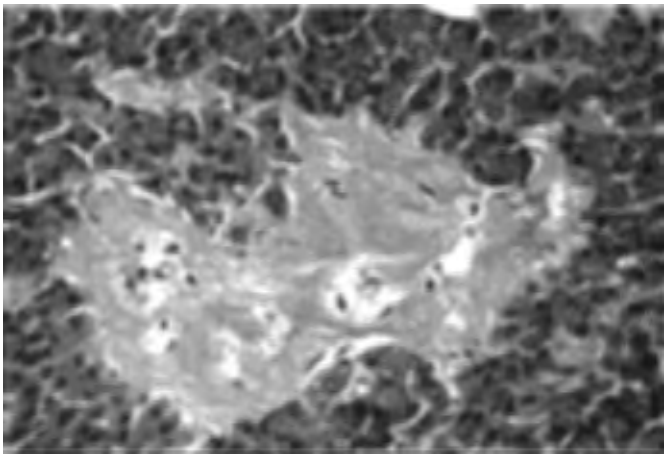

FIGURE 58.59 Islet amyloidosis in a cat. Homogenous eosinophilic amyloid replaces most of the normal islet cells. There are a few hypertrophic, vacuolated islet cells that remain. H&E stain.

species that are prone to islet amyloidosis have sequences of amino acids 20–29 of amylin that favor formation of a β-sheet configuration and amyloid formation in the interstitium of the islets. Overproduction of amylin, decreased catabolism, or aberrant enzymatic processing may also contribute to amyloid formation. Amyloidosis of the islet predisposes to the development of T2DM, but not all individuals with islet amyloidosis have DM.

29.3. Islet Cell Degeneration and Loss

Degeneration of islet cells may occur with certain chemicals or drugs, islet inflammation, or β-cell dysfunction associated with chronic T2DM. Obesity and overeating are common causes of β-cell dysfunction, degeneration, and eventual loss due to apoptosis. In the early stages of obesity and metabolic syndrome there is peripheral insulin resistance with increased total insulin secretion, but the first phase of insulin secretion may be impaired. The pathophysiology of β-cell loss with T2DM is not well understood, but it is insidious. Potential causes of β-cell dysfunction include glucose toxicity, lipotoxicity, increased secretory demand due to insulin resistance, inflammation, and inflammatory cytokines (such as interleukin-1). By the time clinical signs are apparent, approximately 50% of the β-cell mass is gone. Islets in obese animals with T2DM have β-cell hypertrophy, vacuolation, cytoplasmic glycogen accumulation, apoptosis, islet congestion, mononuclear cell infiltration, and fibrosis with collagen deposition. Some islets may have irregular shapes with cellular hypertrophy and hyperplasia in addition to the degenerative, inflammatory and fibrotic changes. Initially, drug therapy (such as metformin) can be effective by stimulating increased insulin secretion from the remaining β cells. There is a strong interest in therapies that will permit recovery of β-cell mass. The thiazolidinediones (such as rosiglitazone, troglitazone, and pioglitazone) are PPAR (peroxisome proliferator activated receptor)-γ agonists and have shown efficacy for preserving β cells, increasing insulin secretion, and protecting β cells from oxidative stress in the early stages of T2DM. Eventually, enough β cells are lost so that the animals develop insulin-dependent DM (IDDM).

29.4. Islet Cell Hyperplasia and Neoplasia

Islet cell hyperplasia and adenomas are common age-related lesions found in multiple species, including rats, mice, dogs, ferrets, and cattle. Islet cell carcinomas occur less commonly. There is a continuum of islet cell hyperplasia with progression to adenoma and carcinoma. Proliferative lesions of the islets cells usually affect the β cells, but can affect the α cells or other cell types. Dietary restriction will reduce the background incidence of islet cell tumors in rats. Pancreatic islet cell hyperplasia is characterized by enlargement of multiple islets (up to 500 μm in diameter), but all islets are not typically involved (Figure 58.60). There is no compression of the adjacent pancreas, and there is lack of a fibrous encapsulation. The islet cells appear normal or may be elongated or spindle-shaped in islets with marked hyperplasia. There is low mitotic activity. Adjacent islets may coalesce to form enlarged, irregularly shaped islets. Islet cell adenomas are characterized by discrete, solitary tumors that are 1–10 mm in diameter.

Adenomas grow by expansion, induce variable compression of the adjacent pancreas, and are well differentiated (Figure 58.61). Adenomas may coalesce with adjacent hyperplastic islets. There may be a partial fibrous capsule. Islet cell carcinomas have invasion into the tumor capsule and adjacent pancreatic tissues

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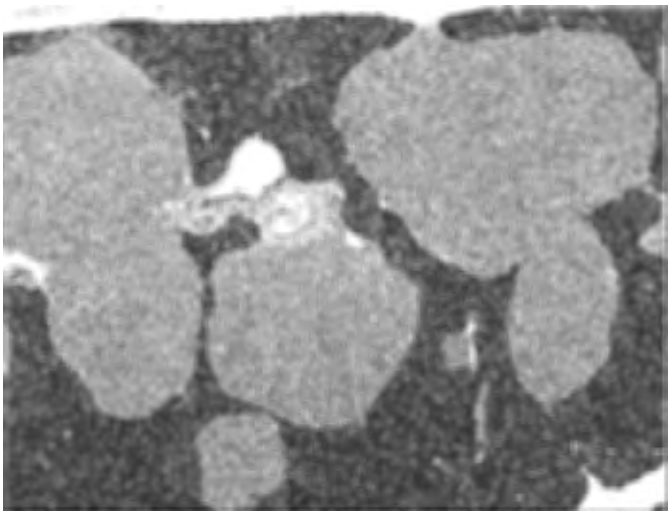

FIGURE 58.60 Islet cell hyperplasia in a 2-year-old B6C3F1 mouse. The islets are variably enlarged due to hyperplasia of β-cells and fusion of multiple islets. Note that some islets remain normal in size. H&E stain.

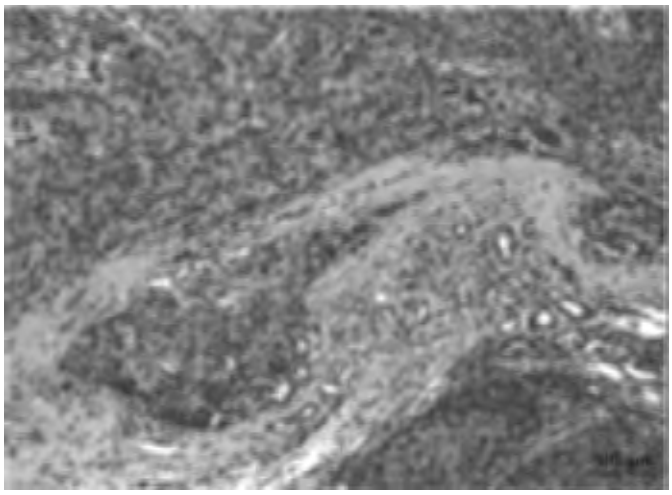

FIGURE 58.62 Islet cell carcinoma in a 2-year-old F344 rat. The islet cell carcinoma is moderately differentiated, invasive into the pancreas interstitium, and has reactive fibrosis, mild hemorrhage and congestion, and secondary proliferation of exocrine ductules. H&E stain.

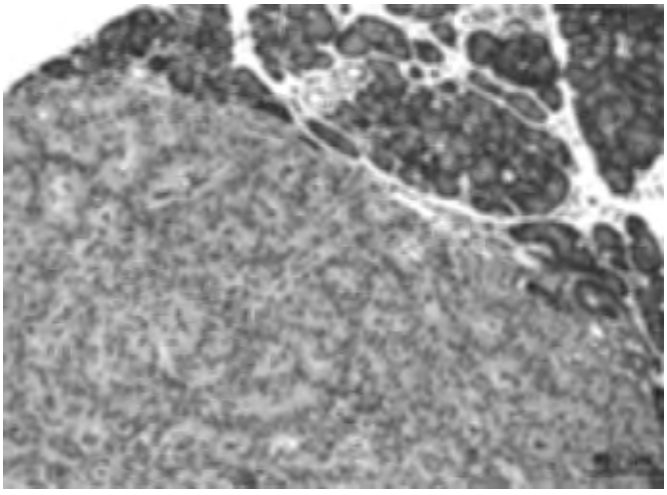

FIGURE 58.61 Islet cell adenoma in a 2-year-old F344 rat. The adenoma is composed of well-differentiated islet cells that have a well-defined margin with mild compression of the adjacent exocrine pancreatic tissue. H&E stain.

(Figure 58.62). Distant metastases may be present in regional lymph nodes or the liver.

Islet cell hyperplasia was reported in transgenic mice that overexpressed the EAT/mcl-1 gene that is related to Bcl-2. Bcl-2 and its analogs bind to the BAD and BAK proteins to inhibit apoptosis. The islet cell hyperplasia in EAT/mcl-1 mice was due to decreased apoptosis of β and α cells and resulted in a mild increase in serum insulin concentration. A few of the mice developed islet cell adenomas.

Glucocorticoids increase blood glucose concentrations and promote insulin resistance. Prolonged administration of glucocorticoids to rats has the potential to increase the incidence of islet cell hyperplasia and neoplasia in 2-year studies. Rats, particularly males, are uniquely sensitive to the non-genotoxic mechanisms of glucocorticoids to induce islet cell tumors. This effect is not thought to have relevance to humans.

Glucagon receptor antagonists or antireceptor antibodies have the potential for treatment of T2DM due to their ability to decrease blood glucose and hepatic production of glucose. Animals with experimental DM treated with functional or structural glucagon receptor antagonists and glucagon receptor knockout mice develop α-cell hyperplasia with prominent hyperglucagonemia. In addition, glucagon-like peptide-1 (GLP-1) levels are increased, which have a beneficial effect on treating the DM. Plasma glucagon concentrations are a useful biomarker for antagonism of the glucagon receptor or its function. Plasma glucagon returns to normal quickly after cessation of antiglucagon receptor therapy even though α-cell hyperplasia may still be present, which tends to regress slowly.

Compounds that increase prolactin secretion increase the incidence of pancreatic islet cell

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hyperplasia in rats and mice. Prolactin secretion is inhibited by dopamine. Therefore, dopamine or dopamine receptor antagonists will increase prolactin secretion. Sulpiride (dopamine receptor antagonist) induced pancreatic islet cell tumors in rats. The combination of naproxen and metoclopramide (structurally related to sulpiride) induced pancreatic islet carcinomas in male rats.

Nafarelin acetate (nasal solution) is a synthetic analog of gonadotropin-releasing hormone (GnRH), which increased pancreatic islet cell adenomas in male and female rats in 2-year dosage studies. Leuprolide acetate (depot dosage formulation) increased pancreatic islet cell adenomas in female rats in 2-year studies. The mechanism by which GnRH and its analogs induces islet cell tumors in rats is unknown. However, it has been reported that GnRH and its receptor are present in the rat pancreas and GnRH agonists increase insulin resistance.

Medroxyprogesterone acetate (MPA) induced a dose-related increase in islet cell adenomas and carcinomas in female rats in a 2-year oral study at 1000 and 5000 μg/kg/day (10–50 times the human dose), but not at 200 μg/day.

Anamú (*Pativeria alliacea*) is a common weed in Columbia cattle-raising areas. Toxic effects in calves include renal and skeletal toxicity and islet cell hyperplasia with hyperinsulinemia and hyperglycemia.

30. EVALUATION OF TOXICITY

30.1. Morphologic Evaluation and Immunocytochemistry

Morphologic evaluation of the pancreatic islets can be assessed using standard sections stained with hematoxylin and eosin. Individual cell types can be identified specifically using immunohistochemical stains for hormones, such as insulin, glucagon, somatostatin, and pancreatic polypeptide. Immunohistochemistry also can be used to identify inflammatory cell types or the deposition of collagen in regions of fibrosis. Quantitative pathology or stereology can be used to measure the effects of compounds on the pancreatic islets. However, correct nonbiased sampling is essential because there is normal variability in the size and distribution of islets in the different species.

30.2. Assay of Circulating Hormones or Chemicals

Measurement of circulating hormones is used to determine the function and clinical significance of islet cell degeneration, loss, hyperplasia, and neoplasia. Islet cell hyperplasia and neoplasia is usually associated with increased secretion of endocrine hormones. For example, functional β-cell or α-cell hyperplasia/tumors would be expected to be associated with hyperinsulinemia and hyperglucagonemia, respectively.

Glucose challenge or tolerance tests are used to measure the ability of the test subject to reduce serum glucose concentration effectively to an oral or intravenous glucose challenge. Usually, a 2-hour oral glucose tolerance test (with a baseline fasted sample) is adequate to diagnose a state of impaired glucose tolerance or diabetes mellitus. The intravenous glucose challenge is useful for identification of prediabetic states.

Serum glycated hemoglobin (HbA1c) is measured to evaluate the average blood glucose concentration over a prolonged period of time. Effective therapeutics for diabetes are expected to reduce the HbA1c concentrations. Serum HbA1c concentrations can be used to predict the incidence and severity of cardiac, renal, and vascular complications associated with diabetes.

PART VIII: SUMMARY AND CONCLUSIONS

The endocrine glands are an important group of organs that are susceptible to chemical-induced changes that occur in short-term and long-term preclinical toxicology studies. It is important to differentiate spontaneous and stress-related changes from compound-induced effects. There are multiple examples of drugs that are approved for human use that have been associated with degenerative or neoplastic changes in the endocrine glands of laboratory animals, but the weight of evidence on the pathogenesis and mode of action has demonstrated a lack of relevance for humans. The mode of action of chemicals on the toxicology of the endocrine glands can be direct or indirect. Therefore, studies on the modes of action require

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knowledge and experimental interrogation of physiological control and feedback mechanisms, hormone distribution and receptors on diverse target cells, the primary endocrine cells, and peripheral metabolism and excretion of hormones. In addition, there are significant interspecies differences in the physiology and pathology of the endocrine glands, which makes it imperative to understand the mode of action of chemicals in order to compare preclinical toxicology findings in different species and predict human relevance. This chapter has reviewed the pathophysiology of the endocrine glands in the species typically used for preclinical toxicology studies, and includes toxicologic mechanisms, classic examples of chemical-induced changes and their modes of action, and spontaneous diseases.

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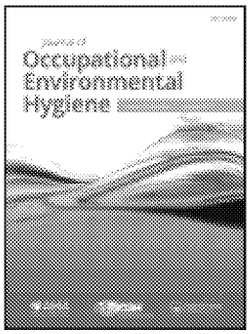

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The Scientific Basis of Uncertainty Factors Used in Setting Occupational Exposure Limits

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The uncertainty factor concept is integrated into health risk assessments for all aspects of public health practice, including by most organizations that derive occupational exposure limits. The use of uncertainty factors is predicated on the assumption that a sufficient reduction in exposure from those at the boundary for the onset of adverse effects will yield a safe exposure level for at least the great majority of the exposed population, including vulnerable subgroups. There are differences in the application of the uncertainty factor approach among groups that conduct occupational assessments; however, there are common areas of uncertainty which are considered by all or nearly all occupational exposure limit-setting organizations. Five key uncertainties that are often examined include interspecies variability in response when extrapolating from animal studies to humans, response variability in humans, uncertainty in estimating a no-effect level from a dose where effects were observed, extrapolation from shorter duration studies to a full life-time exposure, and other insufficiencies in the overall health effects database indicating that the most sensitive adverse effect may not have been evaluated. In addition, a modifying factor is used by some organizations to account for other remaining uncertainties—typically related to exposure scenarios or accounting for the interplay among the five areas noted above. Consideration of uncertainties in occupational exposure limit derivation is a systematic process whereby the factors applied are not arbitrary, although they are mathematically imprecise. As the scientific basis for uncertainty factor application has improved, default uncertainty factors are now used only in the absence of chemical-specific data, and the trend is to replace them with chemical-specific adjustment factors whenever possible. The increased application of scientific data in the development of uncertainty factors for individual chemicals also has the benefit of increasing the transparency of occupational exposure limit derivation. Improved characterization of the scientific basis for uncertainty factors has led to increasing rigor and transparency in their application as part of the overall occupational exposure limit derivation process.

Keywords adjustment factor, occupational exposure, risk assessment, uncertainty factor

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INTRODUCTION

The use of uncertainty factors (UFs) in establishing exposure limits extends back at least as far as 1954, when Lehman and Fitzhugh[1] proposed a 100-fold factor (which they referred to as a "margin of safety") for extrapolating from animal toxicity data to safe levels of human exposure to food additives and pesticide residues. The UF concept is integrated into health risk assessments for all aspects of current public health practice, including in deriving health-based occupational exposure limits (OELs). The concept has widespread application, but the nomenclature for UFs varies among organizations—other terms for this concept include safety factors or assessment factors.[2,3] Despite the widespread acceptance of the UF approach for risk assessment, implementing the concept varies significantly among organizations. In addition, there are unique aspects of occupational risk assessment that impact UF application. Methods for establishing OELs were initially proposed decades ago and have seen ongoing modification and application.[2,4–8] In these articles, the authors briefly described the sources of uncertainty in the limit-setting process and suggested possible default UFs, but none presented specific scientific support for the UFs recommended for establishing OELs.

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The conceptual basis for the application of UFs is that chemical toxicity usually follows a predictable pattern with increasing dose—from no significant effects, to minimal effects (not necessarily adverse) within the range of biological compensation, to clearly toxic effects, and finally to overt disease and/or death.[9] The use of UFs is predicated on the assumption that sufficiently reducing exposure from levels known to be toxic will yield an exposure level that is safe for at least the great majority of the exposed population, including vulnerable subgroups. Organizations that publish OEL values differ in how to approach chemicals with a toxic mode of action (MOA) that suggests the lack of a biological threshold. This issue is most commonly encountered in the context of genotoxic carcinogens and mutagens, which are frequently considered to be exceptions to the general rule that a safe dose exists for most toxicants. Such chemicals are often treated as posing some degree of excess risk—though possibly very slight—at any dose greater than zero. In many organizations, development of acceptable exposure levels for chemicals that lack a biological threshold is based on dose-response modeling and low-dose extrapolation rather than application of UFs.[10] The field is moving to increased use of mode of action analysis to evaluate implications of the nature of the toxic response for its likely dose-response behavior. The result is an integrated risk assessment approach for cancer and non-cancer endpoints and use of methods that best reflect biological understanding.[11] Whether for cancer or non-cancer endpoints, considering UFs is often a critical step in OEL development.

This article describes the application of the UF approach for OEL setting and the issues encountered. Key points of emphasis covered in this manuscript include the following.

- Risk assessment organizations generally agree in the areas of variability and uncertainty addressed in setting OELs. However, the quantitative application of UFs and the level of documentation provided in an OEL rationale vary widely.
- The current standards of practice for setting OELs are changing with a general shift toward including chemical-specific adjustments and increasing documentation and transparency of the UF selection.
- An understanding of the sources of variability and uncertainty addressed in an OEL are essential to its informed use and interpretation by the risk manager.
- Application of advances in toxicology, including results of new testing strategies beyond traditional animal testing, is having a growing impact in informing mode-of-action and dose-response assessments, including in quantitative UF application.

APPLICATION AND DESCRIPTION OF UNCERTAINTY FACTORS

Uncertainty Factors Application for Occupational Exposure Limit Derivation

UFs enter the OEL process after a point of departure (PoD) has been determined,[12] and prior to establishing the OEL. The PoD refers to the dose or concentration from the health effects study that is the starting point for extrapolation to the OEL. Regardless of whether the PoD represents a no-effect level (e.g., a no-observed-adverse-effect level [NOAEL] or benchmark dose [BMD]) or an effect level (e.g., a lowest-observed-adverse-effect-level [LOAEL]) in the exposed population or animal dose group, in general there will be some uncertainty in how the PoD relates to the true no-adverse-effect level in the broader working population. This is particularly evident when the PoD is based on animal data. The application of UFs is intended to result in an OEL that is protective of all adverse chemical-related effects.

For the purposes of calculation, health-based OELs are derived by dividing the PoD for the critical effect by various uncertainty or adjustment factors to extrapolate to the "true" no-effect level in the worker population of interest (Equation (1)). In general, the OEL can be derived with greatest confidence from a high quality epidemiology or controlled inhalation exposure study in human volunteers. However, the PoD is typically derived from a single key study of a relatively small number of animals or humans, possibly via another route of exposure.[12] For purposes of this discussion, the PoD is defined as a mg/kg dose, because oral-dosing data are more commonly available than inhalation data. If other units are used (such as mg/m^3 exposure concentration), or if the PoD is related to local or site-of-entry effects, dose unit adjustments may be required.[13] UFs have been defined for each of the main sources of uncertainty typically considered. These factors are multiplied together to form a composite uncertainty factor (UF_C) that is used in the denominator of the general equation used to set health-based limits.

Equation (1) Calculation of an OEL for systemic effects

$$\mathrm{OEL(mg/m^3)} = \frac{\mathrm{PoD} \times \mathrm{BW}}{\mathrm{UF_C} \times \mathrm{TK} \times \mathrm{MF} \times \mathrm{V}}$$

where :
OEL = Occupational Exposure Limit
PoD = Point of Departure for Extrapolation (mg/kg − day)
BW = Body Weight (kg)
UF_C= Composite Uncertainty Factor
TK = Toxicokinetic adjustment
MF = Modifying Factor
V = Volume of air breathed during workshift (m^3)

Basis for Individual Uncertainty Factors

Regardless of the organization involved, there are common considerations related to biological variability and database uncertainty that all, or nearly all, OEL-setting organizations consider. Table I highlights the common considerations using the nomenclature of EPA,[14] which will be carried forward in this article. The specific nomenclature used and specific individual factors and their default values vary among organizations (Table II). Note that most expert committees do not publish specific information on the values they assign to UFs, if any are explicitly used.[15,16]

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TABLE I. UFs Used in OEL-setting, and the Rationale for Their Use

Factor	Area of Uncertainty	Basic Principle
UF_A	Animal to Human	Adjusts for differences in sensitivity between animals and the average human, when the PoD is based on animal studies
UF_H	Average Human to Sensitive Human	Adjusts the PoD for the difference between the average human and the most sensitive applicable subpopulation
UF_L	LOAL-to-NOAEL	Adjusts for uncertainty in the value of the PoD as an estimate of the threshold for the onset of effects, if based on a LOAEL rather than a benchmark dose or a NOAEL
UF_S	Short-term to Long-term Exposure	Adjusts for the possibility of identifying a lower PoD for chronic toxicity when extrapolating from a study of shorter duration
UF_D	Database Insufficiency	Adjusts for the possibility of identifying a lower PoD (or more sensitive effect) if additional studies were available

A conceptual visualization of each area of uncertainty is shown in Figure 1. Here, UFs are seen as differing extrapolations, either among dose-response curves for different types of data, or between different points along the same dose response curve. Two of the UFs (UF_A and UF_H) deal specifically with biological variability. In the context of biological variability, research to gain more information helps to characterize the inherent variability and may increase or decrease the value of the factor needed. For example, UF_A accounts for uncertainty in extrapolating from experimental animals to human toxicity when the PoD is based on animal bioassay data. This adjustment to the PoD allows for the possibility that the human response may occur at a lower dose than the animal response. Similarly, UF_H allows for variation in kinetics and dynamics in the dose-response relationship for humans, adjusting the PoD estimate from the average human to a sensitive or susceptible human subpopulation. Together these two factors have the effect of converting a NOAEL in animals to a NOAEL for the sensitive human population. In many cases, there are other inadequacies in the source data for the PoD and the resulting uncertainties are addressed using three additional factors, UF_L, UF_S, and UF_D. Jointly, these three factors account for the possibility that a lower PoD would have been found if more complete data had been available. Reducing these uncertainties by applying more data will provide a better PoD and reduce overall uncertainty. UF_L is applied to extrapolate from a LOAEL to a NOAEL within the same dose-response curve. UF_S shifts the PoD from a short-term study to a lower value, which might be observed given a longer study. UF_D is intended to adjust for missing data (such as the lack of developmental or reproductive toxicity assays) that might have identified more sensitive endpoints. Each of these areas of uncertainty is detailed below.

Addressing Sources of Uncertainty Using Science-based Adjustment Factors

Dourson and Stara,[17] Naumann and Weideman,[18] and Dourson et al.[19] summarized the scientific bases for the uncertainty factors discussed below. The use of UFs is a scientific judgment based on a policy decision to determine a safe concentration/dose. This judgment should be based on a systematic approach. Toxicological expertise is normally required to determine the appropriate UFs to apply, and

TABLE II. Default Uncertainty/Assessment Factors for Workers[A]

Factor	ECHA	ECETOC	TNO/RIVM	Other[B]
UF_A	AS–$BW^{0.75}$ 2.5 (TD)	AS–$BW^{0.75}$	AS 3 (TD)	NS
UF_H	5	3	3	NS
UF_L	1	3 or BMD	1–10 or BMD	NS
UF_S	2–6	2–6	10–100	NS
UF_D	1	NA	1	NS
MF	NA	NA	NS	NS

[A]Adapted from ECHA (2008) Table R.8-19. Guidance on information requirements and chemical safety assessment. Chapter R.8: Characterization of dose [concentration]-response for human health.[41]

[B]ACGIH—American Conference of Governmental Industrial Hygienists Threshold Limit Values; American Industrial Hygiene Association Guideline Foundation Workplace Environmental Exposure Levels; SCOEL—Scientific Committee on Occupational Exposure Limits

Abbreviations: ECHA—European Chemicals Agency; ECETOC – European Centre for Ecotoxicology and Toxicology of Chemicals; TNO/RIVM—National Institute of Public Health and the Environment (in cooperation with TNO Nutrition and Food research); AS—Allometric Scaling ($BW^{0.75}$); NS – Not Specified; TD – Toxicodynamics

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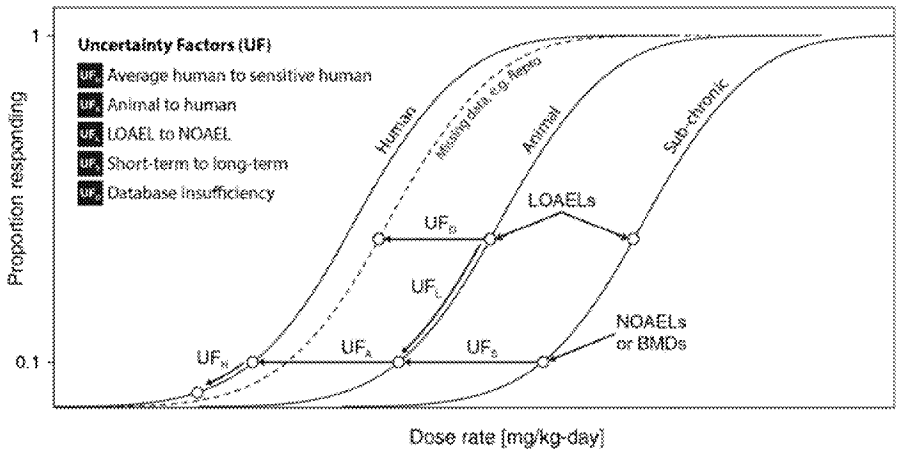

FIGURE 1. A conceptual illustration of uncertainties that are commonly considered in noncancer risk assessment for OEL setting. UFs are seen as differing extrapolations among dose response curves for either experimental animal to human (UF_A), average to sensitive humans (UF_H), LOAEL to NOAEL (UF_L), shorter-term to longer-term (UF_S), or as an adjustment for database insufficiency (UF_D).

alternative factors may be used if supported by the available data for a specific chemical and a sound scientific rationale. For this reason, to achieve robust derivation of an OEL, full consideration must be given to the underlying rationale for and intent of each key area of uncertainty, along with how it can be best addressed with the available data. UFs discussed below address the major sources of variability and uncertainty when setting safe levels of exposure for humans. Note that the values may differ depending on the segment of the population being protected. Reference doses (RfDs) established by the U.S. Environmental Protection Agency (EPA) are designed to protect the whole population, including the young, elderly, and infirm, throughout their entire lifetime. OELs are intended to protect workers, who are generally considered healthy adults, during their working lifetime. They should also protect the offspring of workers and not lead to premature illness or death beyond working life.

Animal-to-Human Uncertainty Factor

The animal-to-human uncertainty factor (UF_A) is used to extrapolate from an animal-based PoD to estimate the corresponding PoD for the "average" human. Application of this factor takes into account the possibility that humans may respond at a lower dose than the animals used in the critical toxicity study. This assumption is viewed as precautionary, because humans can be equally sensitive or even less sensitive than animals for a given response. This factor is used in the absence of data to demonstrate these latter possibilities, and replacement with data is preferred, when possible. A default uncertainty factor as high as 10 is typically used in the absence of chemical-specific data;[19] see below for further information.

The UF_A is often conceptualized as being composed of a sub-factor for toxicokinetic (TK) differences between species (i.e., differences in the internal dose to the target tissues for toxicity), and a sub-factor for toxicodynamic (TD) differences in the sensitivity (i.e., differences in the response of the target tissue to a given internal dose). This division of the UF_A into sub-factors differs among organizations. Notably, the WHO applies a sub-factor of 4 for toxicokinetics and 2.5 for toxicodynamics;[20, 21] while the EPA typically uses equal sub-factors of $\sqrt{10}$, or approximately 3.[22] The TK sub-factor may require adjustment under certain circumstances, such as when inhalation dosimetry models are used or when the PoD is related to direct contact (portal of entry) effects. This adjustment might also be relevant for the interindividual TK sub-factor (discussed below).

Recognizing that interspecies differences reflect both toxicokinetics and toxicodynamics opens the possibility of refining this factor with known differences in physiology. One approach to address TK differences is through allometric scaling. Allometric scaling provides an estimate of differences in physiological parameters and basal metabolism (and therefore chemical metabolism and elimination) based on ratios of body weight or surface area. An FDA guidance document on setting doses in initial clinical trials includes species-specific extrapolation factors for estimating human-equivalent doses, based on allometric scaling.[23] These are sometimes used for setting OELs for pharmaceuticals. The use of allometric scaling based on body surface area, or body weight, to the 2/3 power ($BW^{0.67}$) provides a human-equivalent dose used to improve clinical trial safety.[23] These species-specific values range from 2 (dog) to 12 (mouse), due to differences in body surface area.[24,25] The EPA uses a factor that more closely aligns with interspecies differences in physiological parameters and metabolism. Allometric scaling using the body weight to the $\frac{3}{4}$ power ($BW^{0.75}$) results in species-specific factors that range from 1.5 (dog) to 7 (mouse).[26] An analysis by Bokkers and Slob[27] suggests that an additional UF of 3.1 should be applied to the allometrically scaled dose, based on the 95th percentile of the allometric extrapolation factor.

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Vermeire et al.[3] reached a similar conclusion. Although not specifically attributed to toxicodynamics by the authors, this additional UF would be numerically similar to the addition of a TD sub-factor to the allometrically scaled TK sub-factor. A recent analysis by Escher et al.[28] supports the use of allometric scaling for interspecies extrapolation, but argues that the remaining uncertainties may be due to factors other than toxicodynamics. In practice, there is no consensus on the need for an additional sub-factor to address toxicodynamic differences, after applying the factor based on these allometric scaling methods.

There are additional advanced dosimetry techniques for refining the UF_A, typically for replacing the TK portion of this factor with data based on physiological differences among species.[13] For assessments based on a PoD adjusted using inhalation dosimetry, another consideration is whether the methods address all key aspects of TK variability. For example, methods that rely only on predicted deposition fractions or surface concentrations, may not fully address other kinetic processes related to metabolism and elimination. In part, such considerations can be addressed using advanced approaches that estimate the pertinent target tissue dose. A recently published physiologically-based pharmacokinetic (PBPK) model for diacetyl[29] illustrates the potential for such models to replace default TK assumptions for interspecies scaling; the model indicates that the lower respiratory tract of a worker exposed to diacetyl may receive exposures up to 40-fold greater than the lower respiratory tract of a rat inhaling the same concentration.

Interindividual (Human) Variability Uncertainty Factor (UF_H)

The interindividual (human) variability uncertainty factor (UF_H) accounts for general physiological and metabolic variation within the human population and the possibility that there may be sensitive subpopulations due to variations in age, sex, genetic susceptibility, pre-existing diseases, etc. In general, the OEL is intended to protect nearly all workers, but may not always be protective of the most susceptible individuals in the population. For example, Johansson et al.[30] showed that experimental data on asthmatics generally have not been explicitly considered when setting OEL values; however, this has changed with several recent ACGIH TLV®s. A complication to defining UF_H is that the fraction of workers "nearly all" refers to is not well documented in the OEL methods or derived OELs published to date. A key consideration is the relative size of the subpopulation that might be affected. A second consideration is the severity of effects that might occur in subpopulations that have a specific unique susceptibility if exposed at the OEL. The possibility for severe reactions would favor coverage by the OEL. Alternatively, the severity of effect along with residual uncertainties regarding susceptible subpopulations may be addressed by addition of a modifying factor (see below).

The UF for interindividual variability is used to adjust PoD values derived from either animal or human data. In the case of human data, it may be the only UF that is required. A default UF as high as 10 is typically used for the general population in the absence of chemical specific data[19] (see below for further information). Like the UF_A, the UF_H is often conceptualized as being composed of two sub-factors, with one sub-factor for TK differences between individuals and the other sub-factor for differences in suceptibility at a given tissue dose (i.e., TD differences). Unlike the UF_A, the TK and TD sub-factors of the UF_H are generally assigned numerical values of $\sqrt{10}$, or approximately 3.[20–22]

The scientific basis for the UF_H has been reviewed.[17–19,31–34] It has been noted that a TK sub-factor of $\sqrt{10}$ may not be adequate for all groups of the population.[32] A TK sub-factor of $\sqrt{10}$ has been shown to be adequate for $\geq$ 99% of healthy adults when polymorphic pathways are not involved; however, sensitive sub-groups may require a larger TK sub-factor.[35] It has also been argued that an overall UF_H of less than 10 should be adequate for setting OELs, on the grounds that workers are expected to be less heterogeneous than the general population because they do not include the very old and very young, and they are not exposed for an entire lifetime.[36,37] Epidemiological studies of working populations generally find that, in the absence of excess exposures to toxicants, workers are healthier than the general population. This is known as the "healthy worker effect," which is well established in occupational epidemiology.[38,39] However, working populations may also include asthmatics, atopic individuals, pregnant women, older workers, and others who may be more susceptible than the average member of the population.

Genetic and epigenetic factors can be an important driver for variability in response to chemicals. This has been well described in the context of variability in metabolism of pharmaceuticals. Implications for similar considerations for occupational chemical exposures are also an important variable in setting OELs, since one of the primary UFs is assigned to address human variability. An approach for systematic consideration of genetic and epigenetics is discussed in detail by Schulte et al.[40]

The European Chemicals Agency recommends[41] a factor of 5 to address interindividual variability in workers and a factor of 10 for the general population when establishing derived no-effect levels (DNELs) (see Table 2). The basis for this distinction is not well documented, although the literature on chemical-specific adjustment factors (CSAF), as discussed below, seems to support this dichotomy. For example, a reanalysis of the TK data for pharmaceutical active ingredients (APIs) analyzed by Naumann et al.[42] and Silverman et al.[43] show that the mean ratios of the bimodal CSAFs for elderly or diseased subpopulations are a factor of 2.2 higher compared with the healthy populations for the same APIs. If similar toxicodynamics are assumed, a 2-fold reduction in the default UFs of 10 for interindividual variability appears justified for workers.

It is a valid scientific question whether a full default value of 10 for the UF_H is appropriate to account for variability

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in susceptibility for working populations, and in the absence of a definitive scientific rationale, organizations may well establish differing policies on this point. The rationale for determining this relies on information on the underlying basis for susceptibility to the specific effects caused by the chemical under study. The types of considerations that are embedded in the selection of UF_H include the following.

- Is the underlying basis for susceptibility more or less prevalent in the worker population than in the general population?
- Is there a basis for assuming that a working population would experience more severe or less severe toxic effects than the general population?

LOAEL-to-NOAEL Uncertainty Factor (UF_L)

When the key study used to derive an OEL includes a LOAEL but not a NOAEL, the preferred procedure is to calculate a benchmark dose (BMD) to use as the PoD for extrapolation.[12] The benchmark dose (lower confidence limit), or BMDL, typically approximates the NOAEL for standard study designs and is used similarly to the NOAEL as a PoD. If a NOAEL or BMDL is used as the PoD, a value of 1 is typically applied for this area of uncertainty in health risk assessments,[14,41] since the goal of the extrapolation is to estimate a concentration that is not associated with adverse effects. However, some datasets may be unsuitable for benchmark dose estimation. If an OEL is based on the LOAEL as the PoD for extrapolation, an additional UF (UF_L) should be applied to estimate a point on the dose-response curve where no effect would be expected to occur.

Some judgment may be required in selecting an appropriate UF_L. Dourson and Stara[17] conducted an empirical analysis of LOAEL:NOAEL ratios, and they found that 96% of the cases they considered fell within a factor of 10. Naumann and Weideman[18] noted that the average LOAEL:NOAEL ratio is approximately 3, based on analyses of a large number of good quality toxicology studies.[44–46] Thus, the choice of a default value for the UF_L may depend, to some extent, whether organizational policy favors an upper-bound estimate of risk or a central tendency estimate. These analyses do have the limitation of analyzing NOAEL and LOAEL ratios, which are pre-defined by study dose-spacing for individual studies. This is an inherent challenge in the PoD selection, and is the reason BMD methods are generally preferred.[12]

In choosing a value other than the default value, several considerations are relevant. The most common basis for using a different value is the severity of the effect at the LOAEL. In addition, some OEL setting processes also incorporate the use of a lowest-observed-effect level (LOEL) as the basis for the PoD. For example, for setting exposure guides for pharmaceutical agents, the most sensitive effect is often based on a pharmacological effect (which is the often the intended effect for patients). However, for workers, such effects are candidates for the PoD, since the effects are not intended outside of therapeutic treatment. In this light, pharmacological effects are typically viewed as "adverse," although clinical study documentation often refers to them as a LOEL rather than a LOAEL. Whether the critical effect is termed a LOAEL or LOEL, the key determinates of UF_L selection remain the same (effect severity and dose-response slope). Dourson and Stara,[17] Naumann and Weideman,[18] and Dourson et al.[19] agree that the severity of the effect at the LOAEL should enter into the choice of UF_L, on the grounds that an effect of minimal toxicological severity, such as fatty liver, suggests that the LOAEL is likely to be close to the NOAEL, while a more severe effect, such as hepatic necrosis, suggests that the LOAEL may be further from the NOAEL. Other considerations can include response incidence, the slope of the dose-response curve, and the MOA (anticipated dose-response behavior). Any residual uncertainties can be addressed with the modifying factor (see below).

Shorter-Term-to-Longer-Term Uncertainty Factor (UF_S)

OELs are intended to protect workers who are exposed for an entire working lifetime, which for animal studies are usually equated to a chronic bioassay (e.g., 2 years for mice and rats). If the PoD is based on sub-chronic data, then the UF_S is applied to adjust for the lack of adequate chronic exposure data based on the consideration that the OEL is intended to be protective of a working lifetime. The default value of the UF_S has been the subject of some debate in the literature. Dourson and Stara[17] argued for a default factor of 10, citing studies that indicated a 10-fold factor would equal or exceed the observed ratio of subchronic-to-chronic effect levels 96% of the time. Naumann and Weideman[18] advocated a default UF_S value of 3, noting that reviews of a large number of high quality toxicology studies suggest that a factor of 3 is typically sufficient to account for the possibility that a lower PoD would have resulted if longer-term studies were conducted.[47–52] Dourson et al.[19] advocate a more flexible approach in which a UF_S of 10 is seen as "a loose upper bound estimate," which may be reduced if the chemical-specific data suggest that a smaller UF_S is warranted.

Assessing the appropriate value of UF_S requires evaluating whether the critical effect that is the basis of the PoD would be expected to increase in either incidence or severity, or to occur at a lower dose, if the study duration was increased. It is possible that a reversible acute effect may be experienced on a chronic basis without any increase in severity. The progression or lack of progression of toxicity from short-term studies to longer studies, such as a 2-week study compared with a 90-day study, may provide some data relevant to assessing the UF_S. The MOA may also provide relevant information. A full assessment of MOA is based on whether the dose would have likely accumulated with longer-term exposure (i.e., TK considerations) *and* whether effects would accumulate and become more severe with longer-term exposure (i.e., TD considerations). For example, a chemical that produces damage via a reactive metabolite (which does not accumulate in the body) and does not show evidence of a progression of

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damage over time may merit a reduced UF_S. Sensory irritant effects are generally not expected to progress with longer exposure duration, and OELs for these are often assigned a UF_S of 1. Chemicals studied for durations longer than the typical 3-month sub-chronic study, but less than chronic duration (e.g., a 6-month or 1-year study) may also merit a $UF_S < 10$. The choice of the UF_S for a particular chemical must include consideration of both the quality of the available data and the MOA of the chemical.

Database Inadequacy (incomplete data) Uncertainty Factor (UF_D)

An additional UF may be required if the overall toxicity database is incomplete, and significant data gaps suggest the possibility that a lower PoD would be identified for unstudied toxic endpoints. Such considerations often arise if data are available from only one species, or if developmental and reproductive toxicity studies have not yet been performed. Although not all OEL-setting organizations include an explicit UF_D, this factor is intended to account for the possibility of finding a lower PoD once the additional studies are performed. Dourson et al.[53] reviewed chronic toxicity data for pesticides and concluded that an adequate database would consist of 2-year studies in the rat and dog, plus reproductive and developmental studies in the rat. It is unusual to have 2-year dog study data for OEL setting purposes; 2-year studies in mice and rats are more common. The available data suggest a UF_D of 3–10 for database inadequacy may be needed if an OEL is based on datasets that lack longer-term studies in two species plus reproductive and developmental studies.[19]

The decision to apply a UF_D should be based on considering the specific types of data available for the chemical in question and judging the likelihood that additional studies would reveal additional toxicity. In some cases, existing data may suggest the possibility of an effect that can only be reliably tested using a special study design that has not been conducted. For example, indications from standard study designs of concerns related to neurological effects or effects on the immune system might suggest the need for special functional or endpoint-specific studies. If absent, such data gaps are often used to support a larger UF_D. It is not unusual in OEL-setting to have the benefit of human studies, which though inadequate for establishing an OEL due to lack of reliable exposure measurements, may shed light on the hazards associated with the chemical. For example, a dataset lacking reproductive and developmental studies in animals may be considered inadequate if the available human data suggest a potential reproductive hazard, but adequate ($UF_D = 1$) if the human data are sufficient to rule out concern for reproductive or developmental toxicity.

A common data gap in setting OELs is the absence of toxicity data from exposure by the inhalation route; many OELs are derived based on oral-dosing studies. This reflects testing designs for actual end-product use scenarios where ingestion and skin application dominate. There are scientific principles that underpin the circumstances when route-to-route extrapolation is biologically justifiable.[54] However, examining the uncertainty generated by such route-to-route extrapolations is necessary. Common considerations include the degree to which an observed systemic toxicity effect is likely to be more sensitive than portal of entry effects. This is a major concern because a very common endpoint for current OELs for industrial chemicals is respiratory tract irritation.[55] In some cases the potential for such effects might be inferred from irritation assays tested via other routes, although relying on correlations between endpoints such as dermal irritancy and irritation of the respiratory tract is not ideal. The need for adjustments for route-dependent bioavailability is also considered. In some cases adjustments for this consideration can be handled via application of dosimetry adjustments or through the use of absorption factors in the OEL equation.[5,13,56] When there are a priori concerns regarding differences in bioavailability via different routes, and data are not available to address quantitative adjustments for route considerations, the UF_D might be increased for this purpose. Alternatively, residual uncertainties regarding bioavailability can be addressed with the modifying factor.

The Composite Uncertainty Factor (UF_C)

The value of typical defaults used varies among organizations. Organizations that derive environmental standards in the United States to protect the general population (e.g., EPA reference doses [RfDs] and Agency for Toxic Substances and Disease Registry [ATSDR] minimal risk levels [MRLs]), tend to favor order of magnitude-based adjustments of 1 or 10, using an intermediate value of 3. Note that in such cases the value of 3 represents one-half of the log10 unit (3.16 rounded to 3) as the minimum increments that are used for the UF adjustments to reflect the level of precision for such an approach. Many European agencies also use a value of 10 to account for a full area of uncertainty, but they tend to favor intermediate values of 2 and 5 in their calculations. In some cases, other alternative defaults are provided for specific data extrapolations (e.g., the assessment factors used for the derived-no-effect level [DNEL] under chemical registration requirements in the EU).

After the numerical values of the various UFs have been determined, they are then multiplied to yield the overall composite UF, UF_C. For example, default values of 4 for UF_A and 10 for UF_H would be multiplied to yield a UF_C of 40, which would then be applied to the PoD as described above. Note that although the value of 3 is used in place of 3.16 during the discussion of default UFs, caution should be applied when multiplying UFs of 3 together. For example, when multiplying two UFs of 3 together, where each factor represents a half-log adjustment (e.g., 3.16×3.16), the product will be 10, not 9. This is also illustrated by multiplying three UFs of 3, that is, $3 \times 3 \times 3$ will equal 30, not 27.

Assuming default values for the UF_A, UF_H, UF_S, and UF_L, the numeric value of the UF_C could conceivably be very large. Although this multiplicative approach to setting the UF_C would be correct if the various UFs were strictly independent of one another, it has been argued that the various UFs are not entirely

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independent and that simply multiplying default factors of 10 can lead to double-counting of some sources of uncertainty. Reducing some of the UFs to adjust for the presumed double-counting has been proposed[57] and verified empirically by Swartout et al.[58] Current EPA guidelines[22] for applying UFs for determining reference doses for the general population also assume that an overall UF_C of 10,000 is likely to be more than adequately protective, and therefore UF_C is limited to a value of 3,000 even if four full areas of uncertainty are present (i.e., the UF_A, UF_H, UF_S, and UF_L are all assigned values of 10). Moreover, if five full areas of uncertainty are present, it is likely that the data are not sufficient to derive an exposure limit with adequate confidence.

Probabilistic approaches for defining combinations of UFs have also been proposed.[58–60] In general, these lead to smaller values of the total UF_C than those obtained by multiplying the default values of UFs. Most recently, Hasegawa et al.[61] proposed an alternative set of default UFs, based on probabilistic combinations of empirically-derived UFs. These alternative UFs are in some cases larger than the usual 10-fold default UFs, but in other cases less than 10. Although these probabilistic combinations of UFs have to date not attained wide regulatory acceptance, they may provide a framework for refining existing practices for establishing the overall UF_C. The impacts of probabilistic UFs in interpreting occupational risk are further described by Waters et al.[62]

The nature and level of documentation of the UF application in deriving the OEL is highly variable among the organizations that publish OELs. The OEL documentation published by leading organizations for Threshold Limit Values (TLV®), Workplace Environmental Exposure Levels (WEELs), and values published by the EU Scientific Committee on Occupational Exposure Limits (SCOEL) describe the rationale for the OEL, noting considerations and uncertainties. An explicit description of the individual UFs or values used in deriving the OEL is not provided, although many cases the composite is given. In contrast, risk values such as environmental or emergency airborne limits established by most government organizations (e.g., EPA Reference Concentrations [RfC], Acute Emergency Guideline Levels [AEGL], limits derived by U.S. state agencies; and EU DNEL values including for occupational scenarios) have a documented uncertainty factor approach, and the values applied to derive a given value are typically enumerated. There are merits and limitations of each approach for calculating a composite UF and enumerating specific default component subfactors (Table III) and hybrid approaches have also been proposed.[63] However, overall the trend is toward increased documentation (at least qualitatively) of the UF choices in the rationale for the OEL.

Toxicokinetic Adjustment Factor

As an alternative to using dosimetry models to modify the PoD,[13] there are additional adjustments that modify the denominator of the OEL equation that some organizations use to reflect specific toxicokinetic considerations. For example, ECHA has predefined assessment factors for correction of the PoD to consider differences in dosing route and the exposure route for the worker exposure scenario.[41] Two other common adjustments applied by some organizations (and in particular the pharmaceutical sector) reflect route-specific bioavailability and extrapolation to address steady-state internal dose conditions.[5] In certain cases, route-to-route extrapolation might be appropriate when attempting to derive an OEL from a study conducted by a route (e.g., oral) that is different from the potential route of exposure (i.e., inhalation). Although direct studies using the relevant route of administration are normally expected, extrapolation using an additional adjustment factor (e.g., bioavailability correction factor) may be useful to ensure adequate protection in certain situations.

Naumann and Weideman[18] and Naumann et al.[56] provided the background on the use of bioavailability correction factors and guidance on when and how they should be applied for OEL development. Absent a validated TK model or a risk assessment requiring the use of a predetermined default, the technical approach most commonly employed for the bioavailability adjustment reflects a ratio of route-specific bioavailability data.[5,64] When the adjustment factor is placed in the denominator of the OEL equation it can be calculated as:

Bioavailability Adjustment Factor (BAF)
= (%systemic bioavailability for the route of interest)/
(%systemic bioavailability for the dose route used
as the basis for the PoD).

For example, if an OEL were derived based on a critical effect derived from an oral dosing study with a systemic bioavailability of 10% and the bioavailability by the inhalation route was 100%; the resulting bioavailability adjustment factor would be 1.0/0.1 or a factor of 10. This adjustment, therefore, serves as an estimate of the inhalation equivalent to the oral PoD on a systemic dose basis, i.e., the OEL is lowered by this factor.

In practice, such adjustments for bioavailability often have significant limitations based on simplifying assumptions that must be made in the absence of route-specific bioavailability data. Several limitations to the application of such a factor include:

- The adjustment is not applicable for OELs based on effects at the portal of entry—in such cases extrapolation across routes is not recommended.
- The adjustment relies on reliable estimates of systemic bioavailability. Surrogates for total systemic dose (e.g., penetration rates) are not adequate metrics for systemic dose since they do not take into account other toxicokinetic properties such as local or hepatic first-pass metabolism.
- If the form of the chemical that causes toxicity (e.g., parent or metabolite) is not known then the bioavailability adjustments based on parent compound may not reflect

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TABLE III. Merits and Limitations of Alternative UF Documentation Approaches

Factor Application and Multiplication Approach
Description: specific default values are assigned for preselected areas of uncertainty. The composite UF is calculated as the product of the default values that are pertinent to the specific data sets being evaluated. Rules for modifying the resulting composite UF may be specified to address overlapping uncertainties.
Advantages:
• Transparency in OEL calculation improves user ability to interpret the OEL and its uncertainties and supports worker risk communication
• Relative impact of different uncertainties on the OEL are clear, allowing for a determination of the value or impact of collecting new data to relieve uncertainty
Disadvantages:
• Rigorous application of default values limits the value offered through the use of expert scientific judgment
• Multiplying default values may yield a composite UF which does not align with the totality of the data set, often requiring significant effort and potential user confusion in explaining departures from pre-assigned defaults
Uncertainty Weight of Evidence Approach
Description: areas of uncertainty are considered in an integrated approach, with particular focus on potential overlapping considerations and the optimum protective composite UF derived when balancing the data from all lines of evidence.
Advantages:
• Provides greater flexibility in deriving the composite UF that takes into account all aspects of the available data using a weight of evidence approach
• Avoids pitfalls of setting OELs that are not appropriate that can result from misapplication of default UF values or overlapping areas of uncertainty
Disadvantages:
• Absence of specific defaults limits transparency in the basis for the OEL, with resulting limitations for user communication and data collection
• Requires a high degree of scientific expertise in OEL development, because the approach is less prescriptive

actual route-specific differences in toxic potency. This consideration is common for chemicals that undergo significant hepatic first-pass metabolism following oral dosing.

- Unless inhalation is the primary route of exposure of concern for consumer or environmental exposures for a chemical, TK data following inhalation is not likely to be available. Thus, the adjustment may be based on empirical bioavailability estimates for the dose route for the critical study that served as the PoD, but the bioavailability for inhalation (the route of interest for the OEL) must be estimated. One approach to address this limitation is to assume 100% inhalation bioavailability (for most small organic molecules) as a precautionary assumption or to estimate a value based on properties such as partition coefficients.
- Estimating inhalation bioavailability for large molecules (e.g., protein therapeutics) adds additional complexity that needs to take into account factors such as molecular weight, the presence of specific receptors that facilitate epithelial transport, and bioavailability from the gastrointestinal tract following mucociliary clearance and swallowing.

Additional factors may be needed to adjust OELs because of the potential for bioaccumulation with repeated exposure. For chemicals with long elimination half-times, plasma levels increase with repeated exposure until they reach steady state. The OEL established using a NOAEL from a short-duration study would need to be adjusted downward by a factor corresponding to the ratio of the plasma level following a short-term exposure (duration equivalent to the study used to identify the PoD) to the expected plasma level at steady state following long-term exposure. This adjustment is of specific utility in setting OELs for pharmaceuticals where single dose or short-duration clinical studies are often available. OELs derived from animal toxicology studies typically require repeat-dose studies where steady-state kinetics apply before an OEL would be derived. This bioaccumulation adjustment is not necessary if the OEL is established using studies of sufficient length such that the steady state at the maintenance dose is achieved, because any accumulation has already been taken into account under such conditions.

Modifying Factor (MF)

This factor adjusts for uncertainties not addressed by the UFs described above, and it allows for explicitly incorporating scientific judgment, especially when there are multiple reviewers. A modifying factor may also be considered if there is a need to address residual uncertainties not covered by the other factors. This factor also allows for scientific judgment to be applied to address the overall quality of the database and relevance of available studies to human risk assessment. Additional factors of <1 to 10 may be used in these cases.[19] Explicitly incorporating a modifying factor (MF), along

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with the rationale for including it, may promote increased transparency of the decision-making process, leading to the final OEL. However, current EPA guidance is to forego the specific use of this factor and incorporate the uncertainties it addresses within another factor, such as UF_D.[22]

Modifying factors may be used to make further adjustments that the reviewer deems necessary to cover situations that other factors do not clearly or adequately address. Some considerations that are often cited for application of a MF include the following.

1. The slope of the dose-response curve for the critical effect.
2. The choice of the critical effect.
3. The severity of the effect.
4. Route-to-route extrapolation.
5. Identification of susceptible subpopulations.
6. Clinical significance of the critical effect.
7. Reversibility of the critical effect.
8. Overall quality of the database.
9. Relevance of the critical effect to workers.
10. Similarity or differences with related chemicals.
11. Lack of independence between individual uncertainty factors.

Chemical-Specific Adjustment Factors (CSAFs) in OEL Setting

Default uncertainty factors, by definition, should be used only in the absence of relevant data. Chemical-specific adjustment factors (CSAFs) are intended to replace the default factors where TK and TD data are available, according to the scheme proposed by Renwick[65–67] and adopted by the World Health Organization/International Programme on Chemical Safety.[21,68]

CSAFs can be used to replace default uncertainty factors to establish safe levels of exposure whenever sufficient chemical-specific data are available. The Renwick scheme provides a framework for incorporating chemical-specific TK and TD data when deriving regulatory and internal health-based limits, such as acceptable daily intake values, tolerable intake values, reference doses/concentrations, and occupational exposure limits. Renwick[67] listed a number of considerations for use of chemical-related data, including whether the chemical itself or a metabolite is the active species, the relevance of the TK or TD data to the critical endpoint, and how representative the data are of the human population. Meek et al.[69] have also proposed guidelines on the use of data-derived adjustment values to replace default uncertainty factors when sufficient chemical-specific data are available.

The distinction between uncertainty and variability should be emphasized. CSAFs are used to characterize the "variability" in TK and TD in a relevant subset of the intended population. To the extent that this subset is representative of the overall population and provides quantitative information on differences (or similarities), the level of "uncertainty" should be reduced considerably. The risk assessors/risk managers should feel more confident that the health-based limit they derive will provide adequate protection for even the most susceptible individuals. There may be residual uncertainties; however, these would obviously be less than when no data are available on potentially important differences. By focusing on TK and TD data for potentially susceptible subpopulations, the risk assessor consciously evaluates data for the segment of the population in greatest need of protection.

Within the CSAF scheme, interindividual differences are assessed using several TK and TD parameters indicative of systemic exposure and pharmacologic activity of the chemicals evaluated. The TK parameters initially chosen as measures

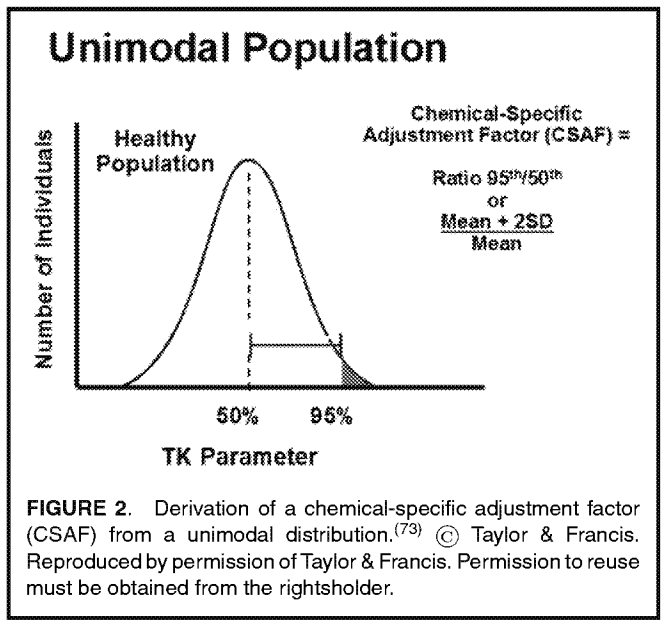

FIGURE 2. Derivation of a chemical-specific adjustment factor (CSAF) from a unimodal distribution.[73] © Taylor & Francis. Reproduced by permission of Taylor & Francis. Permission to reuse must be obtained from the rightsholder.

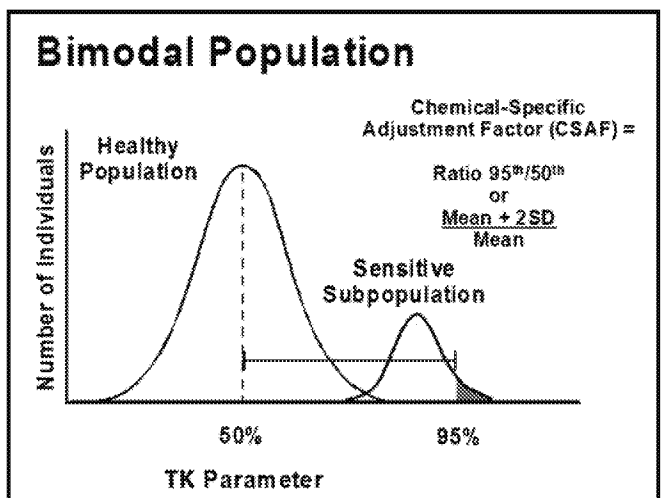

FIGURE 3. Derivation of a chemical-specific adjustment factor (CSAF) from a bimodal distribution.[73] © Taylor & Francis. Reproduced by permission of Taylor & Francis. Permission to reuse must be obtained from the rightsholder.

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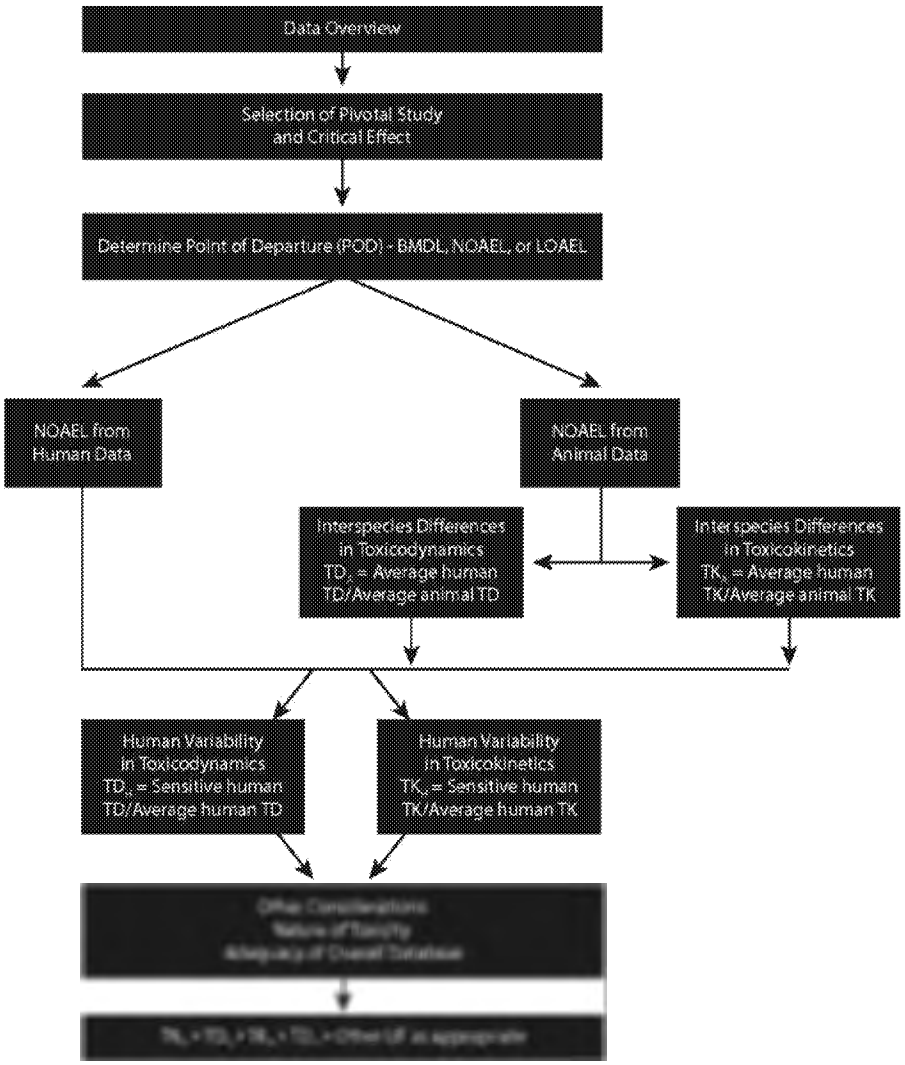

FIGURE 4. Application of the CSAF approach with additional uncertainty factors for exposure limit setting. Adapted from WHO/IPCS (2005).[21]

of internal dose were peak plasma concentration (C_{max}), area under the curve of blood concentration by time (AUC), steady-state plasma concentration (SS), and elimination or clearance rates. The first two, C_{max} and AUC, are considered the best indicators of body burden and systemic exposure because they are direct measures of the amount of chemical in the blood, although clearance rates are also commonly used metrics in the CSAF method.

The general guidelines[21,69] for evaluating TK and TD data include some important considerations for choosing CSAFs. For assessing interindividual differences in TK, (1) the TK parameter used should be directly related to the critical effect (e.g., AUC vs. C_{max}), (2) the data should be generated with the dose and route most relevant to the recommended health-based limit, and (3) data from human subjects should be representative of the expected variability in the population being protected (workers, in this case age 18–65), including any sensitive subpopulations. For assessing interindividual differences in TD, (1) the measured endpoint must be directly related to the critical effect used to derive the recommended exposure limit, (2) the data must include a quantitative comparison of the response in tissues at a similar level of response from average and sensitive humans, and (3) a sufficient number of individuals must be included to define interindividual variability. The WHO/IPCS guidance documents [21,68] provide examples of how CSAFs are derived for all four sub-factors.

The method for estimating interindividual differences and deriving CSAFs relies on the ratio of the tail of the distribution to the central tendency for the TK or TD parameter of interest. The approach is based on the premise that if a subpopulation (e.g., tail of the distribution) is sufficiently different (i.e., more susceptible based on significantly higher C_{max} or AUC values for a given dose), their level of exposure needs to be

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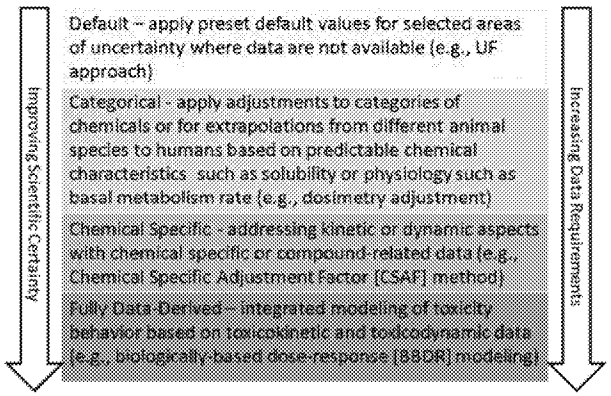

FIGURE 5. Hierarchy of approaches to address uncertainty, and the correspondence of greater scientific certainty to an increased requirement for the incorporation of chemical- and species-specific data into the risk assessment process.

adjusted downward to conform to the normal (average) healthy individual. Where two or more distinct subpopulations exist, the ratio of the upper tail of the most sensitive subpopulation over the mean of the healthy population is used to derive an appropriate adjustment factor. This is illustrated graphically in Figure 2 (transformed unimodal distribution) and Figure 3 (transformed bimodal distribution).

In practice, evaluating interindividual differences typically relies on papers presenting only summary statistics (e.g., mean and standard deviation) for TK and/or TD parameters. A CSAF is calculated by dividing the mean plus two standard deviations by the mean (Mean + 2SD)/Mean). Occasionally, standard errors need to be converted to standard deviations by multiplying the former by $\sqrt{N}$.

A case study was published to illustrate how CSAFs can be used to derive occupational exposure limits.[70] The case study involved timolol maleate, a non-selective beta-adrenergic blocking agent, and it shows how a polymorphism in oxidative metabolism by CYP2D6 with two distinct phenotypes (i.e., poor and extensive metabolizers) was evaluated to take this potential susceptibility into account when establishing the OEL. The prevalence of poor metabolizers (up to 9% of selected subpopulations) is high enough to indicate careful consideration of this potential susceptibility when establishing the OEL for timolol maleate. A chemical-specific adjustment factor (CSAF) for kinetics of 9.8 was calculated for this bimodal distribution by dividing the value corresponding to two standard deviations above the mean AUC value for poor metabolizers by the mean AUC for the extensive metabolizers, ($Mean_{Poor}$ + 2SD)/$Mean_{Ext}$), as shown in Figure 3. When combined with a CSAF for dynamics of 1.2, this yields a composite CSAF of 12 to address human variability in sensitivity. The CSAF was applied to the extrapolated no-effect level for clinically significant cardiovascular effects (with correction for oral bioavailability) to establish an occupational exposure limit (OEL) for timolol maleate that is expected to be protective of nearly all workers, including those who may be poor metabolizers.

It is important to note that use of CSAFs does not automatically imply a reduced "safety factor" used in setting health-based limits. Sometimes the available data for a given chemical suggest a high level of interindividual variability. Note that, in the example above, the composite uncertainty factor derived using the $CSAF_{H\text{-}TK}$ combined with the default for toxicodynamics ($CSAF_{H\text{-}TD}$) of 3.2 would have been 31, which is 3-fold higher than the default value of 10. The overall process for applying the CSAF approach in the context of setting exposure limits is shown in Figure 4.

CONCLUSION

The application of UFs in deriving OELs has evolved considerably in recent decades. Although originally seen as a somewhat arbitrary safety factor, based primarily on professional judgment, application of UFs has moved towards an increasingly strong dependence on scientific evidence —first in regard to scientifically-based defaults,[17,18,31,71] and more recently in developing chemical-specific values.[21,42,43,70,72] Increasingly, it is the application of chemical-specific data that is seen as the preferred "default" method, with the application of standard values of UFs limited to those situations in which an OEL must be derived in the absence of chemical-specific data. Thus, there is a preferred hierarchy of UF approaches (Figure 5). Despite this trend for the majority of industrial chemicals detailed TK data to support CSAF calculation may not be available.

This article outlines the scientific basis for the most commonly used UFs, (i.e., the UFs for animal-to-human extrapolation, intra-individual variability, extrapolation from shorter-term to longer-term exposures, extrapolation from a LOAEL to a NOAEL, and adjustments for an incomplete database). Because the application of scientific judgment cannot always be avoided in the OEL-setting process, such judgments should be open and transparent, and they should systematically evaluate key areas of uncertainty, incorporating chemical-specific data when available, and clearly documenting the rationale for final decisions that are used in the OEL. The science of UF application is continuing to improve. Advancements in the scientific development of UF application include the following:

- greater expectation for transparency and description of the basis for UF selection, including the quantitative values that were applied;
- improved application and access to more robust methods (e.g., CSAFs and bioavailability correction factors) because tools are better, access to data is greater, and familiarity with methods is increasing;
- an understanding of the basis for human variability in sensitivity (e.g., genetics and epigenetics), and more complete

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consideration in OEL setting, with a goal of moving toward fully data-derived methods of OEL derivation; and

- application of advances in toxicology, including computational toxicology approaches to refine UF values in a quantitative manner.

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ED_005043_00251365-00015

Appointment

From: Nickerson, William [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=148F2C1C05B54F358E29C59B841664AA-WNICKER]
Sent: 7/10/2018 3:11:05 PM
To: Ex. 6 Personal Privacy (PP)

Subject: Accepted: FW: TWG - Perchlorate
Location: Conference Call

Start: 7/11/2018 6:00:00 PM
End: 7/11/2018 7:00:00 PM
Show Time As: Busy

Appointment

From: Penman, Crystal [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=93662678A6FD4D4695C3DF22CD95935A-PENMAN, CRYSTAL]
Sent: 4/5/2016 3:22:28 PM
To: Shapiro, Mike [Shapiro.Mike@epa.gov]; Lousberg, Macara [Lousberg.Macara@epa.gov]; Grevatt, Peter [Grevatt.Peter@epa.gov]; mike.a.mcneill@nasa.gov; Southerland, Elizabeth [Southerland.Elizabeth@epa.gov]
CC: WENNERBERG, LINDA S. (HQ-LD020) [linda.s.wennerberg@nasa.gov]; james.leatherwood-1@nasa.gov; Lape, Jeff [lape.jeff@epa.gov]

Subject: Meeting with NASA
Attachments: Background information for NASA Meeting 5-2-16.docx
Location: 1201 Constitution Ave NW, Washington DC 20640 WJCE 3233 WjCE Please call for escort 202-564-5700

Start: 5/3/2016 5:00:00 PM
End: 5/3/2016 5:45:00 PM
Show Time As: Busy

Background information for N...

Meeting with NASA
WJCE 3233
5/3/2016 1:00 PM

Purpose: The Director (James Leatherwood) and Deputy Director (Mike McNeill) of NASA's Environmental Management Division have requested a meeting to discuss a number of OW regulatory actions that have the potential to impact on NASA and other federal agencies. Specifically, NASA is seeking to participate in interagency review of the following actions:

- Water quality criteria for specific chemicals,
- UCMR4 proposed rule,
- Section 610 Review of the Ground Water Rule,
- Regulations Implementing Section 1417 of the Safe Drinking Water Act: Prohibition on Use of Lead Pipes, Solder, and Flux, , and
- NPDWR Perchlorate.

Interagency Review Status of Actions:

- **Water Quality Criteria:** EPA's water quality criteria documents, as with most independent science documents, are not typically reviewed by OMB under Executive Order 12866.
    - Water quality criteria documents are highly technical effects assessments. They are based solely on data and scientific judgements on the relationship between pollutant concentrations and ecological or human health effects.
    - They do not set policy, and they do not reflect consideration of economic impacts or the technological feasibility of meeting the pollutant concentrations in ambient water.
    - Water quality criteria are recommendations; states and tribes are not required to adopt the specific criteria that EPA publishes. They are developed according to scientifically accepted criteria development methodologies and undergo rigorous internal and external peer reviews.

- **Unregulated Contaminant Monitoring Rule (UCMR 4) for Public Water Systems:** The NPRM (published 12/15/15) was determined by OMB to be non-significant. Anticipate submitting request for non-significant determination for the final action.

- **Regulations Implementing Section 1417 of the Safe Drinking Water Act: Prohibition on Use of Lead Pipes, Solder, and Flux:** The proposed action was determined non-significant by OMB on 10/30/15.

- **610 Review of National Primary Drinking Water Regulations: Ground Water Rule:** Section 610 reviews are not considered significant for purposes of OMB review. If based on the analysis conducted during a 610 review, the agency were to decide to revise a regulation, it would initiate a separate rulemaking process that would be subject to interagency under EO 12866.

- **National Primary Drinking Water Regulations: Regulation of Perchlorate**: The proposed action was determined to be significant by OMB on 10/11/2011.

Additional Background on Actions:

OST:

Numeric Nutrient Criteria for Lakes and Reservoirs of the Continental United States

- **External Abstract:** Nitrogen and phosphorus pollution degrades the conditions of water bodies worldwide, and in lakes and reservoirs, the effects of excess nitrogen and phosphorus are often particularly evident. High levels of nutrient loading can stimulate excess growth of algae that can impair the use of a lake by aquatic life, and for recreation and as a source of potable water. In this document, EPA recommends, under Section 304(a) of the Clean Water Act, numeric criterion values for total nitrogen and total phosphorus that protect aquatic life, recreation, and potable water uses in lakes and reservoirs of the United States. The ranges of criterion values provided here facilitate the development of locally applicable nutrient water quality standards by States and Tribes.

Draft Recommended Field-Based Methods for States to Develop Aquatic Life Criteria for Specific Conductivity

- **External Abstract:** EPA's development of a methodology for deriving chronic ambient water quality criteria for conductivity is being driven by 1) the need to protect aquatic life from the deleterious effects of waters with elevated salinity as assessed by conductivity and 2) the recent progress in the development of a field-based methodology for assessing impacts of waters with elevated ionic strength on aquatic life that has been extensively peer-reviewed. This document addresses the need to have scientifically defensible conductivity criteria for use by states in permitting and water quality standards development. The new conductivity criteria will reflect this field-based methodology to derive chronic freshwater criteria for broader application to each State and/or Tribe that wishes to adopt it into their water quality standards.

Update of Aquatic Life Ambient Estuarine/Marine Water Quality Criteria for Copper

- **External Abstract:** Exposure to elevated concentrations of copper negatively impacts the survival, growth, development, and reproduction of aquatic life in estuarine and marine environments. EPA is updating its national recommended ambient water quality criteria for copper in estuarine/marine environments in order to reflect the latest scientific information. In addition to the inclusion of new laboratory aquatic toxicity test data, the updated criteria uses a saltwater biotic ligand model (BLM) to provide updated acute and chronic water quality criteria for copper that are applicable to estuarine and marine waters. This BLM includes a characterization of copper binding to natural organic matter in marine systems and considers copper accumulation on the surface membranes of embryo-larval stages of several sensitive marine invertebrates. Once finalized, EPA's water quality criteria for copper in estuarine/marine environments will provide recommendations to states and tribes authorized to establish water quality standards under the Clean Water Act.

Selenium Ambient Water Quality Criteria for the Protection of Aquatic Life

- **External Abstract:** The revised criteria will be expressed as a fish tissue residue criterion that may be used by States and Tribes in their water quality standards. Interest in the criteria is mounting because of mountain top mining operations, water re-use in arid agricultural areas, and ESA Section 7 consultation at both the State (California) and Federal levels. EPA's revised criterion recommendation for selenium poses unique

implementation questions, which EPA intends to address in this implementation guidance. The aquatic life criterion recommendation for Selenium is based on a fish tissue rather than water column concentration. This is because diet is the primary route of exposure for fish, and water concentrations poorly correlate with effects. The draft selenium implementation guidance will provide information on how states/tribes could express their selenium criterion in their water quality standards and the recommended way to utilize that criterion in water management programs such as NPDES, assessment/listing and TMDL programs. As part of this guidance, EPA is providing a mechanism to translate a fish tissue criterion to a water column concentration that we expect will facilitate the development of appropriate permit limits.

Cyanotoxin Ambient Water Quality Criteria for Recreational Waters

- **External Abstract:** Cyanobacteria, common to freshwater and marine ecosystems, can form scums or "blooms" at the surface of a water body under certain conditions (e.g., high nutrient concentrations and high light intensity). These blooms can produce toxic compounds (cyanobacterial toxins or "cyanotoxins") that are harmful to the environment, animals and human health. Toxins are released to the water column as the bacteria die and elevated levels can persist in the water after the bloom has disappeared. Humans and animals exposed to the toxins via ingestion, inhalation or dermal pathways can experience adverse health effects including liver damage, kidney damage, neurological damage, gastrointestinal illness, skin irritation, and other effects depending on the dose and the type of cyanotoxin. Harmful algal blooms have been reported in ambient waters in most coastal and inland states. At least 36 states have reported cyanotoxin poisonings and/or health advisories. In this document, EPA recommends, under Section 304(a) of the Clean Water Act, numeric criterion values for the cyanotoxins, microcystins and cylindrospermopsin. These recommended criteria will facilitate the development of nationally-consistent water quality standards by States and Tribes.

OGWDW

Unregulated Contaminant Monitoring Rule (UCMR 4) for Public Water Systems

- **External Abstract:** The Safe Drinking Water Act (SDWA), as amended in 1996, requires that the U.S. Environmental Protection Agency (EPA) establish criteria for a program to monitor not more than 30 unregulated contaminants every five years. EPA published the first Unregulated Contaminant Monitoring Rule (UCMR 1) in the Federal Register on September 17, 1999 (64 FR 50556), the second (UCMR 2) on January 7, 2007 (72 FR 367), and the third (UCMR 3) on May 2, 2012 (77 FR 26072). This action meets the SDWA requirement by establishing the terms for the next cycle of monitoring, and identifying the new unregulated contaminants to be monitored during the UCMR 4 period of 2017-2021.
- **Status in Fall 2015 Reg Agenda:** NPRM Publication = 6/00/2015; Final Publication = 12/00/2016
- **Status in Spring 2016 Reg Agenda (NOT YET PUBLIC):** NPRM Published = 2/15/2015; Final Publication = 2/00/2018
- **Status of OMB Significance Determination:** Anticipate submitting request for Non-significant determination; significance request to be submitted. NPRM (published 12/15/15) was determined by OMB to be non-significant.

610 Review of National Primary Drinking Water Regulations: Ground Water Rule

- **External Abstract:** *(NOTE: Please note that the abstract reflects the Spring regulatory agenda and the result of our analysis. NASA cannot share this result with others.)*
- EPA published the Ground Water Rule (GWR) in the Federal Register on November 08, 2006. The purpose of the rule is to provide for increased protection against microbial pathogens in public water systems that use ground water sources. EPA is particularly concerned about ground water systems that are susceptible to fecal contamination since disease-causing pathogens may be found in fecal contamination. The GWR applies to public water systems that serve ground water. The rule also applies to any system that mixes surface and ground water if the ground water is added directly to the distribution system and provided to consumers without treatment. EPA reviewed this action in the fall of 2015 through the spring of 2016, pursuant to section 610 of the Regulatory Flexibility Act (5 U.S.C. 610). As part of this review, EPA solicited comments for consideration on the following factors: (1) The continued need for the rule; (2) the nature of complaints or comments received concerning the rule; (3) the complexity of the rule; (4) the extent to which the rule overlaps, duplicates, or conflicts with other federal, state, or local government rules; and (5) the degree to which the technology, economic conditions or other factors have changed in the area affected by the rule. EPA has completed its review and concluded that the rule needs no revisions at this time to minimize impacts on small entities. The results of the review are summarized in a report and placed in the Water Docket under Docket ID No. EPA-HQ-OW-2015-0541. You can access the docket at the Federal eRulemaking Portal: [HYPERLINK "http://www.regulations.gov"].
- **Status in Spring 2016 Reg Agenda (NOT YET PUBLIC):** NPRM Publication = 10/31/2015; Final Publication = 4/20/2016

Regulations Implementing Section 1417 of the Safe Drinking Water Act: Prohibition on Use of Lead Pipes, Solder, and Flux

- **External Abstract:** The Reduction of Lead in Drinking Water Act was enacted on January 4, 2011, to amend Section 1417 of the Safe Drinking Water Act (SDWA or Act) respecting the use and introduction into commerce of lead pipes, plumbing fittings or fixtures, solder and flux. The 2011 "Reduction of Lead in Drinking Water Act" revised Section 1417 to:
    - Redefine "lead free" in SDWA Section 1417(d) to
        - lower the maximum lead content of plumbing products such as pipes and fixtures from 8.0% to 0.25%;
        - establish a statutory method for the calculation of lead content; and
        - eliminate the requirement that lead free products be in compliance with voluntary standards established in accordance with SDWA 1417(e) for leaching of lead from new plumbing fittings and fixtures.
    - Create exemptions in SDWA Section 1417(a)(4) from the prohibitions on the use or introduction into commerce for:
        - "pipes, pipe fittings, plumbing fittings or fixtures, including backflow preventers, that are used exclusively for nonpotable services such as manufacturing, industrial processing, irrigation, outdoor watering, or any other uses where the water is not anticipated to be used for human consumption;" (SDWA 1417(a)(4)(A))

        - "toilets, bidets, urinals, fill valves, flushometer valves, tub fillers, shower valves, service saddles, or water distribution main gate valves that are 2 inches in diameter or larger." (SDWA 1417(a)(4)(B)
    - The Community Fire Safety Act of 2013 further amended Section 1417 of SDWA to exempt fire hydrants from the prohibitions on use and introduction into commerce of pipes, fittings, and fixtures that are not lead free. EPA will propose regulations to codify and assist in the implementation of these amendments to Section 1417 of SDWA.
- **Status in Spring 2016 Reg Agenda (NOT YET PUBLIC):** NPRM Publication = 12/15/2016; Final Publication = 4/30/2016
- **Status of OMB Significance Determination:** Proposed action determined Non-significant by OMB on 10/30/15.

National Primary Drinking Water Regulations: Regulation of Perchlorate

- **External Abstract:** The EPA is developing a national regulation for perchlorate in drinking water. The Safe Drinking Water Act describes the EPA's requirements for regulating contaminants. In accordance with these requirements, the EPA will consider the Science Advisory Board's guidance on how to best interpret perchlorate health information to derive a Maximum Contaminant Level Goal for perchlorate. The agency is also evaluating the feasibility and affordability of treatment technologies to remove perchlorate from drinking water and will examine the costs and benefits of a Maximum Contaminant Level (MCL) and alternative MCLs. The EPA is also seeking input through informal and formal processes from the National Drinking Water Advisory Council, the Department of Health and Human Services, State and Tribal drinking water programs, the regulated community (public water systems), public health organizations, academia, environmental and public interest groups, and other interested stakeholders on a number of issues relating to the regulation.
- **Status in Fall 2015 Reg Agenda:** NPRM Publication = 2/00/2016; Final Publication = 10/00/2017
- **Status in Spring 2016 Reg Agenda (NOT YET Public):** NPRM Publication = 4/00/2019; Final Publication = 8/00/2021
- **Status of OMB Significance Determination:** The proposed action was determined to be significant by OMB on 10/11/2011.

Message

From: Shapiro, Mike [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2C70AF880BA747B5A8B6BAA45A040125-MSHAPIRO]
Sent: 3/31/2016 12:15:05 PM
To: Lousberg, Macara [Lousberg.Macara@epa.gov]; Evalenko, Sandy [Evalenko.Sandy@epa.gov]
CC: Gude, Karen [Gude.Karen@epa.gov]
Subject: RE: NASA Requests for Meeting on Office of Water Pending Actions

I have not responded yet. But I did send an email to Crystal Penman asking her to set up a meeting with the NASA folks, just forgot to copy you. Since Crystal is out this week I don't think she followed up, but you may want to check with Damara to see if she has done anything. As I indicated, I don't have to be part of the meeting if I'm not needed for protocol reasons.

Mike

Michael Shapiro
Deputy Assistant Administrator, Office of Water
US EPA, 4101M
1200 Pennsylvania Ave., NW
Washington, DC 20460
202-564-5700

From: Lousberg, Macara
Sent: Thursday, March 31, 2016 7:58 AM
To: Shapiro, Mike <Shapiro.Mike@epa.gov>; Evalenko, Sandy <Evalenko.Sandy@epa.gov>
Cc: Gude, Karen <Gude.Karen@epa.gov>
Subject: RE: NASA Requests for Meeting on Office of Water Pending Actions

Sure. Have you responded to Mr. McNeill or should I send him a note?

From: Shapiro, Mike
Sent: Wednesday, March 30, 2016 8:56 PM
To: Evalenko, Sandy <Evalenko.Sandy@epa.gov>; Lousberg, Macara <Lousberg.Macara@epa.gov>
Cc: Gude, Karen <Gude.Karen@epa.gov>
Subject: RE: NASA Requests for Meeting on Office of Water Pending Actions

Thanks, based on your responses I think a meeting with the NASA folks makes sense. I don't really know how their hierarchy works, but I am happy to participate if that is appropriate. We should certainly have reps from OST and OGWDW. Macara, could you work on setting something up? Thanks.

Mike

Michael Shapiro
Deputy Assistant Administrator, Office of Water
US EPA, 4101M
1200 Pennsylvania Ave., NW
Washington, DC 20460
202-564-5700

From: Evalenko, Sandy
Sent: Wednesday, March 30, 2016 6:42 PM
To: Lousberg, Macara <Lousberg.Macara@epa.gov>

Cc: Shapiro, Mike <Shapiro.Mike@epa.gov>; Gude, Karen <Gude.Karen@epa.gov>
Subject: Re: NASA Requests for Meeting on Office of Water Pending Actions

Sorry for a late response. Macara is correct.

1. Conductivity criteria or most independent scientific documents are not typically reviewed by OMB under the EO. The implementation guidance for the methyl-mercury criteria is the only criteria document with an interagency review since I've been in OW.

2. OMB determined the UCMR4 NPRM and the lead safe free act NPRM to be non-significant. Most likely Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

Is NASA interested in getting data on a chemical or pollutant or something?
For the Lead Safe Free Act rule, the program is Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

3. I think that Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

4. NPDWR Perchlorate Ex. 5 Deliberative Process (DP) Both will have an interagency review.

I don't know what the intersection with NASA for our actions. It might be helpful to know what their interests or concerns are wrt our regs. Do you know who in ORD talked with NASA? I could ask Bob Fegley on ORD Ex. 5 Deliberative Process (DP) experience and discussions with NASA.

Let me know how I can assist.
Sandy

Sent from my iPhone

On Mar 23, 2016, at 8:48 AM, Lousberg, Macara <Lousberg.Macara@epa.gov> wrote:

> HI Mike. I looked these actions up in the Action Tracker report and Mr. McNeill is correct in most cases.
>
> Water quality criteria for specific chemicals: most show that we are proposing for them to Ex. 5 Deliberative Process (DP)
>
> Ex. 5 Deliberative Process (DP)
>
> In Sandy's absence, I'm cc'ing Karen Gude in case she knows something more/different from what I outlined above.

From: Shapiro, Mike
Sent: Tuesday, March 22, 2016 10:50 PM
To: Lousberg, Macara <Lousberg.Macara@epa.gov>
Cc: Evalenko, Sandy <Evalenko.Sandy@epa.gov>
Subject: Fwd: NASA Requests for Meeting on Office of Water Pending Actions

What do you think of this note? Don't most of these actions go through interagency review?

Mike

Sent from my iPhone

Begin forwarded message:

> **From:** "Mcneill, Mike A (HQ-LD020)" <mike.a.mcneill@nasa.gov>
> **Date:** March 22, 2016 at 4:30:06 PM EDT
> **To:** "shapiro.mike@epa.gov" <shapiro.mike@epa.gov>
> **Subject: NASA Requests for Meeting on Office of Water Pending Actions**
>
> Dear Dr. Shapiro,
>
> Please allow me to introduce myself, I am the Deputy Director of NASA's Environmental Management Division (EMD). James Leatherwood, the EMD Director, and I request the opportunity to discuss with you a group of pending Office of Water actions. These actions, such as water quality criteria for specific chemicals, the UCMR4 proposed rule, the Ground Water Rule review, implementing regulations of SDWA prohibitions on lead pipes and solder, and perchlorate, have the potential to impact on NASA and other Federal agencies. Currently, no established interagency dialogue or review process is available in these Office of Water pending actions. We welcome starting a dialogue on these and related actions.
>
> Based on the success of the EPA IRIS process's interagency review, NASA requests the Office of Water consider **Ex. 5 Deliberative Process (DP)**
> Ex. 5 Deliberative Process (DP) As with the IRIS process, many of the identified Office of Water actions result in science policy decisions on action levels and implementation of regulations that potentially impact federal agencies in mission execution. Comparable federal agency experience with specific IRIS decisionmaking found interagency review strengthened the process, especially information sharing and collegiality among the participating federal agencies. We welcome discussing the targeted use of interagency review to support sound science in the Office of Water's decision making and promote communication and information sharing among federal agencies.
>
> I request that we discuss this matter. I am including the contact information of my scheduler, Ms. Bridget Mackall (bridget.d.mackall@nasa.gov, 202/358-2030) and my contact information (mike.a.mcneill@nasa.gov, 202/359-1886) should you prefer to call me directly. The NASA team looks forward to working with you and your team.
>
> Respectfully,
> Mike A. McNeill
>
> ==========
> Mike A. McNeill, P.E.
> Deputy Director
> Environmental Management Division

NASA Headquarters
Suite 2T89, Room 2T81
300 E Street SW
Washington, DC 20546-0001
(202) 358-1886 FAX (909) 380-8607
"Enabling environmentally sound mission success"

Message

From: Shapiro, Mike [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=2C70AF880BA747B5A8B6BAA45A040125-MSHAPIRO]
Sent: 3/28/2016 3:04:29 AM
To: Crystal Penman [penman.crystal@epa.gov]
Subject: FW: NASA Requests for Meeting on Office of Water Pending Actions

Crystal,

Please set up the requested meeting – see the incoming from Mike Mcneill below. Participants for OW should include Macara Lousberg, representatives from OGWDW and OST. Thanks.

Mike

Michael Shapiro
Deputy Assistant Administrator, Office of Water
US EPA, 4101M
1200 Pennsylvania Ave., NW
Washington, DC 20460
202-564-5700

From: Lousberg, Macara
Sent: Wednesday, March 23, 2016 8:48 AM
To: Shapiro, Mike <Shapiro.Mike@epa.gov>
Cc: Evalenko, Sandy <Evalenko.Sandy@epa.gov>; Gude, Karen <Gude.Karen@epa.gov>
Subject: RE: NASA Requests for Meeting on Office of Water Pending Actions

HI Mike. I looked these actions up in the Action Tracker report and Mr. McNeill is correct in most cases.

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Ex. 5 Deliberative Process (DP)

In Sandy's absence, I'm cc'ing Karen Gude in case she knows something more/different from what I outlined above.

From: Shapiro, Mike
Sent: Tuesday, March 22, 2016 10:50 PM
To: Lousberg, Macara <Lousberg.Macara@epa.gov>
Cc: Evalenko, Sandy <Evalenko.Sandy@epa.gov>
Subject: Fwd: NASA Requests for Meeting on Office of Water Pending Actions

What do you think of this note? Don't most of these actions go through interagency review?

Mike

Sent from my iPhone

Begin forwarded message:

From: "Mcneill, Mike A (HQ-LD020)" <mike.a.mcneill@nasa.gov>
Date: March 22, 2016 at 4:30:06 PM EDT
To: "shapiro.mike@epa.gov" <shapiro.mike@epa.gov>
Subject: NASA Requests for Meeting on Office of Water Pending Actions

Dear Dr. Shapiro,

Please allow me to introduce myself, I am the Deputy Director of NASA's Environmental Management Division (EMD). James Leatherwood, the EMD Director, and I request the opportunity to discuss with you a group of pending Office of Water actions. These actions, such as water quality criteria for specific chemicals, the UCMR4 proposed rule, the Ground Water Rule review, implementing regulations of SDWA prohibitions on lead pipes and solder, and perchlorate, have the potential to impact on NASA and other Federal agencies. Currently, no established interagency dialogue or review process is available in these Office of Water pending actions. We welcome starting a dialogue on these and related actions.

Based on the success of the EPA IRIS process's interagency review, NASA requests Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) As with the IRIS process, many of the identified Office of Water actions result in science policy decisions on action levels and implementation of regulations that potentially impact federal agencies in mission execution. Comparable federal agency experience with specific IRIS decisionmaking found interagency review strengthened the process, especially information sharing and collegiality among the participating federal agencies. We welcome discussing the targeted use of interagency review to support sound science in the Office of Water's decision making and promote communication and information sharing among federal agencies.

I request that we discuss this matter. I am including the contact information of my scheduler, Ms. Bridget Mackall (bridget.d.mackall@nasa.gov, 202/358-2030) and my contact information (mike.a.mcneill@nasa.gov, 202/359-1886) should you prefer to call me directly. The NASA team looks forward to working with you and your team.

Respectfully,
Mike A. McNeill

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