To: Campo-Moreno, Pablo (campomp)[campomp@UCMAIL.UC.EDU]
From: Conmy, Robyn
Sent: Wed 1/14/2015 11:51:23 AM
Subject: RE: oil projects update

Hey Pablo,

How about Jan 16 at 11 am?

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

From: Campo-Moreno, Pablo (campomp) [mailto:campomp@UCMAIL.UC.EDU]
Sent: Sunday, January 11, 2015 9:01 AM
To: Conmy, Robyn
Subject: Re: oil projects update

Robyn,

I can meet any time on the 16th, 20th, or 21st.

Regards,

Pablo Campo-Moreno, Ph.D.
Research Assistant Professor
University of Cincinnati
Department of Biomedical, Chemical and Environmental Engineering
766 ERC
Cincinnati, OH 45221-0012
PH:(513)-556-3637
Fax: (513)-556-4162
campomp@ucmail.uc.edu

It is a capital mistake to theorize before one has data. Insensibly one begins to twist facts to suit theories, instead of theories to suit facts.

The adventures for Sherlock Holmes

On Jan 9, 2015, at 3:17 PM, Conmy, Robyn wrote:

> Thank you for sending. I am on travel a fair this month. Unfortunately, my open days are 16th, 20th, morning of 21st, and the week of the 26th.
>
> <>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>
>
> Robyn N. Conmy, Ph.D.
>
> Research Ecologist
>
> USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

From: Campo-Moreno, Pablo (campomp) [mailto:campomp@UCMAIL.UC.EDU]
Sent: Wednesday, January 07, 2015 12:52 PM
To: Conmy, Robyn
Cc: Raghu Venkatapathy
Subject: oil projects update

Dear Robyn,

Hope you had a good time during the holidays. Attached please find the updated QAPP for WA 4-05, task 2.3 (round robyn test for solidifiers). Section 4 has been modified to accommodate level 1 QA requirements for assessments. In WA 4-05, Task 1.1, I found an anionic dispersant in Finasol yielded an accurate mass of 422.2335 with an error of 0.87 ppm and a molecular formula C20H38O7S (98% probability). These results strongly indicate that DOSS is present in Finasol, so we are preparing to run a biodegradation experiment as we did with C9500 and ANS at the low concentration level. In Task 1.2 Ruta is almost done with the litaratue review on dilbit. She also completed the short tests with different fresh water cultures Edie provided, dilbit, and ANS. Unfortunately, we did't se alkanes and PAHS (GC/MSMS) nor TPH removal (UV-VIS) in any of the treatments. If possible, I'd like to meet with you at your early convenience to talk about how to proceed in theses projects specially if all of them will be considered level 1 QA.

Best regards,

Pablo Campo-Moreno, Ph.D.
Research Assistant Professor
University of Cincinnati
Department of Biomedical, Chemical and Environmental Engineering

766 ERC
Cincinnati, OH 45221-0012
PH:(513)-556-3637
Fax: (513)-556-4162
campomp@ucmail.uc.edu

It is a capital mistake to theorize before one has data. Insensibly one begins to twist facts to suit theories, instead of theories to suit facts.
The adventures for Sherlock Holmes

To: Baldwin, Maxanne[Baldwin.Maxanne@epa.gov]; Clark, Patrick[Clark.Patrick@epa.gov]
From: Conmy, Robyn
Sent: Mon 5/2/2016 3:32:45 PM
Subject: Fwd: Finasol OSR 52 Sample Request

Not sure how we can purchase this as they don't accept purchase cards? PR??

Sent from my iPhone

Begin forwarded message:

From: Malcolm Gore <malcolm.gore@clearcoastllc.com>
Date: May 2, 2016 at 11:19:03 AM EDT
To: "Conmy, Robyn" <Conmy.Robyn@epa.gov>
Subject: Re: Finasol OSR 52 Sample Request

Hello Robyn
This has been processed our side. We cannot accept credit cards and would ask for a check or direct transfer be made as per the invoice.
Please advise on payment timing
Thank you.
Malcolm
Malcolm Gore
President

+1 832 244 1533
malcolm.gore@clearcoastllc.com
www.clearcoastllc.com

From: "Conmy, Robyn" <Conmy.Robyn@epa.gov>
Date: Thursday, April 28, 2016 at 1:02 PM
To: malcolm gore <malcolm.gore@clearcoastllc.com>
Subject: RE: Finasol OSR 52 Sample Request

Yes we have received the invoice and I have provided our purchase card holder with the information. He will be contacting your office for payment.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Malcolm Gore [mailto:malcolm.gore@clearcoastllc.com]
Sent: Thursday, April 28, 2016 1:43 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: Re: Finasol OSR 52 Sample Request

Hi Robyn

I have given the go ahead on this. Please confirm receipt of the invoice sent yesterday.

Malcolm

Malcolm Gore

President

CLEAR COAST

+1 832 244 1533

malcolm.gore@clearcoastllc.com

www.clearcoastllc.com

From: "Conmy, Robyn" <Conmy.Robyn@epa.gov>
Date: Tuesday, April 26, 2016 at 11:05 AM
To: malcolm gore <malcolm.gore@clearcoastllc.com>
Subject: RE: Finasol OSR 52 Sample Request

Thanks for the update!

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Malcolm Gore [mailto:malcolm.gore@clearcoastllc.com]
Sent: Monday, April 25, 2016 5:45 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: Re: Finasol OSR 52 Sample Request

Apologies for the delay Robyn

Awaiting some shipping information. Should have this to you in the morning.

Malcolm

Malcolm Gore

President

CLEAR COAST

+1 832 244 1533

malcolm.gore@clearcoastllc.com

www.clearcoastllc.com

From: "Conmy, Robyn" <Conmy.Robyn@epa.gov>
Date: Monday, April 25, 2016 at 10:05 AM
To: malcolm gore <malcolm.gore@clearcoastllc.com>
Cc: Peter Egan <peter.egan@total.com>
Subject: RE: Finasol OSR 52 Sample Request

Hello Malcolm,

Thank you for getting back with me regarding the purchase. Yes, the information below is correct, with a mail stop at room 166. Can you send to me the invoice via email so I can provide to the purchase card holder to make the purchase?

Thank you in advance,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Malcolm Gore [mailto:malcolm.gore@clearcoastllc.com]
Sent: Monday, April 25, 2016 10:57 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Egan <peter.egan@total.com>
Subject: Finasol OSR 52 Sample Request

Hello Robin

I am writing with respect to your email below regarding a sample request of Total Finasol OSR 52.

Clear Coast is the custodian of the product stored here in Houston for Total.

I will arrange the sample as requested.

Please confirm the following:

Contact:

Robyn Conmy 513-569-7090 (office)

Deliver to:

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

Quantity:

2 US Gallons.

On receipt of confirmation I will get the samples drawn, packaged and dispatched.

Regards

Malcolm

Malcolm Gore

President

CLEAR COAST

+1 832 244 1533

malcolm.gore@clearcoastllc.com

www.clearcoastllc.com

From: Conmy, Robyn
Sent: Monday, April 18, 2016 10:47 AM
To: 'peter.egan@total.com' <peter.egan@total.com>
Cc: Wilson, Gregory <Wilson.Gregory@epa.gov>; Principe, Vanessa <Principe.Vanessa@epa.gov>
Subject: purchase of Finasol OSR52

Mr. Egan,

This follows up our telephone conversation of April 11.

As discussed, EPA is seeking to acquire a number of dispersant products listed on the NCP Subpart J Product Schedule for the purposes of both toxicity and efficacy testing. The product testing is intended to further inform currently proposed regulatory actions under Subpart J of the National Contingency Plan. The products will in addition be used to support our general research in the area of oil spill response.

Your product Finasol OSR52 has been identified as one of interest, as it is commonly stockpiled in the U.S. Not only could the product be encountered when presented with a response situation, but including this dispersant product in toxicity and efficacy testing studies will also allow for comparison and consistency with other existing and ongoing studies by EPA and other federal agencies. EPA's Office of Research and Development (ORD) will be conducting the dispersant studies.

At this time we are seeking 2 U.S. gallons of the product.

We appreciate your time and attention to this request.

Respectfully,

Robyn Conmy

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

To: Mike Fulton - NOAA Federal[mike.fulton@noaa.gov]
From: Conmy, Robyn
Sent: Mon 7/6/2015 7:03:13 PM
Subject: RE: following up

Ok – on my calendar. Just give me a call at the office number below.

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Mike Fulton - NOAA Federal [mailto:mike.fulton@noaa.gov]
Sent: Monday, July 06, 2015 2:38 PM
To: Conmy, Robyn
Subject: Re: following up

Thanks Robyn. How about tomorrow at 11:30?

Mike

On Mon, Jul 6, 2015 at 1:29 PM, Conmy, Robyn <Conmy.Robyn@epa.gov> wrote:

Hi Mike,

I am in this week but out next week. I have time on Tuesday between 11-2 ET and Wednesday anytime in the morning.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Mike Fulton - NOAA Federal [mailto:mike.fulton@noaa.gov]
Sent: Monday, July 06, 2015 1:24 PM

To: Conmy, Robyn
Subject: Re: following up

Hi Robyn,

I hope you're well and had a great holiday. Would you have time for a chat about shoreline cleaners sometime in the next several days? Thanks.

Mike

On Mon, Feb 9, 2015 at 2:48 PM, Conmy, Robyn <Conmy.Robyn@epa.gov> wrote:

OK. If you are running behind, then we can try for Wednesday…

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

From: Mike Fulton - NOAA Federal [mailto:mike.fulton@noaa.gov]
Sent: Monday, February 09, 2015 2:28 PM

To: Conmy, Robyn
Subject: Re: following up

Thanks Robyn. I have another meeting in the morning, but I'll try to call at 10:30.

Mike

On Mon, Feb 9, 2015 at 2:24 PM, Conmy, Robyn <Conmy.Robyn@epa.gov> wrote:

Hi Mike,

I am available tomorrow morning between 9:30 -11 am ET.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

From: Mike Fulton - NOAA Federal [mailto:mike.fulton@noaa.gov]
Sent: Monday, February 09, 2015 10:15 AM
To: Conmy, Robyn
Subject: Re: following up

Hi Robyn,

I hope you're well. Would you have time for a quick call sometime this week? I've got a couple of dispersant (finasol, corexit) questions.

Mike

On Tue, Sep 23, 2014 at 11:18 AM, Mike Fulton - NOAA Federal <mike.fulton@noaa.gov> wrote:

Thanks Robyn. I think we are about to receive the oil we requested from BP. Fingers crossed.

Mike

On Fri, Sep 19, 2014 at 10:13 AM, Conmy, Robyn <Conmy.Robyn@epa.gov> wrote:

Hi Mike,

I heard from EPA OEM that the oil purchase is approved but that it hasn't been ordered. That is the only information I have received. I did speak with Debbie Payton on Wednesday and she mentioned that her office has gotten some oil that may be available to you. I hope that is the case. There is some irony that we are all trying to conduct oil research that is critical for regulation, yet cannot access the oil. Sigh.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

To: BColon@versar.com[BColon@versar.com]
Cc: Schubauer-Berigan, Joseph[Schubauer-Berigan.Joseph@epa.gov]; Gilliland, Alice[Gilliland.Alice@epa.gov]
From: Conmy, Robyn
Sent: Thur 4/9/2015 1:53:49 PM
Subject: RE: BSEE Peer Review on the Testing of Four Dispersants in Simulated Arctic Conditions

Hello Ms. Colon,

I am happy to serve as a reviewer for the BSEE report.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

From: Gilliland, Alice
Sent: Thursday, April 09, 2015 8:17 AM
To: Conmy, Robyn
Cc: Schubauer-Berigan, Joseph
Subject: FW: BSEE Peer Review on the Testing of Four Dispersants in Simulated Arctic Conditions

Robyn,

Can you please reach out to Betzy Colon next week (after you return) if you have time to be a peer reviewer on this?

Thanks,

Alice

From: Colon, Betzy [mailto:BColon@versar.com]
Sent: Wednesday, April 08, 2015 3:32 PM
To: Gilliland, Alice
Subject: BSEE Peer Review on the Testing of Four Dispersants in Simulated Arctic Conditions

Hi Alice,

I tried leaving a message on your phone but not sure if I was successful. I may have hung up before the message was saved. The reason I contacted you is because we are conducting a peer review for the Bureau of Safety and Environmental Enforcement (BSEE) on dispersants and wanted to see if someone from your team might be interested in participating as a reviewer. I know Dr. Venosa used to conduct research in this area but not sure if someone from EPA has taken over this research after he retired.

I provided specific information below on the peer review. Feel free to forward to anyone on your team who may be able to participate in this peer review.

Thank you,

Betzy

Bethzaida Colon

Environmental Scientist

Environmental Services Group

VERSAR

Direct Line: (703) 642-6727

Mobile: (352) 514-5471

Fax: (703) 642-6809

Email: bcolon@versar.com

Visit us at: www.versar.com

>>>

My name is Betzy Colon and I work for an environmental consulting firm that is supporting the Department of the Interior (DOI) Bureau of Safety and Environmental Enforcement (BSEE) in conducting an external peer review of the draft document titled "Comparative Testing of Corexit EC9500A, Finasol OSR 52, Accell Clean DWD, and ZI 400 at Ohmsett in a Simulated Arctic Environment." The peer review will be a letter peer review with no teleconference or in-person meeting required. I am writing to see if you would be interested in participating in the peer review scheduled to begin in early May.

BACKGROUND:

BSEE has requested an external peer review of the draft report entitled "Comparative Testing of Corexit EC9500A, Finasol OSR 52, Accell Clean DWD, and ZI 400 at Ohmsett in a Simulated Arctic Environment," which was prepared by BSEE. Part of BSEE's research is committed to ensuring that functional, safe, and environmentally responsible oil spill response methods are identified and used under appropriate conditions. Understanding oil spill response technologies for use in the Arctic is crucial for the U.S. government and industry to develop robust spill response plans. In February of 2014, BSEE conducted independent dispersant effectiveness testing to compare available formulations. Several products were tested under mesoscale simulated arctic conditions at the Ohmsett facility. The study was conducted in order to better understand and compare the effectiveness of various dispersants under simulated Arctic test conditions. Four dispersants were selected from the EPA's NCP Product Schedule and tested on an Alaskan crude oil: Corexit® EC9500A, Finasol® OSR 52, Accell® Clean DWD, and ZI 400.

The objective of this letter-style peer review is for BSEE to receive written comments from individual experts on the scientific merit of the report, appropriateness of the methods used, quality of the data, and the overall strengths and limitations of the study.

LENGTH OF PAPERS:

-

The draft document to be reviewed contains approximately 25 pages of main text, including tables and figures, and 55 pages of references and appendices.

-

DUTIES OF REVIEWER:

-

Your primary function as a peer reviewer would be to evaluate and provide written comments on the document and answer seven charge questions.

We are identifying approximately six to seven scientific experts from which five will be selected to serve as peer reviewers. The reviewers will be senior scientists with expertise/experience in oil spill response in Arctic waters and a demonstrated understanding of the methods utilized to

understand the efficacy/effectiveness of chemical dispersant use.

TIMELINE:

We are expecting to select reviewers within the next few weeks in preparation to begin the review in early May. Reviewers will have approximately six weeks to complete their reviews and prepare written comments, following receipt of the materials and charge questions.

-

COMPENSATION:

An honorarium is being provided for the peer review and will be discussed if you are interested and available to participate in the review.

-

NEXT STEPS:

If you are interested in participating, please provide the following information:

1. An electronic copy of your CV.

2. Complete contact information (address, phone number, email).

3. Whether you will be entering this agreement as a consultant or a subcontractor through your company (subcontract - only applicable for those people that work for companies).

Once I've received the information requested above, I will send you our conflict of interest questions for you to answer and return via e-mail, along with forms requiring your signature. Before participating, you will need to confirm that there are no conflict of interest issues, either real or perceived.

We are hoping to make selections within the next few weeks and, as a result, would appreciate a prompt response from you.

Thanks, and I look forward to hearing from you.

Betzy

Bethzaida Colon

Environmental Scientist

Environmental Services Group

VERSAR

Direct Line: (703) 642-6727

Mobile: (352) 514-5471

Fax: (703) 642-6809

Email: bcolon@versar.com

Visit us at: www.versar.com

To: Zhang, Yu (zhang4y5)[zhang4y5@mail.uc.edu]; Holder, Edith[holder.edith@epa.gov]
Cc: Campo-Moreno, Pablo (campomp)[campomp@UCMAIL.UC.EDU]; Zhuang, Mobing (zhuangmg)[zhuangmg@mail.uc.edu]
From: Conmy, Robyn
Sent: Mon 6/8/2015 7:07:21 PM
Subject: RE: Result of Finasol Experiment

Thank you for sending and for the update!

Pablo, can you send to Edie and I the figures from the dilbit and ANS degradation results? We didn't walk away with our hard copies. Also, Ruta's SARA plots would be helpful too.

Thanks,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Zhang, Yu (zhang4y5) [mailto:zhang4y5@mail.uc.edu]
Sent: Monday, June 08, 2015 1:34 PM
To: Conmy, Robyn; Holder, Edith
Cc: Campo-Moreno, Pablo (campomp); Zhuang, Mobing (zhuangmg)
Subject: Result of Finasol Experiment

Hi! Robyn and Edith.

Please find the Finasol result in the attachment. Let me know if there is any problem with it. Thanks!

Regards,

Yu

To: Campo-Moreno, Pablo (campomp)[campomp@UCMAIL.UC.EDU]
From: Conmy, Robyn
Sent: Fri 1/9/2015 8:17:31 PM
Subject: RE: oil projects update

Thank you for sending. I am on travel a fair this month. Unfortunately, my open days are 16th, 20th, morning of 21st, and the week of the 26th.

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

From: Campo-Moreno, Pablo (campomp) [mailto:campomp@UCMAIL.UC.EDU]
Sent: Wednesday, January 07, 2015 12:52 PM
To: Conmy, Robyn
Cc: Raghu Venkatapathy
Subject: oil projects update

Dear Robyn,

Hope you had a good time during the holidays. Attached please find the updated QAPP for WA 4-05, task 2.3 (round robyn test for solidifiers). Section 4 has been modified to accommodate level 1 QA requirements for assessments. In WA 4-05, Task 1.1, I found an anionic dispersant in Finasol yielded an accurate mass of 422.2335 with an error of 0.87 ppm and a molecular formula C20H38O7S (98% probability). These results strongly indicate that DOSS is present in Finasol, so we are preparing to run a biodegradation experiment as we did

with C9500 and ANS at the low concentration level. In Task 1.2 Ruta is almost done with the litaratue review on dilbit. She also completed the short tests with different fresh water cultures Edie provided, dilbit, and ANS. Unfortunately, we did't se alkanes and PAHS (GC/MSMS) nor TPH removal (UV-VIS) in any of the treatments. If possible, I'd like to meet with you at your early convenience to talk about how to proceed in theses projects specially if all of them will be considered level 1 QA.

Best regards,

Pablo Campo-Moreno, Ph.D.
Research Assistant Professor
University of Cincinnati
Department of Biomedical, Chemical and Environmental Engineering
766 ERC
Cincinnati, OH 45221-0012
PH:(513)-556-3637
Fax: (513)-556-4162
campomp@ucmail.uc.edu

It is a capital mistake to theorize before one has data. Insensibly one begins to twist facts to suit theories, instead of theories to suit facts.
The adventures for Sherlock Holmes

To: Holder, Edith[holder.edith@epa.gov]
From: Conmy, Robyn
Sent: Mon 4/4/2016 6:34:31 PM
Subject: dispersants

I was looking for volumes of the 4 dispersants that we are using for the hypersaline DE experiments. I found Accell clean and Finasol but not the Corexit or Dispersit. Do you kow the volume we have? The tox testing will require 400 ml of each. Given the waiver issue with Corexit and Finasol, I'm not sure how we can get more since it is for tox testing.

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

To: Barron, Mace[Barron.Mace@epa.gov]
From: Conmy, Robyn
Sent: Fri 4/29/2016 11:46:05 AM
Subject: IOSC

Hey Mace,

Are you planning on attending the IOSC conference in May 2017? Short abstracts are due this May 20th. I am submitting one (can you serve as a STICS technical reviewer BTW?) and would encourage you to do the same on the oil tox program. We should have some great results by then! If you are interested, oils can cover most if not all of the travel (provided we have our 2017 budget by May next year).

In other news, we have procured Corexit and Finasol for the tox work!!!! They should be shipped to us next week. 2 gallons of each. I can't believe it. Still working on remaining 2 but this can get hydrosphere started.

R

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

To: CJ Beegle-Krause[CJ.Beegle-Krause@sintef.no]
From: Conmy, Robyn
Sent: Wed 12/21/2016 2:41:50 PM
Subject: RE: book chapter on dispersants in DWH
Book_Chapter_Perspectives_Manuscript_FINAL.docx

Hey CJ! I have a hard copy but not the final proof in electronic form. Attached is the final draft sent to editor that should be the exact technical content, but may have had some grammatical error edits. I am sending to you with the understanding that you will not circulate, as it isn't the galley proof. But for your purposes, and for citing, this should be sufficient.

And I will certainly accept the trade! ☺ Sounds like interesting work.

Happy Holidays,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: CJ Beegle-Krause [mailto:CJ.Beegle-Krause@sintef.no]
Sent: Wednesday, December 21, 2016 9:08 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: book chapter on dispersants in DWH

Hi Robyn,

Do you have a copy of the book chapter "Science-Based Decision Making on the Use of Dispersants in the Deepwater Horizon Oil Spill"? I am writing a paper for IOSC on the need for integrated ocean observing systems in the Gulf of Guinea, and would like to have a good reference for dispersant use issues during DWH...so I would like to read the chapter, as searching for a potential reference, this one popped up.

I can trade you my latest paper on deep ocean dissolved oxygen levels and oil development in Angola. Working on an MPB paper for a suite of example areas.

Best regards,

CJ

CJ Beegle-Krause, Ph.D.
Senior Researcher

SINTEF Materials and Chemistry
Monitoring and Modeling / Environmental Technology
Mobile: +47 93002631
www.sintef.no

Science-Based Decision Making on the Use of Dispersants in the Deepwater Horizon Oil Spill

[1]Albert D. Venosa, [2]Paul T. Anastas, [3]Mace G. Barron, [1]Robyn N. Conmy,

[4]Marc S. Greenberg, and [4]Gregory J. Wilson

U.S. Environmental Protection Agency
[1]Office of Research and Development
Cincinnati, OH 45268

[2]Yale University, Department of Chemistry
New Haven, CT 06520

[3]U.S. Environmental Protection Agency
Office of Research and Development
Gulf Breeze, FL

[4]U.S. Environmental Protection Agency
Office of Solid Waste and Emergency Response
Washington, D. C.

Key words: Deepwater Horizon, oil spill, dispersants, EPA, research

ED_001324_00001243-00001

Abstract

The oil spill from the Deepwater Horizon (DWH) drilling rig that occurred on April 20, 2010, tragically cost 11 lives and was the second largest environmental disaster involving crude oil ever recorded in history. The total volume of oil spilled was estimated by the Federal Interagency Solutions Group at 780 million L, second only to the intentional release in the Persian Gulf in 1991, which was estimated at 950 to 1,270 million L (http://www.incidentnews.gov/incident/6786). This chapter summarizes the authors' scientific perspectives in the aftermath of the DWH spill response. It is organized into seven sections, including an introduction, a brief history of dispersant use as a spill mitigation treatment, a summary of what is known about dispersion effectiveness from laboratory and wave tank research, a brief review of the toxicity of dispersants and dispersed oil, a review of monitoring technology developments in surface waters and in the deep sea, a discussion of fate and transport of the dispersed oil plume, and a summary of a comprehensive oil spill research strategy developed by EPA's Office of Research and Development (ORD) to fill knowledge gaps. This research strategy has been provided to the Interagency Coordinating Committee on Oil Pollution Research (ICCOPR) as an aid in stimulating collaboration and cross-disciplinary research among the 13 federal agencies charged with developing and conducting advanced oil spill response technologies for remediating future oil

ED_001324_00001243-00002

spills.

Introduction

The explosion of the Deepwater Horizon oil rig in the Gulf of Mexico on April 20, 2010 led to the tragic loss of 11 human lives. It also unleashed the largest oil-related environmental disaster in United States history and the second largest in recorded history.

A failed blowout preventer below the Deepwater Horizon drilling rig caused an unprecedented amount of oil and associated gas to discharge continuously over a period of three months. Spill response capabilities were tested to their limits by the continuous and rapid flow of crude oil from the sea floor. Responders turned to information from previous incidents and all available scientific literature to help formulate effective response approaches and deal with unprecedented circumstances.

Lessons learned from the 1989 Exxon Valdez incident in Alaska and other major oil spills have taught us that conventional mitigation techniques such as booming, skimming, and mechanical recovery cannot be fully relied upon to prevent oil from reaching shorelines and damaging sensitive ecosystems during a large-scale spill. They also offer insights into the conditions and circumstances in which dispersant application may be more or less effective. Early in the Exxon

ED_001324_00001243-00003

Valdez spill, for example, alternative response techniques including the use of dispersants and *in-situ* burning were attempted to minimize spread of the surface slick. Four attempts to apply dispersants over the course of four days were deemed ineffective due to circumstantial and operational challenges such as equipment failure and lack of sufficient wave action at sea to adequately mix the dispersant with the oil. As a result, dispersant application was discontinued (1).

While all oil spill cleanup techniques have associated environmental tradeoffs, those related to the decision to use dispersants may be especially complex. Unlike mechanical recovery, which physically removes oil from the environment, dispersant application drives oil from the surface into the water column. This keeps oil away from shore and transfers exposure risk from water fowl and shoreline species to planktonic and pelagic species. But at the same time, this technique delays eventual removal of oil fractions that are amenable to natural biodegradation processes. In 2005, the U.S. National Research Council acknowledged that, indeed, "one of the most difficult decisions that oil spill responders and natural resources managers face during a spill is evaluating the environmental trade-offs associated with dispersant use" (1).

The depth at sea and continuous flow of oil from the Deepwater Horizon (DWH) well also posed entirely new challenges for oil spill responders and

ED_001324_00001243-00004

decision-makers. The point-source of discharge was situated approximately 1,500m below the water surface and oil rising to the surface continued to spread over an ever-increasing area. Subsea dispersant application was raised as a potential response option to overcome these unprecedented circumstances (2). There are several practical advantages associated with the application of dispersant directly at the point-source of discharge. First, dispersant can be applied directly to fresh oil, before lighter oil fractions are lost and before oil has time to significantly weather and emulsify with water as a result of naturally present surface active agents. Second, direct injection at the point-source increases the probability that applied dispersant will encounter all or most of the released oil. This significantly increases efficiency of treatment and decreases the amount of chemical added to the environment. In contrast, a slick on the water surface may be fragmented and stringy, resulting in wasted chemicals in unnecessary quantities when applied from overhead.

Prior to the DWH incident, most (if not all) existing oil spill response knowledge was based on surface spills and surface applications of dispersant. The behavior of dispersants subsea was (and still is) less understood and previous research had not focused on the duration or quantity of dispersant used during the DWH incident. Recognizing the unique nature of this challenge and response, the U.S. Environmental Protection Agency and the U.S. Coast Guard issued a

ED_001324_00001243-00005

Directive requiring BP to implement a monitoring and assessment plan for both subsurface and surface applications of dispersants as part of the BP oil spill response. The goal of the monitoring program was to evaluate daily the effects of dispersant application at the source of discharge. This required an understanding of the fate and transport of dispersed oil. As a result, monitoring efforts were extensive and multifaceted. Coordinated efforts included dissolved oxygen monitoring to ensure hypoxia was not taking place, fluorometric measurements to track the oil plume, Laser *In-Situ* Scattering and Transmissometry (LISST) sensor measurements to assess particle size distribution, oil chemistry analysis of collected samples, monitoring of currents and advective movement of the plume, conductivity/temperature/depth (CTD) profile data collection, and Rototox analyses to estimate acute toxicity from the dispersed plume.

Several government agencies and stakeholders were involved in this monitoring effort, including the National Oceanic and Atmospheric Administration (NOAA), the U.S. Geological Survey (USGS), the Department of Fisheries and Oceans Canada (DFO), academic researchers funded by the National Science Foundation (NSF), BP response contractors, and the states bordering the Gulf of Mexico. As a result of this collaborative effort, the response community has learned a great deal about subsea dispersion, the behavior of dispersed oil plumes as they advect, how best to monitor oil plumes, and the acute

ED_001324_00001243-00006

toxicity of certain dispersants. But only those questions immediately relevant to the spill response were able to be addressed in the midst of the crisis and its immediate aftermath. Many more important scientific questions remain. This paper provides the authors' perspective on deep sea dispersant injection and identifies what research must be undertaken to answer the questions raised by the DWH oil spill tragedy.

Brief History and Evolution of Dispersants for Oil Spill Mitigation

According to Etkin (3), 367 of the 408 oil spills that occurred globally in the period 1966-69 were treated with dispersants. The first recorded use was on an off-shore tanker spill in Germany in 1966 (4). Of those, the most well-known major spill where dispersants were used for treatment occurred in 1967 during the Torrey Canyon incident, which spilled approximately 1 million barrels (bbl) of crude oil (144 million L) off the western coast of Cornwall, England. Over 10,000 bbl (1.6 million L) of surfactants (alkylphenols) and solvents (aromatic hydrocarbons) were sprayed onto the slick for 14 days following the release. This resulted in extreme toxicity to marine life due to the toxic nature of the chemicals comprising the dispersant. The toxicity of the dispersant was deemed mostly due to the toxicity of the solvents in the mixture, not the surfactants (5). However, alkylphenols today are known to be endocrine disruptors, which exert their effects

ED_001324_00001243-00007

chronically rather than acutely and at low concentrations. The Torrey Canyon incident spurred European, American, and Canadian governments to sponsor research programs to develop more effective and less ecologically harmful dispersants and application equipment.

One infamous spill incident where dispersants were used for mitigation was the Ixtoc well blowout off the Gulf of Mexico in 1979, where over 530 million L of crude oil spewed into the sea over a period of 10 months [for perspective, the DWH spill reportedly spewed 780 million L into the Gulf over an 87-day period (6)]. Dispersant use by Mexican officials was reported to be successful, but dispersants were not used in U.S. waters because the oil was too weathered to expect success (3). The second generation dispersants produced in the 1970s were less toxic but also considerably less effective. One of the first of the third generation of dispersants that emerged in the 1980s was Corexit 9527, a product then manufactured by Exxon. Its toxicity was substantially reduced while its efficacy in dispersing oil into the water column was significantly greater. This was followed by Corexit 9500 in the mid-1990s, and this dispersant is still the predominant one in supply in much of the U.S., being the one most used in the DWH spill. Other dispersants were produced in the 1990's and 2000's, leading to 15 products currently listed on the National Contingency Plan Product Schedule (NCPPS)

ED_001324_00001243-00008

(http://www.epa.gov/emergencies/content/ncp/tox_tables.htm#dispersants).

Dispersant Efficacy and Dispersion Effectiveness

The term "efficacy" is generally defined as the capacity to produce a specific effect under highly controlled conditions. "Effectiveness" describes an effect produced under non-controlled conditions. In the latter sense, the researcher cannot create a control to use as a comparative baseline. So, when we describe how well a product disperses oil, we use efficacy to describe oil dispersion in a controlled laboratory setting such as a flask containing oil, water, and a dispersant, compared to a control flask containing oil and water but without dispersant. In the field, there can be no control. We describe how effective a chemical disperses oil in the field by taking measurements that may indirectly indicate effectiveness such as particle size distribution, buoyancy, analytical measurements, and fluorescence, and then comparing these data to measurements from historical, controlled laboratory experiments.

To be considered for use in the United States as an efficacious oil spill mitigation agent, a dispersant product must be listed on the NCPPS. Listing on the NCPPS is obtained by passing test protocols identified by EPA: the manufacturer must provide data on the Swirling Flask Test (SFT), and standard aquatic toxicity assays. These tests are described in Appendix C, Subpart J of 40

ED_001324_00001243-00009

CFR part 300, series 900 (6). Listing of a dispersant on the product schedule has been contingent on the dispersant being at least 45% effective in dispersing Prudhoe Bay and South Louisiana crude oils in the SFT laboratory test.

After promulgation of the SFT in 1994, unexpectedly large discrepancies were discovered between the data submitted by product vendors and data generated by EPA contractors (7). EPA's Office of Research and Development (ORD) conducted an in-depth laboratory study to determine the cause of this anomaly. Results indicated that a baffled flask provided substantially superior results both in terms of the degree of dispersion and variability among replicates, mainly due to the over-and-under type of mixing induced by the flask's baffles. Hence, the Baffled Flask Test (BFT) was concluded to be the superior protocol for testing and listing dispersant products on the NCPPS (8, 9). Soon after, ORD began to work with EPA's Office of Emergency Management to develop a proposed rule that incorporated changes to the existing protocol.

A follow-on study was conducted to develop a pass-fail decision rule. The approach is based on calculating the lower 95% confidence limit from the mean of all replicate measurements that must be greater than a set efficacy level. The higher the variance, the lower is the probability of passing the criterion. This approach ensures that only the most effective dispersants will be selected for

ED_001324_00001243-00010

inclusion on the product schedule and that a high degree of confidence will accrue in making these decisions. When all the results were in, a more stringent threshold was proposed (10), although ongoing research may suggest that the ultimate threshold may be slightly changed to account for physically dispersed oil.

Although other laboratory testing procedures are available in the literature, the BFT is the only one that has undergone quantitative mixing energy calibration. A hot wire anemometer was used to quantify and compare the turbulence in the baffled flask (BF) and the swirling flask (SF). The velocity gradient and energy dissipation rate were computed based on these measurements. Mixing in the BF was found to be much more uniformly distributed than in the SF. The BF average energy dissipation rate was about two orders of magnitude higher than in the SF. Also, in the BF, the size of the microscales approached the size of oil droplets observed at sea, which means that the turbulence in the BF closely resembles the turbulence occurring at sea during breaking waves (i.e., a moderately energetic sea state). Hence, the BF was deemed preferable for dispersant efficacy testing in the laboratory (11).

In an effort to better understand the effectiveness of the eight dispersants on the NCCPS that at the time were readily available in the U.S. market, EPA conducted efficacy tests in a laboratory using the BFT in a controlled situation

ED_001324_00001243-00011

using reference S. Louisiana Crude (SLC) oil. Testing was conducted at the two temperatures in the Gulf: 5 °C to represent temperature conditions for the deep sea dispersant injection and 25 °C to represent the temperature in the top 5 m where surface application was performed. The BFT was modified to test performance only on SLC oil at the two temperatures and to expand the number of replicates from 4 to 6 to increase the statistical power. This modification was designed because only one oil was being tested rather than two. Based on the laboratory findings presented above, it appears that only 3 of the 8 dispersants would have provided satisfactory SLC dispersion effectiveness (at a volumetric DOR of 1:25) (12). Corexit 9500 was one of the three dispersants giving satisfactory results. This conclusion assumes that the BFT is moderately predictive of field results, which may not be an accurate assumption since other factors (e.g., advection, dilution, and current flow) not testable in a closed flask may play a major role in determining performance in the field.

Dispersion effectiveness is ultimately determined by the dispersed oil droplet size distribution (4, 13). Droplets < 70 μm in diameter have small rise velocities and tend to remain suspended in the water column. They also become widely dispersed in the water column by turbulent diffusion and are more rapidly biodegraded due to the high surface area to volume ratio (14). In addition, the oil droplets get surrounded by surfactant molecules, forming

ED_001324_00001243-00012

swollen micelles or microemulsions, which tend to repel each other, further reducing the probability of recoalescence. Conversely, large oil droplets with diameters in the hundreds of μm tend to recoalesce and resurface unless strong mixing energy exists to overcome their buoyancy. Therefore, smaller droplets are much more favorable from the perspective of oil spill mitigation. Laboratory and field measurements suggest that for effective dispersion of oil in which the dispersed oil droplets remain suspended in the water column, average droplet sizes have to be less than 50–70 μm (15, 16).

Unpublished LISST measurements of the dispersed oil plume in the deep sea during the DWH spill at 1,100 to 1,300 m below the surface showed that most of the plume consisted of particle sizes ranging from 2.5 to 70 μm in diameter. Furthermore, light scattering measurements of water samples collected from that plume displayed the characteristic bimodal distribution typical of chemically- as opposed to physically-dispersed oil (17, 18). This suggests, but does not scientifically prove, that the oil in the deep zone was likely chemically dispersed due to the injection of dispersant into the oil exiting from the riser tube. Although it is plausible that the extreme turbulence of the oil as it exited the well may have caused extensive physical dispersion without the need for chemical dispersant use, review of reported data and information in the literature leads us to determine that it is less likely and that the application of dispersants in the deep sea was

ED_001324_00001243-00013

successful in dispersing the oil at the source.

Toxicity of Dispersants

Laboratory Testing. EPA performed two phases of laboratory dispersant toxicity testing during the DWH spill to supplement existing data available on the NCP product schedule. Phase 1 involved testing of eight dispersants using both standard toxicity bioassays with a fish and an aquatic invertebrate, as well as *in-vitro* mammalian cell line assays. Phase 2 of EPA's testing determined the acute toxicity of the 8 dispersants mixed with SLC to 2 Gulf of Mexico estuarine species. *In-vitro* testing was focused on determining if any of the eight commercial dispersants had estrogenic or androgenic activity, as well as any activity in other biological pathways using a large battery of cell line assays. Overall, no activity was observed in any androgenic assay, 2 dispersants showed a weak estrogenic signal in 1 assay, and all dispersants showed minimal cytotoxicity (19).

The ecotoxicity testing approach used consistent test methodologies within a single laboratory in assessing the relative acute toxicity of the eight dispersants, including Corexit 9500A (identical to Corexit 9500), the predominant dispersant applied during the DWH spill. Static acute toxicity tests were

ED_001324_00001243-00014

performed using the mysid shrimp (*Americamysis bahia*) and the inland silversides (*Menidia beryllina*). For all eight dispersants in both test species, the dispersants alone were less toxic (LC50s: 3 to > 5,600 ppm) than the dispersant-SLC mixtures (0.4 to 13 mg TPH/L). SLC alone was in general similarly toxic to mysids (LC50: 2.7 mg/L) and *Menidia* (LC50: 3.5 mg/L) as the dispersant-SLC mixtures. The results were consistent with data available on the NCPPS and indicated that the toxicity of Corexit 9500A was generally similar to other available dispersants when tested alone and as a mixture with SLC (20). The results of EPA's dispersant toxicity testing were made publicly available on the EPA Internet site (http://www.epa.gov/bpspill) and facilitated the EPA Administrator's advice and support to the Incident Commander of the decision regarding dispersant use during the DWH spill response.

In-Field Monitoring. EPA conducted extensive monitoring of the nearshore environment and communities during the emergency response to the DWH spill that included sampling and testing of marine surface water and sediments. Because EPA recognized early that dispersant usage was an environmental trade-off not to be taken lightly, this monitoring was important because it provided relevant data for detecting any measurable environmental impacts from this usage. Markers of dispersants such as dipropylene glycol butyl ether (DPBE) and dioctylsulfosuccinate (sodium salt) (DOSS) were included in

ED_001324_00001243-00015

the chemical analysis, and few detections of these compounds were found in water and sediment samples. All such detections were below aquatic life benchmarks for water that were based on biological effects data (http://www.epa.gov/bpspill; OSAT, 2010). Toxicity testing associated with samples collected in Alabama, Mississippi, and Florida using estuarine and marine organisms [mysid shrimp (*Americamysis bahia*); inland silversides (*Menidia beryllina*); sea urchin (*Arbacia punctulata*); polychaete worms (*Neanthes arenaceodentata*); and amphipods (*Leptocheirus plumulosus*)] resulted in observed toxicity in 1 surface water and 3 sediment samples. However, these findings could not be attributed to the DWH spill because, among many factors, the analytical results did not exceed ecological screening values, the water samples were negative for petroleum and dispersant analytes, and the observed sediment toxicity was inconsistent across test species and likely due to effects from grain size (http://www.ils-inc.com/news/announcements/ils-to-assess-bp-oil-spill-disaster) .

In addition, as a part of the directive issued by the USCG and EPA to BP to implement monitoring and assessment of the subsurface and surface applications of dispersants, daily 24-h acute toxicity testing of rotifers was conducted. Rotifers were exposed to offshore water samples collected near the wellhead and response operations. A critical variable for monitoring the long-term

ED_001324_00001243-00016

effects of the spill was daily measurements of dissolved oxygen, which would indicate consumption of oil by microbial activity. If DO approached 2 mg/L, which was considered hypoxic in the deep sea, considerations would be made by the Unified Command to suspend dispersant injection at the wellhead. Fortunately, this never occurred. The results from over 1,000 sample test results conducted on samples taken from May 29-August 26, 2010 indicated limited observations of toxicity. Approximately 89% of the samples were not toxic (survival > 90%), 10% were marginally toxic (survival range < 90% to ≥ 75%), and 1% showed survival < 75 %. Collectively, these monitoring results were helpful for daily decisions on the use of dispersants during the spill, including the reduction in dispersant application rate as the response unfolded.

Monitoring of Dispersants on the Surface and in the Deep Sea

Monitoring in surface waters. Dispersion effectiveness during an oil spill is most commonly monitored using guidance set forth by the non-regulatory SMART (Special Monitoring of Applied Response Technologies) protocols (21, 22). The protocols establish a monitoring system for rapid collection of real-time, scientifically-based information to assist decision-making during dispersant and *in-situ* burning operations. Its primary focus determines if dispersant application is relatively effective or ineffective, where degree of effectiveness is a secondary

ED_001324_00001243-00017

focus but is often difficult to address quantitatively in the field. The ultimate objective of SMART is to determine whether or not further chemical dispersion should be carried out during an oil spill.

To meet this objective, relative effectiveness (RE) must be defined. According to the SMART protocol, RE describes the amount of oil that the dispersant puts into the water column relative to the amount of oil that remains in the surface slick. A dispersant is considered "relatively effective" if half the oil is dispersed. Extensive laboratory tests have been conducted to determine the extent of chemical dispersion on various oils, but these studies do not represent real world conditions for reasons already mentioned. An inherent problem occurs when attempting to calculate this in the field, as plumes and slicks are heterogeneous and patchy, and no reliable way exists to accurately measure thickness of either. An added complication is apparent when oil is not from a spill of known volume but from a leak and/or continuous spewing source, making a mass balance of oil challenging (23). Environmental factors such as conductivity or salinity, water temperature, pressure, sea state, and weathering also influence extent of both physical and chemical dispersion. The extent of these influences is poorly understood in real world situations.

SMART lays out a tiered, structured approach, where Tier I is reliant on

ED_001324_00001243-00018

visual criteria from aircraft or ships to confirm the formation of dispersed oil (generally as a yellow or coffee-colored plume below the water's surface). This is followed by Tier II, which calls for real-time fluorometric monitoring of the underwater plume and discrete sample analysis of oil concentrations at 1-m depth. Tier III expands on this and uses additional fluorometers to monitor multiple depths between 1 and 10 m and collection of ancillary environmental data on water temperature, conductivity or salinity, pH, dissolved oxygen, and turbidity. These monitoring techniques are sufficient for the majority of spills although false positives and negatives may occur, affecting confirmation of dispersion effectiveness (24). Since the establishment of SMART, reviews have highlighted the limitations of the methods (25) and subsequent updates to the protocols in 2006 and 2008.

Monitoring in the deep sea. In the case of the DWH oil spill, the existing SMART protocols, as written, provided insufficient guidance for examining dispersant effectiveness. This was due to the unprecedented nature of the spill, given the extreme depth of the wellhead leak within the ocean. This posed unique challenges to the monitoring efforts, where oil that remained in the subsurface plume (between 1,000-1,500 m) could not be tracked via common methods such as aerial surveys or shallow-towed fluorometers. Tracking during the DWH emergency response effort, therefore, required a modified monitoring approach,

ED_001324_00001243-00019

using vertical profiling packages to extend fluorometric measurements and water sample collection to the deep ocean floor. Sampling was conducted in a radial pattern to initially confirm the existence and location of a subsurface plume resulting from dispersant application at depth. Added to the measurement suite was a LISST sensor to measure suspended particle or droplet size to indicate degree of dispersion (26). Since the LISST cannot differentiate between chemical and physical dispersion, multi-wavelength fluorescence analyses of discrete samples to calculate Fluorescence Intensity Ratios (FIR) were used (27). Discrete and *in-situ* fluorescence analyses both revealed the presence of what was termed the "fluorescence anomaly" due to elevated values above background natural organic matter in the deep ocean. Although there has been much debate over which fluorometer is best-equipped (wavelengths, sensitivity, calibration) to measure dispersed oil, all sensors used during the response efforts were capable of measuring some portion of the oil. Validation of any anomalies was confirmed with chemical analyses (PAHs, TPH, BTEX) during the response effort.

The DWH oil spill possessed both surface and subsurface plume components (Figure 1). The response efforts at the surface were informed by the SMART monitoring program, along with hydrodynamic ocean circulation models and innovative remote sensing techniques (28). At depth, the response effort used various indicators to detect and track the subsurface plume, where assessment was

ED_001324_00001243-00020

conducted by a collaborative team of scientists from federal, academic, and industrial organizations who were tasked with providing rapid response analysis of data (Joint Analysis Group membership at http://ecowatch.ncddc.noaa.gov/jag). The multi-pronged approach required by the unique demands of the spill call to light the need for further research in monitoring technologies and the need for updating the living guidance documents that make up SMART.

ED_001324_00001243-00021

Fate and Transport of Dispersants and Dispersed Oil

Understanding the transport and subsequent fate of oil and dispersed oil is important in aiding the decision-making process for response to an oil spill of any magnitude. These processes have a direct influence on the concentration and, therefore, the effects exerted on the ecosystem. The whole strategy behind use of dispersants is predicated on creating tiny droplets of oil, driving them into the water column where exposure of water fowl to the toxic and smothering slick is limited, and rendering the droplets amenable to more rapid biodegradation due to the high surface area imparted by the dispersant treatment. Use of chemical dispersants also changes the behavioral variation of entrained oil droplets compared to natural dispersion. As pointed out by NRC (1), movement of oil in the environment involves surface transport (mostly spreading, which determines the shape, thickness, and location of the slick, all of which affect decisions on using dispersants), vertical transport into the water column (which is responsible for the initial dilution after dispersant application), and horizontal transport or advection (which is responsible for the ultimate dilution of the oil mitigating the effect on fish and enhancing its biodegradation).

EPA in concert with DFO-Canada has conducted substantial research in the recent past on the importance of mixing energy in achieving the small particle size distribution needed for permanent dispersion in the bulk water column (29,

ED_001324_00001243-00022

30). This work was conducted at the wave tank co-owned by EPA and DFO and located at DFO's Bedford Institute of Oceanography in Dartmouth, Nova Scotia (30). In this study, the wave tank was operated in flow-through mode to simulate advective dilution of the dispersed oil plume in the sea. This led to the development of the term "dynamic dispersion effectiveness" (DDE), which reflects both dispersion of oil into the water column and transport and dilution of the dispersed oil droplets through the water column. DDE differs from the dispersant efficacy (DE) obtained in bench-scale flask tests, where only the contact efficiency between oil and dispersant is measured in small enclosed surroundings during which unlimited collision frequency between oil droplets and eddies may occur. The flow pattern was clearly different when the wave tank was operated in flow-through mode compared to batch mode. In particular, the backflow near the bottom of the wave tank during batch operation was overcome by the forward current in the flow-through system, which purged the smaller dispersed oil droplets that were suspended in the water column out of the wave tank. This reduced the inter-drop collision frequency that would cause recoalescence and resurfacing of the smaller dispersed oil droplets, while retaining the larger oil droplets floating at the surface to maintain high droplet-eddy collision frequency for the breakage of droplets into small particles (31).

Figure 2 summarizes the effect of dispersant type and wave energy on the

ED_001324_00001243-00023

average dispersed oil droplet volume mean diameter (VMD) for Alaska North Slope crude oil. In the absence of chemical dispersant (Figure 2A), the oil droplet sizes remained large and highly variable (VMD ~ 150–400 μm) under the coherent, non-breaking wave condition but were rapidly reduced in size and variability (VMD ~ 150–200 μm) under breaking wave conditions. In the presence of the chemical dispersant Corexit 9500 (Figure 2B), the dispersed oil droplet sizes remained large but considerably reduced in variability (VMD ~ 300 μm) under coherent wave conditions. These sizes were dramatically reduced (VMD ~ 50 μm) under breaking wave conditions within 10 min and maintained at this small size for the rest of the experiment (30). In the presence of Dispersit SPC1000 (Figure 2C), the droplet sizes of the dispersed oil achieved the same low VMD even under non-breaking waves, although they began recoalescing after 20-30 min, while the chemically dispersed oil droplets remained stable. Once the small particle size is achieved, especially in the deep sea as observed in the Gulf oil spill, the droplets are in effect neutrally buoyant, do not recoalesce, and remain at depth as advection continues to dilute the plume and biodegradation ensues.

Future Oil Spill Research as a Result of Lessons Learned

It is critical that EPA strengthen its knowledge base and expertise in oil spill response and prevention. As with all environmental protection issues, Agency

ED_001324_00001243-00024

decisions related to oil spill prevention, preparedness, and response must be informed by sound science and research of the highest caliber. The Agency's Office of Research and Development (ORD) has developed a comprehensive oil spill research strategy to fill knowledge gaps, focusing on potential human and environmental risks from oil spills and the application of dispersants, surface washing agents, bioremediation agents, and other mitigation measures. The goal of this strategy is to identify research that would provide environmental managers with the tools, models, and methods needed to mitigate the effects of oil spills in all environments, emphasizing the coastal and inland environments. The research needs identified will fill knowledge gaps in four areas to:

- Develop a better understanding of the impacts of oil spills and dispersant application on the environment.
- Develop a better understanding of the shoreline, coastal, and inland environmental impacts of oil spills, including non-petroleum oils.
- Develop innovative technologies to mitigate the impact of oil spills.
- Address the technical needs of the communities impacted by the DWH oil spill.

Such research would provide a greater understanding of the short- and long-term impacts on the environment and human health associated with the DWH oil spill. Research is proposed to develop innovative technologies to increase the use

ED_001324_00001243-00025

of green or more benign approaches to mitigate surface and subsurface oil spills and restore environments impacted by oil spills. The strategy informs not only specific decisional endpoints necessary to address the effects and impacts of oil spills, including the DWH oil spill, but also identifies research to inform future scenarios involving oil spills that would potentially impact areas vital to a community's well-being. This includes all environments that are critical to a region's economy, commerce, personal livelihood, ecological sustainability, and the overall welfare of an area's inhabitants.

The research outlined in the strategy can inform agencies and academia of the knowledge gaps and needs associated with oil spill remediation and should take on the same integrated transdisciplinary approach as all of EPA's research programs by engaging scientists across a spectrum of disciplines, including engineering, health sciences, ecology and ecotoxicity, chemistry, microbiology, and more. The strategy calls for close interaction between scientists across agencies, coordinated leveraging of resources and ensuring no duplication of efforts.

Summary

To effectively pursue its mission of protecting human health and the environment, EPA must base all of its decisions and actions on sound science. At the same time, during an environmental disaster, the ability to rapidly respond and

ED_001324_00001243-00026

make decisions is critical. Given the unprecedented nature of the DWH oil spill, the magnitude and ongoing nature of the event, and the continuous and rapid flow of crude oil from the sea floor, the coordinated Federal DWH oil spill response faced significant challenges. Seeking to continuously base all decisions on sound science, responders and decision-makers turned to scientists for guidance on the use of dispersants and the interpretation of field monitoring information. Scientific experts provided input based on years of research and experience and also identified needs, gaps, and outstanding questions. Many lessons were learned through this event. These lessons have been thoroughly captured, with steps toward improvement already under way.

EPA has developed a comprehensive oil spill research strategy to highlight knowledge gaps and research needs. The goal of the strategy is to encourage relevant research in advance of such events to improve the response by providing credible, applicable information to responders and decisions makers. This information would help inform effective response approaches both in the planning and operational stages, improve the overall body of scientific knowledge, and inform new and innovative approaches to environmental protection and disaster response.

References

1. NRC, *Oil spill dispersants: efficacy and effects*. National Academies

ED_001324_00001243-00027

Press, Washington, D.C., 2005.

2. E. Kintisch, "An Audacious Decision in Crisis Gets Cautious Praise," *Science* **329,** 735-736 (2010).

3. D.S. Etkin, "Factors in the dispersant use decision-making process: historical overview and look to the future," *Proc 21st Arctic and Marine Oilspill Program Technical Seminar*, Environment Canada, Ottawa, ON pp. 281-304, (1998).

4. A. Lewis, D.C Byford, and P.R. Laskey, "The significance of dispersed oil droplet size in determining dispersant effectiveness under various conditions," *Proc. International Oil Spill Conference*, American Petroleum Institute, Washington, D.C., (1985)

5. NRC, *Oil in the sea: inputs, fates, and effects*. National Academies Press, Washington, DC, USA, 601 pp., 1985.

6. U.S. Environmental Protection Agency, "Swirling Flask Dispersant Effectiveness Test, Revised Standard Dispersant Toxicity Test, and Bioremediation Agent Effectiveness Test," *Appendix C, Part 300, Series 900, 40 CFR Ch. I*, pp. 224-246, (2003).

7. J.R. Clayton, J.R. Payne, J.S. Farlow, and C. Sarwar, *Oil Spill Dispersants*

ED_001324_00001243-00028

Mechanisms of Action and Laboratory Tests, CRC press, Boca Raton, Florida, 103 pp., 1993.

8. G.A. Sorial, A.D. Venosa, K.M. Koran, E. Holder, and D.W. King, "Oil Spill Dispersant Effectiveness Protocol. Part I: Impact of Operational Variables," *J. Env. Eng. ASCE,* **130**(10), 1073-1084, (2004a).

9. G.A. Sorial, A.D. Venosa, K.M. Miller, E. Holder, and D.W. King, "Oil Spill Dispersant Effectiveness Protocol - Part II. Performance of the Revised Protocol," *ASCE J. Env. Eng.* **130**(10), 1085-1093, (2004b).

10. A.D. Venosa, D.W. King, and G.A. Sorial, "The baffled flask test for dispersant effectiveness: a round robin evaluation of reproducibility and repeatability," *Spill Sci. & Technol. Bul.* **7**(5-6), 299-308, (2002).

11. V.J. Kaku, M.C. Boufadel, and A.D. Venosa, "Evaluation of Mixing Energy in Laboratory Flasks Used for Dispersant Effectiveness Testing," *ASCE J. Env. Eng.* **132**(1), 93-101, (2006).

12. A.D. Venosa, and E.L. Holder, "Laboratory-Scale Testing of Dispersant Effectiveness of 23 Oils Using the Baffled Flask Test," Marine Poll. Bulletin, *in press, (2012).*

13. P.S. Darling, D. Mackay, N. Mackay, P.J. Brandvik, "Droplet Size

ED_001324_00001243-00029

Distributions in Chemical Dispersion of Oil Spills: Toward a Mathematical Model," *Oil Chem. Poll.* **7**(3):173–198, (1990).

14. M. Li and C. Garrett, "The Relationship Between Oil Droplet Size and Upper Ocean Turbulence," *Mar. Poll. Bul.* **36**, 961-970, (1998).

15. T. Lunel, "Dispersion: Oil Droplet Size Measurement at Sea, *Proc Sixteeth Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*, Environment Canada, Ottawa, ON, p. 1023-1056, (1993).

16. T. Lunel, in *The Use of Chemicals in Oil Spill Response*, P. Lane (ed.), American Society for Testing and Materials, p. 240, 1995.

17. Z. Li, K. Lee K., T. King, M.C. Boufadel, and A.D. Venosa, "Assessment of Chemical Dispersant Effectiveness in a Wave Tank Under Regular Nonbreaking and Breaking Wave Conditions," *Mar. Poll. Bul.* **56**(5), 903–912, (2008).

18. Z. Li, K. Lee K., T. King, M.C. Boufadel, and A.D. Venosa, "Evaluating Chemical Dispersant Efficacy in an Experimental Wave Tank: 2, Significant Factors Determining In-Situ Oil Droplet Size Distribution," *Env. Eng. Sci.*, **26**(9), 1407-1418, (2009).

19. R.S. Judson, M.T. Martin, D.M. Reif, K.A. Houck, T.B. Knudsen, D.M.

ED_001324_00001243-00030

Rotroff, M. Xia, S. Sakamuru, R. Huang, P. Shinn, C.P. Austin, R.J. Kavlock, D.J. Dix,. "Analysis of Eight Oil Spill Dispersants Using Rapid, In Vitro Tests for Endocrine and Other Biological Activity." *Env. Sci. Technol.* **44,** 5971-5978, (2010).

20. Hemmer, M., M.G. Barron, and R. Greene, "Comparative Toxicity of Eight Oil Dispersants, Louisiana Sweet Crude Oil (LSC) and Chemically Dispersed LSC to Two Aquatic Test Species," *Env. Toxicol. Chem.* **30**(10), 2244-2252, (2011).

21. Special Monitoring of Applied Response Technologies, U.S. Coast Guard, National Oceanic and Atmospheric Administration, U.S. Environmental Protection Agency, Centers for Disease Control and Prevention, and Minerals Management Service, available on-line at http://docs.lib.noaa.gov/noaa_documents/648_SMART.pdf (2003).

22. N. Barnea and R. Laferrier, 'Smart Scientific Monitoring of Advanced Response Technologies," in *Proceedings of the Sixteenth International Oil Spill Conference*, American Petroleum Institute, Washington, D.C., 1265-1268, (1999).

23. M.F. Fingas, in *Surfactants: Fundamentals and Applications to the Petroleum Industry*, L. L. Schramm (ed.),Cambridge University Press, pp.

ED_001324_00001243-00031

461-539, 2000.

24. M.F. Fingas, "Review of monitoring protocols for dispersant effectiveness," In *Report for Prince William Sound Regional Citizens' Advisory Council*. Anchorage, AL, 33 pp., 2003.

25. R.H. Goodman, "Is SMART really that smart?" *Proc. of the Twenty-Sixth Arctic and Marine Oilspill Program Technical Seminar*, Environment Canada, Ottawa, ON, pp. 779-786, 2003.

26. Z. Li, et al. "Effects of Chemical Dispersants and Mineral Fines on Crude Oil Dispersion in a Wave Tank Under Breaking Waves," *Mar. Poll. Bul.*, **54**(7), 983-993, (2007).

27. J.B.C. Bugden, C.W. Yeung, P.E. Kepkay, and K. Lee, "Application of Ultraviolet Fluorometry and Excitation–Emission Matrix Spectroscopy (EEMS) to Fingerprint Oil and Chemically Dispersed Oil in Seawater," *Mar. Poll. Bul.*, **56,** 677-685, (2008).

28. C. Hu, "An Empirical Approach to Derive MODIS Ocean Color Patterns Under Severe Sun Glint," *Geophys Res Letters*, **38**, doi:10.1029/2010GL045422, (2011).

29. Z. Li, K. Lee, T. King, M.C. Boufadel, and A.D. Venosa, "Oil Droplet

ED_001324_00001243-00032

Size Distribution as a Function of Energy Dissipation Rate in an Experimental Wave Tank," *2008 International Oil Spill Conference*, American Petroleum Institute, 621-626, 2008a.

30. Z. Li, K. Lee, T. King, M.C. Boufadel, and A.D. Venosa, "Assessment of Chemical Dispersant Effectiveness in a Wave Tank Under Regular Nonbreaking and Breaking Wave Conditions," *Mar. Poll. Bul.* **56**(5), 903–912, (2008b).

31. C. Tsouris, L.L. Tavlarides, "Breakage and Coalescence Models for Drops in Turbulent Dispersions," *AICHE J*, **40**(3), 395–406, (1994).

ED_001324_00001243-00033

Figure Legends:

Figure 1. Conceptual diagram of monitoring efforts on the surface and in the deep sea.

Figure 2. Dispersed Alaska North Slope Crude oil droplet size subjected to: (A) physical dispersion, (B) dispersion by Corexit 9500, and (C) dispersion by Dispersit SPC 1000. Open circles are for droplets dispersed under regular non-breaking waves and solid circles are under breaking wave conditions (from Li et al., 2009).

Table Legends:

Table 1. Decision context, key science questions, and anticipated outcomes from EPA's Research Strategy.

ED_001324_00001243-00034

Figure 1.

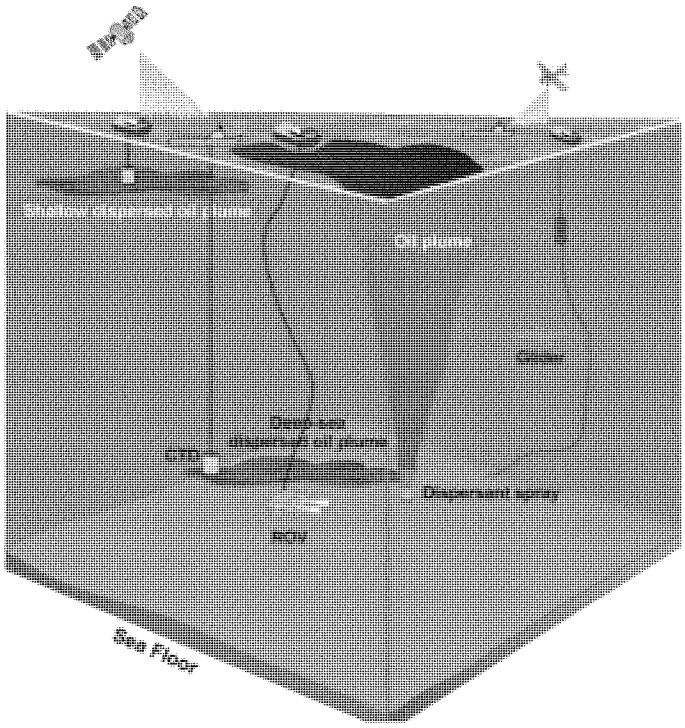
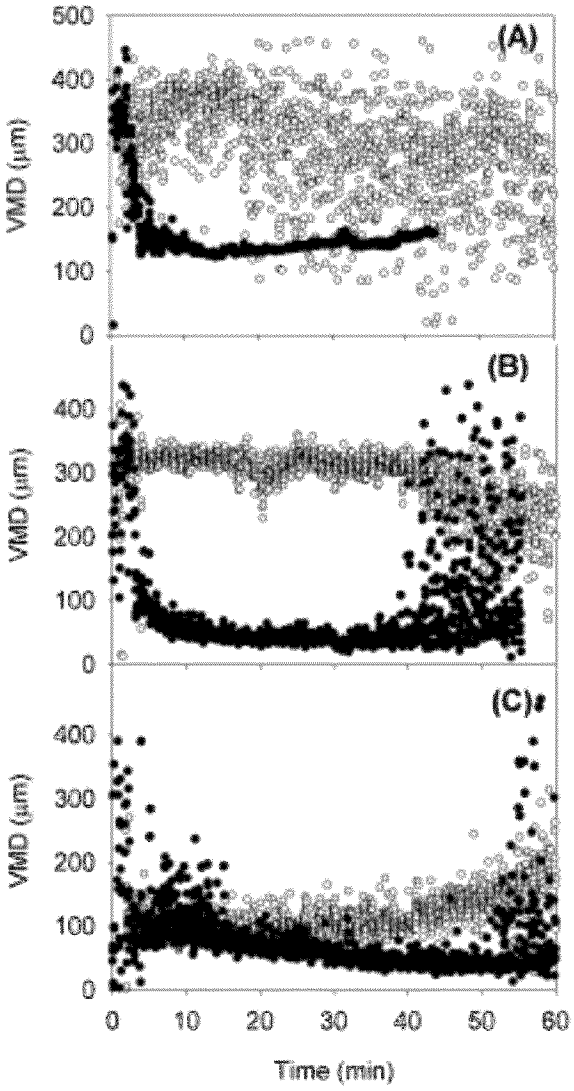


Figure 2.



ED_001324_00001243-00036

Table 1.

Decision Context: What question is the decision-maker asking?	**Key Science Questions: What research will answer that question?**	**Anticipated Outcomes: How will this research inform the overall decision?**
Which dispersants are the most efficacious for particular situations?	When is the use of dispersants most effective and what are the key parameters under which spilled oil is dispersible, such as temperature, mixing energy?	Inform Subpart J regulatory actions. Inform selection of the most effective dispersant on a spill-by-spill basis.
What regulatory actions under Subpart J are needed for dispersants?	What alternative dispersants are available? How effective are they? How toxic are they?	Inform Subpart J regulatory actions.
Do dispersants bioaccumulate in aquatic organisms?	What data are available on dispersant bioaccumulation? Are more studies needed on dispersant bioaccumulation?	Research will provide information about whether dispersants can bioaccumulate through a food chain.
How long do dispersed oil and dispersants from surface, subsurface, and deepwater applications remain in the environment?	What is the environmental fate and transport of dispersed oil and dispersants from surface, subsurface, and deep water scenarios?	Research will provide information on the environmental persistence of dispersants and dispersed oil, which will inform an evaluation of long-term human health and ecological impacts.
What happens to dispersants and dispersed oil when used on deep sea applications?	What are the key variables needed to better understand the coalescence and resurfacing of dispersed oil droplets to develop models for tracking the movement of dispersed oil plumes at the surface, subsurface, and in the deep sea?	Research will provide information to decision-makers on how much oil is dispersed chemically versus physically. If extreme turbulence is all that is needed, then chemical dispersants may not be required.
What methods are available to track dispersed oil in the deep sea?	How can EPA collaborate to improve the SMART protocol for monitoring dispersed oil in the environment, especially the use of innovative and advanced fluorometric techniques?	Improved methods will allow managers to better track dispersed oil plumes and inform remediation decisions.
Are oil dispersant products or chemically dispersed oil chronically toxic to aquatic flora and fauna?	What are the ecotoxicological effects of oil dispersant products and chemically dispersed oil?	Research will be used in ecological risk assessments to inform management decisions on the best products for dispersing oil into the water column.
Will oil dispersant products be toxic to aquatic species when injected at the surface or underwater to mitigate spill impacts from deep sea blowouts?	What are the ecotoxicological effects of dispersants in surface and deep sea injection exposures?	Research will be used in ecological risk assessments to inform management decisions for deploying the least toxic dispersants for mitigating oil spills.

ED_001324_00001243-00037

Will the effective use of dispersants reduce the impacts of the spill to shoreline and water surface resources without significantly increasing impacts to water-column and benthic resources? (NRC, 2005)	What are the comparative ecotoxicological effects of dispersants in surface and deep sea injection exposures versus shoreline?	The dispersant ecological risks will be compared to coastal ecological risks from oil spills in a variety of scenarios. This comparative assessment will address key questions on dispersant use and provide information needed to conduct a net environmental benefit analysis.
What green chemistry methods are available to use as effective dispersants?	What dispersants can be produced that have a "lighter" environmental footprint than petroleum-based products?	Dispersants will be proposed that have reduced life cycle assessment (LCA) ecological impacts to make available "green" options to decision-makers.

ED_001324_00001243-00038

To: Mike Fulton - NOAA Federal[mike.fulton@noaa.gov]
From: Conmy, Robyn
Sent: Mon 7/6/2015 5:29:51 PM
Subject: RE: following up

Hi Mike,

I am in this week but out next week. I have time on Tuesday between 11-2 ET and Wednesday anytime in the morning.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Mike Fulton - NOAA Federal [mailto:mike.fulton@noaa.gov]
Sent: Monday, July 06, 2015 1:24 PM
To: Conmy, Robyn
Subject: Re: following up

Hi Robyn,

I hope you're well and had a great holiday. Would you have time for a chat about shoreline cleaners sometime in the next several days? Thanks.

Mike

On Mon, Feb 9, 2015 at 2:48 PM, Conmy, Robyn <Conmy.Robyn@epa.gov> wrote:

OK. If you are running behind, then we can try for Wednesday…

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

From: Mike Fulton - NOAA Federal [mailto:mike.fulton@noaa.gov]
Sent: Monday, February 09, 2015 2:28 PM

To: Conmy, Robyn
Subject: Re: following up

Thanks Robyn. I have another meeting in the morning, but I'll try to call at 10:30.

Mike

On Mon, Feb 9, 2015 at 2:24 PM, Conmy, Robyn <Conmy.Robyn@epa.gov> wrote:

Hi Mike,

I am available tomorrow morning between 9:30 -11 am ET.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

From: Mike Fulton - NOAA Federal [mailto:mike.fulton@noaa.gov]
Sent: Monday, February 09, 2015 10:15 AM
To: Conmy, Robyn
Subject: Re: following up

Hi Robyn,

I hope you're well. Would you have time for a quick call sometime this week? I've got a couple of dispersant (finasol, corexit) questions.

Mike

On Tue, Sep 23, 2014 at 11:18 AM, Mike Fulton - NOAA Federal <mike.fulton@noaa.gov> wrote:

Thanks Robyn. I think we are about to receive the oil we requested from BP. Fingers crossed.

Mike

On Fri, Sep 19, 2014 at 10:13 AM, Conmy, Robyn <Conmy.Robyn@epa.gov> wrote:

Hi Mike,

I heard from EPA OEM that the oil purchase is approved but that it hasn't been ordered. That is the only information I have received. I did speak with Debbie Payton on Wednesday and she mentioned that her office has gotten some oil that may be available to you. I hope that is the case. There is some irony that we are all trying to conduct oil research that is critical for regulation, yet cannot access the oil. Sigh.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL RISK MANAGEMENT RESEARCH LABORATORY
CINCINNATI, OH 45268

OFFICE OF
RESEARCH AND DEVELOPMENT

February 9, 2015

Ms. Caroline Laikin
Bureau of Safety and Environmental Enforcement
Acquisitions Operations Branch
381 Elden Street, HE 2306
Herndon, VA 20170-4817

Ms. Caroline Laikin,

Please find the enclosed White Paper in response to the Bureau of Safety and Environmental Enforcement's (BSEE) current Broad Agency Announcement #E15PS00027, Topic 7, "Proposed Research on Oil Spill Response Operations in the US Outer Continental Shelf". This submission from the U.S. Environmental Protection Agency, entitled "Characterizing Dispersant Effectiveness of Crude Oils at High Salinities: Implications for Subsea Spill Preparedness", is provided in one-electronic pdf form. If there are any questions regarding this submission, please contact Dr. Robyn Conmy.

Thank you,

Robyn N. Conmy, Ph.D.
U.S. EPA
Office of Research and Development
National Risk Management Research Laboratory
Phone: 513-569-7090
Conmy.robyn@epa.gov

Recycled/Recyclable • Printed with Vegetable Oil Based Inks on 100% Recycled Paper (40% Postconsumer)

To: Holder, Edith[holder.edith@epa.gov]
From: Conmy, Robyn
Sent: Fri 4/1/2016 2:27:54 PM
Subject: RE: endicott and dorado

That's fine. But I have to ask – why do you have to download the pictures to a computer first? Is your text or email feature not working on your phone?

Also, what would be a ball park number for the volume of specific dispersants that we have in the lab? DO we have liters or milliliters of products such as Corexit, finasol, dispersit, accell, etc??? We may be needing to do tox work on dispersants alon to support subpart J decision rule. That would mean we need 1 L of each of whatever products OEM chooses to test. I am thinking we do not have that much of any to spare! And we can't buy more b/c manufacturers require a waiver where we say we won't conduct tox testing with it. I don't see how they are allowed to require this.

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Holder, Edith
Sent: Friday, April 01, 2016 10:23 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: RE: endicott and dorado

I have taken pictures. The only reliable way I have to send pictures from my phone is to download them to my home computer. Life was too crazy last night for me to remember to do that. I will send pictures by Monday.

From: Conmy, Robyn
Sent: Thursday, March 31, 2016 3:06 PM
To: Holder, Edith <holder.edith@epa.gov>
Subject: endicott and dorado

What was the volume of oil that you said we had?

And can you send to me the picture of the old reference oil ampule label? Or tell me where to find them in the lab?

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

To: Malcolm Gore[malcolm.gore@clearcoastllc.com]
From: Conmy, Robyn
Sent: Thur 4/28/2016 6:02:08 PM
Subject: RE: Finasol OSR 52 Sample Request

Yes we have received the invoice and I have provided our purchase card holder with the information. He will be contacting your office for payment.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Malcolm Gore [mailto:malcolm.gore@clearcoastllc.com]
Sent: Thursday, April 28, 2016 1:43 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: Re: Finasol OSR 52 Sample Request

Hi Robyn

I have given the go ahead on this. Please confirm receipt of the invoice sent yesterday.

Malcolm

Malcolm Gore

President

+1 832 244 1533

malcolm.gore@clearcoastllc.com

www.clearcoastllc.com

From: "Conmy, Robyn" <Conmy.Robyn@epa.gov>
Date: Tuesday, April 26, 2016 at 11:05 AM
To: malcolm gore <malcolm.gore@clearcoastllc.com>
Subject: RE: Finasol OSR 52 Sample Request

Thanks for the update!

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Malcolm Gore [mailto:malcolm.gore@clearcoastllc.com]
Sent: Monday, April 25, 2016 5:45 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: Re: Finasol OSR 52 Sample Request

Apologies for the delay Robyn

Awaiting some shipping information. Should have this to you in the morning.

Malcolm

Malcolm Gore

President

CLEAR COAST

+1 832 244 1533

malcolm.gore@clearcoastllc.com

www.clearcoastllc.com

From: "Conmy, Robyn" <Conmy.Robyn@epa.gov>
Date: Monday, April 25, 2016 at 10:05 AM
To: malcolm gore <malcolm.gore@clearcoastllc.com>
Cc: Peter Egan <peter.egan@total.com>
Subject: RE: Finasol OSR 52 Sample Request

Hello Malcolm,

Thank you for getting back with me regarding the purchase. Yes, the information below is correct, with a mail stop at room 166. Can you send to me the invoice via email so I can provide to the purchase card holder to make the purchase?

Thank you in advance,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Malcolm Gore [mailto:malcolm.gore@clearcoastllc.com]
Sent: Monday, April 25, 2016 10:57 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Egan <peter.egan@total.com>
Subject: Finasol OSR 52 Sample Request

Hello Robin

I am writing with respect to your email below regarding a sample request of Total Finasol OSR 52.

Clear Coast is the custodian of the product stored here in Houston for Total.

I will arrange the sample as requested.

Please confirm the following:

Contact:

Robyn Conmy 513-569-7090 (office)

Deliver to:

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

Quantity:

2 US Gallons.

On receipt of confirmation I will get the samples drawn, packaged and dispatched.

Regards

Malcolm

Malcolm Gore

President

+1 832 244 1533

malcolm.gore@clearcoastllc.com

www.clearcoastllc.com

From: Conmy, Robyn
Sent: Monday, April 18, 2016 10:47 AM
To: 'peter.egan@total.com' <peter.egan@total.com>
Cc: Wilson, Gregory <Wilson.Gregory@epa.gov>; Principe, Vanessa <Principe.Vanessa@epa.gov>
Subject: purchase of Finasol OSR52

Mr. Egan,

This follows up our telephone conversation of April 11.

As discussed, EPA is seeking to acquire a number of dispersant products listed on the NCP Subpart J Product Schedule for the purposes of both toxicity and efficacy testing. The product testing is intended to further inform currently proposed regulatory actions under Subpart J of the National Contingency Plan. The products will in addition be used to support our general research in the area of oil spill response.

Your product Finasol OSR52 has been identified as one of interest, as it is commonly stockpiled in the U.S. Not only could the product be encountered when presented with a response situation, but including this dispersant product in toxicity and efficacy testing studies will also allow for comparison and consistency with other existing and ongoing studies by EPA and other federal agencies. EPA's Office of Research and Development (ORD) will be conducting the dispersant studies.

At this time we are seeking 2 U.S. gallons of the product.

We appreciate your time and attention to this request.

Respectfully,

Robyn Conmy

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

Oil plume simulations: Tracking oil droplet size distribution and fluorescence within high-pressure release jets

R.N. Conmy[1], **B. Robinson** [2], **T. King** [2], **M. Boufadel**[3], **S. Ryan**[2], **C. McIntyre**[2], **M. I. Abercrombie**[4], **K. Lee** [2,5]

[1]U.S. Environmental Protection Agency, Office of Research and Development, 26 MLK Drive West, Cincinnati, OH 45255, USA

[2] Bedford Institute of Oceanography, Fisheries and Oceans Canada, 1 Challenger Dr, Dartmouth, NS, B2Y 4A2, Canada

[3] New Jersey Institute of Technology, Department of Civil and Environmental Engineering, University Heights, Newark, NJ 07102, USA

[4]University of South Florida, College of Marine Science, 140 7th Ave South, St. Petersburg, FL 33701, USA

[5] Commonwealth Scientific and Industrial Research Organisation, Oceans and Atmosphere Flagship, PO Box 1130, Bentley WA 6102, Australia

ABSTRACT

Optical measurements have been used during oil spill response for more than three decades to determine oil presence in slicks and plumes. Oil surveillance approaches range from simple (human eyeball) to the sophisticated (sensors on AUVs, aircraft, satellites). *In situ* fluorometers and particle size analyzers were deployed during the Deepwater Horizon (DWH) Gulf of Mexico oil spill to track shallow and deep subsea plumes. Uncertainties regarding instrument specifications and capabilities during DWH necessitated performance testing of sensors exposed to simulated, dispersed oil plumes. Seventy two wave tank experiments were conducted at the Bedford Institute of Oceanography. Simulated were oil releases with varying parameters such as oil release rate, oil temperature (reservoir temp ~ 80 °C), water temperature (<8 °C and >15 °C), oil type, dispersant type (Corexit 9500 and Finasol OSR52) and dispersant

ED_001324_00001251-00001

to oil ratio (DOR). Plumes of Alaskan North Slope Crude, South Louisiana Crude and IFO-120 oils were tracked using *in situ* fluorescence, droplet size distribution (DSD), total petroleum hydrocarbons (TPH) and benzene-toluene-ethylbenzene-xylene (BTEX). Results offer valuable information on the behavior and dispersibility of oils over a range of viscosity, DOR and environmental conditions. Findings have implications for fate and transport models, where DSD, chemistry and fluorescence are all impacted by release variables. This research was supported by the Bureau of Safety and Environmental Enforcement.

INTRODUCTION

The 2010 *Deepwater Horizon* (DWH) oil spill highlighted the pressing need for a better understanding of the interaction of chemical dispersants and crude oil at ocean depth. Early in the blowout release, partial emulsification of oil and heterogeneous slicks were observed as it rose to the surface from 1500-m depth (JAG report, 2010). As a countermeasure, Sub-Sea Dispersant Injection (SSDI) was used, where dispersants were injected directly at the release point as a possible means to increase dispersion efficiency and to potentially reduce the amount of dispersant needed if applied at the air-sea interface (CRRC Report, 2010). Potential advantages of this application during a continuous oil release included the fact that the fresh (unweathered) oil was considered well suited for dispersion, operators were able to inject the dispersant directly into the oil stream thereby maximizing dispersant/oil contact, sufficient control of DOR (Dispersant-to-Oil Ratio) could be maintained, injection may minimize the need for surface application because of reduced oil surfacing, and optimized subsurface application would likely promote formation of smaller and stable oil droplets, enhancing biodegradation (Lee et al., 2009). The Unified Area Command (UAC) coordinated a large-scale environmental

ED_001324_00001251-00002

monitoring program to track and characterize dispersed subsurface oil plumes based on field data and transport modeling outputs. Oil Droplet Size Distribution (DSD) and fluorescence intensity from submersible sensors were used as an indication of Dispersion Effectiveness (DE) onboard vessels, where concentrations were monitored to evaluate oil dispersion (presence of small droplets ≤ 70 μm) based on previous studies for surface dispersant applications (Li et al., 2009a). Monitoring provided sound evidence of the presence of oil-bearing small particles both in surface waters and in the subsurface plume (JAG Report, 2010). SSDI correlated with a higher proportion of low molecular weight compounds in subsea waters compared to surface, suggesting that SSDI may have promoted the formation of small oil droplets in the deep sea and thus would have the potential for enhancing the weathering and dissolution of oil in the water column.

Uncertainties still exist, however, regarding the effectiveness of this application. For example, assumptions of the optimal DOR are based on empirical data mostly obtained from bench-scale experiments designed for testing at standard temperatures and pressures (STP), whereas conditions at a wellhead on the ocean floor or anywhere along a riser could be significantly different. Hence, DOR for direct injection needs to be better understood. Although theoretical analyses and experiments suggest that oil jet breakup is insensitive to the absolute value of hydrostatic pressure for incompressible liquid-liquid systems (Masutani and Adams 2000), the effects of several ambient environmental factors on SSDI, including high release pressure, high oil temperature, low water temperature, and the presence of methane and suspended sediments in the oil plume and/or surrounding water column remain to be clarified. Improved understanding on the influence of these factors and the interaction of oil and chemical

ED_001324_00001251-00003

dispersant under a range of turbulent regimes at depth is required for informed decision-making for future SSDI use.

For evaluating DE, standard laboratory tests are inherently limited in simulating operational performance under realistic environmental conditions in part due to space constraints that are critical for transport and dilution efficiency (NRC, 2005). Meso-scale tanks capable of generating waves and flume flows to simulate ocean conditions help to address this issue. One such tank facility at the Bedford Institute of Oceanography (BIO; Dartmouth, Nova Scotia) has been used previously to assess chemical dispersant effectiveness for various oil types as a function of energy dissipation rate and particle size distribution (Li et al. 2009b) and demonstrated that the effectiveness of a dispersant is strongly dependent on wave conditions, dispersant type, and oil type (Wickley-Olsen et al. 2007; Li et al. 2008; Lee et al. 2009). A strong correlation has been established between dispersion effectiveness and *in situ* DSD within the hydrodynamic regime, particularly energy dissipation rate, under a variety of wave conditions (Li et al. 2008; Li et al. 2009b), where flume mode can simulate the effects of underwater currents on dispersion and dilution of oil (Li et al. 2009a; Li et al. 2010). Experiments have also shown the reliability of fluorescence intensity as a proxy for oil concentration within oil dispersions (Conmy et al., 2014).

Presented here are results from a series of high pressure jet releases of oil within the BIO flume tank to assess the operational performance of SSDI. DSD and fluorescence intensity were used as a proxy for dispersion efficiency for treatments with (chemically-enhanced dispersion) and without (physical dispersion) chemical dispersants. The objective of this work was to evaluate the effects of water temperature and dispersant-to-oil ratio on DE of three oils at high

ED_001324_00001251-00004

reservoir temperatures. Findings help (1) to determine the applicability of existing *in situ* monitoring technologies for SSDI and (2) inform efforts to integrate DSD into deepwater blowout plume formation models that enable prediction of the dispersed oil droplets under high-flow subsurface release velocities. DWH spill monitoring program DSD and fluorescence data played a significant role in supporting emergency response operations, fate and transport modeling, and impact assessment. Findings from this study have implications for efforts in support of future deep-water spill preparedness.

METHODS

Flume Tank Description - Oil dispersion experiments were conducted using Alaska North Slope crude oil (ANS), South Louisiana Crude (SLC) and Intermediate Fuel Oil 120 (IFO 120) with Corexit 9500 and Finasol OSR 52 chemical dispersants in the 32m long flow-through carbon steel flume tank (31,000 L) at BIO. Seawater was pulled from the Bedford Basin (50cm below surface) via two smaller centrifugal pumps (110 gpm), and filtered through high-flow polypropylene bag filters (5 and 25 μm) to a fiberglass holding tank (25,000 L) that supplies seawater for the system to ensure that a constant flow rate is maintained. Two high-flow centrifugal pumps (600 gpm) provided flow to generate horizontal water currents in the tank, where flow gauges on influent and effluent pipes were monitored and valve adjustments made to obtain a balanced flow rate throughout experiments. Water current velocities were measured in the tank using an ADV (Nortek Vectrino) to maintain consistent horizontal water current velocities (3.5 cm/s) at all measured depths. At the completion of experiments, oil absorbent pads removed oil from the water surface. Effluent wastewater was filtered over layers of polypropylene PomPom Oil-Mops to remove insoluble oil from the water prior to discharging, where effluent samples were collected and PomPom's were changed if oil concentrations exceed

ED_001324_00001251-00005

the minimum guidelines (10 ppm) for wastewater discharge in Canada.

Subsurface Oil Injection System - A custom subsurface oil injection system was used to generate dispersed oil plumes in the tank (Figure 1), consisting of a 2 L stainless steel pressure vessel and a series of valves and pressure gauges, fastened to the outer wall of the tank. Inside the tank, a fitting connects the outer assembly to a nozzle (2.4 mm), which extends mid-width perpendicular to the tank wall (9 m downstream from the influent pipe) and is angled at the tip to direct the discharge plume downstream and use horizontal length of the tank to capture the plume movement. For each experiment, oil or oil/dispersant premix is added to the pressure vessel in order to reduce the influence of any additional confounding factor of mixing effectiveness. Copper coil within the pressure vessel connects to a water bath to permit oil heating to 80°C, and then pressurized (40 psi for ANS and SLC; 60 psi for IFO 120) with compressed Nitrogen. A ball valve connected to the vessel controls the oil release through the subsurface nozzle into the tank. Release time and total volume (determined by mass) of oil injected are recorded. After each experiment, the subsurface injector system was cleaned by flushing with toluene, acetone and fresh water.

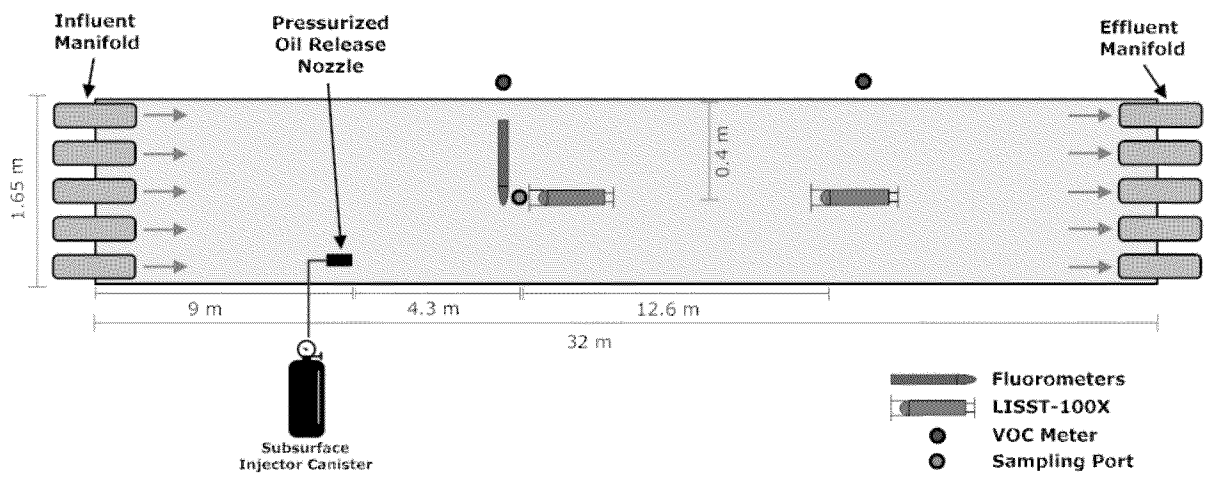


Figure 1. Schematic diagram showing the location of the subsurface injector and *in situ* instrumentation submerged within the tank.

Submersible Sensor Deployment - Sensors were mounted on an aluminum frame located 4.3 m from the oil release point with their pressure windows and at a depth of 0.4 m. The instruments were attached to a crosspiece support bar, so that they were all located the same distance downstream from the oil release point with the pressure window pointed directly towards the bottom of the tank. Data acquisition systems were used to control and collect data with real-time displays from the *in situ* fluorometers. A total of six *in situ* hydrocarbon fluorometers were evaluated during this study; Chelsea Technologies Group UV Aquatrackas - Refined and Crude models, Turner Designs Group Cyclops - Fine and Crude models, Sea Bird – Wet Labs Inc ECO-FLU, and GmBH Trios. Sampling rates and units of signal intensity varied for each instrument with one reading every 5 seconds for GmBH (μg/L PAH), every 3 seconds for Cyclops (auto-gain feature; mV), and every 1 second for the ECO and Aquatrackas (μg/L QSDE, μg/L perylene and μg/L carbazole, respectively. Oil droplet size was measured *in situ* using two LISST-100X (Laser *in-situ* Scattering and Transmissometry) particle size analyzers (Sequoia Scientific, Inc), measuring particle size from 2.5 – 500 μm. The first LISST (upstream LISST) was located immediately after the fluorometer package support frame at a distance of 5.1 m from the oil release point and the second LISST (downstream LISST) was located at 16.9 m from the oil release point and both at a depth of 0.4 m (Figure 1). Placement was informed by the numerical modeling efforts conducted previously in the tank to maximize oil droplet detection without saturating the instrument. Real-time data was provided by LISST-SOP data acquisition software (ver. 5) with a sample acquisition rate of one measurement every 3 seconds.

ED_001324_00001251-00007

Underwater video of oil droplets and the transport of the plume were captured using a GoPro Hero4 digital camera, as well as a Sony RX100 III digital camera with underwater housing.

Submersible Sensor Data Processing - LISST data files were processed using a statistically-based quality control script written using the R statistical package. This script identifies and removes "Over Range" samples (defined as 0 μL/L) and outliers. Outliers are defined as any reading that is greater than the moving mean (5 data points before and after the targeted time point) of the dataset multiplied by four times the standard deviation (over the same interval as the moving mean). Due to the potential for one or more extreme outliers to skew both the moving mean and standard deviation calculations for points around them, this outlier detection routine is run iteratively, excluding previously flagged points, until no more outliers are detected. The script then calculates the Total Particle Concentration (TPC), Volume Mean Diameter (VMD), and Particle Size Concentration (PSC). It then detects the plume curve (if present) and time-normalizes the data based on that location. Data are presented as Droplet Size Distribution (DSD). Plots presented include data 2 minutes before and 8 minutes after the start of the plume curve. Data from the Downstream LISST were normalized so that the plume began at t = 5 min in order to visually convey that the plume was detected in the tank roughly 3 min after detection by the LISST further upstream near point of injection release. Similar to the LISST data, a script was used to detect outliers in the fluorometry data. Curve detection was performed and the data was time-normalized to include 2 minutes of data before, and 8 minutes of data after the start of the plume curve. The baseline of the plume curve was calculated using data points observed in the first minute preceding the start of the curve and this baseline was subtracted from the data. Finally, factory calibration factors were applied to the data.

Experimental Design -. Seawater temperature and salinity in the tank were recorded

using a handheld probe (YSI Incorporated, Yellow Springs, OH). Prior to experiments, the tank was operated in recirculation mode (10 minutes) to allow current flow to stabilize. Five minutes prior to oil injection, data-logging on all instruments was started and background seawater samples were collected. After oil injection, the real-time readout of the fluorometer signal was monitored for the first spike in signal intensity (usually after 2 minutes), and bottle chemistry sample collection began. The experiments ran for 12 minutes and the tank was cleaned using Big Orange Degreaser, to prevent any potential contamination between experiments. Instrument windows were cleaned using disposable alcohol wipes. Water samples were returned to the lab and stored at 4°C.

Discrete Chemistry Samples - Water samples for chemical analysis were collected at various time points throughout the experiments. Three ¼" stainless steel tubes were attached to the aluminum fluorometer frame, so that the end of the tube was located at the same depth as the instrument pressure windows (0.4 m). These were attached via silicon tubing to a digital peristaltic pump with a 120 mL/min flow rate. Samples were analyzed for Total Petroleum Hydrocarbon (TPH) using a method developed by DFO in-house (Cole et al., 2007; King et al., 2015) for Gas Chromatograph (GC) - Flame Ionization Detector (FID) analysis. An eight point calibration was generated using standards prepared from the appropriate crude oil stock that was used to generate the TPH samples (e.g. ANS, IFO 120 and SLC). Peak quantification was performed using relative response factors. Samples were analyzed for benzene, toluene, ethylbenzene and xylene (BTEX) using EPA Method 8240 (purge and trap), modified by running a GC - Mass Spectrometer (MS) in selected ion monitoring mode to include ethylbenzene. BTEX standards were prepared in 40 mL purge and trap vials; and samples and standards were analyzed using this method, along with sample blanks and duplicate samples.

ED_001324_00001251-00009

TPH and BTEX results are not shown here in detail but can be found in the EPA 600/F-16/250 Report (2016).

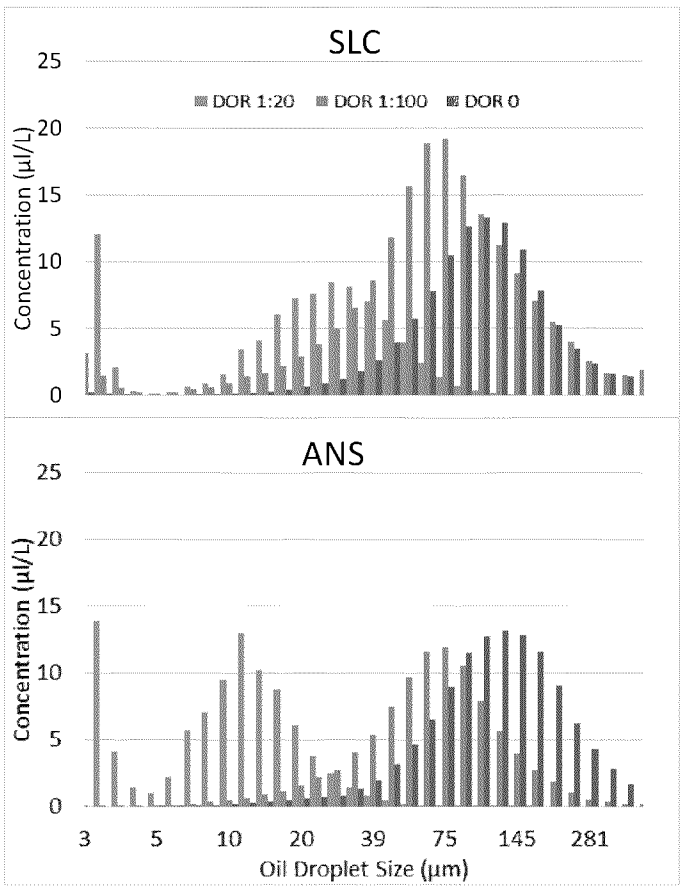
RESULTS & DISCUSSION

A total of 72 experiments were conducted in replicate, where water ranged in temperature from 4.9 - 20.3 °C, with 11 °C serving as the cut off between warm and cold water tests. Previous experiments within the BIO flume tank facility have assessed dispersion effectiveness as a function of energy dissipation rate and particle size distribution (Li et al. 2009a) and demonstrated that DE is strongly dependent on wave conditions, dispersant type, and oil type (Lee et al. 2009) and highly correlated with *in-situ* droplet size distribution (Li et al. 2008; Li et al. 2009a). Here, DE was evaluated via response monitoring tools (fluorescence and particle size analyzers) during high-pressure subsurface injection experiments.

Dispersant-to-Oil Ratio (DOR) Effects- Addition of either Corexit 9500 or Finasol OSR 52 chemical dispersants to Alaskan North Slope (ANS), IFO 120 and South Louisiana Crude (SLC) oils resulted in a decrease in the Volume Mean Diameter (VMD) and shifted the Droplet Size Distribution (DSD) to smaller droplets for warm water treatments (Figure 2). For ANS, dispersions created without chemical dispersants or Dispersant-to-oil ratio (DOR) = 1:100 yielded VMD larger than 70 μm and exhibited unimodal DSD. Dispersions created with DOR = 1:20 yielded VMD between < 70 μm size range with a bimodal distribution. SLC oil was more dispersible compared to ANS for treatments with and without chemical dispersant. Bimodal distribution was observed during DOR = 1:20 and some DOR = 1:100 experiments indicating that the jet release of this particular oil into warm water produced smaller droplets than the ANS. During the DWH spill, droplets ≤ 70μm were considered small enough to remain in suspension

ED_001324_00001251-00010

and were monitored as a proxy for evaluating oil dispersion efficiency. This suggests that produced SLC droplets from a DOR = 1:20 or 1:100 dispersant injection, and ANS droplets at DOR = 1:20 would likely remain dispersed in the presence of mixing energy given the larger proportion of small droplet sizes observed. In general, Corexit 9500 produced slightly smaller droplets compared to Finasol OSR 52 for warm water treatments. The addition of Corexit 9500 or Finasol OSR 52 to IFO 120 during warm temperature experiments resulted in a shift in DSD and a decrease in VMD; however bimodal distribution was not achieved and even DOR = 1:20 did not yield VMD less than 70 μm in most cases. This suggests that dispersant addition to this oil at warm temperatures would not yield droplet sizes that would likely remain in suspension.



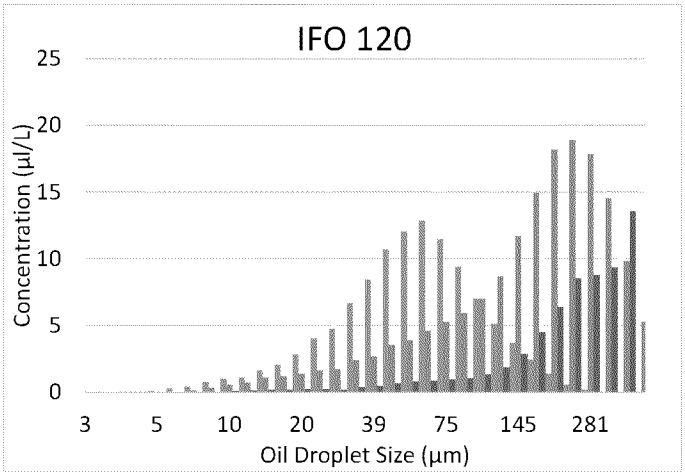


Figure 2. LISST DSD for SLC, ANS and IFO 120 with Corexit 9500 at three DOR treatments in warm water. Histograms represent averages obtained at the 2 minute time stamp during each experiment.

Water Temperature Effects - For ANS, dispersion with < 70 µm droplet VMD was observed for the DOR = 1:20 treatments at both cold and warm water temperatures (Figure 3). Water temperature did not appear to influence the DSD or VMD for this lighter crude oil. However, a temperature effect was observed on the Total Particle Concentration (TPC), where lower temperatures were coincident with fewer particles dispersed within the plume for a given volume of oil injected. This may be due to enhanced density differences between the oil and water at colder temperatures, thus quicker rise time of the produced droplets. For the addition of Corexit 9500 or Finasol OSR 52 to IFO 120 at cold water temperatures, smaller droplet sizes were not observed, where DOR = 1:20 remained above 200 µm. This suggests that dispersant addition to this oil at cold temperatures would not yield droplet sizes that would likely remain in suspension.

ANS IFO 120

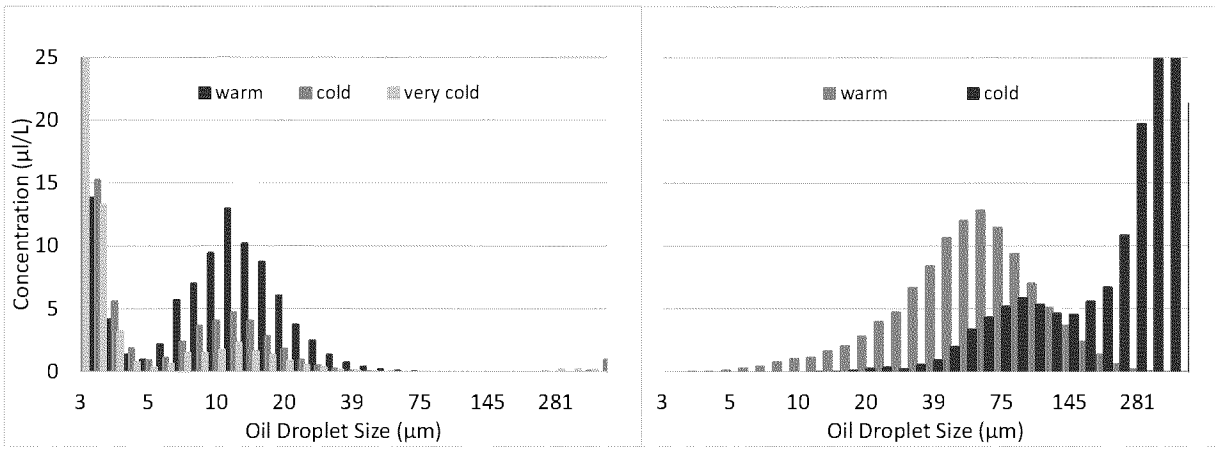


Figure 3. LISST DSD for ANS and IFO 120 with Corexit 9500 at DOR = 1:20 and varying water temperature.

For experiments conducted at water temperatures < 5 °C, the LISST particle size analyzer yielded unexpected DSD where even a unimodal distribution was not measured. Chemistry and *in situ* fluorescence data indicate that the oil was in fact dispersed adequately. This suggests potential operational problems < 5 °C, even though it is within the operating temperature (manufacturer manual). Additional testing of cold water temperature limits is recommended.

Upstream and Downstream Particle Fractionation - Histograms representing the particle concentration for a given size class from the upstream and downstream LISSTs suggest dilution and fractionation of particles within the plume. The downstream LISST positioned further from the jet release serves as an indication of plume evolution through the tank. For all oil and DOR treatments, dilution in particles (decrease in TPC) within the plume expectedly occurred. Also evident is a shift to smaller particles for all DOR treatments as the plume moves through the tank, where size fractionation was dependent on DOR treatment (Figure 4). Physical dispersion (DOR 0) treatments for lighter oils were found to have the largest shift in

DSD and VMD between the upstream and downstream LISSTs suggesting the largest effect of particle fractionation in the plume. Where the decrease in TPC suggests plume dilution in the tank, the DSD shift to smaller particles suggests that within each experiment larger droplets were removed from the plume within 6 minutes of the oil release, most likely rising to the surface of the tank. The effect was least observed in the DOR 1:20 treatments where dilution occurred but the presence of smaller droplets (with lower rise times) at the point of release most likely didn't allow for fractionation. The effects of fractionation were observed in warm and cold treatments.

Time series particle size data allows for evaluating plume dynamics over the duration of the experiments. Figure 5 depicts time series contour plots of the plume, where colored contours represent the particle concentration (normalized to max value for comparison purposes) for given droplet sizes through time. These plots allow for ascertaining how the DSD shifts over the duration of the release as the plume moves in the horizontal direction in the tank. For ANS, data suggest that the leading edge of the plume, as detected by the upstream LISST, exhibited a wide range in DSD during the first few minutes after release. As the plume advances down the tank, the DSD becomes narrower with a shift in the VMD for the DOR = 1:100 and 0 treatments. DSD remained similar for the DOR = 1:20 treatment. Warm and cold water treatments exhibit similar trends.

ED_001324_00001251-00014

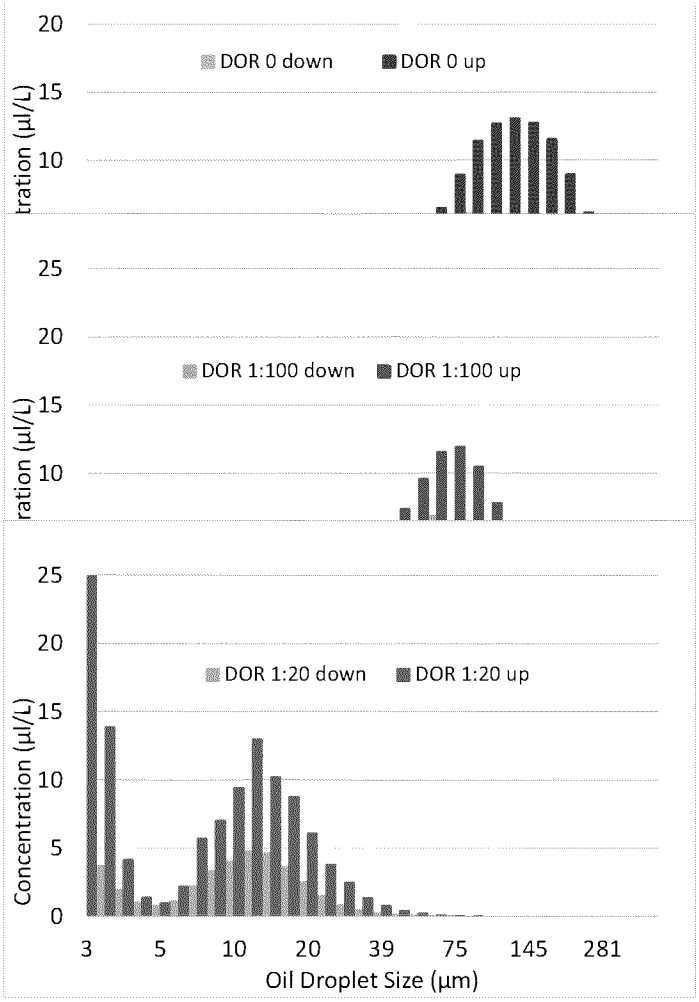


Figure 4. Upstream and downstream LISST Droplet Size Distribution and particle concentration for ANS with Corexit 9500 during warm water treatments.

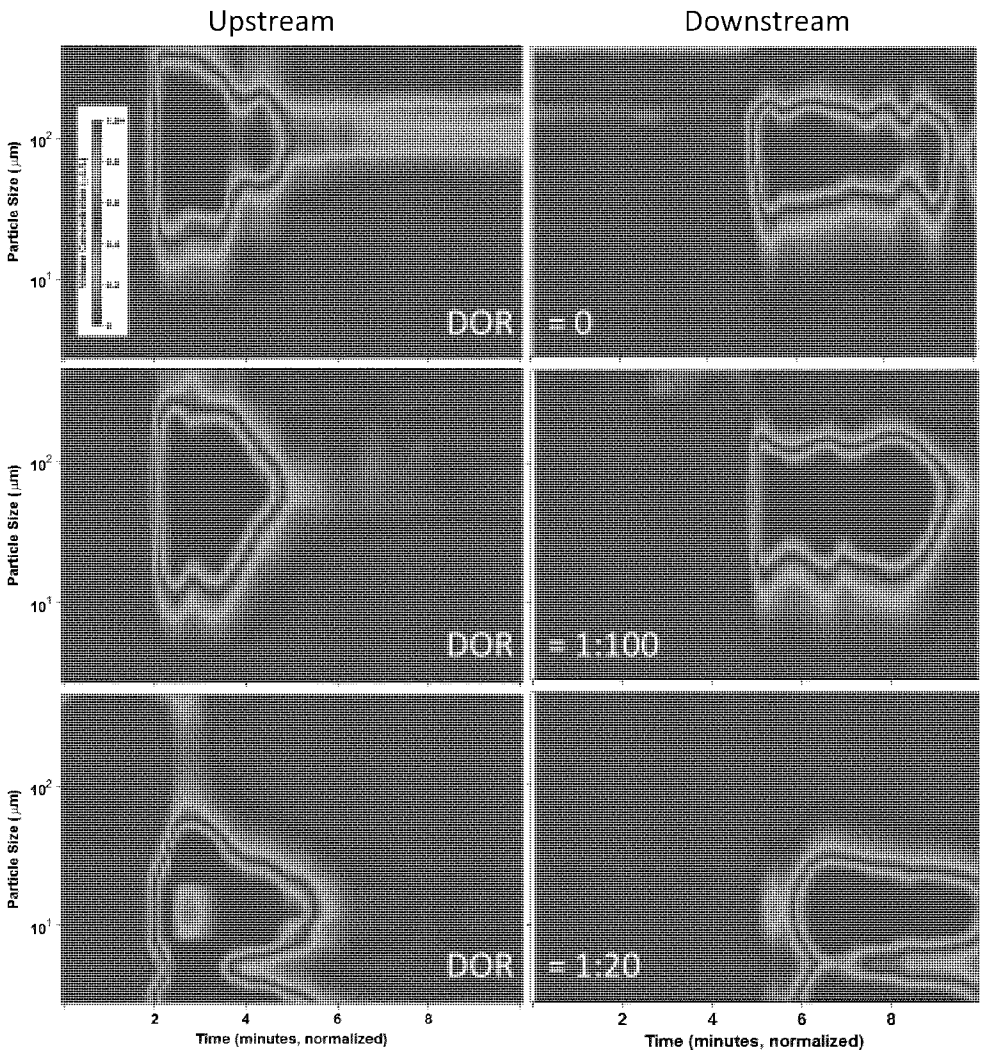


Figure 5. Time series contours of droplet size distribution and particle concentration from the upstream (left panels) and downstream (right panels) LISSTs for ANS with Corexit 9500 during warm water treatments.

<u>Plume Fluorescence Intensity</u> – In addition to the plume transport of particles (oil droplets), dissolved hydrocarbons were also measured via discrete samples and by proxy using fluorescence intensity. The reliability of *in situ* fluorescence measurements as a proxy for oil concentration within physically and chemically dispersed oil has been demonstrated previously

within the tank (Conmy et al., 2014). Here, *in situ* fluorescence serves as a good proxy for oil concentration during the subsurface injection experiments. However, heterogeneity of the produced plumes and the short time scale of experiments (~10 min) led to difficulties in correlations between the plume particle size analyses and chemistry results. This is in part due to discrete samples representing 15 second averages as opposed to instantaneous measures given by fluorometers and particle size analyzers. Thus, although general trends were similar, exact matchups between *in situ* data streams and discrete samples proved to be challenging (Figure 6). Given the experimental design, fluorescence was found to be better suited for correlation with total particle concentration (Figure 7). Depicted are the in situ fluorescence and TPC data during a warm water experiment of ANS with (left) and without (right) Corexit 9500. When exposed to dispersant, fluorescence estimates of dissolved hydrocarbons was higher compared to oil alone treatments, and signal persisted for longer periods of time. Whereas a sharper decay in the fluorescence intensity and the TPH values were observed for the physically dispersed oil.

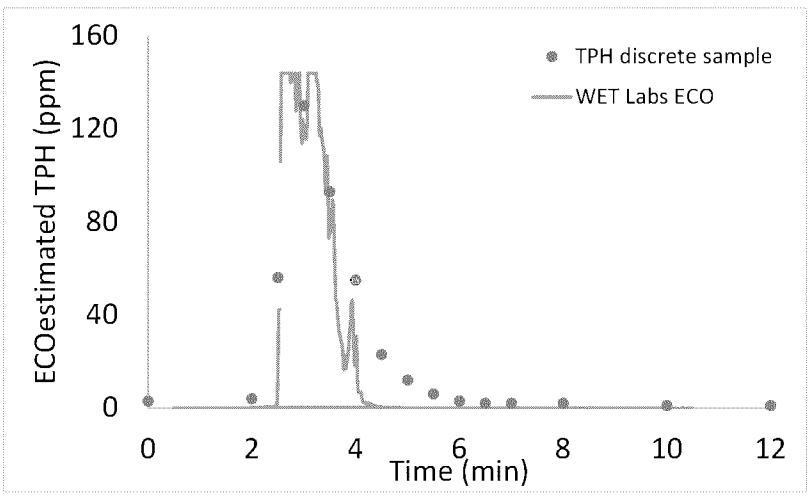


Figure 6. Fluorescence intensity and TPH samples for a warm water treatment using SLC

at DOR = 1:20.

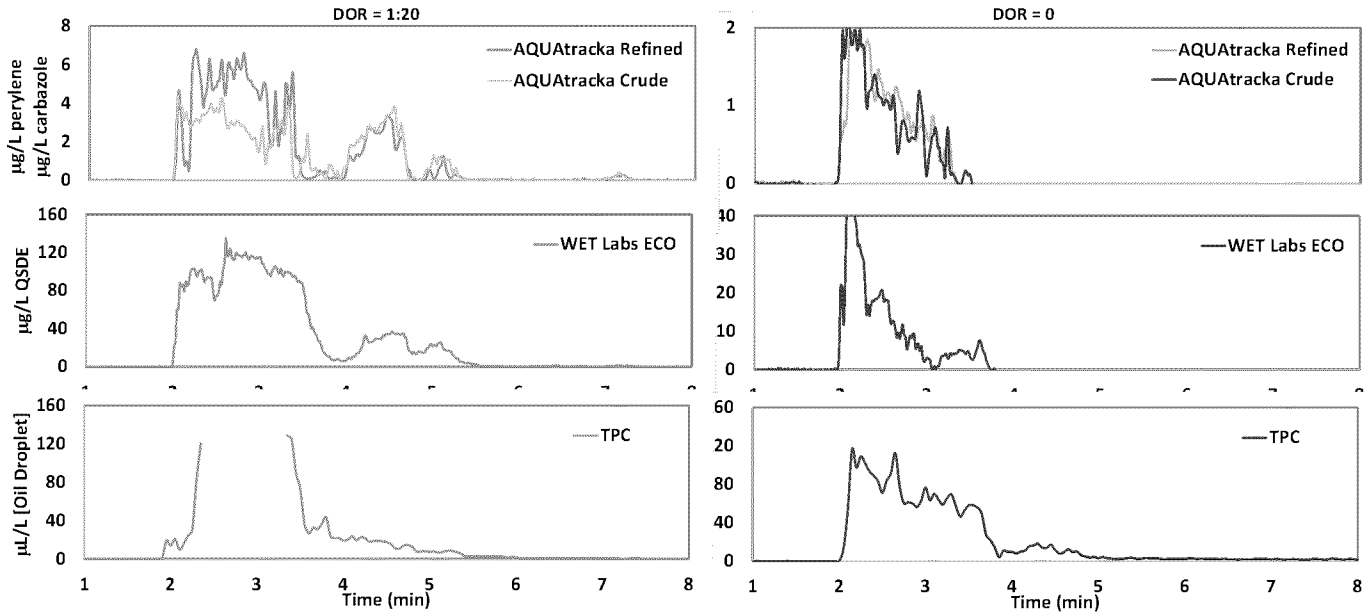


Figure 7. Fluorescence intensity and TPC as a function of time for ANS warm water treatment.

CONCLUSIONS

In situ oil droplet size distribution, particle concentration and fluorescence data from this study are essential for better understanding dispersion effectiveness and for refining droplet formation and trajectory models, such as modified Weber Number technique (Johansen et al., 2013) and the VDROP-J model (Zhou et al., 2014). Thus, findings from this study have implications for predicting the behavior of oil and plume formation, decision-making pertaining to SSDI as a countermeasure, and assessing the transport and fate of slicks and subsurface plumes in support of emergency response operations during any future deepwater spills.

ED_001324_00001251-00018

REFERENCES

Conmy, R. N., et al. (2014) Submersible Optical Sensors Exposed to Chemically Dispersed Crude Oil: Wave Tank Simulations for Improved Oil Spill Monitoring. Environ. Sci. Technol. 48(3): 1803-1810.

Cole, M.G., T. King, K. Lee (2007) Analytical technique for extracting hydrocarbons from water using sample container as extraction vessel in combination with a roller apparatus. Anal. Chem., 77: 2210-17.

CRRC Report (2010) Coastal Response Research Center Deepwater Horizon Dispersant USE Meeting Report, May 26-27, 2010, 108 pp.

EPA 600/F-16/250 Federal Report (2016) Dispersant Effectiveness, In-Situ Droplet Size Distribution and Numerical Modeling to Assess Subsurface Dispersant Injection as a Deepwater Blowout Oil Spill Response Option *and* Evaluation of Oil Fluorescence Characteristics to Improve Forensic Response Tools; IAA No. E12PG00037 for DOI BSEE, 144 pp.

Joint Analysis Group Report (2010) Review of Data to Examine Subsurface Oil in the Vicinity of DWH MC-252, May 19-June 19, 2010; 29 pp.

Johansen, O., et al. (2013) Droplet breakup in subsea oil releases - Part 2: Predictions of droplet size distributions with and without injection of chemical dispersants. Mar. Poll. Bull. 73(1): 327-335.

Lee, K., Li, Z., Boufadel, M.C., Venosa, A.D. and Scott Miles, M. (2009) Wave tank studies on

ED_001324_00001251-00019

dispersant effectiveness as a function of energy dissipation rate and particle size distribution. Final report submitted to NOAA/CRRC/UNH, 67 pp.

Li, Z., Lee, K., King, T., Boufadel, M.C. and Venosa, A.D., (2008) Oil droplet size distribution as a function of energy dissipation rate in an experimental wave tank 2008 International Oil Spill Conference. American Petroleum Institute, Washington D.C., Savannah, GA, pp. 621-626.

Li, Z., Lee, K., King, T., Boufadel, M.C. and Venosa, A.D., (2009a) Evaluating crude oil chemical dispersion efficacy in a flow-through wave tank under regular non-breaking wave and breaking wave conditions. Mar. Poll. Bull., 58: 735-744.

Li, Z., Lee, K., King, T., Boufadel, M.C. and Venosa, A.D., (2009b) Evaluating Chemical Dispersant Efficacy in an Experimental Wave Tank: 2, Significant Factors Determining *In Situ* Oil Droplet Size Distribution. Environ. Engin. Sci., 26(9): 1407-1418.

Li, Z., Lee, K., King, T., Boufadel, M.C. and Venosa, A.D., (2010) Effects of temperature and wave conditions on chemical dispersion efficacy of heavy fuel oil in an experimental flow-through wave tank. Mar. Poll. Bull., 60(9): 1550-9.

NRC Report (2005) National Research Council Oil Spill Dispersants: Efficacy and Effects, Washington, DC, 400 pp.

Wickley-Olsen, E., Boufadel, M.C., King, T., Li, Z., Lee, K., Venosa, A.D. (2007) Regular and breaking waves in wave tank for dispersion effectiveness testing. In: Proceedings of the 30th Arctic and Marine Oil spill Program (AMOP) Technical Seminar, Edmonton, AB, Canada. Environment Canada, Ottawa, Ontario, Canada, pp. 161–187.

Zhao, L., et al. (2014). Evolution of droplets in subsea oil and gas blowouts: Development and validation of the numerical model VDROP-J. Mar. Poll. Bull., 83(1): 58-69.

ED_001324_00001251-00020

To: Ian Walsh[ian@wetlabs.com]
From: Conmy, Robyn
Sent: Fri 9/4/2015 6:11:27 PM
Subject: FW: [Ocean-Color] NASA Arctic scoping study DRAFT report - open for comments
ATT00001.txt
2016 gulf spill conference abstract_conmy.docx

Here is the Arctic COLORS Field Campaign Proposal that we discussed. Comments are due Sept 17. Also, my GoMRI abstract is attached.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Ocean-Color [mailto:ocean-color-bounces@oceancolor.sci.gsfc.nasa.gov] **On Behalf Of** gene carl feldman
Sent: Friday, August 28, 2015 7:58 PM
To: ocean-color@seawifs.gsfc.nasa.gov
Subject: [Ocean-Color] NASA Arctic scoping study DRAFT report - open for comments

Subject: NASA Arctic scoping study DRAFT report - open for comments

Dear Colleagues,

The Arctic-COLORS Draft Report is available for community comment (Aug. 18 – Sept. 17, 2015).

This document is a draft report for the NASA Ocean Biology and Biogeochemistry (OBB) Program describing a candidate Field Campaign program called Arctic-COLORS (Arctic-COastal Land Ocean interactions). The goal of the proposed field campaign is to determine present and future impacts of terrigenous, atmospheric and oceanic fluxes on ecology, biogeochemistry, biodiversity and ecosystem services of the Arctic coastal zone. This scoping study report describes and justifies the science objectives, design and implementation plan of an integrative and interdisciplinary field campaign, modeling and remote sensing program. The Arctic-COLORS science team is requesting comments from the community prior to final submission of the report to NASA. This is an opportunity for you to refine the science and scope of the Arctic-COLORS program. After the open comment period, our team will revise and submit the final eport to NASA, which will proceed with a peer-review and programmatic evaluation of Arctic-COLORS. Your comments can help us improve the report and thus increase the likelihood of NASA to approve funding for this program. Feel free to contact us if you have any questions.

- **View the Arctic-COLORS Report: https://neptune.gsfc.nasa.gov/uploads/Arctic-COLORS_Report.pdf**
- **Comment Submission e-mail: arctic-colors@lists.nasa.gov**
- **Website link: http://arctic-colors.gsfc.nasa.gov**

The Arctic-COLORS draft report is posted for a 30-day comment period open to all. You may submit your comments using the "Comment Submission Form" link above. Please refer to a page and paragraph number (e.g. Page 15, 2nd paragraph) in your comment if applicable.

- Section 1 describes the motivation for the Arctic-COLORS program.
- Section 2 describes the engagement of the broader scientific community.
- Section 3 describes overarching objective and science questions.
- Section 4 describes the science plan - study domain, research phases and timeline of the field campaign, measurements, remote sensing, and modeling activities.
- Section 5 describes the implementation plan.

We look forward to your feedback and comments, and we thank you for your contributions to this effort.

The Arctic-COLORS PIs,

Carlos Del Castillo, Marjorie Friedrichs, Peter Hernes, Antonio Mannino, Patricia Matrai, Joseph Salisbury, and Maria Tzortziou

Antonio Mannino, Ph.D.

Oceanographer

NASA Goddard Space Flight Center

Ocean Ecology Lab

Mail Code 616.1

Building 22 Room 250

Greenbelt, MD 20771

Phone: 301-286-0182

Lab Phone: 301-286-2920

e-mail: antonio.mannino@nasa.gov

http://neptune.gsfc.nasa.gov/osb/

Subsea oil plume simulations: Tracking oil droplet size distribution and fluorescence within high release jets

R.N. Conmy[1], **B. Robinson**[2], **T. King**[2], **M. I. Abercrombie**[3], **S. Ryan**[2], **C. McIntyre**[2], **M. Boufadel**[4] **K. Lee**[2,5]

[1]U.S. Environmental Protection Agency, Office of Research and Development, NRMRL

[2] Bedford Institute of Oceanography, Dept. Fisheries and Oceans Canada

[3]University of South Florida, College of Marine Science

[4] New Jersey Institute of Technology

[5] CSIRO Australia

Optical measurements have been used during oil spill response for more than three decades to determine oil presence in slicks and plumes. Oil surveillance ranges from simple (human eyeball) to the sophisticated (sensors on AUVs, aircraft, satellites). In situ fluorometers and particle size analyzers were deployed during the Deepwater Horizon (DWH) Gulf of Mexico oil spill to track shallow and deep subsea plumes. Uncertainties regarding instrument specifications and capabilities necessitated performance testing of sensors exposed to simulated, dispersed oil plumes. Presented here are results of 72 wave tank experiments conducted at the Bedford Institute of Oceanography. Examined were simulations of subsea releases with varying parameters such as oil release rate, oil temperature (reservoir temp ~ 80 °C), water temperature (<8 °C and >15 °C), oil type, dispersant type (Corexit 9500 and Finasol OSR52) and dispersant to oil ratio (DOR). Plumes of Alaskan North Slope Crude, South Louisiana Crude and IFO-120 were tracked using in situ fluorescence, droplet size distribution (DSD; LISST 100X), total petroleum hydrocarbons (TPH), benzene-toluene-ethylbenzene-xylene (BTEX) and excitation-emission matrix spectroscopy. Results offer valuable information on the behavior and dispersibility of oils over a range of viscosity and composition. Findings have implications for fate & transport models, where DSD, chemistry and fluorescence are all impacted by release variables. Research supported by the Bureau of Safety and Environmental Enforcement.

To: Clark, Patrick[Clark.Patrick@epa.gov]
From: Conmy, Robyn
Sent: Wed 4/27/2016 11:33:47 AM
Subject: FW: Invoice for Finasol sample
Clearcoast inv 150212.pdf

Attached is the invoice to purchase dispersants for the oils program. Please charge to oil.

Thanks!

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Admin Clearcoast [mailto:admin@clearcoastllc.com]
Sent: Tuesday, April 26, 2016 6:15 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Malcolm Gore <malcolm.gore@clearcoastllc.com>
Subject: Invoice for Finasol sample

Robyn,

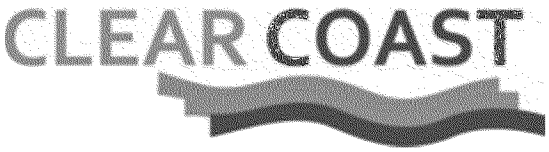
Please fid attached an invoice for sample of Finasol ORS 52 as requested.

Please be advised payment should be made on receipt of product.

Please do not hesitate to contact us if you need any further information

Many thanks for your order

Admin



www.clearcoastllc.com

To: Barron, Mace[Barron.Mace@epa.gov]
From: Conmy, Robyn
Sent: Fri 4/1/2016 1:39:03 PM
Subject: RE: "Final" tox testing details for subpart J

Which 4 dispersants? Our choice or did OEM specify? We are conducting a series of BFTs this summer targeting Corexit 9500, Finasol OSR52, Accell Clean DWD and Dispersit SPC1000. Just an FYI in case specific ones haven't been identified.

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Barron, Mace
Sent: Thursday, March 31, 2016 5:10 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: RE: "Final" tox testing details for subpart J

Its plenty of $.

I just sent email to you, Greg and Vanessa asking about the oil+dispersant test.

I suspect we will have a call!

From: Conmy, Robyn
Sent: Thursday, March 31, 2016 2:20 PM
To: Barron, Mace <Barron.Mace@epa.gov>
Subject: FW: "Final" tox testing details for subpart J

In looking more closely at the tests, do you think $100K will be enough to run one oil and the 4 dispersants alone? I do think it's odd that no oil + dispersant will be run too.

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Barron, Mace
Sent: Thursday, March 31, 2016 3:01 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: FW: "Final" tox testing details for subpart J

Found it: had put in gomri folder because I was summarizing as part of my tampa talk.

I will keep looking for others.

So you are right, there was no oil+dispersant testing, but there is dispersant only testing with 4 dispersants.

From: Principe, Vanessa
Sent: Monday, December 14, 2015 11:14 AM
To: Barron, Mace <Barron.Mace@epa.gov>
Cc: Wilson, Gregory <Wilson.Gregory@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: FW: "Final" tox testing details for subpart J

Hey Mace:

Is this what you have in mind (see forwarded email below)?

Also attached is a PDF of the Sub J proposed rule. Specific to toxicity testing for dispersants, the preamble discussion on what we proposed is in pages 3404-3405. The rule text for what we proposed is at 3425 and Appendix C, the Tox protocols for dispersants is at 3434-3436 (Section 3).

Let us know if this answers your question or if you need anything else.

V & G

From: Barron, Mace
Sent: Monday, May 18, 2015 12:39 PM
To: Principe, Vanessa <Principe.Vanessa@epa.gov>
Cc: Wilson, Gregory <Wilson.Gregory@epa.gov>
Subject: "Final" tox testing details for subpart J

Acute: Oil only (2 oils):

*mysid (2)

*menidia (2)

**Oil WAF characterization (2 oils):

TPH in 100% WAF (can extrapolate TPH to test concentrations based on dilution…eg., LC50 = 20% WAF= 0.2TPH100%).

Acute: dispersant only (4 dispersants)

*mysid (4)

*menidia (4)

Subchronic dispersant only (4 dispersants):

*urchin development (4)

*mysid 7 day (4)

*menidia 7 day (4)

-

My guess is $200K for all the above. Please confirm the list, then we can send to Robyn.

Environmental Science & Technology

Article

pubs.acs.org/est

Submersible Optical Sensors Exposed to Chemically Dispersed Crude Oil: Wave Tank Simulations for Improved Oil Spill Monitoring

Robyn N. Conmy,[†,*] Paula G. Coble,[‡] James Farr,[□] A. Michelle Wood,[§,◆] Kenneth Lee,[//,¶] W. Scott Pegau,[⊥] Ian D. Walsh,[#] Corey R. Koch,[#] Mary I. Abercrombie,[‡] M. Scott Miles,[▽] Marlon R. Lewis,[○] Scott A. Ryan,[//] Brian J. Robinson,[//] Thomas L. King,[//] Christopher R. Kelble,[§] and Jordanna Lacoste[○]

[†]National Risk Management Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, 26 West Martin Luther King BoulevardCincinnati, Ohio 45268, United States

[‡]College of Marine Science, University of South Florida, St. Petersburg, Florida 33701, United States

[□]Office of Response and Restoration, National Oceanic and Atmospheric Administration, 1305 East-West Highway, Silver Spring, Maryland 20910, United States

[§]Atlantic Oceanographic & Meteorological Laboratory, National Oceanic and Atmospheric Administration, 4301 Rickenbacker Causeway, Miami, Florida 33149, United States

[//]Department Fisheries and Oceans Canada, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada

[⊥]Oil Spill Recovery Institute, 300 Breakwater Avenue, Cordova, Alaska 99574, United States

[#]WET Labs, Inc., PO Box 518, Philomath, Oregon 97370, United States

[▽]School of Coast and Environment, Louisiana State University, Baton Rouge, Louisiana 70803, United States

[○]Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada

* Supporting Information

In situ fluorometers were deployed during the Deepwater Horizon (DWH) Gulf of Mexico oil spill to track the subsea oil plume. Uncertainties regarding instrument specifications and capabilities necessitated performance testing of sensors exposed to simulated, dispersed oil plumes. Dynamic ranges of the Chelsea Technologies Group AQUAtracka, Turner Designs Cyclops, Satlantic SUNA and WET Labs, Inc. ECO, exposed to fresh and artificially weathered crude oil, were determined. Sensors were standardized against known oil volumes and total petroleum hydrocarbons and benzene-toluene-ethylbenzene-xylene measurements - both collected during spills, providing oil estimates during wave tank dilution experiments. All sensors estimated oil concentrations down to 300 ppb oil, refuting previous reports. Sensor performance results assist interpretation of DWH oil spill data and formulating future protocols.

INTRODUCTION

SMART (special monitoring of applied response technologies) protocols use real-time fluorescence monitoring in decision-making during oil spill dispersant operations[1] to determine efficacy of chemically enhanced dispersion and dispersed oil transport.[2] Chemical dispersants applied to oil slicks (in the presence of mixing energy) reduce the oil−water interfacial tension, forming small oil droplets (<100 um) that are less likely to recoalesce,[3] removing oil from the water surface and suspending neutrally buoyant oil droplets as a subsurface plume. This reduces risk to shorelines and air−water interface biota and also increases microbial biodegradation rates of oil.[4−6] Under SMART, in situ monitoring of surface waters (<2 m) and below (2 - 10 m) evaluates efficacy. Monitoring requires rapid, reliable, easy-to-operate in situ fluorometers. Validation with oil/water samples yields oil mapping with finer temporo-spatial resolution not achieved through coarse discrete measurements alone.

Fluorescence has been an oil spill monitoring tool for more than 30 years.[7,8] Measurements are inherently sensitive, conducted without laborious extraction techniques, time

Received: September 20, 2013
Revised: December 17, 2013
Accepted: December 30, 2013

 © XXXX American Chemical Society

ED_001324_00001261-00001

efficient, chemistry dependent and indicators of hydrocarbon concentration.[9,10] Low viscosity physically dispersed crude oils typically have two broad UV peaks with excitation (Ex) between 240 and 300 nm and emission (Em) centered at ~350 nm (low molecular weight polycyclic aromatic hydrocarbons, LMW PAH, <3 benzene rings) and ~450 nm (higher MW PAH, >3 benzene rings).[11,12] Fluorescence signatures discriminate oils[11−14] but are complicated by varying concentration, degree of chemical and physical dispersion, oil age and state (fresh, weathered, mousses),[11,14,15] and interference of fluorophores with spectro-chemical similarities. Such is the case with colored dissolved organic matter (CDOM) and oil aromatic fractions, which exhibit broad fluorescence peaks and complex spectra. However, crude oils have peak maxima at slightly shorter wavelengths relative to aquatic CDOM (humics, fulvics), facilitating discrimination.

Optical oil detection is a function of matching Ex/Em wavelengths with oil (center wavelength (CWL) and filter bandwidth), power of the light source, and detector sensitivity. Numerous submersible fluorometers are commercially available, each with unique configurations for detecting compounds[16] (and references therein), thus expanding their use in spill response efforts and SMART protocols.[17] Reviews of commercial off-the-shelf (COTS) sensors[17−19] highlight the need for robust calibrations, operational procedures and scalability testing.[20] Reviews have targeted easy-to-conduct, low-cost bench-scale testing. Often tests are conducted in the absence of dispersant, in small flasks with insufficient mixing energy to physically disperse oil into water,[21] at elevated oil concentrations irrelevant to field observations, with limited oil type and weathering states, and without calibration to standards or hydrocarbon concentrations. Thus there are confidence challenges in establishing saturation cutoffs and detection limits relevant for field applications. Effectiveness in quantitative monitoring also remains unclear.[19] Hence, caution must be exercised when scaling up experimental designs to real-world conditions and decision-making. Rather, a combination of laboratory, wave tank, and field studies has been recommended to establish the best degree of confidence for detecting chemically dispersed oil during environmental spills.[22]

SMART provides guidance on surface, not deepwater spill response like the 2010 Deepwater Horizon spill (DWH) where ~800 million liters of light Louisiana sweet crude oil was released from 1500 m ocean depth. Seven million liters[23] of chemical dispersant (Corexit© 9527, 9500A) were applied to reduce environmental impacts of oil, and aid worker safety. The response effort proved challenging partly due to the inability to visually inspect the subsea oil plume. The horizontal, vertical and temporal extent of, and heterogeneity of the plume equated to a "needle-in-the-haystack" situation with grab sampling too coarse to capture its magnitude and extent. Sample number limitations and oil stratification within bottles during the 1−2 h profiler transit time also contributed to challenges in quantifying oil.[24] Thus, responders and scientists were evermore reliant on submersible sensors in lieu of visual observations to provide critical tracking measurements. Initial plume tracking between 1000 and 1400 m was conducted using WET Labs, Inc. ECO fluorometers. Their accessibility and wavelength configuration- wide filter bandwidth and ExEm370/460 nm CWL (Supporting Information (SI) Table S2) to capture broad nature of the peaks- is appropriate for crude oil fluorescence detection. Chelsea Technologies Group AQUAtracka fluorometers were deployed to monitor the transport and fate of oil at large distances from the wellhead and after well capping (latter response, low concentrations).[24] This unit employs filters (ExEm239/360 nm CWL) to capture peak fluorescence from petroleum aromatic fractions with elevated sensitivity (higher signal-to-noise) due to a xenon lamp light source and a photomultiplier tube (PMT) detector; possibly aiding MC252 oil tracking which has a small fraction of highly fluorescing molecules (~20%).[24]

In the wake of the spill many questions remain regarding oil quantification and fate of the subsea oil plume, as well as sensor applicability to detect oil (dynamic range, detection limits, and wavelength configuration).[25] Effects of concentration, dispersant to oil ratio (DOR), dispersant effectiveness,[26] and fluorescence signatures[12] contribute to uncertainties in selecting sensors. Moreover, concerns persist on sensor reliability and applicability of laboratory calibrations using flasks, high oil concentrations and insufficient mixing energies to real-world conditions[19,25] To address uncertainties the performance of select COTS in situ fluorometers deployed during DWH (Chelsea Technologies Group, Turner Designs, WET Labs, Inc.) and a UV spectrophotometer (Satlantic) was evaluated in the Bedford Institute of Oceanography (BIO) wave tank. This work is timely, as findings will assist in the calibration and interpretation of DWH fluorescence measurements. It also coincides with the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) amendments pertaining to fluorometer protocols, reflecting the need for improved confidence in sensors for estimating spilled oil concentrations.

EXPERIMENTAL SECTION

Wave Tank Description. Experiments were conducted at the BIO, Center for Offshore Oil, Gas and Energy Research (COOGER; Dartmouth, Nova Scotia) wave tank, measuring 32 m long, 0.6 m wide, and 2 m high (1.5 m water depth; 28 800 L volume) (SI Figure S1). Water from the Bedford Basin of Halifax Harbor was pumped into the tank through a coarse (25 μm pore size) and fine (5 μm pore size) serial filtration system. Breaking and nonbreaking waves (computer-controlled flat-type wave maker) provided mixing energies to achieve dispersant effectiveness observed in the field. The tank was drained and cleaned with Big Orange detergent and seawater (tank walls, bottom, wave maker and absorbers) after each experiment to remove all oil and surfactants, and sensor optical windows were also cleaned.

Experimental Design. Experiments were conducted using MC252 crude oil (Discoverer Enterprise drillship recovered, May 31−June 7, 2011), and Corexit© 9500 chemical dispersant (1:25 DOR). Artificially weathered oil was generated by bubbling with nitrogen for 36 h (average time responders typically begin dispersant) resulting in mass reduction by 7%. Experimental duplicate and triplicate treatments were run with random design, reducing effects from confounding factors such as wind, rain, temperature, salinity, and seawater quality (SI Table S1). Prior to experiments, oil and dispersant mixtures were prepared in 1 L of seawater by orbital shaker agitation (20 min). Fluorometers (WET Labs, Inc. ECO, Chelsea Technologies Group AQUAtracka, Turner Designs Cyclops) and a UV spectrophotometer (Satlantic SUNA) were deployed at the same cross-sectional position within the tank 20 m from the wave generator (10 m downstream from oil release). Configuration specifications are shown in SI Table S2 and Figure S1. A laser in situ scattering and transmissometry particle size analyzer (Sequoia Scientific LISST-100X, C-Type,), 1 m

ED_001324_00001261-00002

downstream from sensors, collected continuous particle size distribution measurements. Sensors were mounted so that optical windows were positioned 65 cm deep in the tank and warmed up for 15 min prior to oil addition and collection of baseline. A stepwise addition experiment (EXP_{ADD}) of chemically dispersed fresh oil (300 ppb to 12 ppm, tank operated in steady-state mode) was conducted to establish response linearity and dynamic range of sensors to MC252 oil. Each addition of oil/dispersant was allowed to homogenize, with wave mixing, for 1 h prior to subsequent oil additions. Water samples were collected during the final 5 min of each step and sensors were cleaned in between steps using lens wipes and solvent. There was no indication of sensor fouling from oil as sensor response was checked pre- and postcleaning. Continuous optical data were collected during the 9 h experiment and manufacturer calibrations applied.

Dilution experiments (EXP_{DIL}) were conducted with 100 mL of fresh or weathered oil added, with or without dispersant (only chemically dispersed results reported here). Maximum concentration in the tank was less than 3 ppm, when fully dispersed. To simulate field conditions, ocean waves were generated with tank in flow-through mode (flow rate of 3.8 ± 0.2 L s^{-1}) allowing transport of dispersed oil away from the slick and dilution to extinction.[27,28] EXP_{DIL} were conducted for 90 min, where discrete oil concentration samples were collected at 15 time points via tubing through an inlet located next to the sensor deployment frame. Three-dimensional excitation emission matrix fluorescence spectroscopy (EEMS) analyses confirmed the position of fluorescence peaks and degree of chemical dispersion.

Submersible Sensor Data Processing. Sensor specific blanks (2 min average) prior to oil addition were subtracted. Manufacturer calibrations yielded data in recommended units (carbazole and perylene for AQUAtrackas, quinine sulfate dihydrate for ECO, uM nitrate for SUNA weighted index of UV absorption 210−370 nm, and none was provided for the Cyclops). Final 5-min signal averages for each concentration during the EXP_{ADD} was regressed (model 1, linear least-squares) to ppm oil, benzene-toluene-ethylbenzene-xylene (BTEX) or total petroleum hydrocarbons (TPH) values. Regressions were applied to EXP_{DIL} fluorescence and absorption data. Sensor performance and vicarious calibration were validated with laboratory chemistry results. LISST-11X dispersed oil droplet size distribution recorded 32 particle size intervals logarithmically spaced from 2.5−500 μm in diameter, with the upper size in each bin 1.18 times the lower. Distribution is expressed as the average volumetric concentration of droplets within each size range interval. Total particle concentration (TPC) represents the sum of all particle concentrations between 2.5 and 500 μm.[27]

Discrete Samples Analyses. Oil/water samples were collected at time points and analyzed for TPH, BTEX and PAH. TPH values were acquired using a GC-FID (gas chromatograph-flame ionization detector, Agilent 6890 and Supelco column). Extraction methods[29] were used and samples were concentrated under nitrogen to a final volume of 1.0 mL prior to analysis. Peak quantification, using the total area under the curve, yielded hydrocarbon concentrations in seawater. A spike and recovery study with blanks and duplicates verified extraction method efficiency. BTEX (volatile hydrocarbons) were measured via GC-MS (GC-mass spectrometry, Agilent 5973) using a modified version[29] of the standard EPA Method 8240 (purge and trap) for oil applications. A 5 mL sample was transferred into the purge chamber and purged with helium for 11 min. The volatiles were trapped and then desorbed at 225 °C for 2 min. The MS was used in selected ion monitoring mode (SIM) for six ions: 77, 78, 91, 92, 105, and 106 amu. BTEX calibration standards were prepared by diluting a certified standard mix (Supelco). Both standard and samples (blanks and duplicates) were purged, trapped and analyzed in the same manner.

EEM and absorbance spectra were collected with a Horiba Scientific AquaLog spectrofluorometer with a CCD detector. Oil/water samples were analyzed for fluorescence peaks within 10 min of collection. Samples within a 1 cm cuvette were scanned over Ex 220−550 nm and Em 213−610 nm (1 s integration), blank subtracted, inner-filter effect corrected, quinine sulfate dihydrate normalized, and Rayleigh scatter masked.[30,11]

RESULTS AND DISCUSSION

An EEM contour plot of chemically dispersed MC252 oil (4 ppm) collected during an EXP_{DIL} illustrates the UV fluorescent peak maxima centered on ExEm270/325 and ExEm260/450 nm

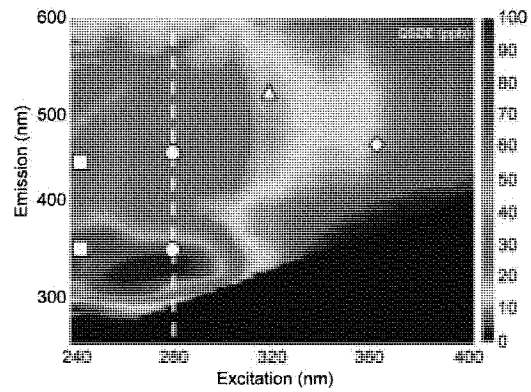


Figure 1. Fluorescence EEM of chemically dispersed fresh MC252 oil (4 ppm) concentration in a EXP_{DIL}. Center Wavelengths (CWL) of Chelsea Technologies Group AQUAtrackas $ExEm_{239/360}$ and $ExEm_{239/440}$ (□), Turner Designs Cyclops $ExEm_{320/510}$ (△), and WET Labs, Inc. ECO $ExEm_{370/460}$ (◊) along with the fluorescence intensity ratio (FIR = $ExEm_{280/340:\ 280/445}$; ○) are overlaid for reference.

(Figure 1). Color map units are ppb QSDE (Quinine Sulfate Dihydrate Equivalents), scaled to peak maximum. CWL of the sensors tested are overlaid illustrating that all sensors were configured to detect some portion of the broad oil signal. Note that the symbols do not take into account wavelength bandwidths, which increases a sensor's ability to detect oil. For reference purposes, white circles on the dotted line represent the fluorescence intensity ratio (FIR) wavelengths used to indicate chemical dispersion of oil.[12]

Manufacturer calibrated sensor response at six cumulative oil concentrations during the EXP_{ADD} is shown in Figure 2 and SI Figure S2. The addition of oil and dispersant formed an oil plume with a sharp leading edge that passed in front of the sensors causing a rapid increase in signal followed by a decrease as the oil mixed/dispersed in the tank. Values at the end of each step (tank homogeneously mixed) are representative of sensor response for a specific oil concentration and established that all sensors detected chemically dispersed oil down to 300 ppb, a critical finding. This is below the laboratory detection limit for GC-FID TPH method, making sensor-estimated oil concentrations evermore valuable for tracking low concentration

ED_001324_00001261-00003

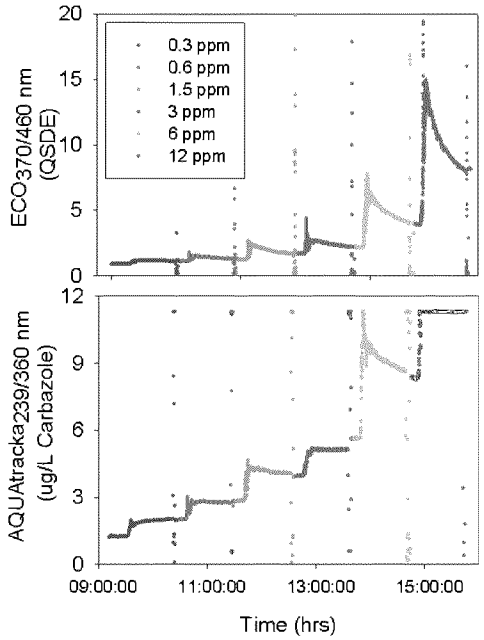


Figure 2. Fluorescence intensity of the ECO and $AQUA_{239/360\ nm}$ sensors exposed to chemically dispersed MC252 crude oil during EXP_{ADD} within the wave tank.

plumes. It also refutes previous notions during the DWH response that certain sensors may not detect oil <1 ppm. This was supported by earlier calibration tests using small flasks which may have been biased by underachieving proper mixing energies negatively impacting dispersant efficiency and solubility of fluorescence molecules into the water phase.[22] Strong linearity of sensor response at the lowest concentrations suggests possible lower detection limits less than 300 ppb (not tested with this experimental design). In any case, sensor response at 300 ppb improves confidence in the instruments deployed for DWH subsea plume monitoring, thereby demonstrating ability to detect low concentrations of oil.

Sensor dynamic range was established during the EXP_{ADD} by regressing fluorescence and absorption averages with oil concentration (Figure 3). Generally, submersible sensors were linearly correlated with TPH, BTEX and oil load, regardless of optical configuration. The ECO was operated in digital mode (no gain control) and the SUNA employed autoadjusting integration time, evidenced by lack of saturation over the entire concentration range. The Cyclops was operated with 100X gain for concentrations ≤6 ppm, but 10X for 12 ppm to avoid saturation. The AQUAtrackas employ BITE (Built in Test Circuitry) to extend calibration intervals. However saturation was exhibited by the $AQUAtracka_{239/360\ nm}$ sensor at 12 ppm oil. This is attributed to higher detector sensitivity, increased power of the light source (flash lamp opposed to LED) and/or wavelength filters closely aligned to the fluorescence maxima of crude oil. The longer wavelength emission AQUA $tracka_{239/440\ nm}$ sensor however did not saturate. Regression equations were employed to calibrate sensors during EXP_{DIL} (Figure 3). This aligns results with response effort measurements: sTPH (speciated TPH; water-insoluble heavy saturate fraction; aromatics > naphthalene; less laborious analytical screening) supplemented with BTEX (more laborious screening) values (Figure 3). All sensors exhibited squared correlation coefficients greater than 0.96. The 12 ppm value for the $AQUAtracka_{239/360\ nm}$ and SUNA (for BTEX) was excluded from the calculation due to saturation. The ECO and BTEX regression excluded values above 1.5 ppm, thereby increasing performance at low concentrations. TPH values for lowest concentration additions are absent because they were below the detection limit of the laboratory-based GC-FID TPH analysis. For all sensors, signal-to-noise ratios permitted detection at low concentrations. Findings demonstrate that all sensors would have been valuable tools during the DWH response given that sTPH and tVOA (total volatile organic analysis; water-soluble fraction; namely BTEX and naphthalene) in field samples were within the linear range of sensors tested in this study.[24] During the spill, sTPH from GC-MS ranged between <1 - 1000 ppb, with subsea plume (900−1300 m) maximum of 485 ppb, 1.2 km from the wellhead and detectable levels extending >10 km from source. In the plume, tVOA concentrations were higher than sTPH, with a maximum of 2112 ppb.

Sensor performance as a function of dispersant presence, oil state, and plume dilution was examined during EXP_{DIL}. TPH and BTEX-calibrated sensor responses for one-fresh and one-weathered, chemically dispersed oil EXP_{DIL} are shown in Figure 4 and SI Figure S3, respectively. Left panels show TPH-calibrated sensor and discrete TPH results (open circles), right panels show BTEX-calibrated sensor and discrete BTEX results (gray circles) over the course of the 90 min experiment. Due to a delayed shipping issue, the $AQUAtracka_{239/440\ nm}$ was operated only during one EXP_{DIL} and appears in the latter figure. EXP_{DIL} particle size distribution (PSD) and concentration of fresh oil droplets corresponding to Figure 4 are shown in Figure 5 (left). Histograms represent a composite of the entire duration of a flow-through experiment (90 min). Presence of particles observed in the <100 um size classes are indicative of chemical dispersion. All experiments using dispersant exhibited oil droplet size ranges consistent with adequate dispersant effectiveness (droplets <100 μm, Figure 5).[26]

For all experiments, MC252 oil state (fresh or weathered) did not appear to influence the sensor response. All sensors responded similarly to both (Figure 4 and SI Figure S3), verified with similar particle size distribution. Like sensor response as a function of oil state lends support for applying regression equations calculated with fresh oil to dilution experiments using weathered oil. Note that oil artificially weathered by 7% may represent oil exposed to physical processes (volatilization due to photo-oxidation, evaporation or dissolution) that occur during a spill of short duration, not necessarily of a prolonged spill with extensive weathering. However, the degradation of light crude oil and the addition of dispersant have been shown to increase the occurrence of longer wavelength fluorescence peaks.[11,15] Hence, sensors may be better capable of detecting extensively weathered oil where wavelength configuration better overlaps with longer fluorescence peaks (Figure 1). This is particularly true for lighter crudes such as MC252 oil that contain a higher proportion of simpler, lower molecular weight hydrocarbons that are more readily physically and biodegraded compared to heavy crudes.[31]

Tank hydrodynamics affected the movement and dispersion of oil within the tank, which varied during dilution experiments. Total particle concentration (TPC) as a function of time was influenced by tank mixing, where experiments exhibited either a gradual or rapid (Figure 5, left and right) decay of oil droplets. Three tests exhibited a gradual increase in signal as oil passed the sensors in the tank followed by gradual decay with dilution (Figure 4). For these cases, maximum values were 4−5 ppm

ED_001324_00001261-00004

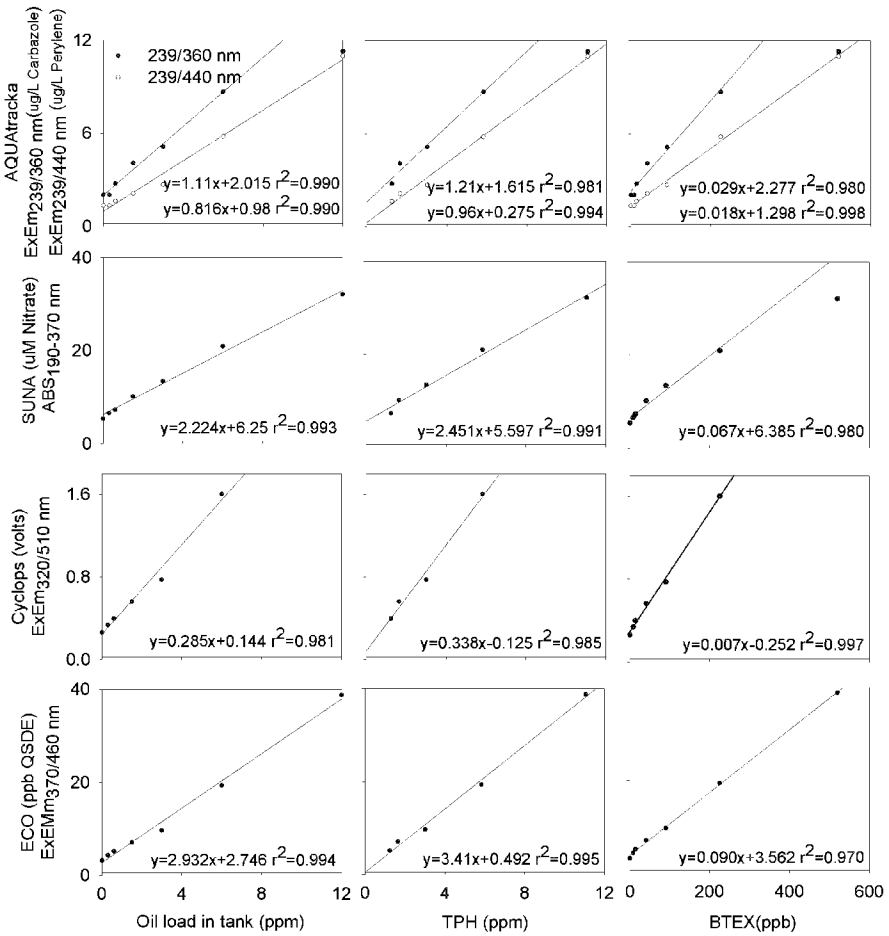


Figure 3. Fluorometric (submersible fluorometers) and spectrophotometric response (SUNA) as a function of oil concentration between 300 ppb and 12 ppm during EXP_{ADD}. Fewer TPH data points due to concentrations below the detection limit of TPH laboratory method.

TPH and 200−250 ppb BTEX, with subsequent plume dilution to 2−3 ppm TPH and 50 ppb BTEX. LISST-100X response (Figure 5, left) indicates like mixing and dispersing conditions in those experiments. Plume dynamics differed in two tests, where steep increases in oil concentration were followed by rapid decay (Figure 6), supported by TPC and droplet size distribution (Figure 5, right) and maximum TPH and BTEX values of 6 ppm and 250 ppb, followed by dilution to concentrationsakin to all experiments. TPH and BTEX-calibrated sensor responses follow the chemistry and oil droplet results in all experiments. Agreement among these parameters is critical as they are used in concert during oil spills to track chemically dispersed oil plumes and confirm degree of dispersant effectiveness.

Sensor response during chemically dispersed EXP_{DIL} was validated with chemistry results to verify sensor performance. Percent differences ($\%_{dif}$) and mean absolute error (MAE) between estimated (from sensors) and actual (chemistry results) oil concentrations were calculated for each time point in experiments, providing performance metrics for estimates of TPH and BTEX for sensors exposed to oil. The MAE and mean, median and average $\%_{dif}$ for each sensor for all time points and for time points excluding 0−10 min are listed in Table 1. Time points <10 min were excluded from calculations due to heterogeneity and timing of the plume leading edge in the tank. The percentage of experiments where calibrated sensor responses were within ±25% and +30% of chemistry results are reported.

Based on $\%_{dif}$, the ECO and AQUAtracka $_{239/440\ nm}$ consistently better estimated BTEX, with 91% and 86% of time points within $30\%_{dif}$, respectively. The AQUAtracka$_{239/440\ nm}$ had the lowest median $\%_{dif}$ of <7.96. These values may be biased, however, as the AQUAtracka$_{239/440\ nm}$ was used in only one flow-through EXP_{DIL}. The ECO median $\%_{dif}$ was slightly higher (12%), but had the highest percentage of time points within $\pm30\%_{dif}$ (91%). Accuracy was also high with MAE of ±17.27 and 12.41 ppb for the ECO and AQUAtracka$_{239/440\ nm}$, respectively. The AQUAtracka$_{239/440\ nm}$, Cyclops and SUNA exhibited similar performance for BTEX where 71−79% values were within $\pm30\%_{dif}$. The AQUAtracka$_{239/360\ nm}$ also exhibited higher accuracy (MAE ± 12.43 ppb), but estimates were less consistent (71% of measurements within $30\%_{dif}$). BTEX consists of single ring aromatics, which are highly volatile and moderately soluble (relative to higher ring no. PAHs) in the shallow water of the wave tank, but any loss did not impact the $\%_{dif}$. Extrapolating this to real-world scenarios, surface spills typically have reduced BTEX or tVOA due to evaporation; however subsea oil plumes would likely retain higher dissolved concentrations.[6] Thus the ECO, AQUAtracka$_{239/360\ nm}$ and AQUAtracka$_{239/440\ nm}$ are each well suited for estimating BTEX in the deep sea, but they do vary with accuracy and precision. Results may differ for medium and

ED_001324_00001261-00005

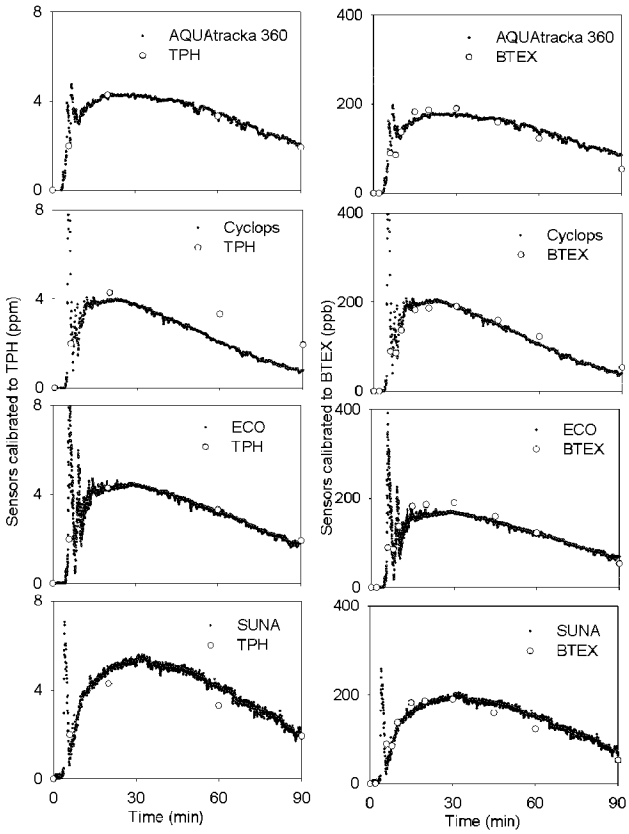


Figure 4. Response of TPH and BTEX-calibrated sensors exposed to chemically dispersed fresh crude oil. TPH and BTEX values from discrete samples are represented by open and gray circles, respectively. Wave tank was operated in flow-through mode (EXP_{DIL}) to observe fluorescence values during the dilution of an oil spill.

heavy oil, however, as BTEX concentrations would vary from the light crude oil tested here.

TPH was better estimated by the SUNA, with respect to both accuracy and precision, followed by the $AQUAtracka_{239/360\ nm}$, with 97% and 84% of time points within 30%$_{dif}$ of actual oil concentrations and MAE of +0.47 and 0.58 ppm, respectively.

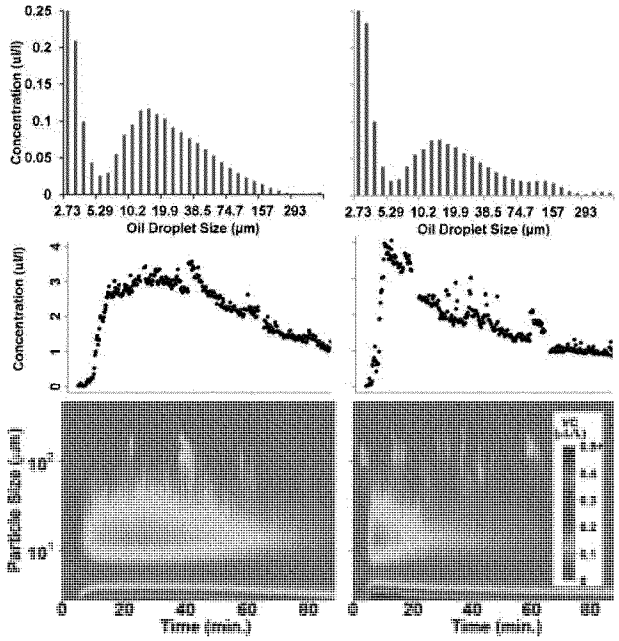


Figure 5. Particle size distribution and concentration within wave tank corresponding to experiments shown in Figures 4 and 6. Histogram plots are composites of entire flow-through experiment. Total particle concentration as a function of time (middle) is shown in scatter plot. Contour plots represent Particle size and volume concentration (VC) as a function of time for chemically dispersed fresh (left) and weathered (right) MC252 crude oil with gradual and rapid decay, respectively.

SUNA had the lowest median %$_{dif}$ of 11% for time points >10 min.

The remaining sensors exhibited median %$_{dif}$ of 18.72−23.87% with fewer values within 30%$_{dif}$ (60−71%) and higher MAE (0.77−0.81 ppm). Compared to BTEX, TPH laboratory analysis captures more compound types which are present at higher concentrations in oil. The SUNA's high performance in estimating TPH may result from absorption, not fluorescence, being measured over a wide wavelength range and oil being comprised of more chromophores than fluorophores. Tank

Table 1. Percent Differences (%$_{dif}$) between Sensors and Chemistry[S]

		TPH (ppm)					BTEX (ppb)				
		ECO 370/460 nm	AQUA 239/360 nm	AQUA 239/440 nm*	Cyclops 320/510 nm	SUNA ABS	ECO 370/460 nm	AQUA 239/360 nm	AQUA 239/440 nm*	Cyclops 320/510 nm	SUNA ABS
all time points	mean %$_{dif}$	31.69	30.24	41.98	40.32	27.92	26.67	30.93	23.14	31.99	30.45
	median %$_{dif}$	23.54	21.15	26.21	25.54	15.68	13.66	20.95	12.34	24.41	23.74
	min %$_{dif}$	0.27	1.96	2.16	1.73	0.76	1.63	3.19	5.29	0.01	0.65
	max %$_{dif}$	183.78	186.85	194.19	199.92	214.04	122.22	128.82	85.95	114.40	140.97
	[MAE]	± 0.91	± 0.67	± 0.78	± 0.90	± 0.68	± 23.53	± 13.46	± 12.41	± 32.22	± 27.68
time points >10 min	mean %$_{dif}$	25.52	19.37	19.22	30.94	13.88	15.54	23.88	14.17	21.11	22.85
	median %$_{dif}$	23.85	18.72	23.87	21.29	11.20	12.00	15.14	7.96	16.67	19.95
	min %$_{dif}$	0.27	1.96	2.16	1.73	0.76	1.63	3.19	5.29	0.01	0.65
	max %$_{dif}$	74.55	53.83	35.22	127.31	46.00	85.57	83.18	31.26	87.71	81.00
	[MAE]	± 0.78	± 0.58	± 0.81	± 0.77	± 0.47	± 17.27	± 12.43	± 12.41	± 29.13	± 24.50
% of >10 min time points	<25%$_{dif}$	56%	72%	57%	56%	90%	86%	68%	86%	64%	63%
	<30%$_{dif}$	69%	84%	71%	60%	97%	91%	71%	86%	79%	74%

[S]Asterisk represents sensor with only one experiment. MAE is mean absolute error.

ED_001324_00001261-00006

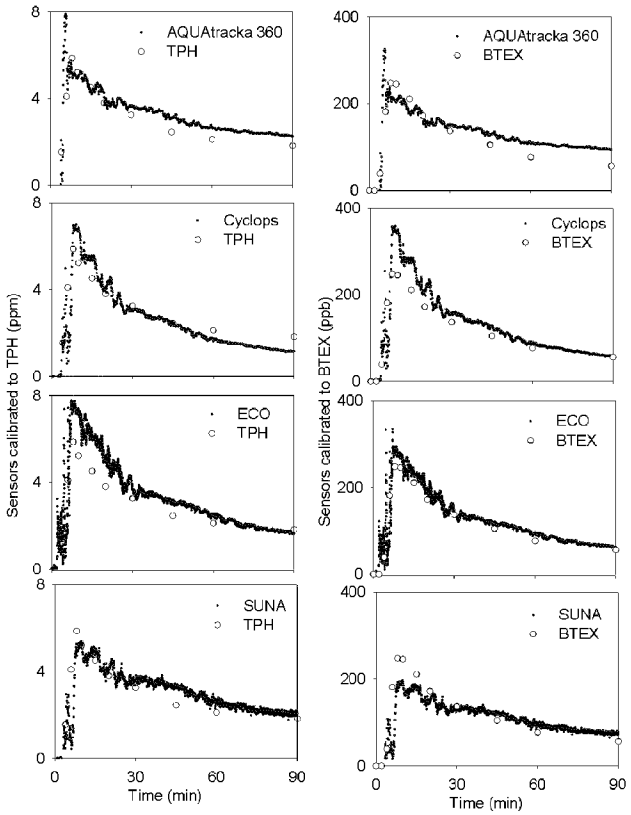


Figure 6. Response of TPH and BTEX-calibrated sensors exposed to chemically dispersed fresh crude oil for dilution experiment with steep increase in particles, followed by rapid decay. TPH and BTEX values from discrete samples are represented by open and gray circles, respectively. Wave tank was operated in flow-through mode (EXP_{DIL}) to observe fluorescence values during the dilution of an oil spill.

results indicate that of the sensors used in the DWH subsea plume, both the $AQUAtracka_{239/360\ nm}$ and ECO would have detected low TPH concentrations. The former may have exhibited better accuracy and precision. However, it could also be speculated that the $AQUAtracka_{239/360\ nm}$ with its narrower dynamic range would have saturated at high concentrations early in the spill and impeded plume tracking. Results are critical and provide evidence that ECO data from early in the spill (high concentrations) can be compared with AQUA data from later in the spill (low concentrations), and that sensors could provide oil concentrations with reasonable accuracy.

Wave tanks simulate natural variability observed in real spills. Thus, vicarious calibration is expected to be more accurate than a laboratory calibration, even though it may suffer from poorer precision. These findings demonstrate the sensitivity of COTS sensors exposed to chemically dispersed MC252 oil and increase confidence using in situ measurements during DWH to fill in the data gaps of coarse discrete samples. During an oil spill, sensor choice should be a function of oil concentration, calibration to TPH or BTEX and trade-offs between accuracy and precision. This work has large implications for plume tracking and understanding fate and transport of spilled oil in the environment.

ASSOCIATED CONTENT

* Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: 513-569-7090; fax: 513-569-7620; e-mail: conmy.rdbyn@epa.gov.

Present Addresses

◆ University of Oregon, Institute of Ecology and Evolution and Institute of Environmental Science, Oregon, USA.

¶ Commonwealth Science and Industrial Research Organization, Wealth from Oceans Flagship, Perth, Australia.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank all funding sources for the experiments at COOGER-BIO (Department of Fisheries and Oceans, Canada): IOOS support (Alliance for Coastal Technology), NOAA's supplemental DWH appropriation, salary support from home institutions, DFO Canada and internal travel support by Horiba JY, Oil Spill Recovery Institute, WET Labs, Inc. This document has been reviewed by the U.S. Environmental Protection Agency National Risk Management Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. This is a contribution from the Land Remediation and Pollution Control Division.

REFERENCES

(1) Report, S. P. *Special Monitoring of Applied Response Technologies Protocol Report*; National Oceanic and Atmospheric Administration, U.S. Coast Guard, U.S. Environmental Protection Agency, 2006; p 43.

(2) Henry, C. B., Roberts, P.O.; Overton, E.B. A primer on in situ fluorometry to monitor dispersed oil. In *International Oil Spill Conference*, 1999; pp 225-228.

(3) Li, M.; Garrett, C. The relationship between oil droplet size and upper ocean turbulence. *Mar. Pollut. Bull.* 1998, *36*, 961−970.

(4) Lessard, R. R.; Demarco, G., The significance of oil spill dispersants. *Spill Sci. Techno. Bull.*, 2000, *6*, (59−68).

(5) Venosa, A. D.; Zhu, X. Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands. *Spill Sci. Technol. Bull.* 2003, *8*, 163−178.

(6) Camilli, R.; Reddy, C. M.; Yoerger, D. R.; Mooy, B. A. S. V.; Jakuba, M. V.; Kinsey, J. C.; McIntyre, C. P.; Sylva, S. P.; Maloney, J. V. Tracking hydrocarbon plume transport and biodegradation at deepwater horizon. *Science* 2010, *330* (6001), 201−204.

(7) Green, D.; Humphrey, B.; Fowler, B. The use of flow-through fluorometery for tracking dispersed oil. In *Internationl Oil Spill Conference*, 1983; pp 473−478.

(8) Page, D. S.; Foster, J. C.; Hotham, J. R.; Pendergasst, E.; Herbert, S.; Gonzalez, L.; Gilfillan, E. S.; Hanson, S. A.; Gerber, R. P.; Vallas, D. Long-term fate of dispersed and undispersed crude oil in two nearshore test spills. In *International Oil Spill Conference*, 1983; pp 479−482.

(9) Keizer, P. D.; D. C., G., Jr. Detection of trace amounts of oil in sea water by fluorescence spectroscopy. *J. Fish. Res. Board Can.* 1973, *30*, 1039−1046.

(10) Østgaard, K.; Jensen, A. Evaluation of direct fluorescence spectroscopy for monitoring aqueous petroleum solutions. *Int. J. Environ. Anal. Chem.* 1983, *14*, 55−72.

ED_001324_00001261-00007

(11) Bugden, J. B. C.; Yeung, C. W.; Kepkay, P. E.; Lee, K. Application of ultraviolet fluorometry and excitation-emission matrix spectroscopy (EEMS) to fingerprint oil and chemically dispersed oil in seawater. *Mar. Pollut. Bull.* 2008, *56* (4), 677−685.

(12) Kepkay, P.; Young, W.; Bugden, J. B. C.; Li, Z.; Lee, K. Ultraviolet fluorescence spectroscopy (UVFS): A new means of determining the effectiveness of chemical dispersants on oil spills. In *International Oil Spill Conference*, Savannah, GA, 2008; pp 639−44.

(13) Li, J.; Fuller, S.; Cattle, J.; Way, C. P.; Hibbert, D. B. Matching fluorescence spectra of oil spills with spectra from suspect sources. *Anal. Chim. Acta* 2004, *514* (1), 51−56.

(14) Christensen, J. H.; Hansen, A. B.; Mortensen, J.; Andersen, O. Characterization and matching of oil samples using fluorescence spectroscopy and parallel factor analysis. *Anal. Chem.* 2005, *77*, 2210−2217.

(15) Zhou, Z.; Liu, Z.; Guo, L. Chemical Evolution of Macondo crude oil during laboratory degradation as characterized by fluorescence EEMs and hydrocarbon composition. *Mar. Pollut. Bull.* 2012, *66*, 164−175.

(16) Conmy, R. N.; Chen, R. F.; Castillo, C. E. D.; Downing, B. Calibrations and quality control of in situ sensors. In *Fluorescence Applications in Aquatic Science*; Cambridge University Press, 2013.

(17) Report, S. L. R. E. R. *Updating the SMART dispersant monitoring protocol: Review of Commercial-Off-The-Shelf instruments*; S.L. Ross Environmental Research, 2008; p 19.

(18) Lambert, P. A literature review of portable fluorescence-based oil-in-water monitors. *J. Hazard. Mater.* 2003, *102*, 39−55.

(19) Fuller, C. B.; Bonner, J. S.; Page, C. A.; Arrambide, G.; M.C. Sterling, J.; Ojo, T. *Field Instruments for Real Time In-Situ Crude Oil Concentration Measurements*; Texas A&M University, 2003; p 10.

(20) Wilde, S. Procedures for monitoring dispersant operations. In *10th Environmental and Scientific Coordinators Workshop* , Queenscliff, Australia, 2001, 2001; pp 163−177.

(21) Srinivasan, R.; Lu, Q.; Sorial, G. A.; Venosa, A. D.; Mullin, J. Dispersant effectiveness of heavy fuel oils using baffled flask test. *Environ. Eng. Sci.* 2007, *24* (9), 1307−1320.

(22) Report, N. R. C. *Oil Spill Dispersants: Efficacy and Effects*; Washington, DC, 2005; p 400.

(23) *Report Oil Budget Calculator: Deepwater Horizon*; Federal Interagency Solutions Group, 2010; p 217.

(24) Report, J. A. G. *Deepwater Horizon Oil Spill: Review of Subsurface Dispersed Oil and Oxygen Levels Associated with the Deepwater Horizon MC252 Spill of National Significance*; National Oceanic and Atmospheric Administration, 2012; p 93.

(25) Report, J. A. G. *Review of Data to Examine Subsurface Oil in the Vicinity of DWH MC-252*; National Oceanic and Atmospheric Administration, May 19−June 19, 2010; p f29.

(26) Lee, K.; Li, Z.; Boufadel, M. C.; Venosa, A. D.; Miles, M. S. *Wave Tank Studies on Dispersant Effectiveness As a Function of Energy Dissipation Rate and Particle Size Distribution*; Department of Fisheries and Oceans Canada, 2009; p 67.

(27) Li, Z.; Lee, K.; King, T.; Boufadel, M. C.; Vensosa, A. D. Evaluating crude oil chemical dispersion efficacy in a flow-through wave tank under regular non-breaking wave and breaking wave conditions. *Mar. Pollut. Bull.* 2009, *58*, 735−744.

(28) Li, Z.; Lee, K.; King, T.; Boufadel, M. C.; Vensosa, A. D. Effects of temperature and wave conditions on chemical dispersion efficacy of heavy fuel oil in an experimental flow-through wave tank. *Mar. Pollut. Bull.* 2010, *60*, 1550−59.

(29) Cole, M. G.; King, T. L.; Lee, K. Analytical technique for extracting hydrocarbons from water using sample container as extraction vessel in combination with a roller apparatus. *Anal. Chem.* 2007, *77*, 2210−17.

(30) Coble, P. G. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Mar. Chem.* 1996, *51*, 325−346.

(31) Atlas, R. M.; Hazen, T. C. Oil biodegradation and bioremediation: A tale of the two worst spills in U.S. history. *Environ. Sci. Technol.* 2011, *45* (16), 67−09−15.

ED_001324_00001261-00008

To: Malcolm Gore[malcolm.gore@clearcoastllc.com]
From: Conmy, Robyn
Sent: Tue 4/26/2016 4:05:46 PM
Subject: RE: Finasol OSR 52 Sample Request

Thanks for the update!

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Malcolm Gore [mailto:malcolm.gore@clearcoastllc.com]
Sent: Monday, April 25, 2016 5:45 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: Re: Finasol OSR 52 Sample Request

Apologies for the delay Robyn

Awaiting some shipping information. Should have this to you in the morning.

Malcolm

Malcolm Gore

President

CLEAR COAST

+1 832 244 1533

malcolm.gore@clearcoastllc.com

www.clearcoastllc.com

From: "Conmy, Robyn" <Conmy.Robyn@epa.gov>
Date: Monday, April 25, 2016 at 10:05 AM
To: malcolm gore <malcolm.gore@clearcoastllc.com>
Cc: Peter Egan <peter.egan@total.com>
Subject: RE: Finasol OSR 52 Sample Request

Hello Malcolm,

Thank you for getting back with me regarding the purchase. Yes, the information below is correct, with a mail stop at room 166. Can you send to me the invoice via email so I can provide to the purchase card holder to make the purchase?

Thank you in advance,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Malcolm Gore [mailto:malcolm.gore@clearcoastllc.com]
Sent: Monday, April 25, 2016 10:57 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Egan <peter.egan@total.com>
Subject: Finasol OSR 52 Sample Request

Hello Robin

I am writing with respect to your email below regarding a sample request of Total Finasol OSR 52.

Clear Coast is the custodian of the product stored here in Houston for Total.

I will arrange the sample as requested.

Please confirm the following:

Contact:

Robyn Conmy 513-569-7090 (office)

Deliver to:

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

Quantity:

2 US Gallons.

On receipt of confirmation I will get the samples drawn, packaged and dispatched.

Regards

Malcolm

Malcolm Gore

President

CLEAR COAST

+1 832 244 1533

malcolm.gore@clearcoastllc.com

www.clearcoastllc.com

From: Conmy, Robyn
Sent: Monday, April 18, 2016 10:47 AM
To: 'peter.egan@total.com' <peter.egan@total.com>
Cc: Wilson, Gregory <Wilson.Gregory@epa.gov>; Principe, Vanessa <Principe.Vanessa@epa.gov>
Subject: purchase of Finasol OSR52

Mr. Egan,

This follows up our telephone conversation of April 11.

As discussed, EPA is seeking to acquire a number of dispersant products listed on the NCP Subpart J Product Schedule for the purposes of both toxicity and efficacy testing. The product testing is intended to further inform currently proposed regulatory actions under Subpart J of the National Contingency Plan. The products will in addition be used to support our general research in the area of oil spill response.

Your product Finasol OSR52 has been identified as one of interest, as it is commonly stockpiled in the U.S. Not only could the product be encountered when presented with a response situation, but including this dispersant product in toxicity and efficacy testing studies will also allow for comparison and consistency with other existing and ongoing studies by EPA and other federal agencies. EPA's Office of Research and Development (ORD) will be conducting the dispersant studies.

At this time we are seeking 2 U.S. gallons of the product.

We appreciate your time and attention to this request.

Respectfully,

Robyn Conmy

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

Biodegradability of Different Initial Concentrations of Alaska North Slope Crude Oil Dispersed with Corexit C9500

Mobing Zhuang[1], Gulizhaer Abulikemu[2], Pablo Campo[3*], William Platten III[4], Makram T. Suidan[5], Albert D. Venosa (retired)[4]and Robyn N. Conmy[4]

1 Department of Biomedical, Chemical and Environmental Engineering, University of Cincinnati, 2901 Woodside Drive, Cincinnati, OH 45221, USA

2 Pegasus Technical Services Inc., 46 E Hollister St, Cincinnati, OH 45219 USA

3 Cranfield Water Science Institute, Cranfield University, Cranfield, Beds, MK43 0AL, UK

4 U.S. Environmental Protection Agency, NRMRL, 26 W. MLK Drive Cincinnati, OH, 45268, USA

5 Faculty of Engineering and Architecture, American University of Beirut, Bechtel Engineering Bldg. - 3rd flr. - Room 308 P.O. Box: 11-0236 Riad El Solh 1107 2020, Beirut, Lebanon

*Corresponding author: Pablo Campo
Tel: +44 (0)1234 754332
E-mail address: p.campo-moreno@cranfield.ac.uk

ED_001324_00001264-00001

ABSTRACT

Following Deepwater Horizon oil spill, in situ concentrations of dispersant and oil were lower than typically studied. To better understand the degradation at low oil and dispersant concentrations observed, laboratory experiments were conducted using Alaska North Slope crude oil (ANS) and the dispersant Corexit 9500 (C9500) to evaluate the removal of selected chemicals including: dioctyl sodium succinate (DOSS) which is an anionic surfactant in C9500, alkanes, and polycyclic aromatics hydrocarbons (PAHs). The study considered the biodegradability of ANS alone, ANS dispersed with C9500 at a dispersant-to-oil ratio of 1:25, and C9500 alone. Oil loads of 1,000 and 40 μL/L were compared at two temperatures with microbial cultures acclimated to the temperature 25 (meso culture) and 5 (cryo culture) °C. The biodegradation rate of DOSS, by the meso culture (25 °C) was approximately an order of magnitude faster in the high concentration experiment than in the low concentration experiment. At the lower temperature, the deep-water culture cryo (5 °C) had limited ability to metabolize DOSS regardless of the initial concentrations. Uptake of oil components was favored by the presence of dispersant as C9500 shortened lag phases and enhanced biodegradation rates. Alkanes and PAHs were degraded more rapidly in the high concentration samples. In the low concentration experiment, the percentage of alkanes persisting was higher than in the high concentration experiment (8-18% vs. below 1%). No significant lag period in PAH biodegradation was observed in the high oil concentration experiment compared to the low oil concentration experiment where a lag period of 12 to 16 d was observed. The extent of biodegradation of some of the less soluble aromatics increased by 4-86% under low oil concentration conditions.

ED_001324_00001264-00002

KEYWORDS

Corexit 9500, biodegradation, initial oil concentration

ED_001324_00001264-00003

INTRODUCTION

To minimize the impact of oil spills, responders adopt the Net Environmental Benefit Analysis, this is, any response technique should decrease the environmental costs rather than increase them (API, 2013). When mechanical recovery or in situ burning cannot achieve the goal of protecting shorelines from oil, the impact of using dispersants is likely outweighed by the benefits, which include keeping oil from sensitive areas and speeding up biodegradation by breaking an oil slick into small droplets (Prince, 2015). Thus, it is important to study the biodegradability of dispersants and dispersed oil under various conditions for better informed use in future applications.

In the aftermath of the Deepwater Horizon blowout in the Gulf of Mexico (GOM), comprehensive monitoring of dispersant Corexit 9500 (C9500) and hydrocarbons in both surface and subsurface environments took place. In samples collected near the Macondo well, concentrations of dioctyl sodium sulfosuccinate (DOSS), an anionic surfactant present in C9500, ranged from 0.4 to 12 μg/L (Kujawinski et al., 2011). After the spillat GOM different locations, DOSS was detected in subsurface waters at concentrations below 40 μg/L, while in one surface water sample close to the wellhead concentration exceeded 200 μg/L (Gray et al., 2014). The combination of turbulence on the GOM surface and the dispersant application could rapidly decrease oil concentrations to below 100 mg/L, dropping even further over time (Lee et al., 2013). Hence, these researchers recommended that biodegradation tests of dispersed oil should be conducted under more dilute concentrations to mimic real conditions.

Most studies published on biodegradation of oil and dispersants prior to the Deepwater incident had been conducted at concentrations well-above the reported in situ levels (Operational

ED_001324_00001264-00004

Science Advisory Team, 2010; Prince, 2015). Several studies assessing biodegradation of oil dispersed by C9500 used initial oil and dispersant concentrations from 100 to 4,500 mg/L, with a dispersant-to-oil ratios (DOR) of 1:10, 1:20, or 1:25 (Campo et al., 2013; Lindstrom and Braddock, 2002; Zahed et al., 2010). A few studies have been published on oil biodegradation with initial oil and dispersant concentrations below 100 μL/L (Brakstad et al., 2015; Prince et al., 2013; Venosa and Holder, 2007). Thus, our objective was to determine how the initial amounts of oil and dispersant affect their biodegradation. We conducted high and low oil concentration biodegradation experiments with Alaska North Slope crude oil (ANS) and C9500 at 5 and 25 °C. C9500 is a dispersant included in the National Contingency Plan Product Schedule and was applied to both the surface and submarine environment in the GOM (Operational Science Advisory Team, 2010). Performance was evaluated by measuring DOSS, and oil chemicals in high and low concentration experiments at 5 and 25 °C are reported in this paper.

MATERIALS AND METHODS

Chemicals and Reagents

The U.S. Environmental Protection Agency (EPA) provided the ANS and the dispersant C9500 (Nalco Naperville, IL) used in this study. Standards for DOSS and its deuterated surrogate (D_{17}-DOSS) were obtained from Aldrich (St. Louis, MO) and Isotec (Miamisburg, OH), respectively. Acetonitrile, deionized ultra-filtered water, and mineral salts were purchased from Fisher Scientific (Pittsburgh, PA). Dichloromethane (DCM) was obtained from Tedia (Fairfield, OH). Sylon CT (Sigma-Aldrich, St. Louis, MO) was used for the deactivation of the glassware to prevent adherence of oil and biomass to the sides of the flasks. The protease cocktail for inhibiting enzymatic activity was also acquired from Sigma-Aldrich (St. Louis, MO).

ED_001324_00001264-00005

Protease Solution Preparation

Following the instruction provided with the protease cocktail, 5 mL dimethyl sulfoxide (DMSO) was added to 1,075 mg lyophilized powder, which was previously stored unopened at -20 °C. The solution was vortexed for ten minutes before the addition of 20 mL deionized water. The resulting solution was clear. The reconstituted protease solution contained the following inhibitors: 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (23 mM), ethylenediaminetetraacetic acid (100 mM), bestatin (2 mM), pepstatin A (0.3 mM), and E-64 (0.3 mM).

Artificial Seawater

GP2 medium was autoclaved at 121 °C for 30 min and then used as the sterile matrix in this study (Spotte et al., 1984). The salts in the GP2 medium, dissolved in DI water, were (expressed in g/L) NaCl (21.03), $MgCl_2 \cdot 6H_2O$ (9.5), Na_2SO_4 (3.52), $CaCl_2 \cdot 2H_2O$ (1.32), KCl (0.61), KBr (0.088), $NaB_4O_7 \cdot 10H_2O$ (0.034), $SrCl_2 \cdot 6H_2O$ (0.02), $NaHCO_3$ (0.17) $FeCl_3 \cdot 6H_2O$ (0.05), $Na_5P_3O_{10}$ (0.297) and KNO_3 (2.89). The GP2 pH was ≈ 7.5.

Microbial Cultures

The meso culture was originally isolated from the surface water of the GOM in the vicinity of the Macondo well. The cryo culturewas isolated from water close to the plume location at a depth of 1,240 m near the wellhead . Both cultures were isolated by EPA's Gulf Breeze, FL research laboratory. Enriched inocula were procured as follows: in 2-L shake flasks, 2.5 mL SLC were added to 500 mL of the original unfiltered GOM seawater supplemented with 2.8 g/L KNO_3, 0.55 g/L NaP_3O_{10}. The flasks were rotated (400 rpm) at 5 °C (cryo culture) and 25

ED_001324_00001264-00006

°C (meso culture). The adequate growth times were 7 d for the meso culture and 17 d for the cryo culture. After these periods, cells were harvested by centrifugation (6,000 × g for 30 min at 4 °C) and the pellets preserved in 10% glycerol at -70 °C. Before shipping to EPA-Cincinnati, 1 mL of each thawed culture were added to shake flasks containing 500 mL GP2 medium fortified with 2.5 mL of SLC. The meso culture was harvested after 6 days of growth and the cryo culture after 29 days. The cultures used in the experiments of this study were grown at EPA (Cincinnati, OH) by transferring the original cultures from EPA's Gulf Breeze to GP2 medium supplemented with South Louisiana crude oil (SLC). The meso culture was harvested after 3 weeks of growth and the cryo culture after 5 weeks. The harvested culture was processed in the same fashion as mentioned previously: centrifugation (6,000 × g for 30 min at 4 °C) and the pellets preserved in 10% glycerol at -70 °C. Upon use in experiments, the cultures were defrosted at room temperature, washed with 0.85% saline solution to remove glycerol, centrifuged, and brought back up to the frozen volume.

Experimental Design

A summary of the experimental design is shown in Tables S1 and S2 for the high and low oil concentration experiments, respectively. For the high concentration experiments, 6 treatments were tested at 5 and 25 °C in triplicate: C9500 dispersant alone, ANS oil alone, ANS dispersed with C9500, and the corresponding killed controls (KCs). The 5 °C experiment required 11 sampling events in triplicate (0, 2, 4, 8, 12, 16, 24, 32, 40, 48 and 56 d), while 9 events were conducted at 25 °C (0, 2, 4, 8, 12, 16, 24, 32 and 40 d). At each sampling event, triplicate shake flasks of each treatment along with KCs were sacrificed, except for the ANS alone KCs which were analyzed at the beginning and end of the experiment.

ED_001324_00001264-00007

The same treatments were included in the low concentration experiment, but both oil and dispersant concentrations were prepared at 4% of the initial loads used in the high concentration experiment. Additionally, a seventh treatment involved the use of triplicate KCs containing the enzyme protease (0.5 mL) to neutralize the enzymatic DOSS hydrolysis. This treatment was included only in the low concentration experiment. The sampling events were conducted similarly to the high concentration experiment with the exception of the protease treatment, which was sampled at 0, 12, 24, 35, 46 and 56 d and 0, 8, 18, 28, 38 and 48 d for the 5 and 25 °C experiments, respectively.

After all treatments were prepared, the appropriate cultures were spiked (0.5 mL) and the flasks were placed on orbital shakers. The shakers were operated at 200 rpm and kept in the appropriate constant temperature rooms until the expected sampling events. All KCs were sterilized by adding 1 mL sodium azide stock (50 g/L) into the 100 mL of GP2 medium.

High Concentration Treatments

To evaluate the biodegradation of dispersed oil, 2 L baffled beakers were used in which 40 μL of C9500 and 1,000 μL of ANS were added to 1,200 mL of GP2 to achieve a volumetric DOR ratio of 1:25. Subsequently, the beaker containing the mixture was shaken at 200 rpm for 10 minutes and then left stationary for an additional 10 min, then the dispersion was transferred to a 20-L continuously mixed carboy. The above procedure was repeated until the volume in the carboy reached 14 L. Subsequently, 100 mL aliquots of the mixtures were transferred to the shake flasks. The second treatment was for the evaluation of oil as the only substrate. ANS (100 μL) was added directly to shake flasks containing 100 mL sterile GP2 medium, which yielded an estimated oil concentration of 1,000 μL/L. The oil added to the ANS alone and dispersed ANS

ED_001324_00001264-00008

treatments aimed to result in the same oil concentration by volume. To evaluate the degradation of dispersant alone, 480 µL of C9500 was added to 14 L of sterile GP2 medium in a continuously mixed carboy; after 30 min, 100 mL aliquots of this mixture were dispensed into shake flasks for this treatment.

Low Concentration Treatments

To prepare the low concentration dispersed oil, a single batch of 1,200 mL GP2 was spiked with 200 µL ANS onto the water surface followed by addition of 8 µL C9500 onto the oil slick, which yielded a volumetric DOR of 1:25 in a 2 L baffled beaker. The shaker was started at a very slow speed and was ramped up to 200 rpm gradually. The beaker was placed on a shaker for 20 min and then remained stationary for 10 minutes. Subsequently, 3 L of the dispersion were poured into a 20 L carboy under continuous mixing and 12 L of sterile GP2 was added for dilution purposes. Overall, the oil and dispersant amounts in this treatment were approximately 4% of that in the high concentration experiment. Finally, shake flasks were filled with 100 mL aliquots of the diluted dispersed oil. Low concentration ANS alone was prepared by spiking approximately 4 µL of oil into shake flasks containing 100 mL sterile GP2, which yields an oil concentration of 40 µL/L (i.e., 4% of the high concentration oil alone experiment). The low concentration dispersant alone treatment was prepared in a single batch of sterile GP2 (14 L) spiked with approximately 20 µL of C9500. The batch was mixed for 30 minutes and, subsequently, 100 mL aliquots were transferred into the shake flasks.

Oil components and Dispersant Analysis

To monitor C9500 degradation, DOSS was measured by liquid chromatography tandem

ED_001324_00001264-00009

mass spectrometry (LC-MS/MS) following the Standard method ASTM D7730−11 (2011). The targeted oil components were pristane (PR), phytane (PH), normal paraffins (n-C_{10-35}) and 2-, 3- and 4-ring PAH compounds and their alkylated homologues (C_{0-4}-naphthalenes, C_{0-3}-fluorenes, C_{0-3}-dibenzothiophenes, C_{0-4}-phenanthrenes/anthracenes, C_{0-4}-napthbenzthiophenes, C_{0-2}-pyrenes, C_{0-4}-chrysenes). The concentrations of these analytes were normalized to hopane present in the oil (Prince et al., 1994). Details of sample preparation methods and oil extraction by DCM can be found elsewhere (Campo et al., 2013). For the high concentration experiment, oil analysis was conducted with a 6890 GC coupled with a 5973 mass spectrometry from Agilent (Palo Alto, CA). In the low concentration experiment, an Agilent (Palo Alto, CA) 7000 GC Triple Quad system was used. The quantification range for the single quad and triple quad mass spectrometry are 1-30 μg/L and 0.5-10 μg/L, respectively. In the case that the concentration of oil hydrocarbons is below the range, we used the quantification results as an estimation of the actual amount. The same column, a DB-5MS column (30m × 0.25mm, 0.25μm film thickness), was used to achieve chromatographic separation of analytes in both instruments. The method was a modification based on EPA Method 8270D (2007).

RESULTS AND DISCUSSION

Removal of DOSS

High Concentration Experiment. Live samples revealed an extremely fast removal rate of DOSS at 25 °C (Fig. 1A-B, open symbols) with extents exceeding 95% after 2 d in dispersed oil (Fig. 1B) and 4 d in C9500 alone (Fig. 1A). The first order rate coefficients were -1.82 ± 0.25 d^{-1} (ANS+C9500) and -0.71 ± 0.024 d^{-1} (C9500 alone). The presence of oil enhanced DOSS biodegradation by a factor of approximately 2.6. Disappearance of DOSS was also observed in

ED_001324_00001264-00010

KCs at 25 °C, with zero order rates of -138 ± 7 and -221 ± 11 μg L^{-1} d^{-1} for C9500 alone (Fig. 1C) and dispersed oil (Fig. 1D), respectively. As monooctyl sulfosuccinate (MOSS) was found in KCs, Campo et al. (2013) explained this abiotic loss in terms of hydrolysis which was confirmed by (Batchu et al., 2014). The presence of oil significantly enhanced DOSS removal in KCs ($p < 0.0001$).

In contrast with the rapid removal of DOSS at 25 °C, the biotic and abiotic processes at 5 °C were much slower. DOSS persisted for 40 d before its concentration decreased in all treatments. The difference in DOSS concentration between live samples and parallel KCs in the dispersed oil treatment was statistically insignificant at days 48 ($p = 0.078$) and 56 ($p = 0.726$). In the C9500 alone treatment, DOSS was significantly lower in live samples than in KCs at days 48 ($p = 0.0015$) and day 56 ($p = 0.023$). These results point to biodegradation as the predominant DOSS removal mechanism as opposed to hydrolysis.

ED_001324_00001264-00011

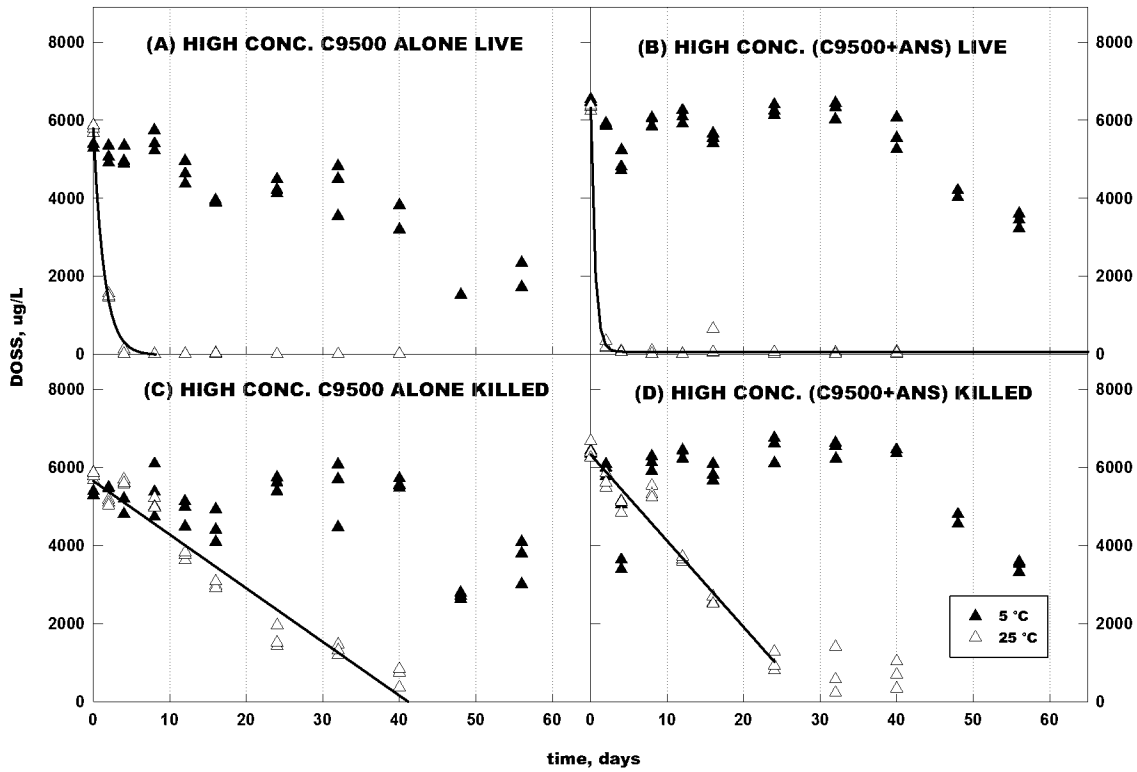


Figure 1. Disappearance of DOSS in high concentration experiment in the absence (A, C) and Presence (B, D) of ANS at 5 °C and 25 °C. Live samples are in panel A and B, whereas killed controls are in panels C and D.

Low Concentration Experiment. At 25 °C and for the dispersed oil treatment, no lag period was observed in DOSS uptake, which showed a first-order constant rate of -0.16 ± 0.015 d^{-1} (Fig. 2B). In the absence of oil (Fig. 2A), we observed an acclimation period of 2 d followed by DOSS biodegradation (-0.07 ± 0.009 d^{-1}). The presence of oil favored DOSS removal as was observed in the high concentration experiment . Removal extents over 95% for DOSS required 32 and 48 d in the presence and absence of oil, respectively. In the corresponding KCs, DOSS concentrations decreased following zero-order kinetics, with constant rates of -4.1 ± 0.3 μg L^{-1} d^{-1} (C9500 alone, Fig. 2C) and -5.1 ± 0.4 μg L^{-1} d^{-1} (C9500 and ANS, Fig. 2D).

For the high concentration experiment at 25 °C and regardless of the presence of oil, the

time series concentration of DOSS in the live samples rapidly declined to zero and clearly separated from the values measured in the corresponding KCs. In the low concentration experiment, it appeared that DOSS disappearance with time in both treatments, C9500 alone and dispersed ANS, overlapped with the abiotic losses observed in the KCs (Fig. 2). To differentiate between microbial activity and hydrolysis, we compared by a *t*-test ($\alpha = 0.05$) the average DOSS concentrations measured in live samples with those values found in the parallel KCs. Live samples significantly departed from the controls after 4 d ($p = 0.0227$) and 16 d ($p = 0.0053$) in the presence and absence of ANS, respectively. At early stages in the experiment, we could not identify the predominant removal mechanism for DOSS but, eventually, microbial uptake prevailed as the main process in terms of rate and extent.

DOSS biodegradation was at both the higher initial concentration and temperature. Nevertheless, based on our low concentration results, it is reasonable to infer that biodegradation of DOSS at the surface of GOM might not happen as rapidly since dilution and emulsification can attenuate the concentration of dispersant. In this fashion, DOSS was detected with a reporting limit of 0.25 $\mu g\ L^{-1}$ and a highest concentration of $229 \pm 16\ \mu g\ L^{-1}$ in surface water samples collected 1-2 months after the spill near the Macondo well (Gray et al., 2014).

DOSS concentration remained unchanged in live samples and KCs under 5 °C and low oiling conditions (Fig. 2, closed symbols). Such finding agreed with studies by Kujawinski et al. (2011) and Gray et al. (2014) who concluded that subsurface degradation of DOSS was minimal. Campo et al. (2013) observed no hydrolysis or biodegradation of DOSS in SLC at 5 °C within 42 d.

ED_001324_00001264-00013

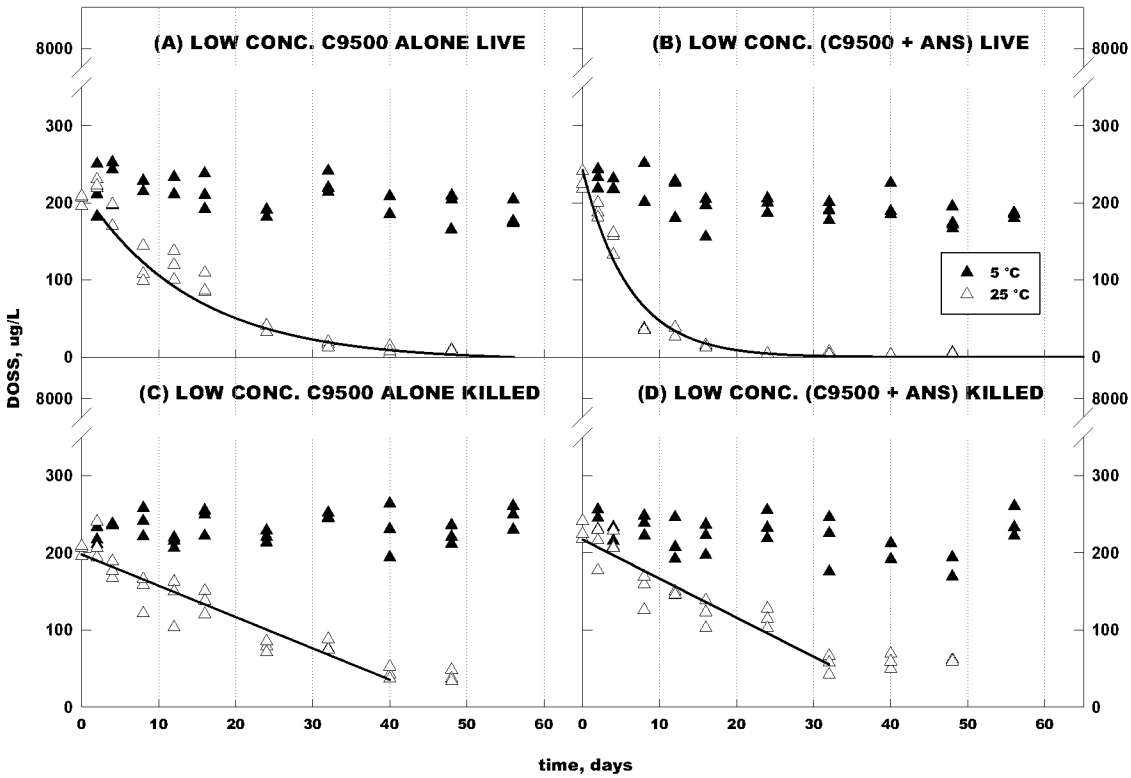


Figure 2. Disappearance of DOSS in low concentration experiment in the absence (A, C) and Presence (B, D) of ANS at 5 °C and 25 °C. Live samples are in panel A and B, whereas killed controls are in panels C and D.

We also included a series of dispersed oil KCs containing a protease cocktail (Fig. 3A), which inhibits enzymatic activity to clarify the abiotic DOSS disappearance. At 25 °C, the addition of inhibitor clearly slowed down the hydrolysis process as the zero-order rate constant in the protease KCs was -3.7 ± 0.4 μg L^{-1} d^{-1} (Fig. 3A) as opposed to -5.1 ± 0.4 μg L^{-1} d^{-1} (Fig. 3B) obtained for the regular KCs (p = 0.021). Such finding suggests the enzymatic nature of DOSS hydrolysis. Nevertheless, different enzymes may be involved in the process so that the protease cocktail could not block all of them. In the protease controls at 5 °C, DOSS persisted throughout the experiment as observed in the regular KCs for that temperature. This indicates that either the lower temperature attenuated DOSS breakdown,the cryo culture lacked the

required enzymes, or a combination of both causes.

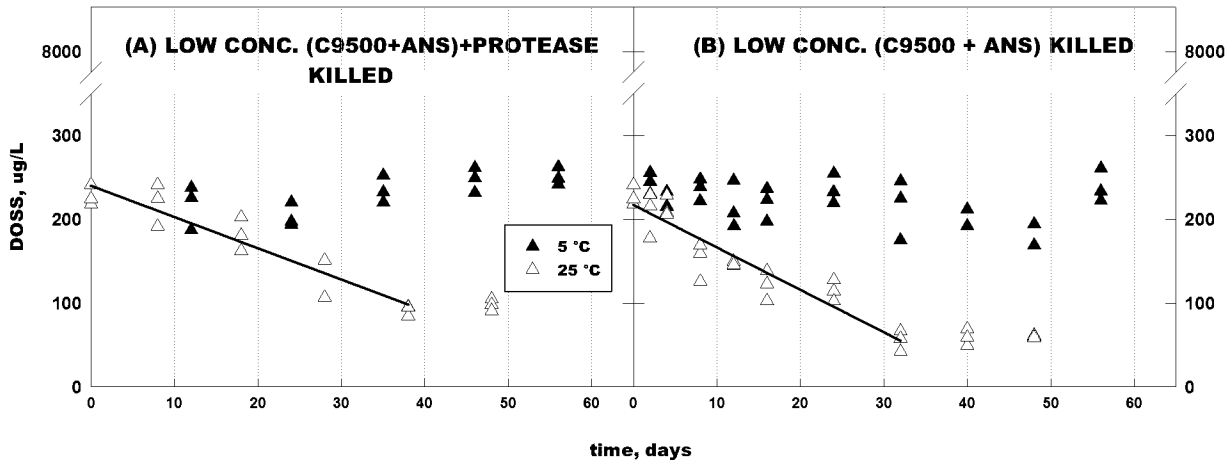


Figure 3. Disappearance of DOSS in low concentration dispersed oil killed controls in the presence (A) and absence (B) of protease at 5 and 25 °C.

Total Alkane Degradation

High Concentration Experiment. Total alkanes at both temperatures are presented in Fig. 4 A-B. At 25 °C, the meso culture metabolized the aliphatic fraction with a first-order rate constants of -0.91 ± 0.10 and -0.85 ± 0.04 d^{-1} in the absence and presence of C9500, respectively. For both treatments, the extent of removals reached 99% after 4 days. As expected, aliphatics degraded slower at 5 °C. In the dispersed oil treatment, this fraction followed first order kinetics (-0.24 ± 0.01 d^{-1}) while, for the oil alone samples, alkanes presented a linear decay during the first 8 d followed by first order kinetics of similar rate (-0.25 ± 0.03 d^{-1}). After days 16 (dispersed oil) and 24 (oil alone), the alkane extent of removal was 99% for the lower temperature.

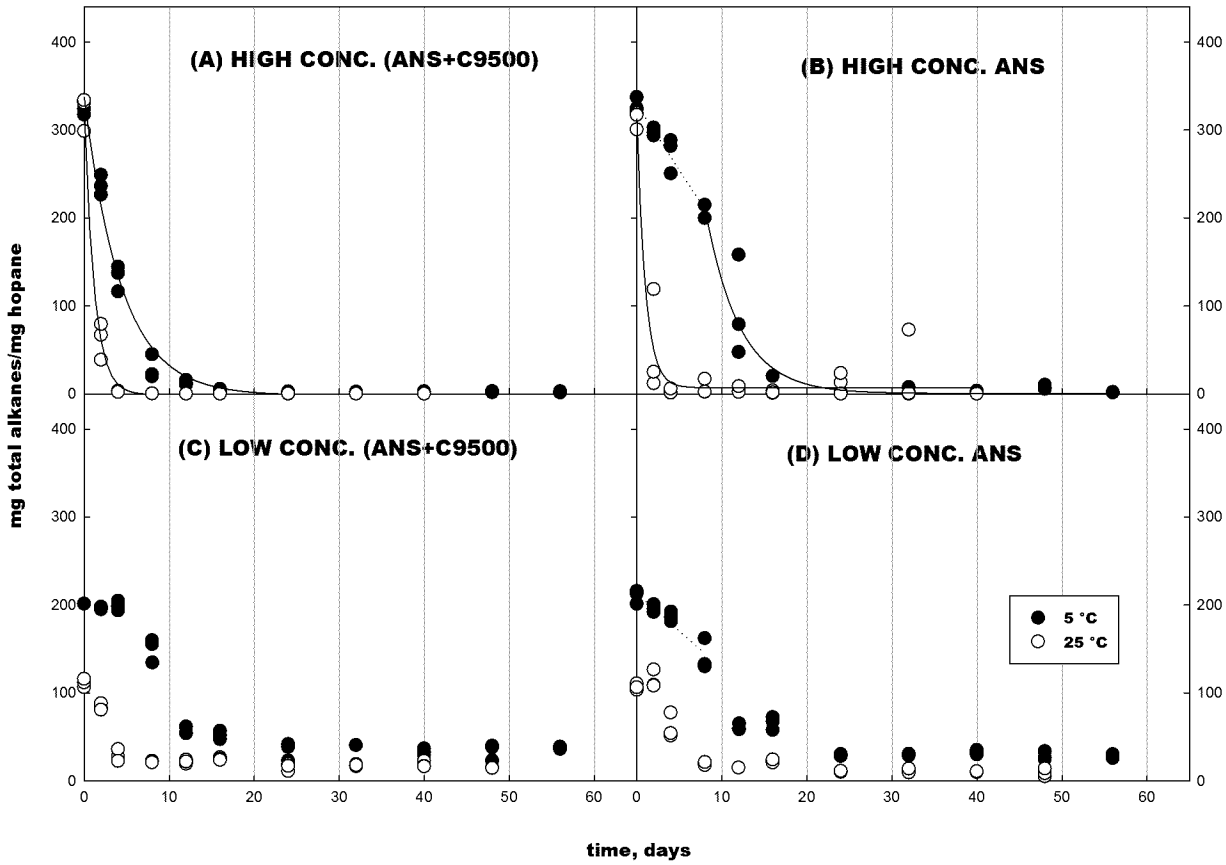


Figure 4. Biodegradation of hopane-normalized total alkanes at both temperatures in high concentration treatments (A, B) and low concentration treatments (C, D).

Low Concentration Experiment. In this experiment, the initial total alkane concentrations were considerably different at 5 and 25 °C (Fig. 4 C and D). The meso culture showed background concentrations of PAHs and hopane, the conservative biomarker to which all the target analytes concentrations are normalized. At time zero, the total alkane concentration normalized to hopane at 25 °C were almost half of the corresponding value at 5 °C.

At 25 °C, the alkane residual concentrations ranged from 8 to 18% of the initial values (Fig. 4 A and B). In the oil alone treatment, alkane biodegradation started after a lag phase of 2

d, while these chemicals were promptly metabolized in the dispersed oil treatment. At 5 °C, the cryo culture required 4 d for acclimation in the dispersed oil. In the absence of dispersant, after a slow and steady linear decay, fast disappearance of alkanes occurred between days 8 and 12. This suggests that C9500 reduced the length of the acclimation phase for alkane uptake.

Table 1 summarizes the actual concentrations of total alkanes (not normalized to hopane) for the first and last sampling event for both the high and low concentration experiments. It is noted that a higher alkane residual amount was observed in the low concentration experiment than the high concentration one.

Table 1. Average and standard deviations (sd) of total alkanes and PAHs concentration in the high and low concentration experiment at first and last sampling event.

			5 °C		25 °C		5 °C		25 °C	
Samples			**ANS alone**		**ANS alone**		**ANS+C9500**		**ANS+C9500**	
			average (µg/L)	**sd**	**average (µg/L)**	**sd**	**average (µg/L)**	**sd**	**average (µg/L)**	**sd**
High Conc.										
	Alkanes	**First**	47,400	604	43,300	9,390	24,600	973	27,200	1260
		Last	201	26	36	42	186	52	0	0
	PAHs	**First**	14,500	110	13,700	3,090	7,310	323	8,790	94
		Last	3,040	281	2,470	300	1,300	74	1,270	158
Low Conc.										
	Alkanes	**First**	2,010	202	1,740	149	1,420	72	1,130	39
		Last	216	21	83	75	225	13	142	4
	PAHs	**First**	482	53	773	25	330	12	453	14
		Last	71	5	88	5	58	10	80	4

Degradation of Individual Alkanes

High Concentration Experiment. Table S3 summarizes the first-order biodegradation

ED_001324_00001264-00017

coefficients for individual alkanes. At 25 °C, among all the compounds monitored only n-C_{10} was biodegraded faster (-2.00 vs. -1.34 d^{-1}) with the addition of dispersant. Branched alkanes exhibited similar rates regardless of the presence of C9500, although higher variability among triplicates was observed in the oil alone samples (Fig. S1 A and B). At 5 °C, consistently higher rates for n-C_{10-27} were observed in the dispersed oil treatment when compared to the oil alone treatment. Also, no lag period for the biodegradation of branched alkanes occurred when C9500 was added, whereas an 8-day lag period occurred in the absence of dispersant.

Low Concentration Experiment. As depicted in Figs. S1-5 (panels C and D), only n-C_{10-16} was completely removed at both temperatures in the low concentration experiment. In this case,a more qualitative approach was used by comparing the disappearance time. At 25 °C in the low oil concentration experiment, n-C_{10} was degraded faster in the presence of dispersant, disappearing completely before day 2, whereas in the absence of C9500, its depletion was achieved only after day 8. The biodegradation of n-C_{10} was also accelerated when C9500 was present at 5 °C, as shown in Table S4 (-0.47 vs -0.63 d^{-1}). At both temperatures, branched alkanes were not degraded faster in the presence of dispersant, as shown in Table S4 and Fig. S1 C-D.

The major mechanism of uptake of water-insoluble substrates (i.e., alkanes) is generally recognized as direct interaction between microbes and the hydrophobic substance (Rojo, 2009; Wentzel et al., 2007) and the effects of surfactant on such process are inconclusive (Bredholt et al., 2002; Bruheim et al., 1999; Tang et al., 1998). Both conducive and inhibitory effects of dispersant on oil biodegradation have been reported (Lindstrom and Braddock, 2002; Prince et al., 2013; Venosa and Holder, 2007). In this study, C9500 exhibited similar effects on the

biodegradation of alkanes in the high and low concentration experiments. Such effects included enhancement of biodegradation rates and shortening of lag periods. Rate enhancements were more notable in the high concentration experiment, whereas C9500 clearly shortened lag phases in the lower concentration tests.

The application of dispersants to an oil slick promotes the formation of small oil droplets by decreasing the interfacial tension between oil and water (Jasper et al., 1978; Li et al., 2009; Lunel, 1995). Consequently, the total interfacial area of oil droplets for microbes to interact with is increased. In the high concentration experiment, exposure of substrates to the microbes is enough that biodegradation can initiate soon after inoculation. In this case, it is likely that dispersants assisted degradation mainly by increasing interfacial area for microbes to interact with oil. For the low concentration experiment, the observed long acclimation phase at both temperatures, especially in the absence of C9500, was possibly due to limited exposure of alkanes to the microorganisms in the beginning. Since the oil load, appearing as an iridescent sheen, is so low, the mixing energy in the flasks could be high enough to produce similar droplet size distribution in the oil alone and dispersed oil treatments. Thus, C9500 may have shortened the lag phase in the low concentration experiment by promoting the attachment of microbes to the oil droplet.

Total Aromatics Degradation

High Concentration Experiment. Fig. 5A-B presents total PAH biodegradation data for the high concentration experiment at both temperatures. Aromatics removals of 42 and 24% occurred by day 2 in the dispersed oil and the oil alone treatment, respectively. This difference in extent was significant ($p < 0.0001$), which indicates a positive effect of the dispersant. At 5 °C,

ED_001324_00001264-00019

the time for attaining maximum level of PAHs removal in non-dispersed treatment took twice as much time as in dispersed oil treatment 16 days vs. 8 days (solid symbols in Fig. 5B and 5A, respectively). The overall PAHs removals in the presence and absence of dispersant were 86% and 82%, respectively.

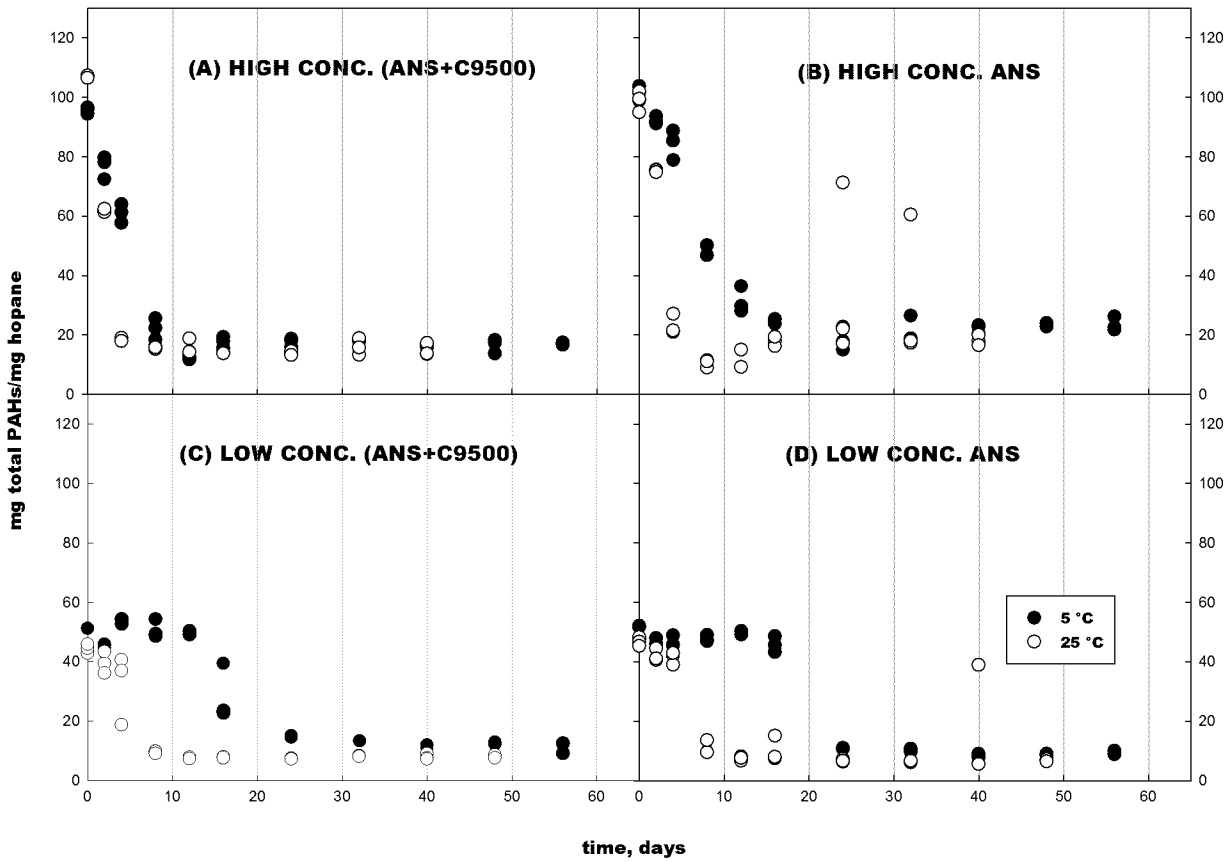


Figure 5. Biodegradation of hopane-normalized total PAHs at both temperatures in high concentration treatments (A, B) and low concentration treatments (C, D).

Low Concentration Experiment. As shown in Fig. 5C-D, when comparing the two low concentration treatments at 25 °C (open symbols), the only difference was in one replicate of dispersed oil on day 4, which had significantly lower total PAH concentration than the parallel diluted oil alone samples. At the high temperature (open symbols), the patterns of PAH degradation were similar in the absence and presence of C9500, regardless of initial

concentration. The treatment without dispersant experienced higher variability. The lag period at the low temperature (closed symbols) was longer than at 25 °C; it lasted12 and 16 days for dispersed oil and oil alone, respectively. For the sampling event on day 16, the extent of PAH removal was 20 to 50% in the replicates of dispersed oil sample, while it was negligible in the treatment without dispersant.

Transport of compounds from non-aqueous phase to aqueous phase is often the most rate-limiting step in the biodegradation of PAHs. Surfactants can enhance the dissolution of PAHs by increasing interfacial area of oil droplet and subsequently increase the bioavailability of PAHs to microorganisms (Haritash and Kaushik, 2009; Mrozik et al., 2003). The effect of C9500 on the biodegradation of total PAHs was less pronounced in the low concentration experiment than in the high concentration experiment, because this effect was observed only in one or more replicates in a single sampling event. This finding is consistent with our previous assumption about low concentration experiment: droplet size distribution in the oil alone and dispersed oil treatments are similar. Dispersion in the oil alone microcosms might have been efficient enough even without the addition surfactant, considering the mixing energy was sufficient (Kaku, 2006) and the initial presence of oil was an iridescent rainbow-colored sheen. Clearly, the comparison between our low concentration dispersed oil samples and oil alone samples indicates that dispersants had little effect on the concentration of PAHs. Nevertheless, most toxicity studies on dispersed oil concluded that the presence of dispersant increased environmental risk for aquatic organisms living in the water column (Milinkovitch et al., 2011; Ramachandran et al., 2004), which is possibly due to their relatively high concentration of oil and dispersant in the laboratory tests.

ED_001324_00001264-00021

Individual Aromatics Degradation

High Concentration Experiment. A first-order model could be used to fit the biodegradation of the individual PAHs that had a negligible fraction persisting by the end of the high concentration experiment. At both temperatures, the biodegradation rates of naphthalene, phenanthrene, fluorine, and dibenzothiophene and their homologues in the high concentration experiment were consistantly higher in the presence of dispersant (Table S5). The greatest enhancement was observed with naphthalene at 25 °C, which was 2.9-fold higher than at 5 °C (-1.94 d^{-1} vs. -0.68 d^{-1}). The surfactant likely assisted the transfer of more soluble compounds from the oil phase to the aqueous phase through the formation of small oil droplets and the resultant increase in interfacial area. Because the bio-uptake of aromatics is mostly from aqueous phase (Haritash and Kaushik, 2009; Mrozik et al., 2003), C9500 promoted the biodegradation of PAHs by increasing their bioavailability in water.

Low Concentration Experiment. Among the low concentration individual PAHs, the time-varying concentration of specific aromatics could be fitted to a first-order kinetics model (rates shown in Table S6), and the results revealed reduced rates in low concentration treaments, especially when C9500 was present (i.e. naphthalene: -0.36 d^{-1} v -1.94 d^{-1}). However, we could not fit a first-order model for the other PAH compounds in the low concentration experiments because of the long lag period and the immediate and nearly complete removal after biodegradation started. As mentioned before, C9500 acted less effectively on the biodegradation of total PAHs in the low concentration experiment, because different PAH concentrations in parallel dispersed oil and oil alone samples was observed only at one or more replicates in a single sampling event (25 °C: day 4, 5 °C: day 16). Nevertheless, C_4-naphthalene, C_{2-4}-

ED_001324_00001264-00022

phenanthrene, C_{2-3}-fluorene, and naphthobenzothiophene were removed at higher extent in the low rather than in the high concentration samples (Fig. S6). The increase in removal extent for the aforementioned aromatics were 6-14%, 4-47%, 14-54%, and 55-86% respectively. Zahed et al. (2010) also observed similar enhancement of crude oil removal extent in low oiling experiments. In the low concentration experiment, the starting PAH concentrations were below the remaining residual found in the high concentration experiment, but the cultures were able to degrade the aromatic compounds to an even lower level, as shown in Table 1. The reasons are a combination of the following: (1) the alkylated structure of these compounds led to less aqueous dissolution and less susceptibility to microbial attack; and (2) their aqueous uptake was primarily diffusion, for which neither energized membrane nor adenosine triphosphate was essential (Bateman et al., 1986). Taking C_2-phenanthrene for example, it was fully degraded in the low concentration experiment, whereas its removal extent ranged from 66% to 94% when the initial oil concentration was high. The enhancement in the extent of removal under diluted conditions was more remarkable at low temperature, which contributed to our conclusion that solubility was the limiting factor for those compounds to be biodegraded. Whether oil concentration is high or low, pyrene, chrysene and naphthobenzothiophene homologues persisted until the last sampling event at both temperatures, as depicted in Figs. S7 and S8. The heavy PAHs, such as chrysene, are likely to be retained on a beach for several years, according to Yin et al. (2015), who conducted research that monitors the submerged oil mats and surface residual oil balls off Alabama's beach affected by the 2010 DWH incident.

ED_001324_00001264-00023

ACKNOWLEDGMENTS

We thank Jan Kurtz and Diane Yates from EPA's Gulf Ecology Division (GED) at Gulf Breeze, FL, who collected the water samples in the GOM and performed the enrichments and provided them for our experiments. The research was a product of the U.S. Environmental Protection Agency's National Risk Management Research Laboratory (NRMRL) and was partially funded by EPA, NRMRL, Cincinnati, OH, under Pegasus Technical Services, Inc. Contract EP-C-11-006.

REFERENCES

2007. U.S. Environmental Protection Agency, EPA 8270D Semivolatile organic compounds by gas chromatography/mass spectrometry (GC/MS), 3rd. ed, Washington, D.C.

API, 2013. Net Environmental Benefit Analysis for effective oil spill preparedness and response. 2013. http://www.api.org/~/media/Files/EHS/Clean_Water/Oil_Spill_Prevention/NEBA/NEBA-Net-Environmental-Benefit-Analysis-July-2013.pdf.

Batchu, S.R., Ramirez, C.E., Gardinali, P.R., 2014. Stability of dioctyl sulfosuccinate (DOSS) towards hydrolysis and photodegradation under simulated solar conditions. Sci. Total. Environ. 481, 260-265.

Bateman, J.N., Speer, B., Feduik, L., Hartline, R.A., 1986. Naphthalene association and uptake in Pseudomonas putida. J. Bacteriol. 166, 155-161.

Brakstad, O.G., Nordtug, T., Throne-Holst, M., 2015. Biodegradation of dispersed Macondo oil in seawater at low temperature and different oil droplet sizes. Marine pollution bulletin 93, 144-152.

Bredholt, H., Bruheim, P., Potocky, M., Eimhjellen, K., 2002. Hydrophobicity development, alkane oxidation, and crude-oil emulsification in a Rhodococcus species. Can. J. Microbiol. 48, 295-304.

Bruheim, P., Bredholt, H., Eimhjellen, K., 1999. Effects of surfactant mixtures, including Corexit 9527, on bacterial oxidation of acetate and alkanes in crude oil. Appl. Environ. Microbiol. 65, 1658-1661.

Campo, P., Venosa, A.D., Suidan, M.T., 2013. Biodegradability of Corexit 9500 and dispersed South Louisiana crude oil at 5 and 25 degrees C. Environ. Sci. Technol. 47, 1960-1967.

D7730−11, A., 2011. Standard Test Method for Determination of Dioctyl Sulfosuccinate in Sea Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). ASTM International 2011. ASTM International.

Gray, J.L., Kanagy, L.K., Furlong, E.T., Kanagy, C.J., McCoy, J.W., Mason, A., Lauenstein, G., 2014. Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. Chemosphere 95, 124-130.

Haritash, A.K., Kaushik, C.P., 2009. Biodegradation Aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. J. Hazard. Mater. 169, 1-15.

Jasper, W.L., Kim, T.L., Wilson, M.P., 1978. Droplet size distributions in a treated oil–water system., in: McCarthy, L.T., Lindblom, G.P., Walter, H.F. (Eds.), Chemical Dispersants for the Control of Oil Spills. American Society for Testing and Materials, Philadelphia, PA.

Kaku, V.J., Boufadel, M.C., Venosa, A.D., 2006. Evaluation of mixing energy in laboratory flasks used for dispersant effectiveness testing. J. Environ. Eng. Div. ASCE 132, 93-101.

Kujawinski, E.B., Kido Soule, M.C., Valentine, D.L., Boysen, A.K., Longnecker, K., Redmond, M.C., 2011. Fate of dispersants associated with the deepwater horizon oil spill. Environ. Sci. Technol. 45, 1298-1306.

Lee, K., Nedwed, T., Prince, R.C., Palandro, D., 2013. Lab tests on the biodegradation of chemically dispersed oil should consider the rapid dilution that occurs at sea. Mar. Pollut. Bull. 73, 314-318.

Li, Z., Lee, K., King, T., Boufadel, M.C., Venosa, A.D., 2009. Evaluating Chemical Dispersant Efficacy in an Experimental Wave Tank: 2—Significant Factors Determining In Situ Oil Droplet Size Distribution. 26, 1407-1418.

Lindstrom, J.E., Braddock, J.F., 2002. Biodegradation of petroleum hydrocarbons at low temperature in the presence of the dispersant Corexit 9500. Mar. Pollut. Bull. 44, 739-747.

Lunel, T., 1995. Understanding the mechanism of dispersion through oil droplet size measurements at sea., in: P. Lane (Ed.), The Use of Chemicals in Oil Spill Response. American Society for Testing and Materials, Philadelphia, PA.

Milinkovitch, T., Kanan, R., Thomas-Guyon, H., Le Floch, S., 2011. Effects of dispersed oil exposure on the bioaccumulation of polycyclic aromatic hydrocarbons and the mortality of juvenile Liza ramada. Sci. Total. Environ. 409, 1643-1650.

Mrozik, A., Piotrowska-Seget, Z., Labuzek, S., 2003. Bacterial Degradation and Bioremediation of Polycyclic Aromatic Hydrocarbons. Pol. J. Environ. Stud. 12, 15-25.

Operational Science Advisory Team, 2010. Summary report for sub-sea and sub-surface oil and dispersant detection: sampling and monitoring. Prepared for Paul F. Zukunft, U.S. Coast Guard.

Prince, R.C., 2015. Oil spill dispersants: boon or bane? Environmental science & technology 49, 6376−6384.

Prince, R.C., Elmendorf, D.L., Lute, J.R., Hsu, C.S., Halth, C.E., Senius, J.D., Dechert, G.J., Douglas, G.S., Butler, E.L., 1994. 17α(H),21β(H)-hopane as a conserved internal marker for estimating the blodegradation of crude oil. Environ. Sci. Technol. 28, 142-145.

Prince, R.C., McFarlin, K.M., Butler, J.D., Febbo, E.J., Wang, F.C., Nedwed, T.J., 2013. The primary biodegradation of dispersed crude oil in the sea. Chemosphere 90, 521-526.

Ramachandran, S.D., Hodson, P.V., Khan, C.W., Lee, K., 2004. Oil dispersant increases PAH uptake by fish exposed to crude oil. Ecotox. Environ. Safe. 59, 300-308.

Rojo, F., 2009. Degradation of Alkanes by Bacteria. Environ. Microbiol. 11, 2477-2490.

Spotte, S., Adams, G., Bubucis, P.M., 1984. GP2 medium is an synthetic seawater for culture or maintenance of marine organisms. . Zoo Biol. 3, 229-240.

Tang, W.C., White, J.C., Alexander, M., 1998. Utilization of sorbed compounds by microorganisms specifically isolated for that purpose. Appl. Microbiol. Biotechnol. 49, 117-121.

Venosa, A.D., Holder, E.L., 2007. Biodegradability of dispersed crude oil at two different temperatures. Mar. Pollut. Bull. 54, 545-553.

Wentzel, A., Ellingsen, T.E., Kotlar, H.K., Zotchev, S.B., Throne-Holst, M., 2007. Bacterial metabolism of long-chain n-alkanes. Appl. Microbiol. Biotechnol. 76, 1209-1221.

Word, J.Q., Clark, J.R., Word, L.S., 2014. Comparison of the Acute Toxicity of Corexit 9500 and Household Cleaning Products. Human Ecol. Risk Assess. 21, 19.

Yin, F., John, G.F., Hayworth, J.S., Clement, T.P., 2015. Long-term monitoring data to describe the fate of polycyclic aromatic hydrocarbons in Deepwater Horizon oil submerged off Alabama's beaches. The Science of the total environment 508, 46-56.

Zahed, M.A., Aziz, H.A., Isa, M.H., Mohajeri, L., 2010. Effect of initial oil concentration and dispersant on crude oil biodegradation in contaminated seawater. Bull. Environ. Contam. Toxicol. 84, 438-442.

***Supporting Information**

Biodegradability of Different Initial Concentrations of Alaska North Slope Crude Oil Dispersed with Corexit C9500

Mobing Zhuang[1], Gulizhaer Abulikemu[2], Pablo Campo[3], William Platten III[4], Makram T. Suidan[5]*, Albert D. Venosa (retired)[4]and Robyn N. Conmy[4]

1 Department of Biomedical, Chemical and Environmental Engineering, University of Cincinnati, 2901 Woodside Drive, Cincinnati, OH 45221, USA

2 Pegasus Technical Services Inc., 46 E Hollister St, Cincinnati, OH 45219 USA

3 Cranfield Water Science Institute, Cranfield University, Cranfield, Beds, MK43 0AL, UK

4 U.S. Environmental Protection Agency, NRMRL, 26 W. MLK Drive Cincinnati, OH, 45268, USA

5 Faculty of Engineering and Architecture, American University of Beirut, Bechtel Engineering Bldg. - 3rd flr. - Room 308 P.O. Box: 11-0236 Riad El Solh 1107 2020, Beirut, Lebanon

*Corresponding author: Makram T. Suidan
Tel: 00961-1-347952
Fax: 00961-1-744462
E-mail address: msuidan@aub.edu.lb

ED_001324_00001265-00001

Table S1. Summary of Experimental Layout for High Concentration Experiment.

Test	Temperature	Treatment	Sampling Events	Sample Replicates	Total Experimental Units (EU)
1	5 °C	C9500 alone	11	3	33
2	5 °C	ANS dispersed by C9500	11	3	33
3	5 °C	alone	11	3	33
4	5 °C	Killed ANS control	1	3	3
5	5 °C	Killed C9500 control	11	3	33
6	5 °C	Killed ANS+C9500 control	11	3	33
				Subtotal EU's	**168**
7	25 °C	C9500 alone	9	3	27
8	25 °C	ANS dispersed by C9500	9	3	27
9	25 °C	ANS alone	9	3	27
10	25 °C	Killed ANS control	1	3	3
11	25 °C	Killed C9500 control	9	3	27
12	25 °C	Killed ANS+ C9500 control	9	3	27
				Subtotal EU's	**138**
			Total EU's for High Concentration Experiment		**306**

ED_001324_00001265-00002

Table S2. Summary of Experimental Layout for Low Concentration Experiment.

Test	Temperature	Treatment	Sampling Events	Sample Replicates	Total Experimental Units (EU)
13	5 °C	C9500 alone	11	3	33
14	5 °C	ANS+C9500	11	3	33
15	5 °C	ANS alone	11	3	33
16	5 °C	Killed ANS control	1	3	3
17	5 °C	Killed C9500 control	11	3	33
18	5 °C	Killed (ANS+C9500) control	11	3	33
19	5 °C	Killed (ANS+C9500) +Protease	6	3	18
				Subtotal EU's	**186**
20	25 °C	C9500 alone	10	3	30
21	25 °C	ANS+C9500	10	3	30
22	25 °C	ANS alone	10	3	30
23	25 °C	Killed ANS control	1	3	3
24	25 °C	Killed C9500 control	10	3	30
25	25 °C	Killed (ANS+C9500) control	10	3	30
26	25 °C	Killed (ANS+C9500) +Protease	6	3	18
				Subtotal EU's	**181**
		Total EU's for Low Concentration Experiment			**357**

ED_001324_00001265-00003

Table S3. First-order biodegradation rate coefficients and standard deviations (sd) of individual alkanes in the high concentration experiment.

Compound	5 °C ANS alone		25 °C ANS alone		5 °C ANS+C9500		25 °C ANS+C9500	
	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})
C10	0.19	0.02	1.34	0.18	0.63	0.03	2.00	0.34
C11	0.15	0.01	1.16	0.15	0.40	0.02	1.30	0.09
C12	0.12	0.01	1.02	0.14	0.29	0.03	0.96	0.06
C13	0.11	0.01	0.94	0.14	0.27	0.03	0.91	0.05
C14	0.11	0.01	0.92	0.14	0.25	0.03	0.82	0.04
C15	0.11	0.01	0.95	0.14	0.24	0.03	0.84	0.05
C16	0.11	0.01	1.01	0.15	0.24	0.03	0.92	0.05
C17	0.11	0.01	1.08	0.16	0.24	0.03	0.97	0.06
PR	0.15	0.03	0.45	0.11	0.07	0.01	0.39	0.04
C18	0.11	0.01	1.06	0.14	0.22	0.02	0.96	0.05
PH	0.15	0.03	0.46	0.12	0.08	0.01	0.43	0.04
C19	0.11	0.01	1.05	0.14	0.22	0.02	0.97	0.06
C20	0.11	0.01	0.99	0.14	0.21	0.02	0.93	0.05
C21	0.10	0.01	0.97	0.14	0.20	0.02	0.91	0.05
C22	0.10	0.01	0.88	0.13	0.19	0.02	0.84	0.04
C23	0.09	0.01	0.84	0.13	0.18	0.02	0.79	0.04
C24	0.09	0.01	0.84	0.14	0.17	0.02	0.79	0.04
C25	0.09	0.01	0.81	0.13	0.15	0.02	0.81	0.03
C26	0.08	0.01	0.81	0.14	0.12	0.02	0.78	0.04
C27	0.08	0.01	0.74	0.13	0.11	0.01	0.71	0.05
C28	0.12	0.02	0.75	0.12	0.12	0.02	0.60	0.09
C29	0.12	0.02	0.81	0.15	0.12	0.02	0.81	0.07
C30	0.10	0.01	0.83	0.14	0.12	0.02	0.74	0.04
C31	0.09	0.01	0.81	0.14	0.09	0.01	0.61	0.05
C32	0.10	0.01	0.81	0.14	0.09	0.01	0.60	0.06
C33	0.09	0.01	0.68	0.09	0.07	0.01	0.56	0.06
C34	0.08	0.01	0.60	0.09	0.05	0.01	0.54	0.05
C35	0.07	0.01	0.53	0.09	0.04	0.01	0.58	0.04

NA: Rate not calculated because the biodegradation of noted compounds did not follow first order kinetics.

ED_001324_00001265-00004

Table S4. First-order biodegradation rate coefficients and standard deviations (sd) of individual alkanes in low concentration experiment.

Compound	5 °C ANS alone		25 °C ANS alone		5 °C ANS+C9500		25 °C ANS+C9500	
	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})
C10	0.47	0.03	0.93	0.16	0.63	0.04	NA1	NA1
C11	0.25	0.02	0.57	0.07	0.25	0.03	0.47	0.02
C12	0.20	0.01	0.38	0.06	0.23	0.02	0.24	0.03
C13	0.18	0.01	0.42	0.06	0.21	0.02	NA2	NA2
C14	0.13	0.01	0.28	0.04	0.15	0.01	NA2	NA2
C15	0.14	0.01	0.35	0.04	0.15	0.01	NA2	NA2
C16	0.14	0.01	0.37	0.04	0.15	0.02	NA2	NA2
C17	0.13	0.01	0.36	0.04	NA2	NA2	NA2	NA2
PR	NA2	NA2	0.04	0.01	NA2	NA2	NA2	NA2
C18	NA2	NA2	0.35	0.04	NA2	NA2	NA2	NA2
PH	NA2	NA2	0.03	0.01	NA2	NA2	NA2	NA2
C19	NA2	NA2	0.32	0.04	NA2	NA2	NA2	NA2
C20	NA2	NA2	0.30	0.04	NA2	NA2	NA2	NA2
C21	NA2	NA2	0.29	0.04	NA2	NA2	NA2	NA2
C22	NA2	NA2	0.17	0.03	NA2	NA2	NA2	NA2
C23	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C24	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C25	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C26	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C27	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C28	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C29	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C30	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C31	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C32	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C33	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C34	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C35	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2

NA1: Rate not calculated because of excessively rapid removal after acclimation.
NA2: Rate not calculated because noted compounds were persisted during the experiment.

ED_001324_00001265-00005

Table S5. First-order degradation rate coefficients and standard deviations (sd) of individual PAHs in high concentration experiment.

Compound	5 °C ANS alone		25 °C ANS alone		5 °C ANS+C9500		25 °C ANS+C9500	
	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})
nap	0.44	0.04	0.68	0.05	0.71	0.02	1.94	0.02
C1-nap	0.23	0.02	0.60	0.04	0.41	0.03	1.84	0.04
C2-nap	0.17	0.02	0.38	0.07	0.26	0.02	0.61	0.03
C3-nap	0.27	0.02	0.35	0.10	0.20	0.02	0.40	0.05
C4-nap	0.13	0.02	0.29	0.10	0.16	0.02	0.35	0.05
phe	0.19	0.01	0.37	0.09	0.26	0.02	0.46	0.05
C1-phe	0.12	0.01	0.34	0.12	0.19	0.02	0.37	0.06
C2-phe	0.05	0.01	0.21	0.07	0.19	0.03	0.27	0.04
C3-phe	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-phe	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
flu	0.19	0.02	0.37	0.10	0.26	0.02	0.44	0.05
C1-flu	0.15	0.02	0.35	0.11	0.21	0.02	0.39	0.06
C2-flu	0.05	0.01	0.20	0.07	0.11	0.01	0.27	0.04
C3-flu	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dbt	0.25	0.01	0.43	0.08	0.44	0.02	0.77	0.03
C1-dbt	0.13	0.01	0.32	0.10	0.22	0.02	0.41	0.04
C2-dbt	0.04	0.01	0.17	0.06	0.11	0.01	0.26	0.04
C3-dbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pyr*	--	--	--	--	--	--	--	--
C1-pyr	0.00	0.00	0.00	NA	NA	0.00	NA	NA
C2-pyr	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

NA: Rate not calculated because noted compounds were persisted during the experiment.
* Not detected in any samples.

ED_001324_00001265-00006

Table S6. First-order degradation rate coefficients and standard deviations (sd) of individual PAHs in low concentration experiment.

Compound	5 °C ANS alone		25 °C ANS alone		5 °C ANS+C9500		25 °C ANS+C9500	
	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})
nap	NA	NA	0.29	0.05	0.43	0.09	0.36	0.03
C1-nap	NA	NA	0.24	0.03	0.44	0.09	0.28	0.03
C2-nap	NA	NA	0.17	0.04	0.40	0.09	0.21	0.04
C3-nap	NA	NA	0.18	0.04	0.31	0.06	0.20	0.04
C4-nap	NA	NA	0.13	0.03	0.15	0.02	0.14	0.02
phe	NA	NA	NA	NA	NA	NA	0.19	0.03
C1-phe	NA	NA	NA	NA	0.18	0.03	0.20	0.03
C2-phe	0.25	0.01	0.14	0.03	0.11	0.01	NA	NA
C3-phe	0.02	0.01	0.02	0.00	0.00	0.00	0.00	0.00
C4-phe	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
flu	NA	NA	NA	NA	0.29	0.06	NA	NA
C1-flu	NA	NA	NA	NA	0.15	0.02	0.00	0.00
C2-flu	0.29	0.03	0.14	0.03	0.21	0.01	0.00	0.00
C3-flu	0.02	0.00	0.03	0.00	0.00	0.00	0.02	0.00
dbt	NA	NA	0.00	0.00	0.31	0.11	0.00	0.00
C1-dbt	NA	NA	NA	NA	0.23	0.05	0.29	0.06
C2-dbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-dbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nbt	0.00	0.00	NA	NA	0.00	0.00	0.31	0.11
C1-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pyr*	--	--	--	--	--	--	--	--
C1-pyr	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.07
C2-pyr	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

NA: Rate not calculated because of excessively rapid removal after acclimation.
*Not detected in any samples.

ED_001324_00001265-00007

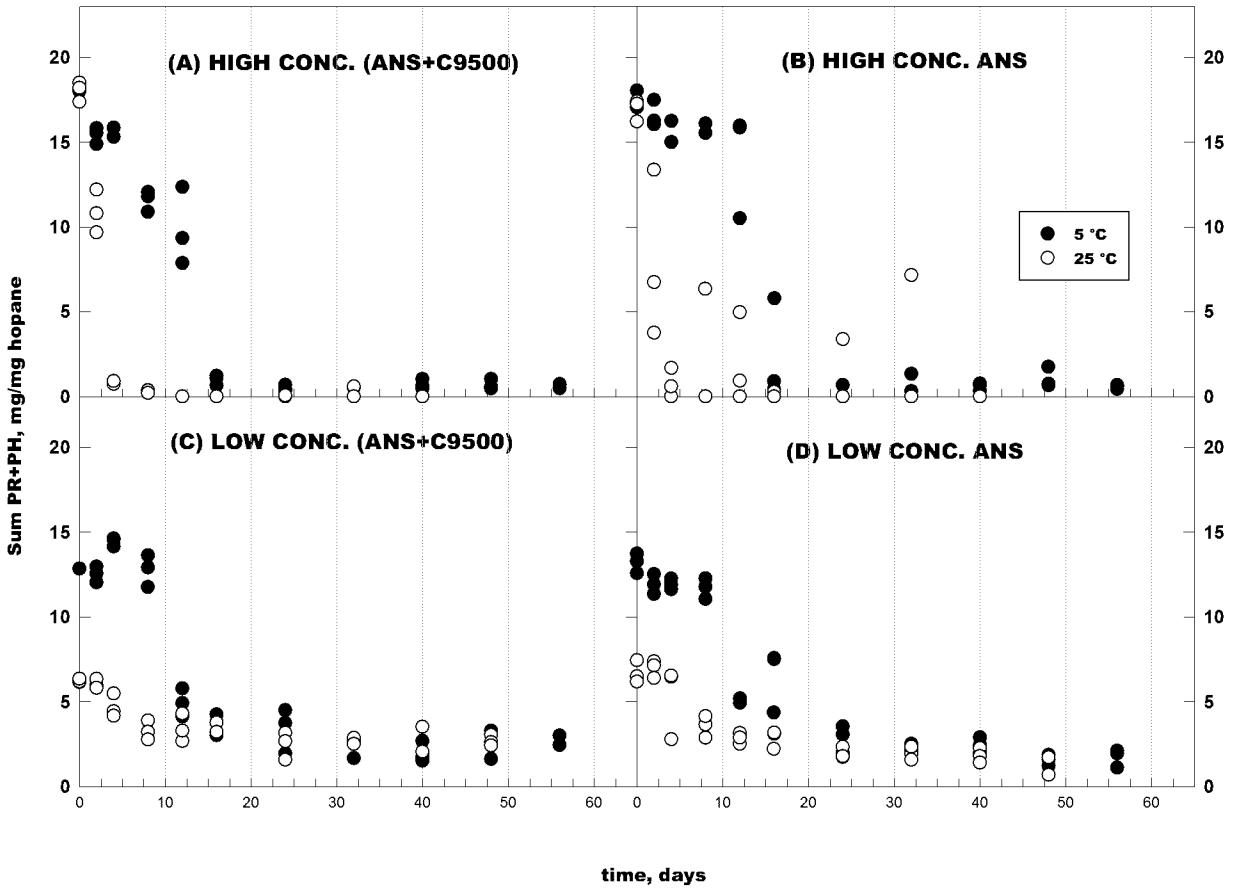


Figure S1. Biodegradation of branched alkanes in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

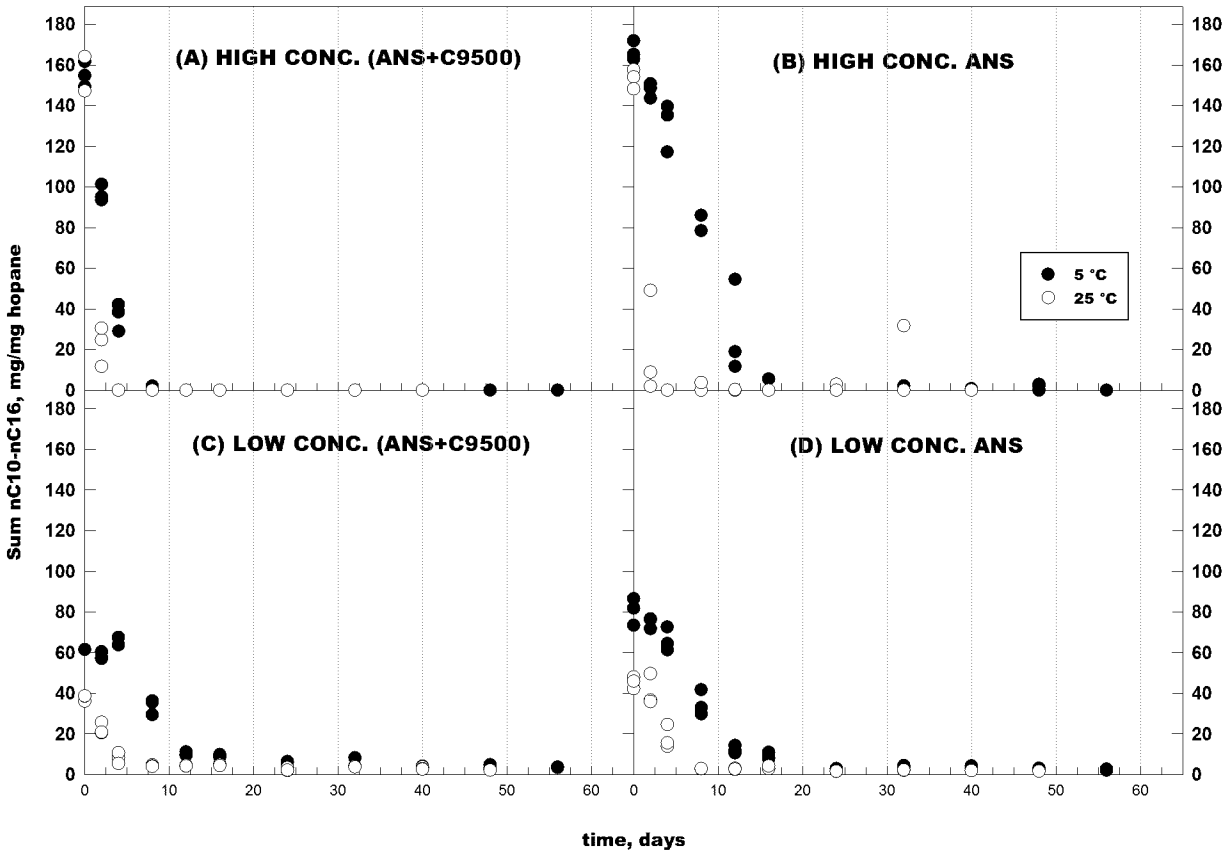


Figure S2. Biodegradation of n-alkanes (nC10-nC16) in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

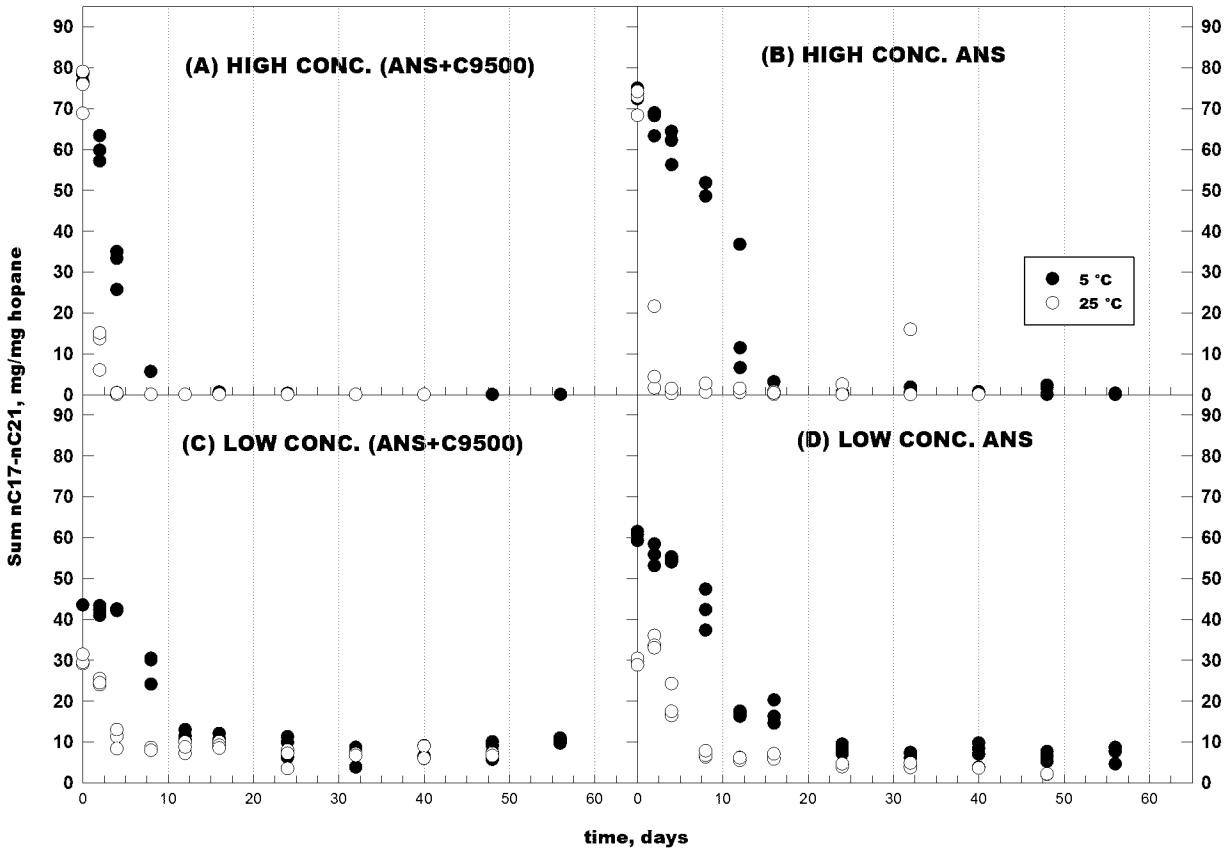


Figure S3. Biodegradation of n-alkanes (nC17-nC21) in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

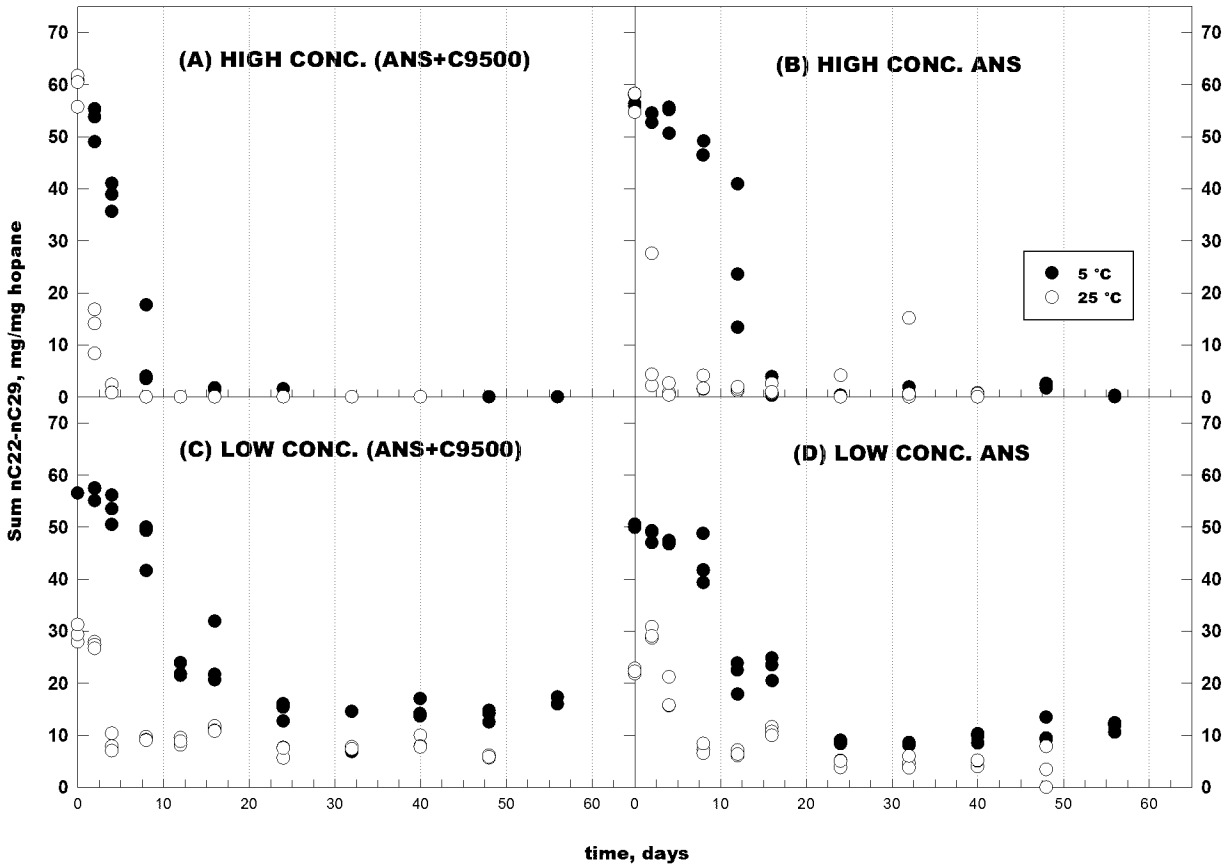


Figure S4. Biodegradation of n-alkanes (nC22-nC29) in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

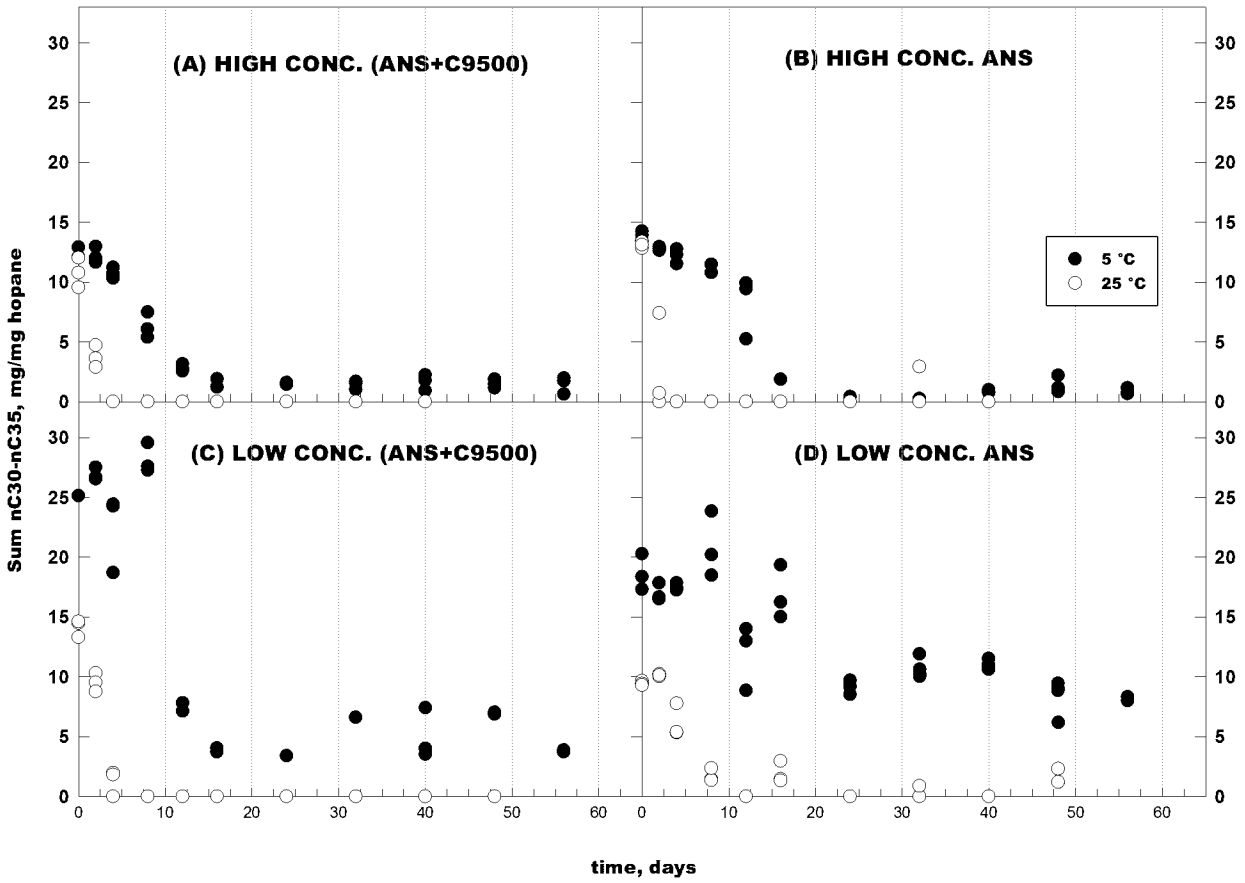


Figure S5. Biodegradation of n-alkanes (nC30-nC35) in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

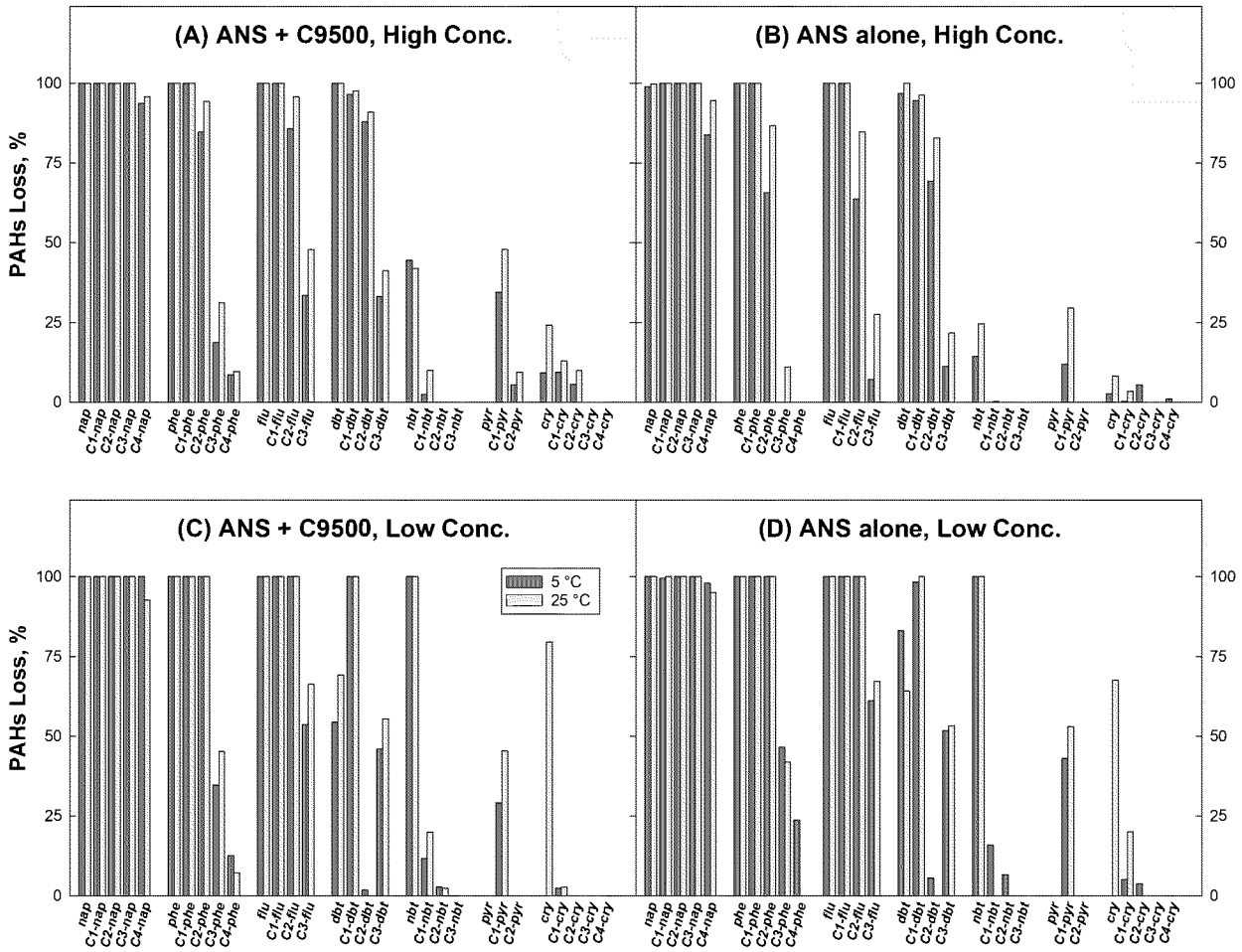


Figure S6. Loss percentage of individual PAH in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C in high concentration (A, B) and low concentration (C, D) experiment.

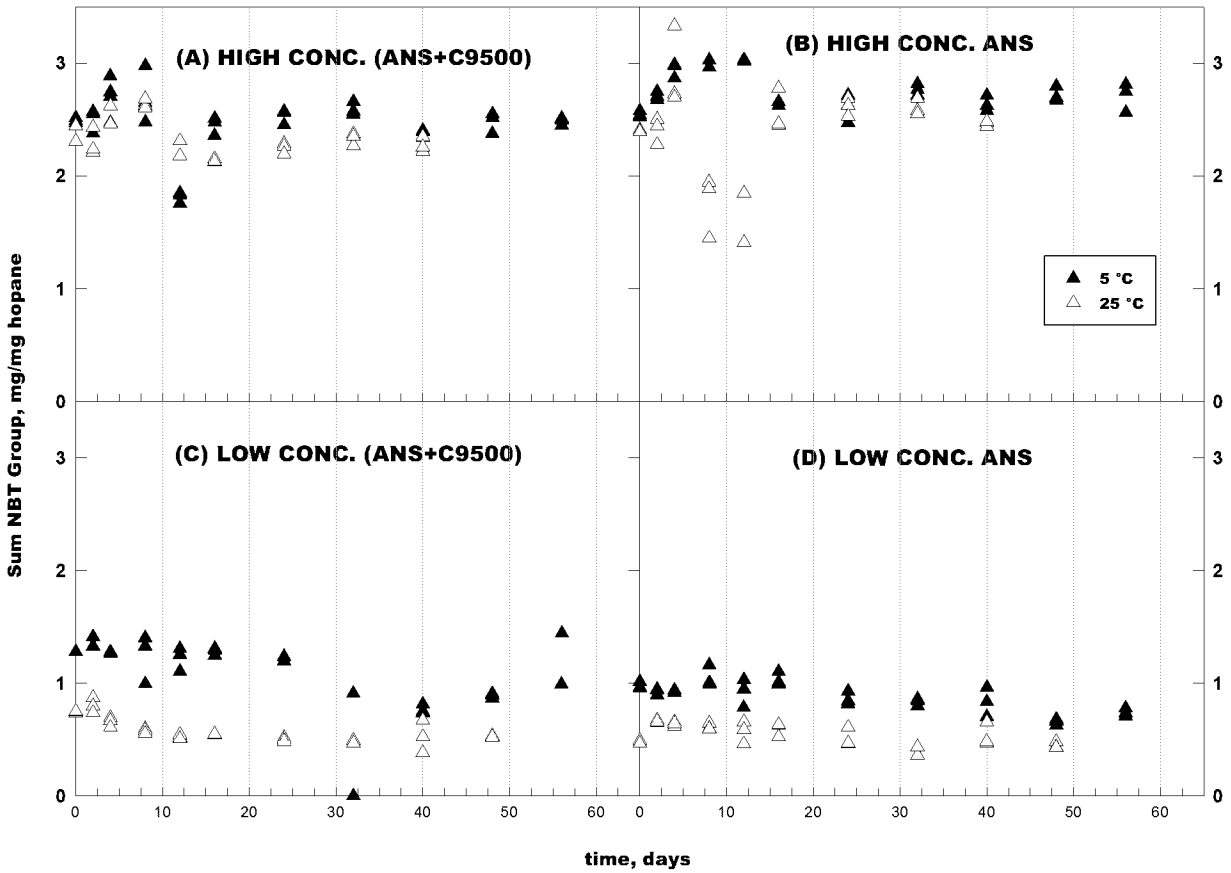


Figure S7. Biodegradation of naphthobenzothiophene homologues in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

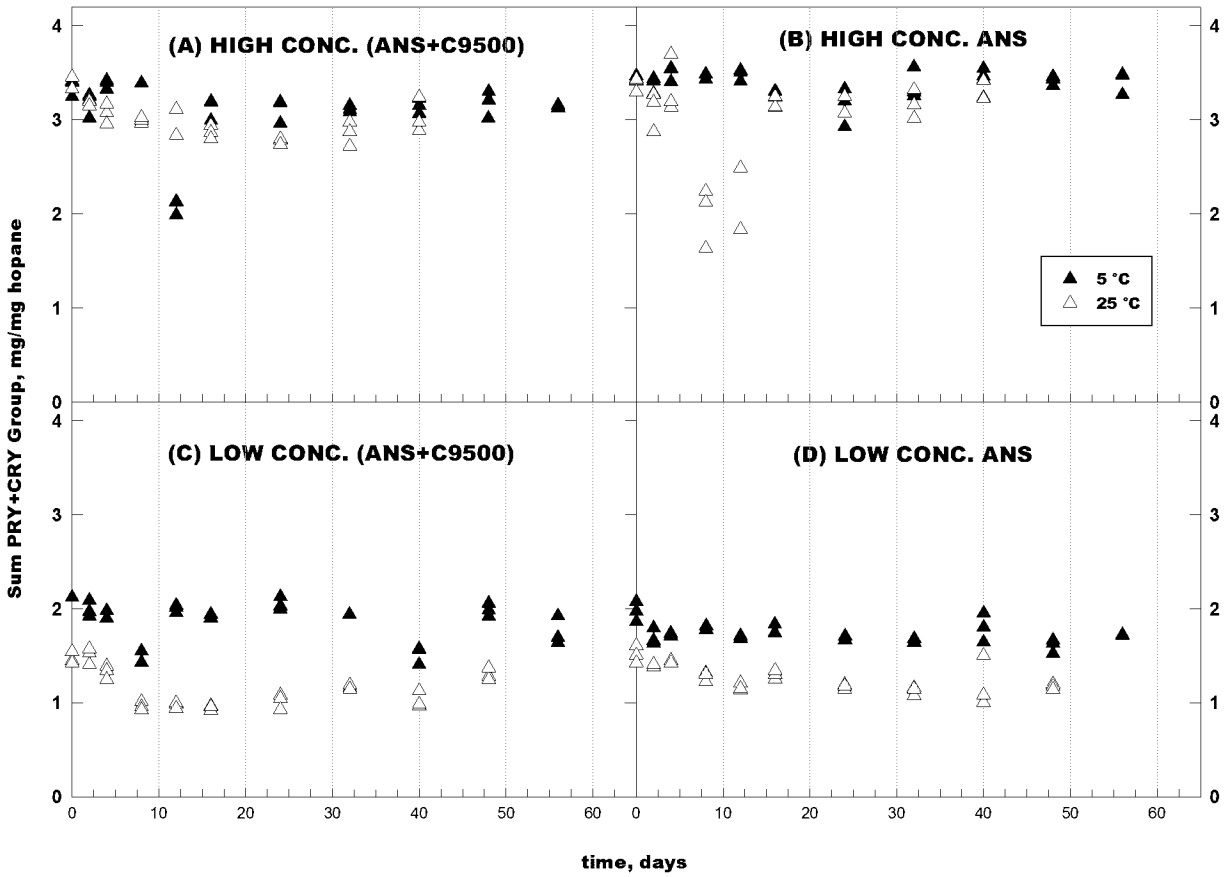


Figure S8. Biodegradation of pyrene homologues + chrysene homologues in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

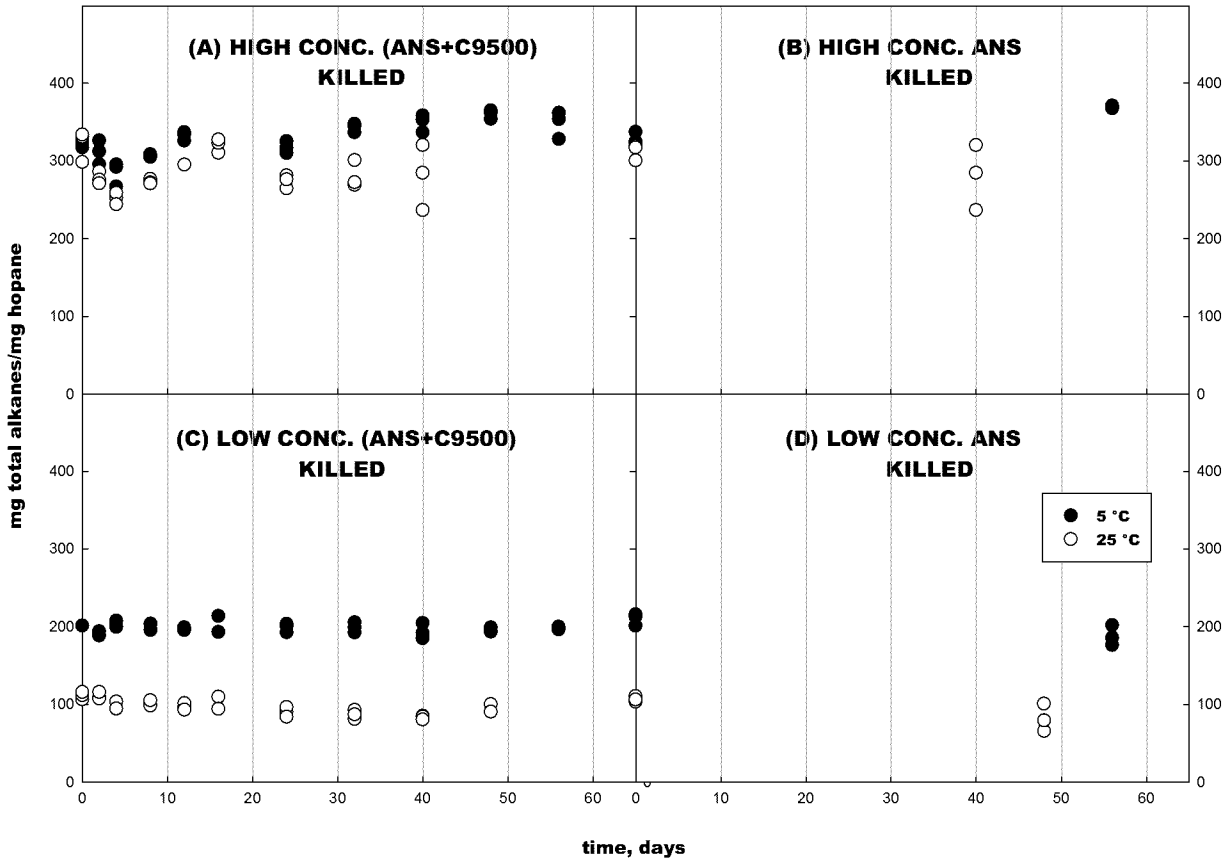


Figure S9. Biodegradation of hopane-normalized total alkanes at both temperatures in high concentration treatments (A, B) and low concentration treatments (C, D) in killed control samples.

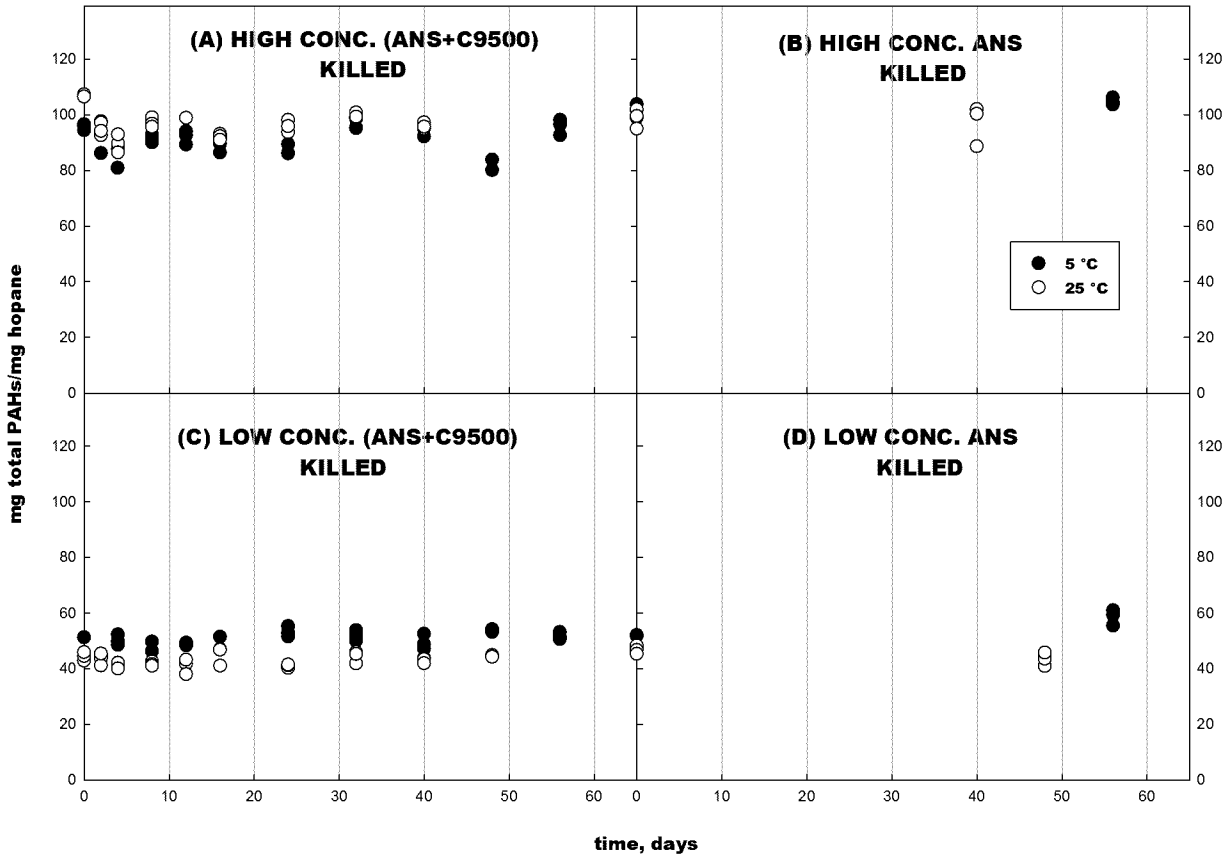


Figure S10. Biodegradation of hopane-normalized total PAHs at both temperatures in high concentration treatments (A, B) and low concentration treatments (C, D) in killed control samples.

Journal of Contaminant Hydrology 131 (2012) 100–118

Contents lists available at SciVerse ScienceDirect

Journal of Contaminant Hydrology

journal homepage: www.elsevier.com/locate/jconhyd

Use of statistical tools to evaluate the reductive dechlorination of high levels of TCE in microcosm studies

Mark Harkness [a,*], Angela Fisher [a,1], Michael D. Lee [b,2], E. Erin Mack [c,3], Jo Ann Payne [d,4], Sandra Dworatzek [e,5], Jeff Roberts [e,5], Carolyn Acheson [f,6], Ronald Herrmann [g,7], Antonio Possolo [h,8]

[a] GE Global Research, One Research Circle, Niskayuna, NY 12309, USA
[b] Terra Systems, Inc., 1035 Philadelphia Pike, Suite E, Wilmington DE 19809, USA
[c] DuPont Corporate Remediation Group, Glasgow 300, P.O. Box 6300, Newark, DE 19714-6300, USA
[d] DuPont, Glasgow 300, P.O. Box 6101, Newark, DE 19714, USA
[e] SiREM, 130 Research Lane Suite 2, Guelph, ON, Canada N1G 5G3
[f] U.S. Environmental Protection Agency, MS 421, 26 W. Martin Luther King Dr., Cincinnati, OH 45268, USA
[g] U.S. Environmental Protection Agency, MS 190, 26 W. Martin Luther King Dr., Cincinnati, OH 45268, USA
[h] Statistical Engineering Division, National Institute of Standards and Technology, Mail Stop 8980, 100 Bureau Drive, Gaithersburg, MD 20899-8980, USA

a r t i c l e i n f o

Article history:
Received 28 April 2011
Received in revised form 24 January 2012
Accepted 25 January 2012
Available online 10 February 2012

Keywords:
Bioremediation
Trichloroethene
DNAPL
Microcosm study
Statistics

a b s t r a c t

A large, multi-laboratory microcosm study was performed to select amendments for supporting reductive dechlorination of high levels of trichloroethylene (TCE) found at an industrial site in the United Kingdom (UK) containing dense non-aqueous phase liquid (DNAPL) TCE. The study was designed as a fractional factorial experiment involving 177 bottles distributed between four industrial laboratories and was used to assess the impact of six electron donors, bioaugmentation, addition of supplemental nutrients, and two TCE levels (0.57 and 1.90 mM or 75 and 250 mg/L in the aqueous phase) on TCE dechlorination. Performance was assessed based on the concentration changes of TCE and reductive dechlorination degradation products. The chemical data was evaluated using analysis of variance (ANOVA) and survival analysis techniques to determine both main effects and important interactions for all the experimental variables during the 203-day study. The statistically based design and analysis provided powerful tools that aided decision-making for field application of this technology. The analysis showed that emulsified vegetable oil (EVO), lactate, and methanol were the most effective electron donors, promoting rapid and complete dechlorination of TCE to ethene. Bioaugmentation and nutrient addition also had a statistically significant positive impact on TCE dechlorination. In addition, the microbial community was measured using phospholipid fatty acid analysis (PLFA) for quantification of total biomass and characterization of the community structure and quantitative polymerase chain reaction (qPCR) for enumeration of Dehalococcoides organisms (Dhc) and the vinyl chloride

* Corresponding author. Tel.: +1 518 0387 5949; fax: +1 518 387 6972.
E-mail addresses: harkness@crd.ge.com (M. Harkness), angela.fisher@research.ge.com (A. Fisher), mlee@terrasystems.net (M.D. Lee), elizabeth-erin.mack@usa.dupont.com (E.E. Mack), Jo-Ann.Payne@usa.dupont.com (J.A. Payne), sdworatzek@siremlab.com (S. Dworatzek), jroberts@siremlab.com (J. Roberts), Acheson.Carolyn@epa.gov (C. Acheson), Herrmann.Ronald@epa.gov (R. Herrmann), antonio.possolo@nist.gov (A. Possolo).
[1] Tel.: +1 518 0387 7392; fax: +1 518 387 6972.
[2] Tel.: +1 302 798 9553; fax: +1 302 798 9554.
[3] Tel.: +1 302 366 6703; fax: +1 302 366 6607.
[4] Tel.: +1 302 366 6719; fax: +1 302 366 6607.
[5] Tel.: +1 519 822 2265; fax: +1 519 822 3151.
[6] Tel.: +1 513 569 7190; fax: +1 513 569 7620.
[7] Tel.: +1 513 569 7741; fax: +1 513 569 7620.
[8] Tel.: +1 301 975 2853; fax: +1 301 975 3144.

0169-7722/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconhyd.2012.01.011

reductase (vcrA) gene. The highest increase in levels of total biomass and Dhc was observed in the EVO microcosms, which correlated well with the dechlorination results.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Enhanced anaerobic biodegradation involves the addition of carbon substrates to the subsurface to stimulate anaerobic bacteria capable of reductively dechlorinating chlorinated solvents like tetrachloroethene (PCE) and trichloroethene (TCE) to the non-toxic end products ethene and ethane. In these processes, chlorinated compounds act as an electron acceptor for the dechlorinating microorganisms, while an electron donor is required to provide energy (Freedman and Gossett, 1989; McCarty, 1994). Hydrogen is generally considered to be the direct electron donor for reductive dechlorination, but is typically produced from the anaerobic fermentation of carbon substrates (Maymo-Gatell et al., 1995). A wide variety of electron donors are commercially available, including soluble donors like sugars, organic acids, and alcohols or insoluble or slow release donors like lactic acid polymers, emulsified vegetable oil (EVO), chitin, and wood chips (AFCEE et al., 2004). These donors ferment via different pathways and at different rates, producing varying levels of hydrogen, and affecting the ability of dechlorinating bacteria to grow and compete with other hydrogen consuming microorganisms (Fennell et al., 1997; Yang and McCarty, 1998).

Dechlorinating bacteria also require basic nutrients like nitrogen, phosphorus, trace minerals, and a circumneutral pH. Nutrients necessary for microbial growth are often present in sufficient quantities in soil and groundwater, but can be limiting in some cases. A diverse microbial population is required to accomplish both the electron donor fermentation and chlorinated solvent dechlorination reactions. Bacteria that can ferment electron donors and those that can dechlorinate TCE to cis-1,2-dichloroethene (cDCE) are present at most chlorinated solvent sites, although the Dehalococcoides (Dhc) group bacteria, which dechlorinate cDCE to vinyl chloride (VC) and ethene, are not present at all sites or may be heterogeneously distributed across individual sites (Fennell et al., 2001; Hendrickson et al., 2002). If Dhc are absent they can be added via bioaugmentation and will grow and proliferate in the subsurface under favorable conditions (Ellis et al., 2000; Harkness et al., 1999).

Other site specific variables that may impact the efficacy of enhanced bioremediation include environmental factors like temperature, pH, and/or the presence of inhibitory co-contaminants such as nitrate, carbon tetrachloride, chloroform, and 1,1,1-trichloroethane (Adamson and Parkin, 2000; Chappelle et al., 1996; Holliger et al., 1993; Nelson et al., 2002; Zhuang and Pavlostathis, 1995). Finally, high levels of chlorinated ethenes themselves may also be inhibitory to reductive dechlorination (Yang and McCarty, 2000a).

Laboratory microcosm studies are a common and usually cost-effective approach to evaluate whether enhanced bioremediation is an appropriate remedial approach for a specific site prior to field implementation (Morse et al., 1998). In addition to evaluating electron donors and determining if nutrients or bacterial populations are limiting, microcosm studies can also assess the impact of environmental factors, microbial distribution, co-contaminants, or concentration of target compounds without the expense and risk associated with field trials. However, testing effects individually is highly inefficient and may miss important interactions between variables, while testing all variables of interest simultaneously can require a large number of bottles, especially when replicates and controls are included. For example, one widely cited protocol for microcosm studies (Morse et al., 1998) recommends thirty bottles per location to evaluate three potential substrates or combinations and the effect of yeast extract and vitamin B_{12} addition with abiotic and unamended controls, all run in triplicate. This problem can be managed by using statistical tools to design microcosm studies and analyze the results. In this case, a fractional factorial experimental design can reduce the number of combinations of the participating variables while still enabling assessment of the effects and interactions that are of primary interest.

As an example of this approach, an extensive microcosm study was performed as part of project SABRE (Source Area BioREmediation), a public/private consortium of twelve companies, two government agencies, and three research institutions whose charter was to determine if enhanced anaerobic bioremediation can result in effective and quantifiable treatment of chlorinated solvent DNAPL source areas. The focus of this 4-year, $5.7 million dollar research and development project was a field site in the United Kingdom (UK) containing a dense non-aqueous phase liquid (DNAPL) source area with groundwater concentrations ranging from 150 to 1100 milligrams per liter (mg/L) TCE.

There were several unique features of the SABRE microcosm study. The program sought to demonstrate this technology in the field on a source area containing TCE DNAPL. Whereas direct biodegradation of the DNAPL itself is unlikely, enhancement in dissolution of the DNAPL phase has been demonstrated in several laboratory studies using PCE (Adamson et al., 2003, 2004; Carr et al., 2000; Cope and Hughes, 2001; Yang and McCarty, 2000b). These results have been reasonably replicated by modeling the dissolution process based on first principles utilizing dechlorination rate constants produced in the laboratory (Seagren et al., 1993, 1994; Sleep et al., 2002). PCE has a relatively low solubility (ca. 150 mg/L) and at this concentration, DNAPL dissolution is relatively slow and toxicity thresholds for the dechlorinating bacteria are generally not exceeded. TCE has a much higher solubility (ca. 1100 mg/L), so dissolution is faster and toxicity issues more pronounced. For these reasons, fewer DNAPL studies have been performed with TCE.

As biodegradation in proximity to DNAPL has been recognized, interest has grown in use of electron donors that can partition into the DNAPL phase (Cápiro et al., 2011; Yang and McCarty, 2002). Donors in this category include EVO and longer chain alcohols. If these donors can be effectively introduced into the subsurface and mixed with the DNAPL, they will slowly dissolve into the water phase where they can be fermented and provide a long-term source of hydrogen at the DNAPL water interface for the dechlorination

process. Because these materials do not require frequent additions, they are typically more economical to apply than non-partitioning substrates due to reduced capital and system maintenance costs (Harkness, 2000).

The six electron donors tested in this study included three soluble donors (lactate, methanol, acetate) and three partitioning donors (EVO, hexanol, butyl acetate). Lactate and methanol were chosen because they are commercially available and widely used in bioremediation applications (AFCEE et al., 2004). Acetate was included because it is used in manufacturing at the SABRE site and is present in site soil and groundwater, where native bacteria may have already acclimated to using it as an electron donor. EVO is commercially available and known to be an effective bioremediation substrate. Hexanol and butyl acetate are more novel choices and were selected based on a literature review which suggested that their physical and chemical properties supported use in this application. These properties included moderate water solubility and octanol water partitioning values, biodegradability, and low cost (internal DuPont memo).

Finally, the microbial community was characterized at the end of the study incubation period by PLFA (phospholipid fatty acid analysis) and two qPCR (quantitative polymerase chain reaction) measurements. PLFA was used to describe the size and structure of the microbial community based on the phospholipids present in cell membranes. Dhc bacteria were quantified via the 16S rRNA gene. The second qPCR measurement, vcrA, provides quantitative information regarding the relative abundance of the gene that codes for the enzyme that dechlorinates vinyl chloride to ethene. These molecular techniques were applied here in an attempt to provide additional insight into the response of the microbial community to the selected experimental variables.

The objectives of the microcosm phase of the SABRE program were to: (1) identify the most effective electron donor from six candidate donors; (2) determine if supplemental nutrients and bioaugmentation are necessary or beneficial to the dechlorination process; and (3) determine if the high levels of TCE present at the site will have a negative impact on the dechlorination process. Four laboratory groups (General Electric [GE], DuPont, SiREM, and Terra Systems) participated in this study.

Because of the number of variables involved, the main experiment was designed as a quarter fractional factorial experiment. Forty-eight treatment combinations were tested in 177 microcosms (including controls). Performance was evaluated based on the time to complete conversion of TCE to ethene. Results were analyzed statistically using ANOVA and a novel technique, survival Weibull regression, which included information from microcosms which did not reach completion. Despite the size and complexity of the study the combination of statistical analyses provided qualitative and in some cases quantitative estimation of all main effects and important 2-factor and 3-factor interactions for the experimental variables under consideration. The ANOVA approach was also applied to the biomass data derived from the PLFA analysis and provided a parallel evaluation of the impact of the same experimental variables.

In a secondary experiment (termed respike experiment), a limited set of the microcosms were respiked with higher levels of TCE more representative of aqueous concentrations that may be encountered at the TCE DNAPL interface. These bottles were also evaluated for extent of TCE dechlorination.

2. Material and methods

2.1. Sample collection and processing

The soil used in this study was obtained from the field site in the UK. Subsurface soil samples were collected in acetate sleeves from the DNAPL source area using a direct push drilling rig. The acetate sleeves were capped and sealed immediately upon retrieval. Site groundwater was also obtained from a nearby monitoring well. Both the soil and the groundwater were packed in iced coolers and sent by courier to DuPont and GE for primary processing.

Upon arrival, the soil was transferred from the sleeves to plastic jars. All further manipulations (i.e. sieving, homogenization, and microcosm construction) of the soil were carried out in a glovebox under an anoxic atmosphere. The composition of glovebox atmosphere varied with laboratory (85–99% nitrogen; 1–10% hydrogen; 0–5% carbon dioxide). The soil was sifted through 0.64 centimeter (cm) screens to remove any large rocks or debris and mixed under anaerobic conditions to homogenize the contents. Approximately 50 percent (%) of the material was too large to pass through the screens and was discarded. The remaining soil was divided into equal parts, placed into jars sealed in the glovebox, and shipped on ice overnight to the other participating laboratories. The groundwater was similarly divided and shipped to the other laboratories.

Upon receiving the soil, each laboratory remixed the soil and performed water extractions to determine the amount of residual TCE present in the soil phase. Each laboratory prepared triplicate mock microcosms consisting of 100 grams (g) of sieved mixed soil with 180 milliliters (mL) of distilled water. The soil and water were shaken and allowed to stand overnight before the water was sub-sampled for TCE. The average TCE concentration on the soil was calculated to be 7.2 mg/kg. This low concentration relative to expected site concentrations was likely due to losses of TCE during sieving and homogenization. Additional soil samples were set aside for microbial analyses and total organic carbon (TOC) testing. The groundwater was sparged with nitrogen or a nitrogen/carbon dioxide mixed gas to remove all the volatile organic compounds (VOCs). The pH was monitored before and after purging to ensure it did not shift significantly due to stripping of carbon dioxide.

2.2. Main experiment set-up

As previously mentioned, the study was designed as a quarter fractional factorial experiment. The variables included four laboratories (DuPont, GE, Terra Systems, and SiREM), six electron donors (lactate, acetate, methanol, EVO, hexanol, and n-butyl acetate), supplemental nutrients (with and without diammonium phosphate and yeast extract), bioaugmentation with dechlorinating microorganisms (with and without KB-1® bacteria), and TCE (low level and high level). If the study were performed as a full factorial design, 192 combinations of these variables would be required to cover the whole experimental space. Instead, the fractional factorial design consisted

of 48 combinations of the experimental variables (the complete list of treatments are provided in Tables S1 and S2 in the Supplemental Materials section). Three replicate bottles were constructed with each combination of variables. Two replicates were run in one laboratory and one in a different laboratory, so that each lab ran 36 amended jars in total. In addition, each laboratory also ran unamended and killed controls, so that the study as designed consisted of 168 bottles. However, nine bottles in the DuPont study were mistakenly bioaugmented during set-up. Although replacement microcosms were prepared, the original nine bottles were retained in the study; so that the final number of bottles was 177 (153 amended bottles plus 24 killed or unamended controls).

The microcosm study was performed in sterile 250 mL glass narrow neck screw-cap bottles. Bottles were sourced from different vendors (Wheaton, Qorpak, Systems Plus, and Fisher Scientific), but were comparable to the Boston round bottles available from Wheaton. All the microcosms were constructed under anaerobic conditions. One hundred grams of soil were dispensed into each bottle with 180 mL of non-sterile groundwater, which left a headspace of approximately 30 mL. The bottles were sealed with Teflon-coated Mininert™ screwcaps after the threads were wrapped with Teflon tape to allow repetitive sampling over the incubation period. Microcosm construction was generally finished within two weeks of receiving soil and groundwater, except for Terra Systems where the soil was stored for four weeks before set-up. The bottles were incubated in the dark either upside-down or side-ways to minimize gas diffusion through the cap. The incubation temperature was controlled to 20–22 °C and monitored during the experiment.

The amount of electron donor added to each bottle was calculated based upon the stoichiometric demand of TCE and the background demand imposed by competitive electron acceptors such as nitrate, iron, and sulfate. Linoleic acid was used as a surrogate for EVO in these calculations. The stoichiometric demand equations assumed complete mineralization of the electron donors to carbon dioxide and complete reduction of all available electron acceptors utilizing the theoretical amount of electrons produced by each donor. The values were calculated assuming average sulfate concentrations of 13.0 mM (1250 mg/L) and iron availability at 10-fold its aqueous concentration based upon field measurements. No nitrate was present, so sulfate was the major electron donor sink in this calculation. An engineering safety factor of 3× was used to account for incomplete mineralization of the donors (e.g., acetate formation) and for competitive processes such as methanogenesis that could not be easily quantified. Equal amounts of each donor were added, based upon electron equivalents, with the exception of magnesium acetate, which was added in three-fold excess due to a computational error.

Soluble electron donors were added to the microcosms every two weeks as concentrated solutions using a gas-tight syringe and sterile technique. Wilclear™ (JRW Bioremediation Technologies) bioremediation grade sodium lactate was added as a 1.0 molar (M) solution in deionized (DI) water. Reagent grade (99%) magnesium acetate tetrahydrate (Sigma-Aldrich) and spectrophotometric grade (99.9%) methanol (Fisher Scientific) were added as 2.0 M solutions in DI water. All soluble amendments were sterilized prior to use by autoclaving or filter sterilization. Partitioning electron donors were added only at the beginning of the study. The EVO used (SRS®, Terra Systems, Inc.) contains 60% soybean oil along with smaller amounts of food grade nonionic emulsifier, sodium lactate, yeast extract, inorganic nutrients, and vitamin B_{12} and was added undiluted. Reagent grade (99+%) 1-hexanol and n-butyl acetate (Sigma-Aldrich) were added as neat compounds. All stock concentrations and amendment amounts are summarized in Table 1.

Supplemental nutrient levels were calculated to produce a carbon:nitrogen:phosphorus ratio of 100:10:1. Reagent grade diammonium phosphate (EM Science) and technical grade yeast extract (Difco) were added once to designated bottles at the beginning of the study. The nutrient solutions were autoclaved or filter sterilized prior to use. Stock concentrations and amendment amounts are shown in Table 1.

The bioaugmented bottles were amended with KB-1® (SiREM), which contains Dhc bacteria (Duhamel et al., 2002, 2004). Bioaugmentation took place two weeks after the beginning of the study to ensure anaerobic conditions were established in the microcosms. Bioaugmentation was performed by replacing 4.0 mL of fluid from each bottle with 4.0 mL of active KB-1® bacteria growing in a low-density liquid culture containing 1×10^{7} to 1×10^{8} Dhc cells/mL.

The killed controls were autoclaved three times on consecutive days and amended with 6.0 mL of a 6% mercuric chloride solution using a gas-tight syringe. The unamended controls did not receive any electron donor or nutrient amendments. Resazurin, a redox-sensitive color indicator, was added to all the bottles at the beginning of the experiment at a concentration of 1 mg/L. No reducing agents were used in this experiment. Neat TCE in the amounts of 150 or 600 μmol was added to the microcosms at the beginning of the study using a glass, gas-tight syringe. Target aqueous phase TCE concentrations were

Table 1
Main experiment amendment table.

Material	Number of additions	Stock solution	Concentration per addition (low TCE)	Concentration per addition (high TCE)
Sodium lactate	10–15	1.0 M	2.8 mM	3.1 mM
Magnesium acetate tetrahydrate	10–15	2.0 M	3.6 mM	3.9 mM
Methanol	10–15	2.0 M	5.7 mM	6.3 mM
EVO (linoleic acid)	1	60% Oil	3.4 mM	3.8 mM
1-Hexanol	1	Neat	11.8 mM	12.2 mM
n-Butyl acetate	1	Neat	10.4 mM	10.8 mM
Ammonium phosphate	1	2.0 M	7.5 mM	7.7 mM
Yeast extract	1	30 g/L	30 mg/L	30 mg/L
KB-1®	1	–	2% by volume	2% by volume

0.76 and 3.04 mM (100 and 400 mg/L), but did not account for sorption to the soil, so that the measured concentrations in the aqueous phase were 0.57 and 1.90 mM (74 and 246 mg/L). The microcosms were shaken well and allowed to equilibrate for 24 h before sampling. The main experiment was run for 203 days.

2.3. Re-spike experiment set-up

As a follow-up to the primary microcosm study, a "respike" experiment was conducted to determine if TCE dechlorination would be observed at higher TCE concentrations more representative of aqueous phase concentrations at the TCE DNAPL water interface. Twenty-nine bottles from the original study that had reached complete dechlorination to ethene were respiked with 1200 μmol of TCE to create target aqueous concentrations of 6.08 mM (800 mg/L). The bottles were selected from all four laboratories and included all six electron donors, along with various combinations of bioaugmentation and nutrient addition (see Table S3 in the Supplemental Materials section for the complete list of treatments). Since sulfate was depleted in the main experiment, a subset of the bottles was amended with sulfate to a target concentration of 13.0 mM (1250 mg/L).

All the bottles were re-amended with electron donor as previously described, using a 3× safety factor (Table 2). Soluble donors were added at 2-week intervals, while partitioning donors were added once. Eight microcosms were amended with sulfate using a 10 molar (M) stock solution of sodium sulfate (Na_2SO_4) in DI water. Where bottles were to receive supplemental nutrients, nutrient levels were calculated as previously described. Bottles were not bioaugmented a second time. Neat TCE was added to each microcosm and the bottles were sampled as previously described. The respike study was run for 210 days.

2.4. Analytical

VOC analyses were performed at the start and at two to four week intervals throughout the study using analytical methods developed in each lab. Samples were obtained by removing 10–1000 microliter (μL) aliquots of liquid from the bottles using glass, gas-tight syringes. TCE, cDCE, VC, and ethene were measured using either headspace or purge and trap auto samplers and a gas chromatograph equipped with a flame ionization detector (GC/FID) or electrolytic conductivity detector (GC/ECD). GC protocols varied between laboratories (see Table S4 in the Supplemental Materials section for the specifics of each method). In each case chlorinated aliphatics and ethene were quantified by comparing peak areas to standard calibration curves generated using water dilutions of a standard mixture containing TCE, cDCE, and VC or by direct injection of known amounts of a standard gas mixture containing ethene.

Sulfate was measured intermittently via ion chromatography using a modified EPA method 300 for sulfate. The modified method is further described in the Supplemental Materials section. All the laboratories routinely monitored pH using pH paper. The TOC in the starting soil was measured using EPA Method SW 846-9060 and the Lloyd Kahn Method (US EPA, 1988).

2.5. Microbial characterization

Soil samples were obtained for PLFA analysis at the beginning and end of the main experiment. Initially, four 10-g samples of wet soil were collected by each lab, placed in glass bottles or plastic centrifuge tubes, and shipped on ice to the United States Environmental Protection Agency (US EPA, National Risk Research Laboratory, Cincinnati, OH). Unfortunately, the biomass levels in these samples were smaller than anticipated and the analytical response was too low to obtain reliable data. As a result, initial biomass values were not available for comparison to post-treatment samples. For the final PLFA samples, 20-g samples of soil were removed from all the microcosms, and shipped as described earlier. A few samples were broken during transport, so the PLFA analysis for the main experiment is based on 139 amended bottles, 11 unamended controls, and 6 killed controls.

PLFA procedures are based on the methods of Findlay (2004) as described in US EPA (2005). The purified phospholipid fatty acid methyl esters (FAMEs) were identified, quantified, and characterized by GC/FID using the MIDI fast-two method (Sasser, 1990). The EVO used in this study contained fatty acids (i.e., oleic acid) but did not contain phospholipids, therefore EVO does not interfere with the FAME/PLFA method (see Supplemental Materials for more information). Total biomass was quantified based on total peak area of the PLFA FAMEs. In addition, the dry weight of the sample was determined and served as a basis for total biomass measurements.

Dhc bacteria and vcrA were measured using qPCR techniques in four samples of the soil at the beginning of the experiment and in 2 g soil samples removed from 18 microcosm

Table 2
Respike experiment amendment table.

Material	Number of additions	Stock solution	Concentration (800 mg/L TCE with 1250 mg/L sulfate)	Concentration (800 mg/L TCE with 0 mg/L sulfate)
Sodium lactate	10	1.0 M	3.2 mM	0.80 mM
Magnesium acetate tetrahydrate	10	2.0 M	3.9 mM	1.8 mM
Methanol	10	2.0 M	6.6 mM	1.9 mM
EVO	1	60% oil	3.9 mM	1.2 mM
1-Hexanol	1	Neat	12.7 mM	1.6 mM
n-Butyl acetate	1	Neat	11.4 mM	1.6 mM
Sodium sulfate	1	10.0 M	10.5 mM	–
Ammonium phosphate	1	2.0 M	7.7 mM	2.3 mM
Yeast extract	1	30 g/L	30 mg/L	30 mg/L

bottles after complete dechlorination was observed. Microcosms were selected to evaluate all experimental variables, although selection was biased toward those electron donors showing the highest levels of dechlorination activity (e.g. EVO, methanol, and lactate). Microcosms sampled for qPCR are noted in Table S1.

DNA analysis was conducted using qPCR techniques as previously described (Scheutz et al., 2008). DNA was extracted from 0.25 g of the soil samples using a PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc.). Cell lyses were performed by adding sediment to the kit bead tube with bead beating performed using a MiniBeadbeater-8 (Biospec Products) at 50% of the maximum setting for 30 s. All subsequent steps were performed according to the manufacturer's instructions. These analyses were performed at SiREM laboratories (Guelph, Ontario).

2.6. Statistical analysis of VOC and biomass data

Mass balance calculations were used to quantify the total amount of chlorinated ethenes in each microcosm bottle. Chlorinated ethenes were measured in the aqueous phase and literature derived equilibrium partitioning coefficients (K_{OC} values) were assigned to TCE, c-DCE, VC, and ethene and used to calculate sediment phase concentrations, based on the measured organic carbon content of 0.6%. Similarly, thermodynamically derived Henry's Law constants were used to estimate the partitioning of these compounds into the headspace. At each time point the number of micromoles of each compound in each compartment (e.g. soil, water, and headspace) were calculated and summed to estimate the total number of micromoles in each bottle. The partitioning and Henry's Law values used are summarized in Table 3, along with a representative calculated distribution of each VOC compound in the various compartments in each bottle. Using this method, 29% of the TCE was found to reside in the soil phase and 61% of the ethene in the headspace. This illustrates the importance of considering all compartments when analyzing and interpreting microcosm data.

The initial number of micromoles of TCE in each bottle was calculated using the median number for total micromoles (TCE, cDCE, VC, and ethene) observed in each bottle over the length of the study. This method was used because the sorbed TCE mass at time zero (T_0) was underestimated in some cases due to partitioning of the TCE into the electron donors themselves. In general, the laboratories hit the target TCE levels, although variability was evident (Figure S1 in Supplemental Materials section). Three outlier values were noted and these bottles were reclassified prior to subsequent statistical analysis of the study data.

Two metrics (responses) were employed to quantify the rate and extent of reductive dechlorination observed in the study. The response of primary interest was the time (measured in days) required for a bottle to reach the ethene endpoint. This response was operationally defined as the time required to remove 98 mol% of the total chlorinated VOCs from the bottle ($D_{0.02}$), resulting in the production of ethene and ethane. This approach measured the overall rate of dechlorination and avoided the complexity associated with calculating and performing statistical analyses on individual degradation rates for TCE, cDCE, and VC.

Normalization was used to reduce variability associated with individual sample points. In this case the molar chlorinated fraction, M_{CL}, was calculated using the total moles of each compound present in all phases (soil, aqueous, headspace) of each bottle

$$M_{CL} = (TCE + DCE + VC)/(TCE + DCE + VC + Ethene). \quad (1)$$

Thus M_{CL} will equal 1 at the beginning of the experiment when TCE is predominant and will approach zero at the end of the study when ethene is produced.

The total VOC data in all the bottles where dechlorination was present displayed a regular, sigmoid pattern when normalized to the starting VOC concentration (Fig. 1). This pattern can be described by means of a logistic function (Pinheiro and Bates, 2000) that expresses M_{CL} as a function of the numbers of days, D, since the beginning of the experiment

$$M_{CL} = 1/(1 + \exp((D - \mu)/\sigma)) \quad (2)$$

where μ is the time in days when M_{CL} reached 0.5 and σ is a scale parameter, such that $-1/(4\sigma)$ is the slope of the straight

Table 3
Partitioning constants and resulting calculated phase distribution of VOCs in microcosm bottles.

Compound	Partitioning constant K_{OC} (mL/g)[a]	Henry's Law constant at 20 °C[b] (L*atm/mol)	VOC distribution in bottles (%)		
			Soil	Water	Headspace
TCE	130	8.06	29.1	67.1	3.8
c-DCE	50	3.26	14.0	84.1	1.9
VC	10	23.6	2.8	83.5	13.7
Ethene	1	228	0.1	38.7	61.2

Note — $K_P = K_{OC} \cdot F_{OC}$ where F_{OC} is the fraction organic carbon in the soil.
[a] Values derived from an internal GE database including multiple literature sources.
[b] Values derived from EPA On-line Tools for Site Assessment Calculation (http://www.epa.gov/athens/learn2model/part-two/onsite/esthenry.html), 2005.

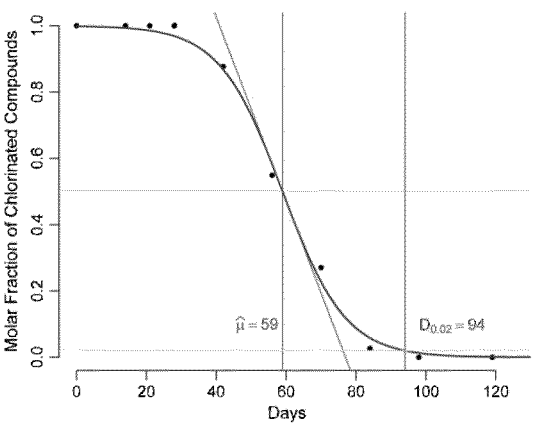
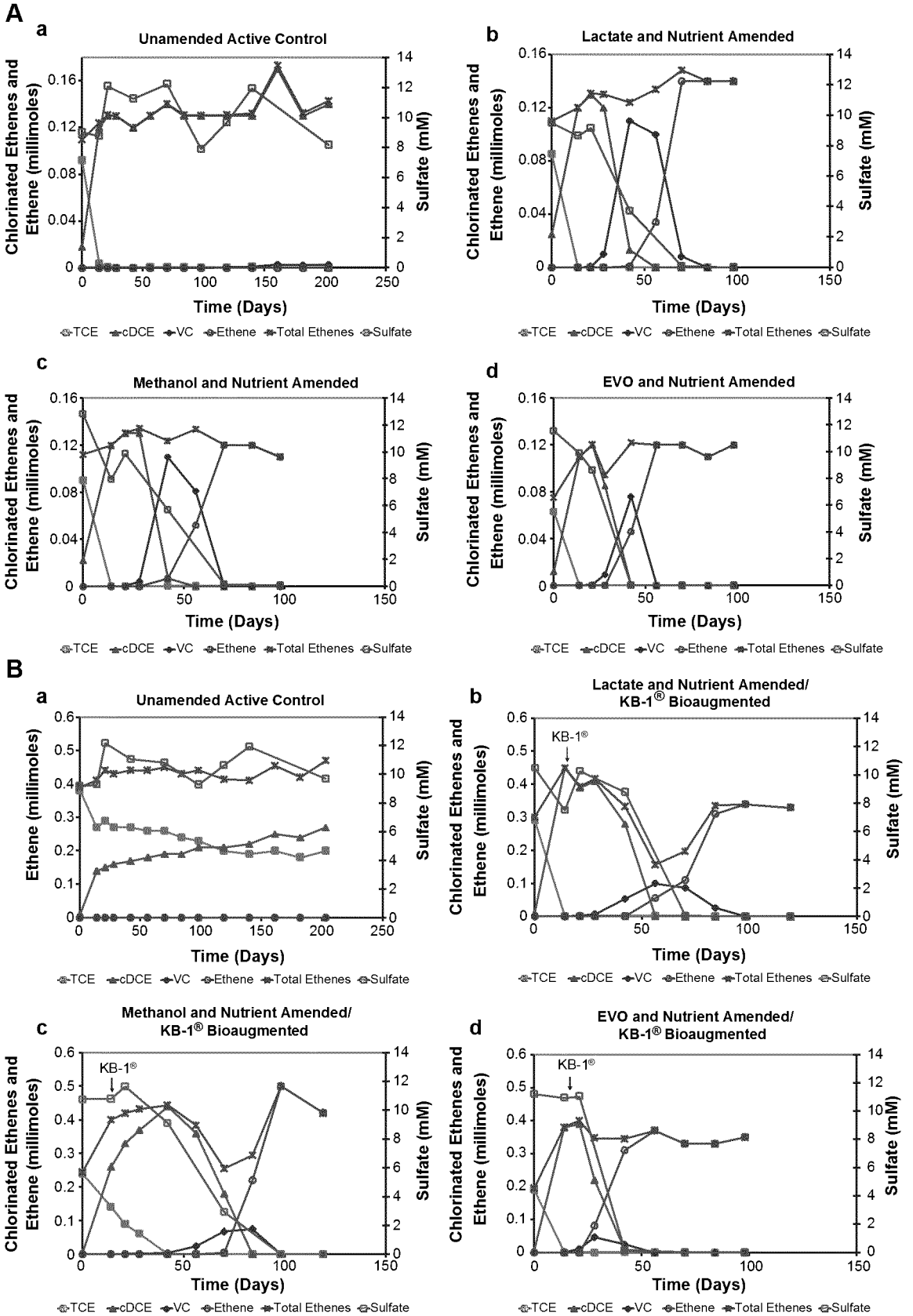


Fig. 1. Example of logistic model (green curve) for dechlorination history, fitted to the data (black dots) from SiREM bottle 17. The molar chlorinated fraction MCL (defined in Eq. (1)) is expressed as a logistic function (Eq. (2)) of the number of days elapsed since the beginning of the experiment. $\hat{\mu}$ represents an estimate of μ in the model. In this case the model was used to estimate that 98% dechlorination ($D_{0.02}$) will have been reached on day 94.


A
a
Unamended Active Control
b
Lactate and Nutrient Amended
c
Methanol and Nutrient Amended
d
EVO and Nutrient Amended
Chlorinated Ethenes and Ethene (millimoles)
Sulfate (mM)
Time (Days)
TCE cDCE VC Ethene Total Ethenes Sulfate
B
a
Unamended Active Control
b
Lactate and Nutrient Amended/ KB-1® Bioaugmented
c
Methanol and Nutrient Amended/ KB-1® Bioaugmented
d
EVO and Nutrient Amended/ KB-1® Bioaugmented
KB-1®
Ethene (millimoles)
Chlorinated Ethenes and Ethene (millimoles)
Sulfate (mM)
Time (Days)
TCE cDCE VC Ethene Total Ethenes Sulfate

line tangent to the graph at D = μ. Use of the logistic function had two valuable features in determining the time required to reach the 98% endpoint (e.g. M_{CL} = 0.02) It allowed interpolation of the data to an exact day between two discreet sampling times when a bottle had achieved complete dechlorination. It also allowed extrapolation to an endpoint in instances when bottles did not achieve complete dechlorination. In the latter case, it was necessary for the bottles to have achieved at least 35% dechlorination to ethene (M_{CL} b 0.65). Otherwise, uncertainty was too high to allow meaningful extrapolation.

A far smaller percentage of bottles reached this criterion in the respike experiment. In this case it was useful to calculate the normalized chlorine number, N_{CL}, defined in terms of molar concentrations as follows

$$N_{CL} \text{ ¼ } 1=3\text{ð}3\text{□½}TCE\text{¬ þ } 2\text{□½}DCE\text{¬ þ ½}VC\text{¬Þ=ð½}TCE\text{¬ þ ½}DCE\text{¬ þ ½}VC\text{¬ þ ½}Ethene\text{Þ:} \quad \text{ð3Þ}$$

N_{CL} gives much better resolution than M_{CL} during the early stages of dechlorination.

ANOVA was used to evaluate the dechlorination data from the main experiment. The experiment was designed so that blocks (laboratories) were fully confounded for the 3-factor interaction of nutrients, bioaugmentation, and TCE. Otherwise, estimates of all main effects, 2-factor, and 3-factor interactions were free of confounding. Only the subset of the bottles where the ethene endpoint was reached or could be estimated provided data for the analysis. Bottles where the time of completion could not be estimated were removed from the data set as censored data.

To exploit all the information the experiment provided, the bottles where the ethene endpoint was not reached and could not be estimated were treated as right-censored observations and both censored and uncensored data were analyzed together using a survival Weibull regression (Meeker and Escobar, 1998). Models of this type are commonly used to perform lifetime studies where a significant number of experimental subjects are expected to survive beyond the duration of the study (e.g., in studies of leukemia patient survival as a function of white blood counts and other physiological attributes). In survival analysis, the time to the 98% endpoint in the censored bottles was assumed to be greater than the maximum value derived from the non-censored data set, which was 353 days. Statistical analyses of chemical data were performed using the R environment for statistical computation and graphics (R Development Team, 2008). Additional details on the survival regression analysis are provided in the Supplemental Materials section.

Due to the limited set of bottles chosen in the respike study, a fully balanced experimental design was not attempted, so that ANOVA could not be applied for most of the primary variables there. However, care was taken in the choice of bottles to allow for statistical analysis of sulfate addition via a linear regression analysis. This was performed by choosing eight sets of replicate bottles and adding sulfate to one in three bottles in each set.

ANOVA was also used to evaluate the biomass data. Total biomass was evaluated using a linear model as a function of seven predictor variables (electron donor, bioaugmentation, median TCE concentration at time 0, nutrient addition, lab, sample day, and chlorine number at the point of sampling). In addition to the main effects of all variables, the three-way and two-way interactions of electron donor, bioaugmentation, and nutrient addition were included. Total biomass values were log transformed so that they could more reasonably be modeled as realized values of Gaussian random variables with constant variance. To compare treatment combinations, the model predicted mean values were calculated for the 48 combinations of electron donor, nutrient addition, and bioaugmentation. The lab effect was averaged and, for purposes of comparability, the chlorine number was set to exactly 0. These predicted values were ranked from highest to lowest. The ranked means were inspected for groupings based on gaps in the distribution of means and the scientific opinion of the authors. Other groups could certainly be considered. The averages across these groups were compared according to the conservative Scheffé p-value (Scheffé, 1953).

Microbial community structure was analyzed based on the relative abundance of 63 specific PLFAs identified in this study. The PLFA data were transformed to a mass percent basis and subjected to multivariate data analysis in order to characterize the relationship between the samples and the categorical variables (Green and Scow, 2000). Hierarchical Cluster Analysis (HCA, Pirouette 3.11 by Infometrix, Inc. Bothell, WA 98011) and Principal Component Analysis (PCA, Pirouette 3.11 by Infometrix, Inc. Bothell, WA 98011) were two exploratory methods used to combine information about the phospholipids present, their abundance, and the categorical variables and identify the differences (PCA) or similarities (HCA) in the data set (see Supplemental Materials for additional details).

3. Results and discussion

3.1. Reductive dechlorination

Anaerobic conditions were established in the amended bottles almost immediately based upon an observed color change in the resazurin dye and appearance of a black iron-sulfide precipitate, indicating that both iron and sulfate reduction was occurring. Sulfate reduction was of particular interest in this study because of the high sulfate levels in the site groundwater. Complete reduction of sulfate was observed between 40 and 100 days in all the lactate and EVO amended bottles and by 120 days in the methanol amended bottles (see Fig. 2A and B for examples). In these cases, sulfate reduction was slowest in bottles containing the high level of TCE in the absence of nutrients or bioaugmentation, but there was

Fig. 2. A. Examples of the sequence of reductive dechlorination under low TCE conditions. Graphs represent (a) unamended active control, (b) lactate and nutrient amended, (c) methanol and nutrient amended, and (d) EVO and nutrient amended. Mass balances are shown for total number of micromoles of chlorinated ethenes and ethene in each bottle. Sulfate levels are also shown. B. Examples of the sequence of reductive dechlorination under high TCE conditions. Graphs represent (a) unamended active control, (b) lactate and nutrient amended/KB-1® Bioaugmented, (c) methanol and nutrient amended/KB-1® Bioaugmented, and (d) EVO and nutrient amended/KB-1® Bioaugmented. Mass balances are shown for total number of micromoles of chlorinated ethenes and ethene in each bottle. Sulfate levels are also shown.

less differential in reduction time between low and high TCE levels in bottles amended with nutrients or bacteria.

Sulfate reduction was more variable in the acetate, butyl acetate, and hexanol-amended bottles. Sulfate was completely reduced with all of these donors in less than 100 days at low TCE levels, but persisted in some of the bottles until the end of the study at high TCE levels. This was particularly true of the hexanol-amended treatments. Once again, high TCE bottles without nutrients and bioaugmentation were the slowest to reduce sulfate. Little sulfate reduction was observed in the autoclaved or unamended controls.

Complete dechlorination of TCE to ethene was observed with all electron donors under various treatment conditions (Fig. 2A and B). In most cases, TCE was dechlorinated in days to weeks in the presence of high sulfate concentrations. Dechlorination of cDCE and VC followed sequentially thereafter and usually occurred concurrently with sulfate reduction, although in some cases VC dechlorination did not begin until all of the sulfate was utilized. The unamended controls supported dechlorination of TCE to cDCE, but did not progress beyond that point in the 203 days of the study. No dechlorination activity was observed in the autoclaved controls. As illustrated by the total ethenes lines in Fig. 2A and B, chlorinated ethene molar balances were usually excellent in both the experimental bottles and the controls, indicating that abiotic losses were minimal.

The reductive dechlorination of PCE and TCE to cDCE is often observed under sulfate-reducing conditions, while the evidence for dechlorination of cDCE and VC in the presence of sulfate is more limited (Bagley and Gossett, 1990; Boopathy and Peters, 2001; Heimann et al., 2005; Hoelen and Reinhard, 2004). The prevalence of PCE and TCE dechlorination may be a result of the wider range of microorganisms capable of mediating these early dechlorination steps, including those that can use sulfate as an electron acceptor (DeWeerd et al., 1991). Otherwise, it appears that sulfate reducing bacteria compete with the dechlorinating bacteria for available hydrogen or produce inhibition by other mechanisms (Chappelle et al., 1996; Nelson et al., 2002; Yang and McCarty, 1998). Our results are similar to those of Hoelen and Reinhard, who observed dechlorination of cDCE and VC to ethene in the presence of 1.3 mM sulfate in microcosms fed with toluene. In our study, rapid fermentation of the added electron donors may have provided sufficient hydrogen to allow both sulfate reduction and reductive dechlorination of cDCE and VC to occur concurrently.

Measured methane concentrations ranged from less than 0.06 to 0.18 mM (1 to 3 mg/L), where the higher levels were typically observed after most of the chlorinated aliphatics were dechlorinated. This is likely because high concentrations of TCE and dechlorination products are inhibitory to the methanogenic bacteria (Maymo-Gatell et al., 1995; Yang and McCarty, 1998, 2000b). This illustrates a significant advantage inherent in performing enhanced bioremediation in source areas, where the dechlorinators can utilize the available hydrogen without competition from methanogens (Yang and McCarty, 2002).

3.2. Analysis of variance

The bulk of the dechlorination data were well described by the logistic model, where 121 out of 153 experimental bottles either reached complete dechlorination to ethene during the 203 days of the study or progressed far enough so that the time of completion could be estimated. When these results were analyzed using a linear ANOVA model, the model was able to fit the data with a multiple R-squared of 77% when adjusted for the number of parameters that were estimated. The residual standard error, estimated based upon 90 degrees of freedom, amounted to 5% of the average value of the response, log $D_{0.02}$.

All five key variables in the experiment (electron donor, bioaugmentation, nutrients, laboratory, and TCE level) had a statistically significant influence on the time to complete dechlorination at a 95% confidence level (Table 4). TCE level is included twice in the ANOVA table, once as a categorical (TCE) and once as a quantitative (TCE_0) variable. The quantitative variable was added to account for differences in starting TCE concentrations among subsets of bottles that had been spiked with either the high or low amounts of TCE. In this case, the quantitative variable had a statistically significant effect on dechlorination rate, while the categorical variable did not (e.g. p-value = 0.11). Statistically significant two-way interactions included those between donor and TCE, donor and bioaugmentation, donor and nutrient addition, and nutrient addition and bioaugmentation.

Box plot summaries of the composite data for the impact of electron donor, bioaugmentation, and nutrient addition as a function of TCE level are shown in Figs. 3 and 4. The data presented in the box plots include only those bottles where a dechlorination endpoint could be estimated using the logistic model. The thick horizontal black line in each boxplot represents the median value. The colored boxes encompass the middle 50% of the data and the whiskers extend to the most extreme data points that are no farther than 1.5 times the inter-quartile range away from the closest end (top or bottom) of each box. The fraction of bottles which reached this level for each data set is shown at the bottom of the figures. The box plots show qualitative differences between treatments and some caution should be exercised when interpreting these figures because the effects are confounded with other variables.

Under low TCE conditions, all the amended treatments except two hexanol bottles reached a point where time to complete dechlorination could be estimated. EVO promoted the fastest dechlorination of TCE to ethene under these conditions, while dechlorination rates between the other donors appeared to be roughly comparable, although the number of censored bottles suggests hexanol was actually the least effective donor (Fig. 3). Differences were more pronounced at high TCE concentrations. Methanol appeared to promote the fastest dechlorination at high TCE concentrations. However, five methanol bottles did not proceed far enough to be included in the analysis. EVO and lactate performed similarly and had only one censored bottle between them. Hexanol and butyl acetate had ten and nine censored bottles, respectively, meaning complete dechlorination was typically not stimulated under high TCE conditions by these amendments.

Both bioaugmentation and nutrient amendment substantially reduced the time required to achieve complete dechlorination of TCE to ethene (Fig. 4). In addition, the number of censored bottles was higher in the non-bioaugmented and non-nutrient amended cases under both low and high TCE

Table 4
Analysis of variance results for time to $D_{0.02}$ in the main experiment.

Factor	Sum of squares	Degrees of freedom	F-value	p-value
Donor	3.06	5	10.23	b0.001
Bioaug	3.08	1	51.43	b0.001
Nutrients	4.56	1	76.10	b0.001
Lab	3.46	3	19.26	b0.001
TCE	0.15	1	2.54	0.11
TCE_0	0.27	1	4.52	0.04
Donor:Bioaug	0.85	5	2.83	0.02
Donor:Nutrients	1.19	5	3.96	0.00
Donor:TCE	2.16	5	7.21	b0.001
Bioaug:Nutrients	0.24	1	3.97	0.05
Bioaug:TCE	2.70E−04	1	4.56E−03	0.95
Nutrients:TCE	0.06	1	0.98	0.33
Residuals	5.39	90		

Notes—Analysis of variance corresponding to the linear model fitted to time to complete dechlorination, expressed as log $D_{0.02}$. All factors are categorical, except TCE_0, which is quantitative. Main effects and two term interactions are shown. p-values indicate level of statistical significance of effect. Type-II tests are calculated according to the principle of marginality, testing each term after all others, except ignoring the term's higher order relatives.

conditions. There was also a statistically significant difference between laboratories (data not shown). This difference was primarily driven by lower dechlorination rates observed in the GE lab. Reasons for this are difficult to discern, but may relate to subtle variations in the set-up conditions, such as soil handling or glove box atmosphere, or to small intra-lab differences in incubation temperatures. For example, the GE glovebox atmosphere differed from the other labs in that it was lower in hydrogen and did not contain carbon dioxide. Although this could have amounted to initial differences of up to 0.1 mmol of hydrogen in the 30 mL bottle headspace due to varying concentrations of hydrogen in the glove boxes, the dechlorination rate did not track glove box hydrogen concentration in all cases. Regardless of the cause, the statistical analyses account for the intra-laboratory variability and still allow conclusions on the effect of the other experimental variables to be drawn, which is a major strength of the statistical approach.

The strongest statistically significant interaction between process variables occurred between electron donor and TCE level (Table 4). Specifically, hexanol and butyl acetate, performed substantially poorer at high TCE levels than at low TCE levels relative to the other donors. Similarly, significant interactions were noted between donors and nutrients and donors and bioaugmentation. In the former case, methanol performance was least sensitive to the presence of nutrients, while EVO performance was most enhanced. In the latter case, bioaugmentation had a disproportionately large positive effect in lactate and EVO amended bottles relative to the other donors (data not shown).

3.3. Survival regression analysis

As previously stated in Section 3.2 the ANOVA analysis did not include the censored data where time to completion could not be accurately estimated. Therefore a survival analysis was performed using a Weibull regression model to incorporate all the data and to quantify differences in dechlorination rates related to the primary variables in the experiment. Fig. 5 illustrates how the probability of survival (which is the inverse of the probability of reaching 98% dechlorination) varies over time for each of the six electron donors using the Weibull survival regression model and is highly descriptive of the temporal progress of dechlorination performance. At low TCE concentrations, EVO supports more rapid dechlorination early in the study, where the EVO bottles diverge from the rest of the bottles at 50–75 days and are the first full set to reach complete dechlorination at 140 days. Lagging dechlorination with hexanol is also evident, as several of these bottles never reach complete dechlorination. At high TCE concentrations, the progression to complete dechlorination is similar for EVO, lactate,

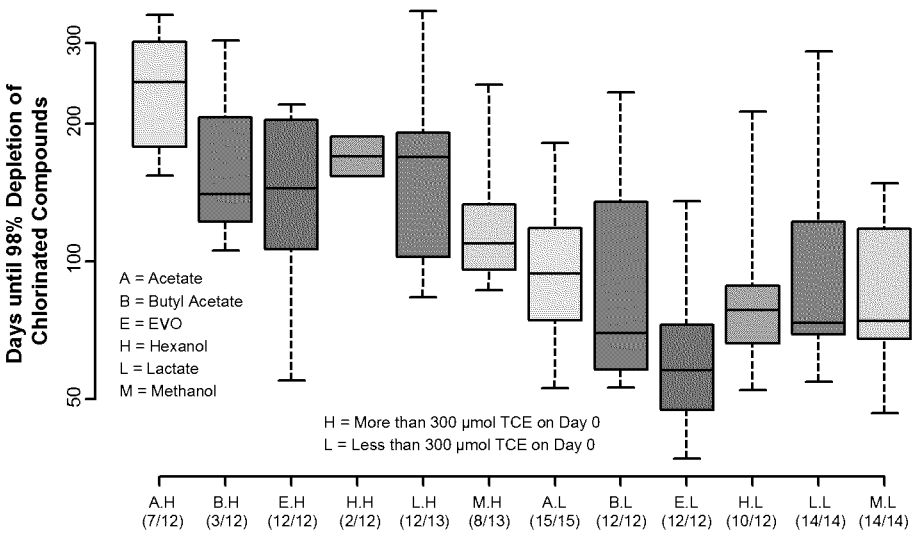


Fig. 3. Boxplots summarizing the numbers of days required to achieve 98% dechlorination ($D_{0.02}$) for various electron donors at high (H) and low (L) TCE concentrations in the main experiment. The fractions at the bottom indicate how many bottles progressed to completion relative to the total number of bottles.

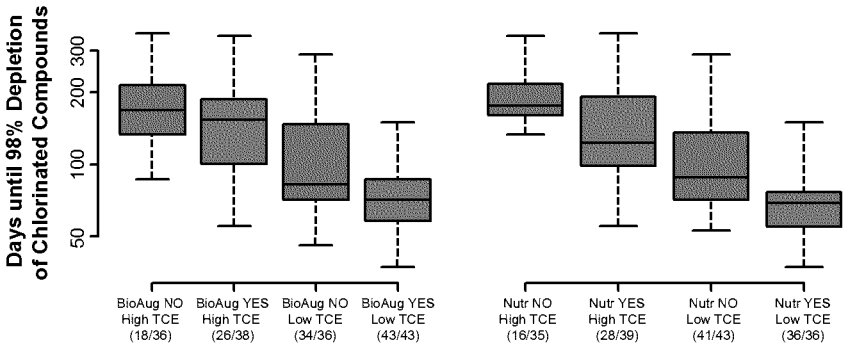


Fig. 4. Boxplots summarizing the numbers of days required to achieve 98% dechlorination ($D_{0.02}$): (left panel) for non-bioaugmented (pink) and bioaugmented bottles (green); and (right panel) without (pink) and with (green) nutrient amendment, both at high and low TCE concentrations in the main experiment. The fractions at the bottom indicate how many bottles progressed to completion relative to the total number of bottles.

and methanol for the first 160 days of the study, before there is differentiation at longer times. Acetate, butyl acetate, and hexanol support substandard dechlorination performance at high TCE levels for most of the study period, although acetate approaches the performance of methanol at the end.

Similar plots show the temporal impact of nutrient addition and bioaugmentation on the time to complete dechlorination (Figs. 6 and 7). At the low TCE concentration, the positive impact of nutrients on dechlorination is most evident around day 80, and all of the nutrient amended bottles reached complete dechlorination by day 150. The difference in dechlorination rate is also evident at the high TCE levels, although a smaller percentage of the bottles reach completion. Similar results were achieved for bioaugmentation at the low TCE level. Although dechlorination in the presence of bioaugmentation was consistently faster at the high TCE level, differences were less pronounced here.

The survival regression also allows the relative performance of the main variables to be quantified, as shown in Table 5. The time to complete dechlorination for all the

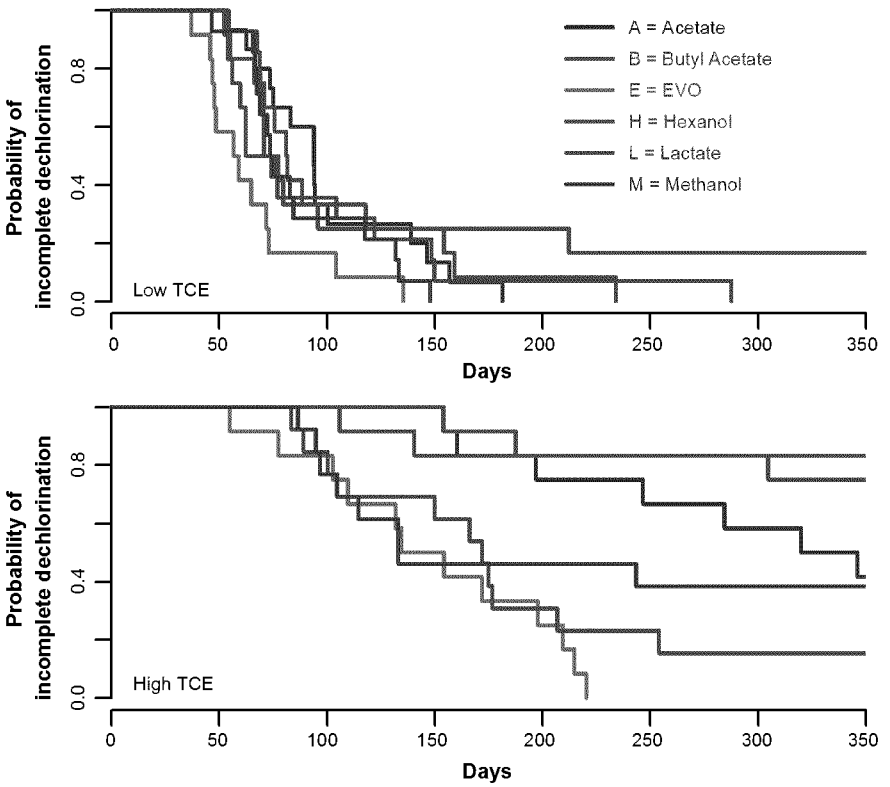


Fig. 5. Estimates of the probability of incomplete dechlorination (probability of not reaching the 98% endpoint) over time, obtained using the Weibull survival regression model that captured the dechlorination history including both censored and uncensored data. The two panels show the results for the six electron donors under low and high initial TCE levels. The horizontal axis measures time in days and the vertical axis shows those probabilities. The earlier the probability reaches zero, the more effective the electron donor.

electron donors was compared to acetate, an arbitrarily chosen reference point. Under low TCE conditions, EVO was the best electron donor, reducing the time to completion by 1.6 times over acetate, but could not be statistically differentiated from methanol, which was 1.4 times faster than acetate, or lactate, which was 1.2 times faster. The performance of butyl acetate and acetate could not be distinguished statistically, but hexanol was slower. Results were more pronounced under high TCE conditions, with EVO and lactate supporting dechlorination 1.6 times faster than acetate. In this case acetate and methanol were statistically indistinguishable, while the performance of butyl acetate and hexanol was substantially worse than acetate.

By similar analyses, bioaugmented bottles reached completion 1.9 times faster than non-bioaugmented bottles under low TCE conditions, while nutrient amended bottles reached completion 2.0 times faster than non-amended bottles at low TCE levels. These results were both statistically significant. In contrast to the donor results, the influence of bioaugmentation and nutrient addition were not statistically significant at high TCE concentrations. This result is discussed more fully in Section 3.5.

Thus the survival regression analysis had several advantages over the ANOVA alone. By incorporating the censored data, it utilized the full data set in the statistical analysis. In addition, it provided temporal information on the progress of dechlorination, quantified relative differences between treatment variables, and provided a means to draw statistical conclusions even with nuanced differences among subsets of the data. Both techniques offer substantial advantages over simple t-tests or other less comprehensive forms of statistical analysis typically applied to microcosm studies.

3.4. Respike experiment

Twenty-nine bottles that went to completion mid-way through the main experiment were subsequently re-spiked with 1200 μmol TCE to evaluate process performance under higher TCE loadings. The measured average starting aqueous phase TCE concentration was 4.05 mM (533 mg/L) in these bottles instead of the target of 6.08 mM (800 mg/L), again reflecting the impact of TCE sorption to the soil. In this case more than half the bottles supported dechlorination of TCE to cDCE and VC after 210 days, although only four bottles completely dechlorinated TCE to ethene. Dechlorination results parsed by electron donor are shown in Fig. 8, expressed in terms of normalized chloride number at 120 days. This time point was chosen because differences in dechlorination rate were relatively more pronounced at that time. Although the effects displayed were not statistically significant because of limitations in the experimental design and are confounded with other variables, EVO again appeared to be superior to the other donors in promoting the most rapid and complete reductive dechlorination. Some of the lactate and methanol bottles were also relatively far advanced at this time, while acetate, butyl acetate, and hexanol promoted little dechlorination. Thus the experimental results were qualitatively similar to those generated under high TCE conditions in the main experiment.

The more limited dechlorination observed in the respike experiment is not surprising. Simulated aquifer experiments

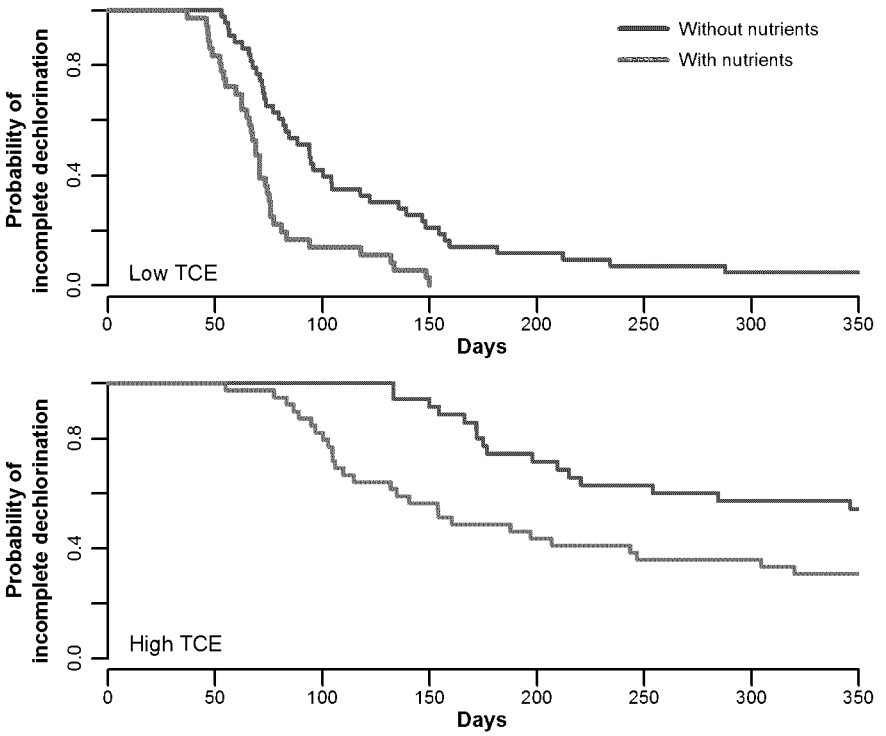


Fig. 6. Survival probabilities obtained using the Weibull survival regression model, shown as a function of nutrient amendment. The horizontal axis measures time in days, and the vertical axis shows probabilities of survival of the chlorinated compounds (probabilities of not having yet reached 98% dechlorination) under low and high initial TCE levels.

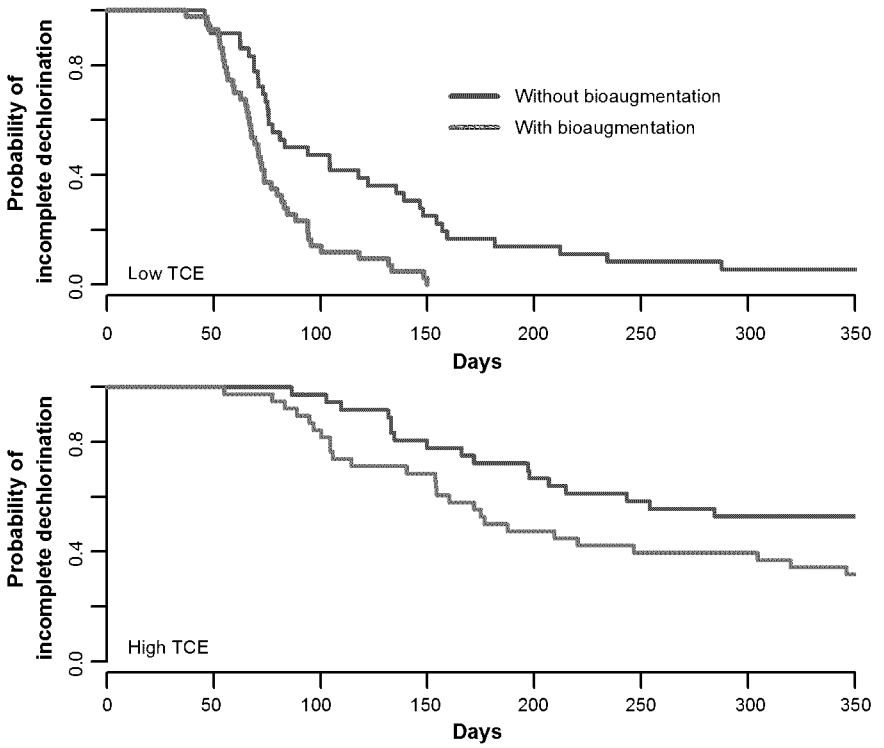


Fig. 7. Survival probabilities obtained using the Weibull survival regression model, as shown a function of bioaugmentation. The horizontal axis measures time in days, and the vertical axis shows probabilities of survival of the chlorinated compounds (probabilities of not having yet reached 98% dechlorination) under low and high initial TCE levels.

performed with PCE DNAPL indicated that dechlorination was limited to cDCE production near the DNAPL interface (Adamson et al., 2003). Microbial characterization performed as part of the Adamson study indicated that Dhc were present, but apparently were unable to further dechlorinate the cDCE in that environment. Inhibition of TCE dechlorination at elevated TCE levels has also been noted in the literature (Hood et al., 2008; Yang and McCarty, 2000a). Yang and McCarty noted that TCE at 2.20 mM (290 mg/L) did not appear to be inhibitory to the dechlorination process, but saturation concentrations (e.g. 8.4 mM or 1100 mg/L) did. Hood and coworkers noted that TCE became inhibitory around 4.87 mM (640 mg/L). Given this information, it is perhaps more surprising that dechlorination in these bottles was as extensive as it was.

Table 5
Comparative effects of main experiment variables derived from survival Weibull analysis.

Low TCE level							
	Electron donor					Bioaugmentation	Nutrients
	B/A	H/A	L/A	M/A	E/A	Y/N	Y/N
	0.88	0.54	1.20	1.4	1.6	1.9	2.0
U	1.1	0.71	1.5	1.8	2.0	2.5	2.6
L	0.69	0.41	0.94	1.1	1.2	1.5	1.6
High TCE level							
	Electron donor					Bioaugmentation	Nutrients
	B/A	H/A	L/A	M/A	E/A	Y/N	Y/N
	0.038	0.059	1.6	1.1	1.6	0.8	1.2
U	0.057	0.092	2.4	1.9	2.4	1.5	2.2
L	0.025	0.039	1.1	0.67	1.1	0.43	0.62

Notes — Differential effects of the electron donors B = butyl acetate, H = hexanol, L = lactate, M = methanol, and E = EVO are relative to A = acetate. Bioaugmentation and nutrient addition are compared to unamended cases. Results are shown separately for low and high TCE environments. A number greater than 1.0 indicates dechlorination was faster than for the comparison treatment. U and L represent upper and lower bounds of the approximate 95% confidence intervals for the differential effects. Confidence intervals that overlap 1.0 indicate effects are not statistically significant.

The enhanced effectiveness of EVO relative to the other donors observed in both the main and respike studies may be partly the result of partitioning of TCE into the distributed soybean oil phase. This partitioning was broadly observed in the EVO bottles at the beginning of the study and had the effect of lowering the aqueous phase concentration of TCE and degradation products, reducing inhibitory effects that may be experienced by the fermenting and dechlorinating bacteria.

Bottles with high original TCE levels (e.g. prior to respiking) appeared to support faster dechlorination in the respike experiment (Fig. 9). This observation suggests the dechlorinating bacteria in these bottles may have been better acclimated to high TCE conditions and so performed better when further challenged. Although these results were not statistically significant due to the unbalanced experimental design, they may be indicative of potential effects.

More importantly, adding sulfate simultaneously with TCE respiking enhanced the rate of dechlorination to a

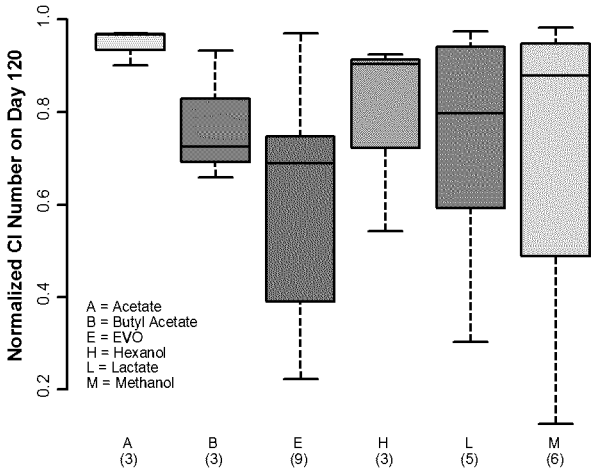


Fig. 8. Boxplots summarizing the values of normalized chloride number for various electron donors in the respike experiment. The numbers in brackets at the bottom are the number of bottles that the boxplots summarize.

statistically significant degree (Fig. 9) After adjusting for effects attributable to differences between laboratories, electron donors, bioaugmentation and nutrient amendments, and level of TCE, the calculated p-values based on fitting a Gaussian linear model to the chloride number were 0.008, 0.03, and 0.04 on sampling days 120, 161, and 182 respectively. The observed enhancement of dechlorination in the presence of sulfate was quite surprising, since as previously stated sulfate reducing bacteria typically compete with the dechlorinating bacteria for available hydrogen or produce inhibition by other mechanisms. Possible explanations for this beneficial interaction include alkalinity formation by the sulfate reduction process, which can act to neutralize acid formed by the reductive dechlorination process (Robinson et al., 2009). Alternatively, it is possible that some synergy exists between the sulfate reducing and dechlorinating bacterial populations, perhaps by sulfate reducing bacteria participating in the co-metabolic

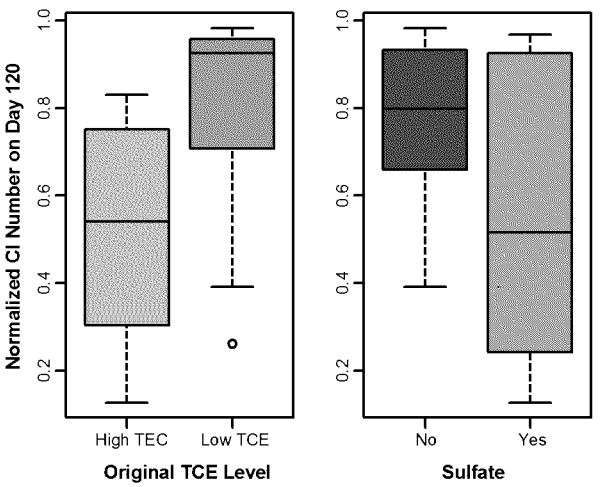


Fig. 9. Boxplots summarizing the values of normalized chloride number as a function of original TCE level and sulfate amendment in the respike experiment.

reductive dechlorination of TCE to cDCE or producing some co-factors that are beneficial to the Dhc bacteria.

Finally, it is possible that some of the inhibition attributed to sulfate reduction in other studies may have been due to hydrogen sulfide formation. Hydrogen sulfide is inhibitory to many bacteria populations important for dechlorination at concentrations ranging from 2 to 5 mM (Maillacheruvu and Parkin, 1996; McCartney and Oleszkiewicz, 1991) and can be a particular problem in microcosm studies because it may accumulate in the bottles. The soil used in this experiment contained a significant fraction of ferric iron, which was reduced to ferrous iron by iron-reducing bacteria and reacted with the hydrogen sulfide to produce black iron sulfide precipitate, effectively removing the hydrogen sulfide from solution. Thus a build-up of hydrogen sulfide was likely not an issue in this study.

Although it did not show superior performance, the fact that acetate supported substantial dechlorination in this study was noteworthy because acetate is not used by Dhc bacteria (Maymo-Gatell et al., 1995) and is not generally considered an effective donor for reductive dechlorination, particularly for cDCE and VC reduction (Cope and Hughes, 2001; Lee et al., 2007). At least one recent study showed acetate alone could support dechlorination of PCE to ethene (He et al., 2002). In that study, it was suggested that syntrophic acetate oxidation may provide hydrogen to support complete dechlorination. At this site, acetate-oxidizing populations may have proliferated where methanogens were inhibited due to high TCE concentrations. Finally, the fact that acetate was present in excess relative to the other donors likely aided the relative dechlorination rate in this case.

The poor performance of hexanol as an electron donor may be the result of substrate inhibition. Hexanol was found to inhibit an anaerobic bacterial consortium at concentrations as low as 2 mM (Playne and Smith, 1983). In this study, hexanol was added at concentrations as high as 11–12 mM. Since the dechlorination process is complex and dechlorinators must rely on other bacteria for various functions, inhibition of any number of bacterial groups (e.g. fermenting bacteria, sulfate reducers) would have a detrimental impact on the whole process. The fact that hexanol performed particularly poorly at high TCE concentrations suggest that substrate and TCE inhibition may have combined to effectively shut down the dechlorination process.

3.5. Microbial characterization

Total biomass measurements were used to characterize the size of the entire microbial community, including fermenting, sulfate-reducing, dechlorinating, and other populations. In the main study, biomass amounts ranged from 0.7 to 180 nanograms (ng) total PLFA/g dry soil and are shown as a function of electron donor in Fig. 10. Once again, other experimental variables are confounded in this graph. Biomass for the unamended and killed controls ranged from 1.1 to 5.6 ng total PLFA/g soil, so that the median biomass in electron donor amended treatments is 2 to 18 times higher than the killed and unamended controls.

The data were statistically analyzed to determine the treatment combinations yielding the highest levels of biomass. Of the seven main variables considered in the

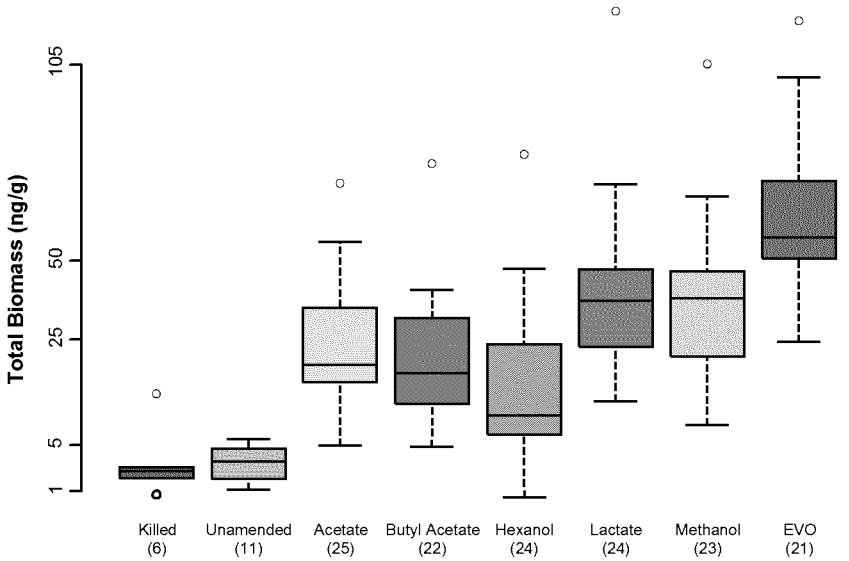


Fig. 10. Box plot summary of total biomass for various electron donors and killed and unamended controls in the main experiment. The numbers in brackets at the bottom are the number of bottles that the boxplots summarize.

analysis, four of the main variables (electron donor, nutrient addition, lab, and chlorine number) and all interaction terms (electron donor–nutrient, electron donor–bioaugmentation, nutrient–bioaugmentation, and electron donor–nutrient–bioaugmentation) were significant at the 95% confidence level (Table 6). Unlike the analysis of chemical data, bioaugmentation was not statistically significant as a main effect but remains in the model due to the significance in the two and three-way interactions.

The preferred treatment combinations can be identified by exploring the highest order interaction, in this case the 3-way interactions of electron donor-nutrient-bioaugmentation. To evaluate the treatment combinations yielding the highest biomass values, biomass was plotted as a function of electron donor with the effects of nutrient and bioaugmentation shown by the colored lines (Fig. 11). If no interaction was observed, one would expect the lines to be parallel in this graph and nutrient amendment and bioaugmentation would produce a consistent effect in all electron donors. In this case lines in the graph cross, often more than once, indicating that the effect of electron donor, nutrients, and bioaugmentation is complex. Three groups (shown in Fig. 11 by the boxes and letters A, B or C) were identified and found to be statistically distinct in terms of total biomass as shown in Table 7. Group (A) had the highest biomass levels and included all the EVO treatments as well as nutrient amended lactate and methanol. It should be noted that the EVO formulation used contains yeast extract, which was part of the nutrient amendment tested in this study. Another group (C) was comprised of hexanol without nutrients. Total biomass in hexanol microcosms without nutrients was very low, similar to the total biomass of unamended and killed controls. Hexanol microcosms with nutrients displayed higher biomass similar to that of some of other electron donors. The remaining group (B) had moderate levels of total biomass and contained all other combinations.

Table 6
Results of statistical analysis of total biomass data.

FACTOR	Sum of squares	Degrees of freedom	F-value	p-value
Donor	8.83	5	31.37	b0.001
Bioaug	0.17	1	3.0	0.086
Nutrients	0.79	1	13.98	b0.001
Lab	3.09	3	18.28	b0.001
Chlorine number	1.67	1	29.72	b0.001
Donor:Bioaug	0.68	5	2.42	0.04
Donor:Nutrients	0.84	5	2.98	0.015
Nutrients:Bioaug	0.41	1	7.19	0.008
Donor:Nutrients:Bioaug	0.75	5	2.66	0.026
Residuals	6.25	111		

Note — The variables sample day and TCE_0 were removed from the model because their P value was >0.1 in the initial analysis. The model was rerun with the remaining variables to calculate the information in this table.

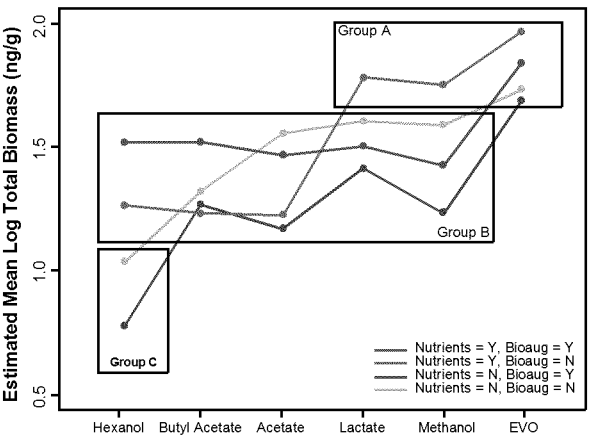


Fig. 11. Estimated total biomass as a function of electron donors, bioaugmentation, and nutrient amendment. The averages across these groups were compared according to the conservative Scheffé p-value. Statistically distinct groups are identified with boxes and the letter A, B, or C.

Table 7
Comparison of total biomass groups in the three way interaction of electron donor, bioaugmentation, and nutrient amendment.

	Difference	Std. error	t-stat	p-value
Group A − Group B	0.4146	0.0504	8.23	8.41E−05
Group B − Group C	0.5178	0.0800	6.47	2.11E−02

Note — The averages across these groups were compared according to the conservative Scheffé p-value.

Bioaugmentation did not appear to affect the differentiation of groups A, B, and C. Since the dechlorinating population is typically a small portion of the total biomass, it is not surprising that bioaugmentation had a limited impact on total biomass (Revesz et al., 2006).

The microbial community structure as determined by the relative abundance of PLFAs was characterized using PCA and HCA. Similar results were obtained using both techniques. Three types of communities were observed by PCA: a large mixed cluster that included bottles amended with lactate, methanol, acetate, and butyl acetate; a cluster of EVO amended bottles; and a cluster of bottles amended with hexanol (Fig. 12). The community structure of the EVO amended bottles was the most distinct in the HCA (data not shown).

The PLFAs which contributed the greatest variance resulting in the differentiation of the EVO communities from the others contained increased proportions of oleic acid

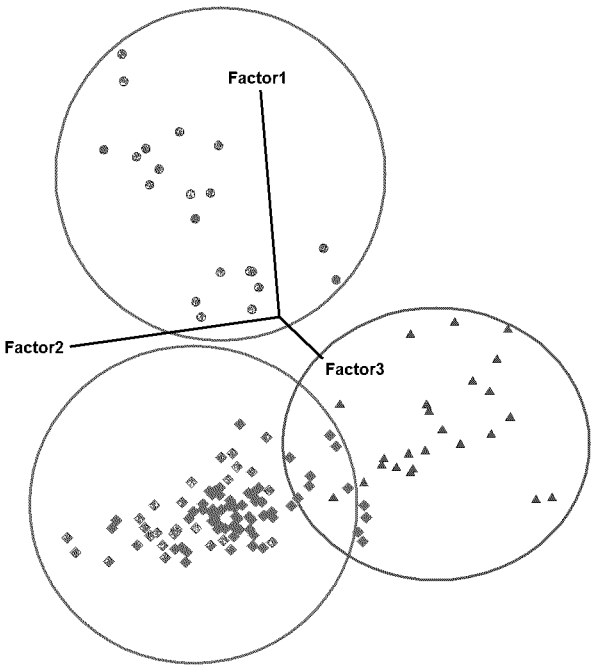


Fig. 12. Community structure based on PCA of PLFAs in the main experiment. Each microcosm is represented by a symbol corresponding to the electron donor (EVO = purple circles, hexanol = green diamonds, and the remaining electron donors [methanol, lactate, acetate, and butyl acetate] = blue triangles). The axes (factors) in the graph were determined by the statistical algorithms of PCA and the PLFA data set. In general, axes are a weighted linear combination of several PLFAs.

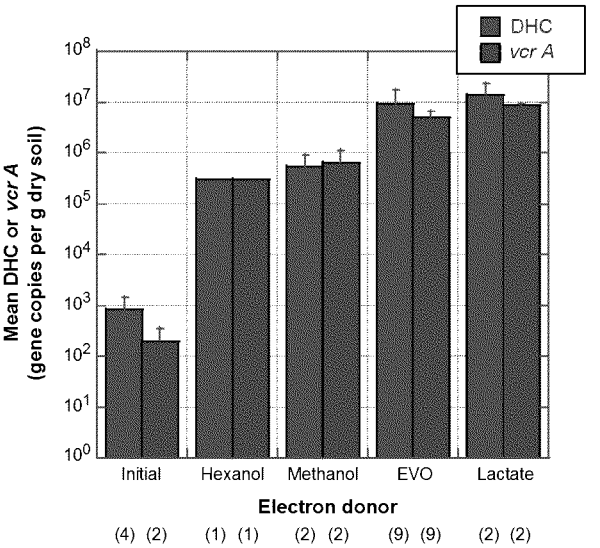


Fig. 13. DHC and vcrA results are shown from the main experiment. The method detection limit was 8×10^{3} copies/g soil for all samples. The numbers in brackets at the bottom are the number of bottles that the bars summarize.

(18:1w9c) and less palmitoleic acid (16:1w7c) in their PLFA distribution. Oleic acid is present in soybean oil and produced during the anaerobic degradation of linoleic acid (Lalman and Bagley, 2000). PLFA sample processing separates phospholipids from hydrocarbons or other cellular debris so the oleic acid measured as part of PLFA analysis was present in the cell membranes as phospholipids. The presence of oleic acid in the cell membranes of the EVO amended microbial community may indicate that their metabolic reaction networks were compatible with this electron donor. In some cases, microbes incorporate exogenous fatty acids into to their cell membranes (Jenkins and Courtney, 2003; Johnsson et al., 1995; Murphy and Perry, 1983). For the microbial community present in this study, more detailed experiments are needed to determine if oleic acid was incorporated directly or whether enhanced levels stem from other biochemical pathways.

A current limitation of the PLFA technique is that it was not possible to connect the different microbial communities observed to specific microbial physiologies such as reductive dechlorination or sulfate reduction because signature phospholipids have not been identified, PLFAs are common to many organisms, or the phospholipids present do not correspond to identified signatures (Green and Scow, 2000). In order to obtain greater benefit from the analysis, it may be useful to combine PLFA with methods such as 16S rRNA assays, denaturing gradient gel electrophoresis, and terminal restriction fragment analysis (Forney et al., 2001) to identify individual microbial functions and better understand the reasons behind the differences observed in the PLFA data.

Such an approach was taken for dechlorinating bacteria, where qPCR was used to measure the levels of 16S ribosomal ribonucleic (rRNA) and vcrA in four samples of starting soil and 18 samples after dechlorination was complete (Fig. 13). Samples were selected with some care in an attempt to

isolate the impact of bioaugmentation on Dhc populations using paired bottles that differed only in the bioaugmentation variable. The initial Dhc densities (based on 16S rRNA qPCR) were 3.6×10^{2} to 1.7×10^{3} gene copies per gram while final densities in amended bottles ranged from 3.0×10^{5} to 3.0×10^{7} gene copies per gram, an increase of 3 to 5 orders of magnitude. When paired samples were examined using a t-test, the cell densities were similar between bioaugmented and non-bioaugmented treatments, suggesting that native and bioaugmented Dhc grew at equivalent rates in these microcosms. The final data also show a strong correlation between 16S rRNA and vcrA values, suggesting that the native Dhc populations are capable of complete dechlorination. Finally, EVO and lactate microcosms displayed higher Dhc levels than the other electron donors. Since sample selection was largely focused on EVO microcosms and was small relative to the experimental variables, statistical interpretation of the electron donor data was not attempted.

An attempt was made to distinguish native Dhc from those present in the KB-1® bioaugmentation culture by monitoring additional reductive dehalogenases (rdhs) known to be present in the KB-1® culture (Waller et al., 2005). Several of the KB-1® rdhs were detected in the non-bioaugmented microcosms and therefore these targets could not be used to distinguish Dhc in KB-1® from native Dhc.

The reason for the variable impact of bioaugmentation on the chemical data at low and high TCE concentration is still difficult to discern given the microbial characterization data. The Dhc measurements were obtained several weeks after dechlorination was complete in each bottle, so bacterial numbers at earlier time points are not known. It is possible the Dhc population was enhanced early in the process in the low TCE bioaugmented bottles, but this advantage relative to the native Dhc population was lost over time. Conversely, KB-1® is a mixed culture containing bacteria including fermenters, sulfate reducers and methanogens. Although differences in Dhc and vcrA were not observed, it is possible that one or a combination of these other bacteria provided a benefit to the dechlorination process in the bioaugmented treatments.

Although there are limitations to the molecular characterization techniques applied in this study, the results indicate that there is a correlation between the chemical and the molecular characterization data. EVO amended microcosms display superior dechlorination at both high and low TCE concentrations and supported a high level of total biomass (Fig. 10), a distinct microbial community (Fig. 11), and elevated Dhc and vcrA levels (Fig. 12). Although it was not clear what organisms were present in the distinct EVO community, it is interesting that such a difference was observed compared to lesser performing electron donors. Lactate and methanol also displayed superior dechlorination performance and supported a similar level of biomass when nutrients were added compared to EVO. These electron donors were less distinct based on microbial community and Dhc values. In contrast, hexanol was ineffective in supporting dechlorination at both high and low TCE concentrations, produced relatively low levels of biomass, particularly in the absence of nutrient addition, and the microbial community structure appeared to be different from that of other electron donors.

4. Conclusions

The results of this study indicate that reductive dechlorination of TCE is feasible at concentrations as high as 3.80 mM (500 mg/L) TCE, similar to the concentrations encountered in the DNAPL source area at the SABRE site. Forty-eight combinations of experimental variables were evaluated using a carefully designed fractional factorial experiment and application of ANOVA and a novel survival Weibull regression to analyze the results. Microcosm performance was primarily evaluated based on chemical data, while microbial characterization data provided supporting information. EVO, methanol, and lactate proved to be superior electron donors in the main study. EVO was selected for field implementation based on the performance in the main study, the respike experiment, and microbial characterization data and because it was judged to have significant operational advantages, including partitioning between the TCE and EVO phases, ease of application, and reduced biofouling compared to soluble substrates that would have to be introduced frequently. Bioaugmentation and nutrient amendment also had statistically significant beneficial effects in the main experiment and were carried forward into field application.

Despite the size and complexity of the study, the statistical tools used in the design and analysis of the experiment provided objective and quantitative comparisons between the experimental variables tested, effectively maximizing the information derived while controlling the experimental effort required. The novel survival regression analysis had several advantages over the ANOVA alone, particularly in providing temporal information on the progress of dechlorination and in quantifying the impact of treatment variables. Although applied to a large and complex microcosm study in this case, the statistical approach described here can be applied to any biotreatability study and may be simplified where necessary for use with smaller, less complex data sets.

Supplementary materials related to this article can be found online at doi:10.1016/j.jconhyd.2012.01.011.

Acknowledgments

Project SABRE is being undertaken by a collaborative team comprising: Acetate Products; Archon Environmental; British Geological Survey; Chevron; CL:AIRE; DuPont; Environment Agency (England and Wales), ESI; General Electric; Geosyntec Consultants; Golder Associates; Honeywell; ICI; Scientifics; SiREM; Shell; Terra Systems; University of Edinburgh; University of Sheffield; and US EPA. SABRE is a UK DTI Bioremediation LINK project with specific funding contributions made by BBSRC, DTI, the Environment Agency, EPSRC and NERC. Additional funding has been provided by SERDP (US Department of Defense). The SABRE team gratefully acknowledges these supporting organizations and the in-kind and direct financial contributions made by each team member. The authors also thank Scientific Advisory Panel member Perry McCarty for his helpful suggestions during the study and Mark Fitzgerald of Neptune and Company for his statistical analysis of the total biomass data. The identification of any commercial product or trade name does not imply endorsement or recommendation by the National Institute of Standards and Technology.

References

Adamson, D.T., Parkin, G.F., 2000. Impact of mixtures of chlorinated aliphatic hydrocarbons on a high-rate, tetrachloroethene-dechlorinating enrichment culture. Environmental Science and Technology 34, 1959–1965.

Adamson, D.T., McDade, J.M., Hughes, J.B., 2003. Inoculation of a DNAPL source zone to initiate reductive dechlorination of PCE. Environmental Science and Technology 37 (11), 2525–2533.

Adamson, D.T., Lyon, D.Y., Hughes, J.B., 2004. Flux and product distribution during biological treatment of tetrachloroethene dense non-aqueous-phase liquid. Environmental Science and Technology 38 (7), 2012–2028.

Air Force Center for Environmental Excellence (AFCEE), Naval Facilities Engineering Center, Environmental Security Technology Certification Program, 2004. Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents. Brooks City-Base, TX.

Bagley, D.M., Gossett, J.M., 1990. Tetrachloroethene transformation to trichloroethene and cis-1,2-dichloroethene by sulfate-reducing enrichment cultures. Applied and Environmental Microbiology 56 (8), 2511–2516.

Boopathy, R., Peters, R., 2001. Enhanced biotransformation of trichloroethylene under mixed electron acceptor conditions. Current Microbiology 42, 134–138.

Cápiro, N.L., Granbery, E.K., Lebrón, C.A., Major, D.W., McMaster, M.L., Pound, M.J., Löffler, F.E., Pennell, K.D., 2011. Liquid–liquid mass transfer of partitioning electron donors in chlorinated solvent source zones. Environmental Science and Technology 45, 1547–1554.

Carr, C.S., Garg, S., Hughes, J.B., 2000. Effect of dechlorinating bacteria on the longevity and composition of PCE-containing nonaqueous phase liquids under equilibrium dissolution conditions. Environmental Science and Technology 34 (6), 1088–1094.

Chappelle, F.S., Haack, P., Adriaens, P., Henry, M., Bradley, P., 1996. Comparison of Eh and H_2 measurements for delineating redox processes in a contaminated aquifer. Environmental Science and Technology 30, 3565–3569.

Cope, N., Hughes, J.B., 2001. Biologically-enhanced removal of PCE from NAPL source zones. Environmental Science and Technology 35 (10), 2014–2021.

DeWeerd, K.A., Concannon, F., Suflita, J.M., 1991. Relationship between hydrogen consumption, dehalogenation, and the reduction of sulfur oxyanions by Desulfomonile tiedjei. Applied and Environmental Microbiology 57 (7), 1929–1934.

Duhamel, M., Wehr, S., Yu, L.Y., Rizvi, H., Seepersad, D., Dwortzek, S., Cox, E.E., Edwards, E.A., 2002. Comparison of anaerobic dechlorinating enrichment cultures maintained on tetrachloroethene, trichloroethene, cis-1,2-dichloroethene and vinyl chloride. Water Research 36, 4193–4202.

Duhamel, M., Mo, K., Edwards, E.A., 2004. Characterization of a highly enriched Dehalococcoides—containing culture that grows on vinyl chloride and trichloroethene. Applied and Environmental Microbiology 70, 5538–5545.

Ellis, D.E., Lutz, E.J., Odom, J.M., Buchanan Jr., R.J., Lee, M.D., Bartlett, C.L., Harkness, M.R., Deweerd, K.A., 2000. Bioaugmentation for accelerated in situ anaerobic bioremediation. Environmental Science and Technology 34 (11), 2254–2260.

Fennell, D.E., Gossett, J.M., Zinder, S.H., 1997. Comparison of butryric acid, ethanol, lactic acid, and propionic acid as hydrogen donors for the reductive dechlorination of tetrachloroethene. Environmental Science and Technology 31, 918–925.

Fennell, D.E., Carroll, A.B., Gossett, J.M., Zinder, S.H., 2001. Assessment of indigenous reductive dechlorinating potential at a TCE-contaminated site using microcosms, polymerase chain reaction analysis, and site data. Environmental Science and Technology 35, 1830–1839.

Findlay, R.H., 2004. Determination of microbial community structure using phospholipid fatty acid profiles, In: Kowalchuk, G.A., DeBruijn, F.J., Head, I.M., Akkermans, A.D.L., Can Elsas, J.D. (Eds.), Molecular Microbial Ecology Manual, 2nd ed. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 938–1004.

Forney, L.J., Liu, W.T., Guckert, J.B., Kumagau, Y., Namkung, E., Nishiharam, T., Larson, R.J., 2001. Structure of microbial communities in activated sludge: potential implications for assessing the biodegradability of chemicals. Ecotoxicology and Environmental Safety 49, 40–53.

Freedman, D.L., Gossett, J.M., 1989. Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. Applied and Environmental Microbiology 55 (9), 2144–2151.

Green, C.T., Scow, K.M., 2000. Aanalysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. Hydrogeology Journal 8, 126–141.

Harkness, M.R., 2000. Economic considerations in enhanced anaerobic biodegradation. In: Wickramananyake, G.B., Gavaskar, A.R., Alleman, B.C., Magar, V.S. (Eds.), Bioremediation and Phytoremediation of Chlorinated and Recalcitrant Compounds. Battelle Press, Columbus, OH, pp. 9–14.

Harkness, M.R., Bracco, A.A., Brennan Jr., M.J., DeWeerd, K.A., Spivack, J.L., 1999. Use of bioaugmentation to stimulate complete reductive dechlorination of trichloroethene in Dover soil columns. Environmental Science and Technology 33 (7), 1100–1109.

He, J.Z., Sung, Y., Dollhopf, M.E., Fathupure, B.Z., Teidjie, J.M., Loffler, F.E., 2002. Acetate versus hydrogen as direct electron donors to stimulate the microbial reductive dechlorination process at chloroethene-contaminated sites. Environmental Science and Technology 36, 3945–3952.

Heimann, A.C., Friis, A.K., Jacobsen, R., 2005. Effects of sulfate on anaerobic chloroethene degradation by an enriched culture under transient and steady-state hydrogen supply. Water Research 39, 3579–3586.

Hendrickson, E.R., Payne, J.A., Young, R.M., Starr, M.G., Perry, M.P., Fahnestock, S., Ellis, D.E., Ebersole, R.C., 2002. Molecular analysis of Dehalococcoides 16S ribosomal DNA from chloroethene-contaminated sites throughout North America and Europe. Applied and Environmental Microbiology 68, 485–495.

Hoelen, T.P., Reinhard, M., 2004. Complete biological dehalogenation of chlorinated ethylenes in sulfate containing groundwater. Biodegradation 15, 395–403.

Holliger, C., Schraa, G., Stams, A.J.M., Zehnder, A.J.B., 1993. A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth. Applied and Environmental Microbiology 59 (9), 2991–2997.

Hood, E.D., Major, D.W., Quinn, J.W., Yoon, W.S., Govaskar, A., Edwards, E.A., 2008. Demonstration of enhanced bioremediation in a TCE source area at Launch Complex 34, Cape Canaveral Air Force Station. Ground Water Monitoring and Remediation 28 (2), 98–107.

Jenkins, J.K., Courtney, P.D., 2003. Lactobacillus growth and membrane composition in the presence of linoleic or conjugated linoleic acid. Canadian Journal of Microbiology 49 (1), 51–57.

Johnsson, T., Nikkila, P., Toivonen, L., Rosenqvist, H., Laakso, S., 1995. Cellular fatty acid profiles of Lactobacillus and Lactococcus strains in relation to the oleic acid content of the cultivation medium. Applied and Environmental Microbiology 61 (12), 4497–4499.

Lalman, J.A., Bagley, D.M., 2000. Anaerobic degradation and inhibitory effects of linoleic acid. Water Research 34 (17), 4220–4228.

Lee, I.S., Bae, J.H., McCarty, P.L., 2007. Comparison between acetate and hydrogen as electron donors and implications for the reductive dehalogenation of PCE and TCE. Journal of Contaminant Hydrology 94, 76–85.

Maillacheruvu, K.Y., Parkin, G.F., 1996. Kinetics of growth, substrate utilization and sulfide toxicity for priopionate, acetate, and hydrogen utilizers in anaerobic systems. Water Environment Research 68, 1099–1106.

Maymo-Gatell, X., Tandoi, V., Gossett, J.M., Zinder, S.H., 1995. Characterization of an H_2-utilizing enrichment culture that reductively dechlorinates tetrachloroethene to vinyl chloride and ethene in the absence of methanogenesis and acetogenesis. Applied and Environmental Microbiology 61 (11), 3928–3933.

McCartney, D.M., Oleszkiewicz, J.A., 1991. Sulfide inhibition of anaerobic degradation of lactate and acetate. Water Research 25 (2), 203–209.

McCarty, P.L., 1994. An overview of anaerobic transformation of chlorinated solvents. Symposium on Intrinsic Bioremediation of Ground Water, EPA/540/R-94/515. US Environmental Protection Agency, pp. 135–142.

Meeker, W., Escobar, L.A., 1998. Statistical Methods for Reliability Data. John Wiley & Sons, New York.

Morse, J.J., Alleman, B.C., Gossett, J.M., Zinder, S.H., Fennell, D.E., Sewell, G.W., Vogel, C.M., 1998. Draft Technical Protocol. A Treatability Test for Evaluating the Potential Applicability of the Reductive Anaerobic Biological In Situ Treatment Technology (RABITT) to Remediate Chloroethenes. ESTCP, pp. 36–52.

Murphy, G.L., Perry, J.J., 1983. Incorporation of chlorinated alkanes into fatty acids of hydrocarbon-utilizing mycobacteria. Journal of Bacteriology 156 (3), 1158–1164.

Nelson, D.K., Hozalski, R.M., Clapp, L.W., 2002. Effect of nitrate and sulfate on dechlorination by a mixed hydrogen-fed culture. Bioremediation Journal 6 (3), 225–236.

Pinheiro, J.C., Bates, D.M., 2000. Mixed-Effects Models in S and S-Plus, Appendix C.7. Springer-Verlag, New York, NY.

Playne, M.J., Smith, B.R., 1983. Toxicity of organic extraction reagents to anaerobic bacteria. Biotechnology and Bioengineering 25, 1251–1265.

R Development Core Team, 2008. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Revesz, S., Sipos, R., Kende, A., Rikker, T., Romsics, C., Meszaros, E., Mohr, A., Tancsics, A., Marialigeti, K., 2006. Bacterial community change in TCE biodegradation detected in microcosm experiments. International Biodeterioration & Biodegradation 58, 239–247.

Robinson, C., Barry, D.A., McCarty, P.L., Gerhard, J.I., Kouznetsova, I., 2009. pH control for enhanced reductive bioremediation of chlorinated solvent source zones. Science of the Total Environment 407 (16), 4560–4573.

Sasser, M., 1990. Identification of bacteria by gas chromatography of cellular fatty acids. Tech. Note #101. Microbial ID, Newark, DE.

Scheffé, H., 1953. A method for judging all contrasts in the analysis of variance. Biometrika 40, 87–104.

Scheutz, C., Durant, N., Dennis, P., Heisterberg, M., Jorgensen, T., Jakobsen, R., Cox, E., Bjerg, P.L., 2008. Concurrent ethene generation and growth of Dehalococcoides containing vinyl chloride reductive dehalogenase genes during a stimulated reductive dechlorination field demonstration. Environmental Science and Technology 42, 9302–9309.

Seagren, E.A., Rittman, B.E., Valocchi, A.J., 1993. Quantitative evaluation of flushing and biodegradation for enhancing in situ dissolution of nonaqueous phase liquids. Journal of Contaminant Hydrology 12, 103–132.

Seagren, E.A., Rittman, B.E., Valocchi, A.J., 1994. Quantitative evaluation of the enhancement of NAPL-pool dissolution by flushing and biodegradation. Environmental Science and Technology 28, 833–839.

Sleep, B.E., Faust, C.R., Durant, N.D., Parker, J.C., Guarnaccia, J.G., Sehayek, L., Harkness, M.R., 2002. Modeling fate and transport of chlorinated organic compounds in the subsurface. Environmental Modeling and Management. Today Media Incorporated, pp. 235–239.

U.S. EPA, 1988. Determination of Total Organic Carbon in Sediment (Lloyd Kahn Method). US Environmental Protection Agency Region II, Edison, NJ USA.

U.S. EPA, 2005. Fatty Acid Methyl Ester (FAME) Analysis. Land Remediation and Pollution Control Division SOP# 05.SOP-M.002.00.

Waller, A.S., Krajmalnik-Brown, R., Löffler, F.E., Edwards, E.A., 2005. Multiple reductive-dehalogenase-homologous genes are simultaneously transcribed during dechlorination by Dehalococcoides-containing cultures. Applied and Environmental Microbiology 71, 8257–8264.

Yang, Y., McCarty, P.L., 1998. Competition for hydrogen within a chlorinated solvent dehalogenating anaerobic mixed culture. Environmental Science and Technology 32, 3591–3597.

Yang, Y., McCarty, P.L., 2000a. Biologically enhanced dissolution of tetrachloroethene DNAPL. Environmental Science and Technology 34 (14), 2979–2984.

Yang, Y., McCarty, P.L., 2000b. Biomass, oleate, and other possible substrates for chloroethene reductive dehalogenation. Bioremediation Journal 4 (2), 125–133.

Yang, Y., McCarty, P.L., 2002. Comparison between donor substrates for biologically enhanced tetrachloroethene DNAPL dissolution. Environmental Science and Technology 36 (15), 3400–3404.

Zhuang, P., Pavlostathis, S.G., 1995. Effect of temperature, pH, and electron donor on the microbial reductive dechlorination of chloroalkenes. Chemosphere 31 (6), 3537–3548.

Biodegradability of Different Initial Concentrations of Alaska North Slope Crude Oil Dispersed with Corexit C9500

Mobing Zhuang[1], Gulizhaer Abulikemu[2], Pablo Campo[3*], William Platten III[4], Makram T. Suidan[5], Albert D. Venosa (retired)[4] and Robyn N. Conmy[4]

1 Department of Biomedical, Chemical and Environmental Engineering, University of Cincinnati, 2901 Woodside Drive, Cincinnati, OH 45221, USA

2 Pegasus Technical Services Inc., 46 E Hollister St, Cincinnati, OH 45219 USA

3 Cranfield Water Science Institute, Cranfield University, Cranfield, Beds, MK43 0AL, UK

4 U.S. Environmental Protection Agency, NRMRL, 26 W. MLK Drive Cincinnati, OH, 45268, USA

5 Faculty of Engineering and Architecture, American University of Beirut, Bechtel Engineering Bldg. - 3rd flr. - Room 308 P.O. Box: 11-0236 Riad El Solh 1107 2020, Beirut, Lebanon

*Corresponding author: Pablo Campo
Tel: +44 (0)1234 754332
E-mail address: p.campo-moreno@cranfield.ac.uk

ED_001324_00001270-00001

ABSTRACT

Laboratory experiments were conducted using Alaska North Slope crude oil (ANS) and the dispersant Corexit 9500 (C9500) to study the biodegradability of ANS alone, ANS dispersed with C9500 at a dispersant-to-oil ratio of 1:25. To determine the role that substrate concentration plays in biodegradation kinetics, oil loads of 1,000 and 40 ppm (v:v) were compared at 25 and 5 °C. The biodegradation rate of dioctyl sodium sulfosuccinate (DOSS), which is an anionic surfactant in C9500, by the meso culture (25 °C) was approximately an order of magnitude faster in the high concentration experiment than in the low concentration experiment. At the lower temperature, the deep-water culture cryo (5 °C) had limited ability to metabolize DOSS regardless of the initial concentrations. Uptake of oil components was favored by the presence of dispersant as C9500 shortened lag phases and enhanced biodegradation rates. Alkanes and PAHs were degraded more rapidly in the high concentration samples. In the low concentration experiment, the percentage of alkanes persisting was higher than in the high concentration experiment (8-18% vs. below 1%). No significant lag period in PAH biodegradation was observed in the high oil concentration experiment compared to the low oil concentration experiment where a lag period of 12 to 16 d was observed. The extent of biodegradation of some of the less soluble aromatics increased by 4-86% under low oil concentration conditions.

KEYWORDS

Corexit 9500, biodegradation, initial oil concentration

ED_001324_00001270-00002

INTRODUCTION

To minimize the impact of oil spills, responders adopt the Net Environmental Benefit Analysis, this is, any response technique should decrease the environmental costs rather than increase them (API, 2013). When mechanical recovery or in situ burning cannot achieve the goal of protecting shorelines from oil, the impact of using dispersants is likely outweighed by the benefits, which include keeping oil from sensitive areas and speeding up biodegradation by breaking an oil slick into small droplets (Prince, 2015). Thus, it is important to study the biodegradability of dispersants and dispersed oil under various conditions for better informed use in future applications.

Makes it sound like the BCA is already foregone conclusion.

In the aftermath of the Deepwater Horizon blowout in the Gulf of Mexico (GOM), comprehensive monitoring of dispersant Corexit 9500 (C9500) and hydrocarbons in both surface and subsurface environments took place. In samples collected near the Macondo well, concentrations of dioctyl sodium sulfosuccinate (DOSS), an anionic surfactant present in C9500, ranged from 0.4 to 12 μg/L (Kujawinski et al., 2011). After the spill, Gray et al. (2014) sampled at GOM different locations and detected DOSS in subsurface waters at concentrations below 40 μg/L, while in one surface water sample close to the wellhead the value exceeded 200 μg/L. In terms of oil concentration, Lee et al. (2013) pointed out that the combination of turbulence on the GOM surface and the dispersant application could rapidly decrease oil concentrations to below 100 mg/L, dropping even further over time. Hence, these researchers recommended that biodegradation tests of dispersed oil should be conducted under more dilute concentrations to mimic real conditions.

ED_001324_00001270-00003

64 Most studies published on biodegradation of oil and dispersants prior to the Deepwater
65 incident had been conducted at concentrations well-above the reported in situ levels (Operational
66 Science Advisory Team, 2010; Prince, 2015). Several studies assessing biodegradation of oil
67 dispersed by C9500 used initial oil and dispersant concentrations from 100 to 4,500 mg/L, with
68 a dispersant-to-oil ratios (DOR) of 1:10, 1:20, or 1:25 (Campo et al., 2013; Lindstrom and
69 Braddock, 2002; Zahed et al., 2010). To our knowledge, few studies have been published on oil
70 biodegradation with initial oil and dispersant concentrations below 100 ppm (Brakstad et al.,
71 2015; Prince et al., 2013; Venosa and Holder, 2007). Thus our objective was to determine how
72 the initial amounts of oil and dispersant affect their biodegradation. We conducted high and low
73 oil concentration biodegradation experiments with Alaska North Slope crude oil (ANS) and
74 C9500 at 5 and 25 °C. C9500 is a dispersant included in the National Contingency Plan Product
75 Schedule and was applied to both the surface and submarine environment in the GOM
76 (Operational Science Advisory Team, 2010). The biodegradation of DOSS, dispersed ANS, and
77 ANS alone in high and low concentration experiments at 5 and 25 °C are reported in this paper.

78 MATERIALS AND METHODS

79 **Chemicals and Reagents**

80 The U.S. Environmental Protection Agency (EPA) provided the ANS and the dispersant
81 C9500 (Nalco Naperville, IL) used in this study. Standards for DOSS and its deuterated
82 surrogate (D_{17}-DOSS) were obtained from Aldrich (St. Louis, MO) and Isotec (Miamisburg,
83 OH), respectively. Acetonitrile, deionized ultra-filtered water, and mineral salts were purchased
84 from Fisher Scientific (Pittsburgh, PA). Dichloromethane (DCM) was obtained from Tedia
85 (Fairfield, OH). Sylon CT (Sigma-Aldrich, St. Louis, MO) was used for the deactivation of the

ED_001324_00001270-00004

glassware to prevent adherence of oil and biomass to the sides of the flasks. The protease cocktail for inhibiting enzymatic activity was also acquired from Sigma-Aldrich (St. Louis, MO).

Protease Solution Preparation

Following the instruction provided with the protease cocktail, 5 mL dimethyl sulfoxide (DMSO) was added to 1,075 mg lyophilized powder, which was previously stored unopened at -20 °C. The solution was vortexed for ten minutes before the addition of 20 mL deionized water. The resulting solution was clear. The reconstituted protease solution contained the following inhibitors: 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (23 mM), ethylenediaminetetraacetic acid (100 mM), bestatin (2 mM), pepstatin A (0.3 mM), and E-64 (0.3 mM).

Artificial Seawater

? define

GP2 medium was autoclaved at 121 °C for 30 min and then used as the sterile matrix in this study (Spotte et al., 1984). The salts in the GP2 medium, dissolved in DI water, were (expressed in g/L) NaCl (21.03), $MgCl_2 \cdot 6H_2O$ (9.5), Na_2SO_4 (3.52), $CaCl_2 \cdot 2H_2O$ (1.32), KCl (0.61), KBr (0.088), $NaB_4O_7 \cdot 10H_2O$ (0.034), $SrCl_2 \cdot 6H_2O$ (0.02), $NaHCO_3$ (0.17) $FeCl_3 \cdot 6H_2O$ (0.05), $Na_5P_3O_{10}$ (0.297) and KNO_3 (2.89). The GP2 pH was ≈ 7.5.

Microbial Cultures

The original cultures were isolated from the surface water of the GOM (meso) in the vicinity of the Macondo well and water close to the plume location at a depth of 1,240 m near the wellhead (cryo) by EPA's Gulf Breeze, FL research laboratory. Enriched inocula were procured

ED_001324_00001270-00005

as follows: in 2-L shake flasks, 2.5 mL SLC were added to 500 mL of the original unfiltered GOM seawater supplemented with 2.8 g/L KNO_3, 0.55 g/L NaP_3O_{10}. The flasks were rotated (400 rpm) at 5 °C (cryo culture) and 25 °C (meso culture). The adequate growth times were 7 d for the meso culture and 17 d for the cryo culture. After these periods, cells were harvested by centrifugation (6,000 × g for 30 min at 4 °C) and the pellets preserved in 10% glycerol at -70 °C. Before shipping to EPA-Cincinnati, 1 mL of each thawed culture were added to shake flasks containing 500 mL GP2 medium fortified with 2.5 mL of SLC. The meso culture was harvested after 6 days of growth and the cryo culture after 29 days. The cultures used in the experiments of this study were grown at EPA (Cincinnati, OH) by transferring the original cultures from EPA's Gulf Breeze to GP2 medium supplemented with South Louisiana crude oil (SLC). The meso culture was harvested after 3 weeks of growth and the cryo culture after 5 weeks. The harvested culture was processed in the same fashion as mentioned previously: centrifugation (6,000 × g for 30 min at 4 °C) and the pellets preserved in 10% glycerol at -70 °C. Upon use in experiments, the cultures were defrosted at room temperature, washed with 0.85% saline solution to remove glycerol, centrifuged, and brought back up to the frozen volume.

Experimental Setup and Sampling

You never actually discuss how much inoculum you add to each 100 mL flask.

A summary of the experimental setup is shown in Tables S1 and S2 for the high and low oil concentration experiments, respectively. For the high concentration experiments, 6 treatments were tested at 5 and 25 °C in triplicate: C9500 dispersant alone, ANS oil alone, ANS dispersed with C9500, and the corresponding killed controls (KCs). The 5 °C experiment required 11 sampling events in triplicate (0, 2, 4, 8, 12, 16, 24, 32, 40, 48 and 56 d), while 9 events were conducted at 25 °C (0, 2, 4, 8, 12, 16, 24, 32 and 40 d). At each sampling event, triplicate shake

ED_001324_00001270-00006

128 flasks of each treatment along with KCs were sacrificed, except for the ANS alone KCs which
129 were analyzed at the beginning and end of the experiment.

130 The same treatments were included in the low concentration experiment, but both oil and
131 dispersant concentrations were prepared at 4% of the initial loads used in the high concentration
132 experiment. Additionally, a seventh treatment involved the use of triplicate KCs containing the
133 enzyme protease (0.5 mL) to neutralize the enzymatic DOSS hydrolysis. This treatment was
134 included only in the low concentration experiment. The sampling events were conducted
135 similarly to the high concentration experiment with the exception of the protease treatment,
136 which was sampled at 0, 12, 24, 35, 46 and 56 d and 0, 8, 18, 28, 38 and 48 d for the 5 and 25 °C
137 experiments, respectively.

138 After all treatments were prepared, the appropriate cultures were spiked (0.5 mL) and the
139 flasks were placed on orbital shakers. The shakers were operated at 200 rpm and kept in the
140 appropriate constant temperature rooms until the expected sampling events. All KCs were
141 sterilized by adding 1 mL sodium azide stock (50 g/L) into the 100 mL of GP2 medium.

142 **High Concentration Treatments**

1mL : 1200mL ? 1:25 ?

143 To evaluate the biodegradation of dispersed oil, 2 L baffled beakers were used in which
144 40 μL of C9500 and 1,000 μL of ANS were added to 1,200 mL of GP2 to achieve a volumetric
145 DOR ratio of 1:25. Subsequently, the beaker containing the mixture was shaken at 200 rpm for
146 10 minutes and then left stationary for an additional 10 min, then the dispersion was transferred
147 to a 20-L continuously mixed carboy. The above procedure was repeated until the volume in the
148 carboy reached 14 L. Subsequently, 100 mL aliquots of the mixtures were transferred to the
149 shake flasks. The second treatment was for the evaluation of oil as the only substrate. ANS (100
150 μL) was added directly to shake flasks containing 100 mL sterile GP2 medium, which yielded an

.10 mL : 100 mL → 1:1000 ratio

This is the only treatment that doesn't involve

estimated oil concentration of 1,000 ppm by volume. The oil added to the ANS alone and dispersed ANS treatments aimed to result in the same oil concentration by volume. To evaluate the degradation of dispersant alone, 480 µL of C9500 was added to 14 L of sterile GP2 medium in a continuously mixed carboy; after 30 min, 100 mL aliquots of this mixture were dispensed into shake flasks for this treatment.

Low Concentration Treatments

.2 mL : 1200 mL ≟ 1:25 ?

To prepare the low concentration dispersed oil, a single batch of 1,200 mL GP2 was spiked with 200 µL ANS onto the water surface followed by addition of 8 µL C9500 onto the oil slick, which yielded a volumetric DOR of 1:25 in a 2 L baffled beaker. The shaker was started at a very slow speed ~~and~~ was ramped up to 200 rpm gradually. shaken for ~~The beaker was placed on a shaker for~~ 20 min and then remained stationary for 10 minutes. Subsequently, 3 L of the dispersion were poured into a 20 L carboy under continuous mixing and 12 L of sterile GP2 was added for dilution purposes. Overall, the oil and dispersant amounts in this treatment were approximately 4% of that in the high concentration experiment. Finally, shake flasks were filled with 100 mL aliquots of the diluted dispersed oil. Low concentration ANS alone was prepared by spiking approximately 4 µL of oil into shake flasks containing 100 mL sterile GP2, which yields an oil concentration of 40 ppm by volume (i.e., 4% of the high concentration oil alone experiment). The low concentration dispersant alone treatment was prepared in a single batch of sterile GP2 (14 L) spiked with approximately 20 µL of C9500. The batch was mixed for 30 minutes and, subsequently, 100 mL aliquots were transferred into the shake flasks.

already on it.

I only have 1.2 L Assume you made many batches

no stationary phase?

Oil components and Dispersant Analysis

.004 mL : 100 mL

4:100 000 = 4×10^{-5}

$\frac{.2 \text{ mL ANS}}{1.2 \text{ L } 1^\circ}$ ~~(1200 mL 1°)~~ $\frac{3 \text{ L } 1^\circ}{(3+12) \text{ L } 2^\circ} = 3.3 \times 10^{-5}$

To monitor C9500 degradation, DOSS was measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) following the Standard method ASTM D7730−11 (2011). The targeted oil components were pristane (PR), phytane (PH), normal paraffins (n-C_{10-35}) and 2-, 3- and 4-ring PAH compounds and their alkylated homologues (C_{0-4}-naphthalenes, C_{0-3}-fluorenes, C_{0-3}-dibenzothiophenes, C_{0-4}-phenanthrenes/anthracenes, C_{0-4}-napthbenzthiophenes, C_{0-2}-pyrenes, C_{0-4}-chrysenes). The concentrations of these analytes were normalized to hopane present in the oil (Prince et al., 1994). Details of oil extraction by DCM and sample preparation methods can be found elsewhere (Campo et al., 2013). For the high concentration experiment, oil analysis was conducted with a 6890 GC coupled with a 5973 mass spectrometry from Agilent (Palo Alto, CA). In the low concentration experiment, an Agilent (Palo Alto, CA) 7000 GC Triple Quad system was used. The quantification range for the single quad and triple quad mass spectrometry are 1-30 μg/L and 0.5-10 μg/L, respectively. In the case that the concentration of oil hydrocarbons is below the range, we used the quantification results as an estimation of the actual amount. The same column, a DB-5MS column (30m × 0.25mm, 0.25μm film thickness), was used to achieve chromatographic separation of analytes in both instruments. The method was a modification based on EPA Method 8270D (2007).

1)

2)

RESULTS AND DISCUSSION

Degradation of DOSS

High Concentration Experiment. Live samples revealed an extremely fast removal rate of DOSS at 25 °C (Fig. 1A-B, open symbols) with extents exceeding 95% after 2 d in dispersed oil (Fig. 1B) and 4 d in C9500 alone (Fig. 1A). The first order rate coefficients were -1.82 ± 0.25 d^{-1} (ANS+C9500) and -0.71 ± 0.024 d^{-1} (C9500 alone). The presence of oil enhanced DOSS

typically in my field rate constants are always positive. ~~It is a rate constant, not~~

194 biodegradation by a factor of approximately 2.6. Disappearance of DOSS was also observed in

195 KCs at 25 °C, with zero order rates of -138 ± 7 and -221 ± 11 μg L^{-1} d^{-1} for C9500 alone (Fig.

196 1C) and dispersed oil (Fig. 1D), respectively. As monooctyl sulfosuccinate (MOSS) was found in

197 KCs, Campo et al. (2013) explained this abiotic loss in terms of hydrolysis which was confirmed

198 by (Batchu et al., 2014). The presence of oil significantly enhanced DOSS removal in KCs ($p <$

199 0.0001).

200 In contrast with the rapid removal of DOSS at 25 °C, the biotic and abiotic processes at 5

201 °C were much slower. DOSS persisted for 40 d before its concentration decreased in all

202 treatments. The difference in DOSS concentration between live samples and parallel KCs in the

203 dispersed oil treatment was statistically insignificant at days 48 ($p = 0.078$) and 56 ($p = 0.726$).

204 In the C9500 alone treatment, DOSS was significantly lower in live samples than in KCs at days

205 48 ($p = 0.0015$) and day 56 ($p = 0.023$). These results point to biodegradation as the predominant

206 DOSS removal mechanism as opposed to hydrolysis.

ED_001324_00001270-00010

If you are analyzing as a first-order loss, you should plot as ln C -v- t, or ln C/Co -v- t. to allow the reader to really see the goodness of fit.

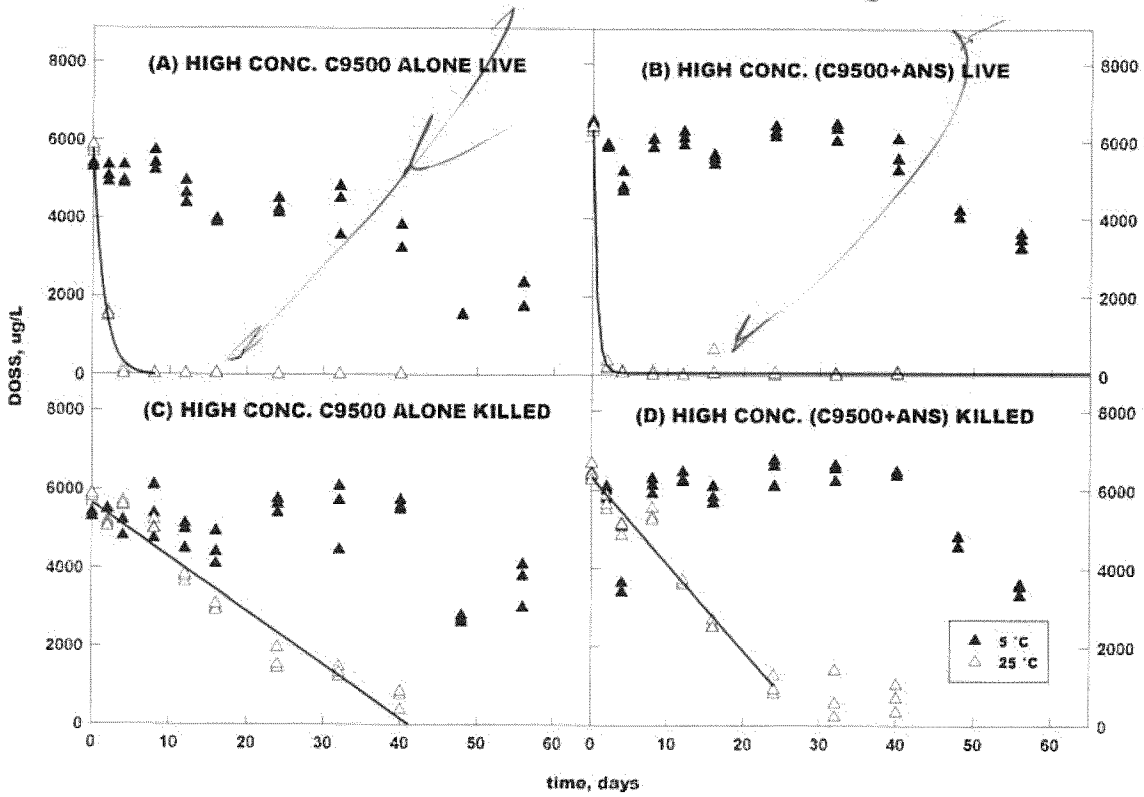


Figure 1. Disappearance of DOSS in high concentration experiment in the absence (A, C) and Presence (B, D) of ANS at 5 °C and 25 °C. Live samples are in panel A and B, whereas killed controls are in panels C and D.

Low Concentration Experiment. At 25 °C and for the dispersed oil treatment, no lag period was observed in DOSS uptake, which showed a first-order constant rate of -0.16 ± 0.015 d^{-1} (Fig. 2B). In the absence of oil (Fig. 2A), we observed an acclimation period of 2 d followed by DOSS biodegradation (-0.07 ± 0.009 d^{-1}). The presence of oil favored DOSS removal as was observed in the high concentration experiment . Removal extents over 95% for DOSS required 32 and 48 d in the presence and absence of oil, respectively. In the corresponding KCs, DOSS concentrations decreased following zero-order kinetics, with constant rates of -4.1 ± 0.3 μg L^{-1} d^{-1} (C9500 alone, Fig. 2C) and -5.1 ± 0.4 μg L^{-1} d^{-1} (C9500 and ANS, Fig. 2D).

How did you conclude this? There seems to be some anomalies w/ the initial point even w/ll KCs.

For the high concentration experiment at 25 °C and regardless of the presence of oil, the time series concentration of DOSS in the live samples rapidly declined to zero and clearly

separated from the values measured in the corresponding KCs. In the low concentration experiment, it appeared that DOSS disappearance with time in both treatments, C9500 alone and dispersed ANS, overlapped with the abiotic losses observed in the KCs (Fig. 2). To differentiate between microbial activity and hydrolysis, we compared by a *t*-test ($\alpha = 0.05$) the average DOSS concentrations measured in live samples with those values found in the parallel KCs. Live samples significantly departed from the controls after 4 d ($p = 0.0227$) and 16 d ($p = 0.0053$) in the presence and absence of ANS, respectively. At early stages in the experiment, we could not identify the predominant removal mechanism for DOSS but, eventually, microbial uptake prevailed as the main process in terms of rate and extent.

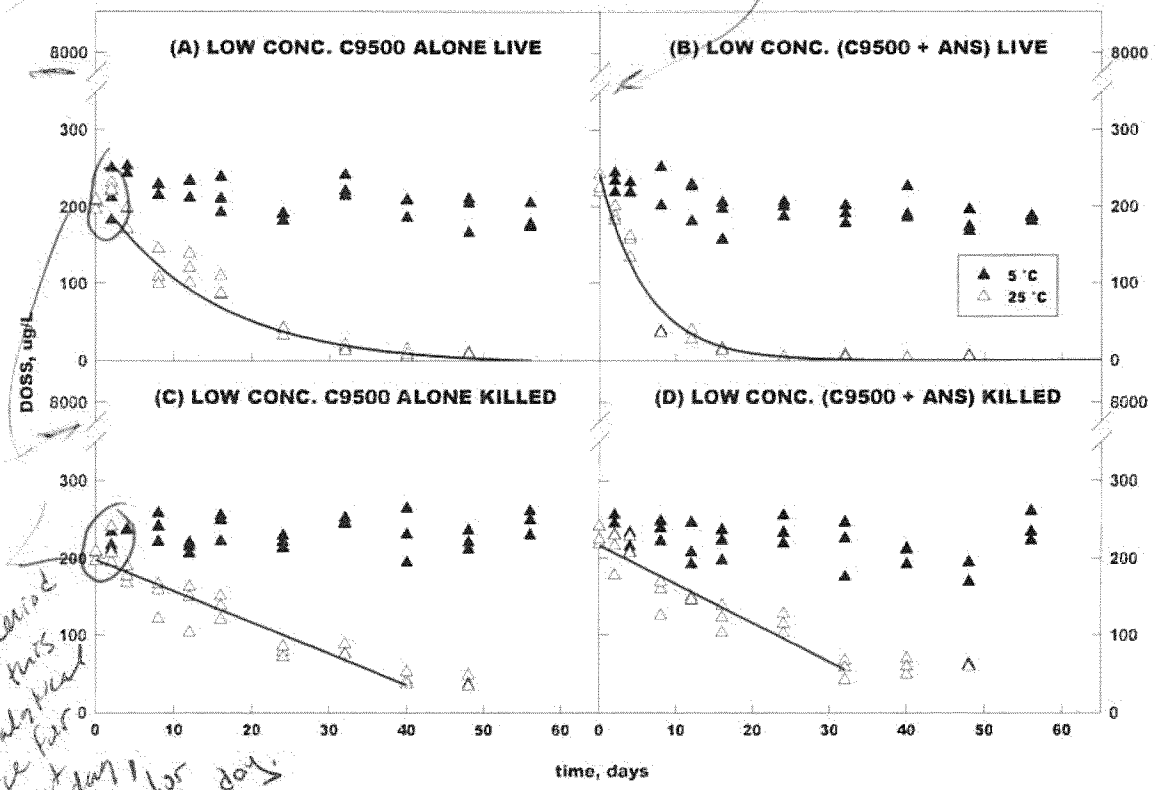
DOSS presented a faster microbial uptake at both the higher initial concentration and temperature. Nevertheless, based on our low concentration results, it is reasonable to infer that biodegradation of DOSS at the surface of GOM might not happen as rapidly since dilution and emulsification can attenuate the concentration of dispersant. In this fashion, DOSS was detected with a reporting limit of 0.25 μg L^{-1} and a highest concentration of 229 ±16 μg L^{-1} in surface water samples collected 1-2 months after the spill near the Macondo well (Gray et al., 2014).

This is discussion out of place.

DOSS concentration remained unchanged in live samples and KCs under 5 °C and low oiling conditions (Fig. 2, closed symbols). Such finding agreed with studies by Kujawinski et al. (2011) and Gray et al. (2014) who concluded that subsurface degradation of DOSS was minimal. Campo et al. (2013) observed no hydrolysis or biodegradation of DOSS in SLC at 5 °C within 42 d.

ED_001324_00001270-00012

Is the broken scale necessary?



Was there really an abiotic acclimation period too? Or is this analytical noise for that day, or for 0-day.

Figure 2. Disappearance of DOSS in low concentration experiment in the absence (A, C) and Presence (B, D) of ANS at 5 °C and 25 °C. Live samples are in panel A and B, whereas killed controls are in panels C and D.

We also included a series of dispersed oil KCs containing a protease cocktail (Fig. 3A), which inhibits enzymatic activity to clarify the abiotic DOSS disappearance. At 25 °C, the addition of inhibitor clearly slowed down the hydrolysis process as the zero-order rate constant in the protease KCs was -3.7 ± 0.4 μg L^{-1} d^{-1} (Fig. 3A) as opposed to -5.1 ± 0.4 μg L^{-1} d^{-1} (Fig. 3B) obtained for the regular KCs ($p = 0.021$). Such finding suggests the enzymatic nature of DOSS hydrolysis. Nevertheless, different enzymes may be involved in the process so that the protease cocktail could not block all of them. In the protease controls at 5 °C, DOSS persisted throughout the experiment as observed in the regular KCs for that temperature. This indicates that either the lower temperature attenuated DOSS breakdown, the cryo culture lacked the required enzymes, or a combination of both causes.

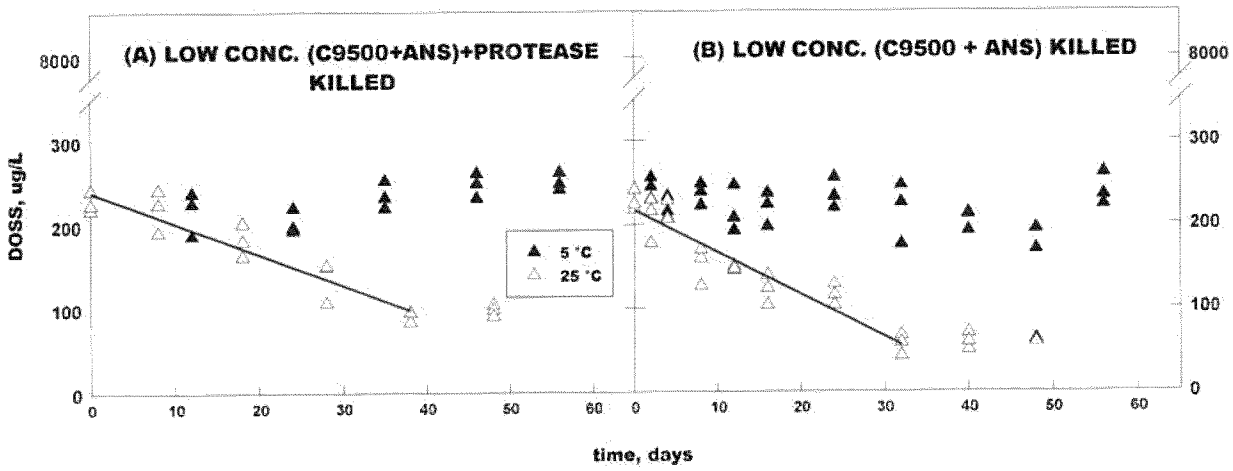
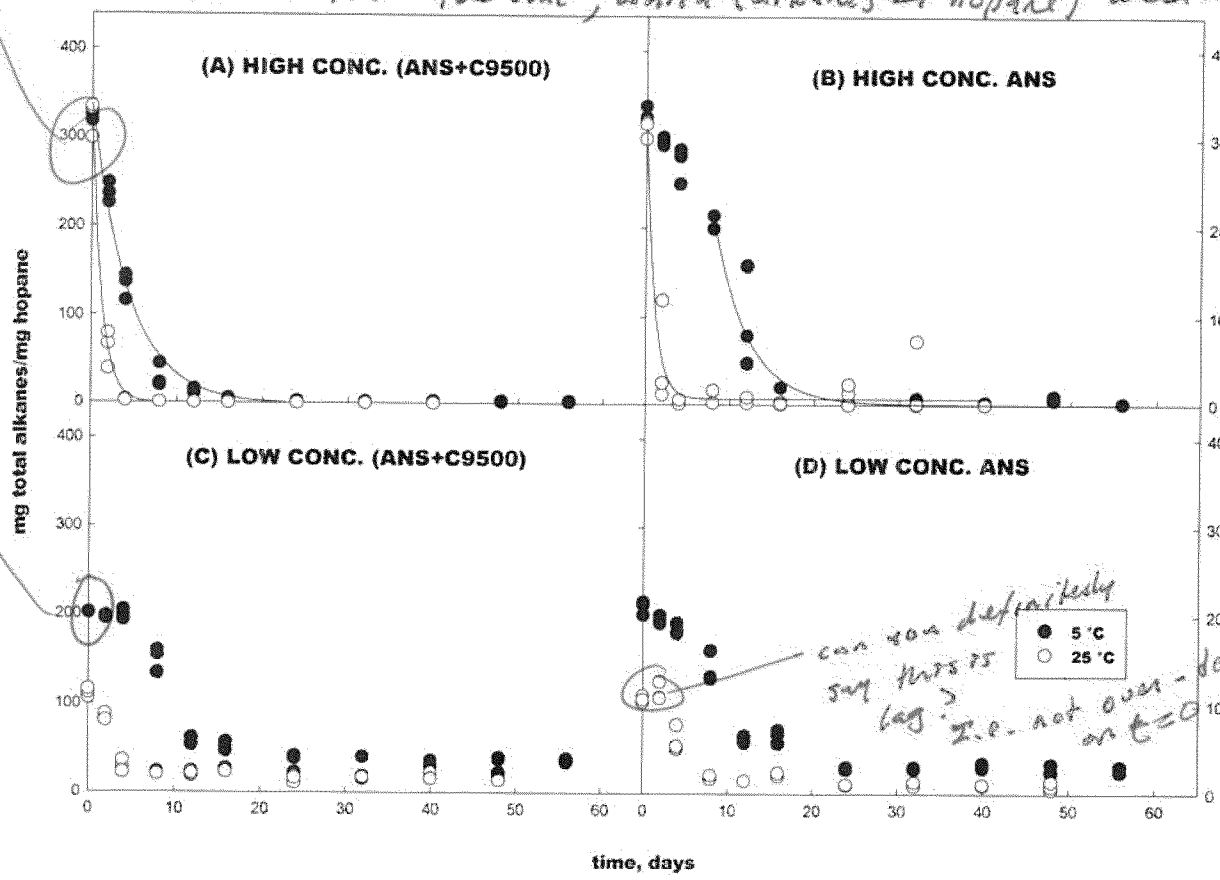


Figure 3. Disappearance of DOSS in low concentration dispersed oil killed controls in the presence (A) and absence (B) of protease at 5 and 25 °C.

Total Alkane Degradation

High Concentration Experiment. Total alkanes at both temperatures are presented in Fig. 4 A-B. At 25 °C, the meso culture metabolized the aliphatic fraction with a first-order rate constants of -0.91 ± 0.10 and -0.85 ± 0.04 d^{-1} in the absence and presence of C9500, respectively. For both treatments, the extent of removals reached 99% after 4 days. As expected, aliphatics degraded slower at 5 °C. In the dispersed oil treatment, this fraction followed first-order kinetics (-0.24 ± 0.01 d^{-1}) while, for the oil alone samples, alkanes presented a linear decay during the first 8 d followed by first order kinetics of similar rate (-0.25 ± 0.03 d^{-1}). After days 16 (dispersed oil) and 24 (oil alone), the alkane extent of removal was 99% for the lower temperature.

why was the ratio of alkanes:hopane different for the 5°C (as well)? The ratio wasn't conserved through the preparation process. If the [~] was supposed to be 4% lower for "low conc", which (alkanes or hopane) were not on target?



can you definitely say this is lag? I.e. not over-dependent on t=0 data?

Figure 4. Biodegradation of hopane-normalized total alkanes at both temperatures in high concentration treatments (A, B) and low concentration treatments (C, D).

Low Concentration Experiment. In this experiment, the initial total alkane concentrations were considerably different at 5 and 25 °C (Fig. 4 C and D). In fact, the meso culture showed background concentrations of PAHs and hopane, the conservative biomarker to which all the target analytes concentrations are normalized. The hopane background levels were comparable to those measured in the initial oil added to the flasks. Hence, at time zero, the total alkane concentration normalized to hopane at 25 °C were almost half of the corresponding value at 5 °C.

So [hopane] was not diluted (whether 1:25 or 1:1200, depending on which is correct) Will this raise red-flags?

This is not very clear. It seems that the $[hopane]_{high}$ was equal to the $[hopane]_{ANS}$ despite the ANS was diluted 1:1200, or 1:25 ...

at what time?

At 25 °C, the alkane residual concentrations ranged from 8 to 18% of the initial values (Fig. 4 A and B). In the oil alone treatment, alkane biodegradation started after a lag phase of 2 d, while these organics were promptly metabolized in the dispersed oil treatment. At 5 °C, the cryo culture required 4 d for acclimation in the dispersed oil. In the absence of dispersant, after a slow and steady linear decay, fast disappearance of alkanes occurred between days 8 and 12. This suggests that C9500 reduced the length of the acclimation phase for alkane uptake.

see graph earlier page.

Table 1 summarizes the actual concentrations of total alkanes (not normalized to hopane) for the first and last sampling event for both the high and low concentration experiments. It is noted that a higher alkane residual amount was observed in the low concentration experiment than the high concentration one. This was especially true considering the combination of high temperature and dispersant application, where the alkane concentrations were less than the detection limit and 142 ± 4 μg L^{-1} in the last sampling event of low concentration samples. One possible explanation is that carbon and energy sources were limited in the low concentration microcosms. Fewer microorganisms provided less microbial surface for hydrophobic compounds to interact with, which resulted in less complete degradation of alkanes.

So presumably the only difference between high and low is the amount of ANS (i.e. for ANS alone). So nutrient limiting theory suggests that the other nutrients (other than alkanes) must be present in ANS. Or maybe there a

Table 1. Average and standard deviations (sd) of total alkanes and PAHs concentration in the high and low concentration experiment at first and last sampling event.

Samples			5 °C ANS alone average (μg/L)	sd	25 °C ANS alone average (μg/L)	sd	5 °C ANS+C9500 average (μg/L)	sd	25 °C ANS+C9500 average (μg/L)	sd
High Conc.	Alkanes	First	47,400	604	43,300	9,390	24,600	973	27,200	126
		Last	201	26	36	42	186	52	0	0
	PAHs	First	14,500	110	13,700	3,090	7,310	323	8,790	94
		Last	3,040	281	2,470	300	1,300	74	1,270	158
Low Conc.	Alkanes	First	2,010	202	1,740	149	1,420	72	1,130	39

I don't assume you intended at the same ocelis/procedure, but have high or low ANS.

difference in the initial inoculum (see p 6).

	Last	216	21	83	75	225	13	142	4
PAHs	First	482	53	773	25	330	12	453	14
	Last	71	5	88	5	58	10	80	4

Degradation of Individual Alkanes

High Concentration Experiment. Table S3 summarizes the first-order biodegradation coefficients for individual alkanes. At 25 °C, among all the compound monitores only n-C_{10} was biodegraded faster (-2.00 vs. -1.34 d^{-1}) with the addition of dispersant. Branched alkanes exhibited similar rates regardless of the presence of C9500, although higher variability among triplicates was observed in the oil alone samples (Fig. S1 A and B). At 5 °C, consistently higher rates for n-$C_{10\text{-}27}$ were observed in the dispersed oil treatment when compared to the oil alone treatment. Also, no lag period for the biodegradation of branched alkanes occurred when C9500 was added, whereas an 8-day lag period occurred in the absence of dispersant.

Low Concentration Experiment. As depicted in Figs. S1-5 (panels C and D), only n-$C_{10\text{-}16}$ was completely removed at both temperatures in the low concentration experiment. In this case, a more qualitative approach was used by comparing the disappearance time. At 25 °C in the low oil concentration experiment, n-C_{10} was degraded faster in the presence of dispersant, disappearing completely before day 2, whereas in the absence of C9500, its depletion was achieved only after day 8. The biodegradation of n-C_{10} was also accelerated when C9500 was present at 5 °C, as shown in Table S4 (-0.47 vs -0.63 d^{-1}). At both temperatures, branched alkanes were not degraded faster in the presence of dispersant, as shown in Table S4 and Fig. S1 C-D.

so straight-chain enhanced w/ dispersant
branched not-enhanced " "

ED_001324_00001270-00017

The major mechanism of uptake of water-insoluble substrates (i.e., alkanes) is generally recognized as direct interaction between microbes and the hydrophobic substance (Rojo, 2009; Wentzel et al., 2007) and the effects of surfactant on such process are inconclusive (Bredholt et al., 2002; Bruheim et al., 1999; Tang et al., 1998). Both conducive and inhibitory effects of dispersant on oil biodegradation have been reported (Lindstrom and Braddock, 2002; Prince et al., 2013; Venosa and Holder, 2007). In this study, C9500 exhibited similar effects on the biodegradation of alkanes in the high and low concentration experiments. Such effects included enhancement of biodegradation rates and shortening of lag periods. Rate enhancements were more notable in the high concentration experiment, whereas C9500 clearly shortened lag phases in the lower concentration tests. ~~[illegible] I would say high and low DNS were not similar. HI: no C9500 effect on~~ quite mixed results hi/low 5°/25°. No consistency.

The application of dispersants to an oil slick promotes the formation of small oil droplets by decreasing the interfacial tension between oil and water (Jasper et al., 1978; Li et al., 2009; Lunel, 1995). Consequently, the total interfacial area of oil droplets for microbes to interact with is increased. In the high concentration experiment, exposure of substrates to the microbes is enough that biodegradation can initiate soon after inoculation. In this case, dispersants assisted biodegradation mainly by increasing interfacial area for microbes to interact with oil. For the low concentration experiment, the observed long acclimation phase at both temperatures, especially in the absence of C9500, was possibly due to limited exposure of alkanes to the microorganisms in the beginning. Since the oil load, appearing as an iridescent sheen, is so low, the mixing energy in the flasks could be high enough to produce similar droplet size distribution in the oil alone and dispersed oil treatments. Thus, C9500 may have shortened the lag phase in the low concentration experiment by promoting the attachment of microbes to the oil droplet.

not at 25°C and [High]

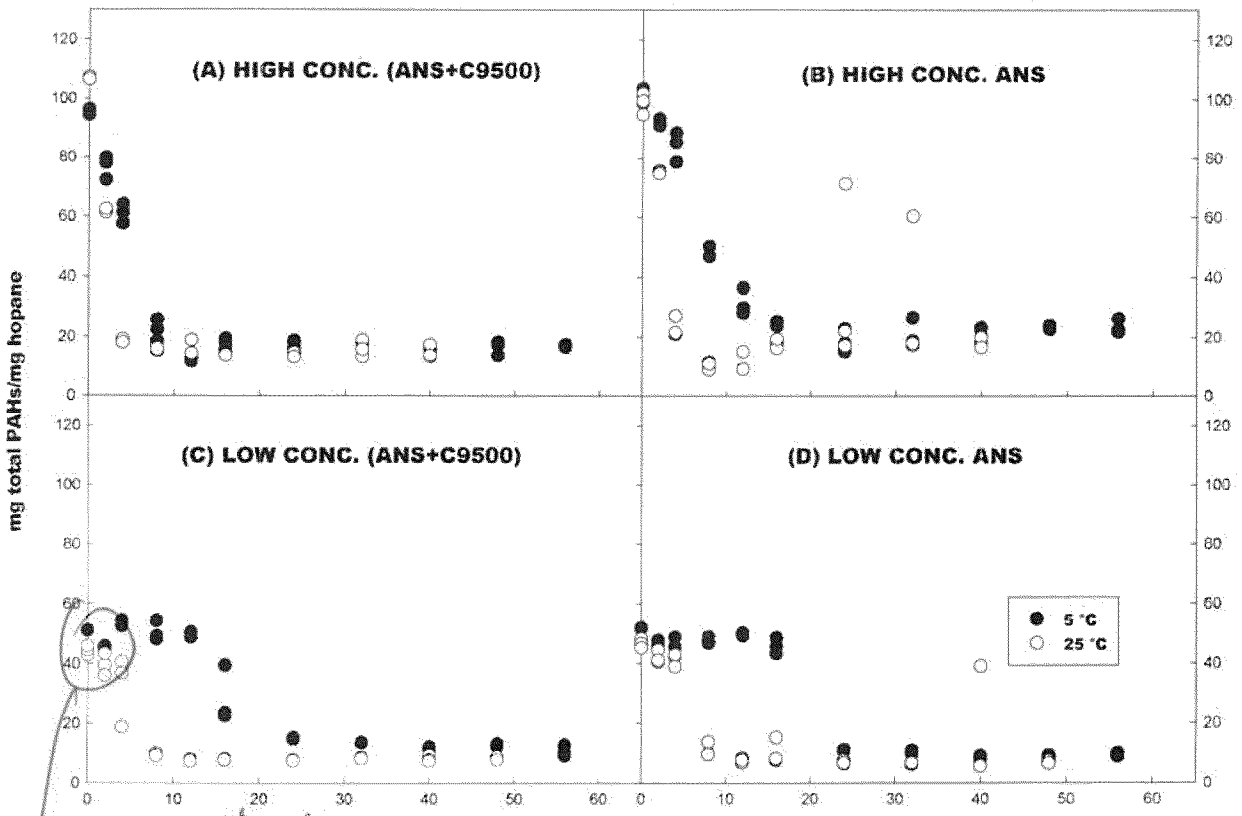
However, you got faster removal w/ C9500. line 307 and line 309

The largest lag-Ø in [low] was seen WITH C9500 at 5°C.

Is a given alkane molecule less likely to encounter a microbe in the [low] exp? I ~~though~~ am guessing the [microbe] is the same.

335 **Total Aromatics Degradation**

336 *High Concentration Experiment*. Fig. 5A-B presents total PAH biodegradation data for
337 the high concentration experiment at both temperatures. Aromatics removals of 42 and 24%
338 occurred by day 2 in the dispersed oil and the oil alone treatment, respectively. This difference in
339 extent was significant ($p < 0.0001$), which indicates a positive effect of the dispersant. At 5 °C,
340 the time for attaining maximum level of PAHs removal in non-dispersed treatment took twice as
341 much time as in dispersed oil treatment 16 days vs. 8 days (solid symbols in Fig. 5B and 5A,
342 respectively). The overall PAHs removals in the presence and absence of dispersant were 86%
343 and 82%, respectively.



344

So while alkanes, the hopane anomalie didn't affect this initial ratio?? This suggests that [hopane] was not the problem w/ the alkanes, as the PAHs are both high, like hopane, in both 5°C 25°C.

Figure 5. Biodegradation of hopane-normalized total PAHs at both temperatures in high concentration treatments (A, B) and low concentration treatments (C, D).

Low Concentration Experiment. As shown in Fig. 5C-D, when comparing the two low concentration treatments at 25 °C (open symbols), the only difference was in one replicate of dispersed oil on day 4, which had significantly lower total PAH concentration than the parallel diluted oil alone samples. At the high temperature (open symbols), the patterns of PAH degradation were similar in the absence and presence of C9500, regardless of initial concentration. The treatment without dispersant experienced higher variability. The lag period at the low temperature (closed symbols) was longer than at 25 °C; it lasted 12 and 16 days for dispersed oil and oil alone, respectively. For the sampling event on day 16, the extent of PAH removal was 20 to 50% in the replicates of dispersed oil sample, while it was negligible in the treatment without dispersant.

Transport of compounds from non-aqueous phase to aqueous phase is often the most rate-limiting step in the biodegradation of PAHs. Surfactants can enhance the dissolution of PAHs by increasing interfacial area of oil droplet and subsequently increase the bioavailability of PAHs to microorganisms (Haritash and Kaushik, 2009; Mrozik et al., 2003). The effect of C9500 on the biodegradation of total PAHs was less pronounced in the low concentration experiment than in the high concentration experiment, because this effect was observed only in one or more replicates in a single sampling event. This finding is consistent with our previous assumption about low concentration experiment: droplet size distribution in the oil alone and dispersed oil treatments are similar. Dispersion in the oil alone microcosms might have been efficient enough even without the addition surfactant, considering the mixing energy was sufficient (Kaku, 2006) and the initial presence of oil was an iridescent rainbow-colored sheen. Clearly, the comparison

Is this a diff. effect than w/ alkanes? (lines 323-334)

ED_001324_00001270-00020

between our low concentration dispersed oil samples and oil alone samples indicates that dispersants had less enhancing effect on the bio-uptake of PAHs after dilution. Nevertheless, most toxicity studies on dispersed oil concluded that the presence of dispersant increased environmental risk for aquatic organisms living in the water column (Milinkovitch et al., 2011; Ramachandran et al., 2004), which is possibly due to their relatively high concentration of oil and dispersant in the laboratory tests.

(relative to environmental concentrations)

Individual Aromatics Degradation

?

so if there was > neg., then the first-order model didn't work. What if there was < neg at the end, but "negligible" by the next-to-last event? Could it be modeled?

High Concentration Experiment. A first-order model could be used to fit the biodegradation of the individual PAHs that had a negligible fraction persisting by the end of the high concentration experiment. At both temperatures, the biodegradation rates of naphthalene, phenanthrene, fluorine, and dibenzothiophene and their homologues in the high concentration experiment were consistantly higher in the presence of dispersant (Table S5). The greatest enhancement was observed with naphthalene at 25 °C, which was 2.9-fold higher than at 5 °C (-1.94 d^{-1} vs. -0.68 d^{-1}). The surfactant likely assisted the transfer of more soluble compounds from the oil phase to the aqueous phase through the formation of small oil droplets and the resultant increase in interfacial area. Because the bio-uptake of aromatics is mostly from aqueous phase (Haritash and Kaushik, 2009; Mrozik et al., 2003), C9500 promoted the biodegradation of PAHs by increasing their bioavailability in water.

Low Concentration Experiment. Among the low concentration individual PAHs, the time-varying concentration of specific aromatics could be fitted to a first-order kinetics model (rates shown in Table S6), and the results revealed reduced rates due to dilution (low vs. high), especially when C9500 was present (i.e. naphthalene: -0.36 d^{-1} v -1.94 d^{-1}). However, we could

This phenom defies the principle of first-order reaction. First-order reactions have rate constants that are not dependent on initial concentration

not fit a first-order model for the other PAH compounds in the low concentration experiments because of the long lag period and the immediate and nearly complete removal after biodegradation started. As mentioned before, C9500 ~~acted less effectively~~ the impact of C9500 ~~the had much less impact~~ on the biodegradation of total PAHs in the low concentration experiment was difficult to assess, because different PAH concentrations in parallel dispersed oil and oil alone samples was observed only at one or more replicates in a single sampling event (25 °C: day 4, 5 °C: day 16). Nevertheless, C_4-naphthalene, C_{2-4}-phenanthrene, C_{2-3}-fluorene, and naphthobenzothiophene were removed at higher extent in the low rather than in the high concentration samples (Fig. S6). The increase in removal extent for the aforementioned aromatics were 6-14%, 4-47%, 14-54%, and 55-86% respectively. Zahed et al. (2010) also observed similar enhancement of crude oil removal extent in low oiling experiments. In the low concentration experiment, the starting PAH concentrations were equal to or below the remaining residual found in the high concentration experiment, but the cultures were able to degrade the aromatic compounds to an even lower level, as shown in Table 1. The reasons are a combination of the following: (1) the alkylated structure of these compounds led to less aqueous dissolution and less susceptibility to microbial attack; (2) their aqueous uptake was primarily diffusion, for which neither energized membrane nor adenosine triphosphate was essential (Bateman et al., 1986). Taking C_2-phenanthrene for example, it was fully degraded in the low concentration experiment, whereas its removal extent ranged from 66% to 94% when the initial oil concentration was high. The enhancement in the extent of removal under diluted conditions was more remarkable at low temperature, which contributed to our conclusion that solubility was the limiting factor for those compounds to be biodegraded. Whether oil concentration is high or low, pyrene, chrysene and naphthobenzothiophene homologues persisted until the last sampling event at both temperatures, as depicted in Figs. S7 and S8. The heavy

This seems to suggest that it is just "abiotic" sorption to the cell wall, and not active uptake, or biodegradation necessarily. And since the ratio PAH:cell area is lower a[low], then voila?

PAHs, such as chrysene, are likely to be retained on a beach for several years, according to Yin et al. (2015), who conducted research that monitors the submerged oil mats and surface residual oil balls off Alabama's beach affected by the 2010 DWH incident.

417 ACKNOWLEDGMENTS

418 We thank Jan Kurtz and Diane Yates from EPA's Gulf Ecology Division (GED) at Gulf
419 Breeze, FL, who collected the water samples in the GOM and performed the enrichments and
420 provided them for our experiments. The research was a product of the U.S. Environmental
421 Protection Agency's National Risk Management Research Laboratory (NRMRL) and was
422 partially funded by EPA, NRMRL, Cincinnati, OH, under Pegasus Technical Services, Inc.
423 Contract EP-C-11-006.

424 REFERENCES

425 2007. U.S. Environmental Protection Agency, EPA 8270D Semivolatile organic compounds by
426 gas chromatography/mass spectrometry (GC/MS), 3rd. ed, Washington, D.C.

427 API, 2013. Net Environmental Benefit Analysis for effective oil spill preparedness and response.
428 2013. http://www.api.org/~/media/Files/EHS/Clean_Water/Oil_Spill_Prevention/NEBA/NEBA-
429 Net-Environmental-Benefit-Analysis-July-2013.pdf.

430 Batchu, S.R., Ramirez, C.E., Gardinali, P.R., 2014. Stability of dioctyl sulfosuccinate (DOSS)
431 towards hydrolysis and photodegradation under simulated solar conditions. Sci. Total. Environ.
432 481, 260-265.

433 Bateman, J.N., Speer, B., Feduik, L., Hartline, R.A., 1986. Naphthalene association and uptake in
434 Pseudomonas putida. J. Bacteriol. 166, 155-161.

435 Brakstad, O.G., Nordtug, T., Throne-Holst, M., 2015. Biodegradation of dispersed Macondo oil
436 in seawater at low temperature and different oil droplet sizes. Marine pollution bulletin 93,
437 144-152.

438 Bredholt, H., Bruheim, P., Potocky, M., Eimhjellen, K., 2002. Hydrophobicity development,
439 alkane oxidation, and crude-oil emulsification in a Rhodococcus species. Can. J. Microbiol. 48,
440 295-304.

441 Bruheim, P., Bredholt, H., Eimhjellen, K., 1999. Effects of surfactant mixtures, including Corexit
442 9527, on bacterial oxidation of acetate and alkanes in crude oil. Appl. Environ. Microbiol. 65,
443 1658-1661.

ED_001324_00001270-00024

Campo, P., Venosa, A.D., Suidan, M.T., 2013. Biodegradability of Corexit 9500 and dispersed South Louisiana crude oil at 5 and 25 degrees C. Environ. Sci. Technol. 47, 1960-1967.

D7730–11, A., 2011. Standard Test Method for Determination of Dioctyl Sulfosuccinate in Sea Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). ASTM International 2011. ASTM International.

Gray, J.L., Kanagy, L.K., Furlong, E.T., Kanagy, C.J., McCoy, J.W., Mason, A., Lauenstein, G., 2014. Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. Chemosphere 95, 124-130.

Haritash, A.K., Kaushik, C.P., 2009. Biodegradation Aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. J. Hazard. Mater. 169, 1-15.

Jasper, W.L., Kim, T.L., Wilson, M.P., 1978. Droplet size distributions in a treated oil–water system., in: McCarthy, L.T., Lindblom, G.P., Walter, H.F. (Eds.), Chemical Dispersants for the Control of Oil Spills. American Society for Testing and Materials, Philadelphia, PA.

Kaku, V.J., Boufadel, M.C., Venosa, A.D., 2006. Evaluation of mixing energy in laboratory flasks used for dispersant effectiveness testing. J. Environ. Eng. Div. ASCE 132, 93-101.

Kujawinski, E.B., Kido Soule, M.C., Valentine, D.L., Boysen, A.K., Longnecker, K., Redmond, M.C., 2011. Fate of dispersants associated with the deepwater horizon oil spill. Environ. Sci. Technol. 45, 1298-1306.

Lee, K., Nedwed, T., Prince, R.C., Palandro, D., 2013. Lab tests on the biodegradation of chemically dispersed oil should consider the rapid dilution that occurs at sea. Mar. Pollut. Bull. 73, 314-318.

Li, Z., Lee, K., King, T., Boufadel, M.C., Venosa, A.D., 2009. Evaluating Chemical Dispersant Efficacy in an Experimental Wave Tank: 2—Significant Factors Determining In Situ Oil Droplet Size Distribution. 26, 1407-1418.

Lindstrom, J.E., Braddock, J.F., 2002. Biodegradation of petroleum hydrocarbons at low temperature in the presence of the dispersant Corexit 9500. Mar. Pollut. Bull. 44, 739-747.

Lunel, T., 1995. Understanding the mechanism of dispersion through oil droplet size measurements at sea., in: P. Lane (Ed.), The Use of Chemicals in Oil Spill Response. American Society for Testing and Materials, Philadelphia, PA.

Milinkovitch, T., Kanan, R., Thomas-Guyon, H., Le Floch, S., 2011. Effects of dispersed oil exposure on the bioaccumulation of polycyclic aromatic hydrocarbons and the mortality of juvenile Liza ramada. Sci. Total. Environ. 409, 1643-1650.

Mrozik, A., Piotrowska-Seget, Z., Labuzek, S., 2003. Bacterial Degradation and Bioremediation of Polycyclic Aromatic Hydrocarbons. Pol. J. Environ. Stud. 12, 15-25.

ED_001324_00001270-00025

478 Operational Science Advisory Team, 2010. Summary report for sub-sea and sub-surface oil and
479 dispersant detection: sampling and monitoring. Prepared for Paul F. Zukunft, U.S. Coast Guard.

480 Prince, R.C., 2015. Oil spill dispersants: boon or bane? Environmental science & technology 49,
481 6376–6384.

482 Prince, R.C., Elmendorf, D.L., Lute, J.R., Hsu, C.S., Halth, C.E., Senius, J.D., Dechert, G.J., Douglas,
483 G.S., Butler, E.L., 1994. 17α(H),21β(H)-hopane as a conserved internal marker for estimating the
484 blodegradation of crude oil. Environ. Sci. Technol. 28, 142-145.

485 Prince, R.C., McFarlin, K.M., Butler, J.D., Febbo, E.J., Wang, F.C., Nedwed, T.J., 2013. The
486 primary biodegradation of dispersed crude oil in the sea. Chemosphere 90, 521-526.

487 Ramachandran, S.D., Hodson, P.V., Khan, C.W., Lee, K., 2004. Oil dispersant increases PAH
488 uptake by fish exposed to crude oil. Ecotox. Environ. Safe. 59, 300-308.

489 Rojo, F., 2009. Degradation of Alkanes by Bacteria. Environ. Microbiol. 11, 2477-2490.

490 Spotte, S., Adams, G., Bubucis, P.M., 1984. GP2 medium is an synthetic seawater for culture or
491 maintenance of marine organisms. . Zoo Biol. 3, 229-240.

492 Tang, W.C., White, J.C., Alexander, M., 1998. Utilization of sorbed compounds by
493 microorganisms specifically isolated for that purpose. Appl. Microbiol. Biotechnol. 49, 117-121.

494 Venosa, A.D., Holder, E.L., 2007. Biodegradability of dispersed crude oil at two different
495 temperatures. Mar. Pollut. Bull. 54, 545-553.

496 Wentzel, A., Ellingsen, T.E., Kotlar, H.K., Zotchev, S.B., Throne-Holst, M., 2007. Bacterial
497 metabolism of long-chain n-alkanes. Appl. Microbiol. Biotechnol. 76, 1209-1221.

498 Word, J.Q., Clark, J.R., Word, L.S., 2014. Comparison of the Acute Toxicity of Corexit 9500 and
499 Household Cleaning Products. Human Ecol. Risk Assess. 21, 19.

500 Yin, F., John, G.F., Hayworth, J.S., Clement, T.P., 2015. Long-term monitoring data to describe
501 the fate of polycyclic aromatic hydrocarbons in Deepwater Horizon oil submerged off Alabama's
502 beaches. The Science of the total environment 508, 46-56.

503 Zahed, M.A., Aziz, H.A., Isa, M.H., Mohajeri, L., 2010. Effect of initial oil concentration and
504 dispersant on crude oil biodegradation in contaminated seawater. Bull. Environ. Contam.
505 Toxicol. 84, 438-442.

ED_001324_00001270-00026

***Supporting Information**

Biodegradability of Different Initial Concentrations of Alaska North Slope Crude Oil Dispersed with Corexit C9500

Mobing Zhuang[1], Gulizhaer Abulikemu[2], Pablo Campo[3], William Platten III[4], Makram T. Suidan[5]*, Albert D. Venosa (retired)[4]and Robyn N. Conmy[4]

1 Department of Biomedical, Chemical and Environmental Engineering, University of Cincinnati, 2901 Woodside Drive, Cincinnati, OH 45221, USA

2 Pegasus Technical Services Inc., 46 E Hollister St, Cincinnati, OH 45219 USA

3 Cranfield Water Science Institute, Cranfield University, Cranfield, Beds, MK43 0AL, UK

4 U.S. Environmental Protection Agency, NRMRL, 26 W. MLK Drive Cincinnati, OH, 45268, USA

5 Faculty of Engineering and Architecture, American University of Beirut, Bechtel Engineering Bldg. - 3rd flr. - Room 308 P.O. Box: 11-0236 Riad El Solh 1107 2020, Beirut, Lebanon

*Corresponding author: Makram T. Suidan
Tel: 00961-1-347952
Fax: 00961-1-744462
E-mail address: msuidan@aub.edu.lb

ED_001324_00001270-00027

Table S1. Summary of Experimental Layout for High Concentration Experiment.

Test	Temperature	Treatment	Sampling Events	Sample Replicates	Total Experimental Units (EU)
1	5 °C	C9500 alone	11	3	33
2	5 °C	ANS dispersed by C9500	11	3	33
3	5 °C	alone	11	3	33
4	5 °C	Killed ANS control	1	3	3
5	5 °C	Killed C9500 control	11	3	33
6	5 °C	Killed ANS+C9500 control	11	3	33
				Subtotal EU's	**168**
7	25 °C	C9500 alone	9	3	27
8	25 °C	ANS dispersed by C9500	9	3	27
9	25 °C	ANS alone	9	3	27
10	25 °C	Killed ANS control	1	3	3
11	25 °C	Killed C9500 control	9	3	27
12	25 °C	Killed ANS+ C9500 control	9	3	27
				Subtotal EU's	**138**
		Total EU's for High Concentration Experiment			**306**

ED_001324_00001270-00028

Table S2. Summary of Experimental Layout for Low Concentration Experiment.

Test	Temperature	Treatment	Sampling Events	Sample Replicates	Total Experimental Units (EU)
13	5 °C	C9500 alone	11	3	33
14	5 °C	ANS+C9500	11	3	33
15	5 °C	ANS alone	11	3	33
16	5 °C	Killed ANS control	1	3	3
17	5 °C	Killed C9500 control	11	3	33
18	5 °C	Killed (ANS+C9500) control	11	3	33
19	5 °C	Killed (ANS+C9500) +Protease	6	3	18
				Subtotal EU's	**186**
20	25 °C	C9500 alone	10	3	30
21	25 °C	ANS+C9500	10	3	30
22	25 °C	ANS alone	10	3	30
23	25 °C	Killed ANS control	1	3	3
24	25 °C	Killed C9500 control	10	3	30
25	25 °C	Killed (ANS+C9500) control	10	3	30
26	25 °C	Killed (ANS+C9500) +Protease	6	3	18
				Subtotal EU's	**181**
		Total EU's for Low Concentration Experiment			**357**

26

ED_001324_00001270-00029

Table S3. First-order biodegradation rate coefficients and standard deviations (sd) of individual alkanes in the high concentration experiment.

Compound	5 °C ANS alone		25 °C ANS alone		5 °C ANS+C9500		25 °C ANS+C9500	
	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})
C10	0.19	0.02	1.34	0.18	0.63	0.03	2.00	0.34
C11	0.15	0.01	1.16	0.15	0.40	0.02	1.30	0.09
C12	0.12	0.01	1.02	0.14	0.29	0.03	0.96	0.06
C13	0.11	0.01	0.94	0.14	0.27	0.03	0.91	0.05
C14	0.11	0.01	0.92	0.14	0.25	0.03	0.82	0.04
C15	0.11	0.01	0.95	0.14	0.24	0.03	0.84	0.05
C16	0.11	0.01	1.01	0.15	0.24	0.03	0.92	0.05
C17	0.11	0.01	1.08	0.16	0.24	0.03	0.97	0.06
PR	0.15	0.03	0.45	0.11	0.07	0.01	0.39	0.04
C18	0.11	0.01	1.06	0.14	0.22	0.02	0.96	0.05
PH	0.15	0.03	0.46	0.12	0.08	0.01	0.43	0.04
C19	0.11	0.01	1.05	0.14	0.22	0.02	0.97	0.06
C20	0.11	0.01	0.99	0.14	0.21	0.02	0.93	0.05
C21	0.10	0.01	0.97	0.14	0.20	0.02	0.91	0.05
C22	0.10	0.01	0.88	0.13	0.19	0.02	0.84	0.04
C23	0.09	0.01	0.84	0.13	0.18	0.02	0.79	0.04
C24	0.09	0.01	0.84	0.14	0.17	0.02	0.79	0.04
C25	0.09	0.01	0.81	0.13	0.15	0.02	0.81	0.03
C26	0.08	0.01	0.81	0.14	0.12	0.02	0.78	0.04
C27	0.08	0.01	0.74	0.13	0.11	0.01	0.71	0.05
C28	0.12	0.02	0.75	0.12	0.12	0.02	0.60	0.09
C29	0.12	0.02	0.81	0.15	0.12	0.02	0.81	0.07
C30	0.10	0.01	0.83	0.14	0.12	0.02	0.74	0.04
C31	0.09	0.01	0.81	0.14	0.09	0.01	0.61	0.05
C32	0.10	0.01	0.81	0.14	0.09	0.01	0.60	0.06
C33	0.09	0.01	0.68	0.09	0.07	0.01	0.56	0.06
C34	0.08	0.01	0.60	0.09	0.05	0.01	0.54	0.05
C35	0.07	0.01	0.53	0.09	0.04	0.01	0.58	0.04

NA: Rate not calculated because the biodegradation of noted compounds did not follow first order kinetics.

ED_001324_00001270-00030

Table S4. First-order biodegradation rate coefficients and standard deviations (sd) of individual alkanes in low concentration experiment.

Compound	5 °C ANS alone		25 °C ANS alone		5 °C ANS+C9500		25 °C ANS+C9500	
	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})
C10	0.47	0.03	0.93	0.16	0.63	0.04	NA1	NA1
C11	0.25	0.02	0.57	0.07	0.25	0.03	0.47	0.02
C12	0.20	0.01	0.38	0.06	0.23	0.02	0.24	0.03
C13	0.18	0.01	0.42	0.06	0.21	0.02	NA2	NA2
C14	0.13	0.01	0.28	0.04	0.15	0.01	NA2	NA2
C15	0.14	0.01	0.35	0.04	0.15	0.01	NA2	NA2
C16	0.14	0.01	0.37	0.04	0.15	0.02	NA2	NA2
C17	0.13	0.01	0.36	0.04	NA2	NA2	NA2	NA2
PR	NA2	NA2	0.04	0.01	NA2	NA2	NA2	NA2
C18	NA2	NA2	0.35	0.04	NA2	NA2	NA2	NA2
PH	NA2	NA2	0.03	0.01	NA2	NA2	NA2	NA2
C19	NA2	NA2	0.32	0.04	NA2	NA2	NA2	NA2
C20	NA2	NA2	0.30	0.04	NA2	NA2	NA2	NA2
C21	NA2	NA2	0.29	0.04	NA2	NA2	NA2	NA2
C22	NA2	NA2	0.17	0.03	NA2	NA2	NA2	NA2
C23	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C24	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C25	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C26	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C27	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C28	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C29	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C30	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C31	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C32	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C33	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C34	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C35	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2

NA1: Rate not calculated because of excessively rapid removal after acclimation.
NA2: Rate not calculated because noted compounds were persisted during the experiment.

ED_001324_00001270-00031

Table S5. First-order degradation rate coefficients and standard deviations (sd) of individual PAHs in high concentration experiment.

Compound	5 °C ANS alone		25 °C ANS alone		5 °C ANS+C9500		25 °C ANS+C9500	
	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})
nap	0.44	0.04	0.68	0.05	0.71	0.02	1.94	0.02
C1-nap	0.23	0.02	0.60	0.04	0.41	0.03	1.84	0.04
C2-nap	0.17	0.02	0.38	0.07	0.26	0.02	0.61	0.03
C3-nap	0.27	0.02	0.35	0.10	0.20	0.02	0.40	0.05
C4-nap	0.13	0.02	0.29	0.10	0.16	0.02	0.35	0.05
phe	0.19	0.01	0.37	0.09	0.26	0.02	0.46	0.05
C1-phe	0.12	0.01	0.34	0.12	0.19	0.02	0.37	0.06
C2-phe	0.05	0.01	0.21	0.07	0.19	0.03	0.27	0.04
C3-phe	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-phe	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
flu	0.19	0.02	0.37	0.10	0.26	0.02	0.44	0.05
C1-flu	0.15	0.02	0.35	0.11	0.21	0.02	0.39	0.06
C2-flu	0.05	0.01	0.20	0.07	0.11	0.01	0.27	0.04
C3-flu	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dbt	0.25	0.01	0.43	0.08	0.44	0.02	0.77	0.03
C1-dbt	0.13	0.01	0.32	0.10	0.22	0.02	0.41	0.04
C2-dbt	0.04	0.01	0.17	0.06	0.11	0.01	0.26	0.04
C3-dbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pyr*	--	--	--	--	--	--	--	--
C1-pyr	0.00	0.00	0.00	NA	NA	0.00	NA	NA
C2-pyr	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

NA: Rate not calculated because noted compounds were persisted during the experiment.
* Not detected in any samples.

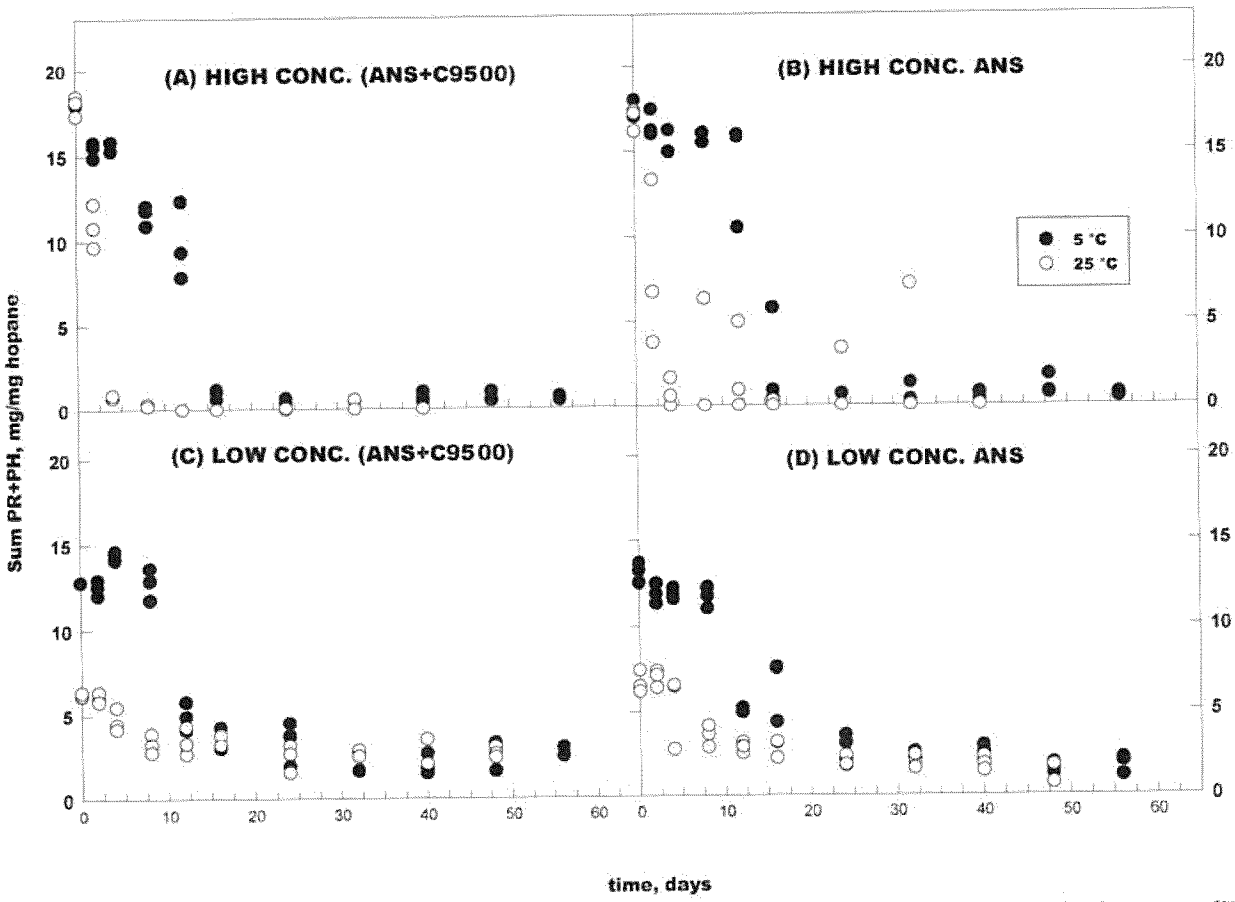
ED_001324_00001270-00032

Table S6. First-order degradation rate coefficients and standard deviations (sd) of individual PAHs in low concentration experiment.

Compound	5 °C ANS alone		25 °C ANS alone		5 °C ANS+C9500		25 °C ANS+C9500	
	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})
nap	NA	NA	0.29	0.05	0.43	0.09	0.36	0.03
C1-nap	NA	NA	0.24	0.03	0.44	0.09	0.28	0.03
C2-nap	NA	NA	0.17	0.04	0.40	0.09	0.21	0.04
C3-nap	NA	NA	0.18	0.04	0.31	0.06	0.20	0.04
C4-nap	NA	NA	0.13	0.03	0.15	0.02	0.14	0.02
phe	NA	NA	NA	NA	NA	NA	0.19	0.03
C1-phe	NA	NA	NA	NA	0.18	0.03	0.20	0.03
C2-phe	0.25	0.01	0.14	0.03	0.11	0.01	NA	NA
C3-phe	0.02	0.01	0.02	0.00	0.00	0.00	0.00	0.00
C4-phe	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
flu	NA	NA	NA	NA	0.29	0.06	NA	NA
C1-flu	NA	NA	NA	NA	0.15	0.02	0.00	0.00
C2-flu	0.29	0.03	0.14	0.03	0.21	0.01	0.00	0.00
C3-flu	0.02	0.00	0.03	0.00	0.00	0.00	0.02	0.00
dbt	NA	NA	0.00	0.00	0.31	0.11	0.00	0.00
C1-dbt	NA	NA	NA	NA	0.23	0.05	0.29	0.06
C2-dbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-dbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nbt	0.00	0.00	NA	NA	0.00	0.00	0.31	0.11
C1-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pyr*	--	--	--	--	--	--	--	--
C1-pyr	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.07
C2-pyr	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

NA: Rate not calculated because of excessively rapid removal after acclimation.
*Not detected in any samples.

ED_001324_00001270-00033



35
36 **Figure S1. Biodegradation of branched alkanes in the presence (A, C) and absence (B,**
37 **D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B,**
38 **whereas low concentration treatments are in panels C and D.**

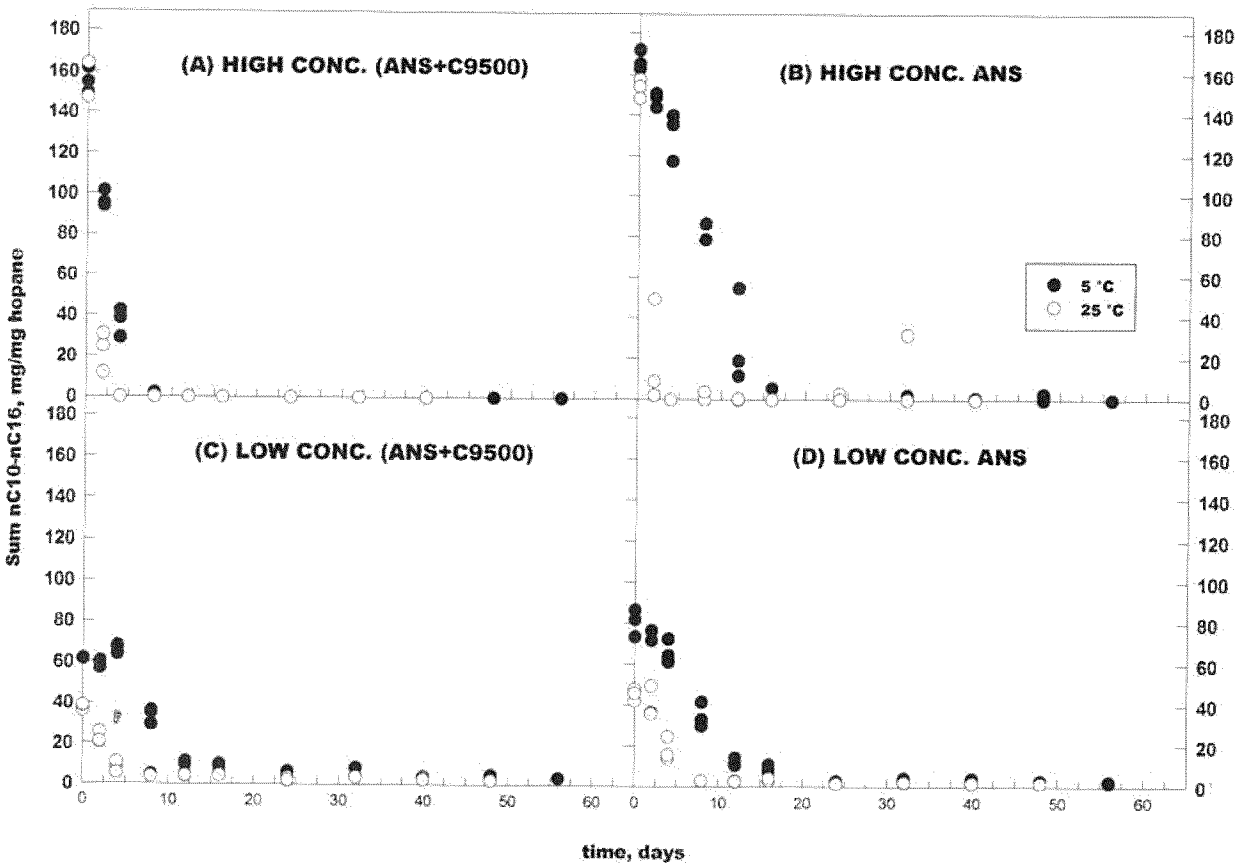
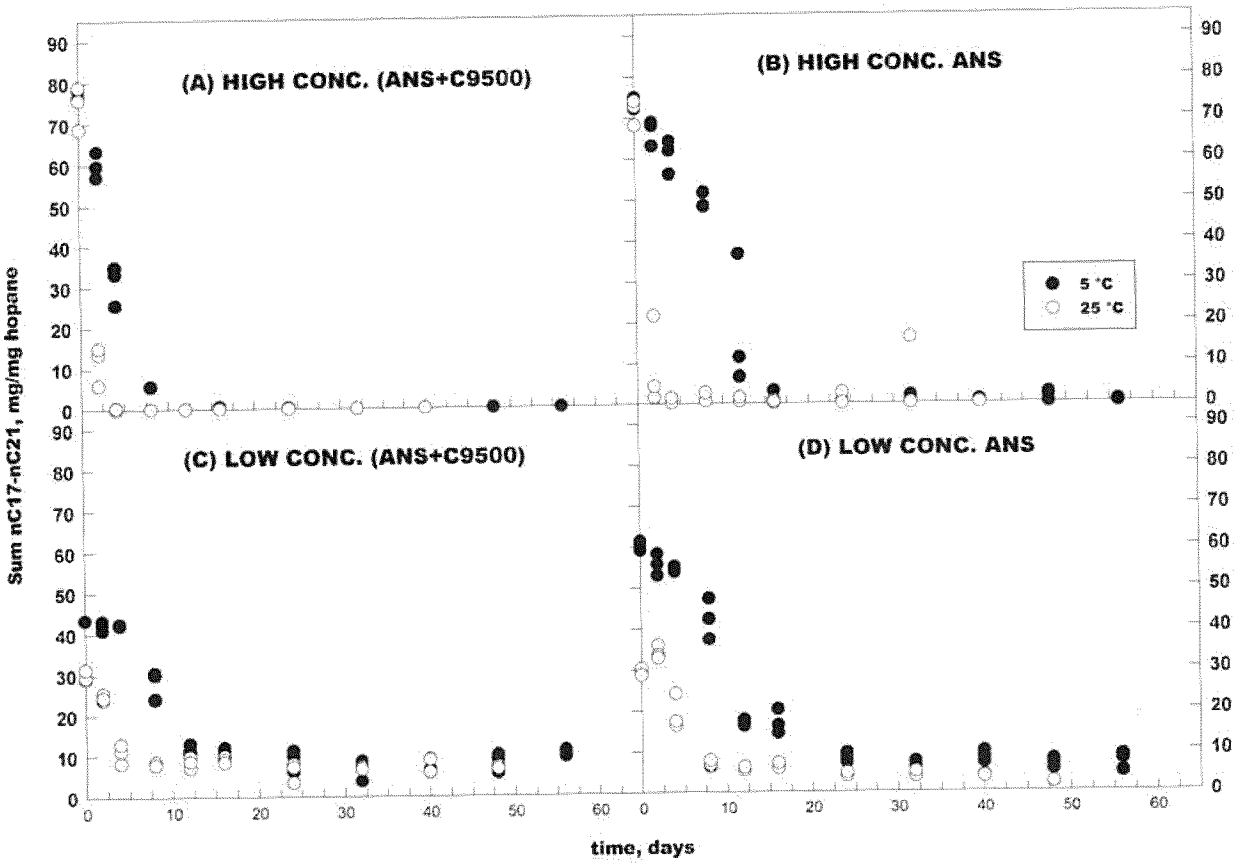


Figure S2. Biodegradation of n-alkanes (nC10-nC16) in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.



43

44 **Figure S3. Biodegradation of n-alkanes (nC17-nC21) in the presence (A, C) and**
45 **absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel**
46 **A and B, whereas low concentration treatments are in panels C and D.**

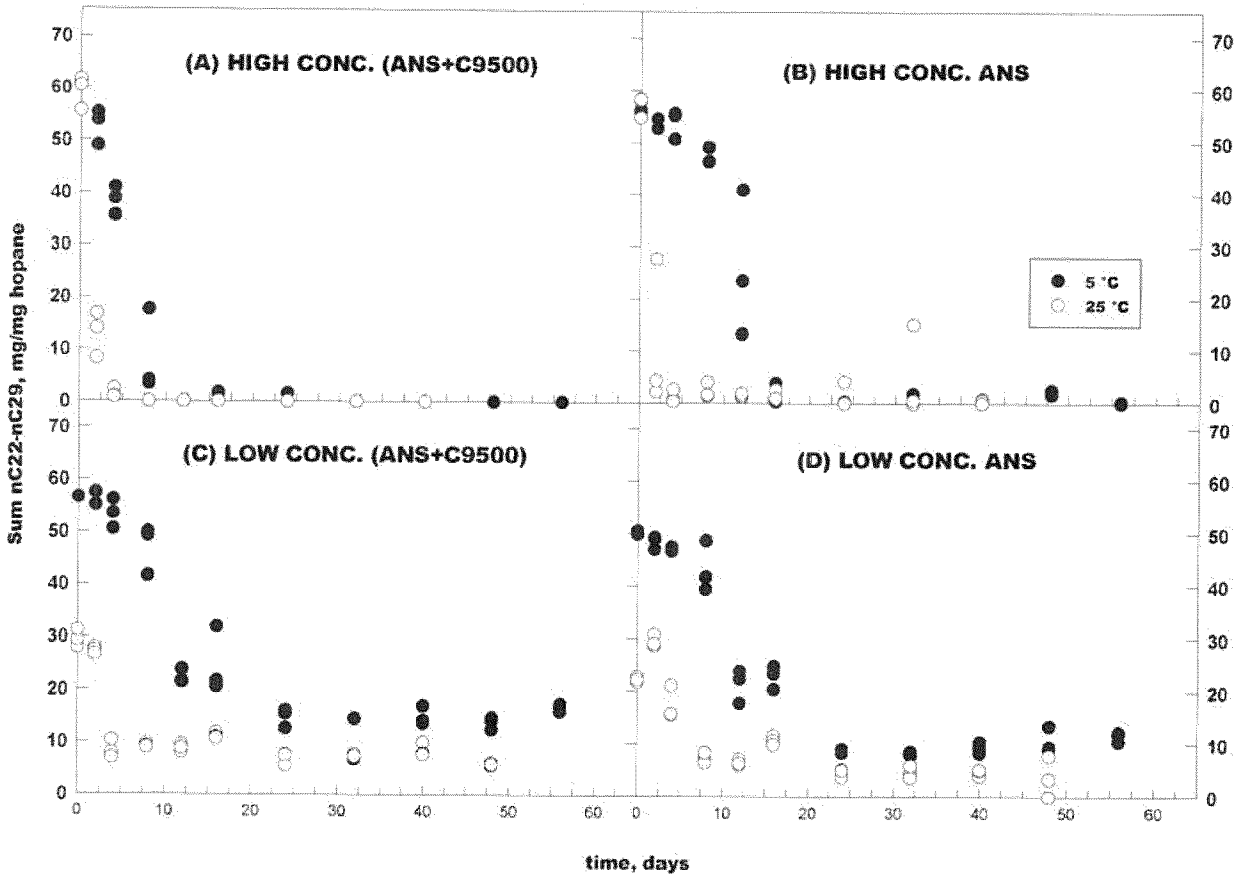


Figure S4. Biodegradation of n-alkanes (nC22-nC29) in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

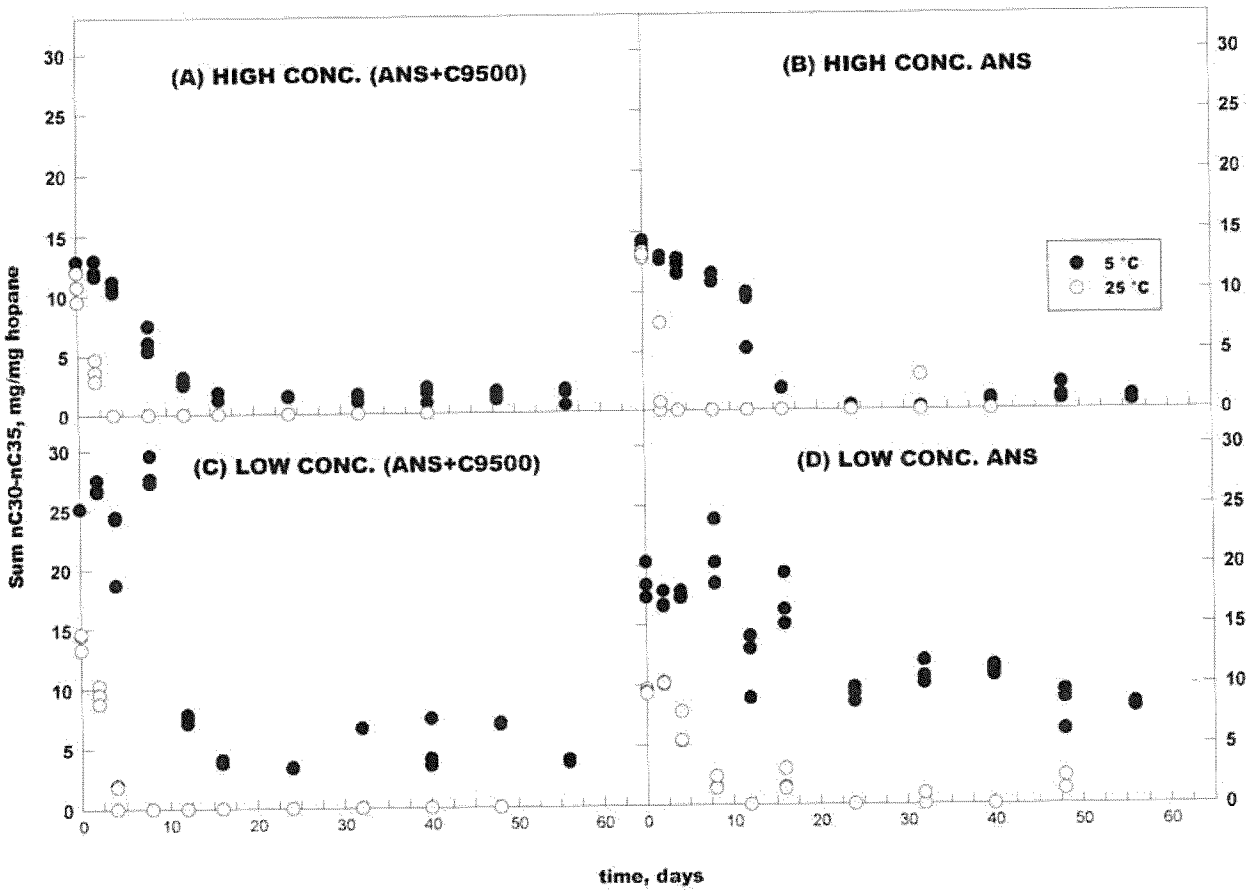


Figure S5. Biodegradation of n-alkanes (nC30-nC35) in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

ED_001324_00001270-00038

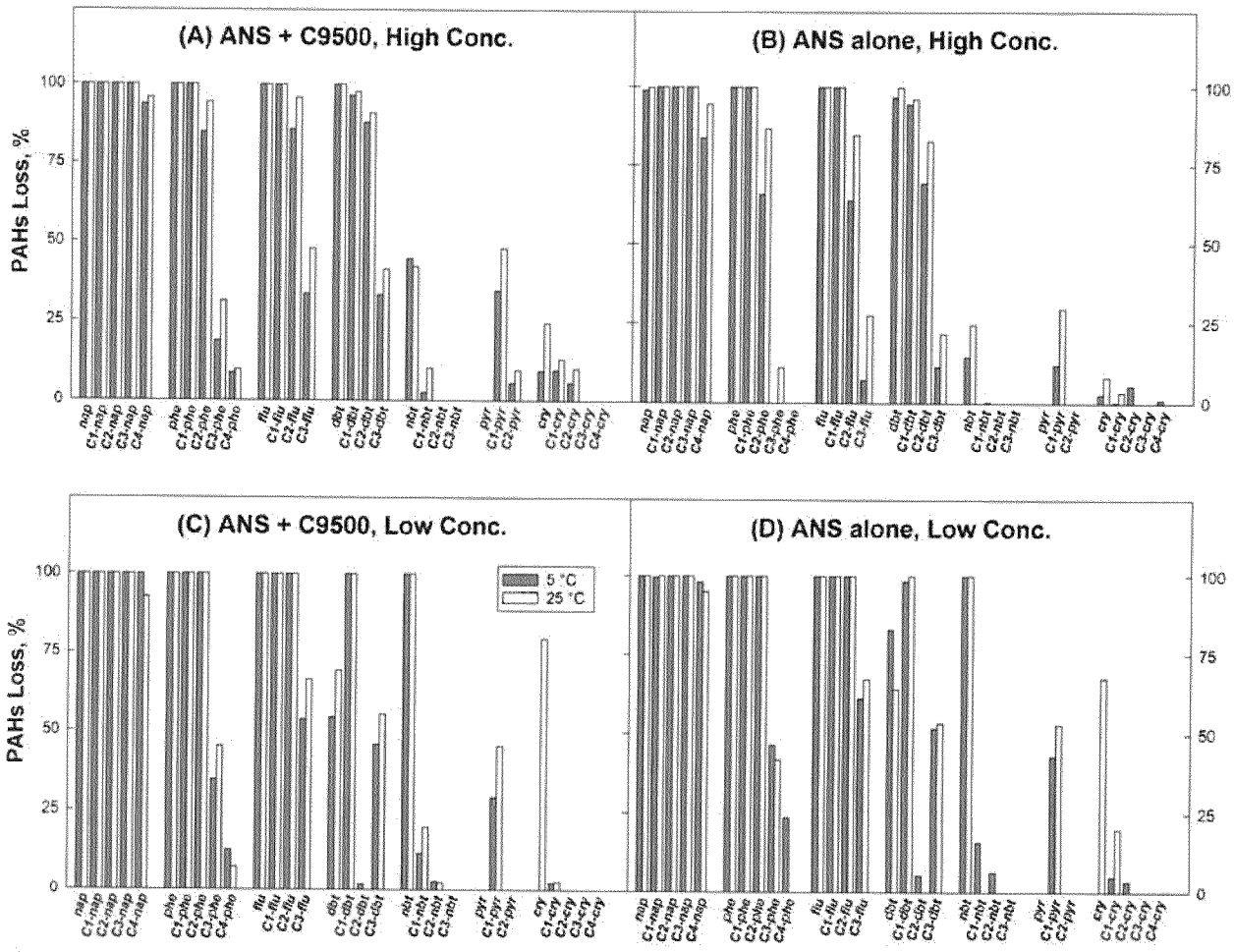


Figure S6. Loss percentage of individual PAH in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C in high concentration (A, B) and low concentration (C, D) experiment.

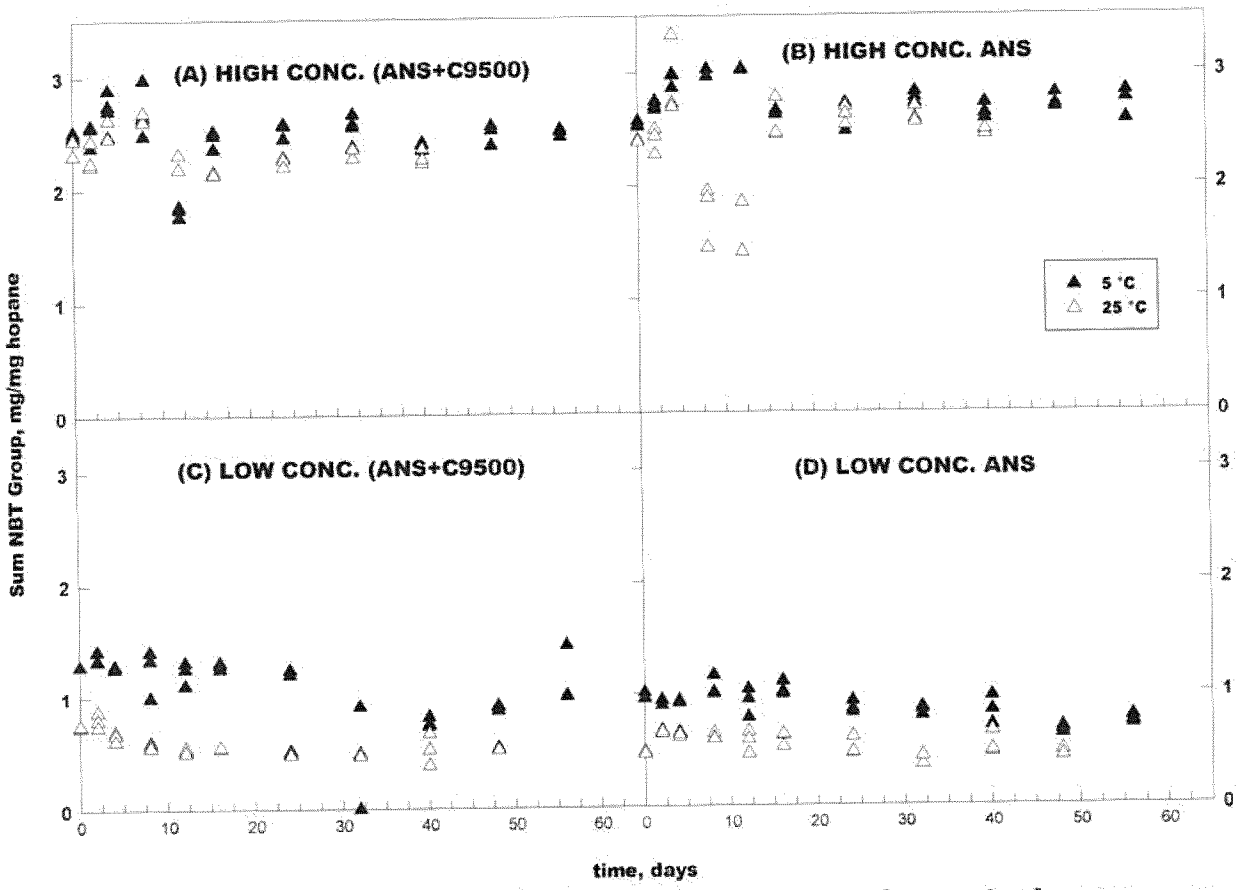


Figure S7. Biodegradation of naphthobenzothiophene homologues in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

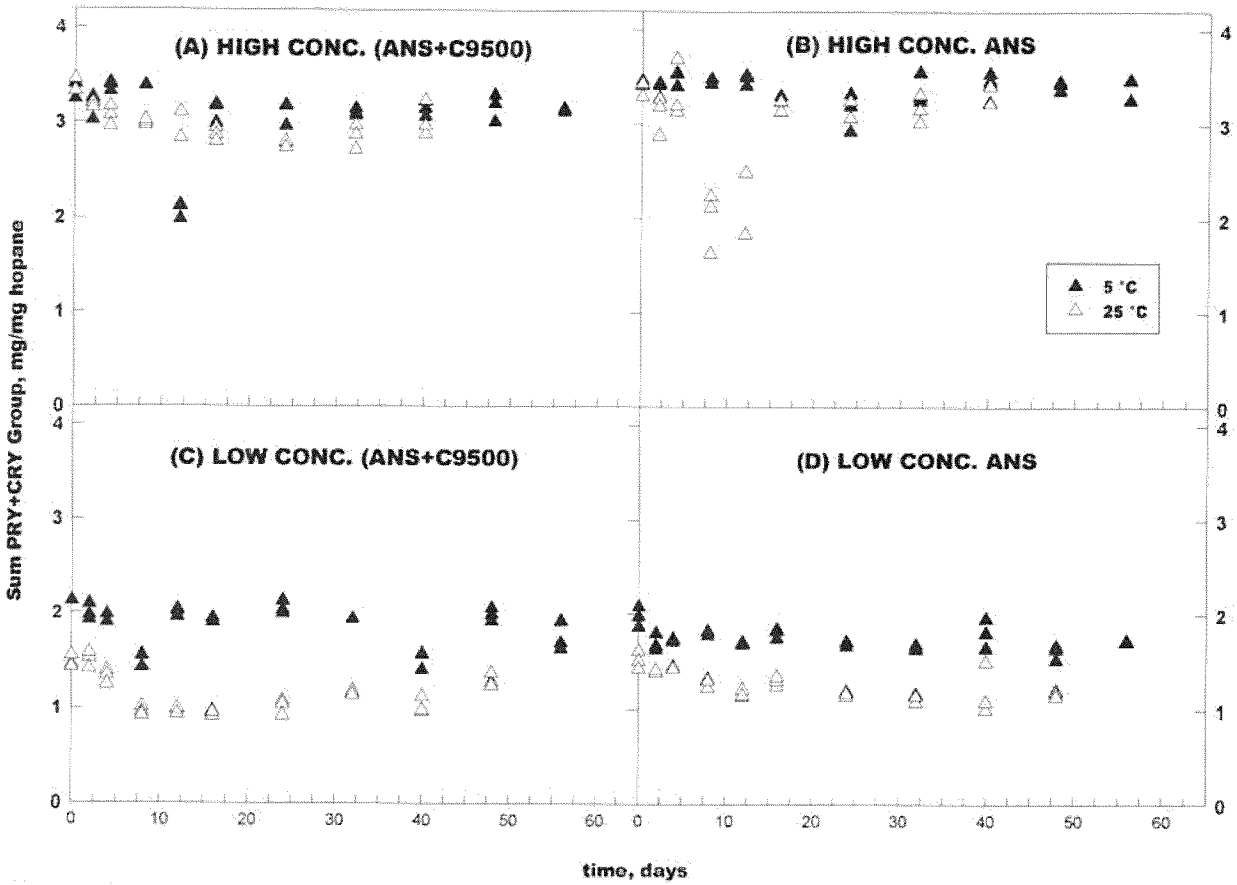


Figure S8. Biodegradation of pyrene homologues + chrysene homologues in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

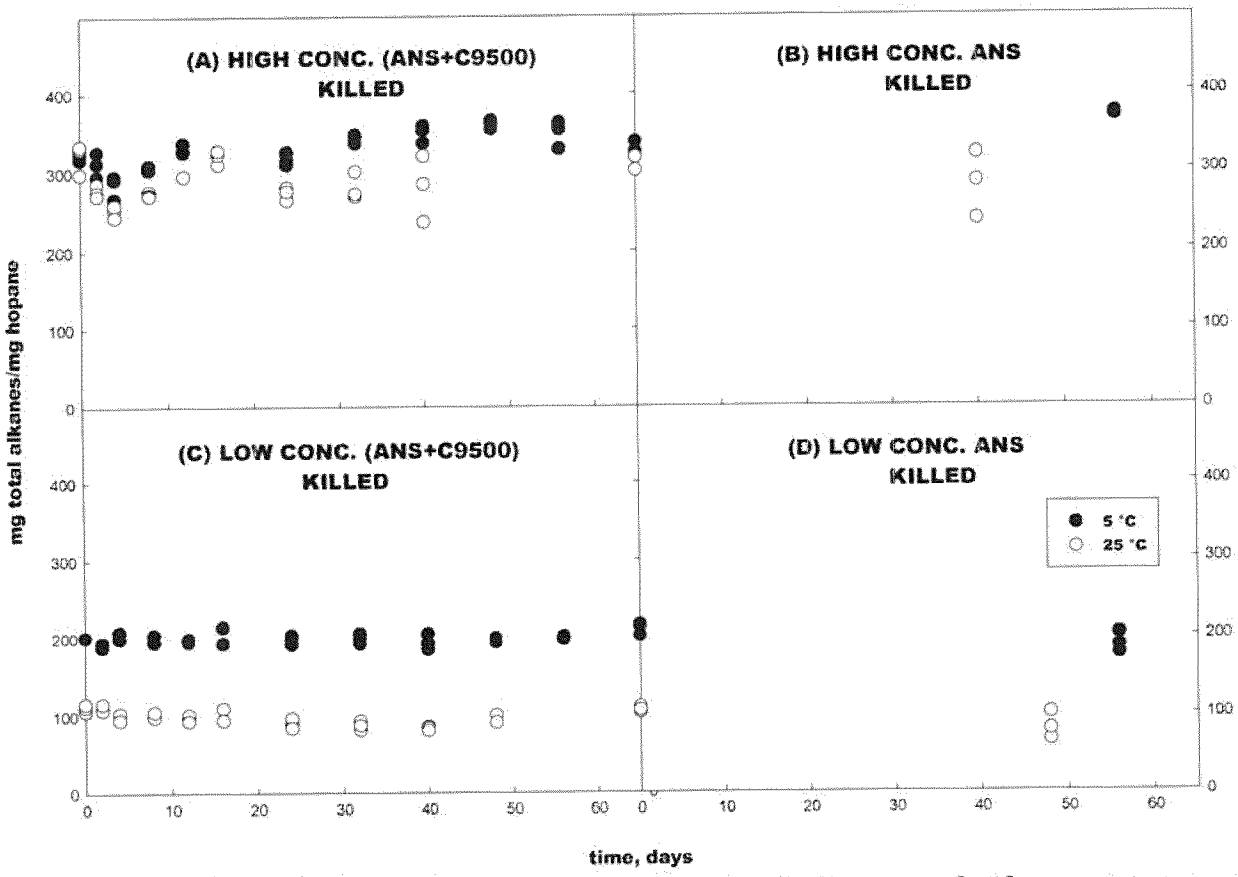


Figure S9. Biodegradation of hopane-normalized total alkanes at both temperatures in high concentration treatments (A, B) and low concentration treatments (C, D) in killed control samples.

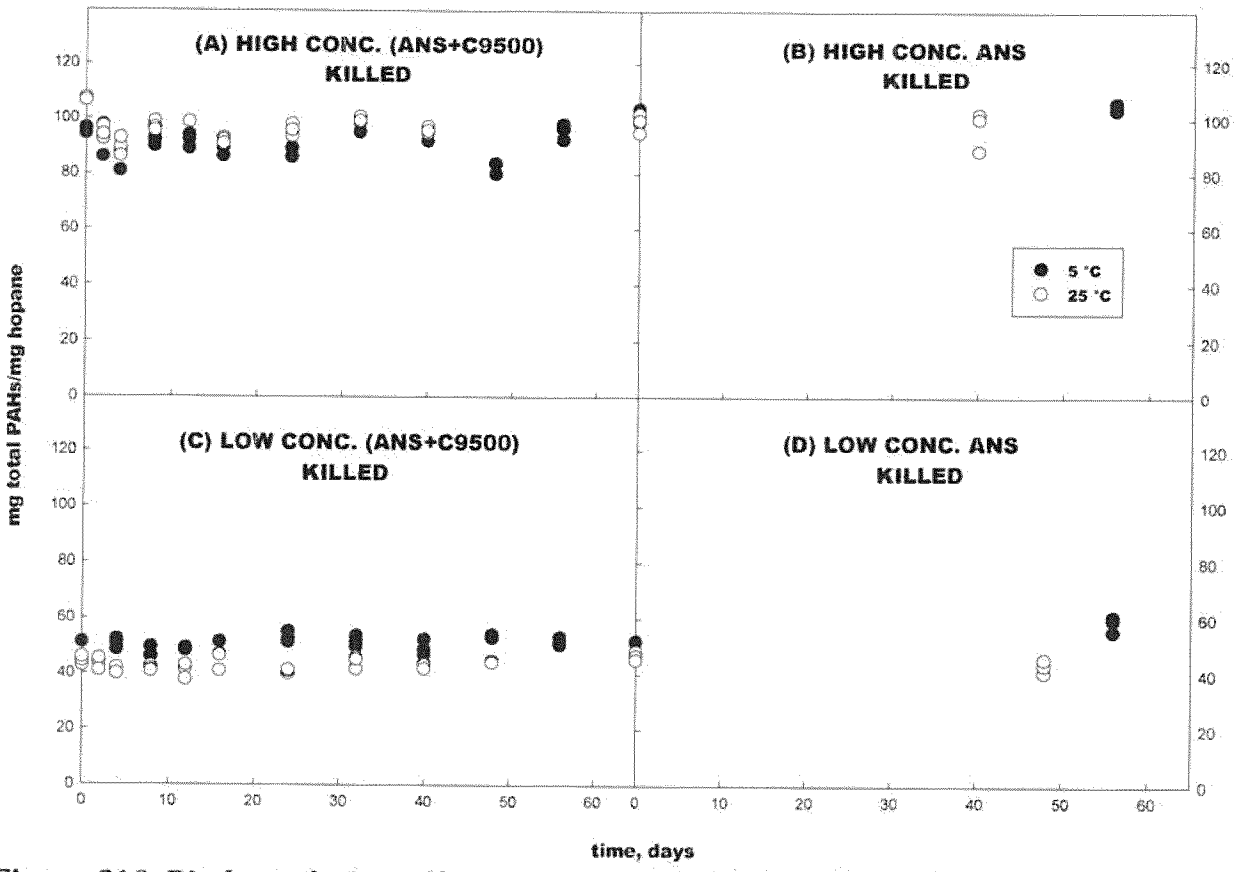


Figure S10. Biodegradation of hopane-normalized total PAHs at both temperatures in high concentration treatments (A, B) and low concentration treatments (C, D) in killed control samples.

Quality Assurance Project Plan
Category III Measurement Project

BIODEGRADABILITY OF DISPERSANT AND DISPERSED OIL AT TWO TEMPERATURES: EFFECT OF USING DILUTED OIL AND DISPERSANT

U.S. Environmental Protection Agency Contract No. **EP-C-11-006**

Work Assignment No. 3-33

Prepared for:
Albert D. Venosa, Ph.D.
Work Assignment Manager

U.S. Environmental Protection Agency
Office of Research and Development
National Risk Management Research Laboratory
26 West Martin Luther King Drive
Cincinnati, OH 45268

Prepared by:
Pablo Campo-Moreno, Ph.D.

University of Cincinnati
School of Energy, Environmental, Biological, and Medical Engineering

Under Subcontract to:

Pegasus Technical Services, Inc.
46 E. Hollister Street
Cincinnati, OH 45219

Revision 0
November 12, 2013

QUALITY ASSURANCE PROJECT PLAN APPROVAL

Concurrences:

1. Pablo Campo-Moreno, Ph.D., University of Cincinnati
   Work Assignment Leader

   _____ _____
   Signature Date

2. Steven Jones, ASQ CQA/CQE, Shaw Environmental & Infrastructure, Inc.
   Pegasus Contract Quality Assurance Manager

   _____ _____
   Signature Date

3. George Sorial, Ph.D., University of Cincinnati
   University of Cincinnati Project Manager

   _____ _____
   Signature Date

4. Karen Koran, Ph.D., Pegasus Technical Services, Inc.
   Project Manager

   _____ _____
   Signature Date

U.S. Environmental Protection Agency Approval for Implementation:

1. Albert D. Venosa, Ph.D., Work Assignment Manager

   _____ _____
   Signature Date

2. Jim Voit, LRPCD Quality Assurance Manager

   _____ _____
   Signature Date

TABLE OF CONTENTS

Section	Name	Revision	Date	Page
0.0	Approval by Project Participants	0	11/2013	2
1.0	Project Description and Objectives	0	11/2013	8
2.0	Organization and Responsibilities	0	11/2013	9
3.0	Scientific Approach	0	11/2013	12
4.0	Sampling Procedures	0	11/2013	16
5.0	Measurement Procedures	0	11/2013	18
6.0	Quality Metrics (QA/QC Checks)	0	11/2013	19
7.0	Data Analysis, Interpretation, and Management	0	11/2013	23
8.0	Reporting	0	11/2013	25
9.0	References	0	11/2013	26
APPENDIX A	Standard Operating Procedures	0	11/2013	27

APPENDIX A

List of Standard Operating Procedures (SOPs)

SOP 1	Preparation of Surrogate Recovery Standard
SOP 2	Preparation od Internal Standard Solution
SOP 3	Preparation of Working Standard, Check Standards, and Oil Standard for GC-MS/MS
SOP 4	Extraction of Oil from Aqueous Samples for GC-MS/MS
SOP 5	GC-MS/MS Method for Analysis of Crude Oil Samples
SOP 6	LC-MS/MS Method for the Analysis of Dioctyl Sulfosuccinate in Seawater
SOP 7	Total DNA Isolation
SOP 8	Quantifying Nucleic Acids With a Nanodrop Nd-1000 Spectrophotometer
SOP 9	Polymerase Chain Reaction (PCR)
SOP 10	PCR Purification With QIAquick Spin Kit
SOP 11	TOPO T/A Cloning

LIST OF TABLES

Table	Title	Page
Table 2.1	Organization/Title Chart	9
Table 2.2	Project Schedule	11
Table 3.1	Experimental Layout	13
Table 4.1	Treatment and Analytical Method	16
Table 4.2	Experimental Sampling and Analytical Procedures to Be Used	17
Table 5.1	Analytical Instruments	18
Table 6.1	Summary of Internal QC Checks for Critical Parameters	20
Table 7.1	Reporting Units	23

LIST OF FIGURES

Figure	Title	Page
Figure 2.1	Organization Chart	10

DISTRIBUTION LIST

Dr. Albert Venosa, Work Assignment Manager, U.S. Environmental Protection Agency
Mr. Jim Voit, LRPCD Quality Assurance Manager, U.S. Environmental Protection Agency
Dr. Jorge Santo Domigo, Project Support for PCR Analysis, U.S. Environmental Protection Agency

Dr. Karen Koran, Project Manager, Pegasus Technical Services, Inc.
Mr. Steven Jones, Contract Quality Assurance Manager, Shaw Environmental & Infrastructure, Inc.
Dr. George Sorial, Manager, University of Cincinnati
Dr. Pablo Campo-Moreno, Work Assignment Leader, University of Cincinnati
Ms. Mobing Zhuang , Project Staff, University of Cincinnati
Ms. Gulizhaer Abulikemo, Project Staff, University of Cincinnati

SECTION 1.0

PROJECT DESCRIPTION AND OBJECTIVES

1.1 Purpose of study

The Deepwater Horizon (DWH) incident in the Gulf of Mexico (GOM) has raised questions not only about the biodegradability of oil in deep and surface waters in that area, but also to answer concerns about the rate of biodegradation of the dispersants *per se* and the dispersed oil. Previous research conducted under U.S. Environmental Protection Agency (EPA) Contract No. EP-C-11-006, Work Assignments (WAs) 1-33 and 2-33, showed that the alkane and polycyclic aromatic hydrocarbons (PAH) fractions in the oil, dispersed oil, and dispersant biodegraded at 5 and 25 °C and the corresponding degradation rates were calculated. Nevertheless, these studies were conducted at substrate concentrations significantly higher than those found in field conditions. Hence, in this project, oil, dispersed oil, and dispersant will be tested at a concentration that is 4.3% of the value previously tested in the aforementioned WAs. The rationale is that normally, in actual field trials, the concentrations of oil and dispersant never reach the levels used in laboratory experiments. The herein described approach will produce environmentally relevant data by determining oil, dispersed oil, and dispersant biodegradation rates at smaller loading concentrations. The EPA is also in need of determining what microbial cultures are responsible for biodegradation at the two temperatures and how these communities change as substrate consumption takes place.

1.2 Project Objectives

The overall aim of this WA is to study biodegradation of oil, dispersed oil, and dispersant at environmentally relevant concentrations. This goal will be achieved through the following specific objectives:

1. To study the rate of biodegradation of Alaska North Slope (ANS) crude oil, dispersant Corexit 9500 (C9500), and ANS dispersed with C9500 at a concentration 4.3% of that used in WAs 1-33 and 2-33.
2. To measure those biodegradation rates at 5 °C and at 25 °C.

1.2_ To investigate the time-dependent changes in the microbial cultures used in the biodegradation experiments.

SECTION 2.0

ORGANIZATION AND RESPONSIBILITIES

2.1 Project Organization

Mr. Michael Moeykens and Mr. Stephen Wright serve as the EPA Project Officers for EPA Contract No. EP-C-11-006. For this WA, Dr. Albert Venosa is the EPA WA Manager responsible for WA planning, providing technical direction related to WA activities, and ensuring that the data received from Pegasus Technical Services, Inc. (Pegasus) and the University of Cincinnati (UC) satisfy the project objectives. Mr. Jim Voit is the EPA Land Remediation and Pollution Control Division (LRPCD) Quality Assurance (QA) Manager responsible for approval of this Quality Assurance Project Plan (QAPP). Dr. Jorge Santo Domigo with EPA will be providing support for polymerase chain reaction (PCR) analysis of samples.

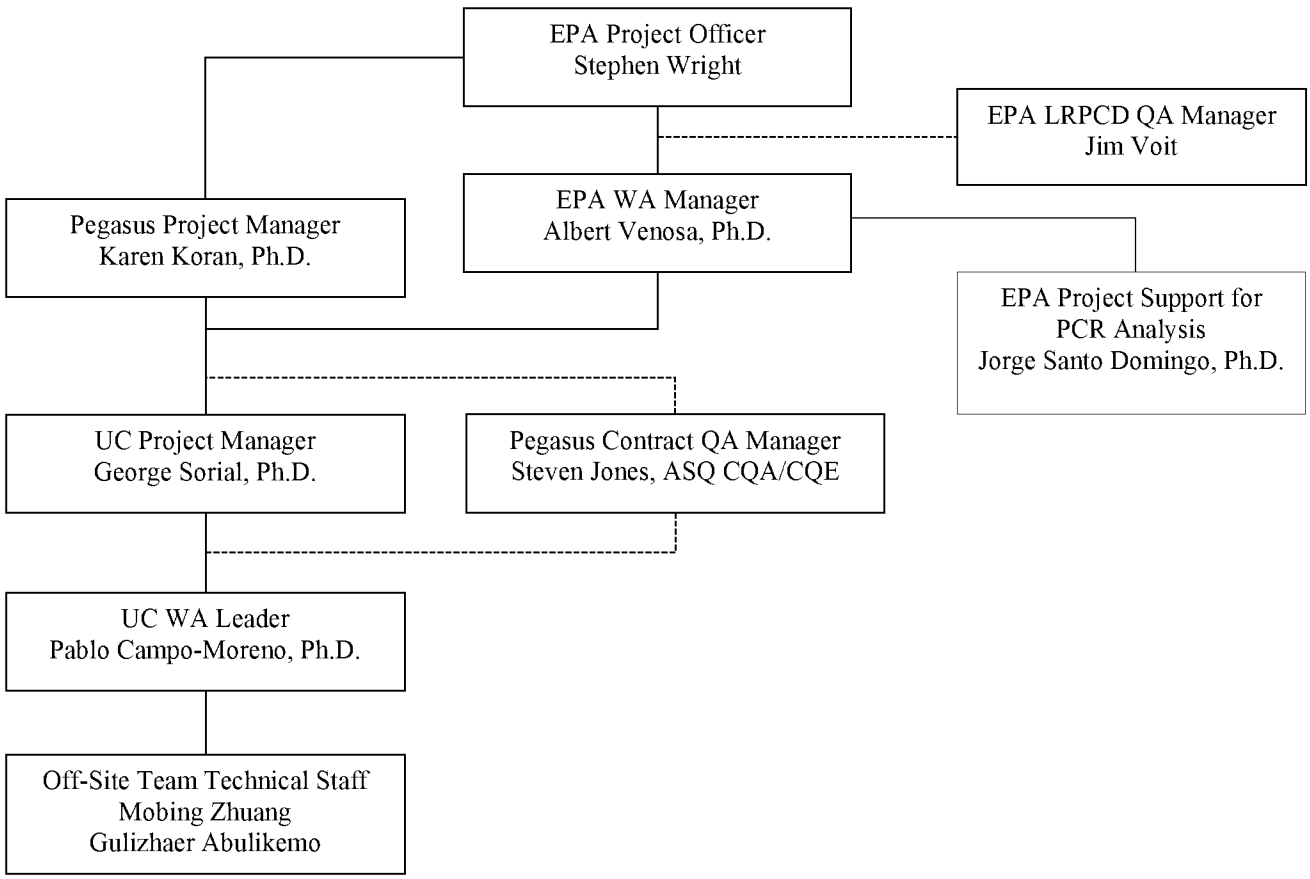
Dr. Karen Koran is the Pegasus Project Manager for this Off-Site WA. Dr. George Sorial with UC (a subcontractor to Pegasus for this off-site WA) is the UC Project Manager. Mr. Steven Jones, ASQ CQA/CQE, with Shaw Environmental & Infrastructure, Inc., is the Pegasus Contract QA Manager responsible for oversight of Pegasus Quality Program implementation, QA review of quality documents and deliverables, and project assessments. Dr. Pablo Campo-Moreno is the UC WA Leader responsible for project planning, coordination of experiments and analyses, interpretation of the data, coordinating internal review of contract deliverables prior to submittal to EPA, and completion of the final report. Ms. Mobing Zhuang and Ms. Gulizhaer Abulikemo, WA staff, will be responsible for assisting the UC WA Leader with the design and maintenance of the experiments as well as chemical analysis.

Table 2.1 Organization/Title Chart

Name	Phone/email	Responsibilities
Mr. Stephen Wright	(513) 569-7610 Wright.Stephen@epa.gov	EPA Project Officer
Mr. Michael Moeykens	(513) 569-7196 Moeykens.Michael@epa.gov	EPA Project Officer
Dr. Albert Venosa	(513) 569-7668 Venosa.Albert@epa.gov	EPA WA Manager
Mr. Jim Voit	(513) 487-2867 Voit.Jim@epa.gov	EPA LRPCD QA Manager
Dr. Jorge Santo Domingo	(513) 569-7085 Santodomingo.Jorge@epa.gov	EPA Project Support for PCR Analysis
Dr. Karen Koran	(513) 569-7304 Koran.Karen@epa.gov	Pegasus Project Manager
Mr. Steven Jones	(513) 782-4655 Steve.S.Jones@CBIFederalServices.com	Pegasus Contract QA Manager
Dr. George Sorial	(513) 556-2987 sorialga@ucmail.uc.edu	UC Project Manager
Dr. Pablo Campo-Moreno	(513) 556-3637 campomp@ucmail.uc.edu	UC (Off-Site) WA Leader

Ms. Mobing Zhuang	(513) 556-3640 zhuangmg@mail.uc.edu	UC Project Staff
Ms. Gulizhaer Abulikemo	(513) 556-3640 abulikgr@mail.uc.edu	UC Project Staff

Figure 2.1. Organization Chart



2.2 Project Schedule

The projected schedule and the main activities are shown in Table 2.2.

Table 2.2 Projected Schedule

Activity	Oct-13	Nov-13	Dec-13	Jan-14	Feb-14	Mar-14	Apr-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14
QAPP Preparation												
Experimental Setup												
Sampling												
Sample Analysis												
Report Writing												
Presentation/ Manuscript												

SECTION 3.0

SCIENTIFIC APPROACH

3.1 Biodegradation study

The objectives will be realized by completing the biodegradation studies of ANS dispersed with C9500. Additionally, degradation of ANS and C9500 as only substrates will be also studied. The experiments will be conducted in 250-mL shake flasks rotating at 200 rpm and sterile GP2 seawater will be the matrix used in the study. Both oil and dispersant will be tested at 5 and 25 °C as described in the subsections below. Time-dependent changes in the microbial communities will be determined as well.

The critical parameters for this project will be the measured time series concentrations for oil components (alkanes and aromatics) and main component of C9500. These values will be use in the calculation of degradation rates and extends of these substances. TPH time series have a semi-quantitative value, since these data give a general idea of how the total extractable hydrocarbons evolve with time, hence this parameter is non-critical. PCR results for microbial determinations are considered non-critical as well.

3.2 Microbial Enrichment Studies

The EPA WA Manager will provide two frozen mixed cultures of oil degrading bacteria to the UC WA Leader. These cultures were previously enriched, grown, and frozen in 5-mL vials containing glycerol by EPA's Gulf Ecology Division at Gulf Breeze, FL. The first mixed culture (denoted as cryo) was isolated from the deep sea of the GOM and enriched on South Louisiana crude oil at 5 °C. The second culture (denoted as meso) was isolated from the top 5 m of the GOM and enriched at 25 °C.

3.3 Experimental setup

The experiments outlined in Table 3.1 will be used for the biodegradation studies to be conducted in order to determine the biodegradation rate of ANS crude oil and C9500. In the 5 °C test, eleven (11) sampling events will take place at 0, 2, 4, 8, 12, 16, 24, 32, 40, 48, and 56 days, while ten (10) sampling events are required at 0, 2, 4, 8, 12,16, 24, 32, 40, and 48 days in the 25 °C experiment. For both temperatures, the following treatments will be set up:

1. C9500 alone.
2. ANS previously dispersed by C9500.
3. ANS alone.

Additionally, killed controls (KCs) will be run in triplicates for each treatment to account for abiotic losses, if any. These KCs include all ingredients as the live microcosms (i.e., oil, dispersant, nutrients, and culture) but they will be sterilized with sodium azide (NaN_3) at a concentration of 500 mg/L per flask. The time zero (T_0) replicates for Tests 1, 2, and 3 (see Table 3.1) will serve as the same T_0 replicates for Tests 8, 9, and 10, respectively. In all the cases, 100 mL of autoclaved GP2 synthetic seawater will be used as medium for microbial activity and the orbital shakers will rotate at 200 rpm. Another set of triplicate KCs will also be

set up with an additional component, a protease enzyme, to break down or inhibit any possible enzyme or exoenzyme previously developed by the culture that may hydrolyze the surfactant dioctyl sulfosuccinate (DOSS), the active ingredient in C9500. The contents from these flasks will be periodically collected for LC-MS/MS analysis.

At a given sampling event, triplicate flasks of each treatment will be sacrificed along with those KCs having dispersed oil and C9500. For oil alone treatments, triplicates of the controls will only be sacrificed on the final event. Before sacrificing any microcosms, 2 mL will be withdrawn from each flask containing live culture and preserved by freezing at -80 °C until analysis for determination of microbial communities, which will be performed by EPA personnel at the EPA Andrew W. Breidenbach Environmental Research Center (AWBERC) under the direction of Dr. Jorge Santo Domingo. Samples will be provided to Dr. Santo Domingo by the UC WA Leader or designee for all nucleic acid-based analyses.

Table 3.1 Summary of Experimental Layout for Two Temperatures.

Test	Temperature	Treatment	Sampling Events	Sample Replicates	Total Exp. Units (EU)
1	5 °C	C9500 alone	11	3	33
2	5 °C	ANS dispersed by C9500	11	3	33
3	5 °C	ANS alone	11	3	33
4	5 °C	Killed ANS control	1	3	3
5	5 °C	Killed C9500 control	10	3	30
6	5 °C	Killed ANS + C9500 control	10	3	30
7	5 °C	Killed ANS + C9500 + Protease	5	3	15
				Subtotal EU's	**177**
8	25 °C	C9500 alone, use T_0 from line 1	9	3	27
9	25 °C	ANS dispersed by C9500, use T0 from line 2	9	3	27
10	25 °C	ANS alone, use T_0 from line 3	9	3	27
11	25 °C	Killed ANS control	1	3	3
12	25 °C	Killed C9500 control	9	3	27
13	25 °C	Killed ANS + C9500 control	9	3	27
14	25 °C	Killed ANS + C9500 + Protease	5	3	15
GP2 with ANS + C9500	**GP2 with C9500**	**GP2 with PBC**		**Subtotal EU's**	**153**
15	**12**	**7**		**Total EU's for C9500**	**330**

3.4 Preparation of treatments

Dispersed oil treatment

To make dispersed oil, the following will be carried out with C9500. The procedure involves making batches of dispersed oil in the baffled flasks and compositing them in a glass carboy or bottle. The composite should be continuously mixed to keep the suspension from settling. The sterile GP2 media will be prepared by dissolving the required amounts of mineral salts in

distilled water. This solution will be autoclaved at 120 °C for 30 minutes in batches of 1 liter. The concentration of each salt (expressed in g/L) will be NaCl (21.03), MgCl2 6H2O (9.5), Na2SO4 (3.52), CaCl2.2H2O (1.32), KCl (0.61), KBr (0.088), NaB4O7.10H2O (0.034), SrCl2.6H2O (0.02), NaHCO3 (0.17) FeCl3.6H2O (0.05), Na5P3O10 (0.297) KNO3 (2.89). A single dispersed oil batch will be prepared at room temperature as follows:

- Measure 1,500 mL of GP2 artificial seawater into a 2-L baffled flask.
- Pipette onto the surface 240 µL ANS with a Brinkmann Eppendorf repeater pipette with a 1 mL tip (3 times at the 4th stop).
- Pipette 10.0 µL (1 time at 5th stop) of C9500 with a Brinkmann Eppendorf repeater plus pipette with a 100 µL tip. This will give a dispersant-to-oil (DOR) volumetric ratio of 1:24.
- Cover with the foil used in autoclaving and place on shaker table.
- Start the shaker at a very slow speed to minimize sloshing and ramp it up to approximately 200 rpm.
- Agitate the flask for 20 minutes.
- Let the flask remain stationary for 10 minutes to allow undispersed oil to float to the surface. Drain the dispersed oil seawater suspension through the bottom stopcock into the carboy being careful not to disturb the top layer of undispersed oil.
- Stop draining when the liquid is still covering the opening to the stopcock. There should be about 200 mL left in the flask.
- For the next preparation, add 1,200 mL of GP2 seawater to the remaining 200 mL of dispersed oil, 200 µL ANS oil (2 times at the 5th stop), and 8 µL C9500 (1 time at the 4th stop). Prepare 3 L of this mixture. Add 12 L of sterile GP2 for a total of 15 L.
- From the time the first batch is drained into the carboy, keep the contents of the carboy well mixed.
- Aseptically dispense 100 mL aliquots of the combined dispersed oil seawater mixture into previously silanized shake flasks.
- Add NaN_3 to the dispersed oil killed controls to a final concentration of 500 mg/L (0.5 mL of a 100 mg/mL stock solution per flask) before adding the culture.
- Incubate both live and killed culture flasks at 5 °C after adding 0.5 mL of the cryo culture; those to be incubated at 25 °C will receive 0.5 mL of the meso culture.
- Place the flasks on the orbital shaker in the corresponding constant temperature room (CTR) at 5 or 25 °C. Set the orbital shakers to 200 rpm.

Non-dispersed oil treatment

The final concentration of oil in the samples should be approximately 28 µg/L as in the dispersed oil treatment. Hence, approximately 4 µL ANS will be pipetted (2nd stop with a 100 µL tip) into each shake flask containing 100 mL sterile GP2. The live and KC flasks will be set up in the same manner as the dispersed oil flasks discussed in the previous section.

Dispersant alone treatment

In order to evaluate the biodegradation of C9500 alone, tests will be conducted where the only

substrate is the dispersant. With this purpose, a single batch of sterile GP2 (12 L) will be spiked with approximately 16 µL of C9500, for this volume yields a similar concentration as in the dispersed oil treatment. This batch should be kept well mixed. From this stock, the experimental units (EUs) for both temperatures will be prepared by transferring 100 mL of the mix into each flask. For the 5 °C experiment, the sample inoculum is the cryo culture whereas the meso will be added to the 25 °C EUs. Additionally, the KCs will be sterilized by adding NaN_3 in a final concentration of 500 mg/L per flask.

SECTION 4.0

SAMPLING PROCEDURES

4.1. Sampling labeling

At the beginning of every experiment, each shake flask will be labeled with temperature (5 or 25 ºC), treatment (oil, oil + dispersant, or dispersant), replicate number (1, 2, or 3), and sampling day. Liquid-liquid extracts, dispersant aliquots, and microbial subsamples for later analysis will be labeled in the same way.

4.2. Sampling Procedures

At T_0 and for any given test, three flasks will be sacrificed per treatment. Before samples are sterilized, a 2 mL-subsample will be withdrawn for further microbial characterization (PCR assays and sequencing analysis) to be conducted at EPA AWBERC. Subsequently, bacterial activity will be stopped by adding NaN_3 to every flask in a final concentration of 500 mg/L. KCs (triplicates) will also be removed for the C9500 alone and dispersed oil treatments. In tests where C9500 alone is present, a 20-mL subsample will be taken for the analysis of its main component, DOSS, by LC-MS/MS as described in SOP 6. In the flasks containing non-dispersed SLC, the 100 mL of seawater will be extracted with methylene chloride and the extracts will be analyzed for alkanes and aromatics by GC-MS/MS (SOP 5). Alkanes will include normal and branched aliphatics ranging in carbon number form 10 to 35 as well as pristine and phytane. Aromatics will include 2-, 3-, 4-, and > 4-ring PAH compounds and their alkylated homologs (i.e. C_{0-4} -naphthalenes, C_{0-3} -fluorenes, C_{0-3} -dibenzothiophenes, C_{0-4} -phenanthrenes/anthracenes, C_{0-4} -napthbenzothiophenes, C_{0-2} -pyrenes, C_{0-4} -chrysenes). Finally, in the EUs for dispersed oil with C9500, after withdrawing the 20-mL subsample for DOSS analysis, the remaining seawater will be extracted with DCM and analyzed for oil components. In addition, the total petroleum hydrocarbons (TPH) will be determined in the solvent extracts with a GC equipped with a flame ionization detector (FID) according to the EPA SW-846 Method 8015C. The SOPs for the analysis of oil components and C9500 are included in Appendix B.

At each subsequent sampling event, three more EUs per treatment along with the corresponding controls will be sampled and the different target compounds analyzed.

Table 4.1. Summary of treatment and the corresponding analytical method.

Treatment	Analysis Method [Analytes]
SLC alone	GC-MS/MS [Oil components]
	TPH [Total extractable hydrocarbons]
Dispersed SLC	GC-MS/MS [Oil components]
	TPH [Total extractable hydrocarbons]
	LC-MS/MS [Dispersant component]
Dispersant alone	LC-MS/MS [Dispersant component]

4.2. Sample Containers, Sample Volumes, Preservation, and Hold Times

Sample containers, sample volumes, preservation, and hold times are shown in Table 4.2.

Table 4.2. *Experimental Sampling and Analytical Procedures to Be Used*

Matrix	Measurement	Sampling/	Analysis Method	Sample Container/ Quantity of each Sample to be analyzed	Preservation / Storage	Holding Time
Organic solvent extract	Alkanes and Aromatics	Grab sample	SOP 5 (see Appendix A)	20 mL glass vials / 1 mL	Store @ 4 °C	6 months
	TPH	Grab sample	EPA SW-846 Method 8015C	20 mL glass vials / 1 mL	Store @ 4°C	6 months
Seawater/ACN[1]	DOSS	Grab sample	SOP 6 (see Appendix A)	60 mL glass vials / 1 mL	Store @ 4 °C	5 days
Seawater	PCR	Grab Sample	EPA Procedure 815-B-04-001	2 mL plastic vials / 2 mL	Store @ -80°C	6 months

[1]ACN, acetonitrile.

SECTION 5.0

MEASUREMENT PROCEDURES

5.1 Measurement Methods

The analyses will be completed with the instrumentation described in Table 5.1.

Table 5.1. *Analytical Instruments*

Analyses	Instrument
Oil Components	Agilent 7890A Gas Chromatograph - 7000 MS triple quadrupole; J&W Scientific DB5-MS column (30 m × 0.25 mm, 0.25 μm)
DOSS	Agilent 1200 Liquid Chromatograph - 6410 MS triple quadrupole; Zorbax Eclipse XDB-C18 Rapid Resolution Column (2.1 × 50 mm, 5 μm)
TPH	Agilent 6890 Gas Chromatograph- Flame Ionization Detector; J&W Scientific DB5-MS column (30 m × 0.25 mm, 0.25 μm)

All chemicals involved in these analytical procedures are of ACS reagent grade or equivalent unless otherwise noted.

The measurement methods are summarized in the Table 5.1. The water quality parameters are appropriate for the matrix/analytes being tested as described in the corresponding SOPs included in Appendix A.

All chemicals involved in these analytical procedures are of ACS reagent grade or equivalent unless otherwise noted. Analytical instruments used for this project are listed in Table 5.1.

Should any method call for modifications, the EPA WA Manager will be notified before modifications are made, and the changes will be documented as amendments to this QAPP.

SECTION 6.0

QUALITY METRICS (QA/QC CHECKS)

Instruments/equipment will be maintained in accordance with the EPA ORD Policies and Procedures Manual, Section 13.4 *Minimum Quality Assurance (QA)/Quality Control (QC) Practices for ORD Laboratories Conducting Research* and in accordance with the SOPs and methods specified in Table 4.2. The quality metrics for this study are summarized below.

6.1 Critical and Non-critical Measurements

The critical measurements for this research study include:

1.1_ Oil components, alkanes and aromatics.
1.2_ DOSS.

The non-critical measures are:

1.1_ TPH
1.2_ Molecular Analyses of Microbial communities.

6.2 QA/QC Objectives

Quality Assurance/ Quality Control (QA/QC) objectives are listed in Table 6-1 and explained in Sections 6.2.1- 6.2.4. For the non-critical measurements, the QC checks and criteria are described in the corresponding SOPs included in Appendix A.

6.2.1 Precision

Precision objectives for all the measurements listed in Table 6-1 are presented as relative percent difference (RPD) for duplicate measurements, and as relative standard deviation (RSD, or coefficient of variance) for three or more measurements.

6.2.2 Accuracy

Accuracy objectives are listed in Table 6-1.

6.2.3 Completeness

Completeness is a measure of valid data obtained from a measurement system compared with valid data that were intended to be obtained to evaluate the performance of the system. The required level of completeness for this study is specified at 85 percent. The laboratory staff will ensure adequate completeness by minimizing mistakes that might lead to sample loss or the generation of data that is unusable for any reason.

If the rejected or missing data are randomly disbursed throughout the study and the variability is less than expected, the quality objectives established may still be achieved; however, if the rejected or missing data are not randomly disbursed, even a high degree of completeness may be inadequate for decision-making purposes.

The corrective action for incompleteness due to failure of QC requirements is statistical readjustment of the data and/or other treatment (e.g. retest or reanalysis, etc. considering the point at which the QC failure occurred).

6.2.4 Comparability

Comparability will be achieved by using the same analytical methods and data reduction/reporting methods throughout the entire course of the project including all the preliminary experiments, parametric studies, and molecular biological assays.

6.3 Internal Quality Control

Table 6-1 summarizes the QC checks of the measurements.

Table 6.1. *Summary of Internal QC Checks for all Parameters*

Measurement/ Matrix/(SOP)	QA/QC Check	Frequency	Acceptance Criteria	Corrective Action
Alkanes & Aromatics (critical parameter)/oily seawater/ (SOP 5)	Air/Water Check	Every run	System is leak free	Make system leak free
	Solvent Blank run in SCAN MODE	Following liner change and at the start of a new run.	No contaminants of interest	Rerun fresh solvent until interfering contamination is removed.
	Autotune	Prior to calibration	Manufacture's specifications	Retune instrument
	Initial calibration (5 or 6 point curve)	Every batch and as required	% RSD of RRFs < 25%	Rerun standard curve, change standards
	Second source calibration check	After initial calibration	RRF within 25% of the average calibration FF of the initial calibration for the selected compounds	Rerun calibration check, change standard, Rerun standard curve
	Calibration check	At the beginning and end of a batch, and every 10 samples.	RRF within 25% of the average calibration FF of the initial calibration	Rerun samples since last valid calibration check, rerun standard curve, and/or change

				standards
	Method Blank	Every batch of extracted samples	Concentration of target compounds less than half of the reporting limit (500 µg/L for petroleum hydrocarbons)	Check for target contamination and rerun samples for the batch.
	Reagent blank	Every 10 samples	Concentration of target compounds less than half of the reporting limit (500 µg/L for petroleum hydrocarbons)	Check for target contamination and rerun samples since last valid blank
	Internal Standard	Every sample	50-200% of target in midrange standard	Discard data point, repeat experiment if there are insufficient data points
	Surrogate spike	Every batch	Surrogate spike recovery within 70-100%	Check standards, rerun spike
Total Petroleum Hydrocarbons by GC/FID (non-critical parameter)/ oily seawater/ (see EPA Method 8015C)	Single point calibration of all target compounds	Beginning of each batch used for screening (See Section 1.3 of EPA Method 80415C)	Compare to known LCS run directly after calibration. LCS must meet criteria for standard to be fit for use	Rerun standard curve, remake/run standards
	Blank	Every batch	Absence of target contaminants	Determine source of contamination. Rerun blank and sample batch
	Laboratory Control Sample	Every batch following calibration and at end of batch	75-125% of known second source standard (LCS concentration at approximate sample concentration)	Rerun standard curve, remake/run standards. Rerun samples since last valid LCS

Measurement/ Matrix/(SOP)	**QA/QC Check**	**Frequency**	**Acceptance Criteria**	**Corrective Action**

DOSS LC-MS-MS (critical parameter) /oily seawater/ SOP 6	Initial calibration (5 or 6 point curve DOSS)	Every batch	Linear fit if $r^2 > 0.98$ Quadratic fit if $r^2 > 0.99$	Rerun standard curve, change standards
	Analytes' retention time	Every sample	± 5% of the retention time of the analyte in a midpoint calibration standard	Rerun calibration curve and compare time shift. Rerun samples since last valid retention time
	Second source calibration check	After initial calibration	± 35% deviation from the nominal concentration of the standard	Rerun standard curve, and/or change standards
	Calibration check	At the beginning and end of a batch, every 20 samples and within 24 h after initial calibration	± 35% deviation from the nominal concentration of the standard	Rerun samples since last valid calibration check, rerun standard curve, and/or change standards
	Method Blank	At the beginning and end of a batch and every 20 samples	Less than half of the reporting limit	Check for target contamination and rerun samples since last valid blank
	Surrogate spike	Every sample	Spike recovery within 50-150%	Check standards, rerun spike
PCR (non-critical parameter)/ oily seawater/ EPA Procedure 815-B-04-001	Positive control	(at least) one per PCR	Quantity (µg/µl) within 10% of control	Repeat PCR
	Negative Control	3 negative PCR tubes per PCR	No band on gel or PCR signal in 2 of 3 tubes	Repeat PCR
	Replicate PCR tubes	3-5 replicate PCR tubes per sample	N/A	N/A

SECTION 7.0

DATA ANALYSIS, INTERPRETATION, AND MANAGEMENT

7.1 Data Reporting

All results will be reduced to the appropriate reporting units designated in the SOPs by the analyst performing the test. The reporting units for each analysis are summarized in Table 7.1. Results will be averaged and the mean and the standard deviation or the range, will be calculated.

Table 7.1
Reporting Units

Measurement	Unit
Alkanes and aromatics	µg analyte/µg hopane
TPH	mg/L
DOSS	µg/L
PCR	quantity (gene copies/µL), number base pairs

Calculations will be carried out on a computer and will be checked initially by the analyst for gross error and miscalculation. The calculations and data entered into computer spreadsheets will be checked by a second analyst for accuracy by printing out the calculation or data spreadsheet and checking the calculation by hand or checking each entry of data from the original. These copied spreadsheets will be initialed and dated by the second analyst doing the checking, noting the errors.

7.2 Data Reduction and Validation

Data reduction procedures are described in the related SOPs. Data will be documented in data log sheets in which sample identification, date and analyst name will be recorded. Hard copy raw data shall be archived with documentation for standard preparation and QC data (calibration check standards, analysis duplicates and analysis blanks).

Data reduction will be computerized to reduce the likelihood of transcription and calculation errors. Computerized calculations will be validated prior to use. After data are calculated in the reporting units, they will be transferred to a Microsoft Excel spreadsheet. The UC WA Leader will review the reduced data to check for coherence and consistency. He will also spot check calculations all the way back to raw data logs at random, at least one per analytical method for each part of the study. Detected errors will be corrected and other data in the same set investigated before it is released to the EPA WA Manager.

7.3 Data Summary and Analysis

When the experimental phase of the project has been completed, the analytical data will be analyzed statistically to show how the data will be used to support the project objectives. Table 3.1 describes a completely randomized design where the treatments are the two temperatures, the

eleven and ten sampling days for 5 and 25 °C, and the three different substrates, i.e., dispersed oil, non-dispersed oil, and dispersant alone. The hypotheses are that, for a given temperature, (1) non-dispersed oil should be less amenable to biodegradation than the dispersed and (2) dispersant alone should experiment a faster degradation that the dispersant and crude oil mix. Additionally, it is anticipated that substrates will be removed in a faster way at 25 °C than at a lower temperature, 5 °C.

The analytical data collected from the experiments will be summarized in two ways: (1) by conducting an analyses of variance (ANOVA) to determine if treatment and block differences exist and (2) by non-linear regression of all the data for each site and for each treatment at the end of the experiment to compare overall first-order biodegradation rates among the various treatments. If the ANOVA reveals significant differences ($p < 0.05$), then treatment differences will be evaluated by the protected least significant difference (LSD) mean separation test. These tests and ANOVA will be done on the sum total of hopane-normalized alkanes, the sum total of hopane-normalized aromatics (PAHs), and for the main component of C9500 and JD-2000 as the dependent variables. For the non-linear regressions, an F-test will be performed to compare the slope coefficients statistically among the various treatments. For these calculations, the variance will be estimated from the sum-of-squared residuals from the fit of the first-order model to the average concentrations (over all replicates) for each treatment at each time point.

Appropriate mean comparison tests, such as the protected LSD test, will depend on whether or not the main effects and/or two-way interactions are statistically significant ($p < 0.05$).

7.4 Data Storage

Laboratory records will be maintained in accordance with Section 13.2, Paper Laboratory Records, of the Office of Research and Development (ORD) Policies and Procedures Manual. Controlled access facilities that provide a suitable environment to minimize deterioration, tampering, damage, and loss will be used for the storage of records. Whenever possible, electronic records will be maintained on a secure network server that is backed up on a routine basis. Electronic records that are not maintained on a secure network server will be periodically backed up to a secure second source storage media, transferred to an archive media (e.g., compact discs, optical discs, magnetic tape, or equivalent), or printed. Electronic records that are to be transferred for retention will be transferred to an archive media or printed, as directed by EPA.

SECTION 8.0

REPORTING

8.1 Monthly Reports

Outputs will be in the form of detailed monthly reports describing progress made in each task. Monthly reports will be prepared by the UC WA Leader, reviewed by the Pegasus Project Manager, and submitted to the EPA every month. Distribution of the monthly report to other agencies will be at the discretion of the EPA WA Manager.

8.2 Presentation/Manuscript

After completion of the experimental period (approximately 10 months), the Team will prepare a presentation for the EPAWA Manager to present to the Water Cluster review panel. In addition, the Team will write a manuscript suitable for publishing the results of the research in a peer-reviewed journal article.

SECTION 9.0

REFERENCES

U.S. Environmental Protection Agency, October 2004. Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples. EPA Document No. 815-B-04-001. Office of Water, Cincinnati, Ohio.

University of Cincinnati, June 2011. Category I Quality Assurance Project Plan, Amendment 2, "Biodegradability of Dispersants and Dispersed oil at Two Temperatures", Revision 0, dated June 10, 2011. EPA Contract No. EP-C-11-066. EPA QA ID: L-15045-QP-1-3.

APPENDIX A

STANDARD OPERATING PROCEDURES (SOPs)

SOP 1. Preparation of Surrogate Recovery Standards

Preparation:

Solvents: Dichloromethane (DCM), Optima (Fisher) grade or equivalent

Reagents: D36-Heptadecane (C16) (Cambridge Isotope Laboratories, Andover, MA)
D50-Tetracosane (C20) (Cambridge Isotope Laboratories, Andover, MA)
D66-Dotriacontane (C32) (Cambridge Isotope Laboratories, Andover, MA)
D10-1-Methylnaphthalene (Cambridge Isotope Laboratories, Andover, MA)
D10-Phenanthrene (Cambridge Isotope Laboratories, Andover, MA)
D10-Pyrene (Cambridge Isotope Laboratories, Andover, MA)
5β-Cholestane (coprostane) (Sigma Chemical Co., St. Louis, MO)

Equipment: Micro-spatula
Small beakers
Glass funnel
Analytical balance (0.0001g)
Vials with Teflon-lined caps
Teflon wash bottle with Optima grade DCM
Volumetric flask (250-mL), class A
Pasteur pipettes

Procedure:

1. Using a calibrated analytical balance, weigh 100 mg (0.100 g) of each reagent into separate small beakers.

2. Dissolve the reagents in their beakers by adding 10 mL DCM. Use a Pasteur pipette to transfer the solutions to a single 250-mL volumetric flask.

3. Wash the beakers 3 or 4 times with DCM. Use a Pasteur pipette to transfer each of the washings to the 250-mL volumetric flask.

4. Dilute the solution to volume with DCM.

5. Use a glass stopper to seal the flask and homogenize the solution by inverting the flask 5 or more times. The final concentration of this solution is 400 ng/L for each of the reagents.

6. Transfer the solution into 40-mL storage vials and cap with Teflon-lined caps. Label with the date of preparation, operator, sample names, and concentrations.

7. Weigh every vial and record the weight on the label. This weight is used to monitor possible evaporation during storage.

8. Store these vials at 0 °C or lower.

9. Before using, allow the solution to come to room temperature, and then shake it well.

10. Weigh the vial before using it, and compare the weight with the last weight recorded on the vial.

11. If the weights are consistent, the integrity of the solution can be assumed. If not, investigate the cause.

Quality Control:

Inject 10 µL of the surrogate stock solution into 1 mL DCM. Add 10 µL of internal standard solution (see SOP 2). Analyze this solution by GC/MS using a calibrated method (see SOP 3 and 4). The expected concentration of each of the corresponding surrogate compounds is 4 ± 2 mg/L. If the measured value does not fall within this range, another independently prepared surrogate solution must be prepared and measured. If the measured concentration of the second surrogate solution is within the allowable tolerance range, it can be assumed that the calibration and instrument conditions are acceptable, and the first surrogate solution will be discarded. If the concentration of the second surrogate solution is also out of range, then the problem may be in the calibration or the instrument. The instrument should be cleaned and/or recalibrated until the problem is resolved.

SOP 2. Preparation of Internal Standard Solution

PREPARATION:

Solvents: Dichloromethane (DCM), Optima (Fisher) grade or equivalent

Reagents: D22 n-dencane (D22 N C10)
D34 n-Hexadecane (D34 N C16)
D42 n-Eicosane (D42 N C20)
D62 n-Triacontane (D62 N C30)
D8-Naphthalene
D10-Anthracene
D12-Chrysene
5α-Cholestane (Sigma-Aldrich, Milwaukee, WI)

All reagents but cholestane obtained from Cambridge Isotope Laboratories, Andover, MA.

Equipment: Micro-spatula
Small beakers
Glass funnel
Analytical balance (0.0001g), calibrated and checked for accuracy
Amber vials with Teflon-lined caps, labeled
Teflon wash bottle with DCM
Volumetric flask (200 mL), class A
Pasteur pipettes

PROCEDURE:

1. Using a calibrated analytical balance, weigh 100 mg (0.100 g) of each of the reagents into separate small beakers.

2. Dissolve the reagents in their beakers by adding 10 mL DCM; using a Pasteur pipette, transfer the solutions to a single 200 mL volumetric flask.

3. Wash the beakers 3 or 4 times with DCM; use a Pasteur pipette to transfer each of the washings to the 200-mL volumetric flask.

4. Dilute the solution to volume with DCM.

5. Seal the flask with a glass stopper and homogenize the solution by inverting the flask 5 or more times. The final concentration of this solution is 500 mg/L.

6. Transfer the solution into 40-mL storage vials and cap with Teflon-lined caps.

7. Label with the date of preparation, operator, sample names, and concentrations.

8. Weigh every vial, and record the weight on the label. This weight is used to monitor possible evaporation during storage.

9. Store this solution at 0°C or lower.

10. Before using, allow the solution to come to room temperature, and then shake it well.

11. Weigh the vial before using it, and compare the weight with the last weight recorded on the vial. If the weights are consistent, the integrity of the solution can be assumed. If not, investigate the cause.

QUALITY CONTROL:

Inject 10 µL of the internal standard solution into 1 mL DCM. Analyze this solution by GC/MS/MS. The only peaks that appear should be the ones corresponding to the internal standards.

SOP 3. Preparation of Working Standards, Check Standards, and Oil Standards for GC/MS/MS

The following SOP applies to the preparation of working standards for GC/MS calibration, check standards, and oil standards. These standards will be used to check the consistency of the instrument prior to and during GC-MS/MS operation.

PREPARATION:

Solvent: Dichloromethane (DCM), Optima (Fisher) grade or equivalent
Stock solutions:

1. Oil analysis mix: 44 compounds, 100 mg/L in hexane/DCM (90/10), Absolute Standards, Inc., Hamden, CT, Part # 90311. For composition see Page 11 of this Appendix.

2. Surrogate solution: 400 mg/L in DCM (see SOP 1).

3. PAH mix: 9 compounds, 1,000 mg/L in DCM, Absolute Standards, Inc., Hamden, CT, Part # 90822. For composition see Page 12 of this Appendix.

4. 1,2-Benzodiphenylene sulfide solution, 2,010 mg/L in DCM, Aldrich Chem. Co., Part # 25,512-2, purity 99%. This solution is required because 1,2-Benzodiphenylene sulfide is the only commercially available source of 1,2-Benzodiphenylene, a PAH considered a target analyte in SOP 5.

5. Hopane solution (100 mg/L in isooctane).

6. Internal standard solution, 500 mg/L in DCM (see SOP 2).

7. Alaska North Slope oil (ANS).

Equipment: Glass storage vials with Teflon-lined caps (2-mL and 40-mL capacity).
Volumetric flasks, Class A, 5-mL, 10-mL, and 100-mL.
Glass syringes capable of dispensing 25-500 µL with an accuracy and precision of ± 1%, or equivalent.
Wheaton repetitive dispenser, Model 411 STEP-PETTE.
Teflon wash bottle filled with Optima grade DCM.
Pasteur pipettes.

The volumes of stock solutions required to make a 20-mg/L working standard are listed in Table 1.

Stock Standards	A	B	C	D	E	
Working standards concentration, mg/L	Oil Analysis Mix (44 compounds, 100 mg/L) µL	Aromatics Mix (9 compounds, 1000 mg/L) µL	1,2-Benzo-diphenylene sulfide (2010 mg/L) µL	Surrogate solution (100 mg/L) µL	Hopane solution (100 mg/L) mL	Volumetric Flask Volume mL
STD 20 (hopane 5)	1000	100	50	250	250	5

Procedure for Working Standards and Check Standards

1. Label a 5-mL volumetric flask as STD20
2. Add 1-2 mL of DCM to the volumetric flask.
3. With glass syringes, add the appropriate volume of stock solution A (as listed in Table 1 to the flasks labeled STD20.
4. Wash the walls of the inner neck of the flasks with several drops of DCM to rinse off the residue of the stock solution into the flasks.
5. Repeat step 3 and step 4 to dispense stock solutions B-E.
6. Dilute to volume with DCM for all the above flasks, seal with glass stoppers, and invert several times to homogenize the solutions.
7. Transfer the solution into 2-mL storage vials, and cap with Teflon-lined caps.
8. Label with date of preparation, operator, sample names, and concentrations.
9. Weigh each storage vial, and record the weight on the label. This weight is used to monitor possible evaporation during storage.
10. Store this solution at 0°C or below.
11. Before using, allow the solution to come to room temperature, and shake it well.
12. Weigh the vial before opening, and compare the weight with the last weight recorded on the vial. If the weights are consistent, the integrity of the solution can be assumed. If not, investigate the cause.

Procedure for Making Oil Standards

In a 100 mL volumetric flask, weigh 0.500 g of the standard ANS, add 1 mL of surrogate

solution (see SOP 1), and bring to volume with DCM. Add 1 mL of internal standard solution (see SOP 2). Follow steps 7 through 12 above, substituting 2-mL storage vials with 40-mL vials.

QUALITY CONTROL

Prepare 5 working standards by making consecutive dilutions of the 20-mg/L stock prepared. Spike the solutions with internal standard (IS) mix (SOP) so that the final IS concentrations is in the middle of the desired calibration range. Analyze the working standards as described in SOP 4 on a tuned GC-MS/MS. Use the MAssHunter software to calculate the average RRF and the relative standard deviation (RSD) of the RRFs for each analyte over the six concentrations. The relative response factor is defined as follows:

$$RRF = \frac{\text{area analyte}}{\text{area internal standard}} \times \frac{\text{concentration internal standard}}{\text{concentration analyte}} \qquad (1)$$

The RSD of the RRFs for all analytes should be 25% or less (EPA Method 525.2).

SOP 4. Extraction of Oil from Aqueous Samples

SCOPE AND APPLICATION: This method is to be used for the extraction of crude oil and dispersed crude oil components, both alkanes and polycyclic aromatic hydrocarbons (PAH), from aqueous samples. The extracts are analyzed by gas chromatography/mass spectrometry (GC/MS) using SOP 5.

METHOD SUMMARY: A separatory funnel liquid-liquid extraction will be used to separate the oil components from seawater with methylene chloride (DCM). The extracted solvent will be concentrated and solvent exchanged into hexane ready for GC/MS analysis.

INTERFERENCES AND POTENTIAL PROBLEMS. From SW-846 Method 3500C: "Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Plastics, in particular, must be avoided because phthalates are commonly used as plasticizers and are easily extracted from plastic materials." Also, from our experience, oil will sorb onto rubber and plastic materials causing a loss of product so O rings, gaskets, and seals should be constructed of PTFE.

HEALTH, SAFETY, AND ENVIRONMENTAL COMPLIANCE: Gloves, lab coats, safety glasses, and fume hoods are required. Samples must be collected and disposed of through appropriate channels. The solvents may not be disposed of in a sink.

SAMPLE PRESERVATION, CONTAINERS, AND STORAGE: Upon receipt of samples, at a specific time point in the experiment, or the conclusion of the experiment, water samples should be refrigerated at 4 °C for analysis within 2 weeks. All containers should be constructed of glass, PTFE, or other DCM-resistant material. The solvent extracts should be stored in tightly sealed glass containers (serum bottles or vials) with solvent-resistant septa. They should be refrigerated at 4 °C and may be stored for up to 6 months.

APPARATUS AND MATERIALS:

1. Separatory funnels (250 mL).
2. Turbo Vap II concentration workstation by Zymark (Hopkinton, MA, USA).
3. Miscellaneous glassware, glass funnels, glass wool, Pasteur pipettes, bulbs, vials, or serum bottles with polytetrafluoroethylene (PTFE)-lined screw or crimp tops.

REAGENTS:

1. Unless otherwise noted, it is assumed that ACS reagent grade chemicals or their equivalent are used.
2. Extraction solvent: dichloromethane (DCM), CH_2Cl_2.
3. Exchange solvent: hexane, C_6H_{12}.
4. Sodium sulfate (granular, anhydrous), Na_2SO_4.
5. Tank of compressed nitrogen gas for concentration by nitrogen Turbo Vap II.
6. Surrogate standard spiking as described in SOP 1.

PROCEDURE:

1. At the specified sampling time or the end of the experiment, each experimental unit will

be removed from the experimental setup. If required, take subsamples for those analytical methods that do not require liquid-liquid extraction; after that add one mL (or a well-known volume so after concentration the recovered concentration is in the middle range of the calibration curve) of the surrogate standard spiking solution onto the sample in the flask.

2. Quantitatively transfer the sample from the shake flask to the separatory funnel. Use 60 mL of DCM to rinse the flask and transfer this rinse solvent to the separatory funnel.
3. Seal and shake the separatory funnel vigorously for 1-2 minutes with periodic venting to release excess pressure.
4. Allow the organic layer to separate from the water for a minimum of 10 minutes. Collect the solvent extract in an Erlenmeyer flask.
5. Repeat the extraction two more times using fresh portions of solvent. Combine the three solvent extracts.
6. Dry the extracts by passing it through a funnel containing a glass wool plug and approximately 10 mL of Na_2SO_4. Transfer the dried extract to the TurboVap II workstation and concentrate to 1-2 mL. Perform a solvent exchange into hexane to remove asphaltenes and quantitatively transfer to a 25-mL volumetric glass. Once the final volume is adjusted, the final extracts can be stored in glass vials sealed with screw caps and Teflon liners.
7. The hexane extracts are ready for the GC/MS injection and analysis (SOP 5).

Method Blank

For every batch of samples a method blank, consisting of 100 mL of GP2 spiked with the surrogate mix will be extracted in the same way that regular samples are treated. Only compounds used as internal standards and surrogates should be detected in these blanks.

SOP 5. GC-MS/MS Method for the Analysis of Crude Oil Samples

INSTRUMENT SPECIFICATIONS:

The instrument used in this procedure is an Agilent 7890A GC coupled with an Agielnt 7000 mass selective detector triple quadrupole and an Agilent 7693 series auto sampler. It is equipped with a DB-5 capillary column by J&W Scientific (30 m, 0.25 mm I.D., and 0.25 μm film thickness), and a split/splitless injection port operating in the splitless mode. Data acquisition occurs either in the MRM (Multi reaction monitoring) mode for quantitative analysis or in SCAN mode for qualitative analysis. In MRM mode, the dwell time of each ion is set to be 10 milliseconds. The number of ions in each ion group should be constant, yielding the same scan rate for each group.

The instrumental conditions for crude oil analysis are summarized in Table 1. Only ultra-high purity helium (99.999% pure) should be used as a carrier gas. A moisture trap, an oxygen trap, and an organic trap are connected in series to the carrier gas line before it enters the column.

TABLE 1--INSTRUMENTAL CONDITIONS FOR CRUDE OIL ANALYSIS

Instrument	Agilent 7890A Gas Chromatograph - 7000 MS triple quadrupole
Column	J&W Scientific DB5-MS column (30 m × 0.25 mm, 0.25 μm)
Carrier Gas	Helium, ultra-high purity grade (99.999%)
Inlet Temperature	300°C
Transfer Line (detector) Temperature	300°C
Oven Temperature Program	35°C for 2 minutes to 310°C at 10°C/minute for 5 min
Flow Rate	Constant flow at 1mL/min. Linear velocity: 36.2 cm/sec.
Injection Volume	1 μL
Split/splitless Mode	Splitless
Total Run Time	34.5 minutes

PROCEDURE:

Preparing the Instrument:

1. Lower the injection port temperature and the oven temperature to 50°C or less to avoid oxidation of the column.
2. Replace the liner with a clean, deactivated liner. Do not touch the liner with bare fingers.
3. Return the injection port and oven to the appropriate temperatures.
4. Wait a few minutes for the temperatures to equilibrate before using the instrument.

Tuning the MSD:

Perform an air/water check. The value reported for the relative abundance of water (*m/z* 18), nitrogen (*m/z* 28), oxygen (*m/z* 32), or carbon dioxide (*m/z* 44) should be less than 10% for a leak-free system.

Tune the MSD using the Autotune program according to the manufacturer's specifications.

Maintaining a logbook:

Maintain an instrument logbook, and make entries for each use. Include the following information in the logbook: operator name, helium cylinder tank pressure, and outlet pressure, vacuum gauge reading, any maintenance performed on the instrument (such as changing the injection port liner, gold seal, guard column, source cleaning), sequence name, data path, samples in order of injection, method information, GC column number, and the Standard Auto Tune report.

Running a Solvent Blank:

Following a liner change or at the start of a new run, an injection of a pure solvent should be run to confirm that the system is free of excessive or interfering contamination. The solvent should be analyzed in SCAN mode, using the same temperature program that is used for sample analysis. If contamination is present, additional samples of fresh solvent should be analyzed until the interfering contamination is removed.

Calibrating With a Multiple-Point Calibration Curve:

A five- or six-point calibration curve is obtained by running five or six working standards (see SOP 3) on the tuned GC-MS/MS instrument. The relative response factor (RRF) for each compound is calculated relative to its corresponding deuterated internal standard as indicated in Table 19. The relative standard deviation (RSD) of the RRFs for each compound should be less than 25%. Running an independently prepared check standard immediately after the calibration standards can validate the accuracy of the calibration curve.

Running Samples:

Once the calibration curve has been validated, samples can be analyzed. Dispense 1,000 µL of sample extract into labeled auto-sampler vials. Add 10 µL of the internal standard solution (see SOP 2) to the extract using a syringe or a positive displacement pipettor. Enter the sample ID into the sequence log table, and run the samples after the standards. Run a check standard every 10 samples to ensure the consistency of the instrument. The RRF for each compound in the check standard should be within 25% of the average RRF obtained in the initial calibration.

Quantification:

Once a calibration table has been generated, each data file is quantified using the

"Calculate and Generate" function in the MassHunter software. Individual peak integration is reviewed manually to ensure proper baseline integration. The quantification of a compound is based on the peak area of the product ion indicated in Table 3 below.

TABLE 3--TARGET COMPOUND LIST

Compound Name	Precursor Ion	Product Ion	Reference Compound for Response Factor	Internal Standard for Quantitation
N D22 C10	66	66	Internal Standard	D22 N C10
n C10	57	57	n C10	
n C11	57	57	n C11	
n C12	57	57	n C12	
n C13	57	57	n C13	
n C14	57	57	n C14	
n C15	57	57	n C15	
N D34 C16	66	66	Internal Standard	D34 N C16
n C16	57	57	n C16	
N D34 C17	66	66	N D34 C17	
n C17	57	57	n C17	
Pristane	57	57	Pristane	
n C18	57	57	n C18	
Phytane	57	57	Phytane	
n C19	57	57	n C19	
N D42 C20	66	66	Internal Standard	D42 N C20
n C20	57	57	n C20	
n C21	57	57	n C21	
n C22	57	57	n C22	
n C23	57	57	n C23	
N D50 C 24	66	66	N D50 C 24	
n C24	57	57	n C24	
n C25	57	57	n C25	
n C26	57	57	n C26	
n C27	57	57	n C27	
n C28	57	57	n C28	
n C29	57	57	n C29	
N D62 C30	66	66	Internal Standard	D62 n C30
n C30	57	57	n C30	
n C31	57	57	n C31	

N D66 C32	57	57	N D66 C32	
n C32	57	57	n C32	
n C33	57	57	n C33	
n C34	57	57	n C34	
n C35	57	57	n C35	
D8 Naphthalene	136	108	Internal Standard	D8 Naphthalene
Naphthalene	128	102	Naphthalene	
D10 1Methylnaphthalene	152	150	D10 1-Methylnaphthalene	
C1 Naphthalene	142	141	C1 Naphthalene	
C2 Naphthalene	156	141	C2 Naphthalene	
C3 Naphthalene	170	155	C3 Naphthalene	
C4 Naphthalene	184	153	C3 Naphthalene	
D10 Anthracene	188	160	Internal Standard	D10 Anthracene
D10 Phenanthrene	188	184	D10 Phenanthrene	
Phenanthrene	178	176	Phenanthrene	
C1 Phenanthrene	192	189	C1 Phenanthrene	
C2 Phenanthrene	206	189	C2 Phenanthrene	
C3 Phenanthrene	220	203	C2 Phenanthrene	
C4 Phenanthrene	234	189	C2 Phenanthrene	
Fluorene	166	165	Fluorene	
C1 Fluorene	165	163	Fluorene	
C2 Fluorene	194	179	Fluorene	
C3 Fluorene	208	193	Fluorene	
Dibenzothiophene	184	139	Dibenzothiophene	
C1 Dibenzothiophene	198	197	Dibenzothiophene	
C2 Dibenzothiophene	212	211	Dibenzothiophene	
C3 Dibenzothiophene	226	225	Dibenzothiophene	
Naphthobenzothiophene	234	202	Naphthobenzothiophene	
C1 Naphthobenzothiophene	248	215	Naphthobenzothiophene	
C2 Naphthobenzothiophene	262	245	Naphthobenzothiophene	
C3 Naphthobenzothiophene	276	274	Naphthobenzothiophene	
Fluoranthene	202	202	Fluoranthene	
D10 Pyrene	212	210	D10 Pyrene	
Pyrene	202	200	Pyrene	
C1 Pyrene	216	215	Pyrene	
C2 Pyrene	230	221	Pyrene	
D12 Chrysene	240	236	D12 Chrysene	D12 Chrysene
Chrysene	228	226	Chrysene	
C1 Chrysene	242	239	Chrysene	
C2 Chrysene	256	239	Chrysene	

C3 Chrysene	270	226	Chrysene	
C4 Chrysene	284	253	Chrysene	
5α-Cholestane	217	95	Internal Standard	5α- Cholestane
5β-Cholestane (Coprostane)	217	95	5β-Cholestane	
Hopane	191	95	Hopane	

Equation 1 is used to calculate the concentration of analytes in units of µg/l:

$$Concentration\ of\ analyte\ (\mu g / L) = \frac{A_{analyte} \times C_{istd}}{A_{istd} \times RRF} \qquad (1)$$

where $A_{analyte}$ is the peak area of the analyte,
C_{istd} is the concentration of the internal standard,
A_{istd} is the area of the internal standard,
RRF is the relative response factor, and

Some analytes are not commercially available or are extremely expensive. In such cases, the RRFs of other compounds (usually the non-alkylated parent or nearest neighbor compound) are used to quantify those analytes. These results will be reported as estimated. For example, the RRF of C3-naphthalene is used to calculate the concentrations of C3- and C4-naphthalenes. See Table 3 for details. The quantification of these alkylated PAHs is relative because it is assumed that the molecular ions of the alkylated PAHs have the same RRFs as the parent compound ions. Nevertheless, these relative concentrations are useful for monitoring the fate of these compounds during the course of any study, as long as their concentrations are measured in a consistent way throughout the study.

Concentration calculations for all target compounds are performed using MassHunter software. Data for each sample can be printed directly using a customized report template. Data can also be automatically entered into a spreadsheet within the MassHunter software. This

spreadsheet file can be opened directly in Excel and modified to perform any additional calculations needed.

QUALITY ASSURANCE QUALITY CONTROL:

The following criteria must be met before any samples are analyzed:

1. Air/water check indicates the system is leak free.
2. AutoTune pass all criteria.
3. Solvent blank scan indicates the GC-MS/MS system is free of interfering contamination.
4. Calibration curve is validated using an independently prepared check standard with a 75-125% recovery criterion.
5. A control chart of a standard oil analysis should be prepared and monitored. Concentrations of the analytes in the control chart should be no more than 25% different from their historical averages.
6. The reporting limit for the petroleum hydrocarbons analyzed by this method is 500 µg/L.

Relative response factors for analytes in the check standards inserted between every 10 samples should be no more than 25 percent different from the average RRF of those same analytes in the calibration curve. Peak shapes should be symmetrical (except for nC10, nC11, nC12, nC13, nC14, and naphthalene, which tail in this method). NC17/pristane, nC18/phytane, and phenanthrene/anthracene should be baseline separated.

SOP 6. LC-MS/MS Method for the Analysis of Dioctyl Sulfosuccinate in Sea Water[1]

1. Scope

1.1 This procedure details dioctyl sulfosuccinate (DOSS) determination in water by direct injection using liquid chromatography (LC) and detection with tandem mass spectrometry (MS/MS). This analyte is qualitatively and quantitatively determined by this method. This method adheres to single reaction monitoring (SRM) mass spectrometry.

1.2 The Detection Verification Level (DVL) and Reporting Range for DOSS are listed in Table 1.

1.2.1 The DVL is required to be at a concentration at least 3 times below the Reporting Limit (RL) and have a signal/noise ratio greater than 3:1. Figure 1 and 2 display the signal/noise ratio of the single reaction monitoring (SRM) transition.

1.2.2 The reporting limit is the concentration of the Level 1 calibration standard as shown in Table 4 for DOSS, taking into account the 50% sample preparation dilution factor.

TABLE 1. Detection Verification Level and Reporting Range

Analyte	DVL (µg/L)	Reporting Range (µg/L)
DOSS	3	10-200

1 This draft analytical procedure was developed by US EPA Region 5 Chicago Regional Laboratory (CRL) in collaboration with the Region 6 Houston Laboratory. It was first developed by CRL, July 12, 2010, and is open to improvement and discussion.

1.3 *This procedure does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 ASTM Standards:

D 1129 Terminology Relating to Water
D 1193 Specification for Reagent Water

2.2 Other Documents

EPA publication SW-846, entitled *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*

3. Terminology

3.1 Definitions:

3.1.1 Detection Verification Level, DVL, n– a concentration that has a signal/noise ratio greater than 3:1 and is at least 3 times below the Reporting Limit (RL).

3.1.2 Reporting Limit, RL, n– the concentration of the lowest-level calibration standard used for quantification.

3.2 Abbreviations:

3.2.1 CH_3CN- acetonitrile

3.2.2 ESI- Electrospray ionization

3.2.3 DOSS- dioctyl sulfosuccinate (CAS # 577-11-7)

3.2.4 LC/MS- liquid chromatography mass spectrometry

3.2.5 ppb– parts per billion, µg/L

3.2.6 mM– millimolar, 1 x 10^{-3} moles/L

3.2.7 NA– no addition

3.2.8 NH_4CO_2H- ammonium formate

3.2.9 ND– non-detect

4. Summary of Procedure

4.1 One surrogate is considered, DOSS-D34. Deuterated DOSS surrogate (DOSS-D34) recovery data are included in this SOP.

4.2 This is a performance based method, and modifications are allowed to improve performance.

4.3 In the lab, the entire collected 20-mL sample is spiked with surrogate, ammonium formate buffer solution and brought to a volume of 40 mL with acetonitrile. This prepared sample is then filtered using a syringe driven filter unit, and analyzed by LC/MS/MS. If visible oil is present, the prepared sample is allowed to settle resulting in an oil layer at the top of the 40 mL solution. A portion of the aqueous (bottom) layer is filtered, leaving the oil layer behind, through a syringe-driven filter assembly and analyzed by LC/MS/MS.

4.4 DOSS and its surrogate are identified by retention time and one SRM transition. The target analyte and surrogate are quantitated using the SRM transitions. The final report issued for each sample lists the concentration of DOSS and the surrogate recovery.

5. Significance and Use

5.1 DOSS has been identified as a possible ingredient in dispersant agents used to treat oil.

5.2 This method has been investigated for use with reagent and sea water for DOSS.

6. Interferences

6.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other apparatuses that produce discrete artifacts or elevated baselines. All of these materials are demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as samples.

6.2 All glassware is washed in hot water with detergent and rinsed in hot water followed by distilled water. The glassware is then dried and heated in an oven at 250°C for 15 to 30 minutes. All glassware is subsequently cleaned with methanol and/or 50% acetonitrile/50% water.

6.3 System contamination and surface binding are problematic as DOSS is a surface active compound. Notable DOSS is associated with the surface of glass containers after one hour. It is important to use sample containers and glassware (treatment preparation glassware, shake flasks, sampling, and analytical glassware) treated with Sylon CT or similar deactivating agent. Thorough rinsing of all lab equipment is necessary to reduce contamination. Carefully analyze blanks to ensure that the method minimizes DOSS carryover.

6.4 All reagents and solvents should be pesticide residue purity or higher to minimize interference problems.

6.5 Matrix interferences may be caused by contaminants in the sample. The extent of matrix interferences can vary considerably from sample source depending on variations of the sample matrix.

6.6 Graduated cylinder- a 50 mL cylinder was used to measure the volume of the sample and total volume. An appropriate cylinder for different sample volumes should be used to accurately measure the sample and prepared sample volumes.

7. Apparatus

7.1 LC/MS/MS System

7.1.1 Liquid Chromatography System- A complete LC system is needed in order to analyze samples. Any system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes, and requirements of the standard may be used.

7.1.2 Analytical Column- Zorbax Eclipse XBD C18 analytical column 2.1 x 50 mm, 3.5 micron particle size (part no.971700-902). Any column that achieves adequate resolution may be used. The retention times and order of elution may change depending on the column used and need to be monitored.

7.1.3 Tandem Mass Spectrometer System- A MS/MS system capable of MRM analysis. Any system that is capable of performing at the requirements in this standard may be used.

7.2 Filtration Device

7.2.1 Hypodermic syringe- A Lock Tip Glass Syringe capable of holding a Millex® HV Syringe Driven Filter Unit PVDF 0.22 µm or similar may be used.

7.2.1.1 A Lock Tip Glass Syringe was used in this test method.

7.2.2 Filter– Millex® HV Syringe Driven Filter Unit PVDF 0.22 µm (Millipore Corporation, Catalog # SLGV033NS) or similar may be used.

7.2.3 Glass Pipette– Small volume pipette for sample container rinses and sample transfer.

8. Reagents and Materials

8.1 Purity of Reagents– High Performance Liquid Chromatography (HPLC) pesticide residue analysis and spectrophotometry grade chemicals shall be used in all tests. Unless indicated otherwise, it is intended that all reagents shall conform to the Committee on Analytical Reagents of the American Chemical Society. Other reagent grades may be used provided they are first determined to be of sufficiently high purity to permit their use without affecting the accuracy of the measurements.

8.2 Purity of Water– Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type 1 of Specification D 1193. It must be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with the analysis.

8.3 Gases– Ultrapure nitrogen.

8.4 Acetonitrile (CH_3CN, CAS # 75-05-8).

8.5 Methanol (CAS # 67-56-1).

8.6 Ammonium Formate (NH_4CO_2H, CAS # 540-69-2).

8.7 2-Propanol (CAS # 67-63-0).

8.8 Dioctyl sulfosuccinate sodium salt (DOSS) (CAS # 577-11-7).

8.9 Deuterated dioctyl sulfosuccinate sodium salt (DOSS-D34, - bis(2-ethylhexyl-D17) sulfosuccinate sodium salt)

9. Hazards

9.1 Normal laboratory safety applies to this method. Analysts should wear safety glasses, gloves, and lab coats when working in the lab. Analysts should review the Material Safety Data Sheets (MSDS) for all reagents used in this method.

10. Sampling

10.1 *Sampling and Preservation*– Grab samples should be collected in 20 mL pre-cleaned and silanized glass vials with Teflon® lined septa caps demonstrated to be free of interferences. This test method is based on a 20 mL sample size per analysis. Store samples at 0°C from sample collection to sample preparation. Analyze the sample within 5 days of collection. After 7 and 10 days of storage at 0°C DOSS recoveries notably decreased (Table 12). Oil in water absorbs DOSS from water and affects surrogate analysis (Table 11). Avoid oil in samples if possible. Samples that have more than one phase may be encountered. Samples may be prepared for analysis using one or a multiple of the following options:

10.1.1 *Whole Sample*: For a 20* mL sample, add 0.2 mL of 20 ppm DOSS surrogate to the sample in the sample container. Cap the container and mix to insure homogeneity. Transfer the contents of the sample container to a graduated cylinder and record the sample volume. Subsequently, add 0.4 mL of 500 mM ammonium formate to the graduated cylinder. Rinse the sample collection vial twice with 6 to 8 mLs of acetonitrile, which is added to the prepared sample to compose ~ 50% acetonitrile solution to ensure quantitative sample transfer. Bring the prepared sample volume to 40 mL using acetonitrile, and then mix thoroughly. If different

sample sizes are used, spiking solution, ammonium formate, and acetronitrile volume shall be adjusted proportionally. The sample must be thoroughly mixed and then allowed to settle. A portion of the aqueous fraction (bottom) should be transferred to a glass syringe for filtration. Filter the sample, using a 0.22 µm PVDF filter, into an autosampler vial or another glass vial and then transfer to an autosampler vial.

10.1.2 Water Subsample*: Collect a 10 mL subsample of the water fraction (bottom) using a needle and a glass syringe. To reduce the oil exposure, invert the vial and tap gently to cause the oil move away from the septum. Insert needle through septum and collect 10 mL of the water layer; place the aliquot removed in a graduated cylinder. Then add 100 µL of 20 ppm DOSS surrogate to the sample in the graduated cylinder. Subsequently, add 0.2 mL of 500 mM ammonium formate to the graduated cylinder. Add 10 mL acetonitrile to the prepared sample to compose ~ 50% acetonitrile solution to ensure quantitative prepared sample transfer. If different sample sizes are used, spiking solution, ammonium formate, and acetronitrile volume shall be adjusted proportionally. Filter the sample, using a 0.22 µm PVDF filter, into an autosampler vial or another glass vial and then transfer to an autosampler vial.

10.1.3 Oil Subsample*: Add 1.5 mL of HPLC grade water to graduated cylinder. Collect a 0.5 mL subsample of oil (top) and place in a graduated cylinder. Then add 20 µL of 20 ppm surrogate to the sample in the graduated cylinder. Subsequently, add 40 µL of 500 mM ammonium formate to the graduated cylinder. Add 2 mL acetonitrile to the prepared sample to compose ~ 50% acetonitrile solution to ensure quantitative prepared sample transfer. If

different sample sizes are used, spiking solution, ammonium formate, and acetonitrile volume shall be adjusted proportionally. The sample should be thoroughly mixed and then allowed to settle. A portion of the aqueous fraction (bottom) should be transferred to a glass syringe for filtration. Filter the sample using a 0.22 µm PVDF filter into another vial (filtered-prepared sample).

*Note- Subsamples of water and oil fractions quantitate only the DOSS in the subsample, the DOSS concentration of subsamples may underestimate the sample DOSS concentration as a result of partitioning (Table 10) and surface binding. DOSS surrogate added to the subsample rather than the received sample and container does not indicate DOSS recovery from the total sample. Additionally, oil in samples resulted in low DOSS recovery, but resulted in higher DOSS recoveries than subsamples (Table 11).

11. Preparation of LC/MS/MS

11.1 LC Chromatograph Operating Conditions

11.1.1 The following specifications were developed for a 6410 Triple Quad Mass equipped with a 1200 Binary HPLC pump, auto sampler, column compartment, and online degasser by Agilent (Palo Alto, CA, USA). Injection volumes of all calibration standards and samples are made at 15 µL volume using a full loop injection. "Full loop" mode is the preferred technique when performing quantitative analyses. Multiple blank samples should be analyzed at the beginning of a run to remove residual DOSS from the system. The first sample analyzed after the calibration curve is a blank to ensure there is minimal DOSS carry-over. The gradient

conditions for the liquid chromatograph are shown in Table 2A. The eluent composition is A: 99% water +1% ACN with 0.1% formic acid and B: 99% ACN +1% water with 0.1% formic acid. Divert the column flow away from the electrospray source for 0 to 2.6 minutes after injection. Flow diversion to waste may be done using the mass spectrometer divert valve, divert tubing configurations vary from manual injection. Test the divert valve configuration and operation prior to analysis. Seawater samples contain nonvolatile salts; the elution from injection to 2.6 minutes after injection is diverted to waste in order to prevent mass spectrometer source contamination. If there is carry-over from one sample to another(greater than half the reporting limit) the initial percentage of acetonitrile should be raised to try and remove the carry-over. This will shorten the elution time of DOSS; therefore, it is necessary reduce the flow diversion and adjust the MRM time. Increasing the initial acetonitrile gradient concentration does not increase the DVL or reporting limit.

TABLE 2A. Gradient Conditions for DOSS Liquid Chromatography

Time (min)	Flow (mL/Min)	%A (water with 0.1% Formic)	%B (ACN with 0.1% Formic)
0-1	0.4	98	2
4	0.4	60	38
7	0.4	20	80
12	0.4	2	98
12.1	0.4	98	2

11.2 LC Sample Manager Conditions:

11.2.1 Wash Solvents– Weak wash is 4.0 mL of 50% water/50% acetonitrile. Strong wash is 2.0 mL of 60% acetonitrile/40% 2-propanol. The strong wash solvent is needed to eliminate

carry-over between injections of DOSS samples. The weak wash is used to remove the strong wash solvent. Instrument manufacturer specifications should be followed in order to eliminate sample carry-over.

11.2.2 Temperatures– Column, 35°C; Sample compartment, 15°C.

11.3 Mass Spectrometer Parameters:

11.3.1 To acquire the maximum number of data points per SRM channel while maintaining adequate sensitivity, the tune parameters may be optimized according to the instrument specifications. Each peak requires at least 10 scans per peak for adequate quantitation. This procedure will contain one surrogate, which is isotopically labeled DOSS, and DOSS which are in one MRM acquisition function to optimize sensitivity. Due to the low pKa of the sulfonate and to avoid bias associated with adducts, DOSS analysis was performed using electrospray negative ionization. Variable parameters regarding retention times, SRM transitions, and cone and collision energies are shown in Table 3. Mass spectrometer parameters used in the development of this method are listed below:

Parameter	**Settings**
Scan Mode	negative
Capillary Voltage	4.0 kV
To Waste	Until 2.6 min
Segments	3
Gas Temperature	350 °C
Gas Flow	6 L/min
Nebulizer	15 psi

TABLE 3. Retention Times, MRM transitions, and DOSS-Specific Mass Spectrometer Parameters

Compound	**Ion**	**Segment**	**Prec. ion**	**Prod. ion**	**Frag (V)**	**CE (V)**	**Multiplier (V)**
DOSS	M-	1	421	81	188	30	600

	Na						
DOSS-D34	M-Na	1	445.4	81	188	30	600

12. 12. Calibration and Standardization

12.1 The mass spectrometer must be calibrated per manufacturer specifications before analysis. In order to obtain accurate analytical values through using this test method within the confidence limits, the following procedures must be followed when performing the test method. (Prepare all solutions in the lab using Class A volumetric glassware.)

12.2 Calibration and Standardization– To calibrate the instrument, analyze six DOSS and surrogate calibration standards; the calibration standards nominal concentrations are detailed in Table 4. A calibration solution is prepared from standard materials or certified solutions. Level 6 calibration solution containing the DOSS and surrogate is prepared and aliquots of that solution are diluted to prepare Levels 1 through 5 and the DVL. The following steps will produce standards with the concentration values shown in Table 4. The analyst is responsible for recording initial component weight, calculating dilutions and preparing appropriate solutions.

12.2.1 Prepare Level 6 calibration stock standard at 200 ppb by adding to a 10 mL volumetric flask individual solutions of the following: 100 µL of DOSS and DOSS (isotopic-labeled), each at 20 ppm in 50% water/50% acetonitrile and dilute to 10 mL with a solution of 5 millimolar ammonium formate in 50% water/50% acetonitrile. The preparation of the stock standard can be accomplished using different volumes and concentrations of stock solutions as is accustomed in the individual laboratory. Depending on the prepared stock concentrations, the solubility at that concentration will have to be ensured.

12.2.2 Aliquots of Level 6 calibration stock standard are then diluted with 5 millimolar acetonitrile in 50% water/50% acetonitrile to prepare the desired calibration levels in 2 mL amber glass autosampler vials. The calibration vials must be used within 24 hours to ensure optimum results. Stock calibration standards are routinely replaced every 7 days if not previously discarded for quality control failure. Calibration standards are not filtered.

12.2.3 Inject each standard and obtain its chromatogram. An external calibration technique is used to monitor the SRM transitions of each analyte. Calibration software is utilized to conduct the quantitation of the target analytes and surrogates using the SRM transition. The quantitation method is set as an external calibration using the peak areas in ppb units. Concentrations may be calculated using the data system software to generate linear regression or quadratic calibration curves. Forcing the calibration curve through the origin is not recommended.

12.2.4 Linear calibration may be used if the coefficient of determination, r^2, is > 0.98 for the analyte (Figure 3). The point of origin is excluded and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards other than the high or low point causes the r^2 of the curve to be < 0.98, this point must be re-injected or a new calibration curve must be regenerated. If any calibration level result is excluded, minimally a five-point curve is acceptable but the reporting range must be modified to reflect this change.

12.2.5 Quadratic calibration may be used if the coefficient of determination, r^2, is > 0.99 for the analyte. The point of origin is excluded, and a fit weighting of 1/X is used in order to give

more emphasis to the lower concentrations (Figure 4). If one of the calibration standards causes the curve to be < 0.99, this point must be re-injected or a new calibration curve must be regenerated. At least six calibration points are required for quadratic regression. Each calibration point used to generate the curve must have a calculated percent deviation less than 25% between the nominal concentration and the regression calculated result.

12.2.6 The retention time window of the SRM transitions must be within 5% of the retention time of the analyte in a midpoint calibration standard. If this is not the case, re-analyze the calibration curve to determine if there was a shift in retention time during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample, refer to the analyte as an unknown.

12.2.7 A calibration check standard (midpoint) must be analyzed at the beginning and end of each run, every 20 samples, or within 24 hours after the initial calibration curve was generated (100 ppb). The end calibration check should be the same calibration standard that was used to generate the initial curve. The regression result from the end calibration check standard must have a percent deviation less than 35% from the target analyte and surrogate nominal concentration. If the results are not within these criteria, the problem must be corrected and either all samples in the batch must be re-analyzed against a new calibration curve or the affected results must be qualified with an indication that they are not within the performance criteria of the test method. If the analyst inspects the vial containing the end calibration check standards and notices that the samples evaporated, affecting the concentration, a new end calibration check standard may be made and analyzed. If this new end calibration check standard has a percent

deviation less than 35% from the calculated concentration for the target analyte and surrogate, the results may be reported unqualified.

TABLE 4. Concentrations of Calibration Standards (PPB)

Analyte/Surrogate	DVL	LV 1	LV 2	LV 3	LV 4	LV 5	LV 6
DOSS	3	10	20	60	100	150	200
DOSS-D34 (Surrogate)	3	10	20	60	100	150	200

12.3 If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., a precision and bias study must be performed to demonstrate laboratory capability (Section 16).

12.3.1 Analyze at least four replicates of a sample solution containing the DOSS and surrogate at a concentration in the calibration range of Levels 3 to 5. The Level 4 concentration of the 6 point calibration curve was used to set the QC acceptance criteria in this method. The matrix and chemistry should be similar to the solution used in this test method. Each replicate must be taken through the complete analytical test method including any sample pre-treatment steps.

12.3.2 Calculate the mean (average) percent recovery and relative standard deviation (RSD) of the four values and compare to the acceptable ranges of the QC acceptance criteria for the Initial Demonstration of Performance in Table 5.

12.3.3 This study should be repeated until the single operator precision and mean recovery are within the limits in Table 5.

The QC acceptance criteria for the Initial Demonstration of Performance in Table 5 are preliminary until a more data and multi-laboratory study is completed. Data generated from a single-laboratory validation from reagent and sea water matrices are shown in the Precision and Bias Section 16. It is recommended that the laboratory generate their own in-house QC acceptance criteria that meets or exceeds the criteria in this standard. A Reference on how to generate QC acceptance criteria is in Method 8000B in EPA publication SW-846.

TABLE 5. Preliminary QC Acceptance Criteria

Analyte	Test Conc. (µg/L) in Reagent Water	Initial Demonstration of Performance			Lab Control Sample	
		Recovery (%)		Precision	Recovery (%)	
		Lower Limit	Upper Limit	Maximum % RSD	Lower Limit	Upper Limit
DOSS	200	50	150	30	50	150
DOSS-D34 (Surrogate)	200	50	150	30	50	150

12.4 Surrogate Spiking Solution (This draft contains DOSS-D34 data):

A surrogate spiking 50% water/50% CH_3CN solution containing DOSS-D34 is added to all samples. A stock surrogate spiking solution is prepared at 20 ppm. Spiking 200 µL of this spiking solution into a 20 mL water sample results in a concentration of 200 ppb of the surrogate in the sample. The result obtained for the surrogate recovery must fall within the limits of Table 5. If the limits are not met, the affected results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.5 Method Blank:

12.5.1 Analyze a reagent water blank with at the beginning and end of every run and every 20 samples. The DOSS measured in the blank should be less than half of the reporting limit. If the concentration of DOSS is found above this level, analysis of samples is halted until the contamination is eliminated, and a blank shows no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method. DOSS has been found to carry-over in LC systems. A rigorous washing of

the injector with stronger wash solvents has been shown to work well as described in this method.

12.6 Laboratory Control Sample (LCS):

12.6.1 To ensure that the test method is in control, analyze a LCS prepared with the DOSS. The LCS is prepared following the analytical method and analyzed with each batch of 20 samples or less. Prepare a stock matrix spiking solution in 50% water/50% acetonitrile containing the DOSS at 20 ppm. Spike 200 µL of this stock solution into 20 mL of water to yield a concentration of 200 ppb for the DOSS in the sample. The LCS result must be within the limits in Table 5. Matrix spiking solutions are routinely replaced every 7 days if not previously discarded for quality control failure.

12.6.2 If the LCS regression result is not within limits listed in Table, analysis of samples is halted until the problem is corrected, and either all samples in the batch must be re-analyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

13. Procedure

13.1 This test method is based upon a 20 mL sample size per analysis. **Any sample size may be used such as a half filled VOA vial as long as the QC spikes and sample preparation volumes are adjusted accordingly.** Samples shall be analyzed within 5 days of collection. If the samples are above 6°C when received or during storage, or not analyzed within 5 days of collection, the data is noted in the case narrative that accompanies the data.

13.2 In the laboratory, the entire 20 mL sample, collected in a 20 mL glass collection vial, is poured into a 50 mL graduated cylinder. The surrogate as described in Section 12 (added to the sample in the original sample container or graduated cylinder depending on sampling described in Section 10.1) and NH_4CO_2H are added to the sample. The vial is rinsed with two 6 to 8 mL portions of acetonitrile to collect DOSS remaining in the collection vial. These 2 portions are added to the 50 mL graduated cylinder. The samples are then diluted to 40 mL final volume with acetonitrile and mixed thoroughly. The prepared sample is filtered through the syringe-driven filter unit fitted with a PVDF filter cartridge into a glass storage vial.

13.3 For samples that are not biphasic, the entire 40 mL volume is filtered through the filtration device described in Section 7.2 and into a pre-cleaned collection vial (such as a VOA vial). A portion of that filtered sample is added to an amber glass LC vial with a Teflon®- lined cap, which is then analyzed. A new filter unit is used for each sample.

13.4 For biphasic samples, the lower aqueous layer is filtered through the filtration device described in Section 7.2 into a pre-cleaned collection vial (such as a VOA vial). The upper oil layer is left behind and is not added to the filtration device. A portion of that filtered sample is added to an amber glass LC vial with a Teflon® lined cap which is analyzed. A new filter unit is used for each sample.

13.5 The syringe must be cleaned between each filtration. It is the analyst's responsibility to ensure that the syringe is clean. A suggested method for cleaning the syringe between filtrations is to first rinse with at least 5 syringe volumes of water, followed by at least 3 volumes of 50% water/50% acetonitrile.

13.6 Once a passing calibration curve is generated, the analysis of samples may begin. An order of analysis may be: method blank, laboratory control sample, method blank, up to 20 samples, followed by an end calibration check standard and a method blank.

14. Calculation or Interpretation of Results

14.1 For DOSS and surrogate analysis, the SRM transitions are identified by comparison of retention times in the sample to those of the standards. Calibration curves are used to calculate the amounts of DOSS and surrogate. Calculate the concentration in µg/L (ppb) for each analyte. The sample concentration was diluted two-fold by the addition of surrogates, ammonium formate, acetonitrile, and target compound spike where applicable. The two-fold dilution must be accounted for when reporting the concentration. DOSS may be reported if present at or above the reporting limit. If the concentration of the analyte is determined to be above the calibration range, the sample is diluted with 5 mM ammonium formate in 50% acetonitrile/50% water to obtain a concentration near the mid-point of the calibration range and re-analyzed. This method uses one surrogate, DOSS-D34, to monitor performance and matrix affect. The surrogate recoveries are provided with all data generated from this test method.

14.1.1 A surrogate is used to monitor the performance of DOSS. If the surrogate meets the quality control criteria in this test method, the data may be reported unqualified for DOSS if all other quality control in this test method are acceptable. If the surrogate does not meet the quality control criteria of the test method, the data is qualified for DOSS.

15. Report

15.1 Determine the results in units of µg/L (ppb) in a water sample. Calculate the concentration in the sample using the linear or quadratic calibration curve. All data that does not meet the specifications in the test method must be appropriately qualified.

16. Precision and Bias

16.1 The determination of precision and bias was conducted through U.S. EPA Region 5 Chicago Regional Laboratory. **More than 50 seawater samples have been analyzed using this procedure without noticeable chromatography effect or increased pressure.**

16.2 This test method was tested by CRL on reagent and sea water. The samples were spiked with the DOSS to obtain a 200 ppb concentration of each as described in Section 12. Table 7 contains the recoveries and standard deviation (SD) for the target compound.

TABLE 7. Single-Laboratory Recovery Data in Reagent Water using chromatography conditions outlined in Table 2A and Table 2B

Precision and Accuracy Samples	Measured ppb from 200 ppb DOSS Spikes in Reagent Water					
	Table 2A			Table 2B		
	Retention time (min)	DOSS measured (ppb)	Percent Recovery	Retentio n time (min)	DOSS measured (ppb)	Percent Recovery
1	6.38	182.6	91.3%	5.41	196.1	98.0%
2	6.37	232.9	116.5%	5.41	232.8	116.4%
3	6.37	224.6	112.3%	5.41	225.8	112.9%
4	6.37	226.8	113.4%	5.41	238.9	119.5%
Average Recovery:		216.7	108.4%		223.4	111.7%
Standard Deviation:		23.0			19.0	
% Relative SD		11.5%			9.5%	

16.3 This test method was tested by CRL on Gulf of Mexico seawater. The samples were spiked with target compound as described in Section 12. Table 8 contains the recoveries for the target compound.

TABLE 8. Single-Laboratory Recovery Data in Gulf of Mexico Sea Water using chromatography conditions outlined in Table 2A and Table 2B

Precision and Accuracy Samples	Measured ppb from 200 ppb DOSS Spikes in Gulf Water					
	Table 2A			Table 2B		
	Retention time (min)	DOSS measured (ppb)	Percent Recovery	Retentio n time (min)	DOSS measured (ppb)	Percent Recovery
1	6.37	189.1	94.6%	5.4	257.2	128.6%
2	6.37	251.4	125.7%	5.4	251.4	125.7%
3	6.37	253.2	126.6%	5.41	263.0	131.5%
4	6.37	257.0	128.5%	5.4	262.4	131.2%
5	6.37	250.7	125.4%	5.4	255.0	127.5%
Average Recovery:		240.3	120.1%		257.8	128.9%
Standard Deviation:		28.7			4.9	
% Relative SD		14.4%			2.5%	

17. Keywords

17.1 Dioctyl Sulfosuccinate; Liquid Chromatography; Mass Spectrometry; Water

SOP 7. Total DNA Isolation

1.0 Scope & Application

This Standard Operating Procedure is applicable to different types of environmental samples including concrete biofilms. The commercially available kit used for this procedure: UltraClean Soil DNA kit from MoBio Laboratories (California, USA).

2.0 Summary of Method

Through various processes including mechanical and chemical lysis, precipitation of PCR inhibitors, separation via centrifugation, DNA-binding, and washing, the final elute contains DNA that can be used for PCR.

3.0 Sample Preservation, Containers, Handling, and Storage

All equipment and supplies are provided by manufacturer and are sterile. Wear gloves at all times, and change them frequently to avoid cross contamination. Following DNA isolation, the DNA may be stored at -20C until analysis.

4.0 Reagents

Proprietary reagents are provided with the kit. Solution S4 contains ethanol and is flammable.

5.0 Equipment and Supplies

Equipment needed for processing samples includes:

- Microcentrifuge (10,000 x g)
- Pipette (50ul – 500ul)
- Vortex
- Solutions S1, S2, S3, S4, S5, IRS Solution
- Spin filter units in 2ml tubes
- Collection tubes (2ml)
-

6.0 Procedure

1. Using sterile forceps, place 0.2 - 1.0 g of microbial biomass in bead solution tubes
2. gently vortex
3. add 60 µl of solution S1, vortex briefly
4. add 200 µl of solution IRS
5. vortex horizontally 10 min, max speed
6. centrifuge 10 000 g, 30 s
7. move supernatant to a new tube (about 400 µl)
8. add 250 µl of solution S2, vortex 5 s
9. incubate in +4° C, 5 min
10. centrifuge tubes 10 000 g, 1 min
11. transfer 450 µl of supernatant to a clean microcentrifuge tube

12. add 900 µl of solution S3, vortex 5 s
13. load 700 µl of sample onto spin column
14. centrifuge columns 10 000 g, 1 min, discard flow through
15. load rest of the supernatant on spin column
16. centrifuge at 10 000 g, 1 min, discard flow through
17. add 300 µl of solution S4
18. centrifuge 10 000 g, 30 s, discard flow through
19. centrifuge again 10 000 g, 1 min
20. place spin filter column to a new tube (avoid splashing any solution S4 onto spin filter)
21. add 50 µl of solution S5 to the center of the white membrane
22. centrifuge 10 000 g, 30 s
23. discard the spin filter, DNA is in tube ready for applications
24. store in -20ºC
25. Measure the DNA concentration using the instruction for the NanoDrop spectrophotometer (manual located in room 675; see attachment A.11)

Calculations
DNA may be quantified using the NanoDrop 1000 spectrophotometer or Qubit Fluorometric Quantitation. Please refer to the NanoDrop SOP or Qubit SOP.

8.0 Quality Control and Quality Assurance
QC samples required in the QA Project Plan must be followed; these may include blanks, and triplicate samples.

9.0 References
N/A

SOP 8. Quantifying Nucleic Acids With a Nanodrop Nd-1000 Spectrophotometer

1.0 Scope & Application

The NanoDrop ND-1000 is a full spectrum (220-750nm) spectrophotometer that measures 1ul samples with high accuracy and reproducibility. In addition the ND-1000 has the capability to measure highly concentrated samples without dilution.
The NanoDrop ND-1000 UV/VIS spectrophotometer can be used to measure:
- Nucleic acid concentration and quality up to 3700ng/ul (dsDNA) without dilution
- Fluorescent dye labeling density of nucleic acid microarray samples
- Purified protein analysis (A280) up to 100 mg/ml
- Bradford assay analysis of protein
- BCA Assay analysis of protein
- Cell density measurements
- General UV/VIS spectrophotometer

2.0 Summary of Method
A 1 or 2ul sample is pipetted onto the end of a fiber optic cable (the receiving fiber). A second fiber optic cable (the source fiber) is then brought into contact with the liquid sample causing the liquid to bridge the gap between the fiber optic ends. The gap is controlled to a 1mm path. A pulsed xenon flash lamp provides the light source and a spectrometer utilizing a linear CCD array is used to analyze the light after passing through the sample. The instrument is controlled by software run on a PC, and the data is logged in an archive file on the PC.

3.0 Sample Preservation, Containers, Handling, and Storage
DNA samples will be preserved at -20C, and RNA samples will be preserved at -70C.

4.0 Reagents
A 70% ethanol solution is used to clean the fiber optic ends between samples, and after completion of all sample analysis.

5.0 Equipment and Supplies
• NanoDrop ND-1000 spectrophotometer
• 70% ethanol – labeled and dated.
• Soft laboratory wipes (Kim Wipes or equivalent)
• pipette (2 -10ul)
• Pipette tips (certified RNase and DNase-free)

6.0 Procedure
1. With the sampling arm open, pipette the sample onto the lower measurement pedestal.
2. Close the sampling arm and initiate a spectral measurement using the operating software on the PC.
3. When the measurement is complete, open the sampling arm and wipe the sample from both the upper and lower pedestals using a soft laboratory wipe.
4. After measuring a large number of samples, it may be necessary to clean the areas around the upper and lower pedestals with a 5% solution of bleach – freshly prepared.
5. Results may be printed, saved to PC, or copied to electronically for archiving.

7.0 Calculations

N/A

8.0 Quality Control and Quality Assurance

All QC samples required in the QA Project Plan must be followed; these may include field blanks, trip blanks, and collection of replicate samples.

9.0 References

NanoDrop ND-1000 Spectrophotometer User's Manual V3.0.1 (rev 16-Apr-04)
Website: http://www.nandrop.com

SOP 9. Polymerase Chain Reaction (PCR)

1.0 Scope & Application

This Standard Operating Procedure is applicable to performing the Polymerase Chain Reaction (PCR) on DNA obtained from prokaryotic and eukaryotic microbiological isolates as well as from environmental samples such as potable water, biofilms, source water, and wastewater as well as enrichment cultures such as the ones associated with this study.

2.0 Summary of Method

The amplification of DNA from any given sample allows for the identification of target genes or sequences, as well as the development of clone libraries. Sample conditions may vary, particularly in environmental samples, so that no universal PCR protocol can be recommended. However, certain methods can be generalized, allowing for further optimization by the researcher.

3.0 Sample Preservation, Containers, Handling, and Storage

All equipment and supplies are provided by manufacturer and are sterile. Wear gloves at all times, and change them frequently to avoid cross contamination. Following DNA isolation, the DNA may be stored at 4C temporarily or -20C long term.

4.0 Reagents

Takara Ex Taq Polymerase, buffer, and dNTP mix (Takara Mirus Bio, Madison, WI).

5.0 Equipment and Supplies

- Thermocycler, e.g. PE9600 or equivalent
- Taq polymerase
- 10x Taq buffer (some buffers includes $MgCl_2$)
- $MgCl_2$ solution (if not included in buffer)
- dNTP's
- 25% acetamide solution or BSA
- PCR grade sterile water (autoclaved SuperQ water or equivalent)
- Primers, one forward and one reverse; varies with each experiment
- pipettes, 1000, 200, 20, and 10 ul sizes (Rainin or equivalent)
- pipet tips, with filters, 1000, 200, 20, and 10 ul sizes
- sterile 2 ml or 5 ml tubes
- PCR tubes, or strips, or 96-well plates
- PCR caps for strips or plates

Ice bucket filled with ice

6.0 Procedure

All PCR tubes, caps, pipet tips, and reagents must be sterile and DNase- and RNase- free. Wear powder-free gloves at all times. Extreme care needs to be exercised when preparing samples for PCR to avoid cross-contamination.

1. Place 10x buffer, $MgCl_2$ solution (if needed), primers, and dNTP's on ice to thaw.
2. Make 25% acetamide fresh, or store at -20*C between uses. If using stored acetamide, place on ice with other solutions to thaw.
3. Once reagents have thawed, make a master mix by placing an aliquot of each reagent (buffer, $MgCl_2$, dNTP's, acetamide, primers, and water) into a sterile tube in proportions dictated by each experiment. Close or seal the tube and place on ice. Return all reagents except water to the -20C freezer
4. Remove Taq polymerase and immediately place on ice. Remove the volume of Taq polymerase required, add to master mix in the tube, reseal the tube, and mix briefly by vortexing or swirling. Place master mix on ice and return the Taq polymerase to the freezer.
5. Aliquot the master mix into PCR tubes, strips, or 96-well plates, using a volume determined for individual protocols. Sample DNA (template) should be added to PCR tubes after the master mix to avoid cross contamination of tubes.

DNA from environmental samples used as a template for PCR should be extracted with a kit (MoBio or equivalent), or by protocols published in the peer- reviewed scientific literature. Freshly extracted DNA may be added to PCR tubes, or DNA may be stored at -20*C until needed. Frozen DNA should be thawed on ice before use. A table containing a typical recipe for PCR is included in this SOP. Further information may be obtained from Ausubel et al., 1988 or a similar protocol reference.

7. Calculations

DNA may be quantified using the NanoDrop 1000 spectrophotometer. Please refer to the NanoDrop SOP. The presence of PCR product is confirmed by agarose gel electrophoresis. Methods for gel electrophoresis are described in Ausubel et al., 1988, and in the Agarose Gel Electrophoresis SOP.

8.0 Quality Control and Quality Assurance

QC samples required in the QA Project Plan must be followed; these may include blanks, and triplicate samples. Each PCR should include a water blank as a negative control, and a positive control of template DNA known to amplify with the primer set in the reaction. Negative controls are critical for PCR as cross contamination is a constant potential problem.

9.0 References

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl, eds. 1988. *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc.

User's Manual, GeneAmp PCR System 9600, Applied Biosystems, Foster City, CA

PCR ASSAY MASTER MIX

PCR master mix		**date**
	one reaction (µl)	**rxn (µl)**
10X buffer	5	
dNTP mix	4	
Taq enzyme	0.25	
F primer 8F	3	
R primer 685	3	
Template	2	
H2O	32.75	
total	**50**	

The following are starting points to use when optimizing PCR conditions for each primer pair:

(1) 94C - 1 minute
(2) 94C - 30 seconds
(3) 55C - 1 minute
(4) 72C - 1 minute
(5) 30 cycles of steps 2 through 4(6) 72C - 2 minutes

SOP 10. PCR Purification With QIAquick Spin Kit

1.0 Scope & Application
Using a microcentrifuge, this protocol is designed to purify single or double stranded DNA fragments from PCR and other enzymatic reactions

2.0 Summary of Method
Fragments ranging from 100bp to 10kb are purified from primers, nucleotides, polymerases, and salts using QIAquick spin columns in a microcentrifuge. The QIAquick method relies on the selective binding properties of a silica gel membrane. DNA adsorbs to the silica membrane in the presence of high salt while contaminants pass through the column. Impurities are washed away, and the pure DNA is eluted.

3.0 Sample Preservation, Containers, Handling, and Storage
DyeEx Spin Kits will be stored dry at room temperature (15-25C). Under these conditions, these kits can be stored for up to 12 months. For longer storage, these kits can be stored at 2-8C. Do not freeze.

4.0 Reagents
All buffers are supplied with the kit. Buffer PB contains guanidine hydrochloride and isopropanol. Buffer PN contains perchlorate and isopropanol. Buffer QC contains guanidine thiocyanate. When working with these reagents, always wear a suitable lab coat, disposable gloves, and protective goggles.

5.0 Equipment and Supplies
Spin columns, collection tubes, and microcentrifuge tubes are supplied with the kits.
All centrifugation steps are performed at 750 x g in a conventional microcentrifuge.

6.0 Procedure
Note: add ethanol (96-100%) to Buffer PE before use. All centrifuge steps are at 13,000rpm (~17,900 x g) in a conventional tabletop microcentrifuge.

1. Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix.
2. Place a QIAquick spin column in a provided 2ml collection tube.
3. To bind DNA, apply sample to the QIAquick column and centrifuge for 30-60seconds.
4. Discard flow through and place the QIAquick column back in the same tube.
5. To wash, add 0.75ml Buffer PE to the QIAquick column and centrifuge for 30-60seconds.
6. Discard the flow through and place the QIAquick column back in the same tube. Centrifuge the column for an additional 1minute.
7. Place QIAquick column in a clean 1.5ml microcentrifuge tube.
8. To elute DNA, add 50ul Buffer EB or UltraPure water to the center of the QIAquick membrane and centrifuge the column for 1minute.

7.0 Calculations
The appropriate speed for individual centrifuges can be calculated as follows:

Rpm = 1000 x √750/1.12r
(r = radius of rotor in mm)

8.0 Quality Control and Quality Assurance

All QC samples required in the QA Project Plan must be followed; these may include blanks, and replicate samples.

Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA.

9.0 References

QIAquick Spin Handbook 07/2002
QIAGEN Inc. 28159 Avenue Stanford, Valencia, CA 91355

SOP 11. TOPO T/A Cloning

1.0 Scope & Application

This standard operating protocol is applicable to cloning any sequence of DNA amplified by PCR. The DNA may be obtained from any organism or environment. Cloning DNA is carried out to produce DNA libraries for sequence analysis. This is particularly applicable to microbial ecology studies in which many environmental organisms of interest are difficult to culture.

2.0 Summary of Method

In TA cloning, the linearized plasmid vector contains single 3' thymidine overhangs. After adding fresh, purified PCR product with single 3' adenosine overhangs, topoisomerase that is covalently bound to the plasmid will ligate product and vector. After transforming competent *E. coli* with the vector and growing transformants on LB/ampicillin agar, plasmids are purified and screened to confirm the presence of the proper size insert.

3.0 Sample Preservation, Containers, Handling, and Storage

Potential problems associated with the TA cloning procedure include the recovery of an insert of an improper size and a low efficiency for the cloning reaction.

To minimize the insertion of an undesired fragment, the PCR product to be cloned should be screened carefully to ensure that it produces a single band on an agarose gel. The product should also be cleaned before cloning to remove small oligonucleotide fragments.

The efficiency of the cloning reaction can be maintained by using fresh PCR product. Once the target sequences are amplified, they immediately should be screened, purified, and ligated with the vector. PCR product may not be stored overnight before cloning; it must be cloned the same day.

4.0 Reagents

TOPO TA Cloning kit (Invitrogen).

5.0 Equipment and Supplies

- Purified PCR product (Qiagen)

A. - Agarose gels, gel box, gel buffer, and power supply
B. - sterile 0.5 ml tubes
C. - pipettes sizes 1000, 200, 20, and 10 ul (Rainin or equivalent)
D. - sterile pipet tips, with filters, sizes 1000, 200, 20 and 10 ul
E. - TOPO TA Cloning kit (Invitrogen) or equivalent

F. - LB agar plates containing 100 ug/ml ampicillin (3-4 per transformation
G. - LB broth containing 100 ug/ml ampicillin
H. - 3 ml culture tubes, pre-sterilized
I. - autoclaved toothpicks
J. - General microbiological supplies, including Bunsen burner, ethanol, plate spreaders, etc.
K. - Plasmid Preparation kit (Qiagen or equivalent)
L. - water bath set at 42 *C
M. - shaking incubator set at 37 *C
N. - non-shaking incubator set at 37 *C

6.0 Procedure

Sample Preparation

For each cloning reaction, fresh PCR product is required. The target gene or sequence should be amplified and the product purified immediately before performing the cloning reaction to obtain a high efficiency. For assistance with PCR protocols, refer to Ausubel et al., 1988 or to PCR SOP. Check the purity of the PCR product by agarose gel electrophoresis (Ausubel et al., 1988). The product should have a single band on the gel. Clean the PCR product using a purification kit (Qiagen or similar type) to remove excess dNTP's and primers.

Cloning reaction

Reagents included in the cloning kit (salt solution, sterile water, vector, and competent *E. coli*) should be thawed on ice before use. SOC medium, also included in the kit, should be warmed to room temperature before cloning.

Each cloning reaction has a total volume of 6 ul. Typically, 2 ul of fresh, purified PCR product is placed in a sterile 0.5 ml tube, along with 2 ul sterile water, 1 ul salt solution, and 1 ul TOPO vector. Mix by stirring with a pipet tip and incubate for 5 minutes at room temperature. Place reaction tube on ice and proceed with the chemical transformation of competent *E. coli* as described in the instruction manual of the Invitrogen TA cloning kit. When working with competent *E. coli*, take great care to mix them *gently* with the ligated vector, to avoid damaging the bacteria.

Analyzing transformants

Colonies that form on LB/ampicillin agar contain transformed bacteria. Isolated colonies are picked with sterile toothpicks and inoculated into sterile tubes containing 2 ml of autoclaved LB/ampicillin broth. Tubes are incubated overnight in a shaking incubator at 37 *C. One ml of grown bacteria is removed for purification using a plasmid preparation kit. The remaining bacterial culture is preserved in glycerol stock.

Purified plasmid should be analyzed by agarose gel electrophoresis, by PCR amplification, by restriction enzyme digestion, or sequencing. These methods of analysis may be used in combination. Methods for restriction enzyme digests are found in Ausubel et al., 1988, as well as the enzyme instruction sheet.

A direct observation of purified plasmid on an agarose gel determines whether or not the proper

size fragment has been inserted into the plasmid. PCR amplification with M13 primers, which are specific to the plasmid, or with primers specific to the cloned fragment, followed by agarose gel electrophoresis, also demonstrates the size of the plasmid insert. A typical PCR master mix for checking transformants is included in this SOP. Carrying out a restriction enzyme digest on the PCR product demonstrates how many plasmids contain the same or similar sequence as an insert. Doing the digest screen before sequencing the plasmid insert prevents sequencing the same fragment repeatedly. Techniques for sequencing DNA are found in Ausubel et al., 1988.

7.0 Calculations

DNA may be quantified using the NanoDrop 1000 spectrophotometer. Please refer to the NanoDrop SOP. The presence of PCR product is confirmed by agarose gel electrophoresis. Methods for gel electrophoresis are described in Ausubel et al., 1988, and in the Agarose Gel Electrophoresis SOP.

8.0 Quality Control and Quality Assurance

QC samples required in the QA Project Plan must be followed; these may include blanks, and triplicate samples. All standard microbiological practices should be observed, including use of aseptic technique and media blanks. Medium containing antibiotics should be stored at 4 *C until needed, and should be used within one to two weeks of being poured. Reagents for cloning reactions should be stored at - 20 *C or -70 *C, as indicated by the kit instructions. Freezer temperature should be monitored regularly and recorded on log sheets.

9.0 References

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl, eds. 1988. *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc.

User's Manual, GeneAmp PCR System 9600, Applied Biosystems, Foster City, CA

Check Transformants (analyze positive clones)

Use M13 PCR assay to confirm the presence of clones. Once the PCR assay is completed load 5µl of the reaction into agarose gel to determine the presence and size of clone inserts. The rest of the reaction will be used for sequencing experiments. Positive M13 clones are sent to Children's Hospital Core Facility, UC for sequencing. At least 20 µl of M13 PCR product are required, so if necessary bring the volume up to desired volume with sterile ddH2O. Follow A.8 protocol if sequencing at Children's.

PCR (25 µL)	per rxn	reactions
Template	2 µl	

10X	2.5µl	
4% BSA	0.4µl	
dNTP	2 µl	
Primer (25pmole/µl)	0.5 µl	
Primer (25pmole/µl)	0.5µl	
dH_2O (total 25µl)	17µl	
Tag enzyme	0.125µl	
Total	25 µl	

To: Mike Fulton - NOAA Federal[mike.fulton@noaa.gov]
From: Conmy, Robyn
Sent: Tue 9/1/2015 7:16:14 PM
Subject: RE: dispersant

Let's say 9:30 ET. Since you will have a group, just call my office number.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Mike Fulton - NOAA Federal [mailto:mike.fulton@noaa.gov]
Sent: Tuesday, September 01, 2015 3:10 PM
To: Conmy, Robyn
Subject: Re: dispersant

By the way, if possible I'd like to have a couple other folks sit in.

Mike

On Tue, Sep 1, 2015 at 3:00 PM, Mike Fulton - NOAA Federal <mike.fulton@noaa.gov> wrote:

Thanks Robyn. Sometime Thursday morning would work.

Mike

On Tue, Sep 1, 2015 at 12:10 PM, Conmy, Robyn <Conmy.Robyn@epa.gov> wrote:

Hi Mike,

Never a pest! I am tied up with meetings today and tomorrow. Would Thursday work for you?

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Mike Fulton - NOAA Federal [mailto:mike.fulton@noaa.gov]
Sent: Tuesday, September 01, 2015 10:34 AM
To: Conmy, Robyn
Subject: Re: dispersant

Hi Robyn,

Sorry to be a pest, but it would be great if we could arrange to chat about dispersants, cleaners and bioremediation agents. Thanks.

Mike

On Wed, Aug 19, 2015 at 9:28 AM, Mike Fulton - NOAA Federal <mike.fulton@noaa.gov> wrote:

> Hi Robyn,
>
> I hope you're well. Would you have some time in the next week or so to discuss our joint interest in dispersants, shoreline cleaners,and bioremediation agents? Thanks.
>
> Mike

> On Thu, Jul 30, 2015 at 2:04 PM, Mike Fulton - NOAA Federal <mike.fulton@noaa.gov> wrote:
>
> > Hi Robyn,
> >
> > Is there a time next week when we could chat about sharing dispersants? Thanks.
> >
> > Mike

> > On Mon, Jul 20, 2015 at 9:21 AM, Mike Fulton - NOAA Federal <mike.fulton@noaa.gov> wrote:
> >
> > > Hi Robyn,
> > >
> > > I just wanted to follow up on the possibility of sharing dispersants/shoreline cleaners...what you may have available to share and what we can share with you. Thanks.
> > >
> > > Mike

On Fri, Jul 10, 2015 at 9:17 AM, Mike Fulton - NOAA Federal <mike.fulton@noaa.gov> wrote:

Hi Robin,

We do have both. I suspect we have enough to share. I'll check and let you know early next week. One caveat...we'd have to the pull the material for you from a larger container. We didn't receive separate aliquots. Can that work?

Mike

On Fri, Jul 10, 2015 at 9:00 AM, Conmy, Robyn <Conmy.Robyn@epa.gov> wrote:

Hi Mike,

I couldn't recall if you said your lab has finasol and corexit in stock for testing? If so, would you have any to share with us? We are running low and trying secure a liter or 2 of each.

Thanks,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

To: Malcolm Gore[malcolm.gore@clearcoastllc.com]
Cc: Peter Egan[peter.egan@total.com]
From: Conmy, Robyn
Sent: Mon 4/25/2016 3:05:26 PM
Subject: RE: Finasol OSR 52 Sample Request

Hello Malcolm,

Thank you for getting back with me regarding the purchase. Yes, the information below is correct, with a mail stop at room 166. Can you send to me the invoice via email so I can provide to the purchase card holder to make the purchase?

Thank you in advance,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Malcolm Gore [mailto:malcolm.gore@clearcoastllc.com]
Sent: Monday, April 25, 2016 10:57 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Egan <peter.egan@total.com>
Subject: Finasol OSR 52 Sample Request

Hello Robin

I am writing with respect to your email below regarding a sample request of Total Finasol OSR 52.

Clear Coast is the custodian of the product stored here in Houston for Total.

I will arrange the sample as requested.

Please confirm the following:

Contact:

Robyn Conmy 513-569-7090 (office)

Deliver to:

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

Quantity:

2 US Gallons.

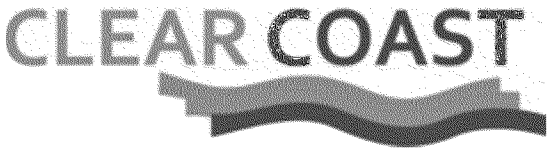
On receipt of confirmation I will get the samples drawn, packaged and dispatched.

Regards

Malcolm

Malcolm Gore

President



+1 832 244 1533

malcolm.gore@clearcoastllc.com

www.clearcoastllc.com

From: Conmy, Robyn
Sent: Monday, April 18, 2016 10:47 AM
To: 'peter.egan@total.com' <peter.egan@total.com>
Cc: Wilson, Gregory <Wilson.Gregory@epa.gov>; Principe, Vanessa <Principe.Vanessa@epa.gov>
Subject: purchase of Finasol OSR52

Mr. Egan,

This follows up our telephone conversation of April 11.

As discussed, EPA is seeking to acquire a number of dispersant products listed on the NCP Subpart J Product Schedule for the purposes of both toxicity and efficacy testing. The product testing is intended to further inform currently proposed regulatory actions under Subpart J of the National Contingency Plan. The products will in addition be used to support our general research in the area of oil spill response.

Your product Finasol OSR52 has been identified as one of interest, as it is commonly stockpiled in the U.S. Not only could the product be encountered when presented with a response situation, but including this dispersant product in toxicity and efficacy testing studies will also allow for comparison and

consistency with other existing and ongoing studies by EPA and other federal agencies. EPA's Office of Research and Development (ORD) will be conducting the dispersant studies.

At this time we are seeking 2 U.S. gallons of the product.

We appreciate your time and attention to this request.

Respectfully,

Robyn Conmy

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

To: Barron, Mace[Barron.Mace@epa.gov]
Cc: Holder, Edith[holder.edith@epa.gov]
From: Conmy, Robyn
Sent: Tue 5/31/2016 6:02:10 PM
Subject: RE: dispersant volume

We will send 1 L of Corexit and Finasol. Thanks!

R

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Barron, Mace
Sent: Tuesday, May 31, 2016 1:16 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: RE: dispersant volume

One liter if that is no problem.

From: Conmy, Robyn
Sent: Tuesday, May 31, 2016 10:30 AM
To: Barron, Mace <Barron.Mace@epa.gov>

Subject: dispersant volume

How much do you want sent to Hydrosphere?

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

To: Mike Fulton - NOAA Federal[mike.fulton@noaa.gov]
From: Conmy, Robyn
Sent: Tue 9/1/2015 4:10:36 PM
Subject: RE: dispersant

Hi Mike,

Never a pest! I am tied up with meetings today and tomorrow. Would Thursday work for you?

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Mike Fulton - NOAA Federal [mailto:mike.fulton@noaa.gov]
Sent: Tuesday, September 01, 2015 10:34 AM
To: Conmy, Robyn
Subject: Re: dispersant

Hi Robyn,

Sorry to be a pest, but it would be great if we could arrange to chat about dispersants, cleaners and bioremediation agents. Thanks.

Mike

On Wed, Aug 19, 2015 at 9:28 AM, Mike Fulton - NOAA Federal <mike.fulton@noaa.gov> wrote:

Hi Robyn,

I hope you're well. Would you have some time in the next week or so to discuss our joint interest in dispersants, shoreline cleaners,and bioremediation agents? Thanks.

Mike

On Thu, Jul 30, 2015 at 2:04 PM, Mike Fulton - NOAA Federal <mike.fulton@noaa.gov> wrote:

Hi Robyn,

Is there a time next week when we could chat about sharing dispersants? Thanks.

Mike

On Mon, Jul 20, 2015 at 9:21 AM, Mike Fulton - NOAA Federal <mike.fulton@noaa.gov> wrote:

Hi Robyn,

I just wanted to follow up on the possibility of sharing dispersants/shoreline cleaners...what you may have available to share and what we can share with you. Thanks.

Mike

On Fri, Jul 10, 2015 at 9:17 AM, Mike Fulton - NOAA Federal <mike.fulton@noaa.gov> wrote:

Hi Robin,

We do have both. I suspect we have enough to share. I'll check and let you know early next week. One caveat...we'd have to the pull the material for

you from a larger container. We didn't receive separate aliquots. Can that work?

Mike

On Fri, Jul 10, 2015 at 9:00 AM, Conmy, Robyn <Conmy.Robyn@epa.gov> wrote:

Hi Mike,

I couldn't recall if you said your lab has finasol and corexit in stock for testing? If so, would you have any to share with us? We are running low and trying secure a liter or 2 of each.

Thanks,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

--

Dr. Michael H. Fulton-Estuaries and Land Use Branch Chief

Center for Coastal Environmental Health and

Biomolecular Research (CCEHBR)

USDOC/NOAA/NOS/NCCOS

219 Fort Johnson Road

Charleston, SC 29412-9110

voice: (843) 762-8576 fax: (843) 762-8700

e-mail: mike.fulton@noaa.gov http://www.chbr.noaa.gov

To: Peter EGAN[peter.egan@total.com]
From: Conmy, Robyn
Sent: Mon 4/25/2016 2:34:00 PM
Subject: FW: purchase of Finasol OSR52

Hello Mr. Egan,

This is a follow up to the message I sent below on April 18th. The EPA would like to procure your product for testing. Please let me know a POC from your organization to arrange the purchase.

Thank you,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Conmy, Robyn
Sent: Monday, April 18, 2016 10:47 AM
To: 'peter.egan@total.com' <peter.egan@total.com>
Cc: Wilson, Gregory <Wilson.Gregory@epa.gov>; Principe, Vanessa <Principe.Vanessa@epa.gov>

Subject: purchase of Finasol OSR52

Mr. Egan,

This follows up our telephone conversation of April 11.

As discussed, EPA is seeking to acquire a number of dispersant products listed on the NCP Subpart J Product Schedule for the purposes of both toxicity and efficacy testing. The product testing is intended to further inform currently proposed regulatory actions under Subpart J of the National Contingency Plan. The products will in addition be used to support our general research in the area of oil spill response.

Your product Finasol OSR52 has been identified as one of interest, as it is commonly stockpiled in the U.S. Not only could the product be encountered when presented with a response situation, but including this dispersant product in toxicity and efficacy testing studies will also allow for comparison and consistency with other existing and ongoing studies by EPA and other federal agencies. EPA's Office of Research and Development (ORD) will be conducting the dispersant studies.

At this time we are seeking 2 U.S. gallons of the product.

We appreciate your time and attention to this request.

Respectfully,

Robyn Conmy

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

Oil plume simulations: Tracking oil droplet size distribution and fluorescence within high-pressure release jets

R.N. Conmy[1], **B. Robinson** [2], **T. King** [2], **M. Boufadel**[3], **S. Ryan**[2], **C. McIntyre**[2], **M. I. Abercrombie**[4], **K. Lee** [2,5]

[1]U.S. Environmental Protection Agency, Office of Research and Development, 26 MLK Drive West, Cincinnati, OH 45255, USA

[2] Bedford Institute of Oceanography, Fisheries and Oceans Canada, 1 Challenger Dr, Dartmouth, NS, B2Y 4A2, Canada

[3] New Jersey Institute of Technology, Department of Civil and Environmental Engineering, University Heights, Newark, NJ 07102, USA

[4]University of South Florida, College of Marine Science, 140 7th Ave South, St. Petersburg, FL 33701, USA

[5] Commonwealth Scientific and Industrial Research Organisation, Oceans and Atmosphere Flagship, PO Box 1130, Bentley WA 6102, Australia

ABSTRACT

Optical measurements have been used during oil spill response for more than three decades to determine oil presence in slicks and plumes. Oil surveillance approaches range from simple (human eyeball) to the sophisticated (sensors on AUVs, aircraft, satellites). *In situ* fluorometers and particle size analyzers were deployed during the Deepwater Horizon (DWH) Gulf of Mexico oil spill to track shallow and deep subsea plumes. Uncertainties regarding instrument specifications and capabilities during DWH necessitated performance testing of sensors exposed to simulated, dispersed oil plumes. Seventy-two wave tank experiments were conducted at the Bedford Institute of Oceanography. Simulated were oil releases with varying parameters such as oil release rate, oil temperature (reservoir temp ~ 80 °C), water temperature (<8 °C and >15 °C), oil type, dispersant type (Corexit 9500 and Finasol OSR52) and dispersant

ED_001324_00001290-00001

to oil ratio (DOR). Plumes of Alaskan North Slope Crude (ANS), South Louisiana Crude (SLC) and IFO-120 oils were tracked using *in situ* fluorescence, droplet size distribution (DSD), total petroleum hydrocarbons (TPH) and benzene-toluene-ethylbenzene-xylene (BTEX). For the lighter SLC, bimodal droplet size with mean diameter < 70 m was achieved for 1:20 and 1:100 DOR, regardless of water temperature. Similarly, the medium ANS crude exhibited mean droplet diameter <70 μm, but was bimodal only for the 1:20 treatment. Bimodal distribution was not achieved with the heavy IFO, but droplet < 70 μm were observed for 1:20 warm waters, indicating poor dispersibility of the high viscosity oil even for jet releases. Results offer valuable information on the behavior and dispersibility of oils over a range of viscosity, DOR and environmental conditions. Findings have implications for fate and transport models, where DSD, chemistry and fluorescence are all impacted by release variables. This research was supported by the Bureau of Safety and Environmental Enforcement.

INTRODUCTION

The 2010 *Deepwater Horizon* (DWH) oil spill highlighted the pressing need for a better understanding of the interaction of chemical dispersants and crude oil at ocean depth. Early in the blowout release, partial emulsification of oil and heterogeneous slicks were observed as it rose to the surface from 1500-m depth (JAG report, 2010). As a countermeasure, Sub-Sea Dispersant Injection (SSDI) was used, where dispersants were injected directly at the release point as a possible means to increase dispersion efficiency and to potentially reduce the amount of dispersant needed if applied at the air-sea interface (CRRC Report, 2010). Potential advantages of this application during a continuous oil release included the fact that the fresh (un-weathered) oil was considered well suited for dispersion, operators were able to inject the

ED_001324_00001290-00002

dispersant directly into the oil stream thereby maximizing dispersant/oil contact, sufficient control of DOR (Dispersant-to-Oil Ratio) could be maintained, injection may minimize the need for surface application because of reduced oil surfacing, and optimized subsurface application would likely promote formation of smaller and stable oil droplets, enhancing biodegradation (Lee et al., 2009). The Unified Area Command (UAC) coordinated a large-scale environmental monitoring program to track and characterize dispersed subsurface oil plumes based on field data and transport modeling outputs for amount and location of oil. Oil Droplet Size Distribution (DSD) and fluorescence intensity from submersible sensors were used as an indication of Dispersion Effectiveness (DE) onboard vessels, where concentrations were monitored to evaluate oil dispersion (presence of small droplets $\leq$ 70 μm) based on previous studies for surface dispersant applications (Li et al., 2009a). Monitoring provided sound evidence of the presence of oil-bearing small particles both in surface waters and in the subsurface plume (JAG Report, 2010). SSDI correlated with a higher proportion of low molecular weight compounds in subsea waters compared to surface, suggesting that SSDI may have promoted the formation of small oil droplets in the deep sea and thus would have the potential for enhancing the weathering and dissolution of oil in the water column.

However, uncertainties still exist regarding the effectiveness of this application. For example, assumptions of the optimal DOR are based on empirical data mostly obtained from bench-scale experiments designed for testing at standard temperatures and pressures (STP). Such experiments do not take into account the effects of natural dispersion during high-pressure jet releases (i.e. deep water blowout) or the range of conditions on ocean floor, anywhere along a riser, or within the water column that could be significantly different. Hence, DOR for direct

ED_001324_00001290-00003

injection needs to be better understood. Although theoretical analyses and experiments suggest that oil jet breakup is insensitive to the absolute value of hydrostatic pressure for incompressible liquid-liquid systems (Masutani and Adams 2000), the effects of several ambient environmental factors on SSDI, including high release pressure, high oil temperature, low water temperature, and the presence of methane and suspended sediments in the oil plume and/or surrounding water column remain to be clarified. Improved understanding on the influence of these factors and the interaction of oil and chemical dispersant under a range of turbulent regimes at depth is required for informed decision-making for future SSDI use.

For evaluating DE, standard laboratory tests are inherently limited in simulating operational performance under realistic environmental conditions in part due to space constraints that are critical for transport and dilution efficiency (NRC, 2005). Meso-scale tanks capable of generating waves and flume flows to simulate ocean conditions help to address this issue. One such tank facility at the Bedford Institute of Oceanography (BIO; Dartmouth, Nova Scotia) has been used previously to assess chemical dispersant effectiveness for various oil types as a function of energy dissipation rate and particle size distribution (Li et al. 2009b) and demonstrated that the effectiveness of a dispersant is strongly dependent on wave conditions, dispersant type, and oil type (Wickley-Olsen et al. 2007; Li et al. 2008; Lee et al. 2009). A strong correlation has been established between dispersion effectiveness and *in situ* DSD within the hydrodynamic regime, particularly energy dissipation rate, under a variety of wave conditions (Li et al. 2008; Li et al. 2009b), where flume mode can simulate the effects of underwater currents on dispersion and dilution of oil (Li et al. 2009a; Li et al. 2010). Experiments have also shown the reliability of fluorescence intensity as a proxy for oil

ED_001324_00001290-00004

concentration within oil dispersions (Conmy et al., 2014).

Presented here are results from a series of high pressure jet releases of oil within the BIO flume tank to assess the operational performance of SSDI. DSD and fluorescence intensity were used as a proxy for dispersion efficiency for treatments with (chemically-enhanced dispersion) and without (physical dispersion) chemical dispersants. The objective of this work was to evaluate the effects of water temperature and dispersant-to-oil ratio on DE of three oils at high reservoir temperatures. Findings help (1) to determine the applicability of existing *in situ* monitoring technologies for SSDI and (2) inform efforts to integrate DSD into deep water blowout plume formation models that enable prediction of the dispersed oil droplets under high-flow subsurface release velocities. DWH spill monitoring program DSD and fluorescence data played a significant role in supporting emergency response operations, fate and transport modeling, and impact assessment. Findings from this study have implications for efforts in support of future deep-water spill preparedness.

METHODS

Flume Tank Description - Oil dispersion experiments were conducted using Alaska North Slope crude oil (ANS), South Louisiana Crude (SLC) and Intermediate Fuel Oil 120 (IFO 120) with Corexit 9500 and Finasol OSR 52 chemical dispersants in the 32m long flow-through carbon steel flume tank (31,000 L) at BIO. Oils were chosen to provide a wide range of viscosity. Seawater was pulled from the Bedford Basin (50cm below surface) via two smaller centrifugal pumps (110 gpm), and filtered through high-flow polypropylene bag filters (5 and 25 μm) to a fiberglass holding tank (25,000 L) that supplies seawater for the system to ensure that a constant flow rate is maintained. Two high-flow centrifugal pumps (600 gpm) provided flow to

ED_001324_00001290-00005

generate horizontal water currents in the tank, where flow gauges on influent and effluent pipes were monitored and valve adjustments made to obtain a balanced flow rate throughout experiments. Water current velocities were measured in the tank using an ADV (Nortek Vectrino) to maintain consistent horizontal water current velocities (3.5 cm/s) at all measured depths. At the completion of experiments, oil absorbent pads removed oil from the water surface. Effluent wastewater was filtered over layers of polypropylene PomPom Oil-Mops to remove insoluble oil from the water prior to discharging, where effluent samples were collected and PomPom's were changed if oil concentrations exceed the minimum guidelines (10 ppm) for wastewater discharge in Canada.

Subsurface Oil Injection System - A custom subsurface oil injection system was used to generate dispersed oil plumes in the tank (Figure 1), consisting of a 2 L stainless steel pressure vessel and a series of valves and pressure gauges, fastened to the outer wall of the tank. Inside the tank, a fitting connects the outer assembly to a nozzle (2.4 mm), which extends mid-width perpendicular to the tank wall (9 m downstream from the influent pipe) and is angled at the tip to direct the discharge plume downstream and use horizontal length of the tank to capture the plume movement. For each experiment, oil or oil/dispersant premix was added to the pressure vessel in order to reduce the influence of any additional confounding factor of mixing effectiveness. It should be noted that pre-mixing would expectedly enhance the dispersion compared to dispersant addition to an oil jet (Nedwed, 2015). Copper coil within the pressure vessel connects to a water bath to permit oil heating to 80°C, and then pressurized (40 psi for ANS and SLC; 60 psi for IFO 120) with compressed Nitrogen. A ball valve connected to the vessel controls the oil release through the subsurface nozzle into the tank. Release time and total

ED_001324_00001290-00006

volume (determined by mass) of oil injected are recorded. After each experiment, the subsurface injector system was cleaned by flushing with toluene, acetone and fresh water.

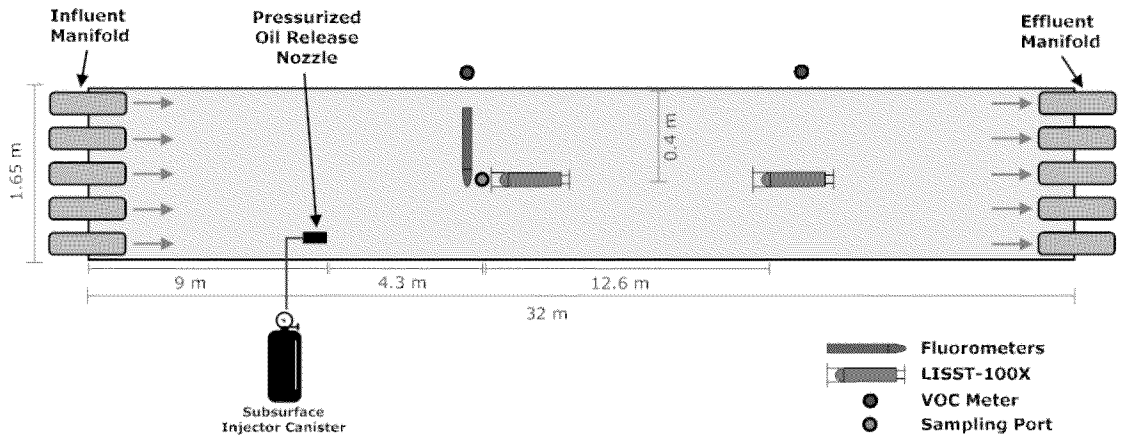


Figure 1. Schematic diagram showing the location of the subsurface injector and *in situ* instrumentation submerged within the tank.

Submersible Sensor Deployment - Sensors were mounted on an aluminum frame located 4.3 m from the oil release point with their pressure windows and at a depth of 0.4 m. The instruments were attached to a crosspiece support bar, so that they were all located the same distance downstream from the oil release point with the pressure window pointed directly towards the bottom of the tank. Data acquisition systems were used to control and collect data with real-time displays from the *in situ* fluorometers. A total of six *in situ* hydrocarbon fluorometers were evaluated during this study; Chelsea Technologies Group UV Aquatrackas - Refined and Crude models, Turner Designs Group Cyclops - Fine and Crude models, Sea Bird – Wet Labs Inc ECO-FLU, and GmBH Trios. Sampling rates and units of signal intensity varied for each instrument with one reading every 5 seconds for GmBH (µg/L PAH), every 3 seconds for Cyclops (auto-gain feature; mV), and every 1 second for the ECO and Aquatrackas (µg/L QSDE, µg/L perylene and µg/L carbazole, respectively. Oil droplet size was measured *in situ*

ED_001324_00001290-00007

using two LISST-100X (Laser *in-situ* Scattering and Transmissometry) particle size analyzers (Sequoia Scientific, Inc), measuring particle size from 2.5 – 500 μm. The first LISST (upstream LISST) was located immediately after the fluorometer package support frame at a distance of 5.1 m from the oil release point and the second LISST (downstream LISST) was located at 16.9 m from the oil release point and both at a depth of 0.4 m (Figure 1). Placement was informed by the numerical modeling efforts conducted previously in the tank to maximize oil droplet detection without saturating the instrument. Real-time data was provided by LISST-SOP data acquisition software (ver. 5) with a sample acquisition rate of one measurement every 3 seconds. Underwater video of oil droplets and the transport of the plume were captured using a GoPro Hero4 digital camera, as well as a Sony RX100 III digital camera with underwater housing.

Submersible Sensor Data Processing - LISST data files were processed using a statistically-based quality control script written using the R statistical package. This script identifies and removes "Over Range" samples (defined as 0 μL/L) and outliers. Outliers are defined as any reading that is greater than the moving mean (5 data points before and after the targeted time point) of the dataset multiplied by four times the standard deviation (over the same interval as the moving mean). Due to the potential for one or more extreme outliers to skew both the moving mean and standard deviation calculations for points around them, this outlier detection routine is run iteratively, excluding previously flagged points, until no more outliers are detected. The script then calculates the Total Particle Concentration (TPC), Volume Mean Diameter (VMD), and Particle Size Concentration (PSC). It then detects the plume curve (if present) and time-normalizes the data based on that location. Data are presented as Droplet Size Distribution (DSD). Plots presented include data 2 minutes before and 8 minutes after the start of the plume curve. Data from the Downstream LISST were normalized so that the plume began at

ED_001324_00001290-00008

t = 5 min in order to visually convey that the plume was detected in the tank roughly 3 min after detection by the LISST further upstream near point of injection release. Similar to the LISST data, a script was used to detect outliers in the fluorometry data. Curve detection was performed and the data was time-normalized to include 2 minutes of data before, and 8 minutes of data after the start of the plume curve. The baseline of the plume curve was calculated using data points observed in the first minute preceding the start of the curve and this baseline was subtracted from the data. Finally, factory calibration factors were applied to the data.

Experimental Design - Seawater temperature and salinity in the tank were recorded using a handheld probe (YSI Incorporated, Yellow Springs, OH). Prior to experiments, the tank was operated in recirculation mode (10 minutes) to allow current flow to stabilize. Five minutes prior to oil injection, data-logging on all instruments was started and background seawater samples were collected. After oil injection, the real-time readout of the fluorometer signal was monitored for the first spike in signal intensity (usually after 2 minutes), and bottle chemistry sample collection began. The experiments ran for 12 minutes and the tank was cleaned using Big Orange Degreaser, to prevent any potential contamination between experiments. Instrument windows were cleaned using disposable alcohol wipes. Water samples were returned to the lab and stored at 4°C.

Discrete Chemistry Samples - Water samples for chemical analysis were collected at various time points throughout the experiments. Three ¼" stainless steel tubes were attached to the aluminum fluorometer frame, so that the end of the tube was located at the same depth as the instrument pressure windows (0.4 m). These were attached via silicon tubing to a digital peristaltic pump with a 120 mL/min flow rate. Samples were analyzed for Total Petroleum Hydrocarbon (TPH) using a method developed by DFO in-house (Cole et al., 2007; King et al.,

ED_001324_00001290-00009

2015) for Gas Chromatograph (GC) - Flame Ionization Detector (FID) analysis. An eight point calibration was generated using standards prepared from the appropriate crude oil stock that was used to generate the TPH samples (e.g. ANS, IFO 120 and SLC). Peak quantification was performed using relative response factors. Samples were analyzed for benzene, toluene, ethylbenzene and xylene (BTEX) using EPA Method 8240 (purge and trap), modified by running a GC - Mass Spectrometer (MS) in selected ion monitoring mode to include ethylbenzene. BTEX standards were prepared in 40 mL purge and trap vials; and samples and standards were analyzed using this method, along with sample blanks and duplicate samples. TPH and BTEX results are not shown here in detail but can be found in the EPA 600/F-16/250 Report (2016).

RESULTS & DISCUSSION

A total of 72 experiments were conducted in replicate, where water ranged in temperature from 4.9 - 20.3 °C, with 11 °C serving as the cut off between warm and cold water tests. Previous experiments within the BIO flume tank facility have assessed dispersion effectiveness as a function of energy dissipation rate and particle size distribution (Li et al. 2009a) and demonstrated that DE is strongly dependent on wave conditions, dispersant type, and oil type (Lee et al. 2009) and highly correlated with *in-situ* droplet size distribution (Li et al. 2008; Li et al. 2009a). Here, DE was evaluated via response monitoring tools (fluorescence and particle size analyzers) during high-pressure subsurface injection experiments.

Dispersant-to-Oil Ratio (DOR) Effects- Addition of either Corexit 9500 or Finasol OSR 52 chemical dispersants to Alaskan North Slope (ANS), IFO 120 and South Louisiana Crude (SLC) oils resulted in a decrease in the Volume Mean Diameter (VMD) and shifted the Droplet

ED_001324_00001290-00010

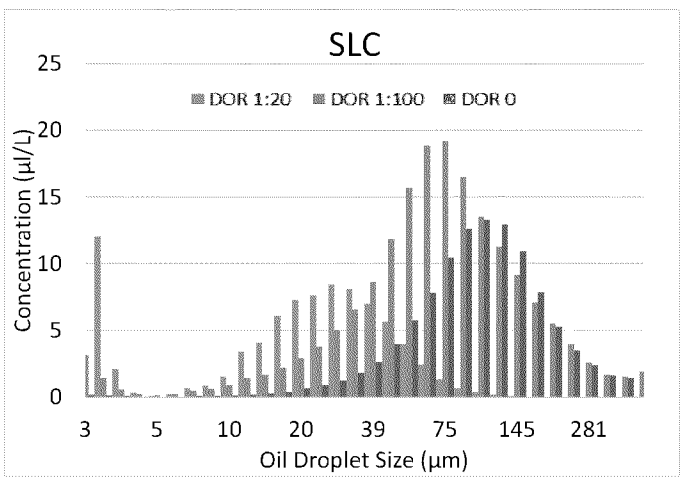
Size Distribution (DSD) to smaller droplets for warm water treatments (Figure 2). For ANS, dispersions created without chemical dispersants or Dispersant-to-oil ratio (DOR) = 1:100 yielded VMD > 70 μm and exhibited unimodal DSD. Dispersions created with DOR = 1:20 yielded VMD < 70 μm size range with a bimodal DSD. SLC oil was more dispersible compared to ANS for treatments with and without chemical dispersant. Bimodal distribution was observed during DOR = 1:20 and some DOR = 1:100 experiments indicating that the jet release of this particular oil into warm water produced smaller droplets than the ANS. During the DWH spill, droplets ≤ 70μm were considered small enough to remain in suspension and were monitored as a proxy for evaluating oil dispersion efficiency (JAG Report, 2010). This suggests that produced SLC droplets from a DOR = 1:20 or 1:100 dispersant injection, and ANS droplets at DOR = 1:20 would likely remain dispersed in the presence of mixing energy given the larger proportion of small droplet sizes observed. In general, Corexit 9500 produced slightly smaller droplets compared to Finasol OSR 52 for warm water treatments (11-20°C). The addition of Corexit 9500 or Finasol OSR 52 to IFO 120 during warm temperature experiments resulted in a shift in DSD and a decrease in VMD; however bimodal distribution was not achieved and even DOR = 1:20 did not yield VMD less than 70 μm in most cases. This suggests that dispersant addition to this oil at warm temperatures would not yield droplet sizes that would likely remain in suspension.

Water Temperature Effects - For ANS, dispersion with < 70 μm droplet VMD was observed for the DOR = 1:20 treatments at both cold and warm water temperatures (Figure 3). Water temperature did not appear to influence the DSD or VMD for this lighter crude oil. However, a temperature effect was observed on the Total Particle Concentration (TPC), where lower temperatures coincided with fewer particles dispersed within the plume for a given volume

ED_001324_00001290-00011

of oil injected. This may be due to enhanced density differences between the oil and water at colder temperatures, thus quicker rise time of the produced droplets. For the addition of Corexit 9500 or Finasol OSR 52 to IFO 120 at cold water temperatures, smaller droplet sizes were not observed, where DOR = 1:20 remained above 200 μm. This suggests that dispersant addition to this oil at cold temperatures would not yield droplet sizes that would likely remain in suspension.

Water Temperature Effects - For ANS, dispersion with < 70 μm droplet VMD was observed for the DOR = 1:20 treatments at both cold and warm water temperatures (Figure 3). Water temperature did not appear to influence the DSD or VMD for this lighter crude oil. However, a temperature effect was observed on the Total Particle Concentration (TPC), where lower temperatures coincided with fewer particles dispersed within the plume for a given volume of oil injected. This may be due to enhanced density differences between the oil and water at colder temperatures, thus quicker rise time of the produced droplets. For the addition of Corexit 9500 or Finasol OSR 52 to IFO 120 at cold water temperatures, smaller droplet sizes were not observed, where DOR = 1:20 remained above 200 μm. This suggests that dispersant addition to this oil at cold temperatures would not yield droplet sizes that would likely remain in suspension.



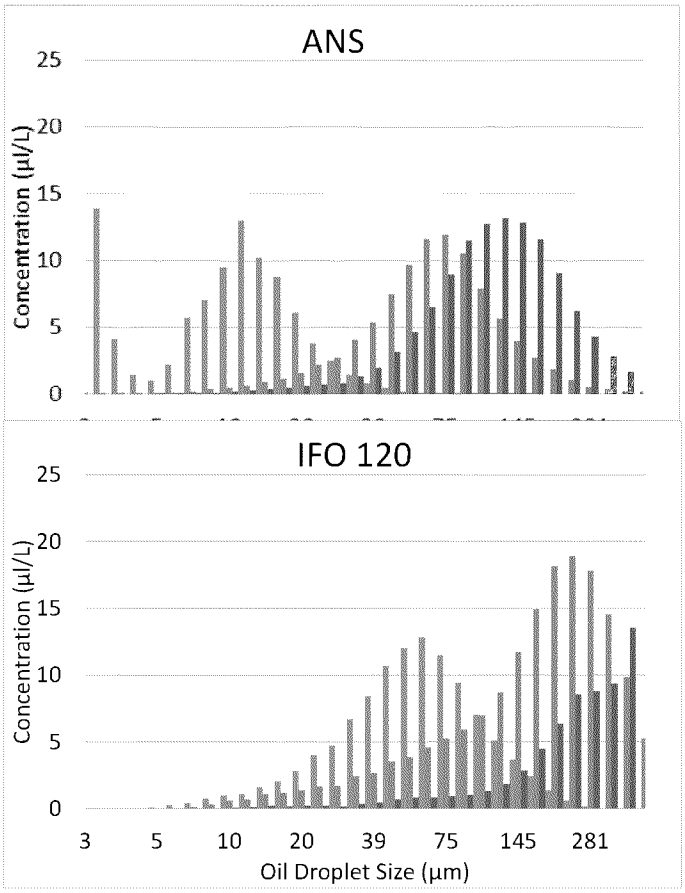


Figure 2. LISST DSD for SLC, ANS and IFO 120 with Corexit 9500 at three DOR treatments in warm water (11-20°C). Histograms represent averages obtained at the 2 minute time stamp during each experiment.

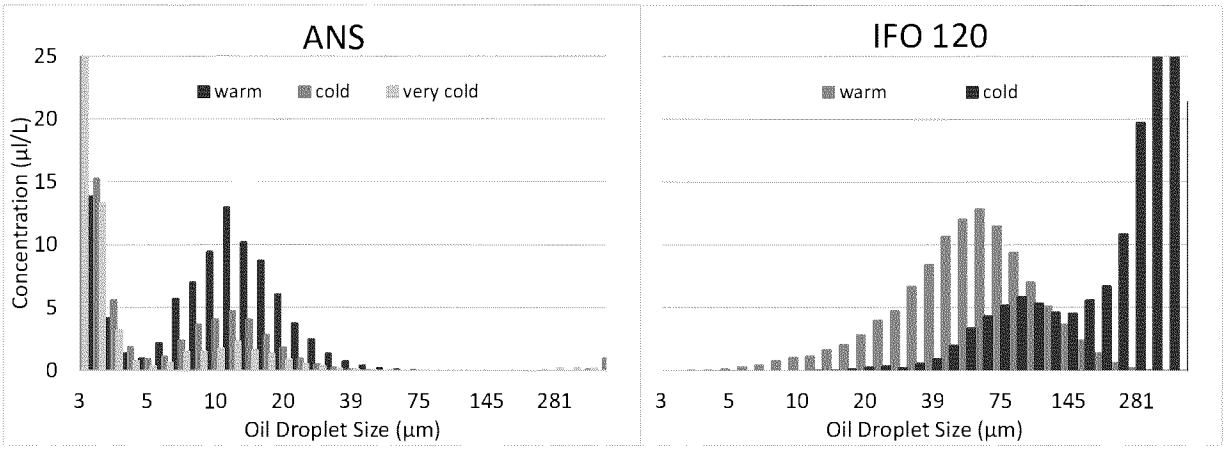


Figure 3. LISST DSD for ANS and IFO 120 with Corexit 9500 at DOR = 1:20 and varying

water temperature. ANS temperatures are 15, 9 and 6 °C for warm, cold and very cold classifications. IFO temperatures are 17 and7 °C.

For experiments conducted at water temperatures < 5 °C, the LISST particle size analyzer yielded unexpected DSD where even a unimodal distribution was not measured. Chemistry and *in situ* fluorescence data indicate that the oil was in fact dispersed adequately. This suggests potential operational problems for this unit < 5 °C, even though it is within the operating temperature (manufacturer manual). Additional testing of cold water temperature limits is recommended. Data collected for < 5 °C temperatures are not included in this paper.

Upstream and Downstream Particle Fractionation - Histograms representing the particle concentration for a given size class from the upstream and downstream LISSTs suggest dilution and fractionation of particles within the plume. The downstream LISST positioned further from the jet release serves as an indication of plume evolution through the tank. For all oil and DOR treatments, dilution in particles (decrease in TPC) within the plume expectedly occurred. Also evident is a shift to smaller particles for all DOR treatments as the plume moves through the tank, where size fractionation was dependent on DOR treatment (Figure 4). Physical dispersion (DOR 0) treatments for lighter oils were found to have the largest shift in DSD and VMD between the upstream and downstream LISSTs suggesting the largest effect of particle fractionation in the plume. Where the decrease in TPC suggests plume dilution in the tank, the DSD shift to smaller particles suggests that within each experiment larger droplets were removed from the plume within 6 minutes of the oil release, most likely rising to the surface of the tank. The effect was least observed in the DOR 1:20 treatments where dilution occurred but the presence of smaller droplets (with lower rise times) at the point of release

ED_001324_00001290-00014

most likely didn't allow for fractionation. The effects of fractionation were observed in warm and cold treatments.

Time series particle size data allows for evaluating plume dynamics over the duration of the experiments. Figure 5 depicts time series contour plots of the plume, where colored contours represent the particle concentration (normalized to max value for comparison purposes) for given droplet sizes through time. These plots allow for ascertaining how the DSD shifts over the duration of the release as the plume moves in the horizontal direction in the tank. For ANS, data suggest that the leading edge of the plume, as detected by the upstream LISST, exhibited a wide range in DSD during the first few minutes after release. As the plume advances down the tank, the DSD becomes narrower with a shift in the VMD for the DOR = 1:100 and 0 treatments. DSD remained similar for the DOR = 1:20 treatment. Warm and cold water treatments exhibit similar trends.

ED_001324_00001290-00015

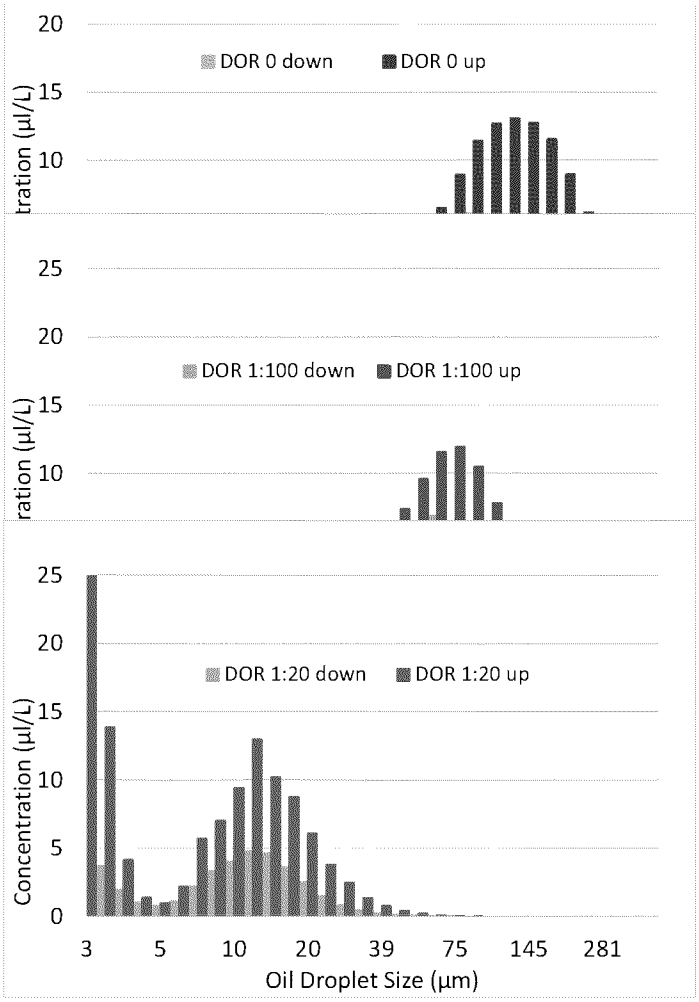


Figure 4. Upstream and downstream LISST Droplet Size Distribution and particle concentration for ANS with Corexit 9500 during warm water treatments.

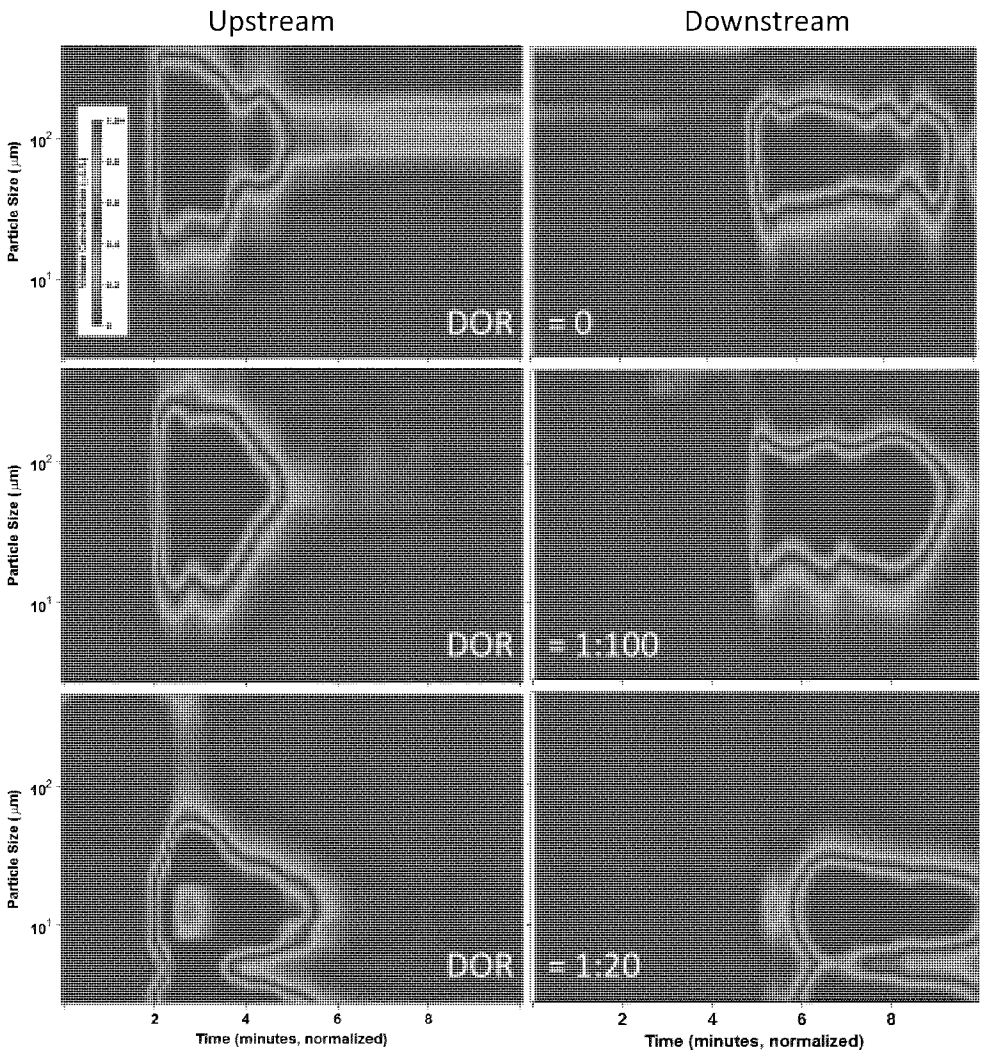


Figure 5. Time series contours of droplet size distribution and particle concentration from the upstream (left panels) and downstream (right panels) LISSTs for ANS with Corexit 9500 during warm water treatments.

Plume Fluorescence Intensity – In addition to the plume transport of particles (oil droplets), dissolved hydrocarbons were also measured via discrete samples and by proxy using fluorescence intensity. The reliability of *in situ* fluorescence measurements as a proxy for oil concentration within physically and chemically dispersed oil has been demonstrated previously

within the tank (Conmy et al., 2014). Here, *in situ* fluorescence serves as a good proxy for oil concentration during the subsurface injection experiments. However, heterogeneity of the produced plumes and the short time scale of experiments (~10 min) led to difficulties in correlations between the plume particle size analyses and chemistry results. This is in part due to discrete samples representing 15 second averages as opposed to instantaneous measures given by fluorometers and particle size analyzers. Thus, although general trends were similar, exact matchups between *in situ* data streams and discrete samples proved to be challenging (Figure 6). Given the experimental design, fluorescence was found to be better suited for correlation with total particle concentration (Figure 7). Depicted are the in situ fluorescence and TPC data during a warm water experiment of ANS with (left) and without (right) Corexit 9500. When exposed to dispersant, fluorescence estimates of dissolved hydrocarbons was higher compared to oil alone treatments, and signal persisted for longer periods of time. Whereas a sharper decay in the fluorescence intensity and the TPH values were observed for the physically dispersed oil.

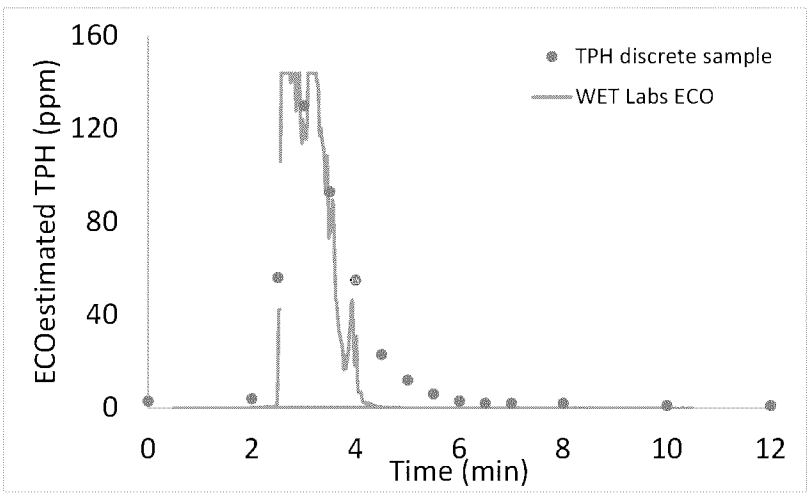


Figure 6. Fluorescence intensity and TPH samples for a warm water treatment using SLC

at DOR = 1:20.

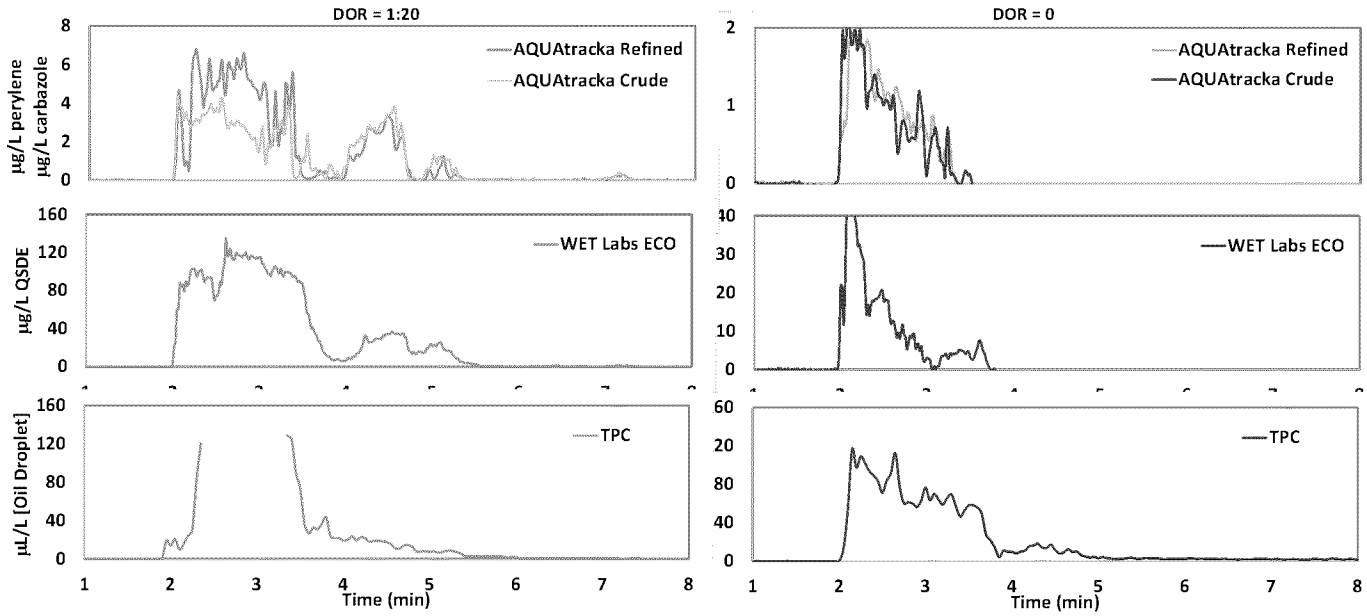


Figure 7. Fluorescence intensity and TPC as a function of time for ANS warm water treatment.

CONCLUSIONS

In situ oil droplet size distribution, particle concentration and fluorescence data from this study are essential for better understanding dispersion effectiveness and for refining droplet formation and trajectory models, such as modified Weber Number technique (Johansen et al., 2013) and the VDROP-J model (Zhou et al., 2014). These results demonstrate that during high pressure releases, light crude oils such as SLC form droplets with VMD < 70 m and bimodal DSD for 1:20 and 1:100 DOR regardless of water temperature. The effect was similar for the medium ANS crude oil, but bimodality was only observed for the 1:20 treatment. Conversely, Bimodal distribution was not achieved with the heavy IFO, but droplet < 70 µm were observed

for 1:20 warm waters, suggesting poor dispersibility of the high viscosity oil even for jet releases. Droplet fractionation observed in the plumes was greatest for the lighter crude and demonstrates the separation that would occur as a plume rises and is advected horizontally in a water column. Thus, findings from this study have implications for predicting the behavior of oil and plume formation, decision-making pertaining to SSDI as a countermeasure, and assessing the transport and fate of slicks and subsurface plumes in support of emergency response operations during any future deep water spills.

ACKNOWLEDGEMENTS

Experiments at Department of Fisheries and Oceans (DFO) Canada – Bedford Institute of Oceanography were funded via Interagency Agreement with the Bureau of Safety and Environmental Enforcement, and internal funding and salary support from DFO Canada and the U.S. Environmental Protection Agency (EPA). This document has been reviewed by the U.S. EPA. The authors thank all funding sources. This document has been reviewed by the U.S. EPA National Risk Management Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. This is a contribution from the Land Remediation and Pollution Control Division.

REFERENCES

Conmy, R. N., et al. (2014) Submersible Optical Sensors Exposed to Chemically Dispersed Crude Oil: Wave Tank Simulations for Improved Oil Spill Monitoring. Environ. Sci. Technol. 48(3): 1803-1810.

Cole, M.G., T. King, K. Lee (2007) Analytical technique for extracting hydrocarbons from water using sample container as extraction vessel in combination with a roller apparatus. Anal. Chem., 77: 2210-17.

CRRC Report (2010) Coastal Response Research Center Deepwater Horizon Dispersant USE

ED_001324_00001290-00020

Meeting Report, May 26-27, 2010, 108 pp.

EPA 600/F-16/250 Federal Report (2016) Dispersant Effectiveness, In-Situ Droplet Size Distribution and Numerical Modeling to Assess Subsurface Dispersant Injection as a Deepwater Blowout Oil Spill Response Option *and* Evaluation of Oil Fluorescence Characteristics to Improve Forensic Response Tools; IAA No. E12PG00037 for DOI BSEE, 144 pp.
Joint Analysis Group Report (2010) Review of Data to Examine Subsurface Oil in the Vicinity of DWH MC-252, May 19-June 19, 2010; 29 pp.

Johansen, O., et al. (2013) Droplet breakup in subsea oil releases - Part 2: Predictions of droplet size distributions with and without injection of chemical dispersants. Mar. Poll. Bull. 73(1): 327-335.

Lee, K., Li, Z., Boufadel, M.C., Venosa, A.D. and Scott Miles, M. (2009) Wave tank studies on dispersant effectiveness as a function of energy dissipation rate and particle size distribution. Final report submitted to NOAA/CRRC/UNH, 67 pp.

Li, Z., Lee, K., King, T., Boufadel, M.C. and Venosa, A.D., (2008) Oil droplet size distribution as a function of energy dissipation rate in an experimental wave tank 2008 International Oil Spill Conference. American Petroleum Institute, Washington D.C., Savannah, GA, pp. 621-626.

Li, Z., Lee, K., King, T., Boufadel, M.C. and Venosa, A.D., (2009a) Evaluating crude oil chemical dispersion efficacy in a flow-through wave tank under regular non-breaking wave and breaking wave conditions. Mar. Poll. Bull., 58: 735-744.

Li, Z., Lee, K., King, T., Boufadel, M.C. and Venosa, A.D., (2009b) Evaluating Chemical Dispersant Efficacy in an Experimental Wave Tank: 2, Significant Factors Determining *In Situ* Oil Droplet Size Distribution. Environ. Engin. Sci., 26(9): 1407-1418.

Li, Z., Lee, K., King, T., Boufadel, M.C. and Venosa, A.D., (2010) Effects of temperature and wave conditions on chemical dispersion efficacy of heavy fuel oil in an experimental flow-through wave tank. Mar. Poll. Bull., 60(9): 1550-9.

Nedwed, T. (2015) Overview of the American Petroleum Institute Joint Industry Task Force Subsea Dispersant Injection Project. Interspill Conference Proceedings.

NRC Report (2005) National Research Council Oil Spill Dispersants: Efficacy and Effects, Washington, DC, 400 pp.

Wickley-Olsen, E., Boufadel, M.C., King, T., Li, Z., Lee, K., Venosa, A.D. (2007) Regular and breaking waves in wave tank for dispersion effectiveness testing. In: Proceedings of the 30th Arctic and Marine Oil spill Program (AMOP) Technical Seminar, Edmonton, AB, Canada. Environment Canada, Ottawa, Ontario, Canada, pp. 161–187.

Zhao, L., et al. (2014). Evolution of droplets in subsea oil and gas blowouts: Development and validation of the numerical model VDROP-J. Mar. Poll. Bull., 83(1): 58-69.

ED_001324_00001290-00021

To: King, Thomas L[Tom.King@dfo-mpo.gc.ca]; Robinson, Brian[Brian.Robinson@dfo-mpo.gc.ca]; boufadel@gmail.com[boufadel@gmail.com]
From: Conmy, Robyn
Sent: Tue 3/31/2015 5:06:18 PM
Subject: FW: Request for Proposal No. E15PS00027 (Broad Agency Announcement)
Conmy-EPA Letter T7-3.pdf
RFP E15PS00027 - Oil Spill Response Research.PDF

FYI – BSEE has requested a full proposal for our hypersaline DE LOI. I am down at USF this week, but will try to arrange a PI call for next week.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

From: Laikin-Credno, Caroline [mailto:caroline.laikin-credno@bsee.gov]
Sent: Monday, March 30, 2015 7:04 PM
To: Conmy, Robyn
Subject: Request for Proposal No. E15PS00027 (Broad Agency Announcement)

The Bureau of Safety and Environmental Enforcement (BSEE) has completed its review of the White Papers submitted under the Broad Agency Announcement No. E1EPS00027 for Proposed Research on Oil Spill Response Operations on the U.S. Outer Continental Shelf.

BSEE has determined that your White Paper has merit. Therefore, we would like your organization to submit a full proposal. A formal letter and a request for proposal are attached.

PLEASE REPLY BACK TO ME ACKNOWLEDGING RECEIPT OF THE RFP.

Thanks,

Caroline Laikin

BSEE Contracting Officer

United States Department of the Interior
BUREAU OF SAFETY AND ENVIRONMENTAL ENFORCEMENT
WASHINGTON, DC 20240-0001

March 30, 2015

U.S. Environmental Protection Agency
Attn: Robyn N. Conmy, Ph.D.
26 W. MLK Drive
Cincinnati, OH 45268

Subject: Broad Agency Announcement (BAA) Solicitation No. E15PS00027 for Proposed Research on Oil Spill Response Operations on the U.S. Outer Continental Shelf, Topic 7 - *"Characterizing Dispersant Effectiveness of Crude Oils at High Salinities: Implications for Subsea Spill Preparedness"*; Request for Proposal

Dear Dr. Conmy:

The Bureau of Safety and Environmental Enforcement (BSEE) has completed its review of the White Paper entitled ***White Paper on Developing an Innovative Dispersant Spray Drift Model*** by your organization in response to the BSEE Broad Agency Announcement under the subject solicitation. At the conclusion of our review of your White Paper, we found your subject matter to have merit and would like your organization to submit a full proposal (Technical, Business and Cost). Please submit your proposal in accordance with the attached Request for Proposal (RFP).

BSEE suggests the following items are considered when preparing your Technical proposal:

a) Consider testing Accell Clean DWD;
b) Keep the test matrix reasonable; test fewer salinities if necessary.

The Government anticipates issuing a Firm Fixed Price (FFP) contract for this effort with an anticipated period of performance not to exceed 12 months. The RFP contains a model standard contract format, which includes terms, conditions, and clauses that are anticipated to be included in contracts that are awarded as a result of the RFP. Specific terms, conditions, and clauses included in the resulting contracts may vary due to the specific circumstances of each award.

Proposals must be submitted in accordance with the instructions provided in Section L of the RFP no later than the deadline for receipt of proposals established in Section L.8. Your proposal will be evaluated based on the factors in Section M of the RFP. This request does not commit the Government to award a contract, to pay any costs incurred in the submission of a proposal, or pay any costs incurred in making necessary studies or designs for its preparation or any subsequent negotiation. In the event you do not intend to respond to this request, I would appreciate receiving a notice to that effect.

If you have any questions regarding this requirement, please submit your inquiries immediately via e-mail to: caroline.laikin-credno@bsee.gov

Sincerely,

//S//

Caroline Laikin-Credno
Contracting Officer

Attachment: RFP E15PS00027

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL RISK MANAGEMENT RESEARCH LABORATORY
CINCINNATI, OHIO 45268

April 30, 2015

Ms. Caroline Laikin
Bureau of Safety and Environmental Enforcement
Acquisitions Operations Branch
45600 Woodland Road
Sterling, VA 20166
Phone: 703-787-1828

Ms. Caroline Laikin,

Please find the enclosed Technical and Cost Proposals in response to the Bureau of Safety and Environmental Enforcement's (BSEE) current Broad Agency Announcement #E15PS00027, Topic 7, "Proposed Research on Oil Spill Response Operations in the US Outer Continental Shelf". This submission is from the U.S. Environmental Protection Agency, along with collaborators from the Department of Fisheries and Oceans Canada and the New Jersey Institute of Technology, entitled "*Characterizing Dispersant Effectiveness of Crude Oils at High Salinities: Implications for Subsea Spill Preparedness*". It is provided in one-original, three-hard copy and one-electronic form. Copies of the signed Standard Form 33 and Standard Form 30 amendments appear within the Cost Proposal and the original is also provided. If there are any questions regarding this submission, please contact Dr. Robyn Conmy.

Thank you,

_____

Robyn N. Conmy, Ph.D.
U.S. EPA Office of Research and Development
National Risk Management Research Laboratory
Oil Research Program Lead
26 MLK Drive West
Cincinnati, OH 45255
Phone: 513-569-7090
Conmy.robyn@epa.gov

To: conmy.robyn@gmail.com[conmy.robyn@gmail.com]
From: Conmy, Robyn
Sent: Mon 3/27/2017 5:25:32 PM
Subject: iosc
IOSC 2017_Conmy et al_plume simulations paper_final.docx

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

Oil plume simulations: Tracking oil droplet size distribution and fluorescence within high-pressure release jets

R.N. Conmy[1], **B. Robinson** [2], **T. King** [2], **M. Boufadel**[3], **S. Ryan**[2], **C. McIntyre**[2], **M. I. Abercrombie**[4], **K. Lee** [2,5]

[1]U.S. Environmental Protection Agency, Office of Research and Development, 26 MLK Drive West, Cincinnati, OH 45255, USA

[2] Bedford Institute of Oceanography, Fisheries and Oceans Canada, 1 Challenger Dr, Dartmouth, NS, B2Y 4A2, Canada

[3] New Jersey Institute of Technology, Department of Civil and Environmental Engineering, University Heights, Newark, NJ 07102, USA

[4]University of South Florida, College of Marine Science, 140 7th Ave South, St. Petersburg, FL 33701, USA

[5] Commonwealth Scientific and Industrial Research Organisation, Oceans and Atmosphere Flagship, PO Box 1130, Bentley WA 6102, Australia

ABSTRACT

Optical measurements have been used during oil spill response for more than three decades to determine oil presence in slicks and plumes. Oil surveillance approaches range from simple (human eyeball) to the sophisticated (sensors on AUVs, aircraft, satellites). *In situ* fluorometers and particle size analyzers were deployed during the Deepwater Horizon (DWH) Gulf of Mexico oil spill to track shallow and deep subsea plumes. Uncertainties regarding instrument specifications and capabilities during DWH necessitated performance testing of sensors exposed to simulated, dispersed oil plumes. Seventy-two wave tank experiments were conducted at the Bedford Institute of Oceanography. Simulated were oil releases with varying parameters such as oil release rate, oil temperature (reservoir temp ~ 80 °C), water temperature (<8 °C and >15 °C), oil type, dispersant type (Corexit 9500 and Finasol OSR52) and dispersant

ED_001324_00001349-00001

to oil ratio (DOR). Plumes of Alaskan North Slope Crude (ANS), South Louisiana Crude (SLC) and IFO-120 oils were tracked using *in situ* fluorescence, droplet size distribution (DSD), total petroleum hydrocarbons (TPH) and benzene-toluene-ethylbenzene-xylene (BTEX). For the lighter SLC, bimodal droplet size with mean diameter < 70 m was achieved for 1:20 and 1:100 DOR, regardless of water temperature. Similarly, the medium ANS crude exhibited mean droplet diameter <70 μm, but was bimodal only for the 1:20 treatment. Bimodal distribution was not achieved with the heavy IFO, but droplet < 70 μm were observed for 1:20 warm waters, indicating poor dispersibility of the high viscosity oil even for jet releases. Results offer valuable information on the behavior and dispersibility of oils over a range of viscosity, DOR and environmental conditions. Findings have implications for fate and transport models, where DSD, chemistry and fluorescence are all impacted by release variables. This research was supported by the Bureau of Safety and Environmental Enforcement.

INTRODUCTION

The 2010 *Deepwater Horizon* (DWH) oil spill highlighted the pressing need for a better understanding of the interaction of chemical dispersants and crude oil at ocean depth. Early in the blowout release, partial emulsification of oil and heterogeneous slicks were observed as it rose to the surface from 1500-m depth (JAG report, 2010). As a countermeasure, Sub-Sea Dispersant Injection (SSDI) was used, where dispersants were injected directly at the release point as a possible means to increase dispersion efficiency and to potentially reduce the amount of dispersant needed if applied at the air-sea interface (CRRC Report, 2010). Potential advantages of this application during a continuous oil release included the fact that the fresh (un-weathered) oil was considered well suited for dispersion, operators were able to inject the

ED_001324_00001349-00002

dispersant directly into the oil stream thereby maximizing dispersant/oil contact, sufficient control of DOR (Dispersant-to-Oil Ratio) could be maintained, injection may minimize the need for surface application because of reduced oil surfacing, and optimized subsurface application would likely promote formation of smaller and stable oil droplets, enhancing biodegradation (Lee et al., 2009). The Unified Area Command (UAC) coordinated a large-scale environmental monitoring program to track and characterize dispersed subsurface oil plumes based on field data and transport modeling outputs for amount and location of oil. Oil Droplet Size Distribution (DSD) and fluorescence intensity from submersible sensors were used as an indication of Dispersion Effectiveness (DE) onboard vessels, where concentrations were monitored to evaluate oil dispersion (presence of small droplets $\leq$ 70 μm) based on previous studies for surface dispersant applications (Li et al., 2009a). Monitoring provided sound evidence of the presence of oil-bearing small particles both in surface waters and in the subsurface plume (JAG Report, 2010). SSDI correlated with a higher proportion of low molecular weight compounds in subsea waters compared to surface, suggesting that SSDI may have promoted the formation of small oil droplets in the deep sea and thus would have the potential for enhancing the weathering and dissolution of oil in the water column.

However, uncertainties still exist regarding the effectiveness of this application. For example, assumptions of the optimal DOR are based on empirical data mostly obtained from bench-scale experiments designed for testing at standard temperatures and pressures (STP). Such experiments do not take into account the effects of natural dispersion during high-pressure jet releases (i.e. deep water blowout) or the range of conditions on ocean floor, anywhere along a riser, or within the water column that could be significantly different. Hence, DOR for direct

ED_001324_00001349-00003

injection needs to be better understood. Although theoretical analyses and experiments suggest that oil jet breakup is insensitive to the absolute value of hydrostatic pressure for incompressible liquid-liquid systems (Masutani and Adams 2000), the effects of several ambient environmental factors on SSDI, including high release pressure, high oil temperature, low water temperature, and the presence of methane and suspended sediments in the oil plume and/or surrounding water column remain to be clarified. Improved understanding on the influence of these factors and the interaction of oil and chemical dispersant under a range of turbulent regimes at depth is required for informed decision-making for future SSDI use.

For evaluating DE, standard laboratory tests are inherently limited in simulating operational performance under realistic environmental conditions in part due to space constraints that are critical for transport and dilution efficiency (NRC, 2005). Meso-scale tanks capable of generating waves and flume flows to simulate ocean conditions help to address this issue. One such tank facility at the Bedford Institute of Oceanography (BIO; Dartmouth, Nova Scotia) has been used previously to assess chemical dispersant effectiveness for various oil types as a function of energy dissipation rate and particle size distribution (Li et al. 2009b) and demonstrated that the effectiveness of a dispersant is strongly dependent on wave conditions, dispersant type, and oil type (Wickley-Olsen et al. 2007; Li et al. 2008; Lee et al. 2009). A strong correlation has been established between dispersion effectiveness and *in situ* DSD within the hydrodynamic regime, particularly energy dissipation rate, under a variety of wave conditions (Li et al. 2008; Li et al. 2009b), where flume mode can simulate the effects of underwater currents on dispersion and dilution of oil (Li et al. 2009a; Li et al. 2010). Experiments have also shown the reliability of fluorescence intensity as a proxy for oil

ED_001324_00001349-00004

concentration within oil dispersions (Conmy et al., 2014).

Presented here are results from a series of high pressure jet releases of oil within the BIO flume tank to assess the operational performance of SSDI. DSD and fluorescence intensity were used as a proxy for dispersion efficiency for treatments with (chemically-enhanced dispersion) and without (physical dispersion) chemical dispersants. The objective of this work was to evaluate the effects of water temperature and dispersant-to-oil ratio on DE of three oils at high reservoir temperatures. Findings help (1) to determine the applicability of existing *in situ* monitoring technologies for SSDI and (2) inform efforts to integrate DSD into deep water blowout plume formation models that enable prediction of the dispersed oil droplets under high-flow subsurface release velocities. DWH spill monitoring program DSD and fluorescence data played a significant role in supporting emergency response operations, fate and transport modeling, and impact assessment. Findings from this study have implications for efforts in support of future deep-water spill preparedness.

METHODS

Flume Tank Description - Oil dispersion experiments were conducted using Alaska North Slope crude oil (ANS), South Louisiana Crude (SLC) and Intermediate Fuel Oil 120 (IFO 120) with Corexit 9500 and Finasol OSR 52 chemical dispersants in the 32m long flow-through carbon steel flume tank (31,000 L) at BIO. Oils were chosen to provide a wide range of viscosity. Seawater was pulled from the Bedford Basin (50cm below surface) via two smaller centrifugal pumps (110 gpm), and filtered through high-flow polypropylene bag filters (5 and 25 μm) to a fiberglass holding tank (25,000 L) that supplies seawater for the system to ensure that a constant flow rate is maintained. Two high-flow centrifugal pumps (600 gpm) provided flow to

ED_001324_00001349-00005

generate horizontal water currents in the tank, where flow gauges on influent and effluent pipes were monitored and valve adjustments made to obtain a balanced flow rate throughout experiments. Water current velocities were measured in the tank using an ADV (Nortek Vectrino) to maintain consistent horizontal water current velocities (3.5 cm/s) at all measured depths. At the completion of experiments, oil absorbent pads removed oil from the water surface. Effluent wastewater was filtered over layers of polypropylene PomPom Oil-Mops to remove insoluble oil from the water prior to discharging, where effluent samples were collected and PomPom's were changed if oil concentrations exceed the minimum guidelines (10 ppm) for wastewater discharge in Canada.

Subsurface Oil Injection System - A custom subsurface oil injection system was used to generate dispersed oil plumes in the tank (Figure 1), consisting of a 2 L stainless steel pressure vessel and a series of valves and pressure gauges, fastened to the outer wall of the tank. Inside the tank, a fitting connects the outer assembly to a nozzle (2.4 mm), which extends mid-width perpendicular to the tank wall (9 m downstream from the influent pipe) and is angled at the tip to direct the discharge plume downstream and use horizontal length of the tank to capture the plume movement. For each experiment, oil or oil/dispersant premix was added to the pressure vessel in order to reduce the influence of any additional confounding factor of mixing effectiveness. It should be noted that pre-mixing would expectedly enhance the dispersion compared to dispersant addition to an oil jet (Nedwed, 2015). Copper coil within the pressure vessel connects to a water bath to permit oil heating to 80°C, and then pressurized (40 psi for ANS and SLC; 60 psi for IFO 120) with compressed Nitrogen. A ball valve connected to the vessel controls the oil release through the subsurface nozzle into the tank. Release time and total

ED_001324_00001349-00006

volume (determined by mass) of oil injected are recorded. After each experiment, the subsurface injector system was cleaned by flushing with toluene, acetone and fresh water.

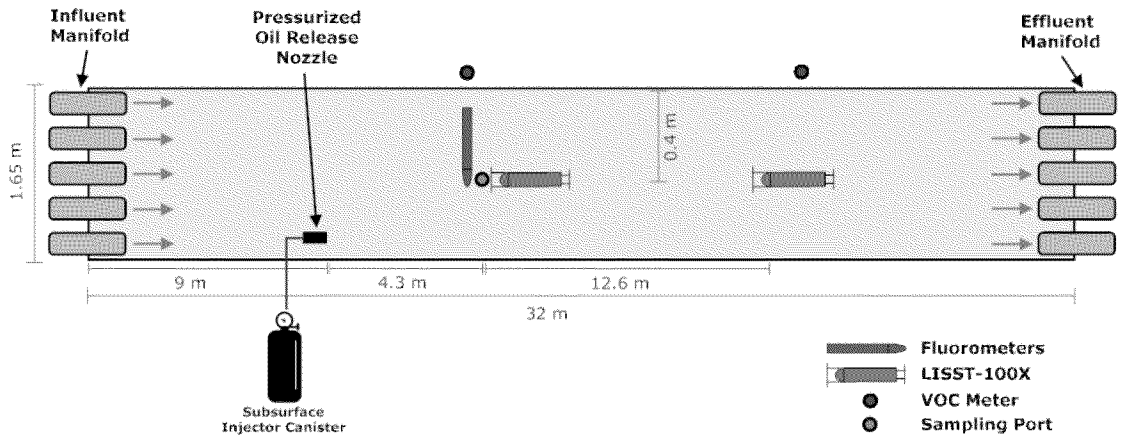


Figure 1. Schematic diagram showing the location of the subsurface injector and *in situ* instrumentation submerged within the tank.

Submersible Sensor Deployment - Sensors were mounted on an aluminum frame located 4.3 m from the oil release point with their pressure windows and at a depth of 0.4 m. The instruments were attached to a crosspiece support bar, so that they were all located the same distance downstream from the oil release point with the pressure window pointed directly towards the bottom of the tank. Data acquisition systems were used to control and collect data with real-time displays from the *in situ* fluorometers. A total of six *in situ* hydrocarbon fluorometers were evaluated during this study; Chelsea Technologies Group UV Aquatrackas - Refined and Crude models, Turner Designs Group Cyclops - Fine and Crude models, Sea Bird – Wet Labs Inc ECO-FLU, and GmBH Trios. Sampling rates and units of signal intensity varied for each instrument with one reading every 5 seconds for GmBH (µg/L PAH), every 3 seconds for Cyclops (auto-gain feature; mV), and every 1 second for the ECO and Aquatrackas (µg/L QSDE, µg/L perylene and µg/L carbazole, respectively. Oil droplet size was measured *in situ*

ED_001324_00001349-00007

using two LISST-100X (Laser *in-situ* Scattering and Transmissometry) particle size analyzers (Sequoia Scientific, Inc), measuring particle size from 2.5 – 500 μm. The first LISST (upstream LISST) was located immediately after the fluorometer package support frame at a distance of 5.1 m from the oil release point and the second LISST (downstream LISST) was located at 16.9 m from the oil release point and both at a depth of 0.4 m (Figure 1). Placement was informed by the numerical modeling efforts conducted previously in the tank to maximize oil droplet detection without saturating the instrument. Real-time data was provided by LISST-SOP data acquisition software (ver. 5) with a sample acquisition rate of one measurement every 3 seconds. Underwater video of oil droplets and the transport of the plume were captured using a GoPro Hero4 digital camera, as well as a Sony RX100 III digital camera with underwater housing.

Submersible Sensor Data Processing - LISST data files were processed using a statistically-based quality control script written using the R statistical package. This script identifies and removes "Over Range" samples (defined as 0 μL/L) and outliers. Outliers are defined as any reading that is greater than the moving mean (5 data points before and after the targeted time point) of the dataset multiplied by four times the standard deviation (over the same interval as the moving mean). Due to the potential for one or more extreme outliers to skew both the moving mean and standard deviation calculations for points around them, this outlier detection routine is run iteratively, excluding previously flagged points, until no more outliers are detected. The script then calculates the Total Particle Concentration (TPC), Volume Mean Diameter (VMD), and Particle Size Concentration (PSC). It then detects the plume curve (if present) and time-normalizes the data based on that location. Data are presented as Droplet Size Distribution (DSD). Plots presented include data 2 minutes before and 8 minutes after the start of the plume curve. Data from the Downstream LISST were normalized so that the plume began at

ED_001324_00001349-00008

t = 5 min in order to visually convey that the plume was detected in the tank roughly 3 min after detection by the LISST further upstream near point of injection release. Similar to the LISST data, a script was used to detect outliers in the fluorometry data. Curve detection was performed and the data was time-normalized to include 2 minutes of data before, and 8 minutes of data after the start of the plume curve. The baseline of the plume curve was calculated using data points observed in the first minute preceding the start of the curve and this baseline was subtracted from the data. Finally, factory calibration factors were applied to the data.

Experimental Design - Seawater temperature and salinity in the tank were recorded using a handheld probe (YSI Incorporated, Yellow Springs, OH). Prior to experiments, the tank was operated in recirculation mode (10 minutes) to allow current flow to stabilize. Five minutes prior to oil injection, data-logging on all instruments was started and background seawater samples were collected. After oil injection, the real-time readout of the fluorometer signal was monitored for the first spike in signal intensity (usually after 2 minutes), and bottle chemistry sample collection began. The experiments ran for 12 minutes and the tank was cleaned using Big Orange Degreaser, to prevent any potential contamination between experiments. Instrument windows were cleaned using disposable alcohol wipes. Water samples were returned to the lab and stored at 4°C.

Discrete Chemistry Samples - Water samples for chemical analysis were collected at various time points throughout the experiments. Three ¼" stainless steel tubes were attached to the aluminum fluorometer frame, so that the end of the tube was located at the same depth as the instrument pressure windows (0.4 m). These were attached via silicon tubing to a digital peristaltic pump with a 120 mL/min flow rate. Samples were analyzed for Total Petroleum Hydrocarbon (TPH) using a method developed by DFO in-house (Cole et al., 2007; King et al.,

2015) for Gas Chromatograph (GC) - Flame Ionization Detector (FID) analysis. An eight point calibration was generated using standards prepared from the appropriate crude oil stock that was used to generate the TPH samples (e.g. ANS, IFO 120 and SLC). Peak quantification was performed using relative response factors. Samples were analyzed for benzene, toluene, ethylbenzene and xylene (BTEX) using EPA Method 8240 (purge and trap), modified by running a GC - Mass Spectrometer (MS) in selected ion monitoring mode to include ethylbenzene. BTEX standards were prepared in 40 mL purge and trap vials; and samples and standards were analyzed using this method, along with sample blanks and duplicate samples. TPH and BTEX results are not shown here in detail but can be found in the EPA 600/F-16/250 Report (2016).

RESULTS & DISCUSSION

A total of 72 experiments were conducted in replicate, where water ranged in temperature from 4.9 - 20.3 °C, with 11 °C serving as the cut off between warm and cold water tests. Previous experiments within the BIO flume tank facility have assessed dispersion effectiveness as a function of energy dissipation rate and particle size distribution (Li et al. 2009a) and demonstrated that DE is strongly dependent on wave conditions, dispersant type, and oil type (Lee et al. 2009) and highly correlated with *in-situ* droplet size distribution (Li et al. 2008; Li et al. 2009a). Here, DE was evaluated via response monitoring tools (fluorescence and particle size analyzers) during high-pressure subsurface injection experiments.

Dispersant-to-Oil Ratio (DOR) Effects- Addition of either Corexit 9500 or Finasol OSR 52 chemical dispersants to Alaskan North Slope (ANS), IFO 120 and South Louisiana Crude (SLC) oils resulted in a decrease in the Volume Mean Diameter (VMD) and shifted the Droplet

ED_001324_00001349-00010

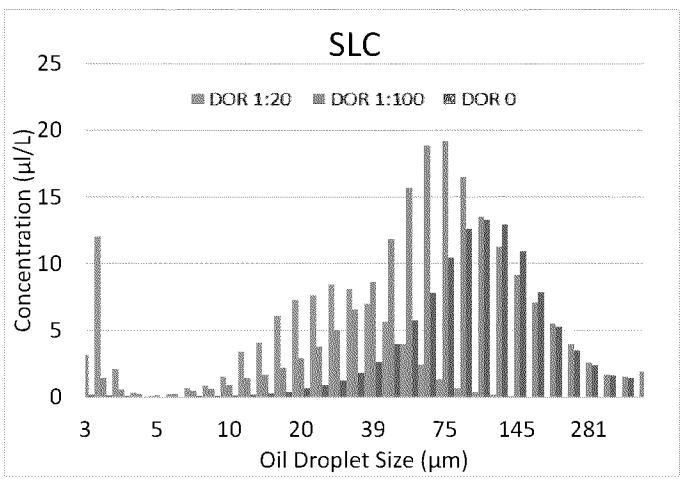
Size Distribution (DSD) to smaller droplets for warm water treatments (Figure 2). For ANS, dispersions created without chemical dispersants or Dispersant-to-oil ratio (DOR) = 1:100 yielded VMD > 70 μm and exhibited unimodal DSD. Dispersions created with DOR = 1:20 yielded VMD < 70 μm size range with a bimodal DSD. SLC oil was more dispersible compared to ANS for treatments with and without chemical dispersant. Bimodal distribution was observed during DOR = 1:20 and some DOR = 1:100 experiments indicating that the jet release of this particular oil into warm water produced smaller droplets than the ANS. During the DWH spill, droplets ≤ 70μm were considered small enough to remain in suspension and were monitored as a proxy for evaluating oil dispersion efficiency (JAG Report, 2010). This suggests that produced SLC droplets from a DOR = 1:20 or 1:100 dispersant injection, and ANS droplets at DOR = 1:20 would likely remain dispersed in the presence of mixing energy given the larger proportion of small droplet sizes observed. In general, Corexit 9500 produced slightly smaller droplets compared to Finasol OSR 52 for warm water treatments (11-20°C). The addition of Corexit 9500 or Finasol OSR 52 to IFO 120 during warm temperature experiments resulted in a shift in DSD and a decrease in VMD; however bimodal distribution was not achieved and even DOR = 1:20 did not yield VMD less than 70 μm in most cases. This suggests that dispersant addition to this oil at warm temperatures would not yield droplet sizes that would likely remain in suspension.

Water Temperature Effects - For ANS, dispersion with < 70 μm droplet VMD was observed for the DOR = 1:20 treatments at both cold and warm water temperatures (Figure 3). Water temperature did not appear to influence the DSD or VMD for this lighter crude oil. However, a temperature effect was observed on the Total Particle Concentration (TPC), where lower temperatures coincided with fewer particles dispersed within the plume for a given volume

ED_001324_00001349-00011

of oil injected. This may be due to enhanced density differences between the oil and water at colder temperatures, thus quicker rise time of the produced droplets. For the addition of Corexit 9500 or Finasol OSR 52 to IFO 120 at cold water temperatures, smaller droplet sizes were not observed, where DOR = 1:20 remained above 200 μm. This suggests that dispersant addition to this oil at cold temperatures would not yield droplet sizes that would likely remain in suspension.

Water Temperature Effects - For ANS, dispersion with < 70 μm droplet VMD was observed for the DOR = 1:20 treatments at both cold and warm water temperatures (Figure 3). Water temperature did not appear to influence the DSD or VMD for this lighter crude oil. However, a temperature effect was observed on the Total Particle Concentration (TPC), where lower temperatures coincided with fewer particles dispersed within the plume for a given volume of oil injected. This may be due to enhanced density differences between the oil and water at colder temperatures, thus quicker rise time of the produced droplets. For the addition of Corexit 9500 or Finasol OSR 52 to IFO 120 at cold water temperatures, smaller droplet sizes were not observed, where DOR = 1:20 remained above 200 μm. This suggests that dispersant addition to this oil at cold temperatures would not yield droplet sizes that would likely remain in suspension.



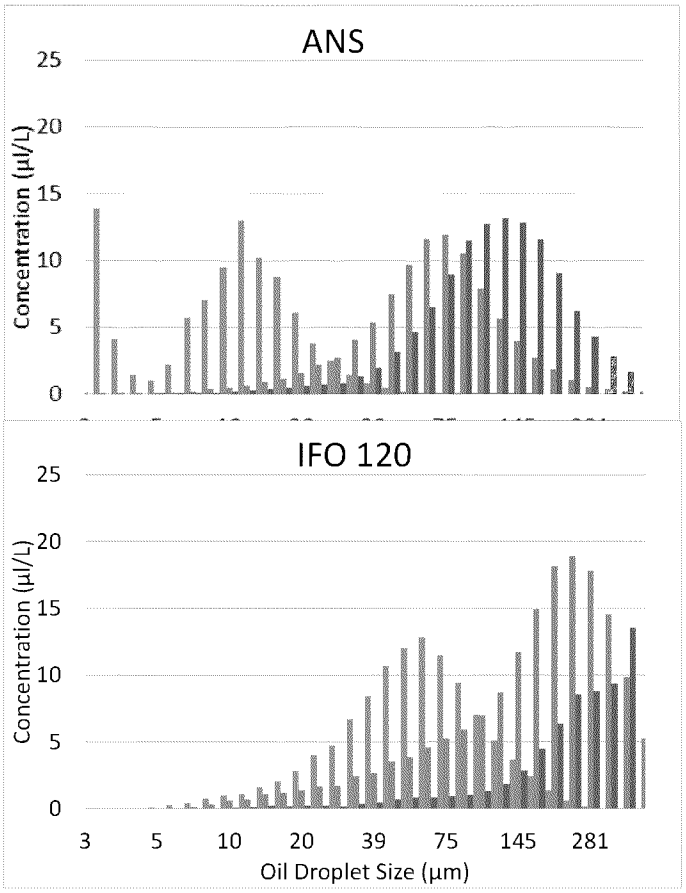


Figure 2. LISST DSD for SLC, ANS and IFO 120 with Corexit 9500 at three DOR treatments in warm water (11-20°C). Histograms represent averages obtained at the 2 minute time stamp during each experiment.

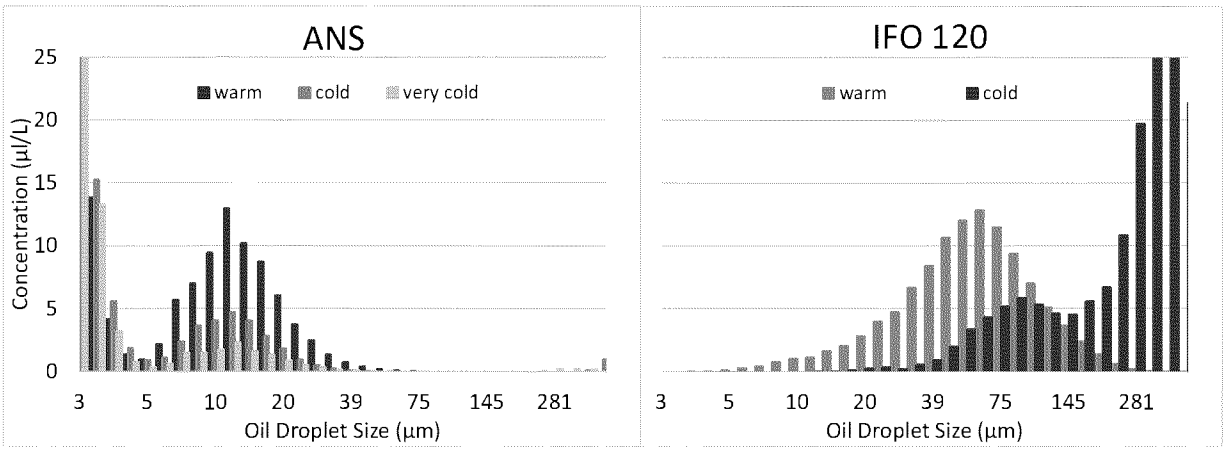


Figure 3. LISST DSD for ANS and IFO 120 with Corexit 9500 at DOR = 1:20 and varying

water temperature. ANS temperatures are 15, 9 and 6 °C for warm, cold and very cold classifications. IFO temperatures are 17 and7 °C.

For experiments conducted at water temperatures < 5 °C, the LISST particle size analyzer yielded unexpected DSD where even a unimodal distribution was not measured. Chemistry and *in situ* fluorescence data indicate that the oil was in fact dispersed adequately. This suggests potential operational problems for this unit < 5 °C, even though it is within the operating temperature (manufacturer manual). Additional testing of cold water temperature limits is recommended. Data collected for < 5 °C temperatures are not included in this paper.

Upstream and Downstream Particle Fractionation - Histograms representing the particle concentration for a given size class from the upstream and downstream LISSTs suggest dilution and fractionation of particles within the plume. The downstream LISST positioned further from the jet release serves as an indication of plume evolution through the tank. For all oil and DOR treatments, dilution in particles (decrease in TPC) within the plume expectedly occurred. Also evident is a shift to smaller particles for all DOR treatments as the plume moves through the tank, where size fractionation was dependent on DOR treatment (Figure 4). Physical dispersion (DOR 0) treatments for lighter oils were found to have the largest shift in DSD and VMD between the upstream and downstream LISSTs suggesting the largest effect of particle fractionation in the plume. Where the decrease in TPC suggests plume dilution in the tank, the DSD shift to smaller particles suggests that within each experiment larger droplets were removed from the plume within 6 minutes of the oil release, most likely rising to the surface of the tank. The effect was least observed in the DOR 1:20 treatments where dilution occurred but the presence of smaller droplets (with lower rise times) at the point of release

ED_001324_00001349-00014

most likely didn't allow for fractionation. The effects of fractionation were observed in warm and cold treatments.

Time series particle size data allows for evaluating plume dynamics over the duration of the experiments. Figure 5 depicts time series contour plots of the plume, where colored contours represent the particle concentration (normalized to max value for comparison purposes) for given droplet sizes through time. These plots allow for ascertaining how the DSD shifts over the duration of the release as the plume moves in the horizontal direction in the tank. For ANS, data suggest that the leading edge of the plume, as detected by the upstream LISST, exhibited a wide range in DSD during the first few minutes after release. As the plume advances down the tank, the DSD becomes narrower with a shift in the VMD for the DOR = 1:100 and 0 treatments. DSD remained similar for the DOR = 1:20 treatment. Warm and cold water treatments exhibit similar trends.

ED_001324_00001349-00015

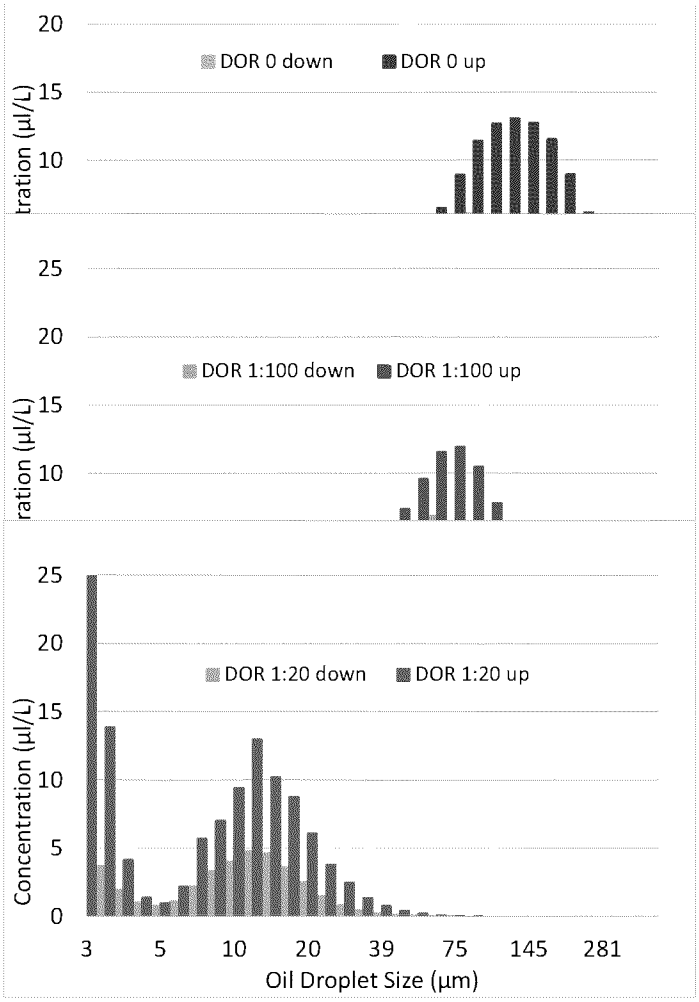


Figure 4. Upstream and downstream LISST Droplet Size Distribution and particle concentration for ANS with Corexit 9500 during warm water treatments.

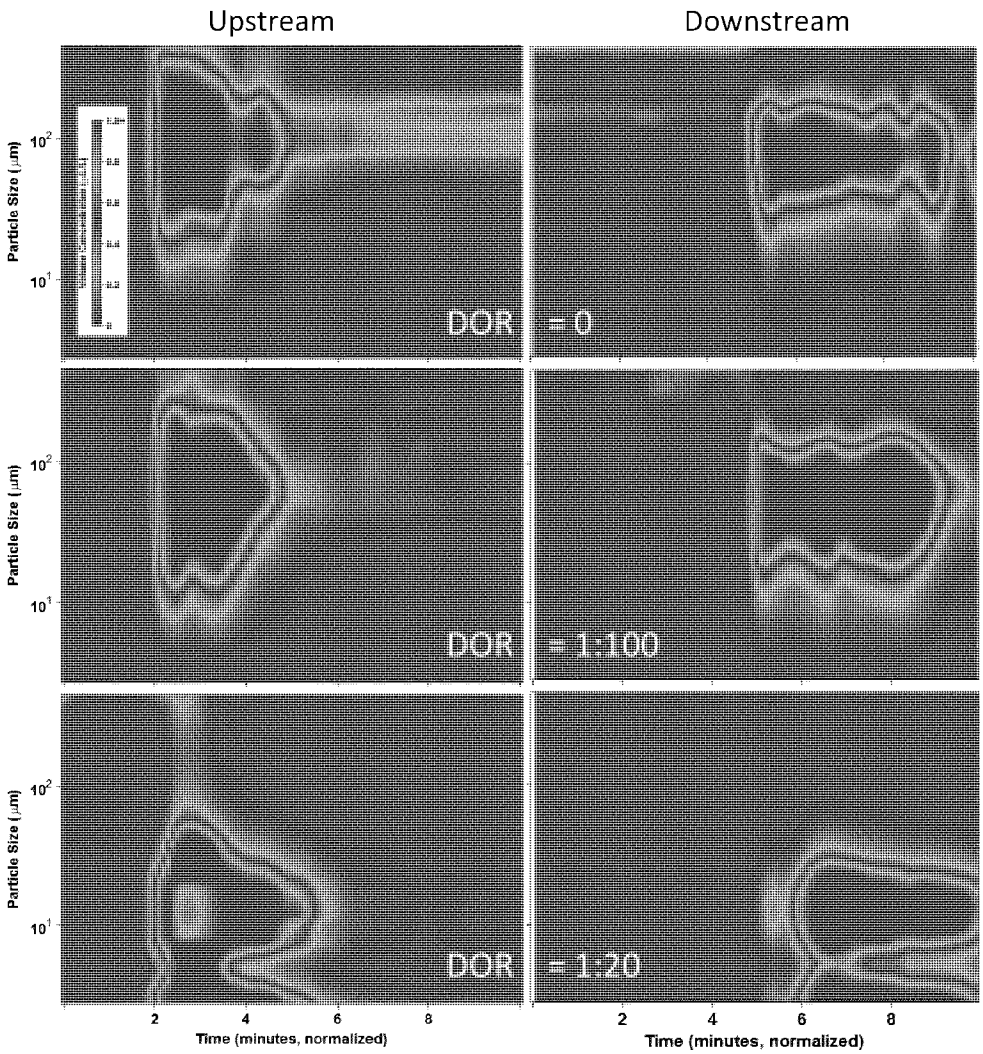


Figure 5. Time series contours of droplet size distribution and particle concentration from the upstream (left panels) and downstream (right panels) LISSTs for ANS with Corexit 9500 during warm water treatments.

<u>Plume Fluorescence Intensity</u> – In addition to the plume transport of particles (oil droplets), dissolved hydrocarbons were also measured via discrete samples and by proxy using fluorescence intensity. The reliability of *in situ* fluorescence measurements as a proxy for oil concentration within physically and chemically dispersed oil has been demonstrated previously

within the tank (Conmy et al., 2014). Here, *in situ* fluorescence serves as a good proxy for oil concentration during the subsurface injection experiments. However, heterogeneity of the produced plumes and the short time scale of experiments (~10 min) led to difficulties in correlations between the plume particle size analyses and chemistry results. This is in part due to discrete samples representing 15 second averages as opposed to instantaneous measures given by fluorometers and particle size analyzers. Thus, although general trends were similar, exact matchups between *in situ* data streams and discrete samples proved to be challenging (Figure 6). Given the experimental design, fluorescence was found to be better suited for correlation with total particle concentration (Figure 7). Depicted are the in situ fluorescence and TPC data during a warm water experiment of ANS with (left) and without (right) Corexit 9500. When exposed to dispersant, fluorescence estimates of dissolved hydrocarbons was higher compared to oil alone treatments, and signal persisted for longer periods of time. Whereas a sharper decay in the fluorescence intensity and the TPH values were observed for the physically dispersed oil.

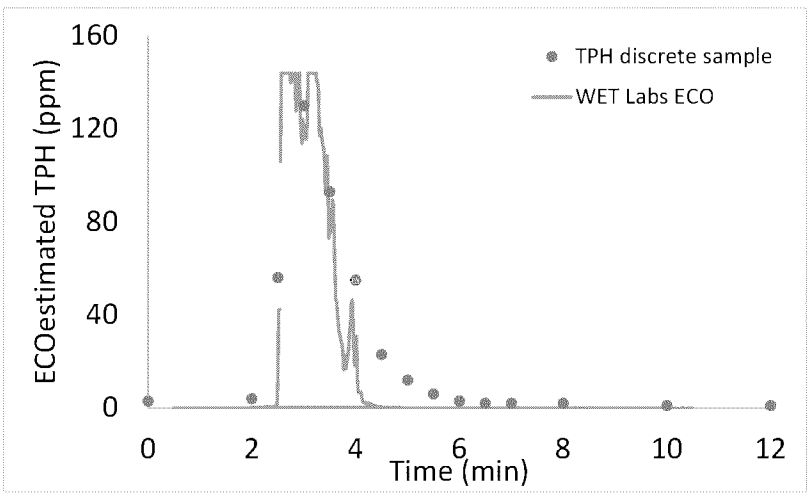


Figure 6. Fluorescence intensity and TPH samples for a warm water treatment using SLC

at DOR = 1:20.

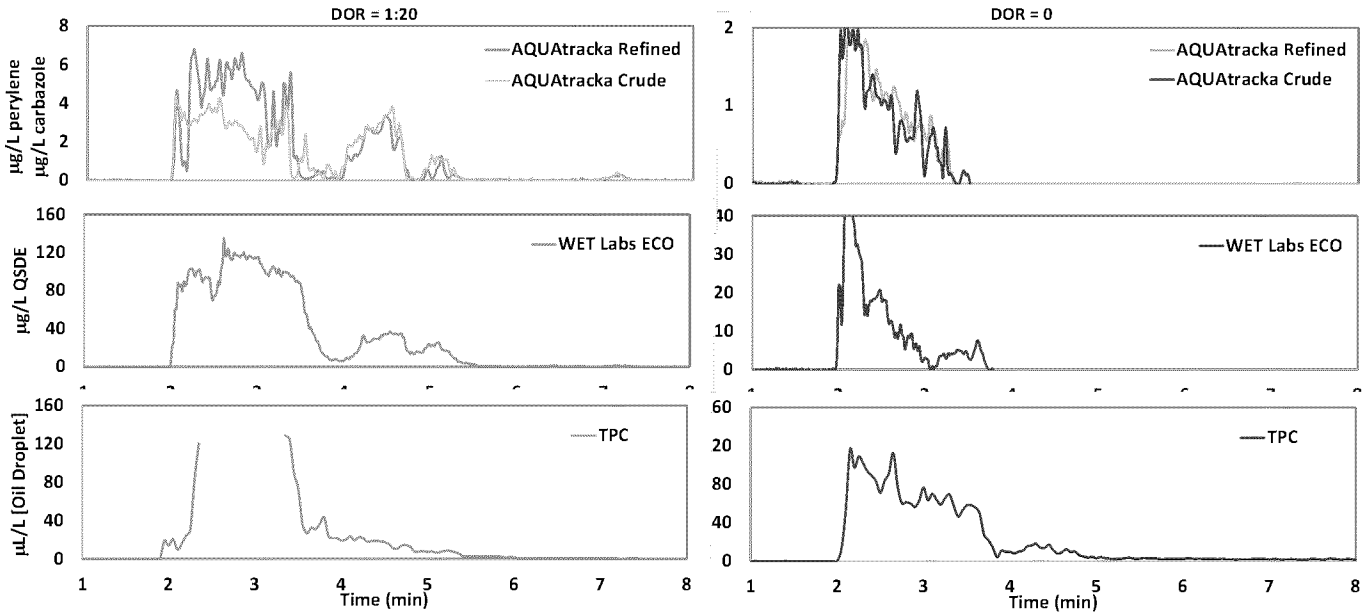


Figure 7. Fluorescence intensity and TPC as a function of time for ANS warm water treatment.

CONCLUSIONS

In situ oil droplet size distribution, particle concentration and fluorescence data from this study are essential for better understanding dispersion effectiveness and for refining droplet formation and trajectory models, such as modified Weber Number technique (Johansen et al., 2013) and the VDROP-J model (Zhou et al., 2014). These results demonstrate that during high pressure releases, light crude oils such as SLC form droplets with VMD < 70 m and bimodal DSD for 1:20 and 1:100 DOR regardless of water temperature. The effect was similar for the medium ANS crude oil, but bimodality was only observed for the 1:20 treatment. Conversely, Bimodal distribution was not achieved with the heavy IFO, but droplet < 70 µm were observed

for 1:20 warm waters, suggesting poor dispersibility of the high viscosity oil even for jet releases. Droplet fractionation observed in the plumes was greatest for the lighter crude and demonstrates the separation that would occur as a plume rises and is advected horizontally in a water column. Thus, findings from this study have implications for predicting the behavior of oil and plume formation, decision-making pertaining to SSDI as a countermeasure, and assessing the transport and fate of slicks and subsurface plumes in support of emergency response operations during any future deep water spills.

ACKNOWLEDGEMENTS

Experiments at Department of Fisheries and Oceans (DFO) Canada – Bedford Institute of Oceanography were funded via Interagency Agreement with the Bureau of Safety and Environmental Enforcement, and internal funding and salary support from DFO Canada and the U.S. Environmental Protection Agency (EPA). This document has been reviewed by the U.S. EPA. The authors thank all funding sources. This document has been reviewed by the U.S. EPA National Risk Management Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. This is a contribution from the Land Remediation and Pollution Control Division.

REFERENCES

Conmy, R. N., et al. (2014) Submersible Optical Sensors Exposed to Chemically Dispersed Crude Oil: Wave Tank Simulations for Improved Oil Spill Monitoring. Environ. Sci. Technol. 48(3): 1803-1810.

Cole, M.G., T. King, K. Lee (2007) Analytical technique for extracting hydrocarbons from water using sample container as extraction vessel in combination with a roller apparatus. Anal. Chem., 77: 2210-17.

CRRC Report (2010) Coastal Response Research Center Deepwater Horizon Dispersant USE

ED_001324_00001349-00020

Meeting Report, May 26-27, 2010, 108 pp.

EPA 600/F-16/250 Federal Report (2016) Dispersant Effectiveness, In-Situ Droplet Size Distribution and Numerical Modeling to Assess Subsurface Dispersant Injection as a Deepwater Blowout Oil Spill Response Option *and* Evaluation of Oil Fluorescence Characteristics to Improve Forensic Response Tools; IAA No. E12PG00037 for DOI BSEE, 144 pp.
Joint Analysis Group Report (2010) Review of Data to Examine Subsurface Oil in the Vicinity of DWH MC-252, May 19-June 19, 2010; 29 pp.

Johansen, O., et al. (2013) Droplet breakup in subsea oil releases - Part 2: Predictions of droplet size distributions with and without injection of chemical dispersants. Mar. Poll. Bull. 73(1): 327-335.

Lee, K., Li, Z., Boufadel, M.C., Venosa, A.D. and Scott Miles, M. (2009) Wave tank studies on dispersant effectiveness as a function of energy dissipation rate and particle size distribution. Final report submitted to NOAA/CRRC/UNH, 67 pp.

Li, Z., Lee, K., King, T., Boufadel, M.C. and Venosa, A.D., (2008) Oil droplet size distribution as a function of energy dissipation rate in an experimental wave tank 2008 International Oil Spill Conference. American Petroleum Institute, Washington D.C., Savannah, GA, pp. 621-626.

Li, Z., Lee, K., King, T., Boufadel, M.C. and Venosa, A.D., (2009a) Evaluating crude oil chemical dispersion efficacy in a flow-through wave tank under regular non-breaking wave and breaking wave conditions. Mar. Poll. Bull., 58: 735-744.

Li, Z., Lee, K., King, T., Boufadel, M.C. and Venosa, A.D., (2009b) Evaluating Chemical Dispersant Efficacy in an Experimental Wave Tank: 2, Significant Factors Determining *In Situ* Oil Droplet Size Distribution. Environ. Engin. Sci., 26(9): 1407-1418.

Li, Z., Lee, K., King, T., Boufadel, M.C. and Venosa, A.D., (2010) Effects of temperature and wave conditions on chemical dispersion efficacy of heavy fuel oil in an experimental flow-through wave tank. Mar. Poll. Bull., 60(9): 1550-9.

Nedwed, T. (2015) Overview of the American Petroleum Institute Joint Industry Task Force Subsea Dispersant Injection Project. Interspill Conference Proceedings.

NRC Report (2005) National Research Council Oil Spill Dispersants: Efficacy and Effects, Washington, DC, 400 pp.

Wickley-Olsen, E., Boufadel, M.C., King, T., Li, Z., Lee, K., Venosa, A.D. (2007) Regular and breaking waves in wave tank for dispersion effectiveness testing. In: Proceedings of the 30th Arctic and Marine Oil spill Program (AMOP) Technical Seminar, Edmonton, AB, Canada. Environment Canada, Ottawa, Ontario, Canada, pp. 161–187.

Zhao, L., et al. (2014). Evolution of droplets in subsea oil and gas blowouts: Development and validation of the numerical model VDROP-J. Mar. Poll. Bull., 83(1): 58-69.

ED_001324_00001349-00021

To: Campo-Moreno, Pablo (campomp)[campomp@UCMAIL.UC.EDU]
From: Conmy, Robyn
Sent: Thur 8/27/2015 1:00:34 PM
Subject: RE: do you have full citation for this in MPB?

I searched chemosphere but cannot locate it there either. Is it still in press or published?

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Campo-Moreno, Pablo (campomp) [mailto:campomp@UCMAIL.UC.EDU]
Sent: Thursday, August 27, 2015 8:53 AM
To: Conmy, Robyn
Subject: Re: do you have full citation for this in MPB?

Robyn,

This paper was rejected by MBP and it was accepted for publication in Chemosphere.

Regards

Pablo

Sent from my iPad

On Aug 27, 2015, at 08:47, Conmy, Robyn <Conmy.Robyn@epa.gov> wrote:

Zuang, M. Abulikemu, P. Campo-Moreno, W.E. Platten, A.D. Venosa, R.N. Conmy (2015) A comparative study on the biodegradability of crude oil dispersed with JD-2000 at 5 and 25 °C. *Marine Pollution Bulletin.*

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

To: Barron, Mace[Barron.Mace@epa.gov]
From: Conmy, Robyn
Sent: Thur 4/21/2016 2:19:43 PM
Subject: RE: DWH source oil...

We find that some of the products eat through the plastic containers over time. We've had a transfer 2 into glass bottles in the last year!

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Barron, Mace
Sent: Thursday, April 21, 2016 9:53 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: RE: DWH source oil...

Since Michael Hemmer retired, and our previous IH guy left, we have been just sitting on old dispersants and the BP oil. Out of the blue the BP contractor asked about their oil: I said we could either transfer it to another EPA facility (you) or send it back.

They asked for it back.

So we are cleaning house on the legacy DWH stuff. Crazy one of the dispersants Z-400 was in a plastic bottle and we found it leaking. All those are going into haz waste.

Hope all is well!

From: Conmy, Robyn
Sent: Thursday, April 21, 2016 8:40 AM
To: Barron, Mace <Barron.Mace@epa.gov>
Subject: RE: DWH source oil...

Sweet! It's like you're having a garage sale. ☺

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Barron, Mace
Sent: Thursday, April 21, 2016 9:32 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: DWH source oil...

Will also send you a couple mL of oil collected at the well head by ROV. We got about 5 mL of the precious during the spill!

We had that chemically characterized as part of the attached work.

Unfortunately, it was stored in same vial (head space), so it may be compromised. You can check it out when you get it.

Mace

To: Ken Trudel[ken@slross.com]
From: Conmy, Robyn
Sent: Mon 11/16/2015 2:13:57 PM
Subject: RE: API Dispersant Review of Dispersant Research Meeting
API Disp Research Rev Interim Report October2015_rc edits.docx

Hi Ken,

Please find my comments on the draft Dispersant Research Report. Overall, I think it is a good start, but perhaps some more time should be given to making the review less subjective. This is no simple task when talking about flaws in publications, but it would serve the community well to better understand how the limitations of an experimental design translate into implications of the research results. When do you expect to have a complete working draft of the published literature review?
Cheers,
Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>
Robyn N. Conmy, Ph.D.
Research Ecologist
USEPA/NRMRL/LRPCD
26 West MLK Drive
Cincinnati, Ohio 45268
513-569-7090 (office)
513-431-1970 (EPA mobile)
727-692-5333 (Personal mobile)
conmy.robyn@epa.gov

-----Original Message-----
From: Ken Trudel [mailto:ken@slross.com]
Sent: Friday, November 13, 2015 3:57 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: Re: API Dispersant Review of Dispersant Research Meeting

Hi Robyn:
Good to hear from you.
I believe that you had already told me that you were not going to be at Clean Gulf. I decided to proceed with it in order to obtain face to face feedback on the interim report sent earlier. Few people ultimately made it to the conference so the meeting was small. We decided to hold a conference call next week to go over the items discussed at the meeting.
I will send minutes of the meeting and information on next weeks call as soon as I can.
Ken

On 09/11/2015 7:22 AM, Conmy, Robyn wrote:
> Hi Ken,
>
> FYI I will not be at Clean Gulf.
>
> Cheers,
> Robyn
>
>
> <>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

> Robyn N. Conmy, Ph.D.
> Research Ecologist
> USEPA/NRMRL/LRPCD
> 26 West MLK Drive
> Cincinnati, Ohio 45268
> 513-569-7090 (office)
> 513-431-1970 (EPA mobile)
> 727-692-5333 (Personal mobile)
> conmy.robyn@epa.gov
>
> -----Original Message-----
> From: Ken Trudel [mailto:ken@slross.com]
> Sent: Friday, November 06, 2015 3:57 PM
> To: Tom Coolbaugh <Thomas.S.Coolbaugh@exxonmobil.com>; Emily Hague
> <Hague@api.org>; victoria.broje@shell.com; Conmy, Robyn
> <Conmy.Robyn@epa.gov>; Gala, William (WGala) <WGala@chevron.com>;
> Cooper, Cortis (CortCooper) <CortCooper@chevron.com>;
> Ken.Lee@csiro.au; Nedwed, Tim J <tim.j.nedwed@exxonmobil.com>; Bess,
> Amanda S <ABess@chevron.com>
> Subject: API Dispersant Review of Dispersant Research Meeting
>
> Hello:
> We have discussed having a meeting of the review panel for this project next week at Clean Gulf. To that end I have booked a meeting room at the Morial Convention Centre Room 346 from
> 5 to 7 pm on Tuesday November 10. Please confirm that you will be able to attend. A preliminary agenda is below.
>
> Agenda
>
> 1. Opening remarks
> 2. Review of Progress to date and "Interim Report"
> a. Progress to date/
> b. Interim report
> c. Revised Screen/Review Protocol
> 3. Where to go from here
> a. Overview of papers to review
> 4. Discussion of Assignments (if appropriate)
> 5. Next Meeting Tentative (Tuesday, November 17th, 2015 at 11:00
> am EST)
>
> I will be traveling to New Orleans on Monday.
>
> See you all in New Orleans.
>
> Regards
>
> Ken
>
>
> --
> Ken Trudel
> SL Ross Environmental Research Ltd.
> Tel: (613) 232-1564; Fax: (613) 232-6660
> 200 - 1140 Morrison Drive, Ottawa, ON CANADA, K2H 8S9
> email: ken@slross.com
> web site: http://www.slross.com

>
>

--
Ken Trudel
SL Ross Environmental Research Ltd.
Tel: (613) 232-1564; Fax: (613) 232-6660
200 - 1140 Morrison Drive, Ottawa, ON
CANADA, K2H 8S9
email: ken@slross.com
web site: http://www.slross.com

IPIECA, OGP and API Review of Newly Published Oil Spill Dispersant Research Papers 2013-15:

INTERIM REPORT: JANUARY 1, 2013 TO OCTOBER 1, 2015

For

American Petroleum Institute

1220 L Street, NW

Washington, DC 20005-4070

CONFIDENTIAL

By

S.L. Ross Environmental Research Limited

1140 Morrison Drive, Suite 200

Ottawa, ON, Canada

October 1, 2015

[Type text]

Acknowledgements

In Preparation

Executive Summary

In Preparation

CONFIDENTIAL

CONFIDENTIAL

Contents

Preface 2
Introduction 2
Overview of Publications 5
Subsea Dispersant Injection (SSDI) 7
Experimental Studies in SSDI 7
SSDI Studies with Problems 8
Fate and Degradation of Dispersed Oil and Dispersants 9
Field Studies During the Macondo Spill 9
Subsurface plumes of oil and dispersant 9
Monitoring for Corexit Dispersants 10
Laboratory Experimental Studies 11
Problems in Studies of the Fate of Dispersed Oil and Dispersants 11
Microbial Degradation 14
Effects of Dispersants, Dispersed Oil and Chemically Dispersed Spills 18
Effects of the Macondo Spill 18
Experimental Toxicology Studies 19
Composition of the Exposure Media 19
Sensitivity to Dispersed Oil 20
Effects of Variables on Toxicity 20
Sublethal Effects of Dispersant and Dispersed Oil 21
Problems in Studies of Effects of Oil and Dispersants 21
Summary of Problems in Effects Research 23
Health 26
Problems with Human Health and Safety Studies 26
Effectiveness 28
Editorial, Policy Etc 28
References 29
Appendix 1 – Bibliography to August 31, 2015 37

CONFIDENTIAL

ED_001324_00001363-00004

Preface

This document is a detailed outline for the report for the American Petroleum Institute project to review recent dispersant research publications. This outline was requested during one of the panel's earlier conference calls. The objective of preparing the document was to prepare a strawman outline for the project report, showing where we were going with the reviews. As you may recall, the objective of the panel was to prepare a document that tells both the good news and bad news about the new dispersant research. The challenge has been to separate the good papers from the bad papers and to prepare a document that discussed each group in a useful way. The main challenge has been to identify the important scientific flaws in the problem papers that lead the authors to incorrect conclusions. After re-reviewing a selection of the papers it appeared that there were two types of problems: a) dispersant specific problems (e.g. using nominal oil concentrations in toxicity studies) and b) basic scientific problems. The document below has sorted the papers into subjects; classified the papers as either problematic or not problematic; developed a classification scheme for some of the problems; and has applied the system to a selection of papers. The problems are discussed in plain language, so please do not circulate this document. Problems will be discussed in a more constructive way in the final project report.

CONFIDENTIAL

Introduction

The Deepwater Horizon Macondo (DWH) oil spill (April to July 2010) in the Gulf of Mexico greatly increased awareness of chemical oil spill dispersants among researchers and the public in general. This heightened awareness of dispersants resulted in a rapid increase in dispersant research and an avalanche of research publications. This project reviewed a series of new publications in order to relate any new findings to the spill response management questions facing response planners and to the research practices and standards developed over the years to help address these management questions. To this end a 12-member panel of experienced dispersant researchers was assembled to review recent research. This interim report is a strawman template for the final report for this project. It considers the 98 papers identified for review from January 1, 2013 to April 30, 2014.

Before embarking on any dispersant research study, it is important for researchers to recognize that chemical oil spill dispersant is a tool whose sole purpose is to manage the behaviour of oil spilled at sea in order to minimize its risks to human and environmental health and to speed environmental recovery. Since the development of dispersants in the 1960s, they have been the

ED_001324_00001363-00005

subject of considerable Research and Development (R&D) to advance the technology.Over the years R&D has come to focus narrowly on three management-oriented questions, namely:

a) What is the effect of chemical dispersants on crude oils oil, and how effective are they in dissipating surface oil slicks and oil discharged from subsea blowouts?
b) What is the fate of chemically-dispersed oil and dispersant?
c) What are the environmental effects of dispersants and chemically-dispersed oil?

Given that dispersants are a response tool to be used under specific spill conditions, research methods have been refined over the years to simulate the specific field conditions that are amenable to their use (e.g., Coelho et al 2013). This helps to maximize the applicability of data generated from controlled experiments to field operations. For example, in studying the toxic effects of mixtures of oil and dispersants at sea, researchers recognized that organisms exposed to dispersed oil would be exposed to an initial spike of high concentrations of toxicant that would quickly decline to background within a matter of hours or days. To simulate this exposure, researchers developed a "spike exposure protocol" to simulate the real-world exposure, (e.g., Singer et al…). The "spike exposure protocol" not only allows workers to extrapolate from research results to predict effects of dispersant use in the field, but it also provides a standard exposure procedure that enables inter-comparison of results in different studies. Dispersant researchers have developed a number of these research protocols, which helps standardize the research and in the interpretation of results for addressing operational questions

CONFIDENTIAL

Prior to DWH, dispersant planning focused solely on application to surface oil slicks. However, the atypical subsea release of oil during DWH allowed for a novel way to deploy dispersants, namely injecting the dispersant subsurface, directly into the discharging oil jet. This gave rise to a series of new research questions concerning the potential behaviour, movement and persistence of the discharged oil, effectiveness of the dispersant and the effects of the chemically versus physically dispersed oil.

Recognizing that there was a large volume of material published in recent years, this project focused solely on review of papers published in peer-reviewed journals, given that they have already been subjected to scrutiny for scientific rigour. In addition, the review is limited to papers published since 2013. The following report provides:

a) an overview of the papers;

b) a summary of the findings of each publication organized by subject area; and
c) a brief discussion of the issues encountered in some research that may limit the usefulness of their results.

CONFIDENTIAL

Overview of Publications

The 98 papers considered for review from January 1, 2013 to April 30, 2014 were considered for inclusion in this interim report (See Literature Cited below). These publications have been organized into categories for purposes of this report (Table 1 below), including:

a) subsea dispersant injection;
b) fate and degradation of oil and dispersants;
c) effects of dispersed oil and dispersants;
d) dispersant phenomenology, effectiveness and products;
e) health effects; and
f) editorials.

The vast majority of new publications have addressed the fate and effects of oil and dispersants, so this report focuses more on these papers.

Literature published from April 2014 to present is listed in Appendix 1 – Bibliography.

CONFIDENTIAL

ED_001324_00001363-00008

Table 1 Papers organized by subject (As of April 2014)

SSDI Research	Fate / Degradation of Dispersed Oil and Dispersants		Effects of Dispersants and Dispersed Oil		Effectiveness, Products, Theory	Health	Editorial
SSDI Research	**Macondo**		**Macondo**		**Theory**	Health	Editorial
Brandvik et al. 2013 Johansen et al. 2013 Nedwed and Coolbaugh 2013	Spier et al. 2013 Mendoza et al. 2013 Zhou et al. 2013 Place et al. 2014 Gray et al. 2013	Perkins et al. 2013 Bejarano et al. 2013 Sammarco et a; 2013	Fitzgerald et al. 2013 Ali et al. 2013 Echols et al. 2013 Montagna et al. 2013	Montagna Moody, Paul Ziervogel Bejarano	King et al. 2013 Wang et al. 2013	D'Andrea and Reddy 2013 Afonne et al. 2013	Adams 2013. Paul, I. 2013. Arnold C.2013 Peavler B. 2013. Perrons, R.K..2013. Schultz, C. 2013 Schrope, M. 2013. Stokstad, E. 2013
	Degradation – Experimental		**Lethality**		**New Products**		
	Lee et al. 2013 Prince and Butler 2013 Prince et al.2013 McFarlin et al.2014 Campo et al.2013	Joo et al. 2013 Batchu et al.2014 Macías-Zamora et al.2013 Vilcaez J et al.2013	Adams et al. 2013 Almeda et al. 2013 Anderson et al 2013 Coelho et al. 2013. Rico-Martínez et al. 2013 Gardiner et al. 2013 Goodbody-Gringley et al. 2013	Kuhl, et al. 2013 Lee KW, et al. 2013 Martin et al. 2013 Radniecki, et al. 2013 Ozhan et al. 2013a Ozhan et al. 2013b Powers et al. 2013	Saha et al. 2013		
	Methods/Equipment		**Sublethal Effects**		**Effectiveness**		
	Conmy et al. 2013 Lavallee et al. 2013 Mendoza,W et al. 2013 Zhou et al. 2013 Van Scoy et al. 2013		**Bioaccumulation** Chase et al. 2013 **Enzyme Induction/Metabolites** Milinkovitch et al. 2013 **Immunosuppresion** Barron et al. 2013 **Performance and Fitness** Claireaux, et al. 2013 Milinkovitch et al. 2013	**Gross Abnormality or Histopatology** Agamy 2013 Agamy 2013 **Growth/Reproduction** Brewton et al. 2013 Zhang et al. 2013 Polli et al. 2013	Venosa et al. 2013 Venkataraman et al. 2013		
	Fate Experimental						
	Ehrenhauser et al. 2013 Liyana-Arachchi et al. 2013 Gong, et al. 2013	Kuhl et al. 2013 Zhou et al. 2013					

CONFIDENTIAL

Subsea Dispersant Injection (SSDI)

Subsea dispersant injection had received very little research attention prior to 2010, but proved to be a key response tool during the 2010 Macondo incident. In Macondo, SSDI played an important role in minimizing the amount of oil from the blowout that reached the sea surface, thus protecting the environment and health and safety of response workers. SSDI will clearly be incorporated into spill-response strategies for future offshore drilling operations where appropriate.

Nedwed and Coolbaugh 2014 (no paper) reported on recent industry research initiatives, which address:

a) Effectiveness of subsea injection - Scaled testing is evaluating injection methods and effects of dispersant-to-oil ratios (DOR). The effect on oil droplet size of DOR, low-solvent dispersants and dispersant injection method and location are being studied. Preliminary research findings include:
    a. Dispersant was effective at reducing droplet size even with low DOR's;
    b. Injection wands were effective;
    c. Removing solvent from dispersants improves dispersant effectiveness; and
    d. Injecting dispersant more than 5 pipe diameters above discharge point was effective.

b) The fate and effects of oil dispersed in deep water;

c) Improved numerical modeling to better predict the fate of dispersed oil in deep water; and

d) Monitoring tools to determine the effectiveness of subsea injection.

CONFIDENTIAL

Experimental Studies in SSDI

Brandvik et al 2013 studied the size distribution of droplets produced in a subsea discharge using a new meso-scale test facility at SINTEF. Brandvik described the facility and presented results from tests with Oseberg Blend crude oil, with and without injection of chemical dispersants (Corexit 9500). Experiments without dispersants examined droplet size distributions generated using exit diameters 0.5 to 3 mm, flow rates 0.2 to 5L/min. with and without injection of air. Effects of dispersants were studied at a single nozzle diameter (1.5 mm) at flow rates of 1.2 to 1.5 L/m, with DORs from 0 to 1:25.

In the experiments without dispersants, with constant nozzle diameter (0.5mm), increasing the flow rate (0.2 to 0.5 L/min.) caused a shift towards smaller droplets. The result was similar when the flow rate was held constant (5 L/min) and the nozzle diameter reduced. Releasing air in combination with the oil (GOR=1:1) gives larger oil droplets. Finally, injecting dispersant into the combined oil and gas release reduced the droplet diameter compared to both a combined release and an oil alone release with the constant flow rate and nozzle diameter. Droplet size declined as proportion of dispersant to oil increases.

The following are in preparation.

ED_001324_00001363-00010

Johansen et al. 2013

SSDI Studies with Problems

None

CONFIDENTIAL

Fate and Degradation of Dispersed Oil and Dispersants

This section considers papers dealing with the environmental fate of dispersants and dispersed oil. Papers have been grouped into:

a) Field studies conducted during the Macondo spill;
b) Experimental studies of oil fate and microbial degradation of dispersed oil and dispersants; and
c) Methods developed to monitor or analyse for dispersants or dispersed oil.

Field Studies During the Macondo Spill

Subsurface plumes of oil and dispersant

One of the key challenges of the Macondo spill was identifying and tracking the subsurface plumes of oil discharged from the seabed. A number of field surveys and modeling studies were completed during the actual event to locate subsurface oil plumes and track the fate of the oil and dispersant they contained. These are summarized in Spier et al. 2013.

Spier et al. 2013 examined analyses of oil in water samples taken offshore at depth from May 9 to June 26 during the spill. They identified the vertical plume of hydrocarbons rising to the surface from the blowout and addressed several research questions, as follows.

a) Concerning the location and direction of subsurface hydrocarbon plumes, Spier et al. identified plumes with mid-depths of 25, 265, 865, and 1175 m and identified the direction of movement of each. Plumes at 25, 265 and 1175 were clearly persistent, but the 865-m-deep plume was not sampled often enough to assess persistence.

b) Spier confirmed the existence of the 1175 m plume that had been predicted in various modeling studies published at the time.

c) Spier tested and confirmed that a plume had formed at 800 m as predicted by models and confirmed it.

d) Spier confirmed that the oil composition of the plumes varied with depth. Their analysis determined that the more soluble compounds were found in the deeper plume. This gave further evidence that the more water soluble compounds were extracted from the oil deeper in the water column leaving the less water soluble ones to dominate in the upper water column.

Mendoza et al. 2013 monitored the subsurface distribution of oil and dispersant within 40 km(?) of the DWH spill site from May 27 to June 4 during the spill. They took water samples using CTD casts and analysed them for HCs using fluorescence techniques (excitation and emission matrix (EEM) fluorescence and parallel factorial analysis (PARAFAC) modeling techniques) (*We need a chemist to assess the usefulness of these techniques*). The benzene and naphthalene-

ED_001324_00001363-00012

like components of crude oil exhibited a maximum at ~1200 m, coinciding with the 1175-m centred

plume identified by others. The maximum fluorescence of the component associated with the dispersant (i.e., Corexit EC9500A) was observed at the same depth. The plume observed at this depth was attributed to the dispersed crude oil from the Deepwater Horizon oil spill[1].

Zhou et al.[2] also studied the distribution of oil in surface and deep waters near the DWH spill site during May and June using fluorescence EEMs techniques coupled with PARAFAC modeling. This group also identified petroleum hydrocarbons to the SW of the DWH site at a depth of 1100 to 1400 m.

The following are in preparation.

Bejarano et al. 2013.

Monitoring for Corexit Dispersants

Prior to Macondo little attention was paid to monitoring the fate of the dispersant during a spill response. During Macondo monitoring the fate and degradation of the dispersant became an issue due to the amount used and concern voiced by the public. During the response itself, attempts to monitor the dispersant focussed on analysing the dioctylsulfosuccinate (DOSS) component of the product. Recently published work involved analysing environmental samples taken during the response and developing methods for analysing components of the dispersants other than DOSS.

CONFIDENTIAL

Place et al. developed an analytical method for detecting all four classes of surfactants discharged in the response to the Macondo spill , as well as the hydrolysis products of DOSS, the enantiomeric mixture ofα-and β-ethylhexyl osuccinate (α -/β-EHSS). The new analytical method was then used to evaluate practices for sample collection, storage, and analysis to identify those that produced high quality data. Place concluded that the method "Large volume, direct injection of seawater followed by liquid chromatography tandem mass spectrometry (LC-MS/MS)" minimized analytical artifacts, analysis time, and both chemical and solid waste. The authors reported the ranges of analyte concentrations observed in Macondo water samples analysed.

Perkins et al. (abstract only) reported on concentrations of the major constituents of Corexit, Span 80, Tween 80, Tween 85, as well as, DOSS and its primary hydrolysis products in depth specific water column samples collected from priority sites in the Gulf of Mexico in 2012. These

[1] Results demonstrate the application of EEM and PARAFAC to simultaneously monitor selected PAH, dispersant-containing and humic-like fluorescence components in the oil spill region in the Gulf of Mexico. Four of the seven derived PARAFAC loadings were associated with the Macondo crude oil components. The other three components were associated with the dispersant, an unresolved component and colored dissolved organic matter (CDOM).
[2] Zhou, LG , A Shiller, S Lohrenz, V Asper, C. Osburn2013 Characterization of oil components from the Deepwater Horizon oil spill in the Gulf of Mexico using fluorescence EEM and PARAFAC techniques Marine Chemistry Volume 148, 20 January 2013, Pages 10–21

values are compared to those for samples collected from similar sites in 2010 and 2011. Additionally, a preliminary method for extracting and quantifying DOSS and degradation products from marine sediment, using LVI-LC-MS/MS, is presented.

Gray et al. 2013 collected water samples at the surface and at depth during two cruises in May/ June 2010 for DOSS analysis. A newly developed LC/MS/MS technique for the determination of dioctylsulfosuccinate (DOSS) in clean and oily sea water was applied to the samples. Although DOSS was present at high concentration in the immediate vicinity of the well where it was being continuously applied, a combination of biodegradation, photolysis, and dilution likely reduced persistence at concentrations exceeding the USEPA aquatic life benchmark beyond this immediate area.

Laboratory Experimental Studies

The following are in preparation.

Ehrenhauser, F –	bubble bursting as fate process
Liyana-Arachchi –	bubble bursting
Gong, -	oil sediment interactions, review
Gong –	effect sed sorption on photodeg and ozonation
Zhou, Z., -	chem evolution by degr inj lab

Problems in Studies of the Fate of Dispersed Oil and Dispersants

The following provides an overview of research issues arising from papers in this subject area. The types of major errors are summarized in Table 2 below. Errors have been divided into dispersant-research-specific errors and scientific errors. The majority of the discussion focuses on the dispersant-specific errors; scientific errors are discussed only when they influence the discussion of dispersant-related processes.

The problems associated with Sammarco et al. 2013 were discussed in Wilson et al 2014. These are scientific problems and are not dispersant-related. They included problems with experimental design, their sampling strategy, chemical and data analysis, and the basis and interpretation for their human health risk conclusions.

Zuijdgeest and Huettel (2012) studied the penetration of chemically treated and untreated oil into intertidal beach sand. Their objective was to gain knowledge about the fate of oil that had been treated with dispersants in offshore areas during the Macondo spill. The authors concluded that "the addition of dispersants permits crude oil components to penetrate faster and deeper into permeable saturated sands, where anaerobic conditions may slow degradation of these compounds, thus extending the persistence of potentially harmful PAHs in the marine environment. Application of dispersants in nearshore oil spills should take into account enhanced penetration depths into saturated sands as this may entail potential threats to the groundwater."

The problem with their study was that they used an incorrect mental model of the contact of spilled dispersant-treated oil with intertidal sand beached in Macondo. In Macondo, although dispersants were being used regularly 40 mi offshore, the only oil that contacted sand beaches

ED_001324_00001363-00014

was patches of heavily weathered, viscous mouse, some of which may have contained trace amounts of some dispersant constituents. This oil would not have penetrated into the sand, even with tidal pumping. On the other hand, Zuijdgeest and Huettel's experiment studied the penetration into sand columns of dispersants, physically-dispersed oil and chemically –dispersed oil in the form of sonically disrupted suspensions of fresh oil in water. These contained micron-sized droplets of fresh oil. This may have simulated suspensions that would form if fresh oil were to be treated with dispersants in the interdidal zone or in shallow nearshore areas. However, it did not simulate Macondo conditions or conditions in any spill where dispersants may be used many miles offshore. In short, the published results and conclusions did not simulate the actual conditions during Macondo, so their published conclusions are misleading.

Kuhl et al 2013. The experiment and paper by Kuhl et al. included a number of dispersant-related and scientific challenges. In short, the authors attempted to use microcosm experiments to study the effects water salinity on the fate and degradation of untreated and dispersant-treated, weathered oil in a shallow nearshore environment. Their specific objectives were:

1) To explore the role of salinity and time on partitioning of PAHs into the aqueous phase from layers of oil, dispersant and chemically dispersed oil applied to the water's surface in microcosms, and to study their subsequent biodegradation and associated toxicity to Fundulus. grandis.

2) To model the role of these variables on mortality; and

CONFIDENTIAL

3) To determine if a common environmental sampling analysis (aqueous PAH concentrations) accurately reflects acute toxicity of dispersed and non-dispersed crude oil.

To this end the authors layered untreated oil, dispersant and oil-dispersant mix on the surface of aquaria containing sediment and water at three salinities and allowed the aquaria to age under quiescent conditions for up to 16 weeks. PAH concentrations in the water and the toxicity of the water to F. grandis were monitored after 1, 4 and 16 weeks. The authors reported that:

a) Compared with undispersed oil, COREXIT exponentially increased the PAH concentrations in the WAF for up to 16 wk;
b) Hopane-normalized concentrations indicated that biodegradation was slowed for the first 4 wk.
c) Dispersed crude oil and COREXIT were acutely toxic following 1 wk of biodegradation with no correlation between PAH concentrations and crude oil WAF mortality.
d) Both dispersant and dispersant oil mixtures remained toxic for at least 4 wk at the lowest salinity tested, suggesting increased sensitivity or reduced biodegradation of toxic components in low-saline environments.
e) At the lowest salinity, oil dispersed with COREXIT was more toxic than either the COREXIT alone or oil alone, even after 16 wk of biodegradation.

There were several general problems with the design in terms of interpretation and application in the real world as follows.

a) The authors had an inappropriate or unlikely mental model of dispersant use in that it is unlikely that oil slicks would be treated with dispersants in shallow, nearshore environments.
b) It is unrealistic for oil in this environment to remain undisturbed for 16 weeks, making observations concerning transfer of PAH from the slick to the water difficult to interpret in terms of real world applicability. This invalidates all conclusions. Further, any oil that mixed into the water column would have diluted quickly by mixing. This invalidates all time-dependent conclusions regarding degradation and toxicity.
c) Preparation of the exposure medium is poorly described in that the extent to which the oil was mixed into the aquaria or not is not described. When an oil-dispersant mixture is applied to a water surface, it does not mix spontaneously, but if disturbed it mixes very readily with the water phase with very little mixing energy. There is no mention in the paper of measures taken to prevent mixing during the experimental set up, nor were water samples taken at time 0 to confirm that there was no initial mixing. Unfortunately accidental mixing at the time of setup could account for the anomalous pattern of mixing observed during later sampling times.
d) The apparent non-linear relation between salinity and oil fate are difficult to assess because of the lack of replication. This is a scientific error and it invalidates most of the conclusions about effects of salinity on any oil fate processes.

Table 2 Summary of Challenges in Fate/Degradation Studies

Fate/Degradation	Sammarco	Kuhl	Zuijdgeest and Huettel 2012.	
Scientific errors	X	X		
Dispersant –specific errors				
Incorrect mental model of dispersant use		X	X	
Incorrect model of environmental behaviour		X	X	
Incorrect method of preparing media		X		

ED_001324_00001363-00016

Microbial Degradation

During the Macondo spill considerable attention was devoted to the biodegradation of the discharged oil and dispersant, as follows.

Lee et al observed that oil that reached the water column in the Macondo spill biodegraded quickly, but that results of some laboratory degradation studies appeared to tell a contradictory story. Lee suggested that the contradictory results may have resulted from study designs that did not reflect the field conditions encountered in both the GOM spill and most oil spills at sea. According to Lee, lab biodegradation studies have at least two important challenges, namely studies must be run using:

a) Dispersed oil concentrations that are as low as they might be expected to be in the field (generally 100 ppm TPH or less); and

b) The dispersions of droplets be as stable as stable as they might be expected to be in the field (droplets are no larger than 70-100 um diameter and are kept in suspension by mild stirring or agitation).

Lee argued that some biodegradation studies used unrealistically high concentrations of dispersed oil in their tests, which produced unrealistic degradation because:

a) Using concentrations of oil or dispersant much higher than in the field inhibited hydrocarbon-degrading bacteria; and

a) Biodegradation at much higher oil concentrations than at sea in closed systems can limit degradation because of depletion of nutrients. Attempts to counteract this by using high initial concentrations of nutrients may lead to an unrepresentative modification of the microbial community.

Prince & Butler 2013 conducted a mesocosm study aimed at showing that the rate of primary biodegradation of an oil slick is dramatically stimulated by effectively dispersing the oil slick into the water column using dispersants. They used methods that adhered to the principles outlined in Lee et al. above. In short, in a lightly stirred environment, an untreated slick remained on the water surface during the entire biodegradation study, while a dispersant-treated slick was dispersed into the water and remained in the water column as small dispersed oil droplets. Clearly both biodegradation and evaporation were occurring in both experiments with and without dispersant, but it is obvious that biodegradation was far more extensive in the dispersed oil system than in the untreated one. Within 7 days, the dispersed oil had lost 50% of its detectable hydrocarbons versus 14 % in the untreated experiment. After 40 days the dispersed experiment had lost 84% versus less than 14% in the untreated one. Equally important, naphthalene was lost through a combination of both evaporation and biodegradation. However, its non-volatile alkyl congeners and the phenanthrenes were eliminated by largely by biodegradation alone. Clearly, in this study, dispersed oil degraded much more rapidly and extensively than undispersed oil.

Prince et al. 2013a conducted a degradation study under conditions described by Lee et al.

ED_001324_00001363-00017

2013. Under those conditions, oil was extensively and rapidly degraded. They showed that biodegradation of dispersed oil was prompt and extensive when oil is present at the ppm levels expected from a successful application of dispersants – more than 80% of the hydrocarbons of lightly weathered Alaska North Slope crude oil were degraded in 60 d at 8° C in unamended New Jersey (USA) seawater when the oil was present at 2.5 ppm. Degradation half-lives for small molecules like naphthalene were 2 d, most PAHs were <5 days, and the most recalcitrant were 18 to 32 days. The half-times for total extractable HCs was 11 to 14 days. The presence of the dispersant, Corexit 9500 at realistic concentrations did not slow degradation.

McFarlin et al. measured the degradation of physically and chemically dispersed Alaska North Slope crude oil (ANSCO) by indigenous Arctic microbiota in seawater at -1°C. Primary biodegradation was measured in standard mesocosm and mineralization in respirometer systems using respirometry and gas chromatography-mass spectrometry (GC-MS) analysis for measurement. Arctic microorganisms degraded both fresh and weathered crude oil in the presence and absence of Corexit 9500 at -1°C, with biodegradation oil losses ranging from 46 to 61% and mineralization losses up to 11% over 60 days. Weathered ANS dispersed with Corexit 9500 underwent a 57% loss in Arctic seawater after 60 days in their experiment, but experienced an 88% loss in New Jersey seawater in the same time, suggesting that in the Arctic, ANS crude oil degrades slightly more slowly than oil intemperate regions. The authors concluded that the biodegradation of oil in Arctic seawater is extensive at -1°C, and that the biodegradation of dilute, dispersed oil is not inhibited by the presence of Corexit 9500.

CONFIDENTIAL

Campo et al studied whether the microbial communities of the GOM were capable of degrading Corexit 9500 or dispersed oil at temperatures typical of GOM surface waters (25°C) and bottom waters (5°C), using C9500 (as represented by DOSS), South Louisiana crude oil (SLC) alone, and SLC dispersed by C9500. Exposure media were prepared in artificial seawater and an inoculate prepared using microbial communities harvested from GOM surface and bottom waters (1500 m) grown on media augmented with crude oil at 25 and 5 deg C. Oil and dispersant concentrations were measured, but were several times higher than at sea. This study revealed that DOSS, spontaneously and quickly hydrolyzed to MOSS and 2-EH at 25 °C but degraded little at 5 °C during the 42 days of the study. The implication was that DOSS may not persist in warm waters, but may persist for a long time in waters at very cold temperatures. Biodegradation of DOSS at 25 °C was faster in the presence of oil than without oil. Alkane biodegradation proceeded rapidly and virtually completely at warm temperature. Degradation rates were 5-fold greater in dispersed oil than in the untreated oil, which agreed with previous findings.The authors concluded that in their experiments crude oil biodegradation in general was readily enhanced by the addition of dispersant, but PAH biodegradation at 5 °C may have been more a function of the inherent solubility of PAHs compared to alkanes rather than due to the influence of the dispersant.

The following are in preparation.

Batchu -	degrad DOSS by photodegr
Joo –	eff disp on oil deg – mesocosm
Macías-Zamora –	eff disp o degrad PAH in sed
Vilcaez J –	model degrad kinetics of oil droplets

Table 3 Summary of Fate and Degradation Papers

DWH	**Fate Experimental**	**Degradation – Experimental**	**Methods/Equipment**
Spier –Plume tracking in DWH Mendoza, vert dist oil and disperant in DWH Zhou Character oil components in water Sammarco distr HCs in water in DWH Gray - fate of DSS in GOM Perkins distr Corexit in water seds Bejarano	Kuhl – eff dispersants/salinity oil weathering Ehrenhauser, F – bubble bursting as fate process Liyana-Arachchi – bubble bursting Gong, - oil sediment interactions, review Gong – effect sed sorption on photodeg and ozonation Zhou, Z., - chem evolution by degr inj lab Zuijdgeest A., and M. Huettel. 2012.	Lee,K. – degrad studies must consider dilution Prince RC, Butler JD. lab dispersed oil Prince RC – lab dispersed oil stimulates degrad McFarlin – degr disp oil Arctic stimulates degrad Campo - eff temp degr Corexit and disp oil **Baelum** – eff oil in bacterial community Batchu - degrad DOSS by photodegr Changkyu – eff disp on oil deg – mesocosm Macías-Zamora – eff disp o degrad PAH in sed Vilcaez J – model degrad kinetics of oil droplets	Van Scoy - SPMDs) Conmy, - Submersible optical sensors Lavallee - Fluorescence fingerprinting Mendoza, - fluorescence and PARAFAC Zhou - EEM and PARAFAC techniques. Place – trace analy surfactants in disp/ water

CONFIDENTIAL

CONFIDENTIAL

Effects of Dispersants, Dispersed Oil and Chemically Dispersed Spills

This section includes all studies dealing with the effects of dispersants and dispersed oil. It includes:

a) Studies of dispersant-related effects observed during actual spills (Macondo); and

b) Experimental studies undertaken to understand the cause-effect relationship between oil exposure and effects and the level of exposure needed to cause these effects.

Effects of the Macondo Spill

Montagna et al. reported on monitoring cruises that were deployed in fall of 2010 to measure potential impacts on benthic invertebrate communities. Sediment samples were taken for chemical, physical and biological analyses. Although the benthic distribution of oil and its effects may have resulted from effects of dispersants on the fate of oil, the paper did not address the potential role of dispersants.

The most severe relative reduction of faunal abundance and diversity extended to 3 km from the wellhead in all directions covering an area about 24 km2. Moderate impacts were observed up to 17 km towards the southwest and 8.5 km towards the northeast of the wellhead covering an area 148 km2.

Benthic effects were correlated to total petroleum hydrocarbon, polycyclic aromatic hydrocarbons and barium concentrations, and distance to the wellhead; but not distance to hydrocarbon seeps. Thus, benthic effects are more likely due to the oil spill, and not natural hydrocarbon seepage.

CONFIDENTIAL

The following are in preparation.

Ziervogel	Enhanced protein hydrolysis
Fitzgerald –	Contam in reef fish
Moody,	Lack of Impact by DWH
Echols–	tox tests on DWH water samples
Montagna	PA Benthic Footprint DWH
Paul	Tox Mutagenicity GOM waters

Experimental Toxicology Studies

The objectives of toxicological experimentation related to oil spill dispersants are to understand:

1) effects caused by oil and dispersants at the organism level; and
2) the level of exposure needed to cause these effects.

These subjects are being studied to improve understanding of the risks posed by spills and dispersants to the fish and wildlife exposed to them. The effects of these potential toxicants at the organism level include both mortality and sublethal effects. Sublethal effects range from innocuous effects like bioaccumulation of hydrocarbons to major injuries like gross anatomical abnormalities and reproductive impairment. Just as important as the effects caused are the levels of exposure needed to cause the effects. Planners assess the risks posed by spills and spill response by comparing the expected levels of environmental contamination during the event to the experimentally derived estimates of levels of exposure needed to cause effects.

Historically toxicological research has employed standardized exposure protocols (e.g., 96-hour LC50) to facilitate comparison of new results to the existing knowledge base. Dispersant work adheres to these protocols to a degree. Because of the nature of petroleum and the way dispersants are used to respond to them, spills produce a particular type of exposure conditions. This posed a problem for planners trying to use standard toxicological data to assess risks in the field. In order to overcome these challenges, over the years, researchers have developed exposure protocols (e.g., spike exposure protocol), and standard methods for preparing and characterizing exposure media in order to simplify the task of using the experimental data to estimate risks in the field (Coelho et al. 2013). While it is not essential to adhere to these standards, adherence does facilitate the use and inter-comparability of research data.

Composition of the Exposure Media

Recently, several authors have attempted to understanding of the roles of different components of dispersions to toxicity.

Gardiner et al. 2013 - In a study of the sensitivity of Arctic species, Gardiner investigated the relative contributions of different components of the exposure media to its toxicity. They used three methods to prepare media: standard WAF, more energetic WAF or BWWAF; and standard CEWAF. They used GC to quantify the chemical composition of the exposure media. They observed that varying prep methods changed the relative composition of the stock exposure media, as well as its apparent toxicity. Specifically, the standard low energy WAF contained only the more soluble components of the oil and few of the insoluble components; higher energy BWWAF contained a higher concentration of the soluble components, but also added some insoluble components; and CEWAF increased the amount of oil and increased the insoluble components to the point where oil the composition of the dispersed oil was similar to the parent oil. In toxicity terms when toxicity is related to oil concentrations expressed as TPH, the different media were ranked as follows in terms of toxicity: WAF>BWWAF>CEWAF. Toxicity correlated most strongly with naphthalene concentrations. Gardiner concluded that toxic components were the naphthalene components, which were soluble and were the most abundant components of

ED_001324_00001363-00022

the soluble components in WAF. Altering the prep method increased the relative amounts of less toxic components in the medium, so that the apparent LC 50 declined when expressed as TPH. Gardiner also concluded that the presence of dispersant in the media did not contribute to the toxicity of the exposure medium.

Anderson prepared media using a standard way and measured an extended group of PAHs in exposure media using GC. They recognized that the CEWAF had higher concentrations of PAH in the media than the WAF and this was reflected in higher levels of mortality in the CEWAF than WAF.

Sensitivity to Dispersed Oil

Gardiner used standard spike exposure method to study sensitivity of arctic species to physically and chemically dispersed oil. The effects thresholds as defined by the LC10 were above TPH concentrations predicted to occur in the field within hours of surface application of dispersants. They also showed that the sensitivity of arctic species under arctic conditions were similar to temperate species.

Goodbody-Gringely studied the effects of physically dispersed and chemically dispersed oil on survivorship and setting behaviour of coral larvae. The purpose was to examine the effects of exposure to both fresh and weathered DWH oil and dispersants using short-term assays (96-hr) to monitor larval survivorship, settlement, and behavioral responses. The author the used spike exposure method and measured exposures using TPH and GC-fid. The methods of preparing WAF and CEWAF were not described.

CONFIDENTIAL

The author concluded that the higher concentrations of oil in the CEWAF tests caused mortality in both species tested. The author failed to acknowledge that the dispersant oil mixture provided the higher oil concentrations in the test and the higher exposures caused the greater mortality. The toxicity thresholds expressed in TPH were consistent with those I other studies.
The author did not comment on the possible risks to Florida from the Macondo spill.

The following are in preparation.

Adams J
Lee KW,
Martin
Radniecki,
Ozhan
Ozhan
Powers

Effects of Variables on Toxicity

Effect of Species

Gardiner showed differences in sensitivity among the following Arctic species: copepod (Calanus glacialis), juvenile Arctic cod (Boreogadus saida), and larval sculpin (Myoxocephalus sp.). They also identified a significant difference in sensitivity between copepods of different ages.

Rico-Martinez attempted to study the toxic effects of physically dispersed oil and chemically dispersed oil on the marine rotifer, Brachionus manjavacas. The authors demonstrated that there were large differences in apparent sensitivity among the species studies.

Life stage

Anderson studied effects of WAF and CEWAF dispersed oil on juvenile mud crabs to identify any long-term effects of acute exposure on long term survival. They concluded that if the juvenile crabs survive initial exposure to oil, there were no latent effects that appeared post-exposure.

Temperature

Gardiner showed that Arctic animals tested at Arctic temperatures were no more or less sensitive to dispersed oil than temperate zone species tested at temperate zone temperatures.

Ultraviolet light

Alameda showed that UVB radiation increased the lethal effects of dispersed crude oil in mesozooplankton communities by 35%.

Sublethal Effects of Dispersant and Dispersed Oil

In preparation

Bioaccumulation

Chase

Enzyme Induction/Metabolites

Milinkovitch T,

CONFIDENTIAL

Immunosuppresion

Barron
Performance and Fitness
Claireaux G,
Milinkovitch T,

Gross Abnormality/Histopatology

Agamy
Agamy

Growth/Reproduction

Brewton
Zhang
Zhang
Polli

Problems in Studies of Effects of Oil and Dispersants

The following provides an overview of research issues arising in several papers. The types of major errors are summarized in Table 4 below. Errors have been divided into dispersant-research-specific errors and scientific errors. The most of the discussion will address the dispersant-specific errors; scientific errors will be discussed only when they influence the

ED_001324_00001363-00024

discussion of dispersant-related processes.

Anderson studied effects of dispersed oil on juvenile mud crabs assessing the acute effects of non-dispersed and chemically dispersed crude oil. Toxicity tests were done using an EPA standard protocol that involved 96-hr static non-renewal assays. Exposure media were prepared in a standard way. All toxicity results were reported in terms of Nominal oil concentrations, though exposure media were analysed for "extended PAH concentrations". Anderson reported that the chemical dispersion of crude oil significantly increased acute toxicity of the WAF in juvenile mud crabs compared to naturally dispersed oil, but they did acknowledge that the concentrations of PAH in the CEWAF were higher than the WAF and that correlated with higher mortality. By failing to report toxicity results based on measured exposure concentrations makes it difficult to use data to assess risk in the field.

Rico-Martinez attempted to study the toxic effects of physically dispersed oil and chemically dispersed oil on the marine rotifer, *Brachionus manjavacas*. They used the ASTM standard exposure protocol and a standard method for preparing exposure media. They did not attempt to characterize their exposure media in any way and reported toxicity results in NOMINAL. One of their stated objectives was to assess the effects of a crude oil and Corexit 9500A mixture at concentrations that are environmentally relevant. This was not actually attempted in the paper and could not have been done as the authors did not measure the oil concentrations in their exposure media at all.

Although it was not part of their study design the authors did attempt to draw conclusions about the contributions of the components of the oil and dispersant in the exposure media. They reported that synergistic toxic effects of the dispersant and oil increased the toxicity of the oil by a factor of 52 times. This line of discussion was not supported by their experimental design because they did not measure the concentrations of oil in their exposure medium. Their conclusion was completely unfounded, but has become often quoted in the literature.

CONFIDENTIAL

Alameda attempted to determine the effects of short-term crude oil exposure on the survival and bioaccumulation of PAHs in natural mesozooplankton assemblages; and assess the lethal effects of dispersant-treated crude oil and dispersant (CorexitEC9500A) on coastal mesozooplankton communities.

They used non-standard 16- and 48- hour static non-replacement toxicity method. They used an apparently standard method for preparing exposure media and verified that the exposure method contained oil droplets of a size ingestible by the zooplankton. They used NOMINAL oil concentrations, but measured the concentrations of PAHs in the parent oil. They used ratios of zooplankton oil concentrations to nominal oil concentrations in exposure media as indices of bioaccumulation.

Mortality of mesozooplankton increased with increasing NOMINAL oil concentration. Median lethal concentrations were reported but were of little use because of NOMINAL exposure concentrations. Authors concluded that dispersant and dispersant- treated oil were 2.3 and 3.4 times more toxic than crude oil alone mesozooplankton, but this is not valid because of NOMINAL exposure concentrations.

In addition, Alameda attempted to study the accumulation of PAHs from exposure media by mixed species zooplankton assemblages, as well as by a single species, A. tonsa. None of the results from this part of the study is useful because the actual composition of the exposure medium is not known. Even the relative accumulation rates estimated are difficult to interpret because the actual relative concentrations of the compounds in the exposure media are not known. The author calculated and reported bioaccumulation factors, but these are of little use because: a) they are based on the body burdens of individuals of many species that have different uptake mechanisms and different clearance rates; and b) there is no way of knowing how close these body burdens are to steady-state. In short, the results and conclusions from this part of the study are of limited value.

Kuhl – The experiment and paper by Kuhl et al. included a number of dispersant-related and scientific challenges. In short, the authors attempted to use microcosm experiments to study the effects water salinity on the fate, degradation and toxicity of untreated and dispersant-treated, weathered oil in a shallow nearshore environment. The specific objectives were:

1) to explore the role of salinity and time on partitioning of PAHs into the aqueous phase from layers of oil, dispersant and chemically dispersed oil applied to the water's surface in microcosms, and to study their subsequent biodegradation and associated toxicity to Fundulus. grandis.

2) to model the role of these variables on mortality; and

3) to determine if a common environmental sampling analysis (aqueous PAH concentrations) accurately reflects acute toxicity of dispersed and non-dispersed crude oil.

CONFIDENTIAL

Some of the challenges in Kuhl are discussed above in the Fate Section. The toxicity data in this study are difficult to interpret. There was a range of toxic effects but toxicity was not correlated with the concentration of any the PAH. Even if toxicity could be linked to the oil, ther results would be difficult to apply because of the experimental design.

Summary of Problems in Effects Research

To this point in the report writing only problems in the experimental studies have been investigated. The most significant sources of problems are listed in Table 4.

One important problem, seen in Kuhl et al., was an incorrect mental model the behaviour of dispersant-treated oil in a nearshore environment. Their experimental setup involved layering dispersant-treated oil on water in a mesocosm and allowing it to sit, undisturbed for 16 weeks to study the effect of the presence of the dispersant on partitioning of PAH from the oil slick into the water. While interesting, this is a completely unrealistic exposure scenario and the results cannot be related to any real-world situation. In the real-world waves or even tidal action would quickly mix the dispersant-treated oil into the water.

A second problem observed in Kuhl, involved monitoring the degradation of dispersant and dispersant-treated oil in a confined system for 16 weeks and expecting to observe changes reflective of a real-world system. In the real world, the entrained HCs and dispersant would dissipate quickly by dilution making any changes observed in the microcosm artefactual.

A third problem, also observed in Kuhl, was their failure to adequately describe the method used to prepare their exposure media. It appears that in different mesocosms, they layered either oil, dispersant or dispersant-treated oil on the water surface. Dispersant-treated oil on a water surface is very unstable and disperses readily with even a mild disturbance. Great care would be needed to prevent the treated oil from mixing into the water. The authors prepared at least nine of such mesocosms. It is possible/likely(?) that some unintentional dispersion might have taken place during the setup of one or more of these mesocosms. This alone might have accounted for the non-linear effects attributed to effects of salinity or aging time. The authors did not describe precautions taken to prevent mixing or take samples at time 0 to demonstrate that no inadvertent dispersion had taken place during setup.

The most common problem in these studies is the failure to measure the oil or HC concentrations in exposure media. In all studies this failure meant that the sensitivity of the test organism to the hydrocarbons is not determined quantitatively and the results cannot be used to assess risk or to be compared with sensitivity values from other studies. In addition, it makes it impossible to relate effects to the different components in the exposure media. For example, in Rico-Martinez, the most frequently cited publication, the authors concluded that elevated mortalities in their oil+dispersant experiment were caused by synergistic toxicity of the oil and dispersant, failing to acknowledge that the true cause was greatly elevated oil concentrations in the dispersant-treated experiment. The failure to measure oil in exposure media invalidated their conclusion. Alameda had the same problem. In Alameda, failure to measure oil concentrations in exposure media invalidated all conclusions in their bioaccumulation study because the actual oil exposure concentrations were completely unknown.

CONFIDENTIAL

Table 4 Summary of Problems in Experimental Effects Papers						
Effects	Anderson	Rico-Martinez	Alameda	Kuhl		
Scientific errors				x		
Dispersant-specific errors						
Incorrect mental model of dispersant use				x		
Incorrect model of environmental behaviour				x		
Inappropriate test protocol						
Inappropriate method for exposure media prep				x		
Inappropriate method for characterizing exposure media	x	x	x			

CONFIDENTIAL

Health

Problems with Human Health and Safety Studies

D'Andrea and Reddy (2013) assessed the adverse health effects of the Gulf oil spill exposure in subjects participating in the clean-up activity along the coast of Louisiana. This retrospective study included subjects that had been exposed and unexposed to the oil spill and dispersant. Using medical charts, clinical data including white blood cell count, platelets count, hemoglobin, hematocrit, blood urea nitrogen, creatinine, alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), and somatic symptom complaints by the subjects were reviewed and analyzed. Authors reported that hematologic analysis showed that platelet counts were significantly decreased in the exposed group compared with those in the group unexposed to the oil spill. Conversely, the hemoglobin (g per dL) and hematocrit (%) levels were significantly increased among oil spill-exposed subjects compared with the unexposed subjects. The authors concluded that clean-up workers exposed to the oil spill and dispersant experienced significantly altered blood profiles, liver enzymes, and somatic symptoms.

This is a poorly designed, executed, analyzed, and interpreted study. Numerous limitations preclude drawing any conclusions about potential health effects in workers associated with the clean-up of the Deepwater Horizon oil spill. The shortcomings include: biased target and control study groups, failure to control for risk factors associated with several outcome measures and which have clearly different occurrence rates in the study groups, absence of quantitative or qualitative exposure data, and consequently, over-interpretation of the data presented. While the authors acknowledge some of these limitations and correctly note that this research does not address possible causal relationship between the spill and worker health outcomes, their conclusion that the study results support the potential for hematological and hepatic toxicity is also without merit.[3]

[3] Many thanks to reviewers: Heidi S. Erickson and Ken Satin, ETC/HES for this review.

ED_001324_00001363-00029

Table 5 Problems with Health and Safety Papers						
Health and Safety	D' Andrea and Redy					
Scientific errors	x					

CONFIDENTIAL

Effectiveness
In preparation

Editorial, Policy Etc
IN Preparation

CONFIDENTIAL

Literature Cited

Adams, E. Eric. 2013. Comment on "Evolution of the Macondo Well Blowout: Simulating the Effects of the Circulation and Synthetic Dispersants on the Subsea Oil Transport" A polemic. This correspondence comments on the droplet size distribution used by the authors to predict the effects of sub-surface dispersants applied to the Macondo well blowout. Environmental Science & Technology (2013), 47(20), 11905 CODEN: ESTHAG; ISSN: 0013-936X[See - Paris CB, Hénaff ML, Aman ZM, Subramaniam A, Helgers J, Wang DP, Kourafalou VH, Srinivasan A.Evolution of the Macondo well blowout: simulating the effects of the circulation and synthetic dispersants on the subsea oil transport. (Environ Sci Technol. 2012 Dec 18;46(24):13293-302. doi: 10.1021/es303197h. Epub 2012 Dec 3]

Adams J, Sweezey M, Hodson PV. Oil and oil dispersant do not cause synergistic toxicity to fish embryos.Environ Toxicol Chem. 2013 Sep 23. doi: 10.1002/etc.2397. [Epub ahead of print]

Afonne OJ, Onyiaorah IV, Orisakwe OE.2013.Toxicity of Chevron Escravos crude oil and chemical dispersant on guinea pig testicular function. J Basic Clin Physiol Pharmacol. 2013 Nov 1;24(4):321-9. doi: 10.1515/jbcpp-2012-0060.

Agamy, E. 2013. Impact of laboratory exposure to light Arabian crude oil, dispersed oil and dispersant on the gills of the juvenile brown spotted grouper (Epinephelus chlorostigma): A histopathological study. Marine Environmental Research, 86:46-55.

Agamy, E. 2013. Sub-chronic exposure to crude oil, dispersed oil and dispersant induces histopathological alterations in the gills of the juvenile rabbit fish (Siganus canaliculatus). Ecotoxicology and Environmental Safety, 92:180-190.

Ali, A.O., C. Hohn, P.J. Allen, L. Ford, M.B. Dail, S. Pruett, L. Petrie-Hanson. 2014. The effects of oil exposure on peripheral blood leukocytes and splenic melano-macrophage centers of Gulf of Mexico fishes. Marine Pollution Bulletin, Available online 6 January 2014

Almeda, R., Z. Wambaugh, Z. Wang, C. Hyatt, Z. Liu, E. Buskey. 2013. Interactions between zooplankton and crude oil: toxic effects and bioaccumulation of polycyclic aromatic hydrocarbons PLoS One (2013), 8(6),

Aman ZM, Paris CB.Response to comment on "evolution of the macondo well blowout: simulating the effects of the circulation and synthetic dispersants on the subsea oil transport".Environ Sci Technol. 2013 Oct 15;47(20):11906-7. doi: 10.1021/es404183y. Epub 2013 Oct 1. No abstract available.

Anderson, S.S., Peterson, C.H., Cherr, G.N., Hampton, S., Blum, M.. 2012. Casual Observations on DWH Dispersant Effects Expose the Lack of Rigorous Science: Response to Rorick and Colleagues. Bioscience 62(12):1010-.

Anderson, JA, Kuhl AJ, Anderson AN. 2014. Toxicity of Oil and Dispersed Oil on Juvenile Mud Crabs, Rhithropanopeus harrisii. Bull Environ Contam Toxicol. 2014 Apr;92(4):375-80.

D'Andrea MA1, Reddy GK.1011. Health consequences among subjects involved in Gulf oil spill clean-up activities. Am J Med. 2013 Nov;126(11):966-74. doi: 10.1016/j.amjmed.2013.05.014. Epub 2013 Sep 17

Arnold C. Studies seek to find answers about Gulf oil spill legacy. Lancet. 2013 Aug 24;382(9893):673-4.

CONFIDENTIAL

Barron, M.G. 2012. Ecological impacts of the deepwater horizon oil spill: implications for immunotoxicity. Toxicol Pathol. 2012;40(2):315-20.

Barron, M.G., M.J. Hemmer, C.R. Jackson. 2013. Development of aquatic toxicity benchmarks for oil products using species sensitivity distributions. Integr Environ Assess Manag. 2013 Apr 2. doi: 10.1002/ieam.1420.

Barsamian, Adam L.; Perkins, Matt J.; Field, Jennifer A.; Blakemore, Paul R. Regioselective syntheses of [13C]4-labelled sodium 1-carboxy-2-(2-ethylhexyloxycarbonyl)ethanesulfonate and sodium 2-carboxy-1-(2-ethylhexyloxycarbonyl)ethanesulfonate from [13C]4-maleic anhydride Journal of Labelled Compounds and Radiopharmaceuticals (2014) Ahead of Print CODEN: JLCRD4; ISSN: 0362-4803

Baelum, J., Borglin, S., Chakraborty, R., Fortney, J.L., Lamendella, R., Mason, O.U., Auer, M., Zemla, M., Bill, M., Conrad, M.E. 2012. Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. Environmental Microbiology 14(9):2405-16.

Batchu Sudha Rani(1,2); Ramirez Cesar E.(1,2); Gardinali Piero R.(1,2) Correspondence(s): Gardinali P.R. Stability of dioctyl sulfosuccinate (DOSS) towards hydrolysis and photodegradation under simulated solar conditions Science of the Total Environment (15 May 2014), Volume 481, Number 1, pp. 260-265, 28 refs. ISSN: 0048-9697 E-ISSN: 1879-1026 DOI: 10.1016/j.scitotenv.2014.02.033 Published by: Elsevier

Bejarano, A.C., Levine, E. and Mearns, A.J. In Press. Effectiveness and potential ecological effects of offshore surface dispersant use during the Deepwater Horizon oil spill: a retrospective analysis of monitoring data. Environmental Monitoring and Assessment: In Press.

Broje V.; Gala W.R.; Nedwed T. 2013. API research on fate and effects of dispersants and dispersed oil in deepwater environment ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013).

Brandvik PJ, Johansen Ø, Leirvik F, Farooq U, Daling PS.Droplet breakup in subsurface oil releases--part 1: experimental study of droplet breakup and effectiveness of dispersant injection. Mar Pollut Bull. 2013 Aug 15;73(1):319-26. doi: 10.1016/j.marpolbul.2013.05.020. Epub 2013 Jun 21.

Brewton RA, Fulford R, Griffitt RJ.Gene Expression and Growth as Indicators of Effects of the BP Deepwater Horizon Oil Spill on Spotted Seatrout (Cynoscion nebulosus). J Toxicol Environ Health A. 2013;76(21):1198-209. doi: 10.1080/15287394.2013.848394.

Campo, P., A. D. Venosa, and M. T. Suidan. 2013.Biodegradability of Corexit 9500 and Dispersed South Louisiana Crude Oil at 5 and 25 °C. Environ. Sci. Technol., 47 (4):1960–1967.

Changkyu Joo, Won Joon Shim, Gi Beum Kim, Sung Yong Ha, Moonkoo Kim, Joon Geon An, Eunsic Kim, Beom Kim, Seung Won Jung, Young-Ok Kim, Un Hyuk Yim. 2013. Mesocosm study on weathering characteristics of Iranian Heavy crude oil with and without dispersants. Journal of Hazardous Materials, 248–249:37-46

Chase, D.A., Edwards, D.S., Qin, G., Wages, M.R., Willming, M.M., Anderson, T.A., Maul, J.D. 2013. Bioaccumulation of petroleum hydrocarbons in fiddler crabs (Uca minax) exposed to weathered MC-252 crude oil alone and in mixture with an oil dispersant. Science of The Total Environment 444:121-127.

Claireaux G, Théron M, Prineau M, Dussauze M, Merlin FX, Le Floch S. Effects of oil exposure

CONFIDENTIAL

and dispersant use upon environmental adaptation performance and fitness in the European sea bass, Dicentrarchus labrax. Aquat Toxicol. 2013 Apr 15;130-131:160-70. doi: 10.1016/j.aquatox.2013.01.004. Epub 2013 Jan 18.

Coelho,G., J.Clark, D.Aurand. 2013.Toxicity testing of dispersed oil requires adherence to standardized protocols to assess potential real world effects. Environmental Pollution, 177:185–188

Conmy, R.N., P.G. Coble, R. Parsons, I. Walsh, C. Koch, M.S. Miles, M. Abercrombie, J. Farr, A.M. Wood, K. Lee, S. Pegau, C. Kelble (2014) Performance of submersible fluorometers exposed to chemically-dispersed crude oil: wave tank simulations for improved oil spill monitoring and evaluation of Deepwater Horizon spill measurements. *Environmental Science and Technology*. 48, 3: 1803-10.

Claireaux G., M. Théron, M. Prineau, M. Dussauze, F.-X. Merlin, S. Le Floch. 2013. Effects of oil exposure and dispersant use upon environmental adaptation performance and fitness in the European sea bass, Dicentrarchus labrax. Aquatic Toxicology 130–131:160-170.

Ehrenhauser, Franz S.; Avij, Paria; Shu, Xin; Dugas, Victoria; Woodson, Isaiah; Liyana-Arachchi, Thilanga; Zhang, Zenghui; Hung, Francisco R.; Valsaraj, Kalliat T. [Reprint Author] Bubble bursting as an aerosol generation mechanism during an oil spill in the deep-sea environment: laboratory experimental demonstration of the transport pathway. Environmental Science-Processes & Impacts, (2014) Vol. 16, No. 1, pp. 65-73. http://rsc.li/process-impacts. ISSN: 2050-7887. E-ISSN: 2050-7895.

Field J.A.; Place B.J.; Perkins M.2013. Analytical tools for unraveling the complex mixture of oil dispersants ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013

Fitzgerald Timothy Patrick and Gohlke Julia M 2014. Contaminant levels in Gulf of Mexico reef fish after the Deepwater Horizon oil spill as measured by a fishermen-led testing program Environmental Science & Technology Just Accepted Manuscript

Fuller, C.B., J. S. Bonner, M. S. Islam, C. Page, T. Ojo, W. Kirkey. 2013.Estimating sub-surface dispersed oil concentration using acoustic backscatter response. Marine Pollution Bulletin, Volume 70, Issues 1–2, 15 May 2013, Pages 140-146 (ExxonMobil Biblio Jul10)

Gardinali P.R.; Echols B.; Rand G.M.; Benkinney M; Stubblefield W.; Pelz O.X..2013. Influence of Deepwater Horizon field-collected oil characteristics and exposure conditions on aquatic toxicity test results ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013)

Gardiner., W.,J.Q. Word, J.D. Word, R.A. Perkins, K.M. McFarlin, B.W. Hester, L.S. Word, and C.M. Ray CM.2013. The acute toxicity of chemically and physically dispersed crude oil to key arctic species under arctic conditions during the open water season. Environ Toxicol Chem. 32(10):2284–2300.

Glover C.M.; Parker A.M.; Linden K.G.; Rosario-Ortiz F.L.2013. Organic matter in ocean water as a proxy for indirect photochemical degradation of the dispersants used in the Deepwater Horizon oil spill ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013)

Gong Yanyan(1); Zhao Xiao(1); Qian Tianwei(1,2); Zhao Dongye(1); O'Reilly S.E.(3) Effects of oil dispersant and oil on sorption and desorption of phenanthrene with Gulf Coast marine sedimentsEnviron. Pollut. (2014), Volume 185, pp. 240-249, 39 refs.

CONFIDENTIAL

Gong, Yanyan; Zhao, Xiao; Cai, Zhengqing; O'Reilly, S. E.; Hao, Xiaodi; Zhao, DongyeA review of oil, dispersed oil and sediment interactions in the aquatic environment: Influence on the fate, transport and remediation of oil spillsMarine Pollution Bulletin (2014) Ahead of Print CODEN: MPNBAZ; ISSN: 0025-326X

Gong Y.; Zhao X.; Zhao D.; O'Reilly S.E.2013. Effects of oil dispersants on sorption capacity and hysteresis of polycyclic aromatic hydrocarbons with Gulf Coast marine sediments ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013),

Gong Y.; Zhao X.; Zhao D.; O'Reilly S.E.2013. Effects of oil dispersants and sediment sorption on photodegradation and ozonation of persistent oil compounds in the Gulf Coast ecosystems. ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013

Goodbody-Gringley G, Wetzel DL, Gillon D, Pulster E, Miller A, et al. 2013. Toxicity of Deepwater Horizon Source Oil and the Chemical Dispersant, Corexit® 9500, to Coral Larvae. PLoS ONE 8(1): e45574. doi:10.1371/journal.pone.0045574

Gray JL, Kanagy LK, Furlong ET, Kanagy CJ, McCoy JW, Mason A, Lauenstein G. 2013.Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. Chemosphere. 2014 Jan;95:124-30. doi: 10.1016/j.chemosphere.2013.08.049. Epub 2013 Sep 17.

Hazen T.C. 2013. Deepwater Horizon oil spill: A systems biology approach to an ecological disaster ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013)

Holder, E.L, R.N. Conmy, A.D. Venosa (2015) Comparative laboratory-scale testing of dispersant effectiveness of 23 crude oils using four different testing protocols. *J. Environ. Protection*. 6: 628-639.Johansen, Ø. P. Brandvik, U. Farooq. 2013. Droplet breakup in subsea oil releases – Part 2: Predictions of droplet size distributions with and without injection of chemical dispersants Marine Pollution Bulletin, 73(1):327-335.

Joo, Changkyu / Shim, Won Joon / Kim, Gi Beum / Ha, Sung Yong / Kim, Moonkoo / An, Joon Geon / Kim, Eunsic / (...) / Yim, Un Hyuk,2013.Mesocosm study on weathering characteristics of Iranian Heavy crude oil with and without dispersants Journal of Hazardous Materials, 248-249, p.37-46, Mar 2013

King, T., J. Clyburne, K. Lee, B. Robinson. 2013.Interfacial film formation: Influence on oil spreading rates in lab basin tests and dispersant effectiveness testing in a wave tank Marine Pollution Bulletin 71 (1–2):83-91.

Kover S.C.(1); Rosario-Ortiz F.L.(1); Linden K.G.(1) Correspondence(s): Linden K.G.(1) Photochemical fate of solvent constituents of Corexit oil dispersants Water Research (2014), Volume 52, Number 1, pp. 101-111, 6 Tables and spectra, 28 refs. CODEN: WATRAG ISSN: 0043-1354 DOI: 10.1016/j.watres.2013.12.013 Published by: Elsevier Ltd, Langford Lane, Kidlington, OX5 1GB, Oxford, United Kingdom

Kuhl, Adam J.; Nyman, J. Andrew; Kaller, Michael D.; Green, Christopher C. 2013. Dispersant and salinity effects on weathering and acute toxicity of South Louisiana crude oilEnviron Toxicol Chem 2013;32:2611-2620. © 2013 SETAC.

Lavallee, JL; Bugden, JBC; Kepkay, PE; Lee, K Fluorescence fingerprinting of oils dispersed in seawater: application of ultraviolet fluorescence (UVF) and excitation-emission matrix spectroscopy (EEMS) Canadian technical report of fisheries and aquatic sciences/Rapport technique canadien des sciences halieutiques et aquatiques [Can.

Tech. Rep. Fish. Aquat. Sci./Rapp. Tech. Can. Sci. Halieut. Aquat.]. no. 2997, 212 pp. 2012. ISSN: 0706-6457 Published by: DFO, Dartmouth, NS (Canada), [mailto:info@dfo-mpo.gc.ca] URL (Document): http://www.dfo-mpo.gc.ca/Library/346586.pdf URL (Document): http://www.dfo-mpo.gc.ca/

Lee KW, Shim WJ, Yim UH, Kang JH.Acute and chronic toxicity study of the water accommodated fraction (WAF), chemically enhanced WAF (CEWAF) of crude oil and dispersant in the rock pool copepod Tigriopus japonicus. Chemosphere 92(9):1161-8.

Lee, K., T. Nedwed, D. Palandro, R. Prince. 2013.Lab tests on the biodegradation of chemically dispersed oil should consider the rapid dilution that occurs at sea Mar. Pollut. Bull. 73 (1) 314-318.

Lewis M, Pryor R.2013.Toxicities of oils, dispersants and dispersed oils to algae and aquatic plants: review and database value to resource sustainability. Environ Pollut. 2013 Sep;180:345-67. doi:

Liyana-Arachchi TP, Zhang Z, Ehrenhauser FS, Avij P, Valsaraj KT, Hung FR.Bubble bursting as an aerosol generation mechanism during an oil spill in the deep-sea environment: molecular dynamics simulations of oil alkanes and dispersants in atmospheric air/salt water interfaces. Environ Sci Process Impacts. 2014 Jan;16(1):53-64. doi: 10.1039/c3em00391d.10.1016/j.envpol.2013.05.001. Epub 2013 Jun 14.

Macías-Zamora JV, Meléndez-Sánchez AL, Ramírez-Álvarez N, Gutiérrez-Galindo EA, Orozco-Borbón MV. 2013. On the effects of the dispersant Corexit 9500© during the degradation process of n-alkanes and PAHs in marine sediments. Environ Monit Assess. 2014 Feb;186(2):1051-61. doi: 10.1007/s10661-013-

Martin JD, Adams J, Hollebone B, King T, Brown RS, Hodson PV. Chronic toxicity of heavy fuel oils to fish embryos using multiple exposure scenarios. Environ Toxicol Chem. 2014 Mar;33(3):677-87. doi: 10.1002/etc.2486. Epub 2014 Jan 24.

McFarlin KM, Prince RC, Perkins R, Leigh MB. Biodegradation of dispersed oil in Arctic seawater at -1°C. PLoS One. 2014 Jan 8;9(1):e84297. doi: 10.1371/journal.pone.0084297. eCollection 2014.3438-2. Epub 2013 Oct 27.

Mendoza,W., D. Riemer and R. Zika 2013. Application of fluorescence and PARAFAC to assess vertical distribution of subsurface hydrocarbons and dispersant during the Deepwater Horizon oil spill Environ. Sci.: Processes Impacts, 2013,15, 1017-1030

Milinkovitch T, Imbert N, Sanchez W, Le Floch S, Thomas-Guyon H. Toxicological effects of crude oil and oil dispersant: biomarkers in the heart of the juvenile golden grey mullet (Liza aurata). Ecotoxicol Environ Saf. 2013 Feb;88:1-8. doi: 10.1016/j.ecoenv.2012.10.029. Epub 2012 Dec 4.

Milinkovitch T, Thomas-Guyon H, Lefrançois C, Imbert N.2013. Dispersant use as a response to oil spills: toxicological effects on fish cardiac performance.Fish Physiol Biochem. 2013 Apr;39(2):257-62. doi: 10.1007/s10695-012-9696-z. Epub 2012 Jul 28.

Montagna PA, Baguley JG, Cooksey C, Hartwell I, Hyde LJ, Hyland JL, Kalke RD, Kracker LM, Reuscher M, Rhodes ACE. 2013. Deep-Sea Benthic Footprint of the Deepwater Horizon Blowout. PLoS ONE 8(8):e70540. Find it Online*

Montagna, P.A., J.G. Baguley, C. Cooksey, and J.L. Hyland. 2013. Deepwater Horizon Oil Spill: Assessment of Potential Impacts on the Deep Soft-Bottom Benthos. Interim Data Summary Report. NOAA Technical Memorandum NOS NCCOS 166. NOAA, National Centers for Coastal Ocean Science, Charleston, SC. 32 p.

ED_001324_00001363-00036

Moody, R.M., J. Cebrian and K. Heck Jr. 2013. Interannual Recruitment Dynamics for Resident and Transient Marsh Species: Evidence for a Lack of Impact by the Macondo Oil Spill PLoS ONE 8(3):e58376

Nedwed T.; Coolbaugh T. 2013. Industry-sponsored subsea dispersant injection research ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013)

Olsen, G.H., C. Klok, A.J. Hendriks, P. Geraudie, L. De Hoop, F. De Laender, E. Farmen, B.E. Grøsvik, B.H. Hansen, M. Hjorth, C.R. Jansen, T. Nordtug, E. Ravagnan, K. Viaene, J. Carroll.2013. Toxicity data for modeling impacts of oil components in an Arctic ecosystem. Marine Environmental Research 90: 9-17.

Ozhan K, Miles SM, Gao H, Bargu S. Relative Phytoplankton growth responses to physically and chemically dispersed South Louisiana sweet crude oil. Environ Monit Assess. 2014 Feb 12. [Epub ahead of print]

Ozhan K, Bargu S. Distinct responses of Gulf of Mexico phytoplankton communities to crude oil and the dispersant corexit(®) Ec9500A under different nutrient regimes. Ecotoxicology. 2014 Apr;23(3):370-84. doi: 10.1007/s10646-014-1195-9. Epub 2014 Jan 28.

Paris CB, Hénaff ML, Aman ZM, Subramaniam A, Helgers J, Wang DP, Kourafalou VH, Srinivasan A. Evolution of the Macondo well blowout: simulating the effects of the circulation and synthetic dispersants on the subsea oil transport. Environ Sci Technol. 2012 Dec 18;46(24):13293-302. doi: 10.1021/es303197h. Epub 2012 Dec 3. (NOTE A 2012 PAPER included because of published comments by AMMAN (2013) and ADAMS (2013))

Paul, I. 2013. Australian revised National Plan policy and process for the recognition, application and monitoring of oil spill control agents, including dispersants. Proceedings of the 36th AMOP Technical Seminar on Environmental Contamination and Response (2013), pp. 797-808.

Paul, J.H., D. Hollander, P. Coble, K. Daly, S. Murasko, D. English, J. Basso, J. Delaney, L. McDaniel, and C. Kovach. 2013. Toxicity and Mutagenicity of Gulf of Mexico Waters During and After The Deepwater Horizon Oil Spill Environmental Science & Technology 47(17):9651-9.

Peavler B. 2013. Environment Arctic: Advancing Arctic oil spill response. Petroleum Review. 67(798): 24-25.

Perkins, M., B. Place, J. Field. 2013. LVI-LC-MS/MS analysis of Corexit in GOM water column and sediments. Abstracts, 68th Northwest Regional Meeting of the American Chemical Society, Corvallis, OR, United States, July 21-24 (2013), NORM-18. American Chemical Society: Washington.

Perrons, R.K..2013. Assessing the damage caused by Deepwater Horizon: Not just another Exxon Valdez Marine Pollution Bulletin 71(1-2):20-2.

Place, Benjamin J.; Perkins, Matt J.; Sinclair, Ewan; Barsamian, Adam L.; Blakemore, Paul R.; Field, Jennifer A. Trace analysis of surfactants in Corexit oil dispersant formulations and seawater Deep Sea Research, Part II: Topical Studies in Oceanography (2014) Ahead of Print CODEN: DSROEK; ISSN: 0967-0645

Polli, J.R.; Zhang, Y; Pan, X. 2013. Investigating crude oil-dispersant mixture-induced reproductive toxicity and its mechanism in C. elegans Abstracts of Papers, 246th ACS National Meeting & Exposition, Indianapolis, IN, ENVR-174.

CONFIDENTIAL

Powers, Sean P.; Hernandez, Frank J.; Condon, Robert H.; Marcus Drymon, J.; Free, Christopher M. 2013. Novel pathways for injury from offshore oil spills: direct, sublethal and indirect effects of the Deepwater Horizon oil spill on pelagic Sargassum communities. PLoS One (2013), 8(9), e74802 CODEN: POLNCL; ISSN: 1932-6203

Prince RC, Butler JD. 2013. A protocol for assessing the effectiveness of oil spill dispersants in stimulating the biodegradation of oil. Environ Sci Pollut Res Int. 2013 Aug 13. [Epub ahead of print]

Prince RC, McFarlin KM, Butler JD, Febbo EJ, Wang FC, Nedwed TJ.The primary biodegradation of dispersed crude oil in the sea. Chemosphere 90(2):521-6.

Radniecki, T.S., M. Schneider, L. Semprini. 2013. The influence of Corexit 9500A and weathering on Alaska North Slope crude oil toxicity to the ammonia oxidizing bacterium, Nitrosomonas europaea Marine Pollution Bulletin 68(1–2): 64-70.

Ramirez, C., S.R. Batchu, P.R. Gardinali. 2013. High sensitivity liquid chromatography tandem mass spectrometric methods for the analysis of dioctyl sulfosuccinate in different stages of an oil spill response monitoring effort. Analytical and Bioanalytical Chemistry 405(12):4167-4175.

Reichert, M.D., L.M. Walker LM. 2013. Interfacial Tension Dynamics, Interfacial Mechanics, and Response to Rapid Dilution of Bulk Surfactant of a Model Oil-Water-Dispersant System. Langmuir 29(6):1857-1867.

Rico-Martínez R, T.W. Snell, T.L. Shearer. 2013. Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A® to the Brachionus plicatilis species complex (Rotifera). Environmental Pollution 173:5-10.

Saha, A., A. Nikova, P. Venkataraman, V.T. John, A. Bose A. 2013.Oil emulsification using surface-tunable carbon black particles. ACS Appl Mater Interfaces5(8):3094-100.

Sammarco, P.W., S.R. Kolian, R.A. Warby, J.L. Bouldin, W.A. Subra, S.A. Porter SA. 2013. Distribution and concentrations of petroleum hydrocarbons associated with the BP/Deepwater Horizon Oil Spill, Gulf of Mexico. Marine Pollution Bulletin: In Press.

Schultz, C. 2013 Monitoring and Modeling the Deepwater Horizon Oil Spill: A Record-Breaking Enterprise.Eos, Transactions American Geophysical Union 94(20):185–186.

Schrope, M. 2013. Researchers debate oil-spill remedy. Nature 493(7433):461.

Spier, C., W. Stringfellow, T.C. Hazen, M. Conrad. 2013.Distribution of hydrocarbons released during the 2010 MC252 oil spill in deep offshore waters Environmental Pollution 173: 224-230.

Stokstad, E. 2013. BP Research Dollars Yield Signs of Cautious Hope. Science 339(6120):636-637.

Venosa, A., E.L. Holder. 2013. Determining the dispersibility of South Louisiana crude oil by eight oil dispersant products listed on the NCP Product Schedule. Marine Pollution Bulletin 66(1–2): 73-77.

Van Scoy AR, Voorhees J, Anderson BS, Philips BM, Tjeerdema RS. 2013.Use of semipermeable membrane devices (SPMDs) to characterize dissolved hydrocarbon fractions of both dispersed and undispersed oil. Environ Sci Process Impacts. 2013 Oct 23;15(11):2016-22. doi: 10.1039/c3em00275f.

Venkataraman P, J. Tang, E. Frenkel, G.L. McPherson, J. He, S.R. Raghavan, V. Kolesnichenko, A. Bose, V.T. John. 2013. Attachment of a hydrophobically modified

CONFIDENTIAL

biopolymer at the oil-water interface in the treatment of oil spills. ACS Appl Mater Interfaces. 2013 May;5(9):3572-80.

Vilcaez J, Li L, Hubbard S. 2013. A new model for the biodegradation kinetics of oil droplets: application to the Deepwater Horizon oil spill in the Gulf of Mexico. Geochemical Transactions 14(1):4. Find it Online*

Wang, W., Y. Zheng, K. Lee2013. Chemical dispersion of oil with mineral fines in a low temperature environment Marine Pollution Bulletin 72(1): 205-12.

Zhang, Y.Q., D.L. Chen, A.C. Ennis, J.R. Polli, P. Xiao, B.H. Zhang, E.J. Stellwag, A. Overton, X.P. Pan. 2013. Chemical dispersant potentiates crude oil impacts on growth, reproduction, and gene expression in Caenorhabditis elegans. Archives of Toxicology 87(2):371-382.

Zhang, Y., Polli, J. Pan, X. 2013. Oil/dispersant induced the aberrant expression of miRNAs in C. elegans: Implications in reproductive toxicity. Abstracts of Papers, 246th ACS National Meeting & Exposition, Indianapolis, IN

Zhang, Y., Pan, X. 2013.Chemical dispersant synergy the Deepwater Horizon oil spill's toxicity on the growth, reproduction, and gene expression of Caenorhabditis elegans. Abstracts of Papers American Chemical Society, (AUG 19 2012) Vol. 244, pp. 37-ENVR. Meeting Info.: 244th National Fall Meeting of the American-Chemical-Society (ACS). Philadelphia, PA, USA. August 19 -23, 2012. Amer Chem Soc, Div Hist Chem; Amer Chem Soc. CODEN: ACSRAL. ISSN: 0065-7727.

Zhang, M. Abulikemu, P. Campo-Moreno, W.E. Platten, A.D. Venosa, **R.N. Conmy** (2015) A comparative study on the biodegradability of crude oil dispersed with JD-2000 at 5 and 25 °C. *Chemosphere.*

Ziervogel, K., and Arnosti, C., 2013. Enhanced protein and carbohydrate hydrolysis in plume-associated deep waters initially sampled during the early stages of the Deepwater Horizon oil spill. Deep Sea Research Part II: Topical Studies in Oceanography: In Press.

Zhao, L., B. Wang, P.M. Armenante, **R.N. Conmy**, M.C. Boufadel (2015) Characterization of turbulent properties in the EPA Baffled Flask for dispersant effectiveness testing. *J. Environmental Engineering.* DOI:10.1061/(ASCE)EE.1943-7870.0001000.

Zhou, Z., Z. Liu, L. Guo. 2013. Chemical evolution of Macondo crude oil during laboratory degradation as characterized by fluorescence EEMs and hydrocarbon composition Marine Pollution Bulletin 66(1–2):164-175.

Zhou, Z., G. Laodong, A.M. Shiller, S.E. Lohrenz, V.L. Asper, C.L. Osburn. 2013. Characterization of oil components from the Deepwater Horizon oil spill in the Gulf of Mexico using fluorescence EEM and PARAFAC techniques. Marine Chemistry Volume 148:10–21.

Zuijdgeest A., and M. Huettel. 2012. Dispersants as Used in Response to the MC252-Spill Lead to Higher Mobility of Polycyclic Aromatic Hydrocarbons in Oil-Contaminated Gulf of Mexico Sand. PLoS ONE 7(11): e50549. doi:10.1371/journal.pone.0050549

ED_001324_00001363-00039

Appendix 1 – Bibliography to August 31, 2015.

Almeda R, Hyatt C, Buskey EJ. 2014. Toxicity of dispersant Corexit 9500A and crude oil to marine microzooplankton. Ecotoxicol. Environ. Saf. 106C:76-85

Almeda R, Baca S, Hyatt C, Buskey EJ. Ingestion and sublethal effects of physically and chemically dispersed crude oil on marine planktonic copepods.Ecotoxicology. 2014 Aug;23(6):988-1003. doi: 10.1007/s10646-014-1242-6. Epub 2014 Apr 23.Free PMC Article

Almeda R, Bona S, Foster CR, Buskey EJ. Dispersant Corexit 9500A and chemically dispersed crude oil decreases the growth rates of meroplanktonic barnacle nauplii (Amphibalanus improvisus) and tornaria larvae (Schizocardium sp.).Mar Environ Res. 2014 Aug;99:212-7. doi: 10.1016/j.marenvres.2014.06.007. Epub 2014 Jun 24.

Almeda R, Connelly TL, Buskey EJ. Novel insight into the role of heterotrophic dinoflagellates in the fate of crude oil in the sea.Sci Rep. 2014 Dec 19;4:7560. doi: 10.1038/srep07560.Free PMC Article

Almeda R, Hyatt C, Buskey EJ. Toxicity of dispersant Corexit 9500A and crude oil to marine microzooplankton.Ecotoxicol Environ Saf. 2014 Aug;106:76-85. doi: 10.1016/j.ecoenv.2014.04.028. Epub 2014 May 14.

Anderson Lively JA, McKenzie J. Toxicity of the dispersant Corexit 9500 to early life stages of blue crab, Callinectes sapidus.Bull Environ Contam Toxicol. 2014 Dec;93(6):649-53. doi: 10.1007/s00128-014-1370-y. Epub 2014 Aug 31.

Almeda R, Wambaugh Z, Chai C, Wang Z, Liu Z, Buskey EJ. Effects of crude oil exposure on bioaccumulation of polycyclic aromatic hydrocarbons and survival of adult and larval stages of gelatinous zooplankton.PLoS One. 2013 Oct 7;8(10):e74476. doi: 10.1371/journal.pone.0074476. eCollection 2013.Free PMC Article

Almeda R, Wambaugh Z, Wang Z, Hyatt C, Liu Z, Buskey EJ. Interactions between zooplankton and crude oil: toxic effects and bioaccumulation of polycyclic aromatic hydrocarbons.PLoS One. 2013 Jun 28;8(6):e67212. doi: 10.1371/journal.pone.0067212. Print 2013.Free PMC Article

Awkerman, JA, Raimondo S, Jackson CR, Barron MG. 2014. Augmenting aquatic species sensitivity distributions with interspecies toxicity estimation models. Environ. Toxicol. Chem. 33(3):688-95.

Bacosa HP, Erdner DL, Liu Z. Differentiating the roles of photooxidation and biodegradation in the weathering of Light Louisiana Sweet crude oil in surface water from the Deepwater Horizon site.Mar Pollut Bull. 2015 Jun 15;95(1):265-72. doi: 10.1016/j.marpolbul.2015.04.005. Epub 2015 Apr 18.

Barsamian AL, Perkins MJ, Field JA, Blakemore PR. Regioselective syntheses of [13C]4-labelled sodium 1-carboxy-2-(2-ethylhexyloxycarbonyl)ethanesulfonate and sodium 2-carboxy-1-(2-ethylhexyloxycarbonyl)ethanesulfonate from [13C]4-maleic anhydride.J Labelled Comp Radiopharm. 2014 May 15;57(5):397-401. doi: 10.1002/jlcr.3189. Epub 2014 Feb 12. Erratum in: J Labelled Comp Radiopharm. 2014 Aug;57(10):625.Free PMC Article

Batchu SR, Ramirez CE, Gardinali PR. Stability of dioctyl sulfosuccinate (DOSS) towards hydrolysis and photodegradation under simulated solar conditions.Sci Total Environ.

ED_001324_00001363-00040

2014 May 15;481:260-5. doi: 10.1016/j.scitotenv.2014.02.033. Epub 2014 Mar 4.

Benner SW, John VT, Hall CK. Intercomparison of oil spill prediction models for accidental blowout scenarios with and without subsea chemical dispersant injection.J Phys Chem B. 2015 Jun 11;119(23):6979-90. doi: 10.1021/acs.jpcb.5b01092. Epub 2015 Jun 1.

Bejarano AC, Clark JR, Coelho GM. Issues and challenges with oil toxicity data and implications for their use in decision making: a quantitative review.Environ Toxicol Chem. 2014 Apr;33(4):732-42. doi: 10.1002/etc.2501. Epub 2014 Feb 25. Review.

Bejarano AC, Barron MG. Development and practical application of petroleum and dispersant interspecies correlation models for aquatic species.Environ Sci Technol. 2014 Apr 15;48(8):4564-72. doi: 10.1021/es500649v. Epub 2014 Mar 28.

Bejarano AC, Barron MG. 2014. Development and Practical Application of Petroleum and Dispersant Interspecies Correlation Models for Aquatic Species. Environ Sci Technol. 2014 Mar 28. [Epub ahead of print]

Bejarano AC, Clark JR, Coelho GM. 2014 Issues and challenges with oil toxicity data and implications for their use in decision making: A quantitative review. Environ Toxicol Chem. 2014 Apr;33(4):732-42. doi: 10.1002/etc.2501. Epub 2014 Feb 25.

Bookstaver M, Bose A, Tripathi A. Interaction of Alcanivorax borkumensis with a Surfactant Decorated Oil-Water InterfaceLangmuir. 2015 Jun 2;31(21):5875-81. doi: 10.1021/acs.langmuir.5b00688. Epub 2015 May 21.

Brewton RA, Fulford R, Griffitt RJ. Gene expression and growth as indicators of effects of the BP Deepwater Horizon oil spill on spotted seatrout (Cynoscion nebulosus). J Toxicol Environ Health A. 2013;76(21):1198-209. doi: 10.1080/15287394.2013.848394.

Cohen JH, McCormick LR, Burkhardt SM. Effects of dispersant and oil on survival and swimming activity in a marine copepod.Bull Environ Contam Toxicol. 2014 Apr;92(4):381-7. doi: 10.1007/s00128-013-1191-4. Epub 2014 Jan 9.

COOLBAUGH, T; DEMICCO, E; KENNEDY, E DISPERSANT-RELATED OIL SPILL RESPONSE COMMUNICATION TOOLS: TOWARD AN ENHANCED APPROACH TO CONVEYING COMPLEX TOPICS IN AN APPROACHABLE MANNER INTERNATIONAL OIL SPILL CONFERENCE [IOSC] (Savannah, GA, 5/5-8/2014) PROCEEDINGS v.2014, no.1, pp.1163-1171, May 2014. (ISSN 2169-3358; Available at http://iosc.org as of 11/7/2014) Laursen April 21 2015

Cornwall W. Critics question plans to spray dispersant in future deep spillsScience. 2015 Apr 3;348(6230):27. doi: 10.1126/science.348.6230.27. Epub 2015 Apr 2. No abstract available.

Daling PS, Leirvik F, Almås IK, Brandvik PJ, Hansen BH, Lewis A, Reed M. Surface weathering and dispersibility of MC252 crude oil.Mar Pollut Bull. 2014 Oct 15;87(1-2):300-10. doi: 10.1016/j.marpolbul.2014.07.005. Epub 2014 Aug 22

Dussauze M, Pichavant-Rafini K, Le Floch S, Lemaire P, Theron M. Acute toxicity of chemically and mechanically dispersed crude oil to juvenile sea bass (Dicentrarchus labrax): Absence of synergistic effects between oil and dispersants.Environ Toxicol Chem. 2015 Jul;34(7):1543-51. doi: 10.1002/etc.2931. Epub 2015 May 7.

Elarbaoui S, Richard M, Boufahja F, Mahmoudi E, Thomas-Guyon H. Effect of crude oil exposure and dispersant application on meiofauna: an intertidal mesocosm

ED_001324_00001363-00041

experiment.Environ Sci Process Impacts. 2015 May;17(5):997-1004. doi: 10.1039/c5em00051c. Epub 2015 May 7.

Fu J, Gong Y, Zhao X, O'Reilly SE, Zhao D. Effects of oil and dispersant on formation of marine oil snow and transport of oil hydrocarbons.Environ Sci Technol. 2014 Dec 16;48(24):14392-9. doi: 10.1021/es5042157. Epub 2014 Dec 4.

Garcia, Tzintzuni I.; Shen, Yingjia; Crawford, Douglas; Oleksiak, Marjorie F.; Whitehead, Andrew; Walter, Ronald B. 2012. RNA-Seq reveals complex genetic response to Deepwater Horizon oil release in Fundulus grandis. BMC Genomics (2012), 13, 474 CODEN: BGMEET; ISSN: 1471-2164

Garr AL, Laramore S, Krebs W. Toxic effects of oil and dispersant on marine microalgae.Bull Environ Contam Toxicol. 2014 Dec;93(6):654-9. doi: 10.1007/s00128-014-1395-2. Epub 2014 Oct 5.

Kleindienst S, Paul JH, Joye SB. Using dispersants after oil spills: impacts on the composition and activity of microbial communities.Nat Rev Microbiol. 2015 Jun;13(6):388-96. doi: 10.1038/nrmicro3452. Epub 2015 May 6. Review.

Kleindienst S, Grim S, Sogin M, Bracco A, Crespo-Medina M, Joye SB. Diverse, rare microbial taxa responded to the Deepwater Horizon deep-sea hydrocarbon plume.ISME J. 2015 Jul 31. doi: 10.1038/ismej.2015.121. [Epub ahead of print]

Koyama, J., Chie Imakado, Seiichi Uno, Takako Kuroda, Shouichi Hara, Takahiro Majima, Hideyuki Shirota, Nathaniel C. Añasco. 2014. Simulated distribution and ecotoxicity-based assessment of chemically-dispersed oil in Tokyo Bay. Marine Pollution Bulletin

Lindo-Atichati, C.B. Paris, M. Le Hénaff, M. Schedler, A.G. Valladares Juárez, R. Müller. 2014. Simulating the effects of droplet size, high-pressure biodegradation, and variable flow rate on the subsea evolution of deep plumes from the Macondo blowout. Deep Sea Research Part II: Topical Studies in Oceanography, In Press, Corrected Proof, Available online 30 January 2014

Marti, M. E.; Colonna, W. J.; Patra, P.; Zhang, H.; Green, C.; Reznik, G.; Pynn, M.; Jarrell, K.; Nyman, J. A.; Somasundaran, P.; Glatz, C. E.; Lamsal, B. P. 2014. Production and characterization of microbial biosurfactants for potential use in oil-spill remediation Enzyme and Microbial Technology (2014), 55, 31-39 CODEN: EMTED2; ISSN: 0141-0229

McDaniel LD, Basso J, Pulster E, Paul JH. Sand patties provide evidence for the presence of Deepwater Horizon oil on the beaches of the West Florida Shelf.Mar Pollut Bull. 2015 Aug 15;97(1-2):67-77. doi: 10.1016/j.marpolbul.2015.06.032. Epub 2015 Jun 20.

Mu J, Jin F, Ma X, Lin Z, Wang J. Comparative effects of biological and chemical dispersants on the bioavailability and toxicity of crude oil to early life stages of marine medaka (Oryzias melastigma).Environ Toxicol Chem. 2014 Nov;33(11):2576-83. doi: 10.1002/etc.2721. Epub 2014 Sep 16.

Not Known ?? Simulation Study of Hydrophobically Modified Chitosan as an Oil Dispersant Additive

Nørregaard RD, Gustavson K, Møller EF, Strand J, Tairova Z, Mosbech A. Ecotoxicological

CONFIDENTIAL

investigation of the effect of accumulation of PAH and possible impact of dispersant in resting high arctic copepod Calanus hyperboreus.Aquat Toxicol. 2015 Jul 19;167:1-11. doi: 10.1016/j.aquatox.2015.07.006. [Epub ahead of print]

Pham PH, Huang YJ, Chen C, Bols NC. 2013. Corexit 9500 inactivates two enveloped viruses of aquatic animals but enhances the infectivity of a non-enveloped fish virus. Appl Environ Microbiol. 2013 Nov 22. [Epub ahead of print]

Pie HV, Mitchelmore CL. Acute toxicity of current and alternative oil spill chemical dispersants to early life stage blue crabs (Callinectes sapidus).Chemosphere. 2015 Jun;128:14-20. doi: 10.1016/j.chemosphere.2015.01.008.

Pietroski JP, White JR, DeLaune RD.Effects of dispersant used for oil spill remediation on nitrogen cycling in Louisiana coastal salt marsh soil.Chemosphere. 2014 Aug 11;119C:562-567. doi: 10.1016/j.chemosphere.2014.07.050. [Epub ahead of print]

Polli, Joseph Ryan; Zhang, Yanqiong; Pan, Xiaoping 2014 Dispersed crude oil amplifies germ cell apoptosis in Caenorhabditis elegans, followed a CEP-1-dependent pathway Archives of Toxicology (2014), 88(3), 543-551 CODEN: ARTODN; ISSN: 0340-5761

Prince RC. Oil spill dispersants: boon or bane?Environ Sci Technol. 2015 Jun 2;49(11):6376-84. doi: 10.1021/acs.est.5b00961. Epub 2015 May 15.

Socolofsky SA, Adams EE, Boufadel MC, Aman ZM, Johansen Ø, Konkel WJ, Lindo D, Madsen MN, North EW, Paris CB, Rasmussen D, Reed M, Rønningen P, Sim LH, Uhrenholdt T, Anderson KG, Cooper C, Nedwed TJ.Mar Pollut Bull. 2015 Jul 15;96(1-2):110-26. doi: 10.1016/j.marpolbul.2015.05.039. Epub 2015 May 26.

Studivan MS, Hatch WI, Mitchelmore CL. Responses of the soft coral Xenia elongata following acute exposure to a chemical dispersant.Springerplus. 2015 Feb 13;4:80. doi: 10.1186/s40064-015-0844-7. eCollection 2015. Free PMC Article

Ramachandran G, Huynh T, Banerjee S, Groth C, Stenzel M, Blair A, Engel L, Sandler D, Kwok R, Stewart P. 0304 The NIEHS GuLF STUDY: Estimate of workers' exposures through the inhalation route on seven response vessels near the well-site during the Deepwater Horizon oil spill.Occup Environ Med. 2014 Jun;71 Suppl 1:A105. doi: 10.1136/oemed-2014-102362.329.

Rial D, Vázquez JA, Murado MA. 2013 Toxicity of spill-treating agents and oil to sea urchin embryos. Sci Total Environ. 2013 Nov 29;472C:302-308. doi: 10.1016/j.scitotenv.2013.11.030. [Epub ahead of print]

Riehm DA, McCormick AV. The role of dispersants' dynamic interfacial tension in effective crude oil spill dispersion.Mar Pollut Bull. 2014 Jul 15;84(1-2):155-63. doi: 10.1016/j.marpolbul.2014.05.018. Epub

Rooker, Jay R. [Reprint Author]; Kitchens, Larissa L.; Dance, Michael A.; Wells, R. J. David; Falterman, Brett; Cornic, Maelle 2013. Spatial, Temporal, and Habitat-Related Variation in Abundance of Pelagic Fishes in the Gulf of Mexico: Potential Implications of the Deepwater Horizon Oil Spill. PLoS One, (OCT 10 2013) Vol. 8, No. 10, pp. Article No.: e76080. http://ukpmc.ac.uk/journals/1217/. ISSN: 1932-6203. E-ISSN: 1932-6203.

Shi, Rujie; Yu, Kewei 2014. Impact of exposure of crude oil and dispersant (COREXIT EC 9500A) on denitrification and organic matter mineralization in a Louisiana salt marsh sediment Chemosphere (2014) Ahead of Print CODEN: CMSHAF; ISSN: 0045-6535

CONFIDENTIAL

Sørensen L, Melbye AG, Booth AM. 2013. Oil droplet interaction with suspended sediment in the seawater column: Influence of physical parameters and chemical dispersants. Mar Pollut Bull. 2013 Nov 18. pii: S0025-326X(13)00670-X. doi: 10.1016/j.marpolbul.2013.10.049. [Epub ahead of print]

Steffy, David A. [Reprint Author]; Nichols, Alfred C.; Morgan, L. Joe; Gibbs, Rachael 2013. Evidence that the Deepwater Horizon Oil Spill Caused a Change in the Nickel, Chromium, and Lead Average Seasonal Concentrations Occurring in Sea Bottom Sediment Collected from the Eastern Gulf of Mexico Continental Shelf Between the Years 2009 and 2011. Water Air and Soil Pollution, (NOV 2013) Vol. 224, No. 11, pp. Article No.: 1756. http://www.springerlink.com/content/100344/. CODEN: WAPLAC. ISSN: 0049-6979. E-ISSN: 1573-2932.

Tansel,B., Mengshan Lee, Jillian Berbakov, Derya Z. Tansel, Urpiana Koklonis 2014. Dispersion of Louisiana crude oil in salt water environment by Corexit 9500A in the presence of natural coastal materials Estuarine, Coastal and Shelf Science, Volume 143, 20 April 2014, Pages 58-64

Temkin AM, Bowers RR, Magaletta ME, Holshouser S, Maggi A, Ciana P, Guillette LJ, Bowden JA, Kucklick JR, Baatz JE, Spyropoulos DD. Effects of Crude Oil/Dispersant Mixture and Dispersant Components on PPARγ Activity in Vitro and in Vivo: Identification of Dioctyl Sodium Sulfosuccinate (DOSS; CAS #577-11-7) as a Probable Obesogen.Environ Health Perspect. 2015 Jul 2. [Epub ahead of print]Free Article

Vikebø FB, Rønningen P, Meier S, Grøsvik BE, Lien VS. Dispersants have limited effects on exposure rates of oil spills on fish eggs and larvae in shelf seas.Environ Sci Technol. 2015 May 19;49(10):6061-9. doi: 10.1021/acs.est.5b00016. Epub 2015 Apr 30.

Xu Y, Duncan FE, Xu M, Woodruff TK. Use of an organotypic mammalian in vitro follicle growth assay to facilitate female reproductive toxicity screening.Reprod Fertil Dev. 2015 Feb 18. doi: 10.1071/RD14375. [Epub ahead of print]

Zhao X, Gong Y, O'Reilly SE, Zhao D. Effects of oil dispersant on solubilization, sorption and desorption of polycyclic aromatic hydrocarbons in sediment-seawater systems.Mar Pollut Bull. 2015 Mar 15;92(1-2):160-9. doi: 10.1016/j.marpolbul.2014.12.042. Epub 2015 Jan 20.

Ortmann AC, Lu Y. Initial community and environment determine the response of bacterial communities to dispersant and oil contamination.Mar Pollut Bull. 2015 Jan 15;90(1-2):106-14. doi: 10.1016/j.marpolbul.2014.11.013. Epub 2014 Dec 6.

Singkran N. Contingency plan improvement for managing oil spills in the coastal waters of Thailand.Mar Pollut Bull. 2014 Dec 15;89(1-2):149-59. doi: 10.1016/j.marpolbul.2014.10.013. Epub 2014 Oct 28.

Pietroski JP, White JR, DeLaune RD. Effects of dispersant used for oil spill remediation on nitrogen cycling in Louisiana coastal salt marsh soil.Chemosphere. 2015 Jan;119:562-7. doi: 10.1016/j.chemosphere.2014.07.050. Epub 2014 Aug 12.

Riehm DA, McCormick AV. The role of dispersants' dynamic interfacial tension in effective crude oil spill dispersion.Mar Pollut Bull. 2014 Jul 15;84(1-2):155-63. doi: 10.1016/j.marpolbul.2014.05.018. Epub 2014 Jun 2.

Wise CF, Wise JT, Wise SS, Thompson WD, Wise JP Jr, Wise JP Sr. Chemical dispersants used in the Gulf of Mexico oil crisis are cytotoxic and genotoxic to sperm whale skin cells.Aquat Toxicol. 2014 Jul;152:335-40. doi: 10.1016/j.aquatox.2014.04.020. Epub 2014 Apr 25.

CONFIDENTIAL

Zhao, L., J. Torlapati, M. C. Boufadel*, T. King, B. Robinson, and K. Lee. 2014. VDROP: A Comprehensive model for generating the droplet size distribution of oils in water. Incorporation of the interfacial tension and oil viscosity. Chemical Engineering Journal, Accepted, 2014.

Zheng M, Ahuja M, Bhattacharya D, Clement TP, Hayworth JS, Dhanasekaran M. 2013 Evaluation of differential cytotoxic effects of the oil spill dispersant Corexit 9500. Life Sci. 2013 Dec 17. pii: S0024-3205(13)00757-1. doi: 10.1016/j.lfs.2013.12.010. [Epub ahead of print]

Zhao L, Boufadel MC, Socolofsky SA, Adams E, King T, Lee K. Evolution of droplets in subsea oil and gas blowouts: development and validation of the numerical model VDROP-Jar Pollut Bull. 2014 Jun 15;83(1):58-69. doi: 10.1016/j.marpolbul.2014.04.020. Epub 2014 Apr 26.

Koyama J, Imakado C, Uno S, Kuroda T, Hara S, Majima T, Shirota H, Añasco NC. Simulated distribution and ecotoxicity-based assessment of chemically-dispersed oil in Tokyo Bay.Mar Pollut Bull. 2014 Aug 30;85(2):487-93. doi: 10.1016/j.marpolbul.2014.04.001. Epub 2014 Apr 20.

Almeda R, Baca S, Hyatt C, Buskey EJ. Ingestion and sublethal effects of physically and chemically dispersed crude oil on marine planktonic copepods.Ecotoxicology. 2014 Aug;23(6):988-1003. doi: 10.1007/s10646-014-1242-6. Epub 2014 Apr 23.Free PMC Article

Shi R, Yu K. Impact of exposure of crude oil and dispersant (COREXIT® EC 9500A) on denitrification and organic matter mineralization in a Louisiana salt marsh sediment.Chemosphere. 2014 Aug;108:300-5. doi: 10.1016/j.chemosphere.2014.01.055. Epub 2014 Feb 26.

Polli JR, Zhang Y, Pan X. Dispersed crude oil amplifies germ cell apoptosis in Caenorhabditis elegans, followed a CEP-1-dependent pathway.Arch Toxicol. 2014 Mar;88(3):543-51. doi: 10.1007/s00204-014-1198-6. Epub 2014 Feb 5.

Ozhan K, Bargu S. Distinct responses of Gulf of Mexico phytoplankton communities to crude oil and the dispersant corexit(®) Ec9500A under different nutrient regimes.Ecotoxicology. 2014 Apr;23(3):370-84. doi: 10.1007/s10646-014-1195-9. Epub 2014 Jan 28.

Marti ME, Colonna WJ, Patra P, Zhang H, Green C, Reznik G, Pynn M, Jarrell K, Nyman JA, Somasundaran P, Glatz CE, Lamsal BP. Production and characterization of microbial biosurfactants for potential use in oil-spill remediation. Enzyme Microb Technol. 2014 Feb 5;55:31-9. doi: 10.1016/j.enzmictec.2013.12.001. Epub 2013 Dec 12. Erratum in: Enzyme Microb Technol. 2014 Jun 10;60:81.

Fitzgerald TP, Gohlke JM. Contaminant levels in Gulf of Mexico reef fish after the Deepwater Horizon oil spill as measured by a fishermen-led testing program.Environ Sci Technol. 2014 Feb 4;48(3):1993-2000. doi: 10.1021/es4051555. Epub 2014 Jan 16.

Gong Y, Zhao X, O'Reilly SE, Qian T, Zhao D. Effects of oil dispersant and oil on sorption and desorption of phenanthrene with Gulf Coast marine sediments.Environ Pollut. 2014 Feb;185:240-9. doi: 10.1016/j.envpol.2013.10.031. Epub 2013 Nov 27.

Rial D, Vázquez JA, Murado MA. Toxicity of spill-treating agents and oil to sea urchin embryos.Sci Total Environ. 2014 Feb 15;472:302-8. doi: 10.1016/j.scitotenv.2013.11.030. Epub 2013 Nov 30.

Roberts JR, Anderson SE, Kan H, Krajnak K, Thompson JA, Kenyon A, Goldsmith WT,

CONFIDENTIAL

McKinney W, Frazer DG, Jackson M, Fedan JS. Evaluation of Pulmonary and Systemic Toxicity of Oil Dispersant (COREXIT EC9500A(®)) Following Acute Repeated Inhalation Exposure.Environ Health Insights. 2015 Feb 9;8(Suppl 1):63-74. doi: 10.4137/EHI.S15262. eCollection 2014.

Zheng M, Ahuja M, Bhattacharya D, Clement TP, Hayworth JS, Dhanasekaran M. Evaluation of differential cytotoxic effects of the oil spill dispersant Corexit 9500Life Sci. 2014 Jan 30;95(2):108-17. doi: 10.1016/j.lfs.2013.12.010. Epub 2013 Dec 17.

CONFIDENTIAL

To: Andrew Remsen[drewremsen@gmail.com]
From: Conmy, Robyn
Sent: Mon 3/30/2015 12:15:37 PM
Subject: papers
campo et al 2013 EST corexit oil degrad.pdf
venosa_holder 2007 biodeg.pdf

Need first page

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

Environmental Science & Technology

Article

pubs.acs.org/est

Biodegradability of Corexit 9500 and Dispersed South Louisiana Crude Oil at 5 and 25 °C

Pablo Campo,[†] Albert D. Venosa,[‡,*] and Makram T. Suidan[§]

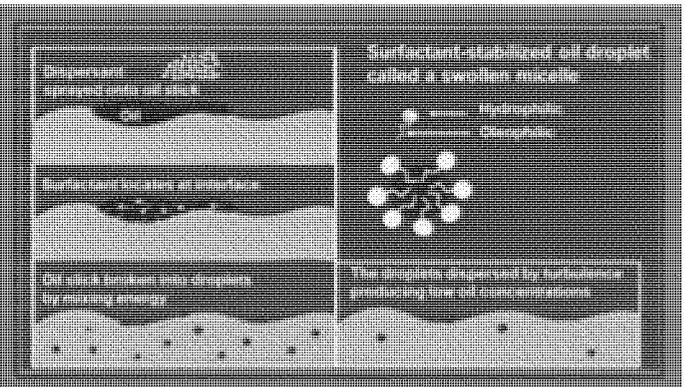
[†]University of Cincinnati, School of Energy, Environmental, Biological and Medical Engineering, Cincinnati, Ohio 45221-0012, United States .

[‡]U.S. Environmental Protection Agency, National Risk Management Research Laboratory, 26 W. Martin Luther King Drive, Cincinnati, Ohio 45268, United States .

[§]American University of Beirut, Faculty of Engineering and Architecture, Office of the Dean, Bechtel Engineering Bldg., Beirut, Lebanon.

* Supporting Information

The reported persistence of the dioctyl sodium sulfosuccinate (DOSS) surfactant in Corexit 9500 in the oil plumes formed during the Deepwater Horizon oil spill has contributed to concerns regarding the biodegradability and bioavailability of dispersed oil and dispersants used as an oil spill countermeasure in the Gulf of Mexico. We studied the biodegradation of DOSS and dispersed South Louisiana crude oil (SLC) in laboratory microcosms. Two oil-degrading cultures from the Gulf of Mexico were isolated, one from the surface (meso) and one from close to the area of the Macondo well (cryo). Each was enriched on SLC, the former at 25 °C, the latter at 5 °C. Results indicated that the meso culture rapidly and completely degraded DOSS, alkanes, and aromatics. The cryo culture metabolized the same compounds but with a lag of 28 d and a remaining residual of ao-alkanes, n-C_{30-35}, and the 4-ring PAHs.



INTRODUCTION

In the aftermath of the April 20, 2010 Deepwater Horizon (DWH) oil blowout in the Gulf of Mexico (GOM), stopping the flow of oil from the well initially dominated the response effort but environmental and human health protection issues contributed to the decision to use dispersants as the primary mitigation action. To aid in accomplishing the response objectives, responders sprayed dispersants onto the growing slick on the surface and injected them directly into the gushing oil at the wellhead. Almost 7 million liters of dispersants [mostly Corexit 9500 (C9500)] were used in the spill, of which approximately 42% was applied at the wellhead.[1] The intended effect of this countermeasure was to disperse the surging oil into tiny, neutrally buoyant droplets that would not easily recoalesce and rise to the surface. A further objective was to accelerate the oil biodegradation rate in the water column due to the higher surface area of the dispersed oil droplets.[2] Injection of dispersant at the wellhead may have contributed to the formation of continuous and some discontinuous plumes of dispersed oil at approximately 1000 to 1250 m depth that persisted for months.[3] Lingering concentrations of sodium 1,4-bis(2-ethylhexoxy)-1,4-dioxobutane-2-sulfonate (common name, dioctyl sulfosuccinate or DOSS), an anionic surfactant present in C9500, were found at the same depth horizons where that oil plume appeared.[4]

Such findings have raised questions about the use of C9500 in deep water, specifically in regards to its inherent biodegradability.[5] With the exception of one recent article,[6] no information has been published about the fate of the active surfactant in C9500 or any other dispersant when subjected to microbial activity in the presence or absence of oil.[7] Data are available in regard to the potential enhancement effect of surfactants and dispersants on oil biodegradation,[8−14] but only one[6] reported the fate of the surfactant during the course of the experiments (although the authors did not report the method they used to measure DOSS). The objective of our work was to determine biodegradation rates of C9500 alone (as represented by DOSS), South Louisiana crude oil (SLC) alone, and SLC dispersed by C9500 under temperature conditions representative of those of the DWH discharge. Experiments were performed in microcosms at 25 and 5 °C, which correspond to average temperatures at depths of approximately 0−5 and 1000−1250 m respectively in the GOM. Two microbial inocula were used in this study after enrichment on SLC, one isolated from near the surface (meso), the other close to the location of

Received: September 25, 2012
Revised: January 29, 2013
Accepted: January 30, 2013
Published: January 30, 2013

 © 2013 American Chemical Society

ED_001324_00001365-00001

the plume above the wellhead (cryo) (about 1240 m deep). Our experimental setup differed substantially from Baelum et al.[6] These researchers enriched their cultures with a mixture of Macondo oil and C9500 at a very high dispersant-to-oil ratio (DOR) of 1:1.67, whereas we initially enriched our cultures on SLC alone in the absence of C9500 and used a substantially lower DOR of 1:25 in the actual dispersed oil treatments.

MATERIALS AND METHODS

Chemicals and Reagents. EPA provided the South Louisiana reference crude oil (SLC) (note, this was not exactly the same oil from the Macondo well but similar in properties and hydrocarbon constituents) and the dispersant Corexit 9500 (Nalco, Naperville, IL) used in this study. DOSS and its deuterated surrogate (D_{17}-DOSS) were purchased from Aldrich (St. Louis, MO) and Isotec (Miamisburg, OH), respectively. Acetonitrile (ACN), deionized ultrafiltered water, and mineral salts were obtained from Fisher Scientific (Pittsburgh, PA). Dichloromethane (DCM) was acquired from Tedia (Fairfield, OH).

Silylation of Glassware. Glassware was deactivated with Sylon CT (Sigma-Aldrich, St. Louis, MO. In this procedure, dimethyldichlorosilane reacts with silanol groups (Si−OH) present on the glass surface so that the formation of hydrogen bonds with both polar molecules or functional groups is averted. The treatment increases the hydrophobicity of the glass and minimizes solute, oil, and biomass adsorption onto the container walls,[15] which occurred in the Baelum et al. article[6] where 30% of the initial oil added was lost to the sides of the glass flasks.

Artificial Seawater. Sterile GP2 medium was prepared by dissolving the required amounts of mineral salts in distilled water.[16] This solution was autoclaved at 120 °C for 30 min in batches of 1 L. The concentrations of each salt (expressed in g/L) were NaCl (21.03), $MgCl_2$ $6H_2O$ (9.5), Na_2SO_4 (3.52), $CaCl_2.2H_2O$ (1.32), KCl (0.61), KBr (0.088), $NaB_4O_7.10H_2O$ (0.034), $SrCl_2.6H_2O$ (0.02), $NaHCO_3$ (0.17) $FeCl_3.6H_2O$ (0.05), $Na_5P_3O_{10}$ (0.297), and KNO_3 (2.89). The GP2 pH was ~7.5.

Microbial Cultures. The cryo culture was isolated from water close to the plume location at a depth of 1240 m near the Macondo wellhead (Lat 28.73834° W, Long −88.386975° N). The meso culture came from water within the top 5 m from the water surface in the vicinity of the wellhead (Lat 28.345667° W, Long −88.777833° N). Both cultures were collected on July 31, 2010. After collection, the water samples were shipped on ice to EPA's Gulf Ecology Division laboratory in Florida for culture enrichment. Both cultures were enriched in 2 L shake flasks containing 500 mL of the original unfiltered GOM seawater supplemented with 2.8 g/L KNO_3, 0.55 g/L NaP_3O_{10}, and 2.5 mL SLC. They were shaken at 400 rpm at 5 °C (cryo culture) and 25 °C (meso culture). After achieving adequate growth (7 days for the meso culture and 17 days for the cryo culture), the cells were harvested by centrifugation at 6000 × g for 30 min at 4 °C. The overlying liquid was decanted, oil removed with sterile swabs, and two consecutive harvesting steps were repeated. The pellets were preserved in 10% glycerol, dispensed in 2 mL aliquots, and frozen at −70 °C. Prior to shipping the cultures to EPA-Cincinnati for use in the biodegradation experiments, the inocula were prepared in shake flasks by adding 1 mL of each thawed culture to 500 mL GP2 medium supplemented with 2.5 mL SLC. The meso culture was harvested after 6 days of growth and the cryo culture after 29 days

Dispersed Oil Treatment. The preparation procedure involved making 700 mL batches of dispersed oil in baffled beakers and, subsequently, compositing them in a 20 L glass carboy under continuous mixing to prevent recoalescence of the dispersed droplets. A given batch was prepared by transferring 700 mL of GP2 into a baffled 1 L beaker followed by 600 μL SLC pipetted onto the water surface and then 24 μL of C9500 onto the oil slick. These volumes yielded a volumetric DOR of 1:25. The mixture was initially shaken at a slow speed to minimize splashing onto the walls of the beaker and ramped up gradually to approximately 150 rpm. After 10 min of shaking at 150 rpm, the beaker was removed from the shaker and held stationary for 10 min to allow undispersed oil to rise to the surface. The dispersed oil seawater suspension was drained through a stopcock placed at the bottom of the flask into the carboy being careful not to disturb the top layer of undispersed oil. Once the volume of dispersed oil suspension reached 14 L, 100 mL aliquots of the composited dispersed oil suspension were dispensed into a series of 120 silanized shake flasks, half of which served as the dispersed oil killed controls (KCs). All flasks were placed on the orbital shakers in corresponding constant temperature rooms set at 5 or 25 °C and maintained at those temperatures for the duration of the experiment.

Nondispersed Oil Treatment. In this treatment, the biodegradation of SLC alone was studied. The final concentration of oil in these samples was approximately the same as in the dispersed oil treatment (i.e., 0.7 g/L).

Dispersant Alone Treatment. To evaluate the biodegradation of C9500 alone at 5 and 25 °C, tests were conducted where the only substrate was the dispersant. A single batch of sterile GP2 (14 L) was spiked with approximately 476 μL of C9500 corresponding to the same concentration as in the dispersed oil treatment. This batch was also kept well mixed. From this stock, which was prepared immediately before the experiment to minimize abiotic loss, the samples for both temperatures were prepared by transferring 100 mL of the mix into each flask.

Experimental Setup and Sampling of Flasks. Table S1 of the Supporting Information summarizes the experimental design layout. In the case of the 5 °C experiment, 11 sampling events took place at 0, 2, 4, 8, 11, 14, 17, 21, 28, 35, and 42 days. For the 25 °C experiment, 9 sampling events were used at 0, 2, 4, 8, 11, 14, 17, 21, and 28 days. For both temperatures, 3 different treatments were studied in triplicate shake flask microcosms, namely, C9500 alone, SLC dispersed by C9500, and SLC alone. The sample volume in each of these treatments was 100 mL. In addition, KCs were necessary for each treatment to account for abiotic losses, if any. For these controls, the sterilant was sodium azide at a final concentration of 500 mg/L. All experimental units were spiked with 0.5 mL of the appropriate culture, which were kept at 4 °C until inoculation. The orbital shakers were set to rotate at 200 rpm (Innova 2300 series digital platforms, New Brunswick, Edison, NJ). At a given sampling event, triplicate flasks of each treatment were sacrificed. In addition, for the treatments with dispersed oil and C9500, KCs were also included. In the case of the KCs of the oil alone treatments, triplicates of the controls were sampled only at the initial and final events.

Oil Components and C9500 Analysis. In tests where C9500 alone was present, a 20-mL subsample was collected for the analysis of its main active component, DOSS, by liquid

ED_001324_00001365-00002

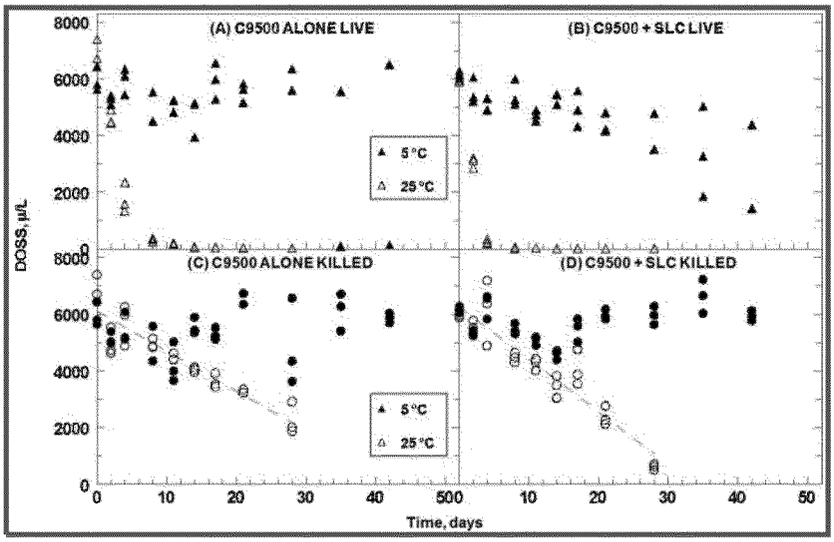


Figure 1. Disappearance of DOSS in the absence (A, C) and presence (B, D) of SLC at 5 and 25 °C. Live treatments are in panels A and B, whereas killed controls are in panels C and D.

chromatography tandem mass spectrometry (LC/MS/MS). In the flasks containing nondispersed SLC, the 100 mL of seawater was extracted with DCM and the extracts analyzed for alkanes and aromatics by GC/MS. Alkanes included normal and branched aliphatics ranging in carbon number from 10 to 35 as well as pristane and phytane. Aromatics included 2-, 3-, and 4-ring PAH compounds and their alkylated homologues (i.e., C_{0-4} -naphthalenes, C_{0-3} -fluorenes, C_{0-3} -dibenzothiophenes, C_{0-4} -phenanthrenes/anthracenes, C_{0-4} -napthbenzothiophenes, C_{0-2} -pyrenes, C_{0-4} -chrysenes). Finally, in the dispersed oil flasks, after withdrawing the 20 mL subsample for DOSS analysis, the remaining seawater was extracted with DCM and analyzed for oil components. This procedure was repeated at each subsequent sampling event.

Hydrocarbon Analysis. We determined that the SLC aromatic fraction comprises napthalenes (72%), phenanthrenes (12%), fluorenes (8%), dibenzothiophenes (6%), and 4-ring compounds napthobenzothiophenes, pyrenes, and chrysenes (2%). After liquid−liquid extraction, oil extracts were filtered through sodium sulfate to dewater the extracts, concentrated under nitrogen gas, and then solvent-exchanged into hexane to a final volume of 10 mL. The GC/MS analysis was performed in a Model 6890 gas chromatograph coupled with a Model 5973 mass spectrometer from Agilent (Palo Alto, CA). A DB-5 MS column [30 m × 0.25 mm I.D., 0.25 µm particle size (J&W, Palo Alto, CA)] was used to achieve chromatographic separation of the alkanes and aromatics. Analyte detection was conducted in the selected ion monitoring mode.[17]

Dioctyl Sulfosuccinate Analysis. DOSS was analyzed in GP2 seawater as described in the ASTM D7730 Standard Method.[18] For this purpose, 20 mL subsamples were collected from those flasks containing C9500 alone or dispersed SLC. These aliquots were spiked with both the surrogate D_{17}-DOSS and an ammonium formate buffer solution, brought to a volume of 40 mL with ACN, and vigorously shaken for 1 min. Finally, portions of these subsamples were withdrawn with a 1 mL syringe and then filtered (0.2 µm) before analysis. DOSS was quantified using a Model 1200 series liquid chromatograph (LC) connected to a Model 6410 tandem Triple Quadrupole Mass Spectrometer (MS/MS) by Agilent (Palo Alto, CA). An analytical column Zorbax Eclipse XBD C_{18}, 2.1 × 50 mm with a particle size of 3.5 µm was used for analyte separation. The binary pump was operated at 0.4 mL/min with two eluents: (A) 99% water + 0.9% ACN + 0.1% of formic acid and (B) 99% ACN + 0.9% water + 0.1% of formic acid. At the beginning of the run (12.1 min), the mobile-phase composition was maintained at 98% A and 2% B during the first min. After that, the gradient's strength was sequentially increased to 38, 80, and 98% B at 4, 7, and 12 min, respectively. DOSS and its deuterated surrogate were detected by tandem mass spectrometry. With the electrospray operated in negative mode, these compounds generated precursor ions (PI) at m/z 421 (DOSS) and 445 (D_{17}-DOSS). Upon fragmentation, both PIs yielded the same product ion m/z 81. For this reason the analytes were monitored and quantified with the selected reactions m/z 421 → m/z 81 and m/z 445 → m/z 81. The electrospray parameters were gas temperature, 350 °C; gas flow, 6 L/min; nebulizer, 15 psi; and capillary voltage, 4 kV. For identification of DOSS byproducts, samples were also injected in scan mode from m/z 50 to m/z 500.

RESULTS AND DISCUSSION

DOSS Degradation. At 25 °C, DOSS biodegradation rates in the absence and presence of SLC were high, and the extent of removal exceeded 99% by days 8 and 14, respectively (open symbols, parts A and B of Figure 1). For these treatments, we calculated the biodegradation rate coefficients by fitting the DOSS data to a first-order model. The first-order rate coefficients were −0.30 ± 0.02/d (C9500 alone) and −0.46 ± 0.03/d (C9500 + SLC). Hence, in the presence of SLC, the DOSS biodegradation rate was enhanced by a factor of 1.5, because our culture, which had been enriched previously on SLC and thus preacclimated to hydrocarbons but not DOSS, likely grew to higher numbers faster and thus metabolized DOSS faster by virtue of the greater population density. This is in stark contrast to the findings of others[6] whose initial enrichments were conducted on mixtures of oil and C9500 at a very high DOR (1:1.67), whereas we enriched our cultures on oil alone. In the Gulf of Mexico waters, oil seeps have been occurring continuously for eons, giving rise to microbial cultures highly adapted to growth on hydrocarbons. Because DOSS is a xenobiotic compound in that environment, one

ED_001324_00001365-00003

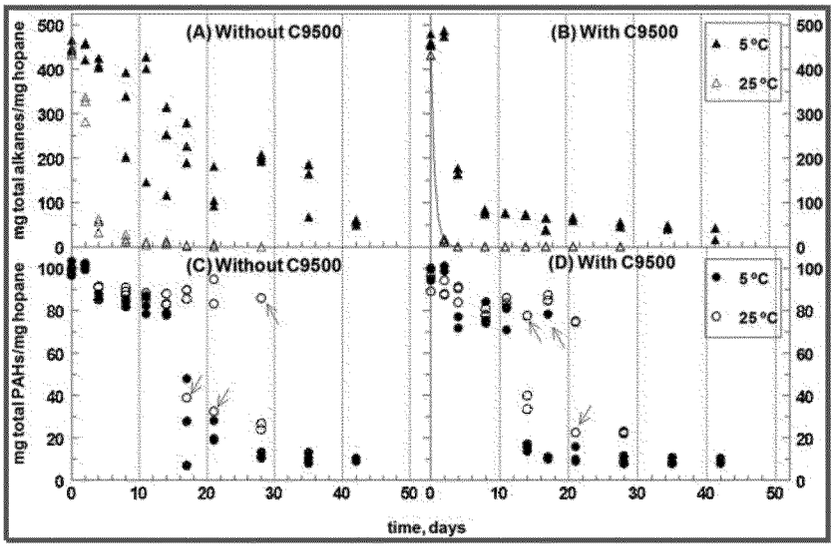


Figure 2. Biodegradation of hopane-normalized total alkanes (A, B) and PAHs (C, D) at both temperatures without (A, C) and with (B, D) C9500 dispersant. The arrows in the figure panels represent statistical outliers as determined by the Grubb's outlier test.[25].

would not expect to find cultures able to degrade DOSS rapidly at 5 °C except after a long adaptation period. Thus, results from our cultures are more representative of the DWH spill conditions because in the GOM dispersant was injected in the deep sea where no previous microbial exposure to DOSS had ever occurred.

KCs run in parallel also revealed a significant DOSS disappearance. At the end of the experiment (28 d), only 10% of the initial DOSS concentration remained in the dispersed SLC controls (open symbols in part D of Figure 1), whereas 33% remained in those for the C9500 alone treatment (open symbols in part C of Figure 1). The linear rates of surfactant loss in the KCs were −185 ± 14 μg/(L d) in the presence of SLC (part D of Figure 1) and −140 ± 12 μg/(L d) in its absence (part C of Figure 1). The abiotic loss can be explained on solvolysis grounds: sulphosuccinates contain ester bonds that are susceptible to hydrolysis.[19] This hydrolysis reaction consists of a staged, acid-catalyzed, nucleophilic attack on the ester bond by water that results in the cleavage of the DOSS molecule to 2-ethylhexoxy-1,4-dioxobutane-2-sulfonate (common name, monooctyl sulfosuccinate or MOSS) and 2-ethylhexanol (2-EH) (Scheme S1 of the Supporting Information).

LC/MS/MS analysis of the KCs in scan mode confirmed the presence of MOSS as a peak eluted at 5.5 min with m/z 309 (part A of Figure S1 of the Supporting Information). The acidic hydrolysis of esters is a reversible process, and the formation of products requires a shift in the reaction to the right.[19] Water displaces the equilibrium toward 2-EH and MOSS. With 2-EH being highly hydrophobic (log P = 2.71), this fatty alcohol strongly partitions to SLC, which forces the equilibrium further to the right, causing the higher disappearance rate achieved in the dispersed oil KCs. The observed zero-order kinetics corresponds either to a catalytic reaction with a multistep mechanism or to an experimental run that did not proceed long enough for the DOSS concentrations to follow the curvature typical of a first-order decay.[20] Clearly, DOSS metabolism takes place at a much faster rate than chemical hydrolysis. When both processes occur at the same time, microbial uptake is facilitated, since the two mechanisms create similar intermediates: DOSS biodegradation always involves a cleavage of the ester bond, and microbial enzymes preferentially and more rapidly attack the same bond as the first step in biodegradation.[21] In our live microcosms, apparent first-order biodegradation rates reflect the overall process for DOSS disappearance, regardless of chemical or biological contributions, with hydrolysis being negligible relative to the biological reaction.

Data obtained at 5 °C contrasted sharply with the 25 °C results, since a 28-d lag period occurred before DOSS biodegradation ensued. As part A of Figure 1 shows, in the absence of SLC, DOSS was depleted by 98% in 1 replicate on day 35 and 2 replicates on day 42, whereas in its presence (part B of Figure 1), the triplicates averaged 61% removal but with a slow, steady decline. These results suggest that the cryo culture needed an acclimation stage to synthesize the enzymes that facilitate DOSS uptake and breakdown. The slow disappearance of DOSS observed with the cryo culture may help explain the results reported in the subsea.[4] DOSS was detected at the same depth horizons as the dispersed oil plumes with concentrations ranging between 0.4 to 12 μg/L within a 300 km distance from the discharge source. Modeling the concentration profiles suggested that DOSS underwater attenuation was due to transport and dilution within the oil plumes and not biodegradation.[4] Again DOSS is a xenobiotic compound in this environment, and its metabolism requires a constant presence and sufficient exposure time to become a microbial substrate.[22] In our experiments with the cryo culture, these requirements were met because of the batch nature of the setup (no continuous dilution possible in a closed system). As parts C and D of Figure 1 show, DOSS also remained unaltered in the KCs run at 5 °C. In general, as temperature declines, so do chemical reaction rates.[20] It is possible that at 5 °C DOSS hydrolysis progressed at such a slow rate that it did not occur within our 42-d experiment, and MOSS was not detected in the LC-MS scans (part B in Figure S1 of Supporting Information).

Hydrocarbon Degradation. Alkanes. Parts A and B of Figure 2 present the hopane-normalized biodegradation results for the total alkanes. Regardless of the presence of dispersant, the effect of temperature was clearly evident. Alkanes disappeared much faster at 25 °C than at 5 °C, and the variability among the 3 replicates at 5 °C (oil alone, part A of Figure 2) was much higher than at 25 °C. We did not model

ED_001324_00001365-00004

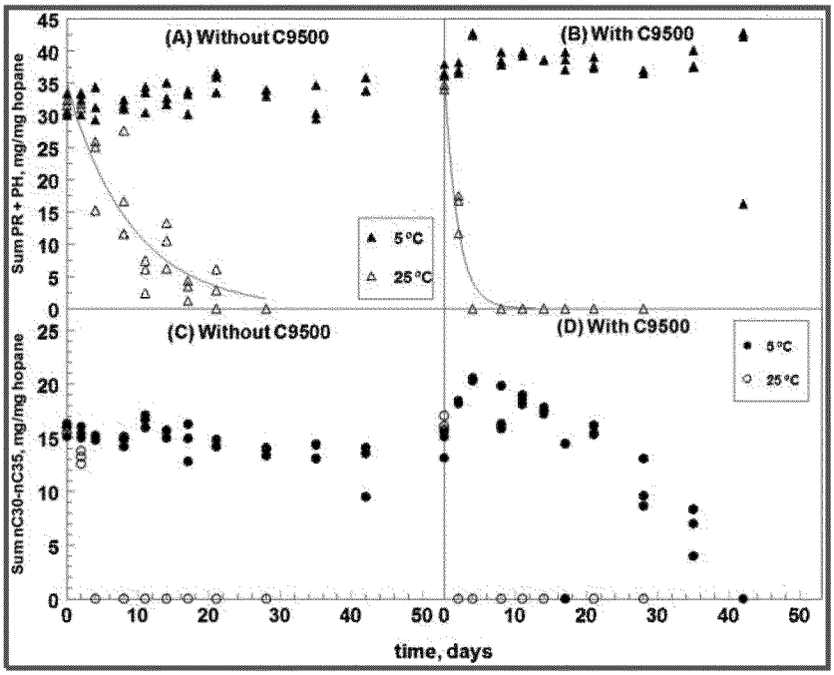


Figure 3. Biodegradation of the branched alkanes (A, B) and n-alkanes > nC_{29} (C, D) in the absence (A, C) and presence (B, D) of C9500.

the low temperature data because the data approached a nonzero asymptote regardless of the absence (part A of Figure 2) or presence (part B of Figure 2) of C9500 (discussed later). Also, a 2 day lag period occurred before biodegradation ensued.

At 25 °C, the biodegradation patterns were markedly different for both dispersant treatments. The alkanes approached nondetectable by day 11 (part A of Figure 2) in the absence of C9500 and by day 4 in its presence (part B of Figure 2). In both cases, no aliphatics were detected at the end of the experiment except for hopane, which remained unchanged (Figure S2 of the Supporting Information) (the reason why hopane was lower at day 0 in the dispersed treatment was that not all of the oil was dispersed in our preparations; thus, this treatment had less oil to begin with). The oil KCs are shown in Figure S3 of the Supporting Information, which shows that no loss in either alkanes or PAHs took place at either temperature.

The first-order biodegradation rate constants for the individual hopane-normalized alkanes at 25 °C were calculated by nonlinear regression. The data are presented in part A of Figure S4 of the Supporting Information for oil without dispersant and S4B for oil in the presence of dispersant. In the dispersed oil treatment (part B of Figure S4 of the Supporting Information), the presence of C9500 enhanced biodegradation to such an extent that all the n-alkanes were degraded before the second sampling event at day 4; thus, we could not collect enough data to estimate their rate constants. For PR and PH, the degradation rates were 6.8- to 5-fold higher respectively over those obtained for the same analytes with the non-dispersed oil.

Upon completion of the 5 °C experiment (42 d), 7% of the initial alkane load remained regardless of the presence or absence of C9500 (parts A and B of Figure 2). This residual amount was due to the persistence of PR and PH (parts A and B of Figure 3) and the high molecular weight n-alkanes (n-C_{30-35}) (parts C and D of Figure 3). However, in the presence of C9500, the n-C_{30-35} alkanes were fully degraded by day 42. As for the nondispersed oil treatment at 5 °C, part A of Figure 2 shows that the total alkane fraction degraded much more slowly than in the presence of C9500 (part B of Figure 2). In the latter case, the extent of biodegradation at day 42 was 93% with a residual fraction comprised exclusively of PR and PH. In the former case, the extent of biodegradation was 87% with the residual fraction comprising PR, PH, and n-alkanes over 29 carbons. The persistence of iso- and n-alkanes at chain lengths >29 carbons in the absence of dispersant suggests that the microorganisms were less able to metabolize branched and higher molecular weight hydrocarbons at this low temperature. The presence of dispersant mitigated this somewhat since only PR and PH were still present at day 42. This indicates that either the microorganisms were less able to hydroxylate the terminal carbon in such structures or that the higher molecular weight n-alkanes and iso-alkanes were less bioavailable to the degrading communities. The above findings also contrast with those of Baelum et al.,[6] who reported only 25 to 60% degradation of oil at 5 °C in the absence or presence respectively of C9500.

As seen in parts A and B of Figure S4 of the Supporting Information, at 5 °C, alkane biodegradation rates were inversely proportional to hydrocarbon chain length for both the dispersed and nondispersed treatments. The comparison of the individual rate coefficients showed that C9500 enhanced by more than 3-fold the biodegradation of the individual alkanes.

Aromatics (PAHs). We were unable to fit a first-order model to the time series data for the biodegradation of the total aromatics (PAHs) (parts C and D of Figure 2). Hence, we used inferential statistics to help explain the observed results. For a given treatment and temperature, we compared the concentration of total PAHs measured at each sampling event by a one-way analysis of the variance (ANOVA) followed by a Newman-Keuls Test (NK).[23,24] This analysis allowed us to detect significant differences among sampling events and identify the data causing such differences. The null hypothesis tested was that the total PAH concentration did not vary with time. We also evaluated the effect of dispersant on total PAH biodegradation by comparing the concentrations measured for

ED_001324_00001365-00005

the nondispersed and dispersed SLC treatments for the same temperature and sampling day. The null hypothesis for these tests was that the dispersant did not affect PAH degradation. Finally, we also identified outliers, marked with arrows in parts C and D of Figure 2, with the Grubb's test.[25] All the statistical analyses were performed with Prism 5 (GraphPad Software, CA).

At 25 °C, total PAH concentrations in the dispersed oil treatment declined in all 3 replicates only in the final sampling event (day 28) (open symbols in part D of Figure 2). This decrease was the only significant difference among sampling events confirmed by the ANOVA ($p < 0.005$). Moreover, based on statistical analysis, the final concentrations for both treatments were not significantly different ($p = 0.078$). These results suggest that the meso culture required a long adaptation period (18−21 d) for enzyme induction, possibly due to DOSS having been completely mineralized long before activation of the PAH degraders, thereby delaying the onset of PAH biodegradation. Also, the method originally used to enrich the two GOM oil degrading cultures, which involved two transfers to fresh medium with each transfer occurring at 6−7 d for the meso culture and 17−29 d for the cryo culture, may have discriminated in favor of alkane degraders. This could help explain why PAH biodegradation did not start until close to the end of the experiment.

Similar to the total alkanes, we also observed a 2-day lag period for total PAH degradation at 5 °C (parts C and D of Figure 2). However, the rate of degradation was greater than at 25 °C, as the cryo culture began degrading PAHs after 14 d for dispersed SLC (part D of Figure 2) and 17 d (part C of Figure 2) for nonchemically dispersed SLC. Furthermore, the 10% residual PAH concentration remaining at day 28 for both treatments was lower than at 25 °C. Student's t-tests revealed differences between treatments at days 4 ($p = 0.0108$) and 14 ($p < 0.0001$) and, in both cases, the dispersed SLC oil produced lower PAH concentrations. This may possibly indicate an enhancement in substrate availability due to DOSS breaking up the oil into tiny droplets. However, despite the statistical differences noted, from a practical standpoint we see little difference in the PAH biodegradation patterns at both temperatures due to the dispersant treatment.

Both the cryo and the meso cultures were able to metabolize the 2- and 3-ring PAHs, but the 4-ring PAHs degraded only negligibly if at all, as depicted in parts C and D of Figure S4 of the Supporting Information. Nonlinear regression analysis was used to estimate first-order biodegradation coefficients for the individual PAHs at both temperatures. The presence of dispersant seemed to improve PAH depletion rates, since the rate constants for the dispersed oil treatment were mostly higher than those estimated with nondispersed SLC. However, the r^2 values in these regressions were relatively low because of the long lag period noted.

The observed biodegradation pattern of the PAHs was complex at both temperatures compared to the alkanes. The variability was considerably higher among the three replicates at the lower temperature. It is well-known that bacteria initiate PAH biodegradation using intracellular dioxygenases.[26,27] Thus, the PAH must be transported into the cell before metabolism can begin. Because petroleum is a complex mixture of thousands of compounds, including the more easily degradable n-alkanes, the relatively high variability in PAH degradation is understandable especially in the presence of another metabolizable substrate in the form of an anionic surfactant such as DOSS.

As for the higher apparent rates of biodegradation of PAHs compared to alkanes at 5 °C, we offer a plausible explanation. Any process or environment that lowers or is near the freezing point of water may result in the formation of crystals. Because the melting point of n-alkanes of carbon number ≥14 is below 5.5 °C and increases with carbon number, all the n-alkanes above tridecane crystallize at 5 °C and below. Thus, the alkanes in this study may have been less available to the microbial communities at the lower temperature compared to the more soluble PAHs. This conclusion could also pertain to the deep water in the Gulf where the temperature is ≤5 °C assuming that the extreme hydrostatic pressure at depth had no effect on biodegradation rates.

From an engineering standpoint, if one assumes that the aqueous solubility of PAHs limits their microbial degradation,[28−32] then it is important to take into consideration mass transfer effects when interpreting our results. Fick's First Law of Diffusion provides that diffusive mass transfer attempts to equalize concentration gradients:

$$Q/t = -DA(C_0 - C_x)/x \quad (1)$$

where Q is the quantity (g/m^3) of substrate diffusing through area A (m^2) per unit of time t (d), and $(C_0 - C_x)/x$ is the PAH concentration gradient where C_0 is the concentration at the source (g/m^3), C_x is the concentration at the sink (g/m^3), x is the diffusion path length (m), and D is the diffusion coefficient, m^2/s. The oil degrading community represents the sink. On the basis of eq 1, several ways exist to optimize mass transfer.[32] First is the production of biosurfactants by the degrading microorganisms that facilitate transmembrane diffusion of PAH into the cell. Application of dispersants containing surfactants also accomplishes this same effect. This, in essence, increases the effective D in eq 1. Surfactants and emulsifiers also tend to increase the interfacial area A resulting in a higher flux of substrate. The rate at which microorganisms degrade a substrate at low concentration depends on their specific affinity toward the substrate[30] defined as the ratio of the maximal substrate uptake rate to the half saturation constant.[33] High specific affinities lead to efficient low-concentration PAH removal from the environment at the cell surface, steeper concentration gradients, and higher substrate transfer rates.

In addition to surfactants affecting Fickian diffusion as discussed above, they also influence dissolution and subsequent uptake of PAHs into the cell by attaching to the oil−water interface where micelles facilitate PAH release from the interface.[28] Alkane degraders are known to produce biosurfactants to increase the bioavailability of the poorly soluble hydrocarbons. However, biosurfactant production is not as common among the PAH degraders,[32,34,35] which means that biosurfactants may not be as important for PAH metabolism as for alkanes. In fact, because surfactants are known to interact directly with cell membranes,[36,37] surfactants such as those in dispersants may negatively affect uptake of PAHs into the cell and subsequent metabolism by disrupting the integrity of the membrane, and it is possible this may even be exacerbated at deep-sea hydrostatic pressures. All of these interactions point to the possible explanation for the observed variability in PAH biodegradation in our microcosms.

The findings from this study revealed that the active surfactant in C9500, DOSS, spontaneously hydrolyzes to MOSS and 2-EH at 25 °C but not 5 °C (at least within 42

ED_001324_00001365-00006

days). In the presence of dispersed SLC oil, its biodegradation at 25 °C was exceedingly fast, much more so than the hydrolytic mechanism, and moderately (approximately 53%) faster than in the absence of oil. The implication is that DOSS will not persist when a dispersant containing that surfactant is applied to the water surface and the temperature is warm. However, if the temperature is near freezing, either in Arctic spills or deep-sea blowouts, our data suggest it is possible for DOSS to persist for a long period of time (at least 42 days, the duration of our experiments) when DOSS-containing dispersants are used for spill response. This conclusion agrees the observations made by Kujawinski et al.,[4] although they concluded that the primary explanation for the lower DOSS concentrations downstream from the wellhead was dilution due to advection rather than biodegradation. Research should be pursued to understand the toxicity of DOSS and its hydrolysis products at cold temperatures.

Alkane biodegradation proceeded rapidly and virtually completely at warm temperature, and its first-order rate coefficient was about 5-fold greater in the presence of dispersed SLC oil than in its absence (1.69 vs 0.34/d). This agreed with previous findings[9] where the rate difference between dispersed and nondispersed oil (PBC) was ~4-fold at 20 °C (0.66 vs 0.17/d). Although these biodegradation rates were somewhat similar in magnitude to experiments conducted during the DWH spill in the Gulf[38] where the half-lives of oil in the field samples ranged from 1.2 to 6.0 days, we believe the data reported in that study[38] were more consistent with dilution than biodegradation. This is especially true when one views our results showing residual PR, PH, and n-alkanes with carbon number >29 remaining after 42 days at 5 °C (Figure 3). Our results were not in agreement with those of Foght and Westlake,[12] who used a different crude oil and a different dispersant, Corexit 9527, which contains the solvent 2-butoxyethanol as an additional ingredient. The C9527 inhibited biodegradation in the first 2 weeks, but after 12 weeks, all alkanes were gone. In our study, PR and PH were still present after 12 weeks at 5 °C (parts A and B of Figure 3), but all other alkanes were nondetectable. It appears that different solvents and surfactants affect biodegradation differently at low temperatures, or the fact that alkanes exist in a semisolid state at near freezing temperature may play a more important role in alkane biodegradation at that temperature. Another study[11] exposed pure cultures of Acinetobacter calcoaceticus and Rhodococcus sp. to 5000 mg/L crude oil and either 100 or 50 mg/L individual surfactants or Corexit 9527 in a Warburg respirometer. They found that DOSS alone either inhibited respiration in both bacteria or enhanced respiration only slightly. Perhaps the concentrations of oil and surfactants used may have been partly responsible for such findings. In our study, the oil concentration was ~700 mg/L and the C9500 was 4% of that by volume, much lower than they reported. In yet another study[10] microcosms were set up to observe biodegradation of Alaska North Slope crude oil at 1400 or 4500 mg/L with C9500 at 1:10 or 1:100 v/v ratios at a temperature of 8 °C. Measurement of the crude oil was only qualitative, but essentially little difference was observed between dispersed and nondispersed oil.

With respect to the PAHs, the long lag period prior to actual biodegradation observed at 5 °C can be explained by the fact that DOSS persisted or degraded slowly during this time period and could have affected PAH metabolism but not the more easily degraded alkanes. We could not fit a first-order model to the total PAH data for either temperature because of the apparent outliers and the shape of the time-series plots. However, at the warm temperature a large decrease in total PAHs occurred after approximately 11−12 days (60%). At the cold temperature, the PAHs declined about 80%. Declines in the nondispersed oil at days 11−12 were much less. Clearly, in our experiments, crude oil biodegradation in general was readily enhanced by the addition of dispersant, but PAH biodegradation at 5 °C may have been more a function of the inherent solubility of PAHs compared to alkanes rather than due to the influence of the dispersant.

ASSOCIATED CONTENT

Supporting Information

Details of our experimental design graphic displaying the hydrolysis of DOSS into MOSS and 2-EH, figures summarizing (1) the chromatograms of DOSS and MOSS in the killed controls, (2) the persistence of hopane for the duration of the experiment, (3) the persistence of total alkanes and PAHs in the killed controls at both temperatures, and (4) a summary of the 1st order biodegradation rate coefficients computed for each individual alkane and PAH in all 4 treatments at both temperatures. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: 513-569-7668, fax: 513-569-7620, e-mail: venosa.albert@epa.gov.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Jan Kurtz of EPA's Gulf Ecology Division (GED) at Gulf Breeze, FL, who collected the water samples in the GOM for culture enrichment, and Diane Yates and Richard Devereux, also of GED, who performed the enrichments and provided them for the experiments. We also acknowledge the able assistance of William Platten III, University of Cincinnati, who assisted in the conduct of the microcosm experiments. The research was a product of the U.S. Environmental Protection Agency's National Risk Management Research Laboratory (NRMRL) and was funded by EPA, NRMRL, Cincinnati, OH, under Pegasus Technical Services, Inc. Contract EP-C-11-006.

REFERENCES

(1) The Federal Integracy Solutions Group; Oil Budget Calculator Science and Engineering Team Oil Budget Calculator Deepwater Horizon; 2010.

(2) National Research Council of the National Academies, Oil Spill Dispersants: Efficacy and Effects: The National Academies Press: Washington, D.C., 2005.

(3) National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling Deep Water. The Gulf Oil Disaster and the Future of Offshore Dirlling. Report to the President; January, 2011.

(4) Kujawinski, E. B.; KidoSoule, M. C.; Valentine, D. L.; Boysen, A. K.; Longnecker, K.; Redmond, M. C. Fate of dispersants associated with the Deepwater Horizon Oil Spill. Environ. Sci. Technol. 2011, 45, 1298−1306.

(5) Quinlan, P. More questions than answers on dispersants a year after Gulf spill. New York Times Apr 22, 2011.

(6) Baelum, J.; Borglin, S.; Chakraborty, R.; Fortney, J. L.; Lamendella, R.; Mason, O. U.; Auer, M.; Zemla, M.; Bill, M.; Conrad, M. E.; Malfatti, S. A.; Tringe, S. G.; Holman, H. Y.; Hazen, T.

ED_001324_00001365-00007

C.; Jansson, J. K. Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. Environmental Microbiology 2012, 14 (9), 2405−16.
(7) Place, B.; Anderson, B.; Mekebri, A.; Furlong, E. T.; Gray, J. L.; Tjeerdema, R.; Field, J. A role for the analytical chemistry in advancing our understanding of the ocurrence, fate, and effects of Corexit oil dispersants. Environ. Sci. Technol. 2010, 44, 6016−6018.
(8) Zahed, M. A.; Aziz, H. A.; Isa, M. H.; Mohajeri, L. Effect of initial oil concentration and dispersant on crude oil biodegradation in contaminated seawater. B. Environ. Contam. Tox. 2010, 84, 438−442.
(9) Venosa, A. D.; Holder, E. L. Biodegradability of dispersed crude oil at two different temperatures. Mar. Pollut. Bull. 2007, 54 (5), 545−553.
(10) Lindstrom, J. E.; Braddock, J. F. Biodegradation of petroleum hydrocarbons at low temperature in the presence of the dispersant Corexit 9500. Mar. Pollut. Bull. 2002, 44, 739−747.
(11) Bruheim, P.; Bredholt, H.; Eimhjellen, K. Effects of surfactant mixtures, including Corexit 9527, on bacterial oxidation of acetate and alkanes in crude oil. Appl. Environ. Microb. 1999, 65 (4), 1658−1661.
(12) Foght, J. M.; Westlake, D. W. S. Effect of the dispersant Corexit 9527 on the microbial degradation of Prudhoe Bay crude oil. Can. J. Microbiol. 1982, 28, 117−122.
(13) Tosmides, H. J.; Hughes, J. B.; Thomas, J. M.; Ward, C. H. Effect of surfactant addition on phenanthrene biodegradation in sediments. Environ. Toxicol. Chem. 1995, 14 (6), 953−959.
(14) Bruheim, P.; Bredholt, H.; Eimhjellen, K. Bacterial degradation of emulsified crude oil and the effect of various surfactants. Can. J. Microbiol. 1997, 43, 17−22.
(15) Armarego, W.; Chai, C. Purification of Laboratory Chemicals, 7th ed.; Butterworth-Heinemann: 2013.
(16) Spotte, S.; Adams, G.; Bubucis, P. M. GP2 medium is an synthetic seawater for culture or maintenance of marine organisms. Zoo Biol. 1984, 3 (3), 229−240.
(17) U.S. Environmental Protection Agency, EPA 8270D Semivolatile organic compounds by gas chromatography/mass spectrometry (GC/MS). 3rd. ed.; Washington, D.C., 2007.
(18) ASTM D7730−11, Standard Test Method for Determination of Dioctyl Sulfosuccinate in Sea Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). ASTM International 2011.
(19) Smith, M. B.; March, J. March's Advanced Organic. Reactions, Mechanisms, and Structure, 6th ed.; John Wiley and Sons: Hoboken, NJ, 2007.
(20) Brezonik, P. L.; Arnold, W. A. Water Chemistry. An Introduction to the Chemistry of Natural and Engineered Aquatic System: Oxford University Press: New York, 2011.
(21) White, G. F.; Russel, N. J. Biodegradation of anionic surfactants and related molecules. In Biochemsitry of Microbial Degradation, Ratledge, C., Ed. Kluwer Academic Publishers: Dordrecht, 1994.
(22) Grady, C. P. L.; Daigger, G. T.; Love, N. G.; Filipe, C. D. M. Biological Wastewater Treatment, 3rd. ed.; CRC Press: Boca Raton, FL, 2011.
(23) Newman, D. The distribution of range in samples from a normal population, expressed in terms of an independent estimate of standard deviation. Biometrika 1939, 31 (1), 20−30.
(24) Keuls, M. The use of the "studentized range" in connection with an analysis of variance. Euphytica 1952, 1, 112−122.
(25) Grubbs, F. Procedures for detecting outlying observations in samples. Tecnometrics 1969, 11 (1), 1−21.
(26) Cerniglia, C. E. Microbial metabolism of polycyclic aromatic hydrocarbons. Adv. Appl. Microbiol 1984, 30, 31−37.
(27) Smith, M. R. The biodegradation of aromatic hydrocarbon by bacteria. Biodegradation 1990, 1, 191−206.
(28) Volkering, F.; Breure, A. M.; Rulkens, W. H. Microbiological aspects of surfactant use for biological soil remediation. Biodegradation 1998, 8, 401−417.
(29) Volkering, F.; Breure, A. M.; Sterkenburg, A.; van Andel, J. G. Microbial degradation of polycyclic aromatic hydrocarbons: Effect of substrate availability on bacterial growth kinetics. Appl. Microbiol. Biotechnol. 1992, 36, 548−522.
(30) Harms, H.; Bosma, T. N. P. Mass transfer limitation of microbial growth and pollutant degradation. J. Ind. Microbiol. 1997, 18, 97−105.
(31) Bosma, T. N. P.; Middeldorp, P. J. M.; Schraa, G.; Zender, A. J. B. Mass transfer limitation of biotransformation: Quantifying bioavailability. Environ. Sci. Technol. 1997, 31, 248−252.
(32) Johnsen, A. R.; Wick, L. Y.; Harms, H. Principles of microbial PAH-degradation in soil. Environ. Pollut. 2005, 133, 71−84.
(33) Button, D. K. Kinetics of nutrient-limited transport and microbial-growth. Microbiol. Rev. 1985, 49 (3), 270−297.
(34) Itoh, S.; Suzuki, T. Effect of rhamnolipids on growth of a Pseudomonas aeruginosa mutant deficient in n-paraffin-utilizing ability. Agric. Biol. Chem. 1972, 36, 2233−2235.
(35) Oberbremer, A.; Muller-Hurtig, R. Aerobic stepwise hydrocarbon degradation and formation of biosurfactants by an original soil population in a stirred reactor. Appl. Microbiol. Biotechnol. 1989, 31, 582−586.
(36) Noordman, W. H.; Janssen, D. B. Rhamnolipid stimulates uptake of hydrophobic compounds by Pseudomonas aeruginosa. J. Appl. Microbiol. 2002, 68, 4502−4508.
(37) Beal, R.; Betts, W. B. Role of rhamnolipid biosurfactants in the uptake and mineralization of hexadecane in Pseudomonas aeruginosa. J. Appl. Microbiol. 2000, 89, 158−168.
(38) Hazen, T. C.; Dubinsky, E. A.; DeSantis, T. Z.; Andersen, G. L.; Piceno, Y. M.; Singh, N.; Jansson, J. K.; Probst, A.; Borglin, S. E.; Fortney, J. L.; Stringfellow, W. T.; Bill, M.; Conrad, M. E.; Tom, L. M.; Chavarria, K. L.; Alusi, T. R.; Lamendella, R.; Joyner, D. C.; Spier, C.; Baelum, J.; Auer, M.; Zemla, M. L.; Chakraborty, R.; Sonnenthal, E. L.; D'haeseleer, P.; Holman, H.-Y. N.; Osman, S.; Lu, Z.; Van Nostrand, J. D.; Deng, Y.; Zhou, J.; Mason, O. U. Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science 2010, 330, 204−208.

ED_001324_00001365-00008

Available online at www.sciencedirect.com
ScienceDirect
Marine Pollution Bulletin 54 (2007) 545–553
MARINE POLLUTION BULLETIN
www.elsevier.com/locate/marpolbul

Biodegradability of dispersed crude oil at two different temperatures

A.D. Venosa [a,*], E.L. Holder [b]

[a] US Environmental Protection Agency, 26 W. Martin Luther King Drive, Cincinnati, OH 45268, United States
[b] University of Cincinnati, Department of Civil and Environmental Engineering, Cincinnati, OH 45221, United States

Abstract

Laboratory experiments were initiated to study the biodegradability of oil after dispersants were applied. Two experiments were conducted, one at 20 ¬C and the other at 5 ¬C. In both experiments, only the dispersed oil fraction was investigated. Each experiment required treatment flasks containing 3.5% artificial seawater and crude oil previously dispersed by either Corexit 9500 or JD2000 at a dispersant-to-oil ratio of 1:25. Two different concentrations of dispersed oil were prepared, the dispersed oil then transferred to shake flasks, which were inoculated with a bacterial culture and shaken on a rotary shaker at 200 rpm for several weeks. Periodically, triplicate flasks were removed and sacrificed to determine the residual oil concentration remaining at that time. Oil compositional analysis was performed by gas chromatography/mass spectrometry (GC/MS) to quantify the biodegradability. Dispersed oil biodegraded rapidly at 20 ¬C and less rapidly at 5 ¬C, in line with the hypothesis that the ultimate fate of dispersed oil in the sea is rapid loss by biodegradation.
Published by Elsevier Ltd.

Keywords: Biodegradation; Biodegradability; Dispersants; Dispersed oil; GC/MS; Crude oil

1. Introduction

Consideration of the use of chemical dispersants as an oil spill countermeasure has grown substantially in recent years due to a number of factors. Modern-day dispersants are much less toxic to aquatic biota than those used in years past. However, concern still exists on the possible toxic effects dispersant use may have on aquatic organisms, especially if dispersants are used in near shore waters. Recently, the National Research Council Ocean Studies Board was commissioned to review what is known about dispersants and formulate recommendations about how best to fill in research gaps as a means to better understand and use this countermeasure technology for treating oil spills off shore and possibly near shore (NRC, 2005).

Once oil is dispersed into the water column, its ultimate fate is mineralization by bacterial and planktonic species suspended in the water. Although much research was done in the 1980s, 1990s, and more recently (Mulkins-Phillips and Stewart, 1974; Bhosle and Row, 1983; Bhosle and Mavinkurve, 1984; Davies et al., 2001; Garcia et al., 2001; Lindstrom and Braddock, 2002; Lindstrom et al., 1999; Foght and Westlake, 1982, 1983, 1987, 1989; Yamada et al., 2003), the literature is still inconclusive in regards to biodegradation effects. One of the conclusions reached in the NRC report was that the effects of chemical dispersion on biodegradation rates have not been systematic or reproducible for a variety of reasons, one of which is the inappropriateness of the experimental systems used to investigate these effects. Often, investigators did not separate dispersed oil from the total system when conducting the biodegradation studies. Thus, it has been difficult to ascribe all effects observed to the dispersed oil per se. Also, in some cases, when information on changes in the oil composition were presented, it was based either on qualitative evidence, i.e., gas chromatograms (Foght and Westlake, 1982; Foght et al., 1983, 1987, 1989) or given for only one incubation time, usually the endpoint (Swannell et al., 1997; Burns et al., 1999; Swannell and Daniel, 1999; Davies et al., 2001; MacNaughton et al., 2003).

* Corresponding author. Tel.: +1 513 569 7668; fax: +1 513 569 7105.
E-mail address: venosa.albert@epa.gov (A.D. Venosa).

0025-326X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.marpolbul.2006.12.013

In this paper, we present results from a study of Prudhoe Bay crude oil (PBC) that was previously dispersed by two different dispersants in artificial seawater. We evaluated two different concentrations of the dispersed oil and exposed the system to two different temperatures (20 ¬C and 5 ¬C).

2. Materials and methods

2.1. Crude oil

Fresh Prudhoe Bay crude oil (PBC) was stored and maintained in our laboratory among other crude oils in our collection.

2.2. Microbial culture

The culture used in the investigation was originally isolated from Disk Island, one of the numerous shorelines contaminated by the Exxon Valdez oil spill in 1989. It was an undefined mixed consortium that was previously grown on PBC for 3 weeks in a 10 L batch. The master culture was harvested by centrifugation, washed twice with saline solution, and mixed with 10% glycerol as a cryoprotectant. The concentrated cultures were dispensed into 5 ml plastic tubes and placed into a ¬ 80 ¬C freezer for storage. On the day of the experiment, several vials were thawed to room temperature and resuspended in sterile saline to be inoculated into the appropriate flasks.

2.3. Artificial seawater

The ingredients of the artificial seawater used, GP2, were reported elsewhere (Haines et al., 2005). The seawater was autoclaved at 121 ¬C for 1 h in 1 L amounts, then transferred in 120 mL volumes to 250 mL baffled trypsinizing flasks for preparation of dispersed oil. Final salinity was 3.5%.

2.4. Residual hydrocarbon analysis

On the day of sampling, a recovery surrogate solution consisting of D_{36}-heptadecane (C_{16}), D_{50}-tetracosane (C_{24}), D_{64}-dotriacontane (C_{32}), D_{10}-1-methylnaphthalene (C_1-naph), D_{10}-phenanthrene (phe), D_{10}-pyrene (pyr), and 5a-cholestane (androstane) in dichloromethane (DCM) was added to each reactor flask to obtain a final surrogate concentration of 4 ng/ l l in the final extract. After addition of 50 mL DCM to each shake flask, the flasks were stirred for 15 min on a magnetic stirring plate. The DCM phase was passed through Na_2SO_4 to remove water. The DCM extracts were then exchanged into hexane under a stream of dry nitrogen. The solvent exchange was performed three times. The hexane extracts were prepared for GC/MS analysis by adding an internal standard mixture of deuterated alkane and aromatic hydrocarbons to yield a final concentration of each standard of 10 ng/ l l. Concentrations of hydrocarbons in the extract were quantified using a Hewlett Packard 6890 Series GC with a 5973 MSD-MS as a detector operated in the selected ion monitoring (SIM) mode. The analytes included 28 alkanes ranging in carbon number from n-C_{10} to n-C_{35} plus pristane, phytane, hopane, and 32 aromatics, consisting of the 2-, 3-, and 4-ring aromatics (naphthalenes, phenanthrenes, dibenzothiophenes, fluorenes, naphthobenzothiophenes, pyrenes, and chrysenes), including their alkylated homologs. The column was a 30 m · 0.25 mm ID with 0.2 l m liquid phase DB5 column (Supelco, Supelco Park, Bellefonte, PA). Alkane and aromatic concentrations were summed to obtain the total alkane and total AROMATIC concentrations in each flask.

2.5. Dispsersants

Two dispersant products were used in the experiments, Corexit 9500 (J. Clark, Exxon-Mobil Corp., Fairfax, VA), and JD2000 (J. Doctor, GlobeMark Resources, Ltd., Houston, TX). Both of these dispersant products are listed on EPA's National Contingency Plan (NCP) product schedule.

2.6. Experimental design

2.6.1. Preparation of dispersed oil

A large batch of dispersed PBC oil was prepared as follows. A volume of 120 mL sterile GP2 seawater was placed into baffled trypsinizing flasks (three flasks for each sampling event plus triplicate uninoculated negative controls). The flasks were fitted with a stopcock at the bottom side to facilitate undisturbed drainage of dispersed oil. To the surface of the seawater, 100 l L PBC was manually dispensed using a Brinkmann Eppendorf repeater pipettor, forming a slick, followed by 4 l L Corexit 9500 directly onto the slick, giving a dispersant-to-oil ratio (DOR) of 1:25. This same procedure was repeated for JD2000. The contents of all flasks were mixed on an orbital shaker (orbital diameter of 2 cm) at 200 rpm for 10 min to provide needed turbulence for dispersion. The mixing was followed by a 10 min quiescent period to allow non-dispersed oil to rise to the surface of the seawater. This was in accord with the baffled flask test for screening commercial dispersant products (Venosa et al., 2002). Following the quiescent period, the stopcock from each flask was opened to allow the dispersed oil to drain into a large continuously stirred aspirator bottle with tubulation (one for Corexit 9500, another for JD2000). Flow of dispersed oil was stopped near the bottom to prevent the recoalesced or non-dispersed oil from draining into the collection vessel. At that point, 100 mL aliquots of dispersed oil were then transferred to a series of 150 mL shake flasks. A second series of flasks of each dispersed oil type was also set up by preparing a 1:10 dilution of the dispersed oil/seawater mixture with fresh sterile seawater.

2.6.2. Treatments

The treatments consisted of triplicate flasks of PBC dispersed with Corexit 9500 for each of the sampling events, a second set of flasks containing PBC dispersed with JD2000, an uninoculated negative control containing dispersed PBC (six flasks) and either Corexit 9500 or JD2000, and another set of flasks containing non-dispersed PBC (no-dispersant control) to compare the biodegradability of dispersed and non-dispersed oil. The same series of flasks was set up for the 1:10 dilution of the dispersed PBC treatments except there was no non-dispersed oil treatment nor an uninoculated control. The null hypotheses being tested were: (1) prior dispersion of crude oil does not enhance its biodegradability in terms of rate and extent, (2) no biodegradability difference exists between the two dispersants being tested, (3) dispersed oil concentration has no effect on biodegradability and (4) temperature plays no role in biodegradability of dispersed oil. To test these hypotheses, simple analyses of variance (ANOVA) were performed using the Microsoft Excel Data Analysis add-in. A separate analysis of variance was conducted for each sampling event within each temperature based on a completely randomized design with a one-way treatment with three levels of dispersant (Corexit 9500, JD2000 and no dispersant) for undiluted experimental units and a one-way treatment with two levels of dispersant (Corexit 9500 and JD2000) for diluted experimental units. The sampling events for the 20 ¬C experiment occurred at Days 0, 3, 7, 14, 21, and 28. For the 5 ¬C experiment, the flasks were analyzed at Days 0, 15, 32, 39, and 46, in anticipation of a slower biodegradation rate.

2.6.3. Procedure

All flasks except the uninoculated negative controls were inoculated on Day 0 with 0.5 mL of the oil-degrading culture, which was previously thawed to room temperature. The triplicate Day 0 flasks were immediately sacrificed by addition of DCM to the flasks to extract the hydrocarbons for GC/MS analysis. The remaining flasks were placed on a rotary shaker and mixed at 200 rpm for the duration of their planned sampling times. For the 5 ¬C experiment, the flasks were all placed in a refrigerated shaker-incubator.

3. Results

3.1. Experiment conducted at 20 ¬C

3.1.1. Total alkanes and aromatics

Fig. 1(a–d) summarizes the time-dependent biodegradation of total alkanes and total aromatics in the 20 ¬C experiment. The figure is divided into four panels: the top panes are the total alkanes undiluted (left) and diluted 1:10 (right), whereas the bottom panes are total aromatics undiluted (left) and diluted 1:10 (right). The reason the concentrations of oil analytes in the non-dispersed samples (open symbols) are higher at Day 0 than those in the dispersed oil samples is that the dispersed oil samples contained only oil that was dispersed by the chemical dispersants after separation from re-coalesced or non-dispersed oil, whereas the non-dispersed oil samples contained all oil originally added to the flasks. It is evident from Fig. 1 that there was at least one difference between the two dispersants: Day 3. This was confirmed by the ANOVAs, which showed that there were statistically significant differences between Corexit 9500 and JD2000 only at Day 3 ($p = 0.045$). This affected the first-order rate coefficients (model shown on each figure panel; see Section 3.1.2), which were calculated using Microsoft Excel's Solver add-in program. The biodegradation rate of Corexit 9500 was about threefold higher than that of JD2000. As for the non-dispersed control, there were statistically significant differences between the control and both of the dispersant treatments at Days 0 and 3, the control being higher in both instances. These differences disappeared from Day 7 onwards, suggesting that biodegradation of the non-dispersed oil with respect to total alkanes was slower than that of the dispersed oil initially, but it caught up eventually.

Fig. 1c displays the undiluted aromatic data. Although the y-intercept value (Day 0) of the non-dispersed oil treatment was approximately 50% higher than those for the dispersed oil treatments, the biodegradation rate coefficients were quite similar in magnitude. This was confirmed by the ANOVA, which revealed no significant statistical differences between the dispersed oils at any sampling time ($p > 0.05$). Again, the major concentration differences were at Days 0 and 3 (p └ 0.01), where the Day 0 concentration of total aromatics for the non-dispersed oil was approximately 22.5 mg/L vs. about 15 mg/L for both dispersant treatments, while the Day 3 levels were about 18 mg/L for the non-dispersed treatment vs. about 10.5 mg/L for the dispersed oil treatments. By Day 14, residual aromatics concentrations in the non-dispersed oil treatment were of similar magnitude to the two dispersed oil treatments.

In regards to the 10·-diluted oil samples (Fig. 1b and d), for all practical purposes, the rate and total residual concentration differences in the diluted samples for total alkanes were very similar for the two dispersants (Fig. 1b). The same is true for the total aromatics (Fig. 1d). Even the first-order biodegradation rate constants for the alkanes and aromatics were similar. The ANOVA uncovered statistically significant differences in total residual aromatics concentrations between Corexit 9500 and JD2000 at Days 7 ($p = 0.027$), 21 ($p = 0.041$), and 28 ($p = 0.013$). However, at these low concentrations, such differences are not of practical importance since the concentrations were close to detection limits at Day 7 and thereafter.

3.1.2. Individual alkanes and aromatics

The biodegradability of the individual analytes was examined by conducting non-linear regression analysis using Microsoft Excel's Solver add-in program. The data were fit to a simple first-order model

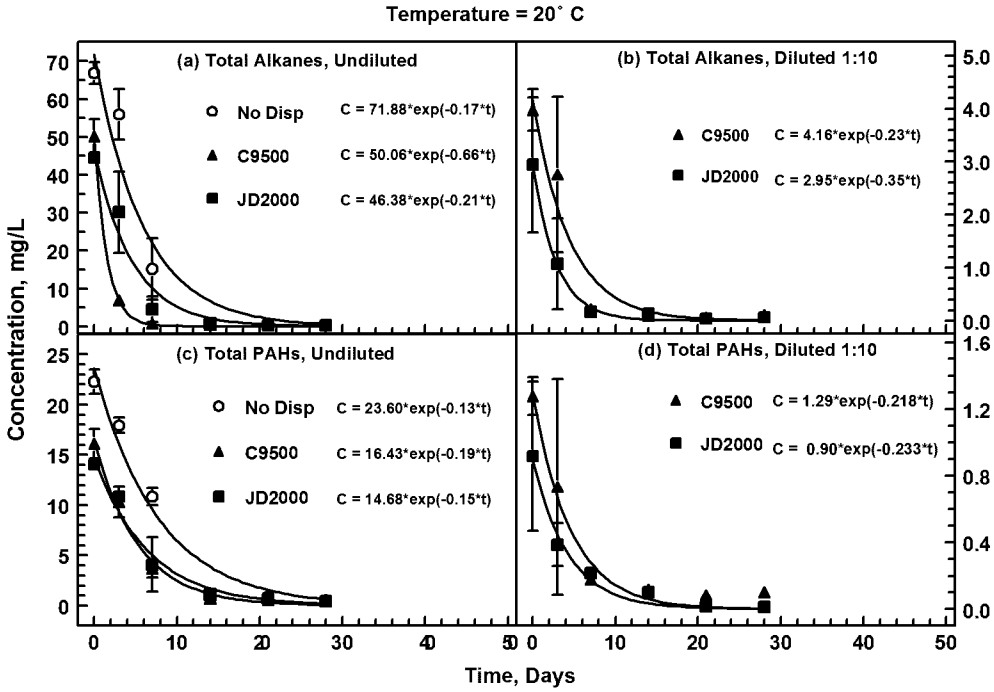


Fig. 1. Biodegradation of total alkanes and total aromatics in crude oil either non-dispersed or dispersed by Corexit 9500 and JD2000 undiluted (a and c) and diluted 1:10 (b and d). Temperature = 20 °C.

$$C_1 = C_0 \cdot \exp(-k \cdot t) \quad (1)$$

where C_1 is the concentration of analyte (mg/L) at any given time t, C_0 is the initial concentration of analyte (mg/L), and k is the first order biodegradation coefficient (day^{-1}).

The rate coefficients were calculated by minimizing the sum of squared errors from all sampling points. Initial concentrations (y-intercepts) for the dispersed oil samples were made equivalent for purposes of determining the rate coefficients since the ANOVAs confirmed no differences between dispersed oil treatments at Day 0. The y-intercept values for the non-dispersed oil treatment were not set to be equivalent to the dispersed oil treatments since the ANOVAs clearly showed significant differences, as discussed in Section 3.1.1 above. Thus, their rate constants were computed separately.

Results of the non-linear regressions are summarized in Fig. 2(a–d) for the 20 °C experiment. First-order rate coefficients are plotted against individual hydrocarbons (undiluted in the left panes and diluted 1:10 in the right panes). In the undiluted samples, the Corexit 9500 total alkane rate coefficients (Fig. 2a) are substantially higher than both the JD2000 samples and the non-dispersed oil samples. This is also evident in Fig. 1a, where concentrations of total alkanes in the Corexit 9500 treatment were significantly lower at Day 3 ($p = 0.045$) than the corresponding JD2000 and non-dispersed oil samples. First-order biodegradation rate coefficients for the JD2000 samples were consistently higher than the non-dispersed oil samples (Fig. 2a). In general, biodegradation rate constants decreased with increasing carbon number in the normal alkanes, a feature that is consistent with biodegradation behavior (Venosa et al., 1996). There were exceptions, however, mostly in the Corexit 9500 data. This is not evident in either of the other two treatments.

In regards to the aromatics data (Fig. 2c), biodegradation rate constants were similar between the two dispersed oil treatments with Corexit 9500 giving slightly higher values than JD2000 for most of the 2- and 3-ring aromatics. Also, rate constants for both dispersed oil treatments were slightly higher than for the non-dispersed oil treatment except for C_2- to C_4-phenanthrene, C_3-fluorene, and all the 4-ring aromatics.

When the dispersed oil treatments were diluted 1:10 (Figs. 2b and 2d), a pattern reversal became evident. Biodegradation rate constants for the JD2000 samples were substantially higher for virtually all analytes, both alkanes and aromatics. This was especially evident for the lower molecular weight analytes.

3.2. Experiment conducted at 5 °C

3.2.1. Total alkanes and aromatics

Fig. 3 summarizes the time-dependent biodegradation of total alkanes and total aromatics in the 5 °C experiment. The total alkanes ANOVA revealed no statistically significant differences between the two dispersed oil treatments at any sampling event. In fact, even the comparison between the non-dispersed oil treatment and the two dispersed oil treatments gave no statistically significant differences in residual concentrations at any sampling event. Although Fig. 3a seems to suggest a moderate difference, the Day 0 and Day 15 variability in the non-dispersed oil

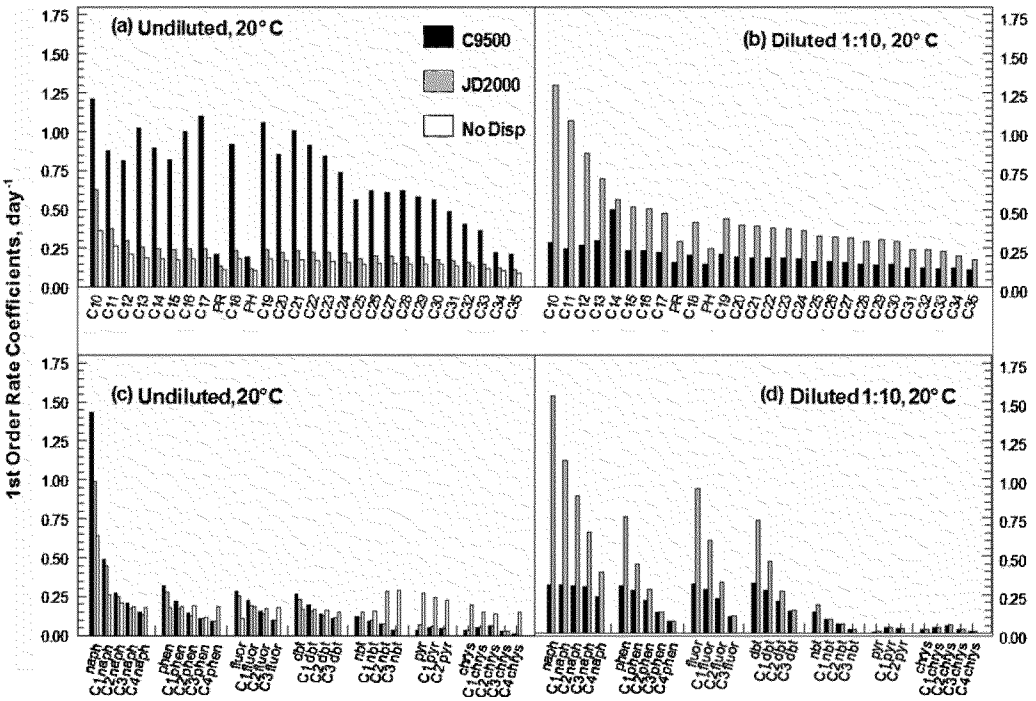


Fig. 2. First-order biodegradation rate coefficients of individual alkanes undiluted (a) and diluted 1:10 (b) and individual aromatics undiluted (c) and diluted 1:10 (d). Temperature = 20 ¬C.

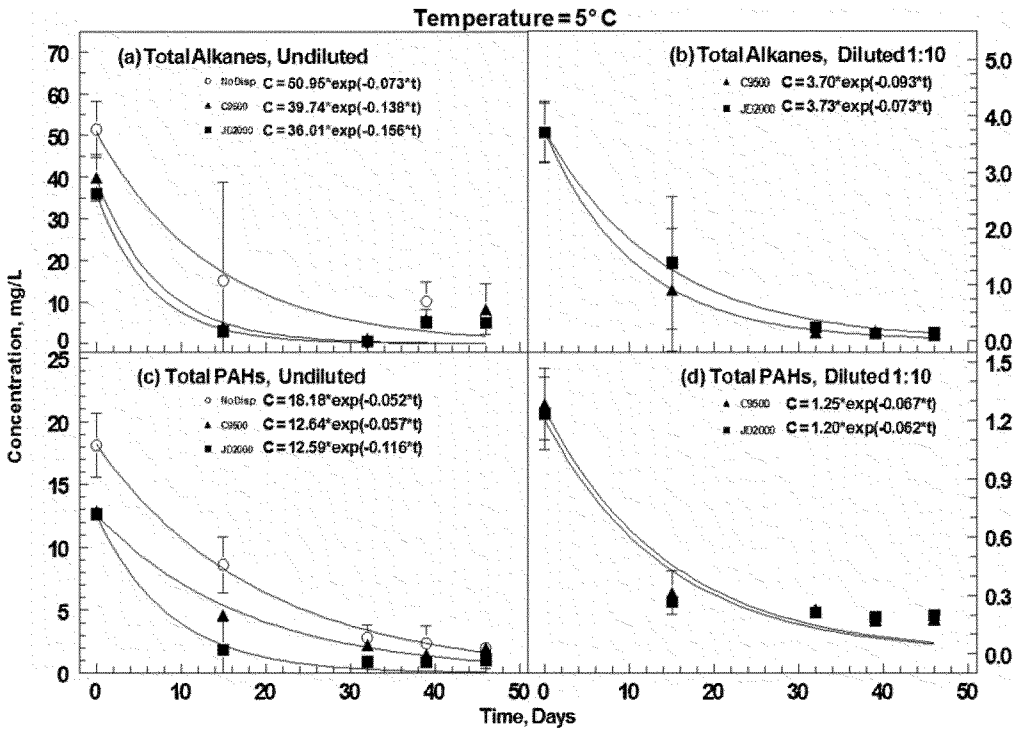


Fig. 3. Biodegradation of total alkanes and total aromatics in crude oil either non-dispersed or dispersed by Corexit 9500 and JD2000 undiluted (a and c) and diluted 1:10 (b and d). Temperature = 5 ¬C.

values was high enough that the apparent rate differences among the three treatments were not statistically significant.

The ANOVA for the undiluted aromatics data (Fig. 3c) showed no significant differences between the two dispersed oil treatments at Days 0 and 15. However, differences were significant at Days 32 ($p = 0.0075$) and 39 ($p = 0.019$). This supports the overall higher first-order biodegradation rate coefficient (¬ 0.116 day¬ 1) for the JD2000 treatment compared to the Corexit 9500 treatment (¬ 0.057 day¬ 1).

The dispersed oil treatments diluted 1:10 (Figs. 3b and 3d) were remarkably similar in biodegradation behavior for both oil fractions as the ANOVAs showed no differences between the two treatments at any sampling event.

3.2.2. Individual alkanes and aromatics

Fig. 4(a–d) summarizes results of the individual analytes in the 5 ¬C experiment. First, it is important to point out an anomaly in the alkane data from this experiment. In Fig. 4a, the rate constants for the normal alkanes C_{22}–C_{30} are substantially lower than for the lower and higher molecular weight analytes for all three treatments (Fig. 4a). This means that the corresponding residual concentrations were substantially higher. Indeed, a close examination of the GC/MS data revealed that these analytes were indeed much higher, but only on Days 39 and 46, than they should have been when considering the levels of all the other analytes. In fact, this is quite evident in Fig. 3a, where the total alkane concentrations on Days 39 and 46 for all three treatments were higher. Checking surrogate recoveries as well as internal standards of all samples at those sampling events as well as having re-injected and re-analyzed the extracts did not reveal the cause of the anomaly. Either there was some systematic laboratory error committed that cannot be detected by reanalysis, or the results are real. The latter possibility seems remote since it is unlikely that only these eight analytes suddenly increased in concentration after Day 32 and remained high through Day 46. A recalculation of the overall rate coefficients after omitting the concentrations of those analytes on Days 32 and 46 does not significantly change the overall rates shown in Fig. 3a. Of course, this is expected since the "error" occurred late in the study, where data points do not exert much influence on calculation of rate constants.

Interestingly, the behavior of the two dispersants at 5 ¬C was almost totally opposite of that at 20 ¬C. The alkane rate coefficients for JD2000 were approximately two- to threefold higher than the non-dispersed control and Corexit 9500 across the board. Corexit 9500 rates were slightly higher than the corresponding rates for the non-dispersed oil control except for analytes C_{31}–C_{35}. The 10··-diluted treatments showed that alkane rates were similar for the two dispersed oil treatments, whereas JD2000 rates were consistently higher than the Corexit 9500 rates for all 2- and 3-ring aromatics. The rate constants for the 4-ring aromatics were very low since the concentrations of those analytes were near detection limits.

4. Discussion

In this study, we reported the effects of temperature (5 ¬C and 20 ¬C) and initial oil concentration on the ability of two different commercial dispersants to enhance the biodegradability of crude oil. We expected to find decreased rates of biodegradation at the lower temperature because of lower bacterial metabolic rates. As shown in Fig. 5(a–d), which summarizes the rate constants for the conditions tested, this expectation was confirmed. With respect to the alkane fraction, the greatest temperature-induced enhancement (an almost fivefold rate increase) occurred with the dispersant

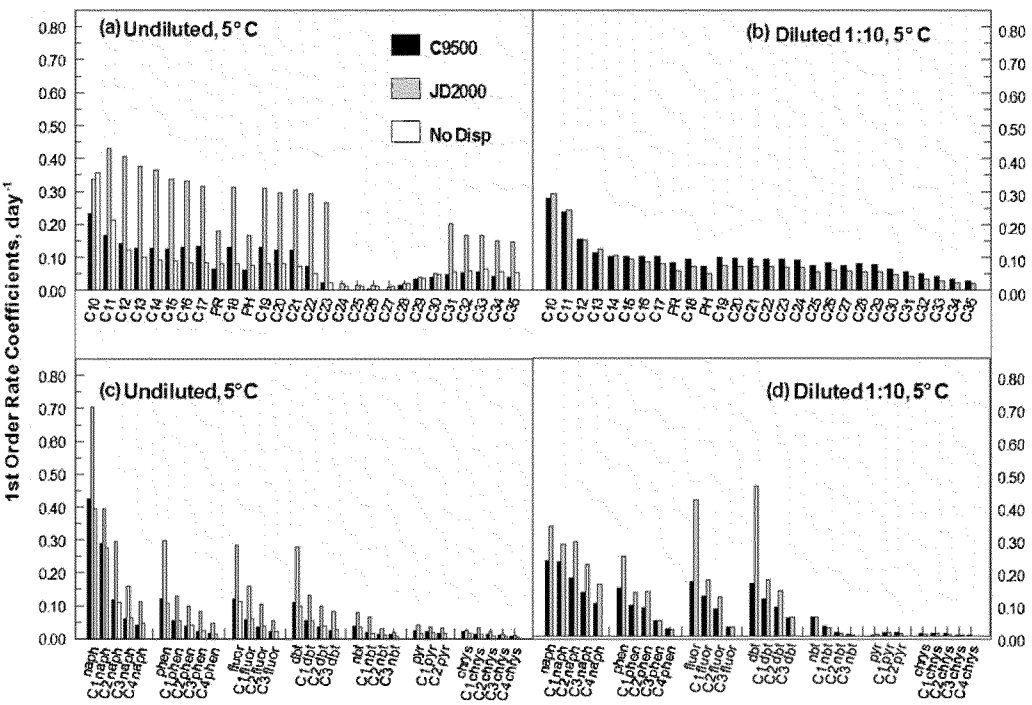


Fig. 4. First-order biodegradation rate coefficients of individual alkanes undiluted (a) and diluted 1:10 (b) and individual aromatics undiluted (c) and diluted 1:10 (d). Temperature = 5 ¬C.

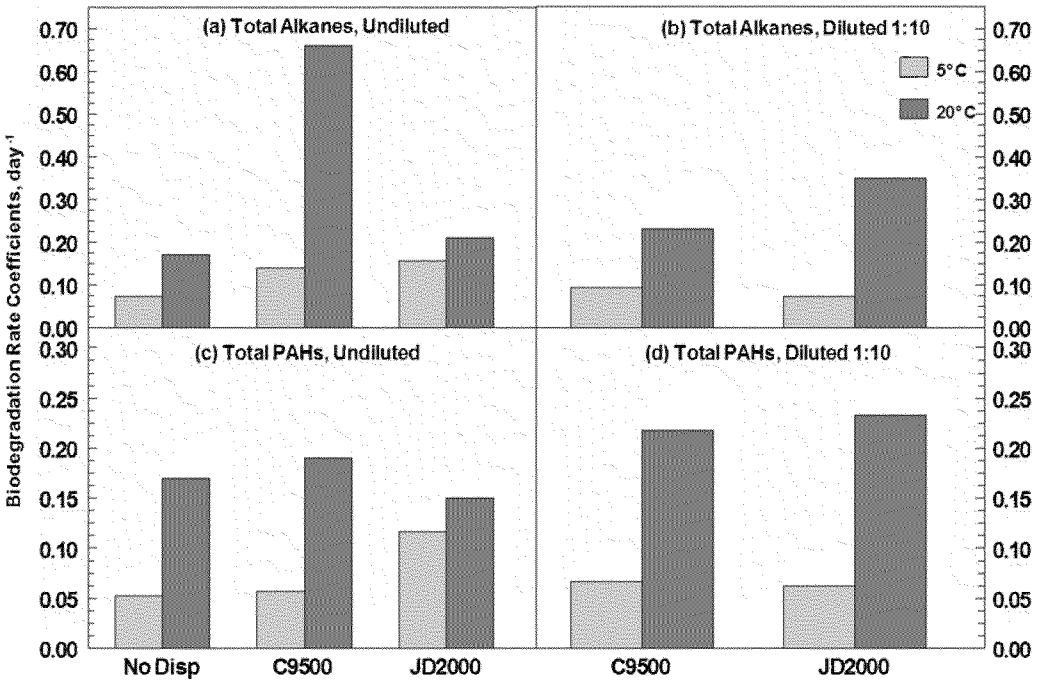


Fig. 5. Summary of rate coefficients for total alkanes undiluted (a) and diluted 1:10 (b) and total aromatics undiluted (c) and diluted 1:10 (d) for the three dispersant treatments.

Corexit 9500 (Fig. 5a). For the aromatics fraction, higher temperature rate enhancements ranged from 29% (JD2000) to more than threefold for the non-dispersed control and Corexit 9500. The lower temperature enhancement by JD2000 was due to the twofold higher overall rate at 5 ¬C compared to the other two treatments (Fig. 5c). For the 10·-diluted samples, rate increases due to temperature were 3.5- to 5-fold for alkanes and >3.5-fold for the aromatics. So, the temperature effect is clear and unambiguous.

We also expected to see an enhancement in the rate but not necessarily the extent of biodegradation due to the action of dispersants compared to the non-dispersed control. In general, this was also observed, and in time, non-dispersed oil always caught up in terms of residual concentrations of analytes, so that eventually the overall extent of biodegradation was about the same in most instances. The reason for the slower startup for the non-dispersed oil was that the microbial culture first had to generate its own biosurfactant to emulsify the oil before substantial biodegradation took place. In our experience, emulsification of oil in closed laboratory flasks usually takes place within 2 days after startup. This is evident by examination of Fig. 1a and 1c, where the Day 3 concentration of total alkanes (Fig. 1a) and total aromatics (Fig. 1c) were higher than the corresponding concentrations of the dispersed oil treatments. Once biodegradation started up, residual concentrations usually caught up to the dispersed oil treatments by Day 7 or 14. This was not evident in the 5 ¬C samples (Fig. 3) because we did not sample them earlier than 14 days after startup. Had we sampled earlier, it is likely the same observation would have been made.

Examination of individual analyte degradation produces a mixed set of observations. In the 20 ¬C experiment, Corexit 9500 stimulated biodegradation rates many-fold more than JD2000 or the non-dispersed control (Fig. 2a). This was not true with the aromatics, however (Fig. 2d). According to Varadaraj et al. (1995) and Bruheim et al. (1999), Corexit 9500 contains two non-ionic surfactants, Tween 80 (eicosethoxy sorbitan monooleate) and Span 80 (sorbitan monooleate) and one anionic surfactant (sodium dioctyl sulfosuccinate). Varadaraj et al. (1995) varied the relative proportions of the two non-ionic surfactants to give a range of hydrophilic–lipophilic balances (HLBs). They studied these various HLBs on the biodegradation rate of Alaska North Slope crude oil (ANS), finding that an HLB of 8 gave the highest enhancement of alkane biodegradation. This HLB was substantially lower than that found by Van Hamme and Ward (1999) using the homologous series of Igepal surfactants. Bruheim et al. (1999) studied the anionic surfactant sodium dioctyl sulfosuccinate to stimulate pure cultures of alkane degraders to degrade oil hydrocarbons. They found that the surfactant was highly inhibitory to pure cultures of the alkane degraders Acinetobacter calcoaceticus and Rhodococcus spp. O94. They also found that the non-ionic surfactants stimulated oil biodegradation rate when tested alone. Bruheim and Eimhjellen (2000) found that these surfactants appeared to affect the permeability of the outer membrane of bacteria. In our study, the rate of biodegradation of alkanes was highly enhanced at 20 ¬C by Corexit 9500, suggesting that the enhancement might have been due to outer membrane permeability effects. The extreme stimulation of biodegra-

dation rates did not occur with JD2000 at the same temperature, suggesting that the surfactants in that product are either different or are in different proportions relative to the other surfactants contained in the product.

However, the role reversal of the two dispersants when the oil mixture was diluted 1:10 was an interesting phenomenon (Fig. 2b). Under this condition, JD2000 enhanced biodegradation rates several-fold more than Corexit 9500. This enhancement was not as great as Corexit 9500 in the undiluted state, but it was much higher than Corexit 9500 at the 1:10 dilution. According to the NRC report (NRC, 2005), direct attachment of bacteria to oil droplets is the mechanism most likely to be affected by chemical dispersion because accumulation of surfactants at the oil–water interface will change its chemical characteristics and possibly interfere with normal attachment properties. This would explain the lower biodegradation rate of the Corexit 9500 treatment in the diluted treatment but does not explain the higher biodegradation rate of the diluted JD2000 mixture. However, it is possible that JD2000 might have been inhibitory to biodegradation in the undiluted treatment, at least to the extent that might have been possible without the inhibition. In other words, biodegradation rates in the JD2000 treatment were still higher in the undiluted samples, but without the putative inhibition they might have been even higher. Research by Zhang and Miller (1992, 1994), Van Hamme and Ward (2001), Bruheim et al. (1997), and Foght et al. (1989) all suggest that adhesion of bacteria to oil droplets is important for enhancement of alkane biodegradation.

According to the NRC report (NRC, 2005), the above mechanism does not apply to aromatics, however, which are much more soluble than alkanes. Biodegradation of aromatics is more likely caused by uptake from the aqueous phase. However, this does not fit with the JD2000 observations from our study (see Figs. 2c and 2d). A huge stimulation in biodegradation of 2- and 3-ring aromatics took place in the JD2000 treatment when the oil–dispersant mixture was diluted 1:10. This did not occur in the Corexit 9500 treatment. If direct uptake of soluble aromatics were the dominant mechanism, then one would not expect to see such a large increase in biodegradation rates when the oil–dispersant mixture was diluted 10-fold. Perhaps JD2000 produced smaller oil droplets than Corexit 9500 under similar mixing conditions in the baffled flasks, thereby increasing the overall surface area and allowing even further solubilization of the aromatics. Particle-size distribution should play a major role in dispersion and subsequent biodegradation, but it was not evaluated in this study.

In the 5 °C experiment, the biostimulatory effect of JD2000 in the diluted compared to the undiluted treatment was much less pronounced but still greater than that caused by Corexit 9500 (Fig. 4d) (note that the y-axis scale of this figure is much lower than that in Fig. 2d). Due to the spreading nature of oil as a slick on the open sea, this might be an advantage for JD2000 as dilution takes place rapidly over time. Clearly, use of dispersants does cause a more rapid biodegradation, which is the ultimate fate of oil that has been dispersed into the water column.

The interplay of surfactants and solvents in commercial dispersant formulations with crude oil is a very complex phenomenon (NRC, 2005). Substantially more research is needed to explain and fully understand chemical dispersion as a countermeasure for treating oil spills. Such factors as the type of oil being treated, the surfactant formulation in commercial dispersants, the HLB, the differences in uptake of the various hydrocarbon constituents of oil, effects of surfactants on bacterial attachment to oil droplets, the effects of dilution, and the vast interrelationships of all these factors are ripe areas for continued further research.

Acknowledgements

We acknowledge the technical assistance given by Mr. Ross Schneider in the conduct of the experiments in the laboratory.

References

Bhosle, N.B., Mavinkurve, S., 1984. Effects of dispersants on microbial growth and biodegradation of crude oil. Mahasagar-Bulletin of the National Institute of Oceanography 17 (4), 233–238.

Bhosle, N.B., Row, A., 1983. Effect of dispersants on the growth of indigenous bacterial population and biodegradation of crude oil. Indian Journal of Marine Sciences 12, 194–196.

Bruheim, P., Eimhjellen, K., 2000. Effects of nonionic surfactants on the uptake and hydrolysis of fluorescein diacetate by alkane-oxidizing bacteria. Canadian Journal of Microbiology 46 (4), 387–390.

Bruheim, P., Bredholt, H., Eimhjellen, K., 1997. Bacterial degradation of emulsified crude oil and the effect of various surfactants. Canadian Journal of Microbiology 43, 17–22.

Bruheim, P., Bredholt, H., Eimhjellen, K., 1999. Effects of surfactant mixtures, including Corexit 9527, on bacterial oxidation of acetate and alkanes in crude oil. Applied and Environmental Microbiology 65 (4), 1658–1661.

Burns, K.A., Codi, S., Pratt, C., Duke, N.C., 1999. Weathering of hydrocarbons in mangrove sediments: testing the effects of using dispersants to treat oil spills. Organic Geochemistry 30, 1273–1286.

Davies, L., Daniel, F., Swannell, R.P.J., Braddock, J.F., 2001. Biodegradability of chemically-dispersed oil. A Report Prepared for the Minerals Management Service. Alaska Department of Environmental Conservation, and the United States Coast Guard, 33 pp.

Foght, J.M., Fairbairn, N.J., Westlake, D.W.S., 1987. Effect of oil dispersants on microbially-mediated processes in freshwater systems. In: Vandermeulen, J.H., Hrudey, S.E. (Eds.), Oil in Freshwater: Chemistry, Biology, Countermeasure Technology. Pergamon Press, Oxford, England, pp. 252–263.

Foght, J.M., Fedorak, P.M., Westlake, D.W.S., 1983. Effect of the dispersant Corexit 9527 on the microbial degradation of sulfur heterocycles in Prudhoe Bay oil. Canadian Journal of Microbiology 61, 623–627.

Foght, J.M., Gutnick, D.L., Westlake, D.W.S., 1989. Effect of emulsan on biodegradation of crude oil by pure and mixed bacterial cultures. Applied and Environmental Microbiology 55 (1), 36–42.

Foght, J.M., Westlake, D.W.S., 1982. Effect of the dispersant Corexit 9527 on the microbial degradation of Prudhoe Bay oil. Canadian Journal of Microbiology 28, 117–122.

Garcia, J.M., Wick, L.Y., Harms, H., 2001. Influence of the nonionic surfactant Brij 35 on the bioavailability of solid and sorbed dibenzofuran. Environmental Science and Technology 35 (10), 2033–2039.

Haines, J.R., Kleiner, E.J., McClellan, K.A., Koran, K.M., Holder, E.L., King, D.W., Venosa, A.D., 2005. Laboratory evaluation of oil spill bioremediation products in salt and freshwater systems. Journal of Industrial Microbiology and Biotechnology 32, 171–185.

Lindstrom, J.E., Braddock, J.F., 2002. Biodegradation of petroleum hydrocarbons at low temperature in the presence of the dispersant Corexit 9500. Marine Pollution Bulletin 44 (8), 739–747.

Lindstrom, J.E., White, D.M., Braddock, J.F., 1999. Biodegradation of dispersed oil using Corexit 9500. Report to the Alaska Department of Environmental Conservation Division of Spill Prevention and Response. 35 pp.

MacNaughton, S.J., Swannell, R.P.J., Daniel, F., Bristow, L., 2003. Biodegradation of dispersed Forties crude and Alaskan North Slope oils in microcosms under simulated marine conditions. Spill Science and Technology Bulletin 8 (2), 179–186.

Mulkins-Phillips, G.J., Stewart, J.E., 1974. Effect of four dispersants on biodegradation and growth of bacteria on crude oil. Applied Microbiology 28 (4), 547–552.

National Research Council, Committee on Understanding Oil Spill Dispersants, 2005. Oil Spill Dispersants: Efficacy and Effects. Ocean Studies Board, National Academic Press, Washington, DC, 378 pp.

Swannell, R.P.J., Daniel, F., 1999. Effect of dispersants on oil biodegradation under simulated marine conditions. In: Proceedings, 1999 International Oil Spill Conference, vol. 212. American Petroleum Institute Washington, DC.

Swannell, R.P.J., Daniel, F., Croft, B.C., Englehardt, M.A., Wilson, D.J., Mitchell, Lunel, T., 1997. Influence of physical and chemical dispersion on the biodegradation of oil under simulated marine conditions. 20th Arctic and Marine Oil Spill Program Technical Seminar, Vancouver, British Columbia, Canada, Environment Canada.

Van Hamme, J.D., Ward, O.P., 1999. Influence of chemical surfactants on the biodegradation of crude oil by a mixed bacterial culture. Canadian Journal of Microbiology 45, 130–137.

Van Hamme, J.D., Ward, O.P., 2001. Physical and metabolic interactions of Pseudomonas sp. strain JA5-B45 and Rhodococcus sp. strain F9-D79 during growth on crude oil and effect of a chemical surfactant on them. Applied and Environmental Microbiology 67 (10), 4874–4879.

Varadaraj, R., Robbins, M.L., Bock, J., Pace, S., MacDonald, D., 1995. Dispersion and biodegradation of oil spills on water. In: 1995 International Oil Spill Conference. American Petroleum Institute, Long Beach, CA.

Venosa, A.D., Suidan, M.T., Wrenn, B.A., Strohmeier, K.L., Haines, J.R., Eberhart, B.L., King, D.W., Holder, E.L., 1996. Bioremediation of an experimental oil spill on the shoreline of Delaware Bay. Environmental Science and Technology 30 (5), 1764–1775.

Venosa, A.D., King, D.W., Sorial, G.A., 2002. The baffled flask test for dispersant effectiveness: a round robin evaluation of reproducibility and repeatability. Spill Science and Technology Bulletin 7 (5–6), 299–308.

Yamada, M., Takada, H., Toyoda, K., Yoshida, A., Shibata, A., Nomura, H., Wada, M., Nishimura, M., Okamoto, K., Ohwada, K., 2003. Study on the fate of petroleum-derived polycyclic aromatic hydrocarbons (aromatics) and the effect of chemical dispersant using an enclosed ecosystem mesocosm. Marine Pollution Bulletin 47, 105–113.

Zhang, Y., Miller, R.M., 1992. Enhanced octadecane dispersion and biodegradation by a Pseudomonas rhamnolipid surfactant (biosurfactant). Applied and Environmental Microbiology 58 (10), 3276–3282.

Zhang, Y., Miller, R.M., 1994. Effect of Pseudomonas rhamnolipid biosurfactant on cell hydrophobicity and biodegradation of octadecane. Applied and Environmental Microbiology 60 (6), 2102–2106.

To: Raghuraman Venkatapathy[raghuraman.venkatapathy@ptsied.com]
Cc: Campo-Moreno, Pablo (campomp)[campomp@UCMAIL.UC.EDU]; Holder, Edith[holder.edith@epa.gov]; BOL-Deshpande, Ruta (deshpars)[deshpars@mail.uc.edu]; BOL-Zhang, Yu (zhang4y5)[zhang4y5@mail.uc.edu]
From: Conmy, Robyn
Sent: Thur 6/4/2015 11:50:16 AM
Subject: Re: Finasol and dilbit results

Yes, the building is shut down. Can we reschedule for Monday morning?

Sent from my iPhone

On Jun 4, 2015, at 6:21 AM, Raghuraman Venkatapathy <raghuraman.venkatapathy@ptsied.com> wrote:

Pablo,

The meeting will likely be cancelled as the main building is still closed (no power since yesterday morning).

Thanks,
raghu

Raghuraman Venkatapathy
On-Site Technical Manager
Pegasus Technical Services, Inc.
On-Site Contractor to U.S. EPA
NRMRL-STD, MS:443
26 W. Martin Luther King Jr. Drive
Cincinnati, OH 45268.
Phone: (513) 569 7077(O)/(513) 549 7880(GV)
Fax: (513) 569 7677
e-mail: raghuraman.venkatapathy@ptsied.com
venkatapathy.raghuraman@epa.gov

On 06/02/2015 10:35 AM, Campo-Moreno, Pablo (campomp) wrote:

Works for me. See you on Thursday at AWBERC
Regards,

Pablo Campo-Moreno, Ph.D.
Research Assistant Professor
University of Cincinnati
Department of Biomedical, Chemical and Environmental Engineering
766 ERC
Cincinnati, OH 45221-0012
PH:(513)-556-3637
Fax: (513)-556-4162
campomp@ucmail.uc.edu

It is a capital mistake to theorize before one has data. Insensibly one begins to twist facts to suit theories, instead of theories to suit facts.
The adventures for Sherlock Holmes

On Jun 2, 2015, at 10:33 AM, Conmy, Robyn <Conmy.Robyn@epa.gov> wrote:

OK. How about Thursday morning at 10?

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Campo-Moreno, Pablo (campomp) [mailto:campomp@UCMAIL.UC.EDU]
Sent: Tuesday, June 02, 2015 10:30 AM
To: Conmy, Robyn
Cc: Holder, Edith; BOL-Deshpande, Ruta (deshpars); BOL-Zhang, Yu (zhang4y5); Venkatapathy Raghuraman
Subject: Finasol and dilbit results

Dear Robyn,

Hope you're doing all right. We finished the biodegradation experiment with Finasol and completed the analysis of the ANS and dilbit samples Edie sent us. I'd like to meet with you at your earlier convenience to discuss our results. This week I'm available any time but Wed from 9 am to 2 pm.

Regards,

Pablo Campo-Moreno, Ph.D.

Research Assistant Professor
University of Cincinnati
Department of Biomedical, Chemical and Environmental Engineering
766 ERC
Cincinnati, OH 45221-0012
PH:(513)-556-3637
Fax: (513)-556-4162
campomp@ucmail.uc.edu

It is a capital mistake to theorize before one has data. Insensibly one begins to twist facts to suit theories, instead of theories to suit facts.
The adventures for Sherlock Holmes

To: Brooks, Rebecca J LT[Rebecca.J.Brooks@uscg.mil]
Cc: Weaver, James (USCG)[James.D.Weaver@uscg.mil]
From: Conmy, Robyn
Sent: Wed 3/22/2017 11:43:07 AM
Subject: RE: [EXTERNAL]IOSC 2017 Paper Extension
IOSC 2017_Conmy et al_plume simulations proceedings paper.docx

I still cannot upload the file. I tried from Explorer and Google Chrome – but no luck. This is exactly what happened in December.

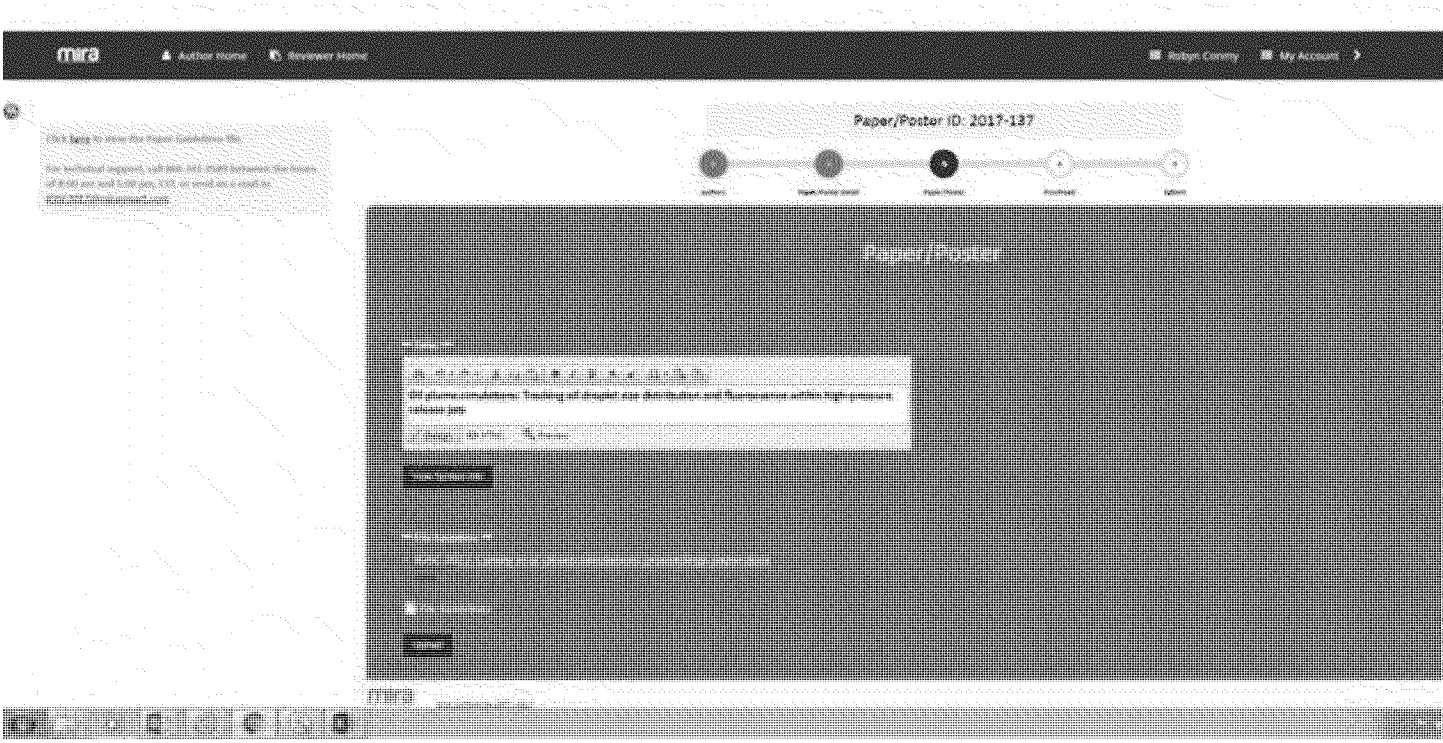
When I select the file to upload and then a blinking yellow circle appears next to the file name and a dark scroll bar under it appears to be only 10% filled suggesting that it cannot upload. When I select the 'Upload' button, an error message box appears saying "submission.mirasmart.com says: Please select file to upload – prevent this page from creating additional dialogs".

So I have attached the paper to this email in the hopes that you can get it into the system.

Cheers,

Robyn

FYI – Last week, before I knew the issue with the missing paper I also sent an email to the mirasystem for help when I couldn't find the reviews. I never received a call or email back from them about the issue. Thought I would let you know in case others were having unresponsive issues with that email on the IOSC mira website.



<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

-----Original Message-----
From: Brooks, Rebecca J LT [mailto:Rebecca.J.Brooks@uscg.mil]
Sent: Wednesday, March 22, 2017 7:15 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Weaver, James (USCG) <James.D.Weaver@uscg.mil>
Subject: RE: [EXTERNAL]IOSC 2017 Paper Extension

Robyn,

Don't worry. I'll send CJ an email explaining what happened and copy you and CDR Weaver.

Why don't you try to upload it again, and if it doesn't work, send me an electronic version. Please also CC CDR Weaver and CJ. We'll get the review knocked out among the Program Committee.

Very respectfully,

LT Becca Brooks

U.S. Coast Guard Headquarters STOP 7516

Office of Marine Environmental Response Policy (CG-MER-3)

2703 Martin Luther King Jr. Ave SE

Washington, DC 20593-7516

Phone: 202-372-2259

-----Original Message-----

From: Conmy, Robyn [mailto:Conmy.Robyn@epa.gov]

Sent: Wednesday, March 22, 2017 7:12 AM

To: Brooks, Rebecca J LT

Cc: Weaver, James D CDR

Subject: [Non-DoD Source] RE: [EXTERNAL]IOSC 2017 Paper Extension

Hi Becca,

Kurt confirmed that my paper was not reviewed by reviewers for his session. So it appears that it was never given to the correct session chair (assuming that is CJ based on our conversation yesterday). I recall that in December, there were issues with the Mira system and I was unable to upload the paper into the system, so I reached out to Kurt (mistakenly) as the chair and provided the paper that way for review. When he realized that the paper wasn't in his session, I was never informed. So this entire time, I thought it was under review.

Have you reached out to CJ yet? If not, I can. Please let me know the next steps from here. I apologize for any inconvenience!

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov <mailto:conmy.robyn@epa.gov>

From: Brooks, Rebecca J LT [mailto:Rebecca.J.Brooks@uscg.mil]

Sent: Tuesday, March 21, 2017 4:12 PM

To: Conmy, Robyn <Conmy.Robyn@epa.gov>

Cc: Weaver, James (USCG) <James.D.Weaver@uscg.mil>

Subject: RE: [EXTERNAL]IOSC 2017 Paper Extension

Robyn,

I think the confusion resides with which submission you were referring to when you spoke with Kurt last time. I'm including the email CDR Weaver originally sent to Kurt notifying him of his responsibilities as a Session Chair. There's a paper in there for which you are listed as a co-chair. He must've thought you meant that one.

This said, I don't believe your paper has been reviewed at all. Maybe Kurt took a look at it outside of the Mira system, so I recommend that you check in with him to make sure that's not the case.

As you know, the Program Committee is planning to undertake some emergency reviews to knock out the last few papers that have not undergone sufficient review. I recommend we add your paper to that list, and we'll leave it in CJ's Session where it's listed right now.

Let me know if this sounds ok, and if you hear anything different from Kurt. Thanks!

-Becca

Very respectfully,

LT Becca Brooks

U.S. Coast Guard Headquarters STOP 7516

Office of Marine Environmental Response Policy (CG-MER-3)

2703 Martin Luther King Jr. Ave SE

Washington, DC 20593-7516

Phone: 202-372-2259

From: Conmy, Robyn [mailto:Conmy.Robyn@epa.gov]

Sent: Tuesday, March 21, 2017 2:13 PM

To: Brooks, Rebecca J LT

Subject: [Non-DoD Source] FW: [EXTERNAL]IOSC 2017 Paper Extension

Hi Becca,

This is the email that I sent to Kurt on 12/19/16 with my full paper. Kurt confirmed that my paper was in his session. So, now I'm totally confused. Where does CJ fit into the mix? Who has the reviews of my paper? How and where can I submit the final paper after I am given the reviews?

I hope this hasn't been problematic for others.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Conmy, Robyn

Sent: Monday, December 19, 2016 7:21 AM

To: Kurt Kriter <KurtKriter@oilspillresponse.com>

Subject: RE: [EXTERNAL]IOSC 2017 Paper Extension

Thanks Kurt. I am a coauthor on 3 other papers and totally goofed and sent this one to Steve. Surprised that he didn't call me out on the mistake last week. In any case, here is the draft.

Thanks again,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Kurt Kriter [mailto:KurtKriter@oilspillresponse.com]

Sent: Monday, December 19, 2016 7:17 AM

To: Conmy, Robyn <Conmy.Robyn@epa.gov>

Subject: RE: [EXTERNAL]IOSC 2017 Paper Extension

Robyn,

No Worries. Yes, you are on my list. Please forward your draft to me as soon as you can.

Regards,

Kurt

Kurt Kriter | Regional Director - Americas | Oil Spill Response USA Inc. | City Centre One, 800 Town & Country Blvd, Suite 300, Houston, TX 77024 |

Tel: +1-281-748-3017 | Email: KurtKriter@oilspillresponse.com <mailto:KurtKriter@oilspillresponse.com> | URL: www.oilspillresponse.com <https://urldefense.proofpoint.com/v2/url?u=http-3A__www.oilspillresponse.com&d=DwMFAg&c=0NKfg44GVknAU-XkWXjNxQ&r=LK0KDUxcbclPKZXy70wY5CvRPc3jI1ye64WJUvVFNYg&m=Mfwxv2Z_BjaQni7RarcKanx
haIBQ&s=bbvn6bpqcR9bw-C5KPj0xcd2Tw3kllQHrIpxCx9bNJY&e=>

A Global Company with a Strong Regional Focus

From: Conmy, Robyn [mailto:Conmy.Robyn@epa.gov]

Sent: Monday, December 19, 2016 6:10 AM

To: Kurt Kriter <KurtKriter@oilspillresponse.com>

Subject: RE: [EXTERNAL]IOSC 2017 Paper Extension

Hi Kurt,

I think I may have goofed. I sent my paper to Steve Lehmann last week thinking that he was my session chair for my first author paper, but now realize that it might be you. Can you tell me if you are the lead for mine? If so I will send ASAP.

Thank,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Kurt Kriter [mailto:KurtKriter@oilspillresponse.com]

Sent: Friday, December 16, 2016 3:23 PM

To: Jill.Rowe@rpsgroup.com; Alicia.Morandi@rpsgroup.com <mailto:Alicia.Morandi@rpsgroup.com> ; Zhengkai.Li@rpsgroup.com; ahwalker@seaconsulting.com; jjoeckel@seaconsulting.com <mailto:jjoeckel@seaconsulting.com> ; mmcpeek@seaconsulting.com; philippe.blanc@total.com; anne.basseres@total.com <mailto:anne.basseres@total.com> ; ronan.jezequel@cedre.fr; Karine.Duboscq@cedre.fr; gaetan.lefloch@univ-brest.fr <mailto:gaetan.lefloch@univ-brest.fr> ; devis.255@gmail.com; Conmy, Robyn <Conmy.Robyn@epa.gov>; p.campo-moreno@cranfield.ac.uk; kanggy@hufs.ac.kr; Ben.Fieldhouse@canada.ca <mailto:Ben.Fieldhouse@canada.ca> ; Alaa.Alsaafin@canada.ca; Kevin.Watson@canada.ca; Sailly.Dave@canada.ca <mailto:Sailly.Dave@canada.ca> ; Caroline.Jung@canada.ca

Subject: RE: [EXTERNAL]IOSC 2017 Paper Extension

Good Afternoon Team,

You will recall the deadline for draft paper submittals is today. A couple of you have sent your papers directly to me, others have uploaded into the Mira system. If you are experiencing any issues, please let me know as soon as possible so we can work them out.

All the best,

Kurt

From: Kurt Kriter

Sent: Monday, November 21, 2016 1:44 PM

To: 'Jill.Rowe@rpsgroup.com' <Jill.Rowe@rpsgroup.com>; 'Alicia.Morandi@rpsgroup.com' <Alicia.Morandi@rpsgroup.com>; 'Zhengkai.Li@rpsgroup.com' <Zhengkai.Li@rpsgroup.com>; 'ahwalker@seaconsulting.com' <ahwalker@seaconsulting.com>; 'jjoeckel@seaconsulting.com' <jjoeckel@seaconsulting.com>; 'mmcpeek@seaconsulting.com' <mmcpeek@seaconsulting.com>; 'philippe.blanc@total.com' <philippe.blanc@total.com>; 'anne.basseres@total.com' <anne.basseres@total.com>; 'ronan.jezequel@cedre.fr' <ronan.jezequel@cedre.fr>; 'Karine.Duboscq@cedre.fr' <Karine.Duboscq@cedre.fr>; 'gaetan.lefloch@univ-brest.fr' <gaetan.lefloch@univ-brest.fr>; 'devis.255@gmail.com' <devis.255@gmail.com>; 'Conmy.Robyn@epa.gov' <Conmy.Robyn@epa.gov>; 'p.campo-moreno@cranfield.ac.uk' <p.campo-moreno@cranfield.ac.uk>; 'kanggy@hufs.ac.kr' <kanggy@hufs.ac.kr>; 'Ben.Fieldhouse@canada.ca' <Ben.Fieldhouse@canada.ca>; 'Alaa.Alsaafin@canada.ca' <Alaa.Alsaafin@canada.ca>; 'Kevin.Watson@canada.ca'

<Kevin.Watson@canada.ca>; 'Sailly.Dave@canada.ca' <Sailly.Dave@canada.ca>; 'Caroline.Jung@canada.ca' <Caroline.Jung@canada.ca>

Subject: FW: [EXTERNAL]IOSC 2017 Paper Extension

Ladies and gentlemen,

Following is a note from the IOSC committee extending the draft paper submittal deadline. If you have any questions, please let me know.

Regards,

Kurt

From: IOSC [mailto:IOSC@courtesyassoc.com]

Sent: Monday, November 21, 2016 11:20 AM

To: IOSC <IOSC@courtesyassoc.com>

Subject: [EXTERNAL]IOSC 2017 Paper Extension

Good day,

Thank you again for your abstract submission to the 2017 International Oil Spill Conference. We look forward to working with you as your paper and presentation are developed, and to the

contributions you will personally make to the IOSC's robust and diverse program.

We would like to inform you that the deadline for Draft Paper submissions has been extended to Friday, December 16, 2016. At that time all draft paper submissions must be submitted in order to allow for Session chair review. Please note that the remaining deadlines for your paper stand:

December 16, 2016 -- draft paper due to Session Chairperson

March 15, 2017 -- final paper due to Session Chairperson

April 12, 2017 - Session Chairperson makes final decision to accept/reject paper

April 26, 2017 - final slide presentation due to Session Chairperson

For those of you serving as Session Chairs, this email is addressed to you for your visibility and to ensure that you are working with the authors assigned to your Session to ensure they are submitting their papers into the system on time. Thank you for your flexibility and continued support.

Please feel free to reach out with any questions you may have.

Sincerely,

IOSC 2017

The information transmitted is intended only for the person or entity to which it is addressed and may contain confidential and/or privileged material. Any review, retransmission, dissemination or other use of, or taking of any action in reliance upon, this information by persons or entities other than the intended recipient is prohibited. If you received this in error, please contact the sender and delete the material from any computer. Any views or opinions expressed in this email are solely those of the author and do not necessarily represent those of the company.

We accept no responsibility for viruses that we may have unintentionally transmitted to you within this email and you should check for viruses before opening this email or any attachment. Those communicating with us by email will be deemed to have consented to us intercepting and monitoring those communications.

Oil Spill Response Limited is registered in England and Wales under number 1808594. The registered office is Lower William Street, Southampton, SO14 5QE, United Kingdom.
http://www.oilspillresponse.com <https://urldefense.proofpoint.com/v2/url?u=http-3A__www.oilspillresponse.com&d=DwMFAg&c=0NKfg44GVknAU-XkWXjNxQ&r=LK0KDUxcbclPKZXy70wY5CvRPc3jI1ye64WJUvVFNYg&m=Mfwxv2Z_BjaQni7RarcKanx
haIBQ&s=bbvn6bpqcR9bw-C5KPj0xcd2Tw3kllQHrIpxCx9bNJY&e=>

The information transmitted is intended only for the person or entity to which it is addressed and may contain confidential and/or privileged material. Any review, retransmission, dissemination or other use of, or taking of any action in reliance upon, this information by persons or entities other than the intended recipient is prohibited. If you received this in error, please contact the sender and delete the material from any computer. Any views or opinions expressed in this email are solely those of the author and do not necessarily represent those of the company.

We accept no responsibility for viruses that we may have unintentionally transmitted to you within this email and you should check for viruses before opening this email or any attachment. Those communicating with us by email will be deemed to have consented to us intercepting and monitoring those communications.

Oil Spill Response Limited is registered in England and Wales under number 1808594. The registered office is Lower William Street, Southampton, SO14 5QE, United Kingdom.
http://www.oilspillresponse.com <https://urldefense.proofpoint.com/v2/url?u=http-3A__www.oilspillresponse.com&d=DwMFAg&c=0NKfg44GVknAU-

XkWXjNxQ&r=LK0KDUxcbclPKZXy70wY5CvRPc3jI1ye64WJUvVFNYg&m=Mfwxv2Z_BjaQni7RarcKanx
haIBQ&s=bbvn6bpqcR9bw-C5KPj0xcd2Tw3kllQHrIpxCx9bNJY&e=>

To: Holder, Edith[holder.edith@epa.gov]; Devi Sundaravadivelu[devis.255@gmail.com]
From: Conmy, Robyn
Sent: Thur 4/21/2016 12:41:44 PM
Subject: RE: dates?

Sounds good. Thanks!

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Holder, Edith
Sent: Wednesday, April 20, 2016 3:29 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>; Devi Sundaravadivelu <devis.255@gmail.com>
Subject: RE: dates?

Devi and I picked 10-11:30 AM. I have reserved a room.

Notes from our discussion:

Burn study: You are going to talk with Brian to ascertain what he needs from us.

I will talk again with Raghu to find out about purge and trap instrument for measuring BTEX of pads and raw oil

I don't understand how he jumped from amount measured on the pad to the total oil budget

Tensiometer: Devi will get a quote for an outside lab to make some measurements.

Explore purchase of instrument, decide what specs we need, how long it would take Pegasus to make a purchase?

Dispersants: acquisition thru OEM slow, have 600 + ml of Accel, Finasol, and Dispersit, but not of Corexit

SWA will schedule group meeting next week

Begin discussion on research plans for next year

Edie

From: Conmy, Robyn
Sent: Wednesday, April 20, 2016 2:55 PM
To: Holder, Edith <holder.edith@epa.gov>; Devi Sundaravadivelu <devis.255@gmail.com>
Subject: dates?

I'm available all next Wednesday for SWA discussion.

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

To: conmy.robyn@gmail.com[conmy.robyn@gmail.com]
From: Conmy, Robyn
Sent: Wed 11/30/2016 6:53:26 PM
Subject: manu
IOSC 2017_Conmy et al_plume simulations paper.docx

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

Oil plume simulations: Tracking oil droplet size distribution and fluorescence within high-pressure release jets

R.N. Conmy[1], **B. Robinson** [2], **T. King** [2], **M. Boufadel**[3], **S. Ryan**[2], **C. McIntyre**[2], **M. I. Abercrombie**[4], **K. Lee** [2,5]

[1]U.S. Environmental Protection Agency, Office of Research and Development, 26 MLK Drive West, Cincinnati, OH 45255, USA

[2] Bedford Institute of Oceanography, Fisheries and Oceans Canada, 1 Challenger Dr, Dartmouth, NS, B2Y 4A2, Canada

[3] New Jersey Institute of Technology, Department of Civil and Environmental Engineering, University Heights, Newark, NJ 07102, USA

[4]University of South Florida, College of Marine Science, 140 7th Ave South, St. Petersburg, FL 33701, USA

[5] Commonwealth Scientific and Industrial Research Organisation, Oceans and Atmosphere Flagship, PO Box 1130, Bentley WA 6102, Australia

ABSTRACT

Optical measurements have been used during oil spill response for more than three decades to determine oil presence in slicks and plumes. Oil surveillance approaches range from simple (human eyeball) to the sophisticated (sensors on AUVs, aircraft, satellites). *In situ* fluorometers and particle size analyzers were deployed during the Deepwater Horizon (DWH) Gulf of Mexico oil spill to track shallow and deep subsea plumes. Uncertainties regarding instrument specifications and capabilities during DWH necessitated performance testing of sensors exposed to simulated, dispersed oil plumes. Seventy two wave tank experiments were conducted at the Bedford Institute of Oceanography. Simulated were oil releases with varying parameters such as oil release rate, oil temperature (reservoir temp ~ 80 °C), water temperature (<8 °C and >15 °C), oil type, dispersant type (Corexit 9500 and Finasol OSR52) and dispersant

to oil ratio (DOR). Plumes of Alaskan North Slope Crude, South Louisiana Crude and IFO-120 oils were tracked using *in situ* fluorescence, droplet size distribution (DSD), total petroleum hydrocarbons (TPH), benzene-toluene-ethylbenzene-xylene (BTEX) and excitation-emission matrix spectroscopy. Results offer valuable information on the behavior and dispersibility of oils over a range of viscosity, DOR and environmental conditions. Findings have implications for fate and transport models, where DSD, chemistry and fluorescence are all impacted by release variables. This research was supported by the Bureau of Safety and Environmental Enforcement.

INTRODUCTION

The 2010 *Deepwater Horizon* (DWH) oil spill highlighted the pressing need for a better understanding of the interaction of chemical dispersants and crude oil at ocean depth. Early in the blowout release, partial emulsification of oil and heterogeneous slicks were observed as it rose to the surface from 1500-m depth (JAG report, 2010). A decision was made to inject dispersants directly at the release point as a possible means to increase efficiency of dispersion and to potentially reduce the amount of dispersant needed if applied at the air-sea interface (CRRC Report, 2010). Large quantities of chemical dispersant were applied via subsurface injection and traditional spraying from aircraft onto the surface oil slick (Oil Budget Calculator, 2010). At a workshop to discuss the use of subsurface chemical dispersants as an oil spill response option, recommendations to the RRT (Regional Response Teams) by spill response and research expert attendees were made on potential advantages of subsurface dispersant injection given the rate of continuous oil release and preliminary evidence of the dispersant efficacy from the DWH spill (CRRC, 2010). Potential advantages of this application included the fact that the fresh (unweathered) oil was considered well suited for dispersion, operators

were able to inject the dispersant directly into the oil stream thereby maximizing dispersant/oil contact, sufficient control of DOR (Dispersant-to-Oil Ratio) could be maintained, injection may minimize the need for surface dispersant application because of reduced oil surfacing and optimized subsurface application would likely promote formation of smaller, more stable droplets of oil, enhancing biodegradation (Lee et al., 2009).

As recommended by the interagency Unified Area Command (UAC) and on-site emergency spill response coordinators, a large-scale environmental monitoring program was implemented to detect and characterize dispersed oil based on field data and plume modeling outputs. This allowed for tracking the subsurface oil plume emanating from the blowout wellhead. Droplet Size Distribution (DSD) analysis using the LISST-100X Laser *in-situ* Scattering and Transmissometry System (Sequoia Scientific Inc. Seattle, WA) and fluorescence intensity from submersible fluorometers were used as an indication of Dispersion Effectiveness onboard the research vessels, where particle concentrations were monitored to evaluate oil dispersion (presence of small droplets ≤ 70 μm) based on previous studies for surface dispersant applications (Li et al., 2009b). Data analysis of the monitoring samples provided sound evidence of the presence of oil-bearing small particles both in surface waters and in the subsurface plume (Lee et al., 2011). Furthermore, a negative correlation between subsurface dispersant injection and low molecular weight compounds in surface waters was observed. In contrast, a strong positive correlation was observed in the subsurface. These results suggest that subsurface dispersant use may have promoted the formation of small oil droplets in the deep sea. This would likely enhance the natural weathering and dissolution of oil in the water column, thus suppressing the presence of oil organic compounds in surface waters.

Although subsurface *in situ* dispersants were used to counter a deepwater spill blowout, much uncertainty still exists in terms of the DE (Dispersion Effectiveness) with this type of application. For example, assumptions of the optimal DOR are based on empirical data mostly obtained from bench-scale experimental protocols that have been designed for testing at standard temperatures and pressures (STP), whereas conditions at a wellhead on the ocean floor or anywhere along a riser beneath the ocean surface could be significantly different. Hence, DOR for direct injection needs to be better understood. Although theoretical analyses and experiments suggest that jet breakup of the oil is insensitive to the absolute value of system hydrostatic pressure for incompressible liquid-liquid systems (Masutani and Adams 2000), the effects of several ambient environmental factors on subsurface dispersant effectiveness, including high release pressure, high oil temperature, low water temperature, and the presence of methane and suspended sediments in the oil plume and/or surrounding water column remain to be clarified. Improved understanding on the influence of these factors on DE and the interaction of crude oil and chemical dispersant under a range of turbulent regimes at depth is required for informed decision-making for future subsurface dispersant use.

For evaluating chemical dispersion effectiveness, standard laboratory tests are inherently limited in simulating real field operational performance due to space constraints that are critical for transport and dilution efficiency (NRC, 2005). To address the need to evaluate chemical dispersion effectiveness under more realistic oceanographic and environmental conditions, a meso-scale wave tank capable of generating breaking and regular non-breaking wave conditions is currently in operation at the Bedford Institute of Oceanography (BIO), Dartmouth, Nova Scotia. This tank facility has been used previously to characterize the tank hydrodynamics and

the efficacy of several oil dispersant formulations on dispersion of different oil types, including fresh and weathered crude oils and heavy fuel oils under breaking wave conditions (Figure 1) (Lee et al. 2009; Li et al. 2008; Wickley-Olsen et al. 2007). Mathematical modeling and experimental measurements have been used in the characterization of the fluid dynamics of the flume tank. In modeling, computer fluid dynamics software packages have been used to conduct numerical simulation of the fluid field and transport phenomena of the flume tank under both non-breaking and breaking wave conditions. Experimentally, wave gauges (WG-50) have been used to monitor wave profiling throughout the flume tank under various hydrodynamic conditions. Acoustic Doppler Velocimetry (ADV) has been employed to evaluate the *in situ* instantaneous three-dimensional velocity distribution, which is used to compute the velocity gradients and energy dissipation rates (ε) in the tank. Using this facility, previous experiments have assessed chemical dispersant effectiveness as a function of energy dissipation rate and particle size distribution (Li et al. 2009a) and demonstrated that the effectiveness of a dispersant is strongly dependent on wave conditions, dispersant type, and oil type (Lee et al. 2009). A strong correlation has been established between dispersion effectiveness and *in-situ* droplet size distribution within the hydrodynamic regime, particularly energy dissipation rate, under a variety of non-breaking wave and breaking wave conditions (Li et al. 2008; Li et al. 2009a). The flume tank has also been operated in flow-through mode to accommodate the effects of underwater currents on dispersion and dilution of oil (Li et al. 2009b; Li et al. 2010). Experiments have also shown the reliability of fluorescence measurements as a proxy for oil concentration within physically and chemically dispersed oil (Conmy et al., 2014). Experimental studies have also been conducted to better understand oil-mineral aggregate (OMA) formation and the influence of mineral fines on the physical and chemical dispersion of oil (Lee et al. 2009).

This paper summarizes results from a project that addresses the operational performance of subsurface injection dispersant use on high pressure releases of oil within the flume tank. Developed methods were focused on monitoring subsurface oil transport by outfitting a new high-flow flume tank at the Department of Fisheries and Oceans Canada (DFO) Bedford Institute of Oceanography (BIO) facility with a new underwater high flow rate oil injection system. In this way, the efficiency of chemical dispersion during high pressure releases within the tank can be quantitatively evaluated and compared to experiments with physical dispersion (without dispersant addition). This work has implications for field response options. To this end, the **objectives** of this work were to:

1) Refine existing equipment, technologies, and methodologies for subsurface dispersant application evaluation and monitoring by measuring dispersed oil concentration, fluorescence, and *in situ* oil droplet size distribution,
2) Evaluate effects of water temperature and dispersant type on dispersion efficacy and dispersed oil droplet size distribution of oil at high temperatures,
3) Evaluate dispersion effectiveness (DE) as a function of oil type and dispersant-to-oil ratio (DOR) for subsurface dispersant injection,
4) Assess the effect of dispersant application on the VOC concentration in air above the air-sea interface of the flume tank,
5) Integrate droplet size distribution into deepwater blowout transport/behavior models to enable prediction of the dispersed oil droplets under high flow subsurface release velocities.

During the DWH spill, small droplet ($d \leq 70\mu m$) concentrations were monitored to aid in

evaluating oil dispersion efficiency. The particle size and distribution data obtained from the field monitoring program during the DWH oil spill had a significant role in supporting emergency oil spill response operations, fate and transport modeling, and impact assessment. Findings from this study will have significant implications in further supporting emergency response operations, spill transport models and assessments for future deepwater spills.

METHODS

RESULTS / DISCUSSION

CONCLUSIONS

TABLES & FIGURES

REFERENCES

10-20 pages

To: Barron, Mace[Barron.Mace@epa.gov]
From: Conmy, Robyn
Sent: Wed 8/26/2015 7:15:43 PM
Subject: review of an abstract.
2016 gulf spill conference abstract_conmy.docx

Hi Mace, Can you review my GoMRI abstract for STICS? It's attached.

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

To: cpodella@abiocat.com[cpodella@abiocat.com]
From: Conmy, Robyn
Sent: Tue 4/19/2016 1:22:57 PM
Subject: Accell Clean DWD

Mr. Podella,

The US EPA is seeking to acquire a number of dispersant products listed on the NCP Subpart J Product Schedule for the purposes of both toxicity and efficacy testing. The product testing is intended to further inform currently proposed regulatory actions under Subpart J of the National Contingency Plan. The products will in addition be used to support our general research in the area of oil spill response.

Your product, Accell Clean DWD has been identified as one of interest for the EPA's Office of Research and Development (ORD) who will be conducting the dispersant studies. At this time we are seeking to procure 2 U.S. gallons of the product.

At this time, can you direct us to a distributor of the product or provide information on how to procure the product directly from Advanced BioCatalytics Corp.?

We appreciate your time and attention to this request.

Respectfully,

Robyn Conmy

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

To: Holder, Edith[holder.edith@epa.gov]
From: Conmy, Robyn
Sent: Tue 11/10/2015 5:39:00 PM
Subject: FW: LISST DSD from BFT dispersion
DispEffectiandDSD ANS and CLB 11-9-2015KingT.docx
TF dispersion effect 11-9- 2015KingT.docx

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Michel Boufadel [mailto:boufadel@gmail.com]
Sent: Tuesday, November 10, 2015 12:36 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: Re: LISST DSD from BFT dispersion

Please find attached. You are a co-author. I just forget to add the name prior to sending it. This work is intimately related to our collaboration on droplets.

Michel

On Tue, Nov 10, 2015 at 12:19 PM, Conmy, Robyn <Conmy.Robyn@epa.gov> wrote:

Hey Michel,

I spoke to Brian Robinson and Tom King yesterday and they mentioned that you have a draft manuscript of DSD from BFT experiments. Would you mind sharing the manuscript with me? I will not distribute. I am curious to see the results before we set up our hypersaline BFTs and LISST sampling.

Regarding the hypersaline work, BSEE will be setting up a Cooperative Agreement directly with you for your portion of the work. After discussions, it appears that this will be the faster and easier option. I separated the cost proposal and sent to the BSEE CO the amount that goes to NJIT ($123,028.50).

And as for the Wave Tank Oil Dispersion report on the FTP site. Because the EPA does not have technical expertise for much of what is in the proposal, it was decided to open up the reviews to a list of people that can provide feedback on various sections. For example, is there someone in your NJIT group that could review yours and Haibo's sections? Can you provide review of Haibo's? There are still EPA reviewers who will look over the whole document for approach, content, flow, policy - but I want to make sure that as many technical people look at it.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

--

Michel C. Boufadel, PhD, PE, BCEE, F. ASCE, D.WRE, P.Hydro.
Director, Center for Natural Resources Development and Protection (NRDP)

Professor, John A. Reif, Jr. Dept. Civil and Environmental Engineering

The New Jersey Institute of Technology

Room 435 Colton Hall

323 MLK Blvd, Newark, NJ 07102-1824

Ph: 973-596-6079

email: boufadel@gmail.com

http://civil.njit.edu/people/Boufadel.php

http://nrdp.njit.edu

To: Sundaravadivelu, Devi[sundaravadivelu.devi@epa.gov]; Zhuang, Mobing (zhuangmg)[zhuangmg@mail.uc.edu]; p.campo-moreno@cranfield.ac.uk[p.campo-moreno@cranfield.ac.uk]
From: Conmy, Robyn
Sent: Fri 11/4/2016 2:49:03 PM
Subject: RE: Dispersant Manuscript- STICS
ANS_Low-high_Appendix.docx
QA Review Form_Zuang_ANS_C9500.pdf
QA verification ANS biodeg C9500.pdf
Zhuang QARF approved_L15045JA3-0_02FEB2015.pdf
ANS_Low_High_manuscript.docx

Here's what we have in STICS from a submission in 2015. It's the same paper (but obviously revised). What I can't seem to remember is if it had been submitted and not accepted, was then revised and is the new submission. If so, then I need to attach the revised manuscript to the old entry and delete the new one I created.

R

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Sundaravadivelu, Devi
Sent: Friday, November 04, 2016 9:57 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>; Zhuang, Mobing (zhuangmg) <zhuangmg@mail.uc.edu>; p.campo-moreno@cranfield.ac.uk
Subject: RE: Dispersant Manuscript- STICS

Hi Robyn,

These are the conference and journal papers that have ever been submitted for this dispersant project. I am not aware of any duplicates. Maybe Pablo or Mobing can recollect if anything was submitted in 2015 with the same title.

Environmental science & technology, 2013

Biodegradability of Corexit 9500 and dispersed South Louisiana crude oil at 5 and 25 C

Interspill Conference, Mar 2015

Biodegradation of Polycyclic Aromatic Hydrocarbons in Alaska North Slope Crude Oil: Effect of Temperature and Dispersant Application

GoMRI Conference , Feb 2016

Biodegradability of Dispersed Heavy Fuel Oil at 5 and 25

Chemosphere, 2016

Effect of dispersants on the biodegradation of South Louisiana crude oil at 5 and 25° C

Current journal paper

Biodegradability of Different Initial Concentrations of Alaska North Slope Crude Oil Dispersed with Corexit C9500 (This was first submitted for technical review on 5/2/2016)

Thanks,

Devi

From: Conmy, Robyn
Sent: Friday, November 04, 2016 9:10 AM
To: Zhuang, Mobing (zhuangmg) <zhuangmg@mail.uc.edu>; Sundaravadivelu, Devi <sundaravadivelu.devi@epa.gov>; p.campo-moreno@cranfield.ac.uk
Subject: RE: Dispersant Manuscript- STICS

Hi All,

I have an odd question. Within our EPA clearance system (STICS) we have an older entry from 2015 that has this exact same title. Was this paper submitted previously and then retooled to be this new version? Or was this older clearance entry redone years ago to become the one published in in Chemosphere in 2016 (but that was for a different oil)?

Just getting clarification so the final review can get completed.

Thanks,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Zhuang, Mobing (zhuangmg) [mailto:zhuangmg@mail.uc.edu]
Sent: Thursday, November 03, 2016 4:56 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>; Sundaravadivelu, Devi <sundaravadivelu.devi@epa.gov>; p.campo-moreno@cranfield.ac.uk
Subject: Re: Dispersant Manuscript- STICS

Dear Dr. Conmy,

I have accepted all changes, and deleted comments from Dr. Carolyn Acheson and my reply to her comments. Please find the clean draft in the attachment, thank you!

Regards,

Mobing Zhuang

From: Conmy, Robyn <Conmy.Robyn@epa.gov>
Sent: Thursday, November 3, 2016 2:41:00 PM
To: Sundaravadivelu, Devi; p.campo-moreno@cranfield.ac.uk; Zhuang, Mobing (zhuangmg)
Subject: RE: Dispersant Manuscript- STICS

Mobing,

Can you send to me a clean draft of the manuscript as well?

Thanks,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Sundaravadivelu, Devi
Sent: Tuesday, October 18, 2016 12:00 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: p.campo-moreno@cranfield.ac.uk; Zhuang, Mobing (zhuangmg) <zhuangmg@mail.uc.edu>
Subject: Dispersant Manuscript- STICS

Hi Robyn,

Please find attached the dispersant manuscript that it ready for STICS entry. All comments have been addressed by the authors.

Thanks,

Devi

---------- Forwarded message ----------
From: **Campo Moreno, Pablo** <P.Campo-Moreno@cranfield.ac.uk>
Date: Wed, Oct 12, 2016 at 4:17 AM
Subject: Re: Skype meeting

To: Devi Sundaravadivelu <devis.255@gmail.com>

Hi Devi,

Attached please find Mobing's manuscript after being reviewed by Makram and me. Could you please check with Robin what should be done next? Also, do you have a current email address for Mobing to send her the files?
Regards,

Pablo

From: Devi Sundaravadivelu <devis.255@gmail.com>
Date: Tuesday, 11 October 2016 at 19:43
To: msuidan <msuidan@aub.edu.lb>, "Suidan, Makram (suidanmt)" <SUIDANMT@ucmail.uc.edu>, "SORIALGA@UCMAIL.UC.EDU" <SORIALGA@ucmail.uc.edu>, "Campo Moreno, Pablo" <P.Campo-Moreno@cranfield.ac.uk>, "Deshpande, Ruta (deshpars)" <deshpars@mail.uc.edu>, "BOL-Zhang, Yu (zhang4y5)" <zhang4y5@mail.uc.edu>
Subject: Skype meeting

Hi All,
Since we are supposed to meet bi-weekly, the next meeting is not scheduled until next Wednesday (Oct. 19th at 9AM).
But in case anybody has any updates and would like to meet tomorrow, please let us know here.

Thanks,
Devi

Land Remediation and Pollution Control Division

Quality Assurance Review

Date Received:	12/17/2014	QA Log Number:	L-15045-JA-3-0
Technical Lead Person:	Robyn Conmy	OMIS Task #:	
Division:	LRPCD	LCO Tracking #:	
Branch:	ESMB	Reviewer:	Jim Voit
Product Type:	Journal Article		
Title:	Biodegradability of Different Initial Concentrations of Alaska North Slope Crude Oil Dispersed with Corexit C9500		

QUALITY ASSURANCE MANAGER'S RECOMMENDATION

● Approved - No deficiencies were identified.

○ Approved with minor revisions -
Observations were identified that must be addressed, but no additional QA review is required. See attached comments for additional instructions regarding an appropriate response.

○ Not Approved, revisions required -
Findings were identified that require resolution. A response must be provided for additional QA review. See attached comments for additional instructions regarding an appropriate response.

○ QA requirements are not applicable

Definitions:

Finding - an identified deficiency that has a significant effect on the ability to attain the project/program objective

Observation - an identified deficience that does not have a significant effect on the ability to attain the project/program objective.

COMMENTS/ATTACHMENTS:

L15045-JA-3-0 review 19DEC2014.docx The research QA category was confirmed as "B" in consultation with NRMRL management . Therefore, the audit requirement finding in the review is not applicable. All other aspects are acceptable.

Jim Voit/CI/USEPA/US
Quality Assurance Manager

01/30/2015
Date

Distribution History:

12/19/2014 11:48 AM Jim Voit sent to: Robyn Conmy Joseph Schubauer-Berigan; copied to: Jim Voit
02/02/2015 11:36 AM Jim Voit sent to: Robyn Conmy Joseph Schubauer-Berigan; copied to: Jim Voit

To: McClellan, Kim[Mcclellan.Kim@epa.gov]
From: Conmy, Robyn
Sent: Fri 11/4/2016 12:56:54 PM
Subject: RE: STICS: Assigned as TIM: #ORD-019331: Biodegradability of Different Initial Concentrations of Alaska North Slope Crude Oil Dispersed with Corexit C9500
Biodegradability of Different Initial Concentrations of Alaska North Slo....docx

Here is the clean draft

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: McClellan, Kim
Sent: Thursday, November 03, 2016 4:31 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Schubauer-Berigan, Joseph <Schubauer-Berigan.Joseph@epa.gov>
Subject: RE: STICS: Assigned as TIM: #ORD-019331: Biodegradability of Different Initial Concentrations of Alaska North Slope Crude Oil Dispersed with Corexit C9500

Hi Robyn,

Another journal article/peer-reviewed record is currently in STICS and has been there since 2014-2015 (you entered the manuscript in STICS on 12/1 7/2014, and the final approver was Cynthia Sonich-Mullin, whom approved it on 6/15/2015).

The original peer-reviewed journal you listed for submitting your manuscript to was Environmental Science &Technology. If Environmental Science & Technology did not accept your manuscript, and you want to submit your manuscript to another peer-reviewed journal, and make some additional changes to this entry in STICS, you do not have to submit a new entry. You can just send me an email and tell me where you want the changes made and who the approvers should be on this entry.

So on the new entry in STICS, the abstract is different, the purpose statement is different, the peer-reviewed journal is different, the tracking and planning section is different. I can make changes to your record without you initiating a new record in STICS.

Did you want me to make the changes to the old record (ORD-011135) with the new information.

I will attach the new attachments to the old record, and resubmit this to Joseph Schubauer-Berigan, Alice Gilliland, Herb Fredrickson, and Cynthia Sonich-Mullin. Is this routing of individuals okay with you?

I noticed on this new entry, you did not get a QA Review from Jim Voit. I will need to have this before I can get your record re-reviewed by the approvers.

If I need any additional information or unable to move your attachments to the new record, I will contact you by email.

Thanks,

Kim

From: ORD_STICS@epa.gov [mailto:ORD_STICS@epa.gov]
Sent: Thursday, November 03, 2016 2:45 PM
To: McClellan, Kim <Mcclellan.Kim@epa.gov>
Subject: STICS: Assigned as TIM: #ORD-019331: Biodegradability of Different Initial

Concentrations of Alaska North Slope Crude Oil Dispersed with Corexit C9500

This e-mail is to inform you that you have been assigned as the TIM on the following clearance submission in STICS:

- Product type, subtype: Journal Article, Peer Reviewed
- Product title: Biodegradability of Different Initial Concentrations of Alaska North Slope Crude Oil Dispersed with Corexit C9500
- Author: Mobing Zhuang
- Initiator: Robyn Conmy
- ORD Tracking Number: Tracking # ORD-019331

The clearance submission is now available for review:

Please click here to access STICS.

Environmental Science & Technology

Article

pubs.acs.org/est

Submersible Optical Sensors Exposed to Chemically Dispersed Crude Oil: Wave Tank Simulations for Improved Oil Spill Monitoring

Robyn N. Conmy,[†,*] Paula G. Coble,[‡] James Farr,[□] A. Michelle Wood,[§,◆] Kenneth Lee,[//,¶] W. Scott Pegau,[⊥] Ian D. Walsh,[#] Corey R. Koch,[#] Mary I. Abercrombie,[‡] M. Scott Miles,[▽] Marlon R. Lewis,[○] Scott A. Ryan,[//] Brian J. Robinson,[//] Thomas L. King,[//] Christopher R. Kelble,[§] and Jordanna Lacoste[○]

[†]National Risk Management Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, 26 West Martin Luther King BoulevardCincinnati, Ohio 45268, United States

[‡]College of Marine Science, University of South Florida, St. Petersburg, Florida 33701, United States

[□]Office of Response and Restoration, National Oceanic and Atmospheric Administration, 1305 East-West Highway, Silver Spring, Maryland 20910, United States

[§]Atlantic Oceanographic & Meteorological Laboratory, National Oceanic and Atmospheric Administration, 4301 Rickenbacker Causeway, Miami, Florida 33149, United States

[//]Department Fisheries and Oceans Canada, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada

[⊥]Oil Spill Recovery Institute, 300 Breakwater Avenue, Cordova, Alaska 99574, United States

[#]WET Labs, Inc., PO Box 518, Philomath, Oregon 97370, United States

[▽]School of Coast and Environment, Louisiana State University, Baton Rouge, Louisiana 70803, United States

[○]Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada

* Supporting Information

In situ fluorometers were deployed during the Deepwater Horizon (DWH) Gulf of Mexico oil spill to track the subsea oil plume. Uncertainties regarding instrument specifications and capabilities necessitated performance testing of sensors exposed to simulated, dispersed oil plumes. Dynamic ranges of the Chelsea Technologies Group AQUAtracka, Turner Designs Cyclops, Satlantic SUNA and WET Labs, Inc. ECO, exposed to fresh and artificially weathered crude oil, were determined. Sensors were standardized against known oil volumes and total petroleum hydrocarbons and benzene-toluene-ethylbenzene-xylene measurements - both collected during spills, providing oil estimates during wave tank dilution experiments. All sensors estimated oil concentrations down to 300 ppb oil, refuting previous reports. Sensor performance results assist interpretation of DWH oil spill data and formulating future protocols.

INTRODUCTION

SMART (special monitoring of applied response technologies) protocols use real-time fluorescence monitoring in decision-making during oil spill dispersant operations[1] to determine efficacy of chemically enhanced dispersion and dispersed oil transport.[2] Chemical dispersants applied to oil slicks (in the presence of mixing energy) reduce the oil−water interfacial tension, forming small oil droplets (<100 um) that are less likely to recoalesce,[3] removing oil from the water surface and suspending neutrally buoyant oil droplets as a subsurface plume. This reduces risk to shorelines and air−water interface biota and also increases microbial biodegradation rates of oil.[4−6] Under SMART, in situ monitoring of surface waters (<2 m) and below (2 - 10 m) evaluates efficacy. Monitoring requires rapid, reliable, easy-to-operate in situ fluorometers. Validation with oil/water samples yields oil mapping with finer temporo-spatial resolution not achieved through coarse discrete measurements alone.

Fluorescence has been an oil spill monitoring tool for more than 30 years.[7,8] Measurements are inherently sensitive, conducted without laborious extraction techniques, time

Received: September 20, 2013
Revised: December 17, 2013
Accepted: December 30, 2013
Published: December 30, 2013

 © 2013 American Chemical Society dx.doi.org/10.1021/es404206y | Environ. Sci. Technol. 2014, 48, 1803−1810

ED_001324_00001412-00001

efficient, chemistry dependent and indicators of hydrocarbon concentration.[9,10] Low viscosity physically dispersed crude oils typically have two broad UV peaks with excitation (Ex) between 240 and 300 nm and emission (Em) centered at ~350 nm (low molecular weight polycyclic aromatic hydrocarbons, LMW PAH, <3 benzene rings) and ~450 nm (higher MW PAH, >3 benzene rings).[11,12] Fluorescence signatures discriminate oils[11−14] but are complicated by varying concentration, degree of chemical and physical dispersion, oil age and state (fresh, weathered, mousses),[11,14,15] and interference of fluorophores with spectro-chemical similarities. Such is the case with colored dissolved organic matter (CDOM) and oil aromatic fractions, which exhibit broad fluorescence peaks and complex spectra. However, crude oils have peak maxima at slightly shorter wavelengths relative to aquatic CDOM (humics, fulvics), facilitating discrimination.

Optical oil detection is a function of matching Ex/Em wavelengths with oil (center wavelength (CWL) and filter bandwidth), power of the light source, and detector sensitivity. Numerous submersible fluorometers are commercially available, each with unique configurations for detecting compounds[16] (and references therein), thus expanding their use in spill response efforts and SMART protocols.[17] Reviews of commercial off-the-shelf (COTS) sensors[17−19] highlight the need for robust calibrations, operational procedures and scalability testing.[20] Reviews have targeted easy-to-conduct, low-cost bench-scale testing. Often tests are conducted in the absence of dispersant, in small flasks with insufficient mixing energy to physically disperse oil into water,[21] at elevated oil concentrations irrelevant to field observations, with limited oil type and weathering states, and without calibration to standards or hydrocarbon concentrations. Thus there are confidence challenges in establishing saturation cutoffs and detection limits relevant for field applications. Effectiveness in quantitative monitoring also remains unclear.[19] Hence, caution must be exercised when scaling up experimental designs to real-world conditions and decision-making. Rather, a combination of laboratory, wave tank, and field studies has been recommended to establish the best degree of confidence for detecting chemically dispersed oil during environmental spills.[22]

SMART provides guidance on surface, not deepwater spill response like the 2010 Deepwater Horizon spill (DWH) where ~800 million liters of light Louisiana sweet crude oil was released from 1500 m ocean depth. Seven million liters[23] of chemical dispersant (Corexit© 9527, 9500A) were applied to reduce environmental impacts of oil, and aid worker safety. The response effort proved challenging partly due to the inability to visually inspect the subsea oil plume. The horizontal, vertical and temporal extent of, and heterogeneity of the plume equated to a "needle-in-the-haystack" situation with grab sampling too coarse to capture its magnitude and extent. Sample number limitations and oil stratification within bottles during the 1−2 h profiler transit time also contributed to challenges in quantifying oil.[24] Thus, responders and scientists were evermore reliant on submersible sensors in lieu of visual observations to provide critical tracking measurements. Initial plume tracking between 1000 and 1400 m was conducted using WET Labs, Inc. ECO fluorometers. Their accessibility and wavelength configuration- wide filter bandwidth and ExEm370/460 nm CWL (Supporting Information (SI) Table S2) to capture broad nature of the peaks- is appropriate for crude oil fluorescence detection. Chelsea Technologies Group AQUAtracka fluorometers were deployed to monitor the transport and fate of oil at large distances from the wellhead and after well capping (latter response, low concentrations).[24] This unit employs filters (ExEm239/360 nm CWL) to capture peak fluorescence from petroleum aromatic fractions with elevated sensitivity (higher signal-to-noise) due to a xenon lamp light source and a photomultiplier tube (PMT) detector; possibly aiding MC252 oil tracking which has a small fraction of highly fluorescing molecules (~20%).[24]

In the wake of the spill many questions remain regarding oil quantification and fate of the subsea oil plume, as well as sensor applicability to detect oil (dynamic range, detection limits, and wavelength configuration).[25] Effects of concentration, dispersant to oil ratio (DOR), dispersant effectiveness,[26] and fluorescence signatures[12] contribute to uncertainties in selecting sensors. Moreover, concerns persist on sensor reliability and applicability of laboratory calibrations using flasks, high oil concentrations and insufficient mixing energies to real-world conditions[19,25] To address uncertainties the performance of select COTS in situ fluorometers deployed during DWH (Chelsea Technologies Group, Turner Designs, WET Labs, Inc.) and a UV spectrophotometer (Satlantic) was evaluated in the Bedford Institute of Oceanography (BIO) wave tank. This work is timely, as findings will assist in the calibration and interpretation of DWH fluorescence measurements. It also coincides with the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) amendments pertaining to fluorometer protocols, reflecting the need for improved confidence in sensors for estimating spilled oil concentrations.

EXPERIMENTAL SECTION

Wave Tank Description. Experiments were conducted at the BIO, Center for Offshore Oil, Gas and Energy Research (COOGER; Dartmouth, Nova Scotia) wave tank, measuring 32 m long, 0.6 m wide, and 2 m high (1.5 m water depth; 28 800 L volume) (SI Figure S1). Water from the Bedford Basin of Halifax Harbor was pumped into the tank through a coarse (25 μm pore size) and fine (5 μm pore size) serial filtration system. Breaking and nonbreaking waves (computer-controlled flat-type wave maker) provided mixing energies to achieve dispersant effectiveness observed in the field. The tank was drained and cleaned with Big Orange detergent and seawater (tank walls, bottom, wave maker and absorbers) after each experiment to remove all oil and surfactants, and sensor optical windows were also cleaned.

Experimental Design. Experiments were conducted using MC252 crude oil (Discoverer Enterprise drillship recovered, May 31−June 7, 2011), and Corexit© 9500 chemical dispersant (1:25 DOR). Artificially weathered oil was generated by bubbling with nitrogen for 36 h (average time responders typically begin dispersant) resulting in mass reduction by 7%. Experimental duplicate and triplicate treatments were run with random design, reducing effects from confounding factors such as wind, rain, temperature, salinity, and seawater quality (SI Table S1). Prior to experiments, oil and dispersant mixtures were prepared in 1 L of seawater by orbital shaker agitation (20 min). Fluorometers (WET Labs, Inc. ECO, Chelsea Technologies Group AQUAtracka, Turner Designs Cyclops) and a UV spectrophotometer (Satlantic SUNA) were deployed at the same cross-sectional position within the tank 20 m from the wave generator (10 m downstream from oil release). Configuration specifications are shown in SI Table S2 and Figure S1. A laser in situ scattering and transmissometry particle size analyzer (Sequoia Scientific LISST-100X, C-Type,), 1 m

ED_001324_00001412-00002

downstream from sensors, collected continuous particle size distribution measurements. Sensors were mounted so that optical windows were positioned 65 cm deep in the tank and warmed up for 15 min prior to oil addition and collection of baseline. A stepwise addition experiment (EXP_{ADD}) of chemically dispersed fresh oil (300 ppb to 12 ppm, tank operated in steady-state mode) was conducted to establish response linearity and dynamic range of sensors to MC252 oil. Each addition of oil/dispersant was allowed to homogenize, with wave mixing, for 1 h prior to subsequent oil additions. Water samples were collected during the final 5 min of each step and sensors were cleaned in between steps using lens wipes and solvent. There was no indication of sensor fouling from oil as sensor response was checked pre- and postcleaning. Continuous optical data were collected during the 9 h experiment and manufacturer calibrations applied.

Dilution experiments (EXP_{DIL}) were conducted with 100 mL of fresh or weathered oil added, with or without dispersant (only chemically dispersed results reported here). Maximum concentration in the tank was less than 3 ppm, when fully dispersed. To simulate field conditions, ocean waves were generated with tank in flow-through mode (flow rate of 3.8 ± 0.2 L s^{-1}) allowing transport of dispersed oil away from the slick and dilution to extinction.[27,28] EXP_{DIL} were conducted for 90 min, where discrete oil concentration samples were collected at 15 time points via tubing through an inlet located next to the sensor deployment frame. Three-dimensional excitation emission matrix fluorescence spectroscopy (EEMS) analyses confirmed the position of fluorescence peaks and degree of chemical dispersion.

Submersible Sensor Data Processing. Sensor specific blanks (2 min average) prior to oil addition were subtracted. Manufacturer calibrations yielded data in recommended units (carbazole and perylene for AQUAtrackas, quinine sulfate dihydrate for ECO, uM nitrate for SUNA weighted index of UV absorption 210−370 nm, and none was provided for the Cyclops). Final 5-min signal averages for each concentration during the EXP_{ADD} was regressed (model 1, linear least-squares) to ppm oil, benzene-toluene-ethylbenzene-xylene (BTEX) or total petroleum hydrocarbons (TPH) values. Regressions were applied to EXP_{DIL} fluorescence and absorption data. Sensor performance and vicarious calibration were validated with laboratory chemistry results. LISST-11X dispersed oil droplet size distribution recorded 32 particle size intervals logarithmically spaced from 2.5−500 μm in diameter, with the upper size in each bin 1.18 times the lower. Distribution is expressed as the average volumetric concentration of droplets within each size range interval. Total particle concentration (TPC) represents the sum of all particle concentrations between 2.5 and 500 μm.[27]

Discrete Samples Analyses. Oil/water samples were collected at time points and analyzed for TPH, BTEX and PAH. TPH values were acquired using a GC-FID (gas chromatograph-flame ionization detector, Agilent 6890 and Supelco column). Extraction methods[29] were used and samples were concentrated under nitrogen to a final volume of 1.0 mL prior to analysis. Peak quantification, using the total area under the curve, yielded hydrocarbon concentrations in seawater. A spike and recovery study with blanks and duplicates verified extraction method efficiency. BTEX (volatile hydrocarbons) were measured via GC-MS (GC-mass spectrometry, Agilent 5973) using a modified version[29] of the standard EPA Method 8240 (purge and trap) for oil applications. A 5 mL sample was transferred into the purge chamber and purged with helium for 11 min. The volatiles were trapped and then desorbed at 225 °C for 2 min. The MS was used in selected ion monitoring mode (SIM) for six ions: 77, 78, 91, 92, 105, and 106 amu. BTEX calibration standards were prepared by diluting a certified standard mix (Supelco). Both standard and samples (blanks and duplicates) were purged, trapped and analyzed in the same manner.

EEM and absorbance spectra were collected with a Horiba Scientific AquaLog spectrofluorometer with a CCD detector. Oil/water samples were analyzed for fluorescence peaks within 10 min of collection. Samples within a 1 cm cuvette were scanned over Ex 220−550 nm and Em 213−610 nm (1 s integration), blank subtracted, inner-filter effect corrected, quinine sulfate dihydrate normalized, and Rayleigh scatter masked.[30,11]

RESULTS AND DISCUSSION

An EEM contour plot of chemically dispersed MC252 oil (4 ppm) collected during an EXP_{DIL} illustrates the UV fluorescent peak maxima centered on ExEm270/325 and ExEm260/450 nm

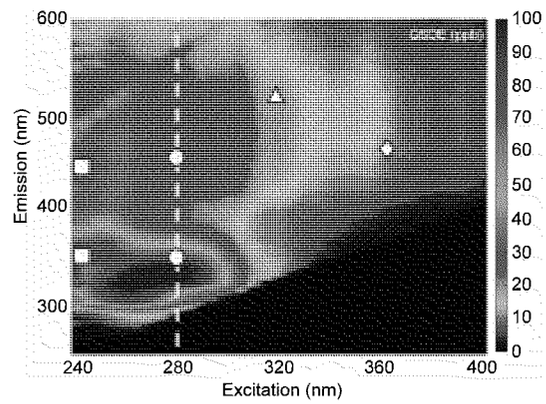


Figure 1. Fluorescence EEM of chemically dispersed fresh MC252 oil (4 ppm) concentration in a EXP_{DIL}. Center Wavelengths (CWL) of Chelsea Technologies Group AQUAtrackas $ExEm_{239/360}$ and $ExEm_{239/440}$ (□), Turner Designs Cyclops $ExEm_{320/510}$ (△), and WET Labs, Inc. ECO $ExEm_{370/460}$ (◊) along with the fluorescence intensity ratio (FIR = $ExEm_{280/340:280/445}$; ○) are overlaid for reference.

(Figure 1). Color map units are ppb QSDE (Quinine Sulfate Dihydrate Equivalents), scaled to peak maximum. CWL of the sensors tested are overlaid illustrating that all sensors were configured to detect some portion of the broad oil signal. Note that the symbols do not take into account wavelength bandwidths, which increases a sensor's ability to detect oil. For reference purposes, white circles on the dotted line represent the fluorescence intensity ratio (FIR) wavelengths used to indicate chemical dispersion of oil.[12]

Manufacturer calibrated sensor response at six cumulative oil concentrations during the EXP_{ADD} is shown in Figure 2 and SI Figure S2. The addition of oil and dispersant formed an oil plume with a sharp leading edge that passed in front of the sensors causing a rapid increase in signal followed by a decrease as the oil mixed/dispersed in the tank. Values at the end of each step (tank homogeneously mixed) are representative of sensor response for a specific oil concentration and established that all sensors detected chemically dispersed oil down to 300 ppb, a critical finding. This is below the laboratory detection limit for GC-FID TPH method, making sensor-estimated oil concentrations evermore valuable for tracking low concentration

ED_001324_00001412-00003

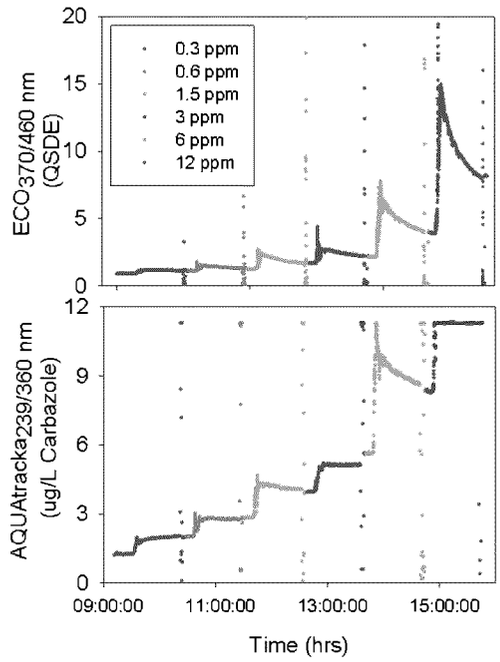


Figure 2. Fluorescence intensity of the ECO and $AQUA_{239/360\ nm}$ sensors exposed to chemically dispersed MC252 crude oil during EXP_{ADD} within the wave tank.

plumes. It also refutes previous notions during the DWH response that certain sensors may not detect oil <1 ppm. This was supported by earlier calibration tests using small flasks which may have been biased by underachieving proper mixing energies negatively impacting dispersant efficiency and solubility of fluorescence molecules into the water phase.[22] Strong linearity of sensor response at the lowest concentrations suggests possible lower detection limits less than 300 ppb (not tested with this experimental design). In any case, sensor response at 300 ppb improves confidence in the instruments deployed for DWH subsea plume monitoring, thereby demonstrating ability to detect low concentrations of oil.

Sensor dynamic range was established during the EXP_{ADD} by regressing fluorescence and absorption averages with oil concentration (Figure 3). Generally, submersible sensors were linearly correlated with TPH, BTEX and oil load, regardless of optical configuration. The ECO was operated in digital mode (no gain control) and the SUNA employed autoadjusting integration time, evidenced by lack of saturation over the entire concentration range. The Cyclops was operated with 100X gain for concentrations ≤6 ppm, but 10X for 12 ppm to avoid saturation. The AQUAtrackas employ BITE (Built in Test Circuitry) to extend calibration intervals. However saturation was exhibited by the $AQUAtracka_{239/360\ nm}$ sensor at 12 ppm oil. This is attributed to higher detector sensitivity, increased power of the light source (flash lamp opposed to LED) and/or wavelength filters closely aligned to the fluorescence maxima of crude oil. The longer wavelength emission AQUA $tracka_{239/440\ nm}$ sensor however did not saturate. Regression equations were employed to calibrate sensors during EXP_{DIL} (Figure 3). This aligns results with response effort measurements: sTPH (speciated TPH; water-insoluble heavy saturate fraction; aromatics > naphthalene; less laborious analytical screening) supplemented with BTEX (more laborious screening) values (Figure 3). All sensors exhibited squared correlation coefficients greater than 0.96. The 12 ppm value for the $AQUAtracka_{239/360\ nm}$ and SUNA (for BTEX) was excluded from the calculation due to saturation. The ECO and BTEX regression excluded values above 1.5 ppm, thereby increasing performance at low concentrations. TPH values for lowest concentration additions are absent because they were below the detection limit of the laboratory-based GC-FID TPH analysis. For all sensors, signal-to-noise ratios permitted detection at low concentrations. Findings demonstrate that all sensors would have been valuable tools during the DWH response given that sTPH and tVOA (total volatile organic analysis; water-soluble fraction; namely BTEX and naphthalene) in field samples were within the linear range of sensors tested in this study.[24] During the spill, sTPH from GC-MS ranged between <1 - 1000 ppb, with subsea plume (900−1300 m) maximum of 485 ppb, 1.2 km from the wellhead and detectable levels extending >10 km from source. In the plume, tVOA concentrations were higher than sTPH, with a maximum of 2112 ppb.

Sensor performance as a function of dispersant presence, oil state, and plume dilution was examined during EXP_{DIL}. TPH and BTEX-calibrated sensor responses for one-fresh and one-weathered, chemically dispersed oil EXP_{DIL} are shown in Figure 4 and SI Figure S3, respectively. Left panels show TPH-calibrated sensor and discrete TPH results (open circles), right panels show BTEX-calibrated sensor and discrete BTEX results (gray circles) over the course of the 90 min experiment. Due to a delayed shipping issue, the $AQUAtracka_{239/440\ nm}$ was operated only during one EXP_{DIL} and appears in the latter figure. EXP_{DIL} particle size distribution (PSD) and concentration of fresh oil droplets corresponding to Figure 4 are shown in Figure 5 (left). Histograms represent a composite of the entire duration of a flow-through experiment (90 min). Presence of particles observed in the <100 um size classes are indicative of chemical dispersion. All experiments using dispersant exhibited oil droplet size ranges consistent with adequate dispersant effectiveness (droplets <100 μm, Figure 5).[26]

For all experiments, MC252 oil state (fresh or weathered) did not appear to influence the sensor response. All sensors responded similarly to both (Figure 4 and SI Figure S3), verified with similar particle size distribution. Like sensor response as a function of oil state lends support for applying regression equations calculated with fresh oil to dilution experiments using weathered oil. Note that oil artificially weathered by 7% may represent oil exposed to physical processes (volatilization due to photo-oxidation, evaporation or dissolution) that occur during a spill of short duration, not necessarily of a prolonged spill with extensive weathering. However, the degradation of light crude oil and the addition of dispersant have been shown to increase the occurrence of longer wavelength fluorescence peaks.[11,15] Hence, sensors may be better capable of detecting extensively weathered oil where wavelength configuration better overlaps with longer fluorescence peaks (Figure 1). This is particularly true for lighter crudes such as MC252 oil that contain a higher proportion of simpler, lower molecular weight hydrocarbons that are more readily physically and biodegraded compared to heavy crudes.[31]

Tank hydrodynamics affected the movement and dispersion of oil within the tank, which varied during dilution experiments. Total particle concentration (TPC) as a function of time was influenced by tank mixing, where experiments exhibited either a gradual or rapid (Figure 5, left and right) decay of oil droplets. Three tests exhibited a gradual increase in signal as oil passed the sensors in the tank followed by gradual decay with dilution (Figure 4). For these cases, maximum values were 4−5 ppm

ED_001324_00001412-00004

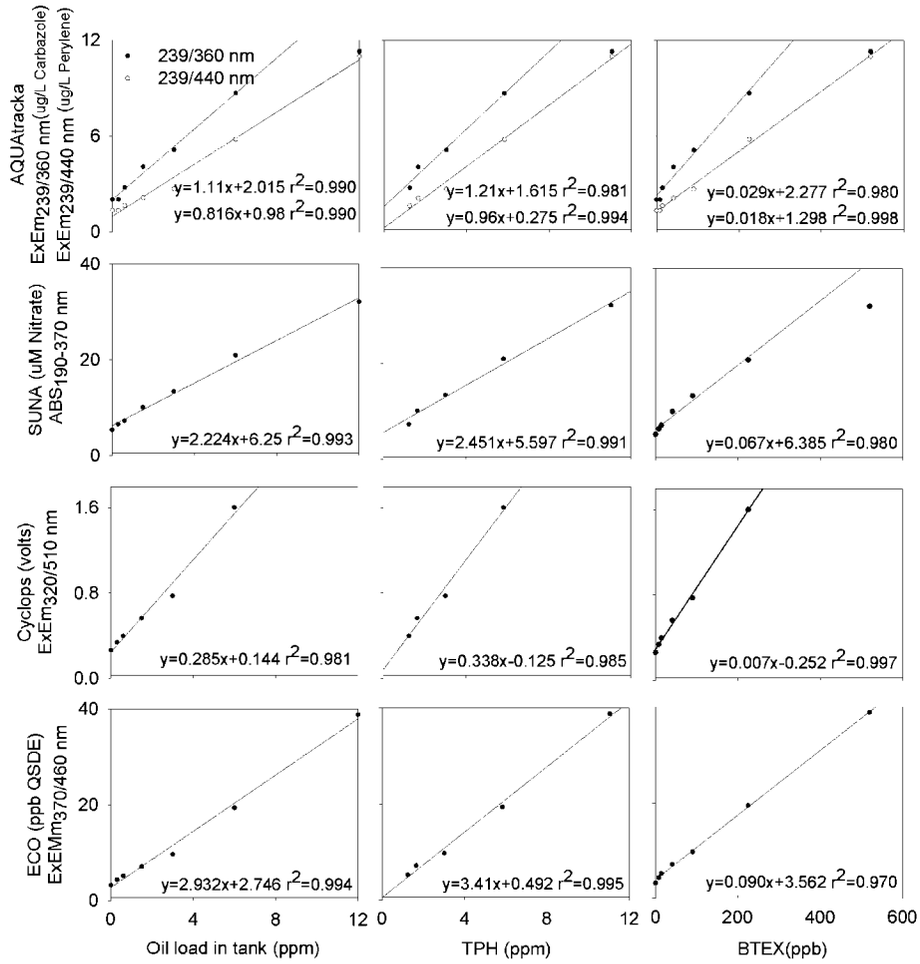


Figure 3. Fluorometric (submersible fluorometers) and spectrophotometric response (SUNA) as a function of oil concentration between 300 ppb and 12 ppm during EXP_{ADD}. Fewer TPH data points due to concentrations below the detection limit of TPH laboratory method.

TPH and 200−250 ppb BTEX, with subsequent plume dilution to 2−3 ppm TPH and 50 ppb BTEX. LISST-100X response (Figure 5, left) indicates like mixing and dispersing conditions in those experiments. Plume dynamics differed in two tests, where steep increases in oil concentration were followed by rapid decay (Figure 6), supported by TPC and droplet size distribution (Figure 5, right) and maximum TPH and BTEX values of 6 ppm and 250 ppb, followed by dilution to concentrations akin to all experiments. TPH and BTEX-calibrated sensor responses follow the chemistry and oil droplet results in all experiments. Agreement among these parameters is critical as they are used in concert during oil spills to track chemically dispersed oil plumes and confirm degree of dispersant effectiveness.

Sensor response during chemically dispersed EXP_{DIL} was validated with chemistry results to verify sensor performance. Percent differences ($\%_{dif}$) and mean absolute error (MAE) between estimated (from sensors) and actual (chemistry results) oil concentrations were calculated for each time point in experiments, providing performance metrics for estimates of TPH and BTEX for sensors exposed to oil. The MAE and mean, median and average $\%_{dif}$ for each sensor for all time points and for time points excluding 0−10 min are listed in Table 1. Time points <10 min were excluded from calculations due to heterogeneity and timing of the plume leading edge in the tank. The percentage of experiments where calibrated sensor responses were within ±25% and +30% of chemistry results are reported.

Based on $\%_{dif}$, the ECO and $AQUAtracka_{239/440\ nm}$ consistently better estimated BTEX, with 91% and 86% of time points within $30\%_{dif}$, respectively. The $AQUAtracka_{239/440\ nm}$ had the lowest median $\%_{dif}$ of <7.96. These values may be biased, however, as the $AQUAtracka_{239/440\ nm}$ was used in only one flow-through EXP_{DIL}. The ECO median $\%_{dif}$ was slightly higher (12%), but had the highest percentage of time points within $\pm30\%_{dif}$ (91%). Accuracy was also high with MAE of ±17.27 and 12.41 ppb for the ECO and $AQUAtracka_{239/440\ nm}$, respectively. The $AQUAtracka_{239/440\ nm}$, Cyclops and SUNA exhibited similar performance for BTEX where 71−79% values were within $\pm30\%_{dif}$. The $AQUAtracka_{239/360\ nm}$ also exhibited higher accuracy (MAE ± 12.43 ppb), but estimates were less consistent (71% of measurements within $30\%_{dif}$). BTEX consists of single ring aromatics, which are highly volatile and moderately soluble (relative to higher ring no. PAHs) in the shallow water of the wave tank, but any loss did not impact the $\%_{dif}$. Extrapolating this to real-world scenarios, surface spills typically have reduced BTEX or tVOA due to evaporation; however subsea oil plumes would likely retain higher dissolved concentrations.[6] Thus the ECO, $AQUAtracka_{239/360\ nm}$ and $AQUAtracka_{239/440\ nm}$ are each well suited for estimating BTEX in the deep sea, but they do vary with accuracy and precision. Results may differ for medium and

ED_001324_00001412-00005

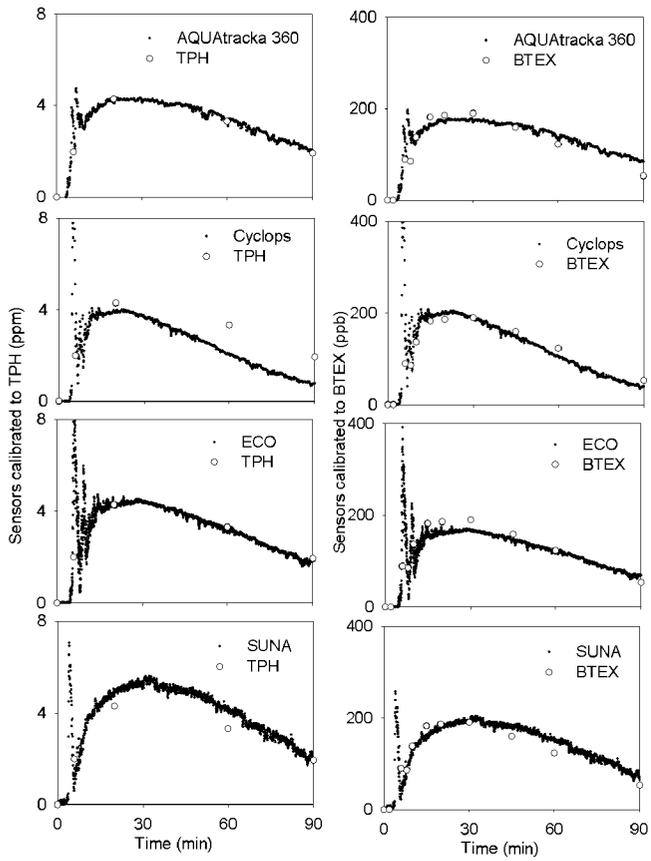


Figure 4. Response of TPH and BTEX-calibrated sensors exposed to chemically dispersed fresh crude oil. TPH and BTEX values from discrete samples are represented by open and gray circles, respectively. Wave tank was operated in flow-through mode (EXP_{DIL}) to observe fluorescence values during the dilution of an oil spill.

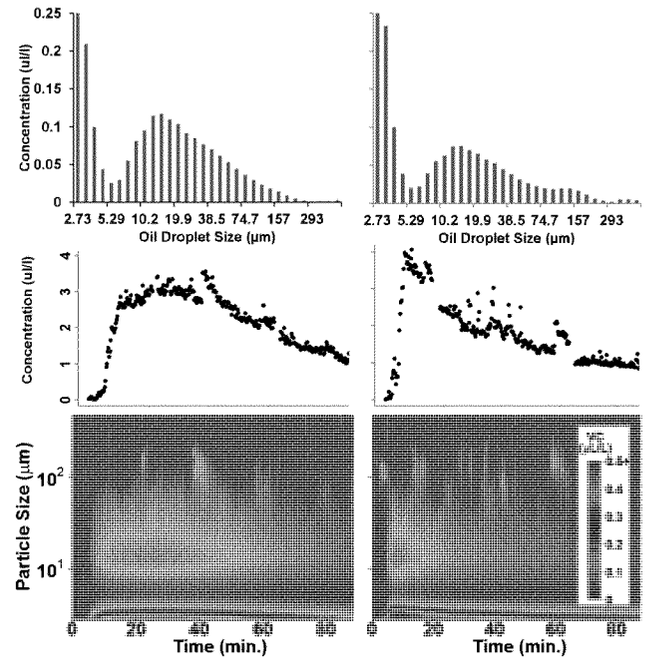


Figure 5. Particle size distribution and concentration within wave tank corresponding to experiments shown in Figures 4 and 6. Histogram plots are composites of entire flow-through experiment. Total particle concentration as a function of time (middle) is shown in scatter plot. Contour plots represent Particle size and volume concentration (VC) as a function of time for chemically dispersed fresh (left) and weathered (right) MC252 crude oil with gradual and rapid decay, respectively.

heavy oil, however, as BTEX concentrations would vary from the light crude oil tested here.

TPH was better estimated by the SUNA, with respect to both accuracy and precision, followed by the AQUAtracka$_{239/360\ nm}$, with 97% and 84% of time points within 30%$_{dif}$ of actual oil concentrations and MAE of +0.47 and 0.58 ppm, respectively. SUNA had the lowest median %$_{dif}$ of 11% for time points >10 min.

The remaining sensors exhibited median %$_{dif}$ of 18.72−23.87% with fewer values within 30%$_{dif}$ (60−71%) and higher MAE (0.77−0.81 ppm). Compared to BTEX, TPH laboratory analysis captures more compound types which are present at higher concentrations in oil. The SUNA's high performance in estimating TPH may result from absorption, not fluorescence, being measured over a wide wavelength range and oil being comprised of more chromophores than fluorophores. Tank

Table 1. Percent Differences (%$_{dif}$) between Sensors and Chemistry[S]

		TPH (ppm)					BTEX (ppb)				
		ECO 370/460 nm	AQUA 239/360 nm	AQUA 239/440 nm*	Cyclops 320/510 nm	SUNA ABS	ECO 370/460 nm	AQUA 239/360 nm	AQUA 239/440 nm*	Cyclops 320/510 nm	SUNA ABS
all time points	mean %$_{dif}$	31.69	30.24	41.98	40.32	27.92	26.67	30.93	23.14	31.99	30.45
	median %$_{dif}$	23.54	21.15	26.21	25.54	15.68	13.66	20.95	12.34	24.41	23.74
	min %$_{dif}$	0.27	1.96	2.16	1.73	0.76	1.63	3.19	5.29	0.01	0.65
	max %$_{dif}$	183.78	186.85	194.19	199.92	214.04	122.22	128.82	85.95	114.40	140.97
	[MAE]	± 0.91	± 0.67	± 0.78	± 0.90	± 0.68	± 23.53	± 13.46	± 12.41	± 32.22	± 27.68
time points >10 min	mean %$_{dif}$	25.52	19.37	19.22	30.94	13.88	15.54	23.88	14.17	21.11	22.85
	median %$_{dif}$	23.85	18.72	23.87	21.29	11.20	12.00	15.14	7.96	16.67	19.95
	min %$_{dif}$	0.27	1.96	2.16	1.73	0.76	1.63	3.19	5.29	0.01	0.65
	max %$_{dif}$	74.55	53.83	35.22	127.31	46.00	85.57	83.18	31.26	87.71	81.00
	[MAE]	± 0.78	± 0.58	± 0.81	± 0.77	± 0.47	± 17.27	± 12.43	± 12.41	± 29.13	± 24.50
% of >10 min time points	<25%$_{dif}$	56%	72%	57%	56%	90%	86%	68%	86%	64%	63%
	<30%$_{dif}$	69%	84%	71%	60%	97%	91%	71%	86%	79%	74%

[S]Asterisk represents sensor with only one experiment. MAE is mean absolute error.

ED_001324_00001412-00006

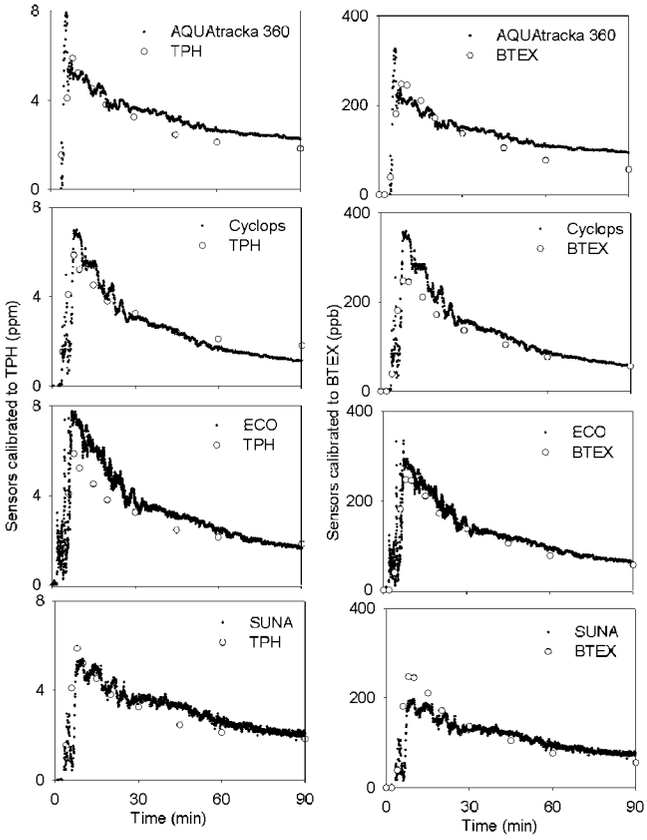


Figure 6. Response of TPH and BTEX-calibrated sensors exposed to chemically dispersed fresh crude oil for dilution experiment with steep increase in particles, followed by rapid decay. TPH and BTEX values from discrete samples are represented by open and gray circles, respectively. Wave tank was operated in flow-through mode (EXP_{DIL}) to observe fluorescence values during the dilution of an oil spill.

results indicate that of the sensors used in the DWH subsea plume, both the $AQUAtracka_{239/360\ nm}$ and ECO would have detected low TPH concentrations. The former may have exhibited better accuracy and precision. However, it could also be speculated that the $AQUAtracka_{239/360\ nm}$ with its narrower dynamic range would have saturated at high concentrations early in the spill and impeded plume tracking. Results are critical and provide evidence that ECO data from early in the spill (high concentrations) can be compared with AQUA data from later in the spill (low concentrations), and that sensors could provide oil concentrations with reasonable accuracy.

Wave tanks simulate natural variability observed in real spills. Thus, vicarious calibration is expected to be more accurate than a laboratory calibration, even though it may suffer from poorer precision. These findings demonstrate the sensitivity of COTS sensors exposed to chemically dispersed MC252 oil and increase confidence using in situ measurements during DWH to fill in the data gaps of coarse discrete samples. During an oil spill, sensor choice should be a function of oil concentration, calibration to TPH or BTEX and trade-offs between accuracy and precision. This work has large implications for plume tracking and understanding fate and transport of spilled oil in the environment.

ASSOCIATED CONTENT

* Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: 513-569-7090; fax: 513-569-7620; e-mail: conmy.robyn@epa.gov.

Present Addresses

◆University of Oregon, Institute of Ecology and Evolution and Institute of Environmental Science, Oregon, USA.

¶Commonwealth Science and Industrial Research Organization, Wealth from Oceans Flagship, Perth, Australia.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank all funding sources for the experiments at COOGER-BIO (Department of Fisheries and Oceans, Canada): IOOS support (Alliance for Coastal Technology), NOAA's supplemental DWH appropriation, salary support from home institutions, DFO Canada and internal travel support by Horiba JY, Oil Spill Recovery Institute, WET Labs, Inc. This document has been reviewed by the U.S. Environmental Protection Agency National Risk Management Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. This is a contribution from the Land Remediation and Pollution Control Division.

REFERENCES

(1) Report, S. P. Special Monitoring of Applied Response Technologies Protocol Report; National Oceanic and Atmospheric Administration, U.S. Coast Guard, U.S. Environmental Protection Agency, 2006; p 43.

(2) Henry, C. B., Roberts, P.O.; Overton, E.B. A primer on in situ fluorometry to monitor dispersed oil. In International Oil Spill Conference, 1999; pp 225-228.

(3) Li, M.; Garrett, C. The relationship between oil droplet size and upper ocean turbulence. Mar. Pollut. Bull. 1998, 36, 961−970.

(4) Lessard, R. R.; Demarco, G., The significance of oil spill dispersants. Spill Sci. Techno. Bull., 2000, 6, (59−68).

(5) Venosa, A. D.; Zhu, X. Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands. Spill Sci. Technol. Bull. 2003, 8, 163−178.

(6) Camilli, R.; Reddy, C. M.; Yoerger, D. R.; Mooy, B. A. S. V.; Jakuba, M. V.; Kinsey, J. C.; McIntyre, C. P.; Sylva, S. P.; Maloney, J. V. Tracking hydrocarbon plume transport and biodegradation at deepwater horizon. Science 2010, 330 (6001), 201−204.

(7) Green, D.; Humphrey, B.; Fowler, B. The use of flow-through fluorometery for tracking dispersed oil. In Internationl Oil Spill Conference, 1983; pp 473−478.

(8) Page, D. S.; Foster, J. C.; Hotham, J. R.; Pendergasst, E.; Herbert, S.; Gonzalez, L.; Gilfillan, E. S.; Hanson, S. A.; Gerber, R. P.; Vallas, D. Long-term fate of dispersed and undispersed crude oil in two nearshore test spills. In International Oil Spill Conference, 1983; pp 479−482.

(9) Keizer, P. D.; D. C., G., Jr. Detection of trace amounts of oil in sea water by fluorescence spectroscopy. J. Fish. Res. Board Can. 1973, 30, 1039−1046.

(10) Østgaard, K.; Jensen, A. Evaluation of direct fluorescence spectroscopy for monitoring aqueous petroleum solutions. Int. J. Environ. Anal. Chem. 1983, 14, 55−72.

ED_001324_00001412-00007

(11) Bugden, J. B. C.; Yeung, C. W.; Kepkay, P. E.; Lee, K. Application of ultraviolet fluorometry and excitation-emission matrix spectroscopy (EEMS) to fingerprint oil and chemically dispersed oil in seawater. Mar. Pollut. Bull. 2008, 56 (4), 677−685.
(12) Kepkay, P.; Young, W.; Bugden, J. B. C.; Li, Z.; Lee, K. Ultraviolet fluorescence spectroscopy (UVFS): A new means of determining the effectiveness of chemical dispersants on oil spills. In International Oil Spill Conference, Savannah, GA, 2008; pp 639−44.
(13) Li, J.; Fuller, S.; Cattle, J.; Way, C. P.; Hibbert, D. B. Matching fluorescence spectra of oil spills with spectra from suspect sources. Anal. Chim. Acta 2004, 514 (1), 51−56.
(14) Christensen, J. H.; Hansen, A. B.; Mortensen, J.; Andersen, O. Characterization and matching of oil samples using fluorescence spectroscopy and parallel factor analysis. Anal. Chem. 2005, 77, 2210−2217.
(15) Zhou, Z.; Liu, Z.; Guo, L. Chemical Evolution of Macondo crude oil during laboratory degradation as characterized by fluorescence EEMs and hydrocarbon composition. Mar. Pollut. Bull. 2012, 66, 164−175.
(16) Conmy, R. N.; Chen, R. F.; Castillo, C. E. D.; Downing, B. Calibrations and quality control of in situ sensors. In Fluorescence Applications in Aquatic Science; Cambridge University Press, 2013.
(17) Report, S. L. R. E. R. Updating the SMART dispersant monitoring protocol: Review of Commercial-Off-The-Shelf instruments; S.L. Ross Environmental Research, 2008; p 19.
(18) Lambert, P. A literature review of portable fluorescence-based oil-in-water monitors. J. Hazard. Mater. 2003, 102, 39−55.
(19) Fuller, C. B.; Bonner, J. S.; Page, C. A.; Arrambide, G.; M.C. Sterling, J.; Ojo, T. Field Instruments for Real Time In-Situ Crude Oil Concentration Measurements; Texas A&M University, 2003; p 10.
(20) Wilde, S. Procedures for monitoring dispersant operations. In 10th Environmental and Scientific Coordinators Workshop , Queenscliff, Australia, 2001, 2001; pp 163−177.
(21) Srinivasan, R.; Lu, Q.; Sorial, G. A.; Venosa, A. D.; Mullin, J. Dispersant effectiveness of heavy fuel oils using baffled flask test. Environ. Eng. Sci. 2007, 24 (9), 1307−1320.
(22) Report, N. R. C. Oil Spill Dispersants: Efficacy and Effects; Washington, DC, 2005; p 400.
(23) Report Oil Budget Calculator: Deepwater Horizon; Federal Interagency Solutions Group, 2010; p 217.
(24) Report, J. A. G. Deepwater Horizon Oil Spill: Review of Subsurface Dispersed Oil and Oxygen Levels Associated with the Deepwater Horizon MC252 Spill of National Significance; National Oceanic and Atmospheric Administration, 2012; p 93.
(25) Report, J. A. G. Review of Data to Examine Subsurface Oil in the Vicinity of DWH MC-252; National Oceanic and Atmospheric Administration, May 19−June 19, 2010; p f29.
(26) Lee, K.; Li, Z.; Boufadel, M. C.; Venosa, A. D.; Miles, M. S. Wave Tank Studies on Dispersant Effectiveness As a Function of Energy Dissipation Rate and Particle Size Distribution; Department of Fisheries and Oceans Canada, 2009; p 67.
(27) Li, Z.; Lee, K.; King, T.; Boufadel, M. C.; Vensosa, A. D. Evaluating crude oil chemical dispersion efficacy in a flow-through wave tank under regular non-breaking wave and breaking wave conditions. Mar. Pollut. Bull. 2009, 58, 735−744.
(28) Li, Z.; Lee, K.; King, T.; Boufadel, M. C.; Vensosa, A. D. Effects of temperature and wave conditions on chemical dispersion efficacy of heavy fuel oil in an experimental flow-through wave tank. Mar. Pollut. Bull. 2010, 60, 1550−59.
(29) Cole, M. G.; King, T. L.; Lee, K. Analytical technique for extracting hydrocarbons from water using sample container as extraction vessel in combination with a roller apparatus. Anal. Chem. 2007, 77, 2210−17.
(30) Coble, P. G. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Mar. Chem. 1996, 51, 325−346.
(31) Atlas, R. M.; Hazen, T. C. Oil biodegradation and bioremediation: A tale of the two worst spills in U.S. history. Environ. Sci. Technol. 2011, 45 (16), 67−09−15.

ED_001324_00001412-00008

Print Form

NRMRL PRODUCT QA/QC VERIFICATION REPORT

A. General Information		
Date Submitted:	6/22/15	
EPA Technical Lead Person:	Robyn Conmy	
Product Title:	Characterizing Light attenuation within Northwest Florida Estuaries: implications for RESTORE Act water quality monitoring	
Product Type: (check one)	Journal Article	☒
	Symposium/Conference Paper	☐
	Report for Publication	☐
	Extended Abstract	☐
	Computer Product/Software/Model/Database	☐
	Scientific Data	☐
	Other (identify):	
Date Review Requested By:	July 9, 2015	

B. QAPP Status		
Was a QAPP required for project activities relating to this product?	☒ Yes	☐ No
Was a QAPP prepared and approved by QA prior to collecting data used to make conclusions in the product? There are 2 QAPPs	☒ Yes	☐ No
If yes, provide the following: QA ID Number: 2011-01-001 2009-01-001 QA Approval Date: 2/3/11 + 8/28/09		
~~If no, explain:~~ Comment: This QAPP was approved through NHEERL Gulf Ecology Division		

C. Data Quality Summary	
Please mark the following as appropriate:	
☒	All project data quality indicator (DQI) requirements were met (i.e., all QA/QC checks specified in the QAPP were performed and acceptance criteria were met).
☐	Some project DQI requirements were not met. Data quality IS NOT adversely affected. (Consult Division QA Manager if needed.)
☐	Some project DQI requirements were not met. Data quality IS adversely affected. (Consult Division QA Manager if needed.)
☐	Not Applicable. Explain:
Discussion of Data Limitations: We had no limitations of data	

D. Signature	
The signature below verifies that the information on this form is accurate and that the product is ready to be subjected to QA review prior to submission for clearance. *(Sign date below and forward to QA Manager with the product documentation.)*	
R N Cy	6/22/15
Technical Lead Person (TLP)	**Date**

09/2008

To: Robinson, Brian[Brian.Robinson@dfo-mpo.gc.ca]; King, Thomas L[Tom.King@dfo-mpo.gc.ca]; mabercrombie@mail.usf.edu[mabercrombie@mail.usf.edu]
From: Conmy, Robyn
Sent: Tue 5/24/2016 3:31:17 PM
Subject: report edits for BSEE
E12PG00037_Draft Final Report__may2016.docx

Hello All,

I have compiled all the technical review edits and comments and have been chipping away at them for some time. It has been a much longer process than I anticipated, b/c each comment raised must be given a response (these are in a separate document that I can share with you if you like). But in the interest of time and to reduce confusion, I have created a semi-clean draft with comments that are relevant to you. Blue highlights represent ones that I need DFO to address. Green highlights represent ones that I need Mary to address.

Please find the attached document for your feedback. Not sure of everyone's schedules of late – but it would be appreciated if you can send this back to me no later than June 10th. If for some reason this is not possible, please let me know.

Thanks,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

To: Barron, Mace[Barron.Mace@epa.gov]
From: Conmy, Robyn
Sent: Wed 10/26/2016 3:14:24 PM
Subject: RE: need 200 mL minimum of dispersant for the lab...

Thanks! Ha!!!! BOSC is all set. I already 'stole' some figures from one of your toxicity presentations ….. but of course your name is listed on the slide as the tech expert for all things tox in SHC 3.62 ☺

I'm in Victoria BC this week and loving life. It will be hard to leave the ocean, mountains, weather, stable government. Sigh.

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Barron, Mace
Sent: Wednesday, October 26, 2016 11:09 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: RE: need 200 mL minimum of dispersant for the lab...

Thank you!

Good luck with BOSC review. Need anything from me?

Mace

From: Conmy, Robyn
Sent: Wednesday, October 26, 2016 9:59 AM
To: Barron, Mace <Barron.Mace@epa.gov>
Subject: FW: need 200 mL minimum of dispersant for the lab...

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Craig Watts [mailto:craig@hydrosphere.net]
Sent: Wednesday, October 26, 2016 9:50 AM
To: Grosser, Robert <Grosser.Robert@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Sundaravadivelu, Devi <sundaravadivelu.devi@epa.gov>; Peter Meyer <pmeyer@hydrosphere.net>
Subject: RE: need 200 mL minimum of dispersant for the lab...

Bob,

Thank you. We will keep an eye out for it.

Regards,

Craig

Hydrosphere research

Providing Environmental & Product Toxicity Testing since 1986

Craig Watts, Lab Director

Hydrosphere Research

11842 Research Circle

Alachua, FL 32615-6817

T (386) 462-7889

www.hydrosphere.net

CONFIDENTIALITY NOTICE: This message may contain privileged and confidential information from Hydrosphere Research. The information is intended to be for the use of the addressee only. Any disclosure, copying, distribution, or use of the contents by anyone but the addressee is prohibited.

From: Grosser, Robert [mailto:Grosser.Robert@epa.gov]
Sent: Wednesday, October 26, 2016 9:28 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>; Craig Watts <craig@hydrosphere.net>
Cc: Sundaravadivelu, Devi <sundaravadivelu.devi@epa.gov>
Subject: RE: need 200 mL minimum of dispersant for the lab...

Craig:

The Accell dispersant will be shipped today. Since you said you did not need it ASAP, it is coming by ground shipping. It should arrive in 3-4 business days.

Let me know if you need anything else.

Thanks.

Bob

Robert Grosser PhD

Environmental Microbiologist

Pegasus Technical Services, Inc.

On-Site Contractor for the US EPA

26 West Martin Luther King Drive

Mail Location 190

Cincinnati, OH 45268

(513) 569-7529 office

(513) 569-7620 fax

From: Conmy, Robyn
Sent: Tuesday, October 25, 2016 6:24 PM
To: Grosser, Robert <Grosser.Robert@epa.gov>; devi.sundaravadivelu@ptsied.com
Subject: FW: need 200 mL minimum of dispersant for the lab...

As per the email from last week, please arrange for 200 ml of accel to be sent to hydrosphere.
Thanks

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Barron, Mace
Sent: Monday, October 24, 2016 5:06 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>; Holder, Edith <holder.edith@epa.gov>
Subject: need 200 mL minimum of dispersant for the lab...

Just fyi on what they used of corexit and finasol.

Please send new dispersant asap!

Thank you,

Mace

From: Peter Meyer [mailto:pmeyer@hydrosphere.net]
Sent: Monday, October 24, 2016 3:41 PM
To: Barron, Mace <Barron.Mace@epa.gov>
Cc: Craig Watts <craig@hydrosphere.net>
Subject: RE: quick question...

Mace,

We ended up using about 160 mLs of each. So if I had to guess…

1) Corexit: We have about 1,200 mLs left over and…

2) Finasol: Probably about 700 mLs left over.

Neither container was full when we received them.

~Peter

From: Craig Watts
Sent: Saturday, October 22, 2016 7:22 PM
To: Barron, Mace <Barron.Mace@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>
Subject: RE: quick question...

Mace,

I am going to have to hand this one off to Peter.

Craig

From: Barron, Mace [mailto:Barron.Mace@epa.gov]
Sent: Friday, October 21, 2016 11:20 AM
To: Craig Watts <craig@hydrosphere.net>

Subject: quick question...

How much dispersant (e.g., corexit) did you guys use in the tox tests?

Do you recall how much we sent you? And how much you got left over?

We are getting ready to send a new dispersant to you and need to know the amount to send.

Thanks!

To: darvin.dwyer@total.com[darvin.dwyer@total.com]
From: Conmy, Robyn
Sent: Mon 4/18/2016 3:31:25 PM
Subject: FW: purchase of Finasol OSR52

Hello Mr. Dwyer,

I had sent this message below to Mr. Peter Egan, but received the out of office reply indicating that you are a POC in his absence. Please read the message below that I sent to Mr. Egan regarding the procurement of Finasol OSR52.

Respectfully,

Robyn Conmy

"Mr. Egan,

This follows up our telephone conversation of April 11.

As discussed, EPA is seeking to acquire a number of dispersant products listed on the NCP Subpart J Product Schedule for the purposes of both toxicity and efficacy testing. The product testing is intended to further inform currently proposed regulatory actions under Subpart J of the National Contingency Plan. The products will in addition be used to support our general research in the area of oil spill response.

Your product Finasol OSR52 has been identified as one of interest, as it is commonly stockpiled in the U.S. Not only could the product be encountered when presented with a response situation, but including this dispersant product in toxicity and efficacy testing studies will also allow for comparison and consistency with other existing and ongoing studies by EPA and other federal agencies. EPA's Office of Research and Development (ORD) will be conducting the dispersant studies.

At this time we are seeking 2 U.S. gallons of the product.

We appreciate your time and attention to this request.

Respectfully,

Robyn Conmy"

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Peter EGAN [mailto:peter.egan@total.com]
Sent: Monday, April 18, 2016 10:48 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: Out of Office: purchase of Finasol OSR52

Hi

I am sorry but I am now out of the office until Monday 11th April. I will have (intermittent) access to Email over this period.

If you need to speak to someone during my absence, Darvin Dwyer (darvin.dwyer@total.com) can be contacted on 713 969 4610.

Best regards,

Peter Egan

Cell: +1 713 297 1996

955.431.041201.SalinityPWS.pdf

Dispersants, Salinity and Prince William Sound

for

Prince William Sound Regional Citizens' Advisory Council (PWSRCAC)
Anchorage, Alaska

by

Merv Fingas
Environmental Technology Centre
Environment Canada

December, 2004

ED_001324_00001435-00001

Abstract

This paper is a summary of the effects of water salinity on chemical dispersion, especially those effects related to effectiveness. Surfactants are the active ingredient in dispersants. The surfactant is more lipophilic, or oil-loving, in freshwater and increases in hydrophilicity (or water-loving) as the salinity rises. The stability of the resulting droplets is also dependent on salinity. This is due to the increasing ionic strength of the water as salinity rises. As the salinity rises above a certain point, which depends on the particular type of surfactant, this increased force results in more surfactant molecules leaving the oil drop entirely. While the theoretical possibility of freshwater dispersants exist, the stability of dispersions in less saline waters would be less.

This report reviews several older dispersant tests. Data from these tests were separated from more recent data because older testing procedures and analytical methods are not as accurate as today's methods. Newer testing is reviewed as well. This testing is marked by the use of analysis by chromatography and very strict protocols in operating the dispersant tests themselves. These tests are marked by having standard deviations less that 10% and often less than 5%. The conclusions from both recent and older studies are the same.

The general surfactant literature was reviewed for the effects of salinity on surfactants and surfactant phenomena. There is a body of literature on the use of surfactants for secondary oil recovery. There are some commonalities among the many findings. Recovery efficiency falls off at both high and low salinities. The salinity at which surfactant efficiency peaks is very dependent on the structure of the specific surfactant. Several studies on the interaction of specific hydrocarbons and surfactants were reviewed. The consensus of these papers is that the solubility of the hydrocarbon increases with increasing salinity and is low at low salinities. The interfacial tension of water and oil changes with surfactant and salinity. The interfacial tension is higher at lower salinities. The optimal interfacial tension is generally achieved at salinities of between 25 to 35 o/oo. A number of physical systems involving surfactants and salinity changes are reported in the literature. Included in these is the finding that the stability of microemulsions is greater at salinities of 25 to 35o/oo. Some workers found that the stability of systems was very low in fresh water or in water with salinities of < 10 o/oo.

Some field studies of dispersant application were conducted in the freshwater environment. While effectiveness was not specifically measured, it was noted in both series of studies that effectiveness may have been low. In the one study, the investigators noted that the surfactants had poor effectiveness and stability in freshwater. In this particular case, the dispersion lasted only for about an hour and the dispersion was limited to a few centimetres. In another case, it was noted that there was oil around the edges of the dispersed pond within a short time of dispersant application.

Some effects studies were conducted under varying salinity conditions. In one study, naphthalene and a,b napthol sulphate uptake were studied under different salinity conditions. There were no significant differences at different salinities, although, naphthalene uptake was somewhat higher under low salinity conditions. Another study examined the induction of hsp60 protein in golden-brown algae. It was found that greater salinity reduced the effects of the simulated oil spills on the algae.

The varying salinities of the waters in Prince William Sound were described and summarized. There are areas around the Sound of low salinity. Dispersant applications in these areas would result in reduced dispersant effectiveness.

The following are the overall conclusions of this study.

a) The effectiveness of conventional and currently available dispersants is very low at 0 o/oo or sometimes they are even completely ineffective. This is consistent with physical studies described in the surfactant literature.

b) Dispersant effectiveness peaks at 20 to 40 o/oo. This may depend on the type of dispersant. Corexit 9500 appears to be less sensitive to salinity but still shows a peak at about 35 o/oo. Corexit 9527 is more sensitive to salinity and appears to peak at about 25 o/oo with some oils and with others at about 35 o/oo.

c) There is a relatively smooth gradient of effectiveness with salinity both as the salinity rises to a peak point of effectiveness and as it exceeds this value.

d) While there is some evidence for a temperature-salinity interaction, as noted in the data of Moles et al., 2002, there are not enough data to make solid conclusions.

e) Recent data are largely taken using Corexit 9527 and Corexit 9500. Since these have the same surfactant packages, there is a concern that the results may be more relevant to these formulations than to others.

f) Observations on two field trials in freshwater appear to indicate that the laboratory tests are correct in concluding very low freshwater effectiveness.

g) There were few studies on the biological effects of oil with varying salinity. There are not sufficient data to reach conclusions.

h) The findings in the dispersant literature summarized in this study are in agreement with the theoretical and basic surfactant literature.

i) The salinity of the waters in Prince William Sound is typically high in the centre of the Sound, but is sometimes low, especially near river outfalls, and in fjords with tidewater glaciers. The salinities in these areas, often less than 15 o/oo, will result in lower dispersant effectiveness.

Summary and Issues

Overall

The relationship between salinity and overall effectiveness of dispersants is reviewed in this document.

Specific Issues

The following is a summary of the specific issues and technical concerns related to salinity and dispersants.

1. It is very clear that salinity changes the effectiveness of conventional oil spill dispersants. In water with low salinity, these products have low effectiveness, even approaching zero.

2. There is very clear agreement on the effect of salinity and the relative changes this causes in dispersant effectiveness. There are a few exceptions, but these are all in the older literature and relate to studies with questionable analytical methods.

3. There are several outstanding questions: whether or not salinity changes any toxicity thresholds and whether there is an interaction between temperature and salinity.

Conclusions

The following are the overall conclusions of this study.

a) In waters with a salinity of 0 o/oo, conventional and currently available dispersant have a very low effectiveness or are sometimes even completely ineffective. This is consistent with physical studies in the surfactant literature.

b) Dispersant effectiveness peaks in waters with a salinity ranging from 20 to 40 o/oo. This may depend on the type of dispersant. Corexit 9500 appears to be less sensitive to salinity but still peaks at about 35 o/oo. Corexit 9527 is more sensitive to salinity and appears to peak at about 25 o/oo with some oils and at about 35 o/oo with others.

c) There is a relatively smooth gradient of effectiveness with salinity both as the salinity rises to a peak point of effectiveness and after it exceeds this value. The curves for this salinity effect appear to be Gaussian.

d) While there is some evidence for a temperature-salinity interaction as noted in the data of Moles et al., 2002, there is not enough data to make solid conclusions.

e) Recent data are almost exclusively measured using Corexit 9527 and Corexit 9500. Since these have the same surfactant packages, there is a concern that the results may be more relevant to these formulations than to all possible formulations.

f) Observations on two field trials in freshwater appear to indicate that the laboratory tests are correct in concluding very low dispersant effectiveness in freshwater.

g) There were few studies on the biological effects of varying salinity and given oil exposure. There are not sufficient data to reach conclusions.

h) The findings in the dispersant literature reviewed here are in agreement with those in the theoretical and basic surfactant literature. The effect of ionic strength and salinity on both hydrophilic-lipophilic balance and stability is the reason for the decreased effectiveness noted at low salinities and the same decrease at high salinities above a certain peak of about 20 to 40 o/oo.

i) The waters in Prince William Sound are sometimes low in salinity, often less than 15 o/oo, especially near river outfalls and in bays. This will result in lower dispersant effectiveness.

List of Acronyms

ANS - Alaska North Slope - Usually referring to the crude oil mixture at the end of the pipeline

ASMB - Alberta Sweet Mixed Blend - a type of crude oil

CTAC - Cetyltrimetyl ammonium bromide (a surfactant)

CTAB - Cetyltrimetyl ammonium chloride (a surfactant)

Corexit 9527 - Brand name of a dispersant from Exxon

Corexit 9500 - Brand name of a dispersant from Exxon

DLVO - Derjaguin Landau Verway Overbeek - A reference to a theory on surfactant stabilization, with each letter referring to the author of the original theory.

DO - Dispersed oil

EPA - US Environmental Protection Agency

EXDET - An Exxon laboratory test for dispersants

GC - Gas Chromatograph, a chemical analytical technique

HLB - Hydrophilic-lipophilic balance

IFP - The French Petroleum Institute - Usually used here as a description of their laboratory test

IFT - Interfacial tension

PAH - Polynuclear Aromatic Hydrocarbons

PWSRCAC - Prince William Sound Regional Citizens' Advisory Council

RSD - Relative standard deviation

WAF - Water-Accommodated Fraction - The sum total of oil in a water sample including physically dispersed and soluble oil

Acknowledgements

The author thanks Lisa Ka'aihue of the Regional Citizens' Advisory Council of Prince William Sound, who is the contract manager for this project. The author also thanks Jennifer Charles for her editorial work and Lloyd Gamble for collecting the references necessary for this report.

Table of Contents

Abstract iii
Summary and Issues v
List of Acronyms vi
Acknowledgements vii

1. Introduction 1
1.1 Objective 1
1.2 Scope 1
1.3 Organization 1

2. Review of Salinity and Dispersant Effectiveness 2

3. Older Laboratory Testing or Colorimetric Measures 3

4. Recent Laboratory Testing 5

5. Salinity Effects in Surfactant Literature 7

6. Field Studies and Effects Studies 9

7. Salinity in Prince William Sound 10

8. Summary 10

9. Conclusions 13

10. References 43

List of Tables

1 Summary of Testing of Salinity Effects on Dispersant Effectiveness 14
2 Data from Belk et al., 1989 15
3 Dispersant Effectiveness Data from Fingas et al., 1991 16
4 Results of Older Salinity Testing 16
5 Results of Salinity Testing from Brandvik and Daling, 1992 17
6 Dispersant Effectiveness Data from Fingas et al., 1994, 1995b 18
7 Data from Moet et al., 1995 18
8 Data from George-Ares et al., 2001 18
9 Dispersant Effectiveness Measured by Blondina et al., 1997 a, b 19
10 Dispersant Effectiveness Measured by Blondina et al., 1999 19
11 Data from Moles et al., 2001, 2002 20
12 Prediction of Temperature and Salinity Interrelationship (Data from Moles et al., 2001, 2002) 20
13 Salinity and Effectiveness (Data from Fingas et al., 2003) 21
14 Summary of Authors and Findings from Surfactant Literature 22
15 Quantitative Data on Salinity Changes from Salinity Literature 23

16 Results of Metabolite Uptake (from Wolfe et al., 1998) 24
17 Recent Salinity Measurement Results 24

List of Figures

1 Salinity and Dispersant Test Results from Belk et al., 1989 25
2 Dispersant E and F Data from Belk et al., 1989 26
3 Dispersant Effectiveness with Salinity from Fingas et al., 1991 26
4 Salinity and Dispersant Effectiveness Data from Byford et al., 1983 and Lehtinen and Vesala, 1984 27
5 Dispersant Effectiveness with Salinity from Brandvik and Daling, 1992 and Brandvik et al., 1995 28
6 Dispersant/Salinity Test on Two Inipol Dispersants - Inipol IFC - A Special Freshwater Dispersant (Data from Brandvik and Daling, 1992 and Brandvik et al., 1995) 29
7 Tests on Dispersant Effects with Varying Salinity (Data from Fingas et al., 1994) 29
8 Dispersant Effectiveness Data for Corexit 9527 and a Light Arabian Crude (Data from Moet et al., 1995) 30
9 Dispersant Effectiveness Data on Prudhoe Bay Crude (Data from Blondina et al., 1997 a,b) 30
10 Effectiveness of Corexit 9500 with Salinity (Data from Blondina et al., 1999) 31
11 Effectiveness of Corexit 9527 with Salinity (Data from Blondina et al., 1999) 31
12 Three-way Relationship of Effectiveness, Salinity, and Temperature for Corexit 9527 and Fresh ANS (Data from Moles et al., 2002) 32
13 Three-way Relationship of Effectiveness, Salinity, and Temperature for Corexit 9500 and Fresh ANS (Data from Moles et al., 2002) 32
14 Three-way Correlation of Corexit 9527 Effectiveness with Fresh ANS (Data from Moles et al., 2002) 33
15 Three-way Correlation of Corexit 9500 Effectiveness with Fresh ANS (Data from Moles et al., 2002) 33
16 Three-way Correlation of Corexit 9527 Effectiveness with Weathered ANS (Data from Moles et al., 2002) 34
17 Three-way Correlation of Corexit 9500 Effectiveness with Weathered ANS (Data from Moles et al., 2002) 34
18 Variation of Dispersant Effectiveness with Salinity (Data from Fingas et al., 2003) 35
19 Measured Zeta Potential Values of Chemically Dispersed Crude Oil Droplets at Selected pH Values (Data from Sterling et al., 2004) 36
20 Effect of Shear Rate on Collision Efficiency (Data from Sterling et al., 2004) 36
21 Variation in Uptake of Naphthalene by Algae (Data from Wolfe et al., 1998) 37
22 Variation in Uptake of a, b Naphthol Sulphate by Algae (Data from Wolfe et al., 1998) 37
23 An Overview of Prince William Sound Showing Detailed Sample Sites 38
24 A Temperature/Salinity Graph from Zaikof Bay Sampling Site 39
25 A Temperature/Salinity Graph from Eaglek Sampling Site 40
26 A Temperature/Salinity Graph from Whale Bay Sampling Site 41
27 A Temperature/Salinity Graph from Simpson Bay Sampling Site 42

1. Introduction

1.1 Objective

The objective of this paper is to address the issue of the effectiveness of dispersants in waters of various salinities, such as are found in Prince William Sound.

1.2 Scope

This paper covers the literature from the inception of the oil spill concern to August of 2004 and focuses primarily on issues related to variations in dispersant effectiveness caused by salinity.

1.3 Organization

The paper begins with a summary and outline of the issues. The overall effects of salinity on dispersant effectiveness are reviewed in Section 2. Laboratory testing or colorimetric measures used in older studies are reviewed in Section 3, while more recent laboratory testing is discussed in Section 4. Section 5 reviews the surfactant literature for studies on the effects of salinity. Field studies and effects studies are discussed in Section 6. The varying salinities found in the waters of Prince William Sound are discussed in Section 7. Section 8 consists of the summary and Section 9, the conclusions of this report.

The tables and figures referred to in the text have been placed at the end of the text, before Section 10, which lists the References.

2. Review of Salinity and Dispersant Effectiveness

Dispersant effectiveness is defined as the amount of oil that the dispersant puts into the water column versus that which remains on the surface. Effectiveness as used in this report will be constant throughout.

Surfactants have varying solubilities in water and varying actions toward oil and water. The parameter used to characterize surfactants is the hydrophilic-lipophilic balance (HLB) (Becher, 1977). HLB is determined using theoretical equations that relate the length of the water-soluble portion of the surfactant to the oil-soluble portion of the surfactant. A surfactant with an HLB between 1 and 8 promotes the formation of water-in-oil emulsions and one with an HLB between 12 and 20 promotes the formation of oil-in-water emulsions. A surfactant with an HLB between 8 and 12 may promote either type of emulsion, but generally promotes oil-in-water emulsions. Dispersants have an HLB in this range.

Dispersants are oil spill treating agents formulated to disperse oil into water in the form of fine droplets. Typically, the HLB of dispersants ranges from 9 to 11. Ionic surfactants can be rated using an expanded scale and have HLBs ranging from 25 to 40. Ionic surfactants are strong water-in-oil emulsifiers, very soluble in water and relatively insoluble in oil, which generally work from the water onto any oil present. Such products disappear rapidly in the water column and are not effective on oil.

Because they are readily available at a reasonable price, however, many ionic surfactants are proposed for use as dispersants. These agents are better classified as surface-washing agents. Some dispersants contain ionic surfactants in small proportions, yielding a total HLB closer to 15 than 10. No studies have been done on the specific effect of this on effectiveness or mode of action. A typical dispersant formulation consists of a pair of non-ionic surfactants in proportions to yield an average HLB of 10 and some proportion of ionic surfactants. Studies have been done on this mixture, one of which used statistical procedures in an attempt to determine the best mixture of the three ingredients.

It is well known in surface science that the hydrophilic portion of a surfactant is strongly affected by the salinity (Becher, 1977). This is a result of ionic strength. The greater the salinity, the greater the ionic strength and thus the greater the stability of the surfactant-stabilized droplet.

Dispersants have long been noted as being less effective in less saline waters. Martinelli and Lynch (1980) noted this as a factor to be considered. Despite this knowledge, several workers presumed that effectiveness was the same or similar in less saline water. Peabody (1982) proposed dispersant use in freshwater and noted that the concerns might be the different toxicity to aquatic species. McAuliffe (1989) developed scenarios for the use of dispersants in the nearshore environment to protect the ecosystem from surface oil damage. The assumption is that there is no reduction in effectiveness with decreasing salinity and that the effectiveness is 100%. Flaherty et al. (1989) reviewed the development of guidelines for using dispersants in fresh water and also did not note any concern about the decrease in effectiveness with decreasing salinity. This indicates that the effect of salinity was not necessarily well known among all oil spill workers, particularly those not involved in full-time research.

3. Older Laboratory Testing or Colorimetric Measures

While studies were conducted on the effectiveness of dispersants early in the history of oil spill dispersants (Martinelli and Lynch, 1980), proper quantitative methods did not appear until the mid-90s. Early methods using colorimetric analysis are in question (Fingas, 1995a). One older colorimetric method of measuring laboratory dispersant effectiveness uses a small aliquot of the dispersion test water, extracts the oil, usually with methylene chloride, and then measures the colour at a specific wavelength. This value is compared to a standard curve and effectiveness calculated. The standard curve was traditionally prepared by injecting the appropriate amount of oil directly into the methylene chloride and measuring colour density. It was found that the traditional approach of preparing standard curves was somewhat in error because the simple addition of water to the extraction process produced some colouration in the methylene chloride, despite drying the extract. This results in inflated effectiveness values.

Experiments comparing correct chromatographic methods and colorimetric methods showed that the latter could yield errors as much as 300 o/oo. More typical medium oils showed errors of only a few percent, but heavy oils again showed significant error because of the different wavelengths at which they are absorbed. Gas chromatography is the only accurate means to analyze for dispersant effectiveness. Many values from effectiveness tests conducted in the past using colorimetric methods are questionable. For this reason, the data relating salinity and effectiveness are separated into those obtained by colorimetric and chromatographic methodologies. All literature surveyed in Sections 3 and 4 is summarized in Table 1. It should be noted that much of the older literature reported data in graph form and not in tables. The numeric data was estimated from the graphs and subsequently re-plotted.

Belk et al. (1989) studied the effectiveness of dispersants in the Labofina laboratory apparatus. They tested several dispersants at a range of salinities and found that all dispersants were less effective at lower salinities. Belk et al. also tested freshwater dispersants and found that these showed similar behaviour and were less effective at lower salinity. These researchers also found that the ionic strength and the type of ion changed the effectiveness of freshwater dispersants. The data for several oils are given in Table 2 and re-plotted as shown in Figure 1. The dispersants tested are not named but are noted alphabetically.

Most of the dispersants show the same tendency, that is the effectiveness decreases to low values near zero salinity. The dispersant tendency also decreases after achieving a maximum of about 20 to 25 degrees salinity. One dispersant, designated 'c', did not behave in quite the same manner, but the authors note that this dispersant is neither typical nor common. Although these data will be compared to recent data, the tendency is the same throughout the data reported in this paper. Figure 2 shows the data for freshwater dispersants. This shows that even the freshwater dispersants have low effectiveness at low salinities and peak at a salinity of about 10 o/oo.

Fingas et al. (1991) studied the effectiveness of dispersant-oil combinations under a variety of salinity conditions using the swirling flask test and colorimetric measurement. They found that the effectiveness peaked at between 40 to 45 o/oo and then dropped rapidly to low values. These data are given in Table 3 and illustrated in Figure 3. This is one of the few data sets to include salinities beyond about 40 o/oo.

Clayton et al. (1992, 1993) reviewed the effect of salinity in the literature and summarized many of the old data. Fritz (1995) also reviews these data. The first numeric results were by Wells and Harris (1979) who report a sharp effect in going from fresh to saltwater. The results are summarized in Table 4 and illustrated in Figure 4 (also includes data from Byford et al., 1983 and Lehtinen and Vesala, 1984). These older data generally show the same tendencies as described by other workers later, although the data are much noisier as would be expected. Byford et al. employed the Labofina test and Lehtinen, the Mackay test.

Brandvik and Daling (1992) and Brandvik et al. (1995) studied the effectiveness of dispersants at low temperatures and salinity for application in the Arctic. They used the IFP test and found that most dispersant-oil combinations showed a large decrease in effectiveness at lower salinities. One dispersant intended for use in freshwater, Inipol IPF, showed the opposite tendency. These data are given in Table 5 and shown in Figures 5 and 6. Most dispersant-oil combinations showed very low effectiveness at low salinities.

Fingas et al. (1994, 1995b) studied the effectiveness of dispersant-oil combinations under a variety of salinity conditions and produced a salinity curve similar to that noted above. They found that the effectiveness peaked at between 30 to 40 o/oo and then dropped rapidly to low values. These data are given in Table 6 and illustrated in Figures 7. This is a data set that again shows a decrease in effectiveness after a peak at about 30 o/oo.

MacKay (1995) reported on tests conducted at Exxon using the Exdet tests. Effectiveness was reported as staying constant, although rising somewhat from the salinity values of 5 through to 35 o/oo. The effectiveness was also reported to be very low in freshwater. These tests were done for Prudhoe Bay crude and Corexit 9527.

Moet et al. (1995) tested the effectiveness of Corexit 9527 on light Arabian crude over a series of salinities, using the Labofina or Warren Springs test. The salinity effect was the same as found by Fingas et al., 1992, 1994, 1995b. The effectiveness peaked at about 33 o/oo and then again decreased. These results are shown in Table 7 and illustrated in Figure 8. Moet's results show the same tendency as results from Fingas et al. (1992, 1994, 1995b) in that the effectiveness peaks at about a salinity of 30 o/oo and then falls rapidly as salinity increases.

George-Ares et al. (2001) tested the effectiveness of various dispersants in river water, distilled water, and water with calcium chloride added. The Exdet apparatus was used to carry out the tests. The lowest effectiveness was found in the distilled water and the effectiveness was higher in the river water. Adding calcium chloride to the dispersant increased the effectiveness above that of the river water. These results, as shown in Table 8, are generally consistent with those noted previously in that a decrease in effectiveness is noted with a decrease in salinity.

Guyomarch et al. (2002) tested the effect of dispersants and variables such as salinity on the aggregate formation with clay. They found that the aggregate particle size increased with increasing salinity.

4. Recent Labor atory Testing

The effectiveness of dispersion at different temperatures and salinity has been measured using various tests. Blondina et al. (1997a, b) measured the effectiveness of dispersing Prudhoe Bay crude at 20°C and 20‰ as 23% for Corexit 9500 and 13% for Corexit 9527, using the EPA swirling flask method. The results also show that, for the same tests, the use of colorimetry as much as doubled the apparent effectiveness. It was concluded that the chromatographic method showed less bias to oils as dependant on their compositions. The results are shown in Table 9 and illustrated in Figure 9. These results are consistent with previously measured results noted in Section 3, namely that dispersant effectiveness is less with lower salinity.

Blondina et al. (1999) also measured the effectiveness of the dispersants Corexit 9527 and Corexit 9500 on several oils. The results are summarized in Table 10 and illustrated in Figure 10 for Corexit 9500 and Figure 11 for Corexit 9527. Blondina and coworkers concluded that the interaction between the salinity of the receiving water and the ability of surfactant-based dispersants to enhance petroleum accommodation into the water column can be both oil- and dispersant-specific. They found that Corexit 9500 was more effective than Corexit 9527 on most oils at most salinities, but the opposite was true in some cases. Corexit 9500 maintained its effectiveness over a wider range of salinities. Blondina et al (1999) concluded that decisions should be made on a specific situation based on the oil, the dispersant, and the salinity of the receiving water.

Moles et al. (2001, 2002) conducted a series of measurements on Alaska North Slope (ANS) oil at lower temperatures and lower salinity. For Corexit 9500 at a temperature of 10°C and 22 °/oo, the effectiveness was 8% for fresh ANS and 2% for weathered ANS. Under the same conditions, Corexit 9527 showed an effectiveness of 10% for the fresh ANS and 5% for the weathered ANS. The effectiveness of Corexit 9500 and Corexit 9527 was tested on Alaska North Slope crude oil at various salinities and temperatures representative of conditions found in Southern Alaskan waters. The oil was weathered to different degrees. Tests were conducted in a swirling flask at temperatures of 3, 10, and 22°C with salinities of 22 and 32 °/oo. Analysis was by GC. The authors concluded that, at the common temperatures found in the estuaries and marine waters of Alaska, the dispersants were largely ineffective. They also found that there was an interactive effect between temperature and salinity. A high effectiveness for 'emulsion', an uncharacterized mixture of oil and water, was attributed to 'osmotic shock' because of the difference in the salinity of the preparation (33 °/oo) and the test salinity. At the combinations of temperature and salinity such as might be typical for Alaska, dispersant effectiveness in the test was less than 10%. The results are summarized in Table 11. The data for the fresh ANS are plotted in Figure 12 for Corexit 9527 and in Figure 13 for Corexit 9500. Both figures generally show the decrease in effectiveness with decreasing salinity. There may be a relationship between temperature, salinity, and effectiveness as shown in these data.

The Moles data (Moles et al., 2001, 2002) were tested for ability to form a consistent relationship between temperature and salinity. This was carried out by correlating the three-dimension factors of effectiveness, salinity, and temperature. The results show that there is a high correlation for the fresh ANS and less so for the weathered and emulsified products. Table 12 shows the three-dimensional linear equation used to identify correlation. This shows that there is a good correlation between all factors, less so for the weathered and emulsified oils. A simple linear

equation is good for the fresh oil case but poor for the weathered and emulsified cases. Figures 14 to 17 show the linear correlations for the six oils. These figures show the three-way correlations as a plane surface or surface of best fit. Individual values are shown as circles and line extensions indicate whether these values are above or below the plane of best fit. It is important to note that such correlation as attempted here would be most valid if there were more data points.

Fingas et al. (2003) studied the effect of resurfacing of dispersed oil. As part of this study, a series of standard tests were conducted with Alberta Sweet Mixed Blend (ASMB) and Alaska North Slope (ANS) crude oils and the dispersants Corexit 9527 and Corexit 9500. Results are shown in Table 13 and illustrated in Figure 18. The same tendencies as Moles et al. (2001, 2002) found for ANS were found in this study, namely that the effectiveness of Corexit 9500 with ANS increases as salinity increases and that of Corexit 9527 generally does as well, but this is variable. The effectiveness of Corexit 9527 appears to peak at a salinity of 25 o/oo. It is not yet known why ANS has shown this tendency in these studies. The ASMB and most other crudes shows the tendency throughout this study that the effectiveness is Gaussian with the peak in this case coming at about 20 o/oo.

Sterling et al. (2004) studied the coalescence of dispersed oil droplets. Theoretical studies were conducted using DLVO theory and kinetic studies were conducted using a laboratory apparatus. Sterling et al. came to the following conclusions.

1. For salinity and pH values found in natural waters, the ζ- potential values of chemical dispersed crude oil were slightly negative. The ζ- potential is a measure of charge between particles and is relevant to dispersants in that a higher ζ- potential indicates a more stable particle and could imply a higher effectiveness. For a fixed pH value, ζ- potential values become marginally more negative with increased water salinity. This is shown in Figure 19. Using DLVO theory, no significant electrostatic energy barrier to droplet coalescence was present. This implies that oil dispersions (including those with dispersants) are unstable over time.

2. Within the tested experimental conditions, the collision efficiency parameter, α, (the probability of successful particle-particle collision) was significantly greater than 0. This result suggests that coalescence kinetics were important in estimating dispersant efficiency in laboratory-scale protocols and may be important in coastal spills. This is shown in Figure 20. The shear rate was the dominant parameter in estimating observed coalescence rates and dispersant efficiencies. This implies that the effectiveness is very dependent on shear rate, but that the resulting emulsions will also be unstable and in fact coalescence occurs faster under some energetic conditions.

3. Salinity had a limited influence on effectiveness values measured in this study. Sterling et al. suggest that salinity has a strong overall effect and thus, because salinity shows a lesser effect on coalescence, that salinity must have a greater effect on initial droplet formation.

5. Salinity Effects in Surfactant Literature

A literature search was conducted of the body of literature on surface chemistry. This search focussed on the effects of salinity on various aspects involving the use of surfactants. The papers are summarized in Table 14 and, where available, numeric results are given in Table 15. The values in Table 15 are given in terms of relative values compared to the value at 0 o/oo.

Davis (1994) reviews the basic surfactant chemistry and physics. He notes that interfacial tension of an oil-water system varies widely with salinity and is generally at a minimum at 15 ppt for many surfactant systems. Davis also provides information on the typical phase changes with changing salinity, including the effects of alkane change length and water fraction. Ysambertt et al. (1997) describes the phase behaviour of emulsions noting that salinity was an important factor in describing phases.

Several authors have studied oil recovery and the effectiveness of surfactants with respect to the salinity of the pore water. Sayyouh et al. (1993) studied the effect of salinity on a surfactant-oil-brine system and found that the stability of the system increased up to the salinity of about 3.8o/oo and then decreased as the salinity rose to 23o/oo . Fjelde and Austad (1994) studied the analysis of salt-tolerant and non-salt-tolerant surfactants, noting that ethoxylated anionic surfactants can tolerate high salinity water. These types of surfactants are not used in oil spill dispersants.

Several authors have tested oil reservoir recovery chemicals and found that increasing salinity increases performance of these surfactants (Austad et al., 1994; Fjelde et al., 1995; Austad and Strand, 1996. Wu et al. (2004) developed a new performance index for surfactants named the relative solubility index. This was used to examine a series of different surfactants at various salinities for oil recovery applications. Drummond and Israelachvili (2002) studied the fundamentals of surface forces and wettability, noting that recovery would be improved with increases in salinity. They also noted that recovery via natural surfactants is improved in high saline waters. Babadagli (2003) found that increasing salinity increased recovery with and without a surfactant. Zhang et al. (2004) studied natural surfactants and found that the recovery from reservoirs was increased with increasing salinity. Liu et al. (2004) studied the effectiveness of oil recovery and noted that increasing salinity increased the partition of surfactant into water. Al-Roomi et al. (2004) studied the use of surfactants to improve the flow properties of oil. Surfactants are used to emulsify oil into the water. Al-Roomi and co-workers found that the dispersion and viscosity reduction improved as surfactant content increased.

Several authors studied the effect of salinity on oil or specific hydrocarbons. Song and Islam (1994) studied the use of surfactant washing for cleaning petroleum from soil. They found that increasing salinity increased the removal or the effectiveness of the surfactant. Watt et al. (1998) studied the formation of a water-in-oil emulsion with a cationic surfactant and diesel oil. They found that the formation tendency increased with salinity up to about 30o/oo salinity and then decreased. Li and Chen (2002) studied the solubilization of PAHs into water with surfactants and found that increasing salinity decreased the cloud point, increased the apparent solubility, and reduced the hydrodynamic radius. Li and Kunieda (2003) studied the effect of having a cationic and an anionic surfactant to dissolve oil and found that salinity increased the effectiveness of the surfactants. Ghannam and Chaalal (2003) tested a vacuum oil recovery system which also used

the surfactant Triton X-100. They found that increasing salinity greatly increased recovery. Moosai and Dawe (2003) studied the theoretical aspects of the use of gas flotation for oily wastewater cleanup. They noted that the flotation improves with salinity and surfactant amount. Chen et al. (2004) studied the change in interfacial tension between hexane and an ionic surfactant. The interfacial tension decreased sharply with a small amount of salinity and rose again slightly and peaked at about 10 ppt. Mollet et al. (1996) also studied interfacial tension but with paraffin oil and sodium linoleate and an in-situ formed surfactant. They found that the optimal IFT occurred with salinities between 10 and 30 ppt.

Some authors studied the solubilization of specific compounds. Chooro et al. (1996) studied the miscellization and adsorption of a zwitterionic surfactant, n-dodecyl betaine, with salt concentrations. These researchers found that the adsorption of the surfactant onto silica gel depended little on temperature, but very much on the salt concentration. Yu et al. (2004) studied the extraction of a bacterial toxin from water using a cationic surfactant. They found that increasing salinity increased the partition of the water portion of the extract. Park and Bielefeldt (2003) studied the partitioning of pentachorophenol into a mineral oil with varying amounts of a nonionic surfactant and found that a higher ionic strength increased the partitioning.

The effects on physical systems of surfactants and varying salinity were investigated by various authors. Abuin et al. (1993) studied the formation of microemulsions with ionic surfactants and found that stability increased with salinity for most CTAC surfactants and decreased if CTAB was the majority surfactant. Hou and Papadopoulis (1996, 1997) studied three-way emulsion droplets and found that the stability of these droplets with surfactant increased significantly with increasing salinity. Kaczmarski et al. (1999) studied the influences of surfactant and salinity on the viscosity of a polymer thickness. The viscosity of the thickener decreased with increasing salinity.

Kjøniksen et al. (1999) studied the formation of gels of ethyl (hydroxyethyl) cellulose with the surfactant sodium dodecyl sulphate (SDS) and found that the intermolecular structure of the gel is increased with increasing salinity. Prosser and Franses (2003) used a thermodynamic/electrostatic model to study sodium dodecyl sulfate/sulfonate systems. They concluded that salinity increases stability by lowering interfacial tension. Sabatini et al. (2003) studied the effect of linker molecules with surfactants in solubilization. They found that solubilization with naphthalenic sulfonates was very saline-dependent and governed the solubility/surfactant concentration relationship.

6. Field Studies and Effects Studies

Some studies focussed on examining the effects of oil dispersed into the freshwater environment. Scott et al. (1979) studied the effects of a freshwater dispersal into a pond. The authors noted that there were significant similarities between the dispersed oil and the non-dispersed oil. The dispersed oil remained in a 3 to 5 cm milky layer only for about an hour after which it separated and formed a slick similar to that of the oil-only pond. This indicates a relatively poor dispersant effectiveness.

Brown and Goodman (1989) report on an extensive study of the effects of oil in the freshwater environment. Several toxicity and behavioural tests are described, but these are not compared to similar species in the saltwater environment. Brown et al. (1990) describe a major field trial of dispersants in the freshwater environment. Three cubic metres of Norman Wells crude oil were spilled on each of two fen lakes. The slick on one lake was treated with the dispersant Corexit 9500. The workers claimed that the dispersant was effective at removing oil from the surface of the one lake but also reported the appearance of thick clumps of oil near the edge of the same pond. The impact of the oil on the fen appeared to be lessened by the use of the dispersant, gauged primarily by the impact on floating vegetation. After one month, there was little impact on either fen. This study concluded that the best response to a spill in such a lake was no response at all.

Clayton et al. (1989) studied chemical and mechanical dispersion in an artificial stream bed. They concluded that the value of added dispersant was tempered by various factors including viscosity of the oil, degree of exposure of sediment surfaces to the oil, sediment substrate characteristics, and water flow characteristics.

Wolfe et al. (1998) studied the uptake of naphthalene by an algae. The oil was Prudhoe Bay crude and the dispersant was Corexit 9527. It was found that the dispersant significantly affected the uptake of naphthalene (by as much as 50%). Salinity, however, did not affect this uptake significantly. The results are shown in Table 16 and Figures 21 and 22. These data show that the uptake of naphthalene and a,b naphthol sulphate are relatively unaffected by salinity. Wolfe et al. (1999) also studied the heat shock protein in *Isochrysis galbana*, a golden-brown algae and primary producer in marine food chains. Wolfe et al. found that the organism efficiently induced the heat shock protein hsp60 in response to elevated temperatures and exposure to low concentrations of petroleum hydrocarbons after a model oil spill and dispersant use. Differences in salinity were found to influence the induction of hsp60 by elevated temperature, WAF and DO preparations, and naphthalene. Increased salinity appeared to decrease the sensitivity of *I. Galbana* to hsp60 induction after exposure to these agents. They suggest that the hsp60 induction may serve as an adaptive function in *I. Galbana* to deal with exposures to oil and dispersants. This also suggests that dispersants/oil may be more toxic at low salinities.

7. Salinity in Prince William Sound

The waters in Prince William Sound vary in salinity (Vaughn et al., 2001; Gay and Vaughn, 2001; Bang and Mooers, 2003). The data generally indicate that the salinity in the middle of the Sound is about 33 o/oo. As one enters areas influenced strongly by river outfalls, however, the salinity drops to that of freshwater. While the range of salinities in the Sound certainly raises concern, the salinity is generally higher than 20 o/oo in the centre of the sound where dispersants are likely to be used.

Figure 23 shows Prince William Sound and the detailed sampling stations. Figure 24 shows varying salinities at the Zaikof Bay station. This figure shows that surface salinities often are 28 o/oo and range only as high as 31.5. This is typical of most of the central portion of the Sound. Figure 25, on the other hand, shows the salinity profiles at Eaglek Bay. The water salinity in this area, which is typical of most of the fringe regions of Prince William Sound, ranges from 20 to 31.5 o/oo. A similar profile is seen in the Whale Bay data as shown in Figure 26. Table 17 shows recent salinity testing results. This data, from Tony Parkin, shows that the outfalls of creeks are very low in salinity. The smaller bays are also very low in salinity. Dispersant application should not be considered in or near such regions where salinity is below 20 o/oo. It must be noted however that salinity varies very much with season and location.

In Alaska, there are three distinct dispersant use zones (Annex I to the Alaska: RRT Dispersant Use Guidelines for Alaska). Zone 1 delineates an area where dispersant use has been preapproved. The On-Scene Coordinator (OSC) is not required to consult with any other agencies prior to the use of dispersants in this zone. In Zone 2, dispersant use can be approved by the OSC, but only with the concurrent approvals from the Environmental Protection Agency (EPA) and the State of Alaska. The use of dispersants is not recommended in a Zone 3 but can be used on a case-by-case basis. Prior to use in a Zone 3, the OSC is required to consult with the Regional Response Team and obtain approvals from the EPA and the State of Alaska.

Generally, Zone 1 in Prince William Sound runs through the center and entrance of the Sound, what is commonly referred to as the "tanker lane." Zone 1 also runs along the southern edge of the entrance to the Sound. The Gulf of Alaska is a Zone 2. Much of the Eastern and Western areas are a Zone 3. Port Valdez is unique in that it has seasonal designations, that change it from a Zone 1 to a Zone 2 depending upon the season, although the eastern edge of the Port is always a Zone 3.

Alaska is unique in the United States in that it has a preapproval zones so close to the nearshore. Many of the preapproval zones in the other states are beyond three nautical miles. This is of concern with respect to salinity, as many of these pre-approval zones are in low salinity zones and the effectiveness of dispersants would be very low in these areas.

8. Summary

Surfactants are the active ingredient in dispersants. Surfactants work to sustain oil droplets in the water by maintaining a portion of the molecule in the oil (lipophilic) and in the water (hydrophilic). The ratio of lipophilic to hydrophilic depends on the ionic strength of the water which relates directly to the salinity. The hydrophilic portion of the surfactant is more

soluble in water with a higher salinity. As salinity rises past a certain point, the surfactant becomes too soluble in the water and has a stronger tendency to partition to the water phase completely. Thus, in theory, the surfactant is more lipophilic in freshwater and increases in hydrophilicity as the salinity rises. The stability of the resulting droplets also depends on salinity due to the increasing ionic strength of the water as salinity rises. This increasing ionic strength results in greater molecular force. Again, as the salinity rises above a certain point, this point being dependent on the particular type of surfactant, this increased force results in more surfactant molecules leaving the oil drop entirely.

There is a theoretical scale of hydrophilic/lipophilic balance or HLB. This is calculated by the type of surfactant present. A surfactant with an HLB of 10 is a dispersant, that is the force of the molecule is equally balanced between hydrophilic and lipophilic tendencies. A surfactant of much greater than 10 is said to form oil-in-water emulsions (dispersions) and one of much lower than 10 can promote the formation of water-in-oil emulsions. The HLB of a surfactant changes with salinity. A low salinity lowers the HLB and vice versa. Thus, it is theoretically possible to design a dispersant with surfactants for lower salinity waters. While this possibility exists, it should be noted that the stability of dispersions is less in less saline waters. Furthermore, it should be noted that there are no recent measurements on freshwater dispersants, indicating that the industry has not pursued this avenue.

This report reviews several older dispersant tests beginning at 1979. These were separated from more recent data because both testing procedures and analytical methods used at that time are not as accurate as today's methods. Some older methods may, in fact, not yield data with sufficient accuracy to discriminate salinity effects. However, for the most part, this review shows that the older data, with a few exceptions, are entirely consistent in the generic conclusions of modern data, while not consistent in the actual numbers.

The following are the findings of several workers.

a) In waters with a salinity of 0 o/oo, most dispersants have a very low effectiveness or are sometimes even completely ineffective.

b) Dispersant effectiveness peaks in water with a salinity from 20 to 40 o/oo.

c) There is a relatively smooth gradient of effectiveness with salinity both as the salinity rises to a peak point of effectiveness and as it exceeds this value.

d) Some early works showed data anomalies, which may have resulted from measurement limitations and difficulties.

e) Studies published earlier than about 1989 are not highly accurate and contain the most anomalies.

Newer testing is also reviewed in this report. This testing is marked by the use of chromatography for analysis and the use of very strict protocols in operating the dispersant tests. These tests are marked by having standard deviations of less that 10% and often less than 5%. These are less than an order-of-magnitude of standard deviations in previous testing.

The followings are the conclusions of the authors of these newer studies.

a) In waters with a salinity of 0 o/oo, most dispersants have a very low effectiveness or are sometimes even completely ineffective.

b) Dispersant effectiveness peaks in water with a salinity from 20 to 40 o/oo. This may depend on the type of dispersant used. Corexit 9500 appears to be less sensitive to salinity, but

still peaks at about 35 o/oo. Corexit 9527 is more sensitive to salinity and appears to peak at about 25 o/oo with some oils and at about 35 o/oo with others.

c) There is a relatively smooth gradient of effectiveness with salinity both as the salinity rises to a peak point of effectiveness and as it exceeds this value. The curves for this salinity appear to be Gaussian as shown in Figures 3, 7, and 8.

d) While there is some evidence for a temperature-salinity interaction as noted in the data of Moles et al., 2002, there is not enough data to make solid conclusions.

e) Recent data are almost exclusively measured using Corexit 9527 and Corexit 9500 and, since these have the same surfactant packages, there is a concern that the results may be more relevant to these formulations than to all possible formulations.

f) The values found in recent tests are much lower than the older tests, however, the trends are the same.

The general surfactant literature was reviewed for salinity effects on surfactants and surfactant phenomena. There is a body of literature on the use of surfactants for secondary oil recovery. There are several commonalities among the many findings. Recovery efficiency falls off at both high and low salinities. The salinity at which surfactant efficiency peaks is very dependent on the structure of the specific surfactant.

Several studies on the interaction of specific hydrocarbons and surfactants were reviewed. The consensus of these papers is that the solubility of the hydrocarbon increases with increasing salinity and decreases at low salinities. The interfacial tension of water and oil changes with surfactant and salinity. The interfacial tension is higher at lower salinities. The optimal interfacial tension is generally achieved at salinities of between 25 to 35 o/oo.

A number of physical systems involving surfactants and salinity changes are reported in the literature. Included in these is the finding that the stability of microemulsions is greater at salinities of 25 to 35 o/oo. Some workers found that the stability of systems was very low in fresh water or waters of salinities of < 10 o/oo. Similar effects were found with gels, polymer thickeners, and linker-molecule solubilization.

Some field studies of dispersant application were conducted in the freshwater environment. While effectiveness was not specifically measured, it was noted in both series of studies that effectiveness may have been low. In the one study, the investigators noted that the surfactants had poor effectiveness and stability. In this particular case, the dispersion lasted only about an hour and the dispersion was limited to a few centimetres. In another case, it was noted that in the dispersed pond, there was oil around the edges within a short time of dispersant application. Effects were monitored in both cases, but could not be compared and were not compared to similar applications at sea.

Some effects studies were conducted under varying salinity conditions. In one study, naphthalene and a,b napthol sulphate uptake were studied under different salinity conditions. There were no significant differences for different salinities, although naphthalene uptake was somewhat higher under low salinity conditions. Another study examined the induction of hsp60 protein in golden-brown algae. It was found that greater salinity reduced the effects of the simulated oil spills to the algae.

The salinity of the water in different parts of Prince William Sound was summarized. There are

areas of low salinity where dispersant application would result in reduced dispersant effectiveness.

9. Conclusions

The following are the overall conclusions of this study.

a) In waters with a salinity of 0 o/oo, conventional and currently available dispersants have a very low effectiveness or are sometimes even completely ineffective. This is consistent with physical studies in the surfactant literature.

b) Dispersant effectiveness peaks in waters with a salinity ranging from 20 to 40 o/oo. This may depend on the type of dispersant. Corexit 9500 appears to be less sensitive to salinity, but still peaks at about 35 o/oo. Corexit 9527 is more sensitive to salinity and appears to peak at about 25 o/oo with some oils and at about 35 o/oo with others.

c) There is a relatively smooth gradient of effectiveness with salinity both as the salinity rises to a peak point of effectiveness and after it exceeds this value. The curves for this salinity effect appear to be Gaussian.

d) While there is some evidence for a temperature-salinity interaction as noted in the data of Moles et al., 2002, there is not enough data to make solid conclusions.

e) Recent data are almost exclusively measured using Corexit 9527 and Corexit 9500. Since these have the same surfactant packages, there is a concern that the results may be more relevant to these formulations than to all possible formulations.

f) Observations on two field trials in freshwater appear to indicate that the laboratory tests are correct in concluding very low dispersant effectiveness in freshwater.

g) There were few studies on the biological effects of varying salinity and given oil exposure. There are not sufficient data to reach conclusions.

h) The findings in the dispersant literature reviewed here are in agreement with those in the theoretical and basic surfactant literature. The effect of ionic strength and salinity on both hydrophilic-lipophilic balance and stability is the reason for the decreased effectiveness noted at low salinities and the same decrease at high salinities above a certain peak of about 20 to 40 o/oo.

i) The waters of Prince William Sound are sometimes low in salinity, often less than 15 o/oo, especially near river outfalls. This could result in lower dispersant effectiveness.

Table 1 Summary of Testing of Salinity Effects on Dispersant Effectiveness

Author	Year	Dispersant Type (s)	Specific Surfactant	Type of Test	Generic Results
Wells and Harris	1979	Commercial	Corexit 9527	Mackay	Effectiveness decreased very much from saltw ater to freshw ater.
Byford et al.	1983	Commercial	Several commercial dispersants	Labofina effectiveness	Saltw ater andf freshw ater dispersants decreased in effectiveness going dow n to zero salinity and decreased after 20 o/oo.
Lehtinen and Ves ala	1984	Commercial	Tw o unidentified dispersants	Mackay	Decreased in effectiveness going dow n to low salinity.
Belk et al.	1989	Several	Designated A to F, both saltw ater and freshw ater dispersants	Labofina effectiveness	Saltw ater and freshw ater dispersants decreased in effectiveness going dow n to zero salinity and decreased after 20 o/oo.
Fingas et al.	1991	Commercial	Corexit 9527 & Enersperse 700	Swirling Flask	Commercial dispersants decreased in effectiveness going dow n to zero salinity and decreased after about 35 o/oo.
Brandvik and Daling	1992	Several	Several commercial dispersants	IFP	Saltw ater and freshw ater dispersants decreased in effectiveness going dow n to 5 0/00 salinity and decreased after 20 o/oo. One Labofina product show ed opposite tendency.
Fingas et al.	1994, 95b	Commercial	Corexit 9527 & Enersperse 700	Swirling Flask	Commercial dispersants decreased in effectiveness going dow n to zero salinity and decreased after about 33 o/oo.
Brandvik et al.	1995	Several	Several commercial dispersants	IFP	Saltw ater and freshw ater dispersants decreased in effectiveness going dow n to 5 0/00 salinity and decreased after 20 o/oo. One Labofina product show ed opposite
Moet et al.	1995	Commercial	Corexit 9527	Labofina effectiveness	Commercial dispersant decreased in effectiveness going dow n to zero salinity and decreased after about 33 o/oo.
Mackay	1995	Commercial	Corexit 9527	Exdet	Effectiveness in distilled w ater w as very low but didn't change much after salinity increased past 5 o/oo.
Blondina et al.	1997 a,b	Commercial	Corexit 9527 and Corexit 9500	Swirling Flask	Effectiveness increased from salinity of 10 up to salinity of 30 for Corexit 9500 and up to 20 o/oo and then decreased otherw ise.
George-Ares et al.	2001	Commercial	Corexit 9500 Enersperse 1037 Basic Freshw ater hipol IPF	Exdet	Effectiveness in distilled w ater could be improved by the addition of calcium chloride.
Moles et al.	2001	Commercial	Corexit 9527	Swirling Flask	Effectiveness increased from salinity of 10 up to salinity of 30 for Corexit 9500 and up to 20 o/oo and then decreased otherw ise.
Guyomarch et al.	2002	Commercial	hipol IP 90	Special - 250 mL beaker	The aggregate size of particles increased w ith salinity.
Fingas et al.	2003	Commercial	Corexit 9527 and Corexit 9500	Swirling Flask	Effectiveness increased from salinity ot 10 up to salinity of 30 for Corexit 9500 and up to 20 o/oo and then decreased otherw ise.

Table 2 Data from Belk et al. (1989)

Actual Values Taken from Graph

Effectivness for Warren Spring Oil at 10°C

Salinity	Disp A	Disp B	Disp C	Disp D
0	5	5	25	5
5	10	8	23	10
10	23	20	22	23
15	60	55	25	25
20	75	65	35	35
25	77	67	47	47
30	80	70	55	58
35	82	72	60	60

Effectivness for Prudhoe Bay Oil at 10°C

Salinity	Disp A	Disp B	Disp C	Disp D
0	10	4	35	3
5	25	12	27	7
10	42	27	22	15
15	57	36	22	22
20	62	42	25	27
25	57	37	28	35
30	55	32	35	45
35	42	25	37	52

Effectivness for Warren Spring Oil at 20°C

Salinity	Disp A	Disp B	Disp C	Disp D
0	0	0	37	2
5	17	11	23	3
10	32	23	18	5
15	47	32	20	10
20	57	42	23	17
25	65	46	27	27
30	70	50	35	40
35	72	52	43	47

Effectivness for Prudhoe Bay Oil at 20°C

Salinity	Disp A	Disp B	Disp C	Disp D
0	12	6	32	3
5	26	17	23	6
10	42	28	22	14
15	55	40	24	20
20	60	45	25	25
25	58	42	30	32
30	50	40	34	37
35	40	32	37	41

Effectivness for Warren Spring Oil at 10°C Freshwater d

Salinity	Disp E	Disp F
0	62	25
5	77	47
10	85	62
15	84	67
20	82	72
25	77	74
30	75	72
35	72	70

Effectivness for Warren Spring Oil at 20°C Freshwater dispersants

Salinity	Disp E	Disp F
0	55	32
5	67	60
10	74	74
15	75	80
20	73	80
25	67	77
30	60	75
35	50	70

Effectivness for Prudhoe Bay Oil at 10°C Freshwater d

Salinity	Disp E	Disp F
0	25	24
5	45	44
10	58	56
15	64	62
20	62	60
25	58	56
30	56	54
35	42	44

Effectivness for Prudhoe Bay Oil at 20°C Freshwater dispersants

Salinity	Disp E	Disp F
0	24	23
5	44	43
10	56	54
15	68	65
20	70	68
25	68	66
30	66	64
35	56	54

Ionic Strength Effects for Dispersant E

Ionic strength mol l-1	Mg	Ca
0.05	40	64
0.1	50	68
0.2	65	70
0.3	80	72
0.4	85	72
0.5	80	68
0.6	72	56

Ionic Strength Effects for Dispersant F

Ionic strength mol l-1	Mg	Ca
0.05	72	72
0.1	76	66
0.2	78	60
0.3	79	56
0.4	76	56
0.5	72	58
0.6	60	62

Table 3 Dispersant Effectiveness Data from Fingas et al., 1991

Salinity o/oo	ASMB Corexit	ASMB Enersperse	Norman Wells Enersperse	Adgo Corexit
0	0	0	0	0
10	8	8	3	14
20	12	11	11	28
30	25	41	30	42
35	30	55	40	43
40	38	68	48	44
50	39	73	39	35
60	41	13	12	33
70	32	6	7	23
80	12	5	5	16
90	9	2	2	7

Table 4 Results of Older Salinity Testing

Oil and Temperature	Dispersant	Effectiveness % at a given salinity Salinity o/oo				
Data from Byford et al., 1983		**0**	**5**	**10**	**22**	**33**
Lago Medio Residue 0°C	Arochem D609	8		11	18	25
	Corexit 9527	10	12	18	30	35
North Slope Crude 0°C	Arochem D609	12	30	34	46	51
	Corexit 9527	12	35		48	52
North Slope Residue 0°C	Corexit 9550	22	61	62	52	50
	Dispolene 34S	15	60	62	58	62
	Finasol OSR5	15	17	20	19	21
	Corexit 9527	25	29	25	26	27
	Experimental	78	70	68	70	79
Data from Lehtinen and Vesala, 1984		**3**	**7**	**12**		
Fresh Russian Crude 15°C	A	60	62	65		
	B	60	55	62		
	C	45	40	47		
Fresh Russian Crude 4°C	A	20	21	30		
	B	10	8	9		
	C	10	12	9		

Table 5 Results of Salinity Testing from Brandvik and Daling, 1992

Oil and Temperature	Dispersant	Effectiveness at Given Salinity % 33 o/oo	5 o/oo
Oseberg 0°C	Dasic NS	80	5
	IKU-9	78	10
	Inipol IPC	76	48
	E-700	76	55
	Dasic LTS	62	12
	E-1075	59	55
	Dasic Freshwater	38	45
	Corexit 9527	36	5
	Disp. 365	30	7
	Corexit 9550	29	20
	OSR 52	26	28
	Inipol IPF	24	50
	Disp. 385	20	24
	OSR 5	15	4
Oseberg 0°C	Enersperse 700	70	69
Weathered	Inipol IPC	68	40
	OSR 52	20	65
	Dasic Freshwater	25	23
	Inipol IPF	18	70
Oseberg 0°C	Enersperse 700	82	55
Weathered 2	Inipol IPC	86	30
	OSR 52	80	30
	Dasic Freshwater	65	58
	Inipol IPF	25	70

Oil and Temperature	Dispersant	33 o/oo	5 o/oo
IFO 0°C	Enersperse 700	67	48
Weathered	Inipol IPC	42	37
	OSR 52	58	54
	Dasic Freshwater	37	38
	Inipol IPF	25	45
Veslefrikk 0°C	Enersperse 700	58	54
w/o	Inipol IPC	80	40
	OSR 52	10	15
	Dasic Freshwater	30	8
	Inipol IPF	8	60
Oseberg 0°C	Enersperse 700	68	58
w/o	Inipol IPC	68	44
	OSR 52	25	35
	Dasic Freshwater	30	35
	Inipol IPF	20	50
IFO 0°C	Enersperse 700	30	4
w/o	Inipol IPC	50	4
	OSR 52	30	4
	Dasic Freshwater	24	10
	Inipol IPF	36	28

Dispersant	Salinity	Effectiveness
IPF Inipol	0.5	38
	1.25	58
	2	78
	2.75	80
	3.5	70
IPC Inipol	0.5	85
	1.25	80
	2	25
	2.75	21
	3.5	18

Table 6 Dispersant Effectiveness Data from Fingas et al., 1994, 1995b

Salinity o/oo	ASMB Corexit 9527	ASMB Enersperse	Norman Wells Enersperse 700	Adgo Corexit
10	8	9	4	14
20	12	11	11	29
30	25	41	31	42
33	32	57	35	39
40	38	68	48	44
50	39	73	39	36
60	41	13	12	35
70	32	6	7	24
80	12	5	5	16
90	10	3	2	6

Table 7 Data from Moet et al., 1995

Salinity	Effectiveness (%)
0	3
20	6
30	16
33	14
40	7
50	3

Table 8	Data from George-Ares et al., 2001					
		Effectiveness in Percent				
Crude Oil	Water	Corexit 9500	Corexit 9500 + Salt	Dasic Freshwater	Enersperse 1037	Inipol IPF
Hydra	Rio de la Plata	49	56,70	71	68	58
	Deionized	32	58	70	70,64	65
Escalante	Rio de la Plata	2	17	27	19	
	Deionized	<5	11 to 22	27	16	7
Canadon Deco	Rio de la Plata	21	25	34	36	
	Deionized	10	42,40	56	37	17

Table 9 **Dispersant Effectiveness Measured by Blondina et al., 1997a, b**

Oil Type	Effectiveness in % at given salinity Salinity (o/oo)					
Prudhoe Bay	**35**	**30**	**25**	**20**	**15**	**10**
Corexit 9500	23	21	22	23	15	12
Corexit 9527	34	29	13	13	9	5

Table 10 **Dispersant Effectiveness Measured by Blondina et al., 1999**

Corexit 9500 **Oil Type**	**Effectiveness in % at given salinity** **Salinity (^{0}/oo)**							
	35	**30**	**25**	**20**	**15**	**10**	**5**	**0**
Arabian Light	38	36	44		31		7	
Arabian Medium	20	24	26	24	11	10		
Forcados	21	31	35	37		26		6
Kuwait	37	38	31		15		5	
Maya	16	11	12		6		3	
Oman	22	20	15		10		3	
Prudhoe Bay	23	21	22	23	15	12		

Corexit 9527 **Oil Type**	**Effectiveness in % at given salinity** **Salinity (^{0}/oo)**						
	35	**30**	**25**	**20**	**15**	**10**	**0**
Arabian Light	23	13	10		6		
Arabian Medium	10	5	7	6	6	3	
Forcados	54	63	55	48		17	6
Kuwait	21	13	7				
Maya	5	4					
Oman	7	5	6				
Prudhoe Bay	34	29	13	13	9	5	

Table 11 Data from Moles et al., 2001, 2002

Oil Type	Temperature	Corexit 9527		Corexit 9500	
		Effectiveness in percent			
		Salinity		Salinity	
	°C	22 ^{0}/oo	32 ^{0}/oo	22 ^{0}/oo	32 ^{0}/oo
Fresh ANS	*3*	8.5	1	10	10
	10	7.9	15	10	22
	22	35	31	16	18
20% evap. ANS	*3*	6.3	6.5	6.3	6.3
	10	1.7	4.1	4.5	2.6
	22	6.3	6.3	6.3	6.3
emulsified' ANS	*3*	26	20	13	23
	10	73	32	42	29
	22	17	20	24	14

Table 12 Prediction of Temperature and Salinity Interrelationship (Data from Moles et al., 2001, 2002)

Equation: Effectiveness = a + b*temperature + c*salinity

Dispersant	Oil	a	b	c	Linear r	Best r^2
9527	fresh ANS	2.6	1.5	-0.15	0.92	0.94
9500	fresh ANS	-2.2	0.34	0.47	0.57	0.72
9527	weathered	2.6	0.026	0.09	0.07	0.85
9500	weathered	6.8	0.025	-0.06	0.07	0.85
9527	emulsion	77	-0.52	-0.15	0.19	0.61
9500	emulsion	37	-0.1	-0.43	0.06	0.68

Table 13 Salinity and Effectiveness (Data from Fingas et al., 2003)

Oil	Dispersant	Salinity	Effectiveness	Std. Dev
ASMB	Corexit 9500	5	21.9	3.4
ASMB	Corexit 9500	10	24.1	1.3
ASMB	Corexit 9500	20	52.8	1.3
ASMB	Corexit 9500	33	43.8	6.5
ASMB	Corexit 9527	5	24.1	2.1
ASMB	Corexit 9527	10	23.3	2.2
ASMB	Corexit 9527	20	54.2	5.5
ASMB	Corexit 9527	33	36.6	3.5
ANS	Corexit 9500	5	19.4	1.1
ANS	Corexit 9500	10	18.8	0.7
ANS	Corexit 9500	20	21.9	1.9
ANS	Corexit 9500	33	34.8	4.7
ANS	Corexit 9527	5	17.1	0.8
ANS	Corexit 9527	10	17.2	1.7
ANS	Corexit 9527	20	24.6	0.8
ANS	Corexit 9527	33	25.9	2.8

ASMB = Alberta Sweet Mixed Blend

ANS = Alaska North Slope Blend

Table 14 Summary of Authors and Findings from Surfactant Literature

Author	Year	Surfactant Type	Specific Surfactant	Type of Test	Generic Results
Abuin et al.	1993	Ionic	CTAB and CTAC	Microemulsion stabilization	Stabilization increases with salinity except for one surfactant
Al-Roomy et al.	2004				
Austad and Strand	1996	Ionic	Exxon RL-3011	Oil behaviour	Salinity has large effect
Austad et al.	2004				
Babadagli	2003	Nonionic	Oxyethanol ethyoxylate	Recovery from reservoir	Recovery increases with salinity
Chen et al.	2004	Ionic	CTAB	Interfacial tension	IFT decreases and then increases
Chooro et al.	1996	Ziwitteronic	N-dodecyl Betaine	Relative solubility	RSD decreases with salinity
Davis	1994	Most		Reviews basics	IFT decreases with salinity
Drummond & Israelachvili	2002	Various		Surface forces	Surface forces decrease with salinity
Fjelde and Austad	1994				
Fjelde et al.	1995	Dual ionic	6EOS & DDBS	Recovery from reservoir	Recovery increases with salinity
Ghannam and Chaalal	2003	Nonionic	Triton X100	Oil spill recovery	Recovery increases with salinity
Hou and Papadopoulis	1996	Nonionic	Tween 80 & Span 80	Droplet stability	Drop stability increases with salinity
Hou and Papadopoulis	1997	Nonionic	Tween 80 & Span 80	Droplet stability	Drop stability increases with salinity
Kaczmarski et al.	1999	Dual ionic & nonionic	SDS & Triton 100	Thickener viscosity	Viscosity lower as salinity increases
Kjøniksen et al.	1999	Ionic	SDS	Gel structure	Salinity increases molecular associations
Li and Chen	2002	Nonionic	Tergitol 15-S-X, Triton X-100, Tween 20, Tween 80	Partitioning of org. into oil	Partitioning increases with salinity
Li and Kunieda	2003	Mixed	Anionic and cationic	Relative solubility	RSD decreases with salinity
Liu et al.	2004				
Mollet et al.	1996	Ionic	Sodium linoleate	Interfacial tension	IFT decreases and then increases
Moosai and Dawe	2003	Various	Various	Gas flotation	Wastewater cleanup improved with salinity
Park and Bielefeldt	2003	Nonionic	Tergitol NP-10	Partitioning of org. into oil	Partitioning increases with salinity
Prosser and Franses	2003	Ionic	SDS & SDSn	Model of IFT equilibrium	Salinity decreases IFT
Sabatini et al.	2003	Ionic	Naphthalene sulphonates	Solubilization	Very salinity-dependent
Sayyouh et al.	1993	Ionic	Sulphonates	Phase behaviour of oil-surfactant-brine	Stability increases up to 3.8% and then decreases as salinity goes to 23%
Song & Islam	1994				
Watt et al.	1998	Cationic	CTAB	Water-in-oil emulsion	Formation improves up to about 30% salinity
Wu et al.	2004	Nonionic	Brij 30	Relative solubility	RSD decreases with salinity
Wu et al.	2004	Nonionic	Tween 20	Relative solubility	RSD decreases with salinity
Wu et al.	2004	Nonionic	Igepol CO210	Relative solubility	RSD decreases with salinity
Ysambertt et al.	1997	Most		Reviews basics	Winsor states affected by salinity
Yu et al.	2004	Cationic	Aliquat-336	Extraction effectiveness	Extraction increases with salinity
Zhang et al.	2004	Natural	Acid fractions	Recovery from reservoir	Recovery increases with salinity

Table 15 Quantitative Data on Salinity Change from Surfactant Literature

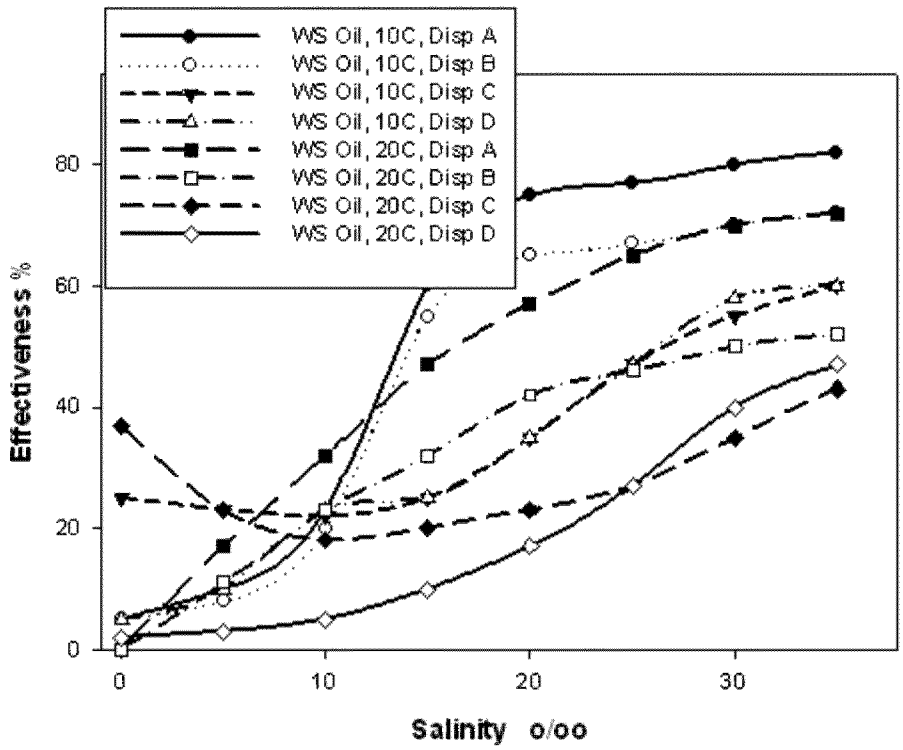
(all data converted to relative effectiveness compared to 0 salinity)

Author and Details	Salinity o/oo	Increase in Effect (from 0)	Change in Solubilty	Reduction in IFT	Notes
Wu et al., 2004	50		4.1		
nonionic	100		5.8		
	50		2.7		
	100		3		
	50		3.4		
	100		5.2		
Chen et al., 2004	1			99.7	
ionic	2			99.4	
	5			99.3	
	7			99.2	
	9			99.1	
	11			99.2	
	15			99.6	
Mollet et al., 1996	5			22	
	10			44	
	20			38	
	30			35	
Kaczmarski et al.,	20	64			surf = .01
1999	30	84			surf = .01
	20	48			surf = .025
	30	77			surf = .025
	20	50			surf = .05
	30	90			surf = .05
Li and Chen, 2002	0.5	50			partitioning
	5	75			partitioning
	10	100			partitioning
	0.5	5			reduced radius
	5	10			reduced radius
	10	17			reduced radius
Ghannam and Chaalal,	10	5			still water
2003	20	150			still water
	30	260			still water
	10	5			circulated water
	20	16			circulated water
	30	36			circulated water
Sayyouh et al., 1993	5			20	
	10			90	
	15			3	
	20			60	
Guyomarch et al.,	10	0			
2002	25	166			
	35	200			
	50	566			

Table 16 Results of Metabolite Uptake (from Wolfe et al., 1998)

	Metabolic Uptake (as percentage recovered)			
	A,b Napthol Sulphate		Napthalene	
Sample	**22 o/oo**	**34 o/oo**	**22 o/oo**	**34 o/oo**
20 C WAF - Control	1	2	98	96
20 C WAF - Exp. Med.	5	8	93	90
20 C WAF - Algae	4	1	80	85
20 C Disp. oil - Control		2	99	96
20 C Disp. oil - Exp. Med.	3	3	96	95
20 C Disp. oil - Algae			95	92
12 C WAF - Control	6	1	91	98
12 C WAF - Exp. Med.	6	3	91	96
12 C WAF - Algae			92	92
12 C Disp. oil - Control	4		94	99
12 C Disp. oil - Exp. Med.	5	3	93	96
12 C Disp. oil - Algae			90	95

Table 17	**Recent Salinity Measurement Results**						
Summer Sampling Data - June 24, 2004							
Location	**Bligh Reef**	**Outer Jack Bay**	**Shoup Bay**	**Gold Creek**	**Mineral Creek**	**Glacial Creek**	**Lowe River**
Latitude	60 47.926	61 02.305	61 07.612	61 07.533	61 07.53	61 06.183	61 05.778
Longitude	146 51.617	146 38.819	146 35.263	146 29.137	146 25.580	146 17.464	146 17.794
Surface Salinity o/oo	26.3	15.3	4.3	2.7	1.2	0.2	0.4
Temperature oC	13.6	15.6	7.2	10.3	10.6	3.6	5.3
Fall Sampling	**September 24, 2004**						
Location		**Outer Jack Bay**		**Gold Creek**			**Lowe River**
Latitude		61 02.210		61 07.710			61 05.779
Longitude		146 40.191		146 28.828			146 17.723
Surface Salinity o/oo		20.6		17.7			2.7
Temperature oC		9.1		8.3			5.9



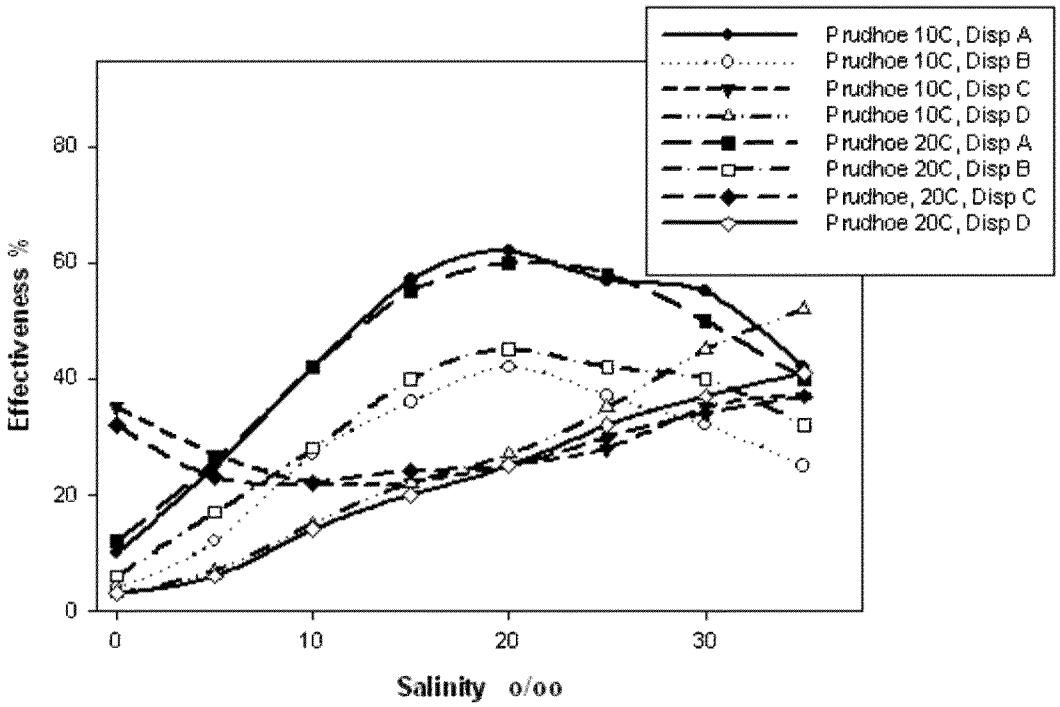


Figure 1 Salinity and Dispersant Test Results from Belk et al., 1989, Warren Springs Oil, Upper Plot: Prudhoe Bay Oil Lower Plot

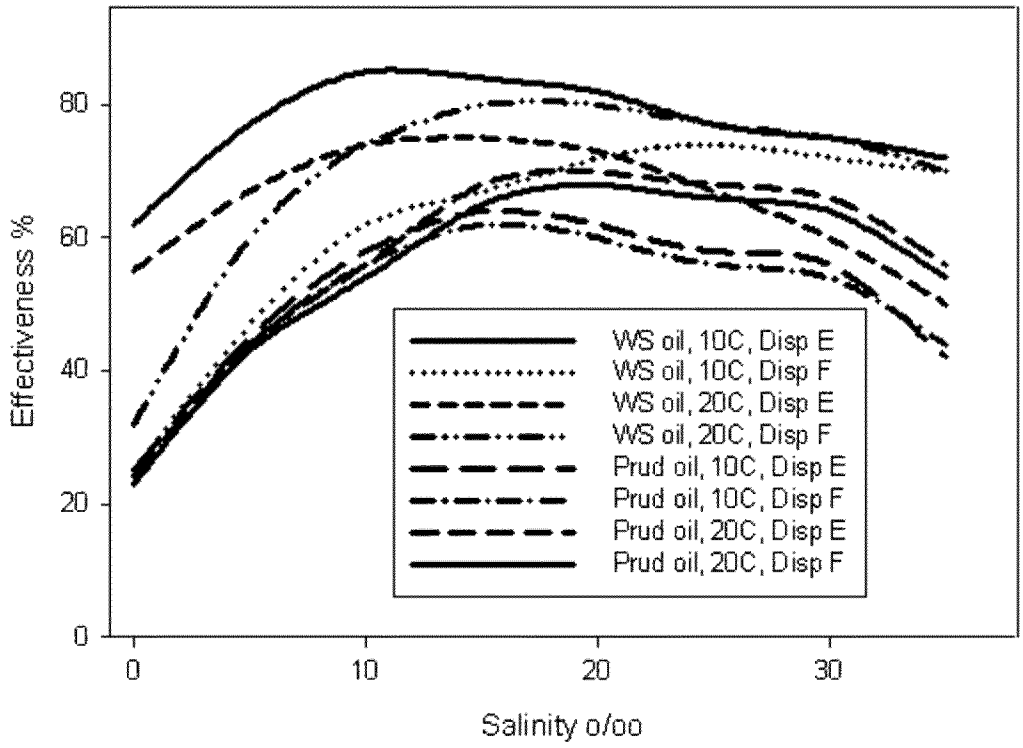


Figure 2 Dispersant E and F Data from Belk et al., 1989

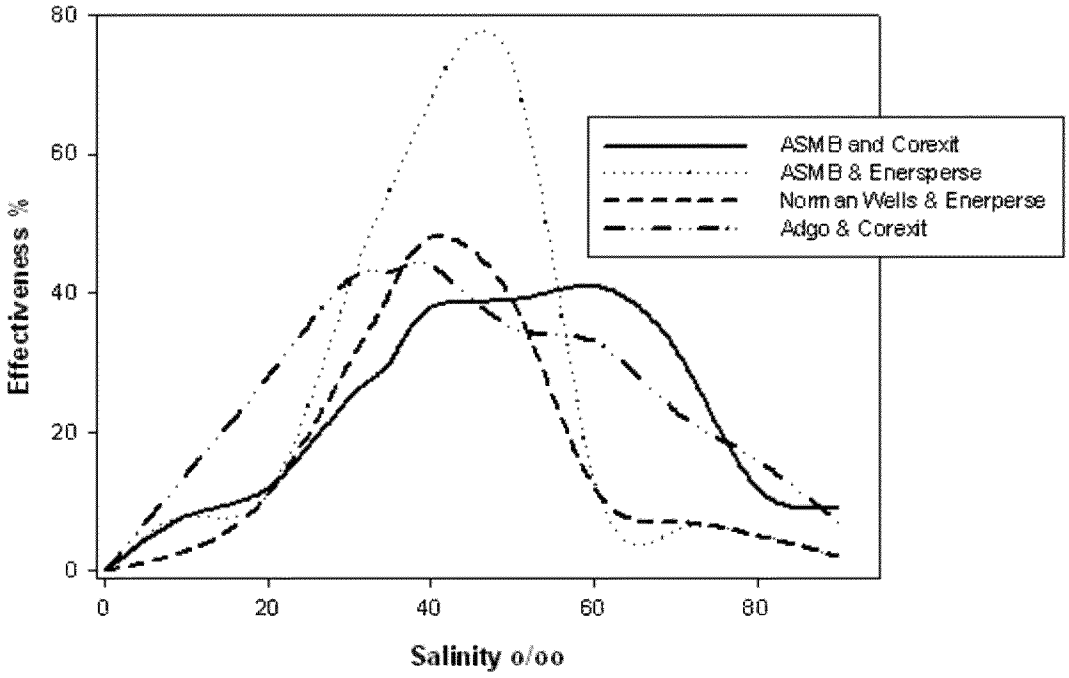


Figure 3 Dispersant Effectiveness with Salinity from Fingas et al. 1991

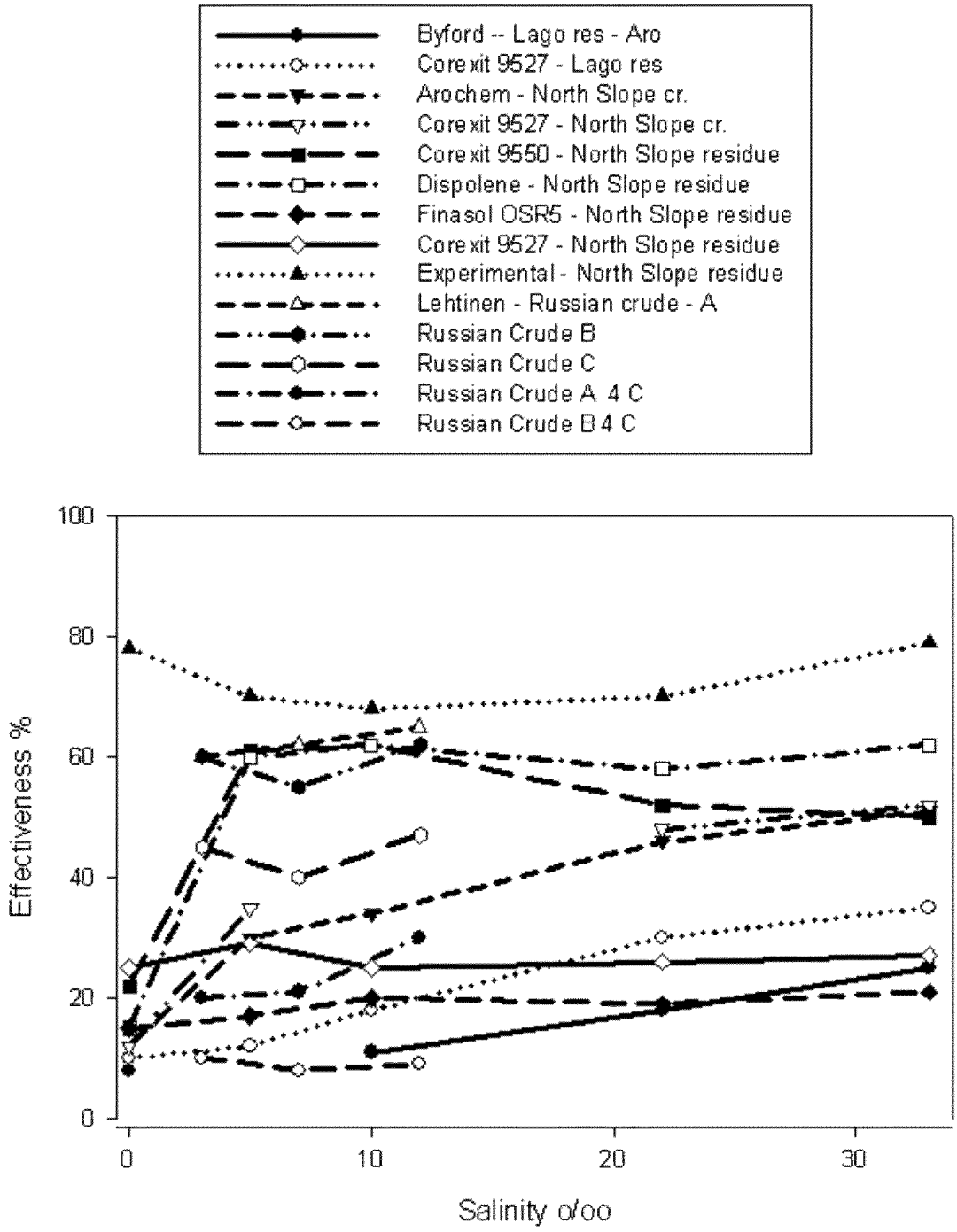
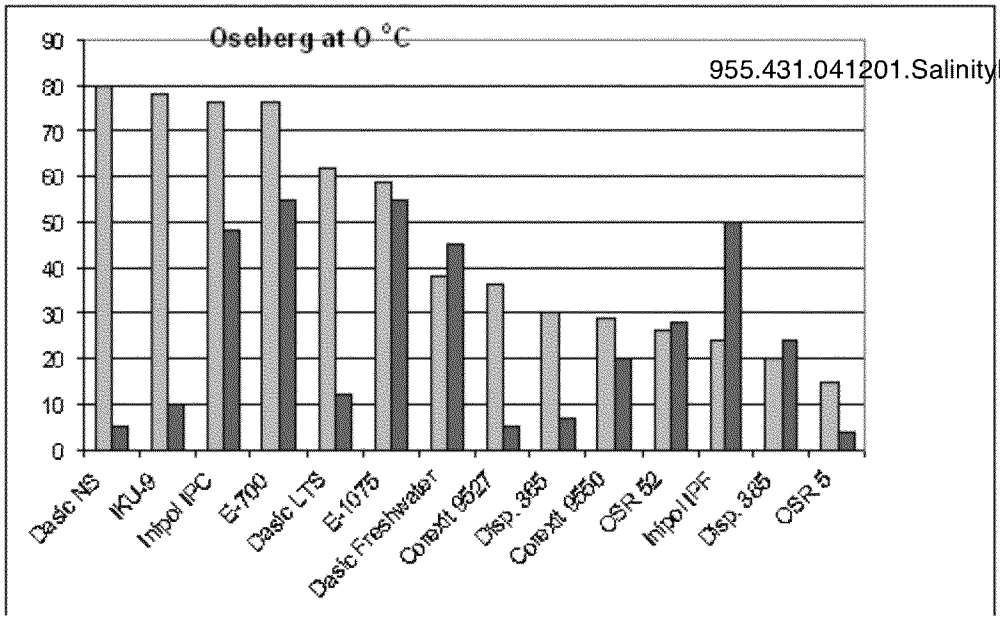
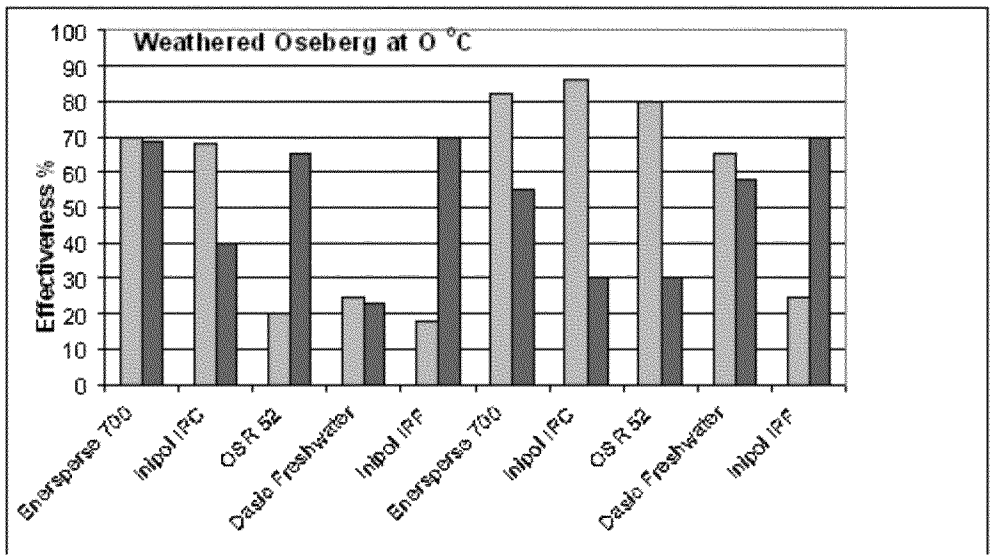


Figure 4 Salinity and Dispersant Effectiveness Data from Byford et al., 1983 and Lehtinen and Vesala, 1984





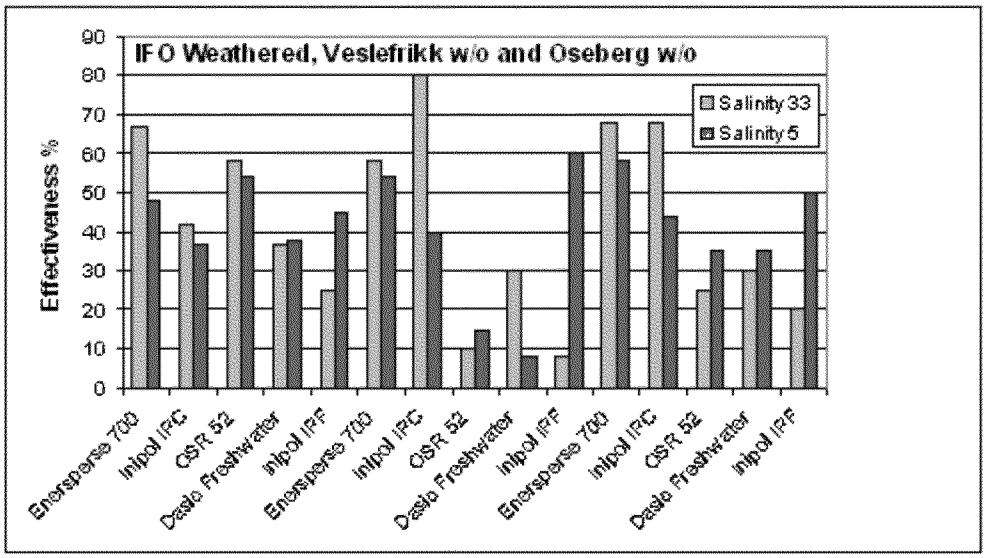


Figure 5 Dispersant Effectiveness with Salinity from Brandvik et al. 1995

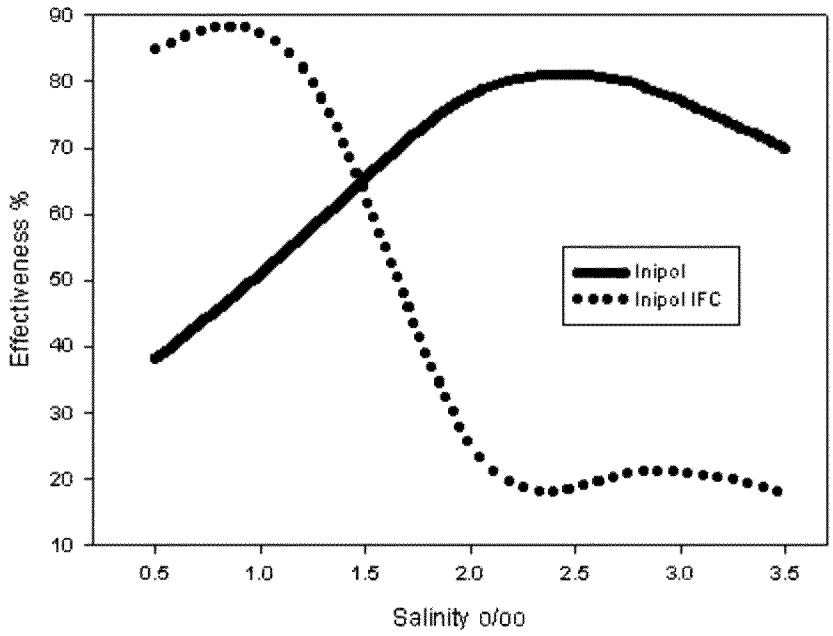


Figure 6 Dispersant/Salinity Test on Two Inipol dispersants - Inipol IFC was a Special Freshwater Dispersant (Data from Brandvik and Daling, 1992)

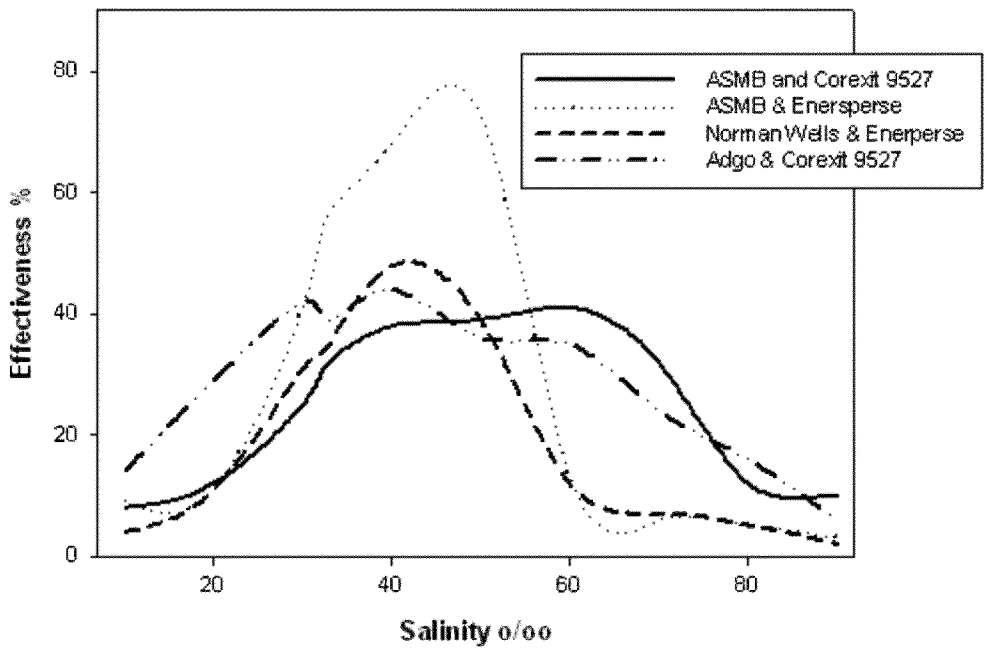


Figure 7 Tests on Dispersant Effects with Varying Salinity (Data from Fingas et al., 1994)

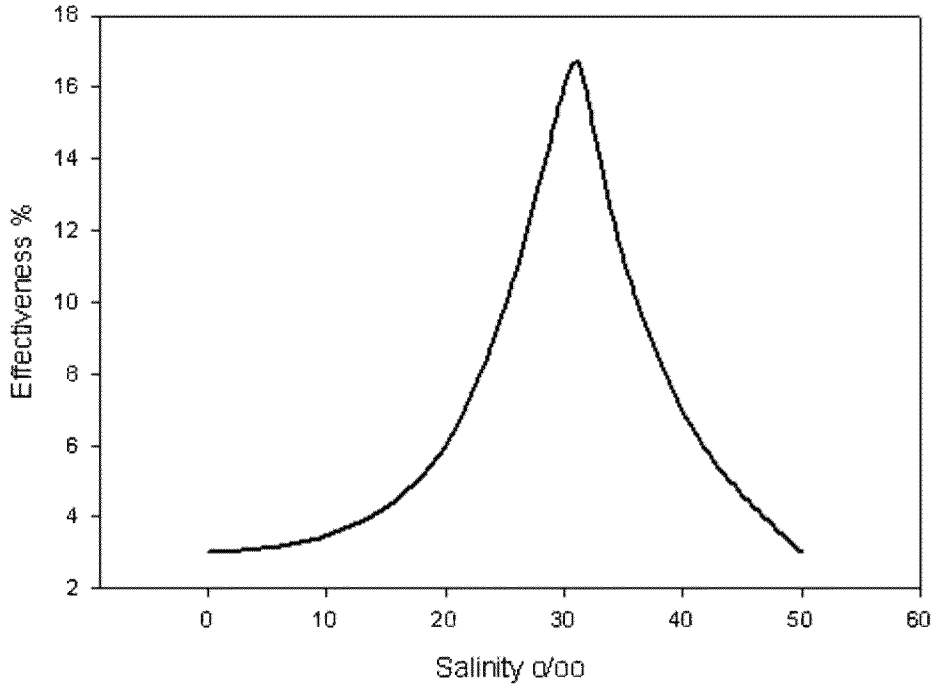


Figure 8 Dispersant Effectiveness Data for Corexit 9527 and a Light Arabian Crude (Data from Moet et al., 1995)

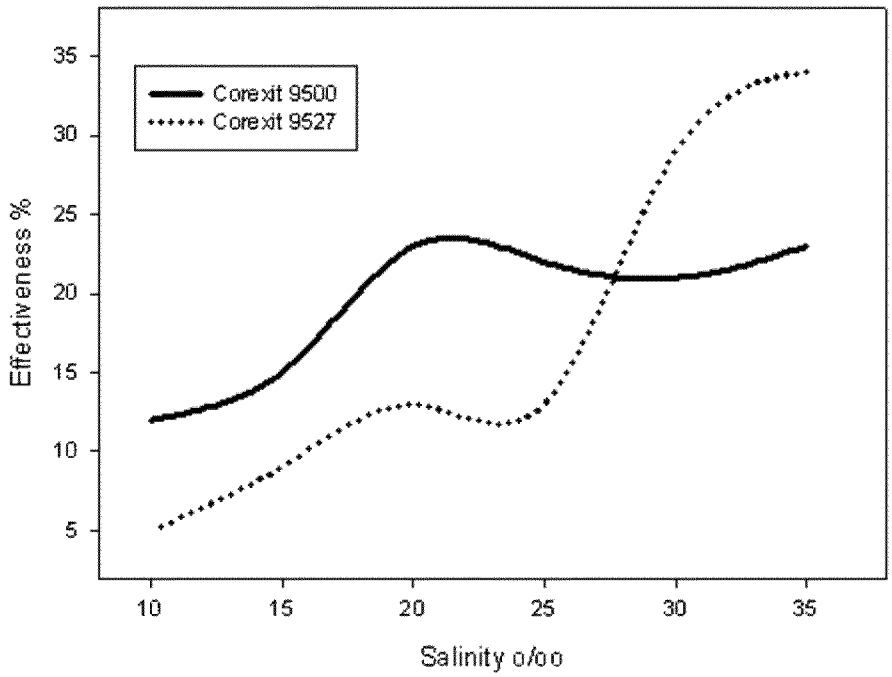


Figure 9 Dispersant Effectiveness Data on ANS Crude (Data from Blondina et al., 1997 a,b)

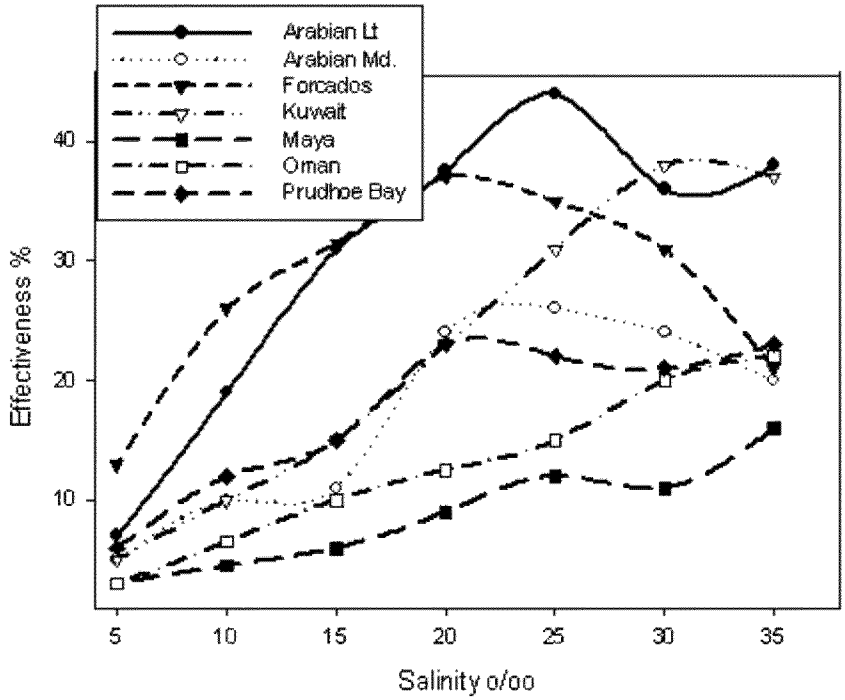


Figure 10 Effectiveness of Corexit 9500 with Salinity (Data from Blondina et al., 1999)

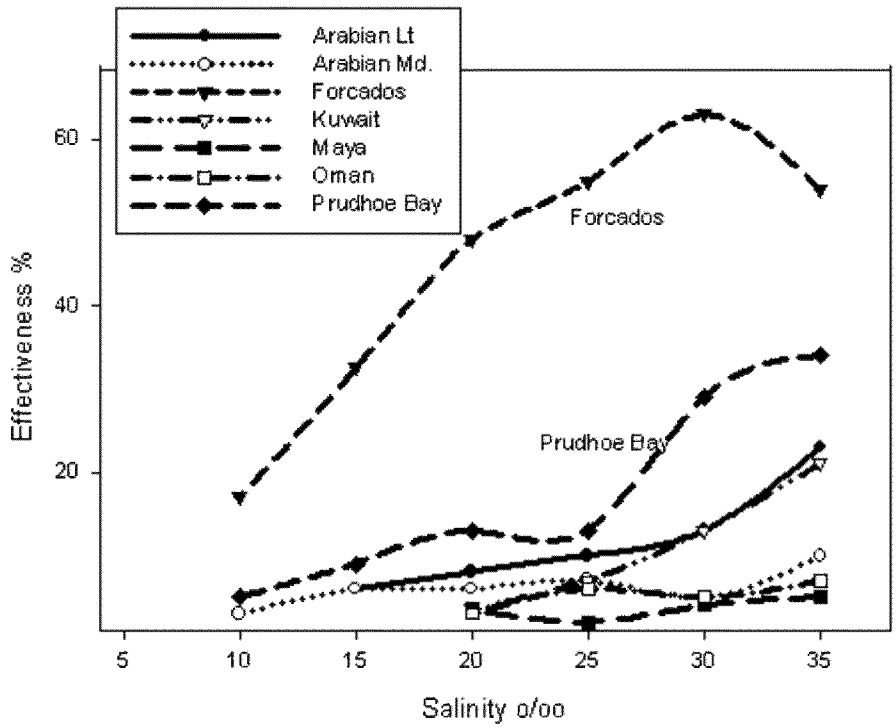


Figure 11 Effectiveness of Corexit 9527 with Salinity (Data from Blondina et al., 1999)

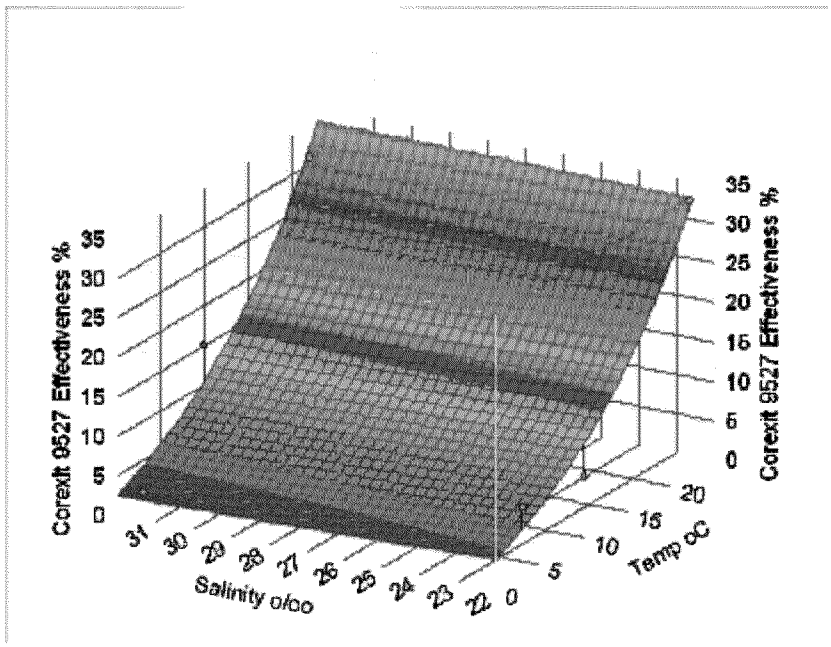


Figure 12 Three-way Relationship of Effectiveness, Salinity, and Temperature for Corexit 9527 and Fresh ANS (Data from Moles et al., 2002)

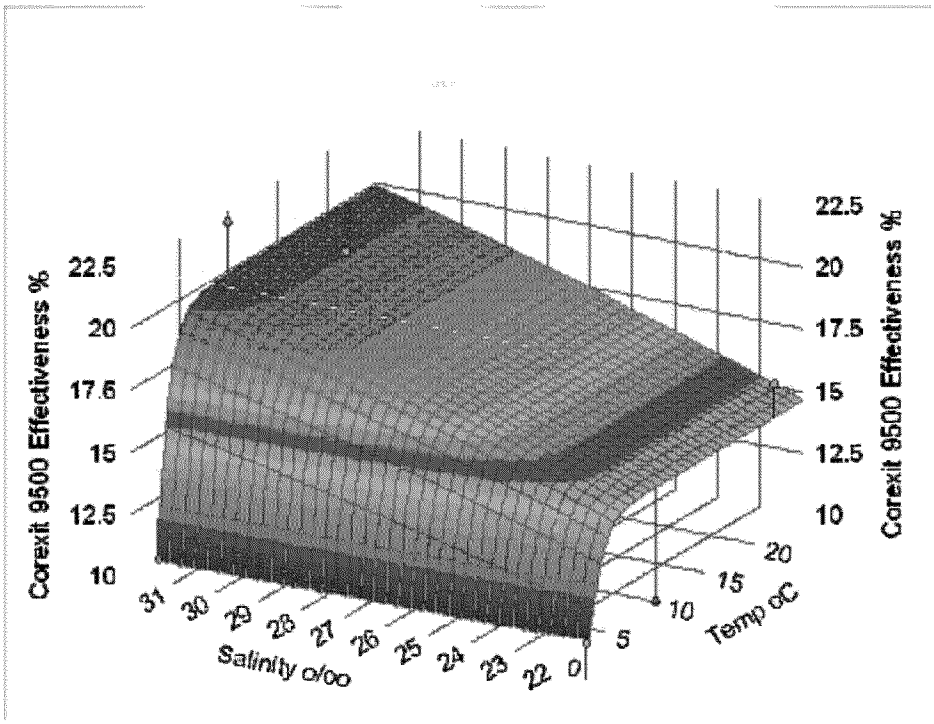


Figure 13 Three-way Relationship of Effectiveness, Salinity and Temperature for Corexit 9500 and Fresh ANS (Data from Moles et al., 2002)

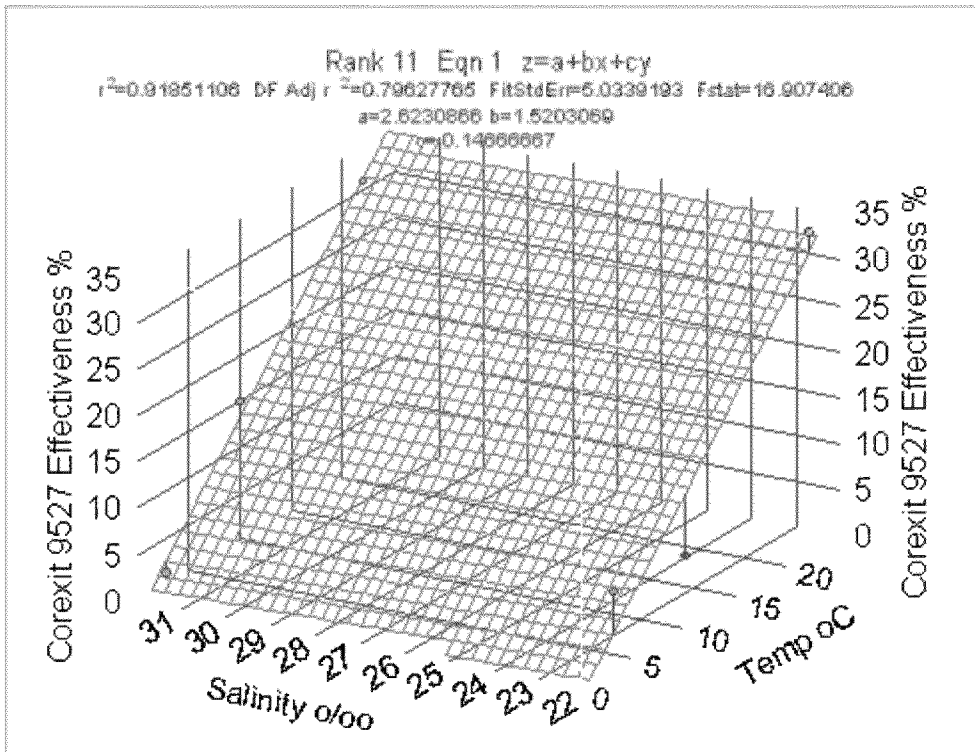


Figure 14 Three-Way Correlation of Corexit 9527 Effectiveness with Fresh ANS (Moles et al., 2002)

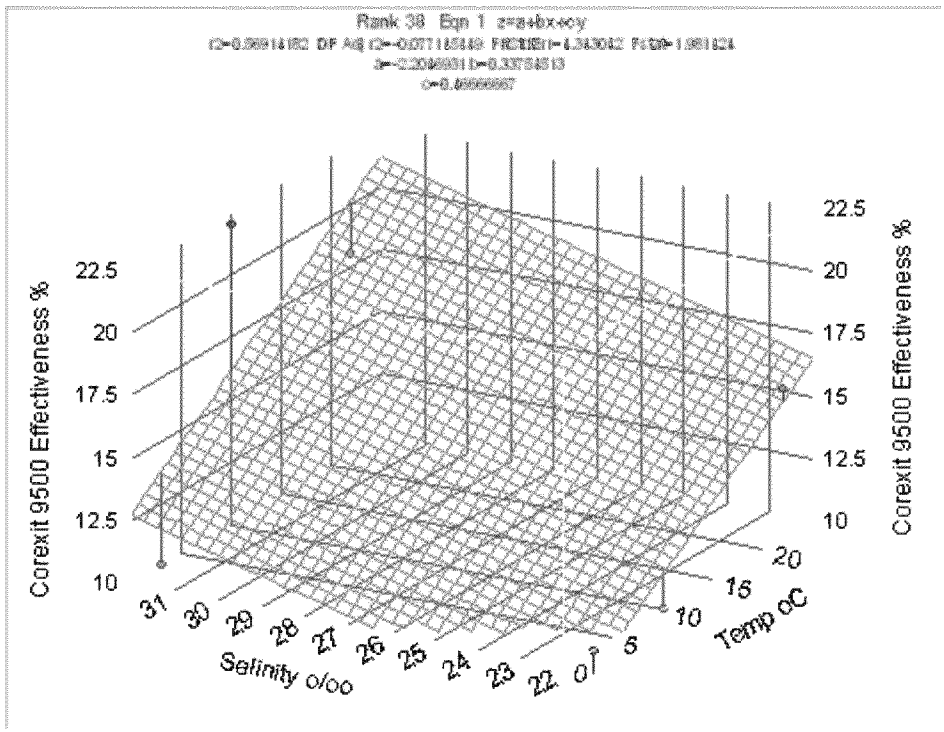


Figure 15 Three-Way Correlation of Corexit 9500 Effectiveness with Fresh ANS (Moles et al., 2002)

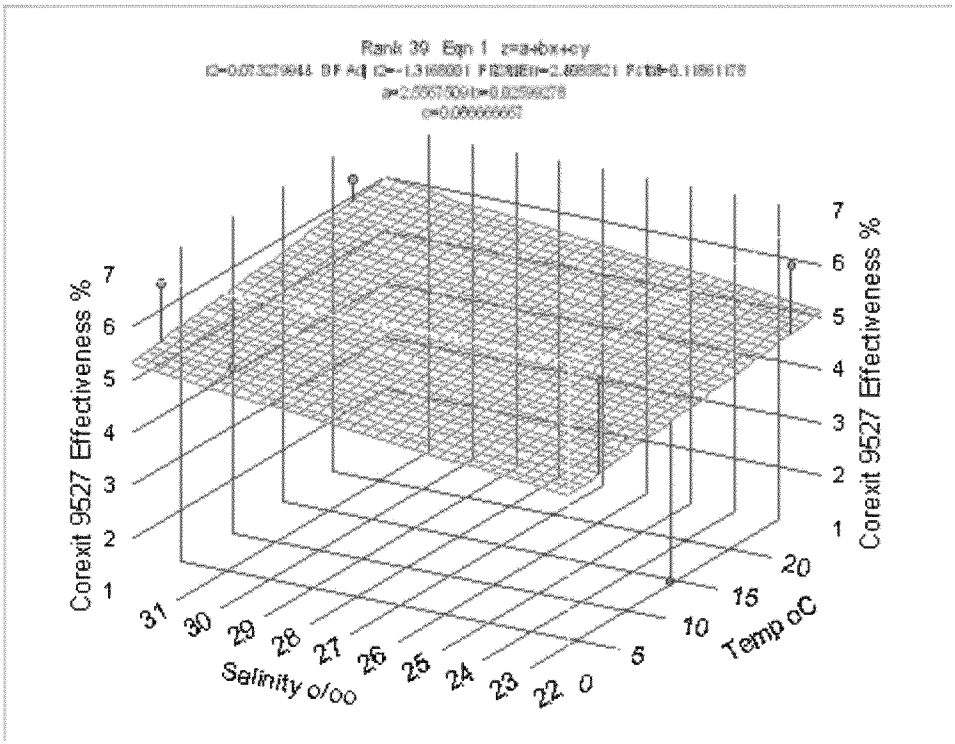


Figure 16 Three-Way Correlation of Corexit 9527 Effectiveness with Weathered ANS (Data from Moles et al., 2002)

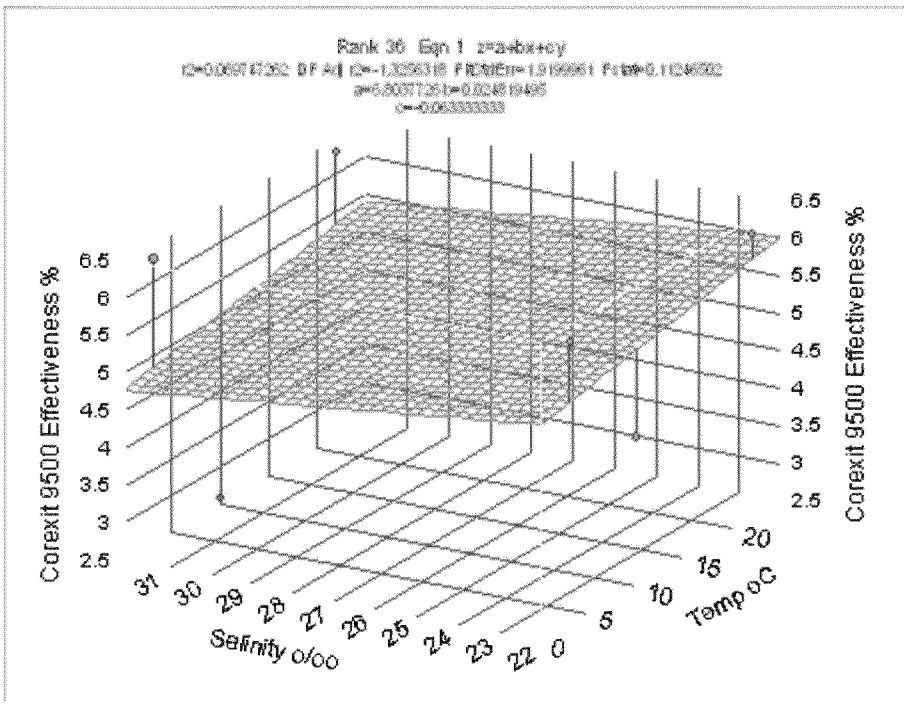


Figure 17 Three-Way Correlation of Corexit 9500 Effectiveness with Weathered ANS (Data from Moles et al., 2002)

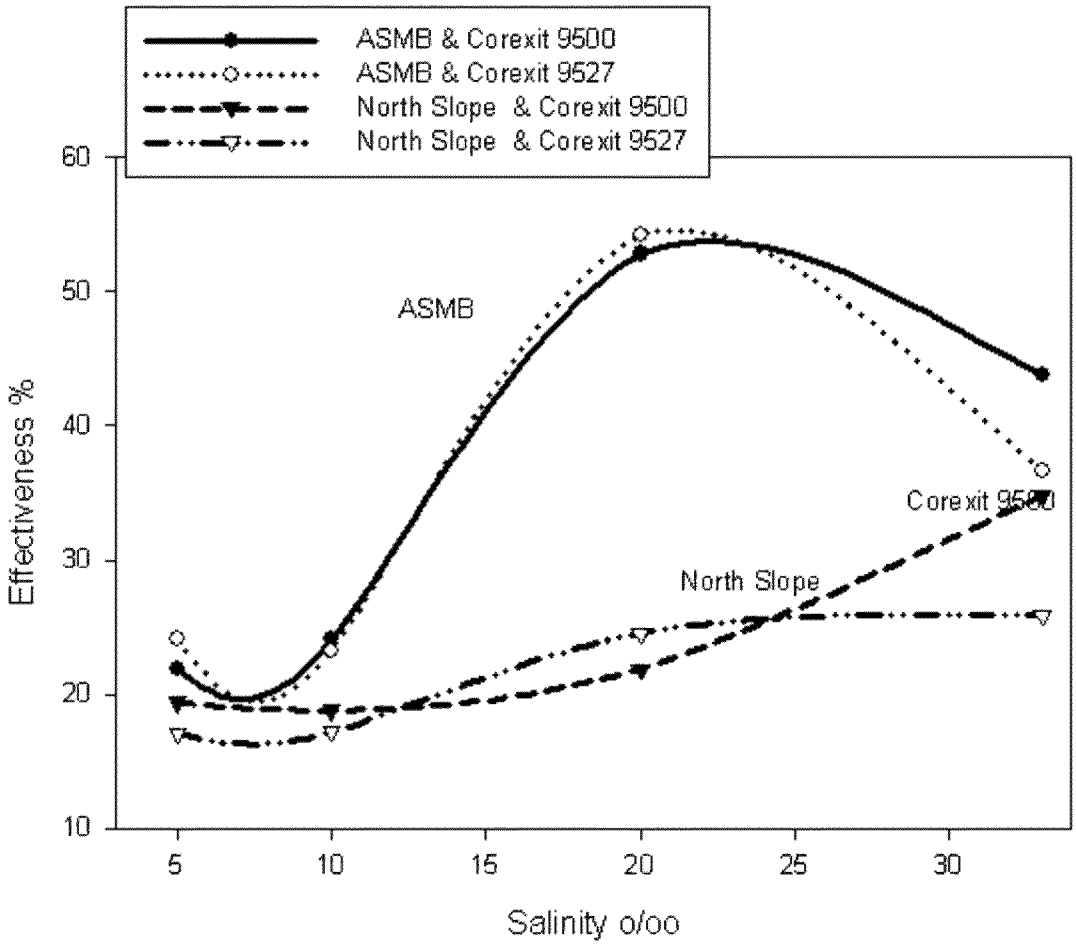


Figure 18 Variation of Dispersant Effectiveness with Salinity (Data from Fingas et al., 2003)

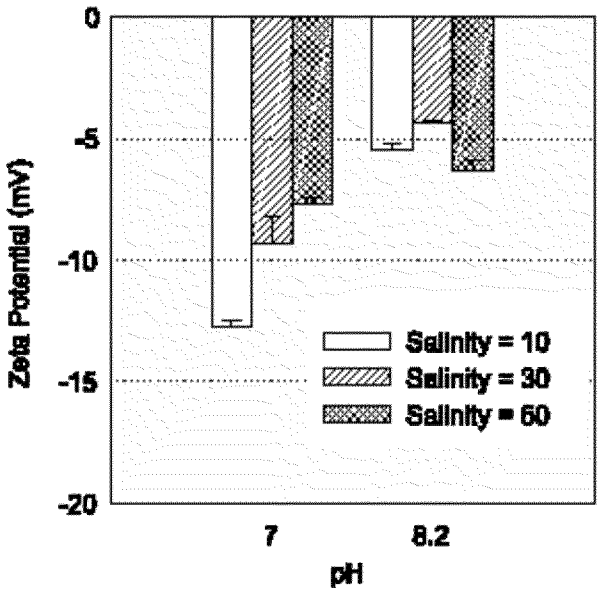


Figure 19 Measured Zeta Potential Values of Chemically Dispersed Crude Oil Droplets at Selected pH Values (Data from Sterling et al., 2004)

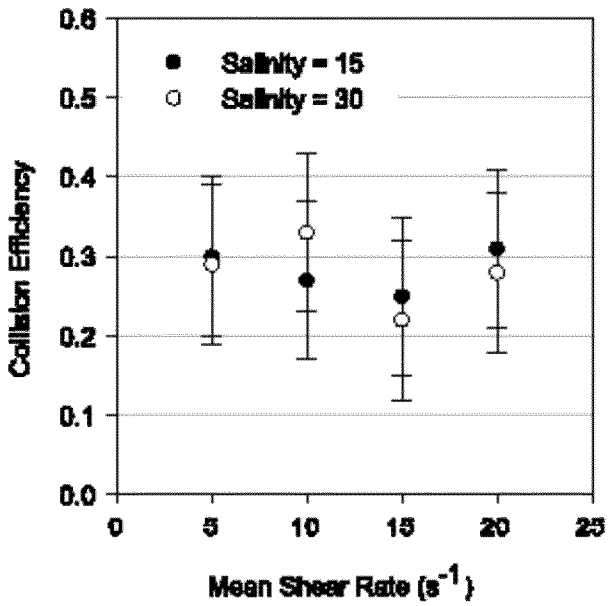


Figure 20 Effect of Shear Rate on Collision Efficiency (Data from Sterling et al., 2004)

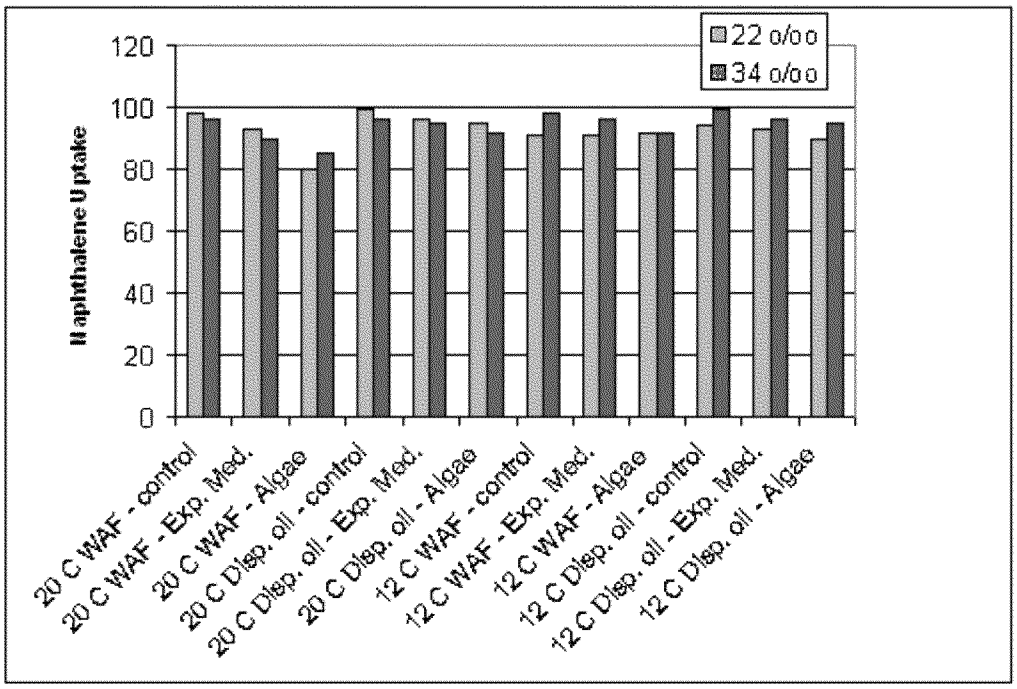


Figure 21 Variation in Uptake of Naphthalene by Algae (Data from Wolfe et al., 1998)

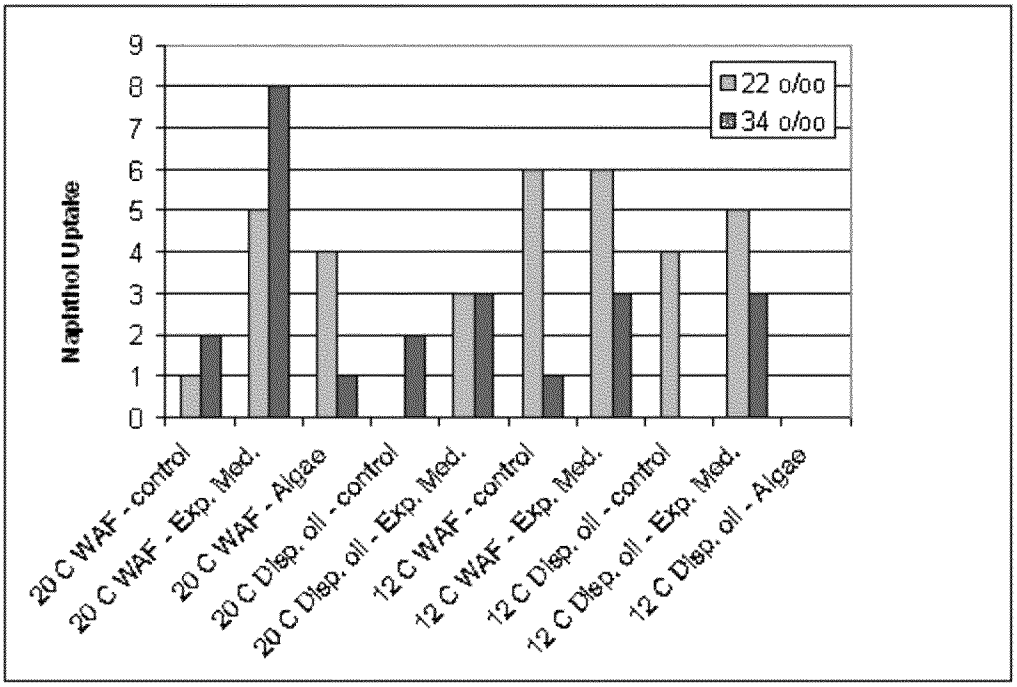


Figure 22 Variation in Uptake of a,b Naphthol Sulphate by Algae (Data from Wolfe et al., 1998)

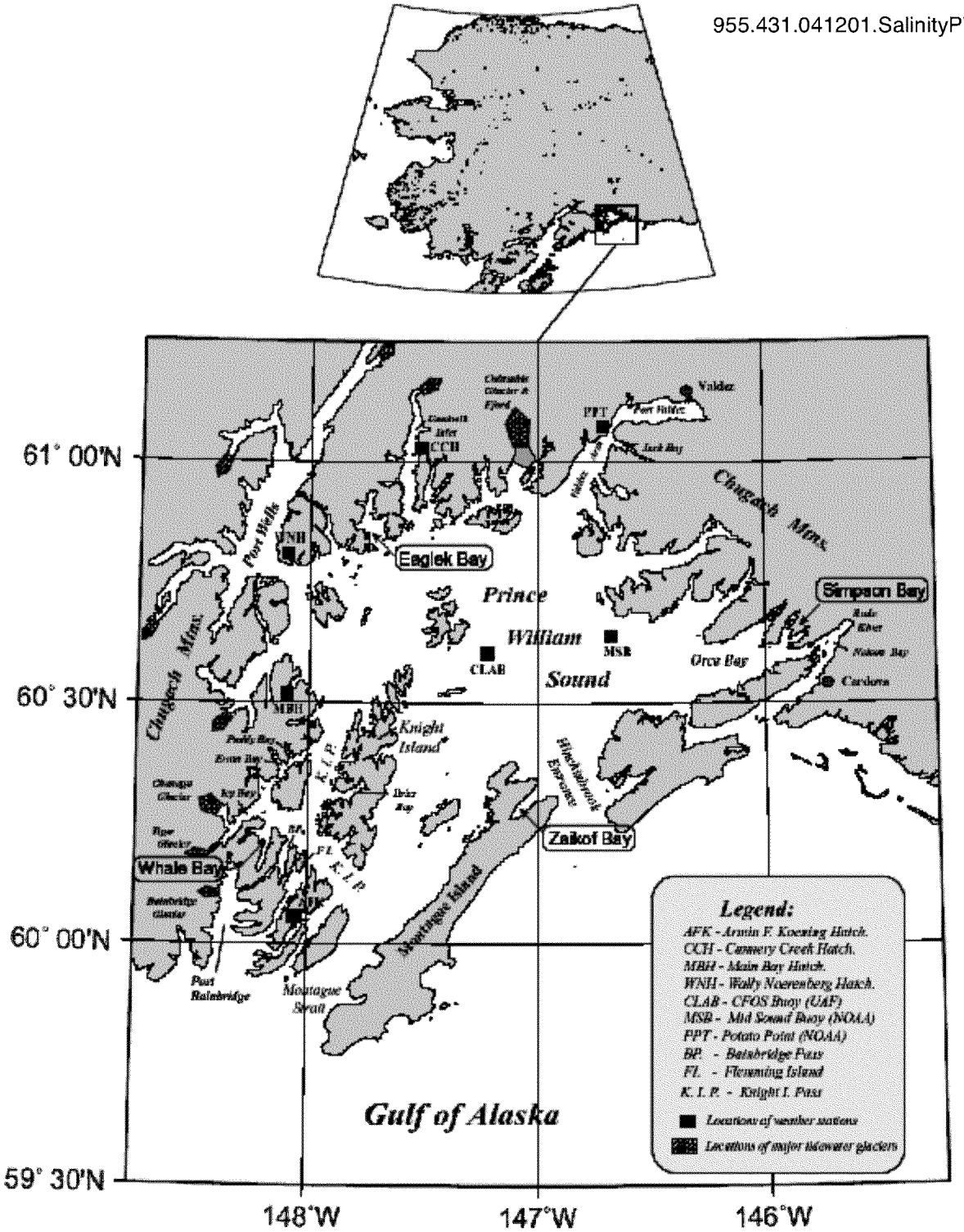


Figure 23 An Overview of Prince William Sound Showing Detailed Sample Sites

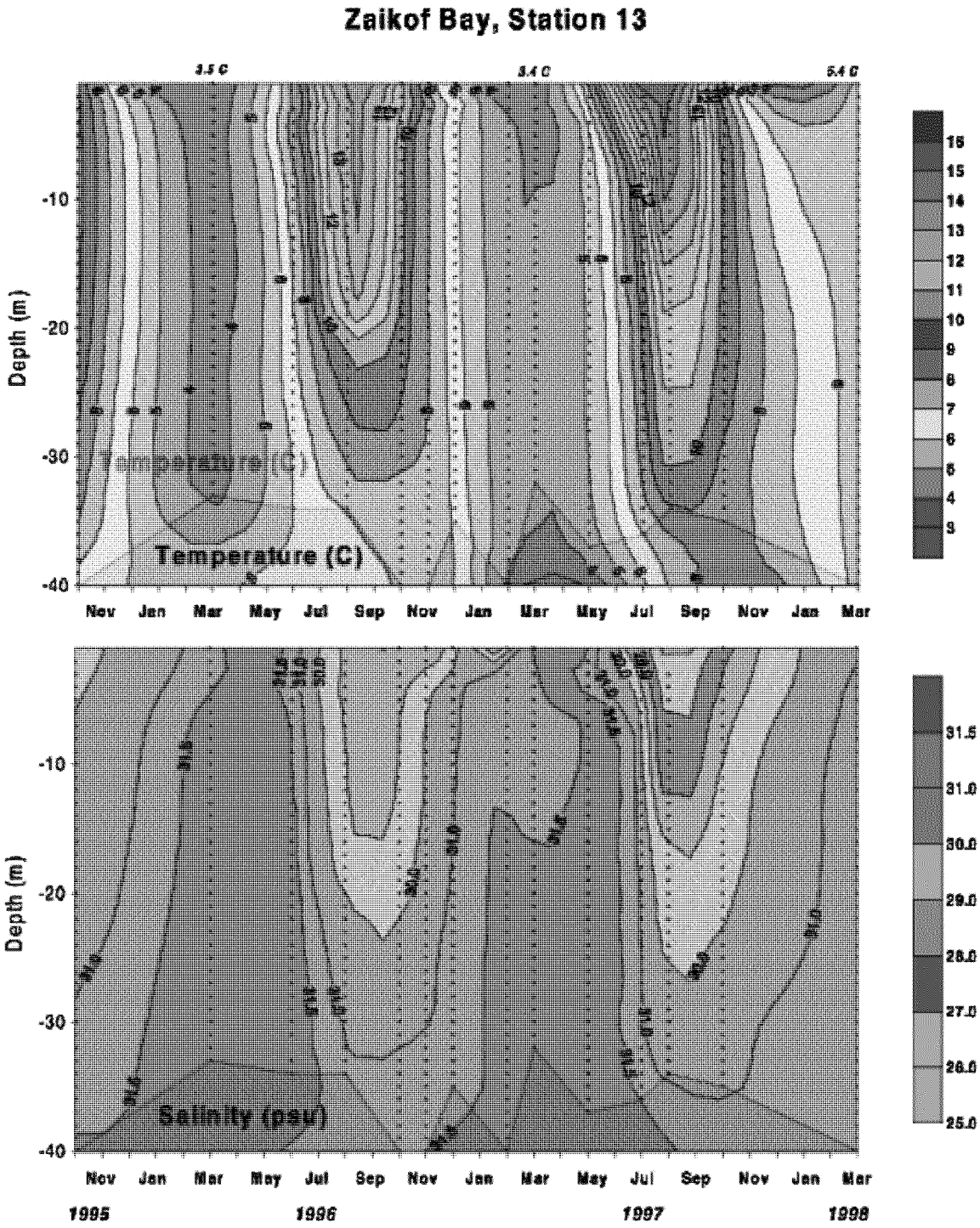


Figure 24 A Temperature/Salinity Graph from Zaikof Bay Sampling Site (Vaughn et al., 2001)

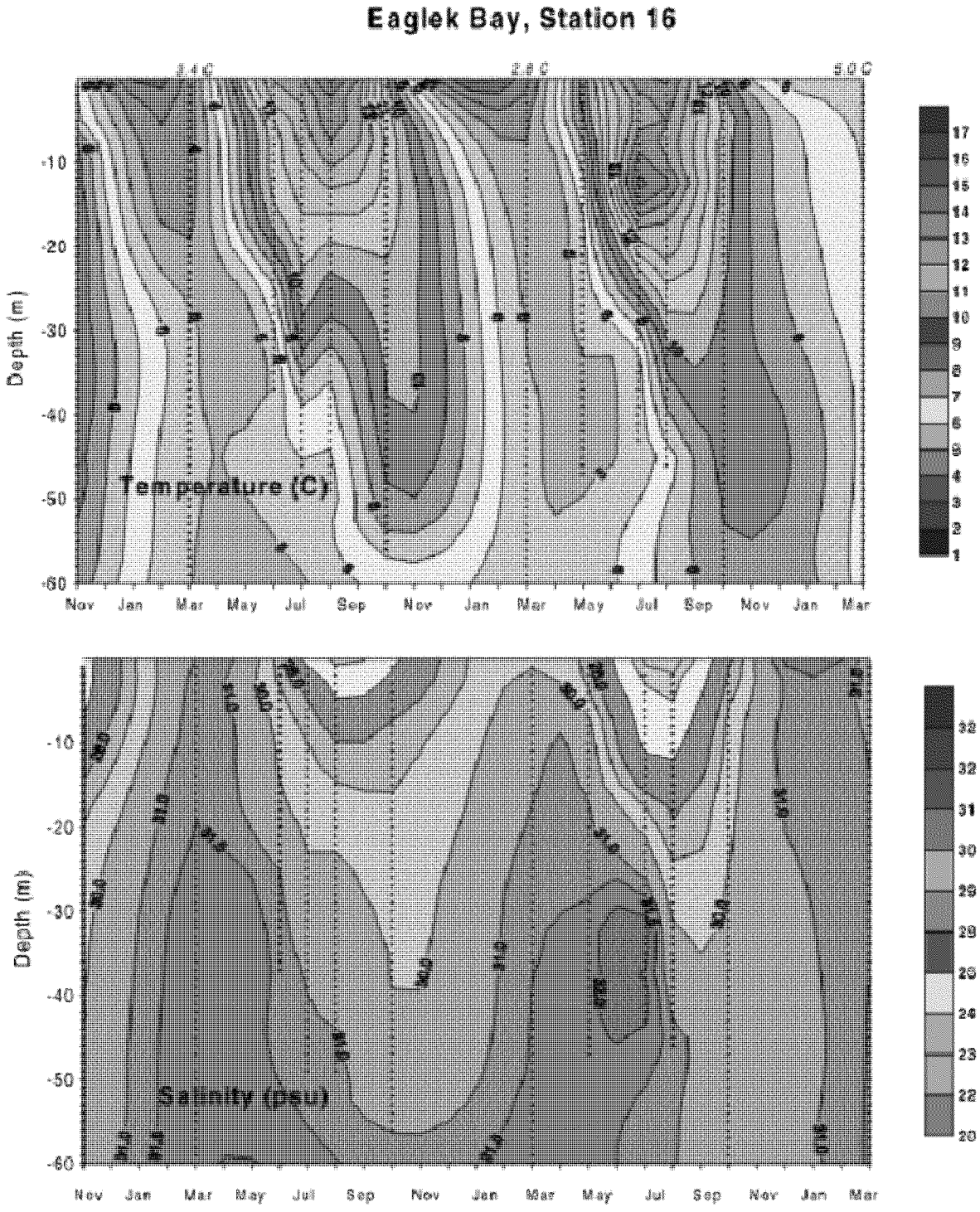


Figure 25 A Temperature/Salinity Graph from Eaglek Bay Sampling Site (Vaughn et al., 2001)

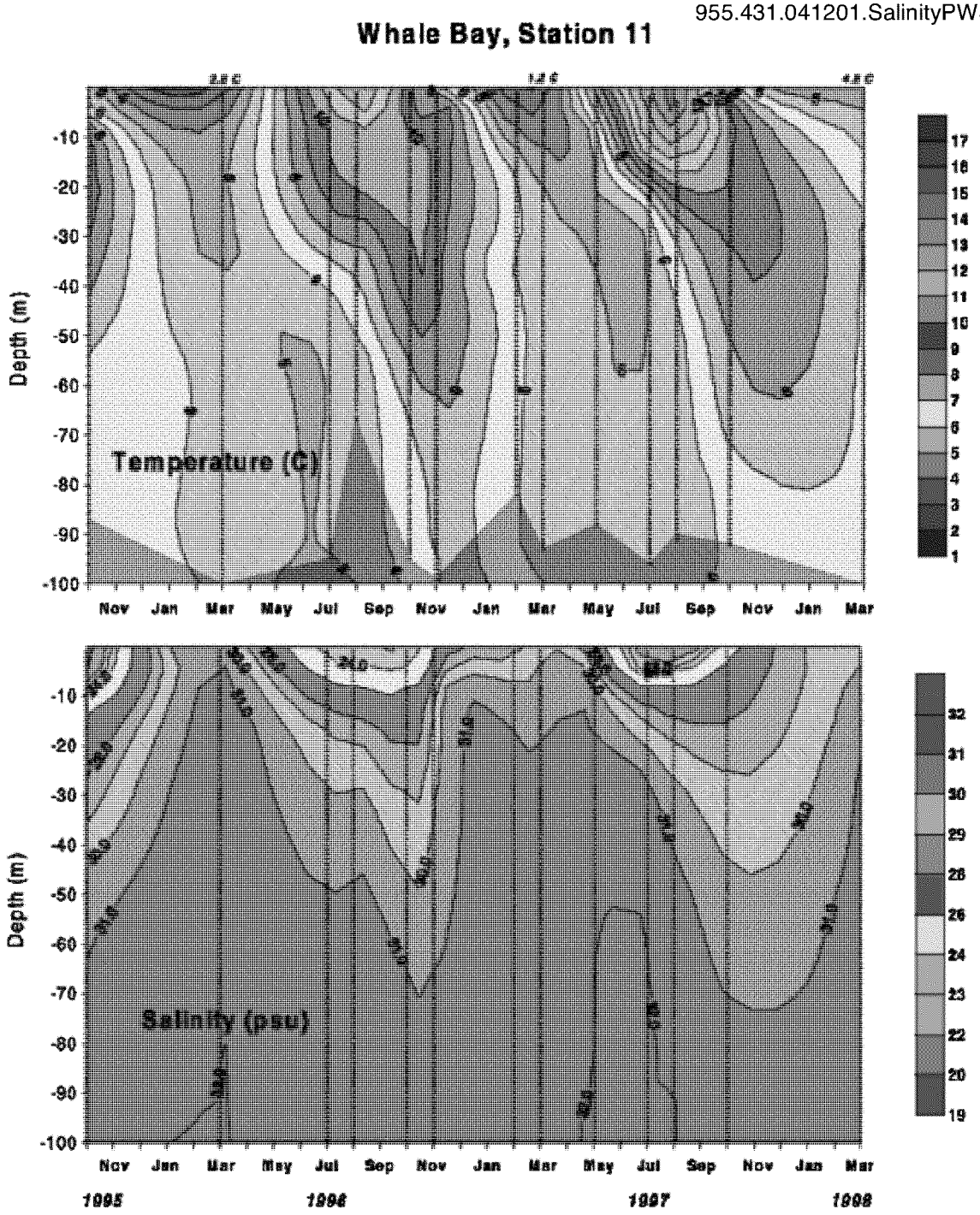


Figure 26 A Temperature/Salinity Graph from Whale Bay Sampling Site (Vaughn et al., 2001)

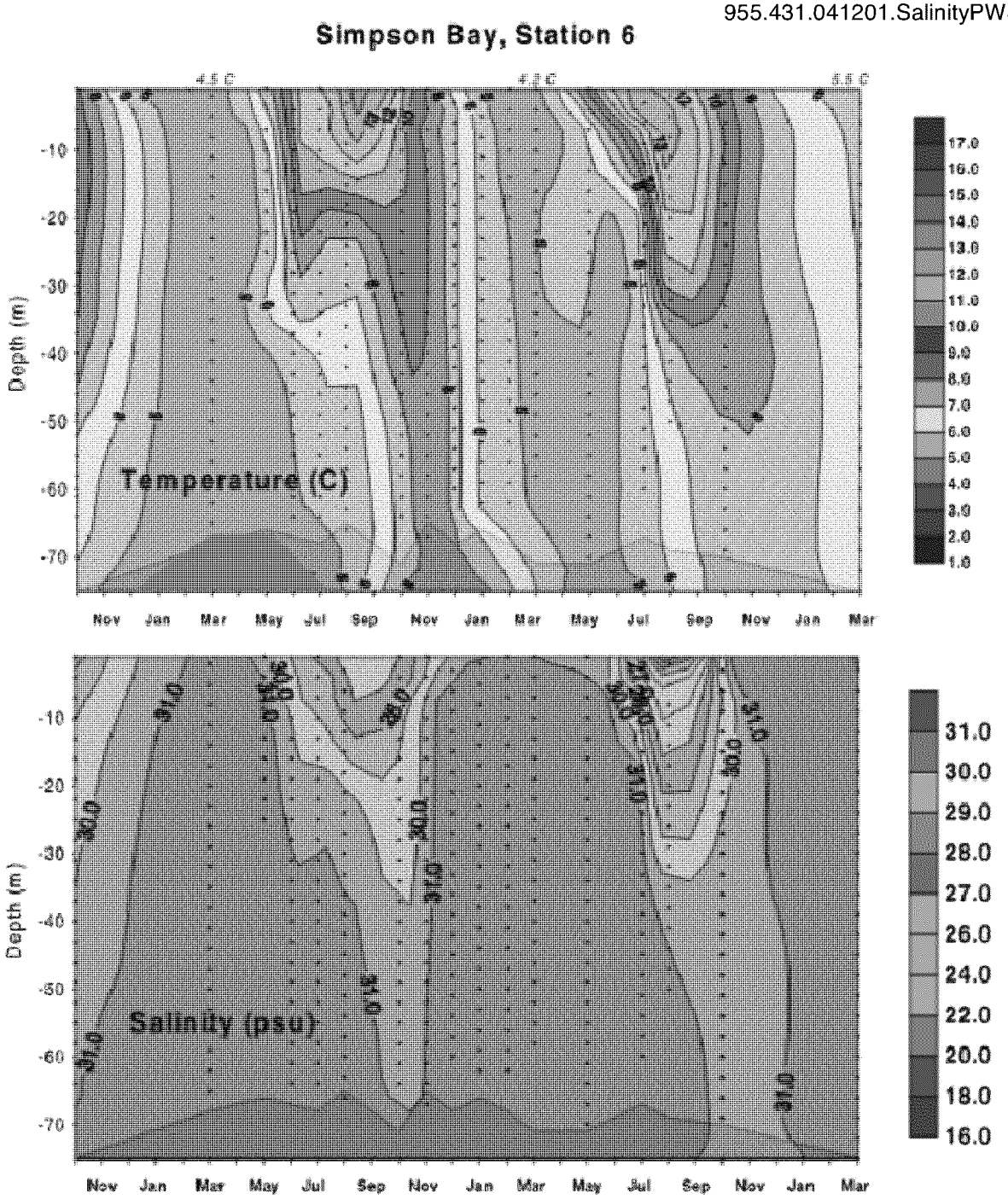


Figure 27 A Temperature/Salinity Graph from Simpson Bay Sampling Site (Vaughn et al., 2001)

10. References

Abuin, E.B., M.A. Rubio and E.A. Lissi, "Solubility of Water in Water-in-Oil Microemulsions Stabilized by Cetyltrimethylammonium: Effects of the Surfactant Counterion, the Nature and Composition of the Oil and the Salinity of the Droplets", *Journal of Colloid and Interface Science*, 158, pp. 129-132, 1993.

Al-Roomi, Y., R. George, A. Egibaly and A. Elkamel, "Use of a Novel Surfactant for Improving the Transportability/transportation of Heavy/viscous Crude Oils", *Journal of Petroleum Science and Engineering*, 42, pp. 234-243, 2004.

Austad, T. and S. Strand, "Chemical Flooding of Oil Reservoirs 4. Effects of Temperature and Pressure on the Middle Phase Solubilization Parameters Close to Optimum Flood Conditions", *Colloids and Surfaces A; Physicochemical and Engineering Aspects*, 108, pp. 243-252, 1996.

Austad, T., I. Fjelde, K. Veggeland and K. Taugbøl, "Physicochemical Principles of Low Tension Polymer Flood", *Journal of Petroleum Science and Engineering*, 10, pp. 255-269, 1994.

Babadagli, T., "Selection of Proper Enhanced Oil Recovery Fluid for Efficient Matrix Recovery in Fractured Oil Reservoirs", *Colloids and Surfaces A; Physicochemical and Engineering Aspects*, 223, pp. 157-173, 2003.

Bang, I. and C.N.K. Mooers, "The Influence of Several Factors Controlling the Interactions between Prince William Sound, Alaska and the Northern Gulf of Alaska", *Journal of Physical Oceanography*, 33, pp. 19-36, 2003.

Becher, P., *Emulsions: Theory and Practice*, Robert E. Krieger Publishing Company, Huntington, NY, 1977.

Belk, J.L., D.J. Elliott and L.M. Flaherty, "The Comparative Effectiveness of Dispersants in Fresh and Low Salinity Waters", in *Proceedings of the 1989 International Oil Spill Conference*, American Petroleum Institute, Washington, DC, pp. 333-336, 1989.

Blondina, G.J., M.L. Sowby, M.A. Ouano, M.M. Singer and R.S. Tjeerdema, "Comparative Efficacy of Two Corexit Dispersants as Measured Using California's Modified Swirling Flask Test", in *Proceedings of the Twentieth Arctic Marine Oilspill Program Technical Seminar*, Environment Canada, Ottawa, ON, pp. 561-573, 1997a.

Blondina, G.J., M.L. Sowby, M.T. Ouano, M.M. Singer and R.S. Tjeerdema, "A Modified Swirling Flask Efficacy Test for Oil Spill Dispersants", *Spill Science and Technology Bulletin*, 4, pp. 177-185, 1997b.

Blondina, G.J., M.M. Singer, I. Lee, M.T. Ouano, M. Hodgins, R.S. Tjeerdema and M.L. Sowby, "Influence of Salinity on Petroleum Accommodation by Dispersants", *Spill Science & Technology Bulletin*, 5(2), pp. 127-134, 1999.

Brandvik, P.J. and P.S. Daling, "Laboratory Testing of Dispersants Under Arctic Conditions", in *Proceedings of the Fifteenth Arctic Marine Oilspill Program Technical Seminar*, Environment

Canada, Ottawa, ON, pp. 123-134, 1992.

Brandvik, P.J., O.O. Knudsen, M.O. Moldestad and P.S. Daling, "Laboratory Testing of Dispersants Under Arctic Conditions", in *The Use of Chemicals in Oil Spill Response,* STP 1252, American Society for Testing and Materials, Philadelphia, PA, pp. 191-206, 1995.

Brown, H.M. and R.H. Goodman, "Dispersants in the Freshwater Environment", in *Oil Dispersants: New Ecological Approaches*, STP 1018, American Society for Testing and Materials, Philadelphia, PA, pp. 31-40, 1989.

Brown, H.M., J.S. Goudey, J.M. Foght, S.K. Cheng, M. Dale, J. Hoddinott, L.R. Quaife and D.W.S. Westlake, "Dispersion of Spilled Oil in Freshwater Systems: Field Trial of a Chemical Dispersant", *Oil and Chemical Pollution*, 6, pp. 37-54, 1990.

Byford, D.C., P.J. Green and A. Lewis, "Factors Influencing the Performance and Selection of Low-Temperature Dispersants", in *Proceedings of the Sixth Arctic Marine Oilspill Program Technical Seminar*, Environment Canada, Ottawa, ON, pp. 140-150, 1983.

Chen, H., L. Han, P. Luo and Y. Ye, "The Interfacial Tension between Oil and Gemini Surfactant Solution", *Surface Science*, 552, pp. L53-L57, 2004.

Chooro, M., N. Kamenka, B. Faucompre, S. Partyka, M. Lindheimer and R. Zana, "Miscellization and Adsorption of a Zwitterionic Surfactant: N-dodecyl Betaine - Effect of Salt", *Colloids and Surfaces A; Physicochemical and Engineering Aspects*, 110, pp. 249-261, 1996.

Clayton, J.R., G.H. Farmer, J.R. Payne, G.D. McNabb, P.C. Harkins, J.S. Evans, N.P. Rottlunda, C.R. Phillips and M.L. Evans, "Effects of Chemical Dispersant Agents on the Behavior and Retention of Spilled Crude Oil in a Simulated Streambed Channel", in *Oil Dispersants: New Ecological Approaches*, STP 1018, L.M. Flaherty (ed.), American Society for Testing and Materials, Philadelphia, PA, pp. 4-24, 1989.

Clayton, J.R., Jr., J.R. Payne, S.-F. Tsang, V. Frank, P. Marsden and J. Harrington, "Chemical Oil Spill Dispersants: Update State-of-the-Art on Mechanism of Action and Laboratory Testing for Performance", EPA/600/S-92/065, United States Environmental Protection Agency, Cincinnati, OH, 6 p., 1992.

Clayton, Jr., J.R., J.R. Payne and J.S. Farlow, *Oil Dispersants: Mechanisms of Action and Laboratory Tests*, C.K. Smoley for CRC Press, Boca Raton, FL, 103 p., 1993.

Davis, H.T., "Factors Determining Emulsion Type: Hydrophile-lipophile Balance and Beyond", *Colloids and Surfaces A; Physicochemical and Engineering Aspects*, 91, pp. 9-241, 1994.

Drummond, C. and J. Israelachvili, "Surface Forces and Wettability", *Journal of Petroleum Science and Engineering*, 33(1-3), pp. 123-133, 2002.

Fingas, M.F., I. Bier, M. Bobra and S. Callaghan, "Studies on the Physical and Chemical Behaviour of Oil and Dispersant Mixtures", in *Proceedings of the 1991 International Oil Spill Conference*, American Petroleum Institute, Washington, DC, pp. 419-426, 1991.

Fingas, M.F., D.A. Kyle and E.J. Tennyson, "Physical and Chemical Studies on Oil Spill Dispersants: Effectiveness Variation with Energy", in *Proceedings of the Fifteenth Arctic and Marine Oilspill Technical Seminar*, Environment Canada, Ottawa, ON, pp. 135-142, 1992.

Fingas, M.F., D.A. Kyle, Z. Wang, F. Ackerman and J. Mullin, "Testing of Oil Spill Dispersant Effectiveness in the Laboratory", in *Proceedings of the Seventeenth Arctic and Marine Oilspill Program Technical Seminar*, Environment Canada, Ottawa, ON, pp. 905-941, 1994.

Fingas, M.F., D.A. Kyle, P. Lambert, Z. Wang and J.V. Mullin, "Analytical Procedures for Measuring Oil Spill Dispersant Effectiveness in the Laboratory", in *Proceedings of the Eighteenth Arctic and Marine Oil Spill Program Technical Seminar*, Environment Canada, Ottawa, ON, pp. 339-354, 1995a.

Fingas, M.F., D.A. Kyle and E.J. Tennyson, "Dispersant Effectiveness: Studies Into the Causes of Effectiveness Variations", in *The Use of Chemicals in Oil Spill Response*, STP 1252, American Society for Testing and Materials, Philadelphia, PA, pp. 92-132, 1995b.

Fingas, M.F., Z. Wang, B. Fieldhouse and P. Smith, "Dispersed Oil Resurfacing with Time", in *Proceedings of the Twenty-Sixth Arctic and Marine Oil Spill Program Technical Seminar*, Environment Canada, Ottawa, ON, pp. 731-742, 2003.

Fjelde, I. and T. Austad, "HPLC Analysis of Salt Tolerant Mixtures of Ethoxylated and Non-ethoxylated Sulfonates Applicable in Enhanced Oil Recovery", *Colloids and Surfaces A; Physicochemical and Engineering Aspects*, 82, pp. 85-90, 1994.

Fjelde, I., T. Austad and J. Miller, "Adsorption VII. Dynamic Adsorption of a Dual Surfactant System onto Reservoir Cores at Seawater Salinities", *Journal of Petroleum Science and Engineering*, 13, pp. 193-201, 1995.

Flaherty, L.M., W.B. Katz and S. Kaufman, "Dispersant Use Guidelines for Freshwater and Other Inland Environments", in *Oil Dispersants: New Ecological Approaches*, STP 1018, L.M. Flaherty (ed.), American Society for Testing and Materials, Philadelphia, PA, pp. 25-30, 1989.

Fritz, D.E., "Effect of Salinity on Chemical Effectiveness", in *Proceedings of a Workshop on the Use of Chemical Countermeasures Product Data for Oil Spill Planning and Response*, NOAA, Seattle, WA, pp. 75-81, 1995.

Gay, S.M. and S.L. Vaughn, "Seasonal Hydrography and Tidal Currents of Bays and Fjords in Prince William Sound, Alaska", *Fisheries Oceanography*, 10(Suppl. 1), pp. 159-193, 2001.

George-Ares, A., R.R. Lessard, K.W. Becker, G.P. Canevari and R.J. Fiocco, "Modification of the Dispersant Corexit 9500 for Use in Freshwater", in *Proceedings of the 2001 International Oil Spill Conference*, American Petroleum Institute, Washington, DC, pp. 1209-1211, 2001.
Ghannam, M.T. and O. Chaalal, "Oil Spill Cleanup Using Vacuum Technique", *Fuel*, 82, pp. 789-797, 2003.

Guyomarch, J., S. Le Floch and F.-X. Merlin, "Effect of Suspended Mineral Load, Water Salinity and Oil Type on the Size of Oil-Mineral Aggregates in the Presence of Chemical Dispersant", *Spill Science and Technology*, 8, pp. 95-100, 2002.

Hou, W. and K.D. Papadopoulis, "$W_1/O/W_2$ and $O_1/W/O_2$ Globules Stabilized with Span 80 and Tween 80", *Colloids and Surfaces A; Physicochemical and Engineering Aspects*, Vol. 125, pp. 181-187, 1997.

Hou, W. and K.D. Papadopoulis, "Stability of Water-in-oil-in-water Type Globules", *Chemical Engineering Science*, 51, pp. 5043-5051, 1996.

Kaczmarski, J.P., M-R. Tarng, Z. Ma and J.E. Glass, "Surfactant and Salinity Influences on Associative Thickener Aqueous Solution Rheology", *Colloids and Surfaces A; Physicochemical and Engineering Aspects*, 147, pp. 39-53, 1999.

Kjøniksen, A-L., B. Nyström and B. Lindman, "Dynamic Light Scattering on Semidilute Aqueous Systems of Ethyl (hydroxyethyl) Cellulose. Effects of Temperature, Surfactant Concentration, and Salinity", *Colloids and Surfaces A; Physicochemical and Engineering Aspects*, 149, pp. 347-354, 1999.

Lehtinen, C.M. and A.M. Vesala, "Effectiveness of Oil Spill Dispersants at Low Salinities and Low Water Temperatures", in *Oil Spill Chemical Dispersants: Research, Experience and Recommendations*, T.E. Allen (ed.), STP 840, American Society for Testing and Materials, Philadelphia, PA, pp. 108-121, 1984.

Li, J.-L. and B.-H. Chen, "Solubilization of Model Polycyclic Aromatic Hydrocarbons by Nonionic Surfactants", *Chemical Engineering Science*, 57, pp. 2825-2835, 2002.

Li, X. and H. Kunieda, "Catanionic Surfactants: Microemulsion Formation and Solubilization", *Current Opinion in Colloid and Interface Science*, Vol. 8, pp. 327-336, 2003.

Liu, Q., M. Dong, W. Zhou, M. Ayub, Y.P. Zhang and S. Huang, "Improved Oil Recovery by Adsorption-desorption in Chemical Flooding", *Journal of Petroleum Science and Engineering*, 2004. (in press)

Martinelli, F.N. and B.W.J. Lynch, "Factors Affecting the Efficiency of Dispersants", LR 363 (OP), Warren Spring Laboratory, Stevenage, 18 p., 1980.

Mackay, D., "Effectiveness of Chemical Dispersants Under Breaking Wave Conditions", in *The Use of Chemicals in Oil Spill Response*, STP 1252, American Society for Testing and Materials, Philadelphia, PA, pp. 310-340, 1995.

McAuliffe, C.D., "The Use of Chemical Dispersants to Control Oil Spills in Shallow Nearshore Waters", STP 1018, American Society for Testing and Materials, Philadelphia, PA, pp. 49-72, 1989.

Moet, A., M.Y. Bakr, M. Abdelmonim and O. Abdelwahab, "Factors Affecting Measurements of the Efficiency of Spilled Oil Dispersion", *Preprints - Symposium on General Papers and Poster*

Session for the Division of Petroleum Chemistry, 210 National Meeting, American Chemical Society, Vol. 40, pp. 564-566, 1995.

Moles, A., L. Holland and J. Short, "The Effectiveness of Corexit 9527 and 9500 in Dispersing Fresh, Weathered and Emulsion of Alaska North Slope Crude Oil Under Subarctic Conditions", Prince William Sound Regional Citizens' Advisory Council, Anchorage, AK, 24 p., 2001.

Moles, A., L. Holland and J. Short, "Effectiveness in the Laboratory of Corexit 9527 and 9500 in Dispersing Fresh, Weathered and Emulsion of Alaska North Slope Crude Oil Under Subarctic Conditions", *Spill Science and Technology Bulletin*, 7, pp. 241-247, 2002.

Mollet, C., Y. Touhami and V. Hornof, "A Comparative Study of the Effect of Ready-Made and In Situ Formed Surfactants on Inter-facial Tension Measured by Drop Volume Tensiometry", *Journal of Colloid and Interface Science*, 178, pp. 523-530, 1996.

Moosai, R. and R.A. Dawe, "Gas Attachment of Oil Droplets for Gas Flotation for Oily Wastewater Cleanup", *Separation and Purification Technology*, 33, pp. 303-314, 2003.

Park, S-K. and A.R. Bielefeldt, "Equilibrium Partitioning of a Non-ionic Surfactant and Pentachlorophenol between Water and a Non-aqueous Phase Liquid", *Water Research*, 37, pp. 3412-3420, 2003.

Peabody, C.H., "Freshwater Dispersant Study", in *Proceedings of the Fifth Arctic Marine Oilspill Program Technical Seminar*, Environment Canada, Ottawa, ON, pp. 265-274, 1982.

Prosser, A.J. and E.I. Franses, "New Thermodynamic/Electrostatic Models of Adsorption and Tension Equilibria of Aqueous Ionic Surfactant Mixtures: Application to Sodium Dodecyl Sulfate/sodium Dodecyl Sulfonate Systems", *Journal of Colloid and Interface Science*, 263, pp. 606-615, 2003.

Sabatini, D.A., E. Acosta and J.H. Harwell, "Linker Molecules in Surfactant Mixtures", *Current Opinion in Colloid and Interface Science*, 8, pp. 316-326, 2003.

Sayyouh, M.H., M.S. Al-Blehed and A.M. Attla, "The Effect of Alkaline and Polymer Additives on Phase Behaviour of Surfactant-oil-brine System at High Salinity Conditions", *Revue de L'Institut Francais du Petrole*, 48, pp. 359-369, 1993.

Scott, B.F., E. Nagy, J.P. Sherry, B.J. Dutka, V. Glooschenko, N.B. Snow and P.J. Wade, "Ecological Effects of Oil-Dispersant Mixtures in Fresh Water", in *Proceedings of the 1979 International Oil Spill Conference*, American Petroleum Institute, Washington, DC, pp. 565-571, 1979.

Song, F.Y. and M.R. Islam, "Effect of Salinity and Rock Type on Sorption Behaviour of Surfactants as Applied in Cleaning of Petroleum Contaminants", *Journal of Petroleum Science and Engineering*, 10, pp. 321-336, 1994.

Sterling, M.S., J.S. Bonner, A.N.S. Ernest, C.A. Page and R.L. Autenreith, "Chemical Dispersant

Effectiveness Testing: Influence of Droplet Coalescence", *Marine Pollution Bulletin*, 48, pp. 969-977, 2004.

Vaughn, S.L., C.N.K. Mooers and S.M. Gay, "Physical Variability in Prince William Sound during the SEA Study (1994-98)", *Fisheries Oceanography*, 10(Suppl. 1), pp. 58-80, 2001.

Watt, S.L., D. Tunaley and S. Biggs, "The Formation of Water-in-oil Microemulsions Using a Concentrated Saline Aqueous Solution", *Colloids and Surfaces A; Physicochemical and Engineering Aspects*, 137, pp. 25-33, 1998.

Wells, P.G. and G.W. Harris, "Dispersing Effectiveness of Some Oil Spill Dispersants: Testing with the 'Mackay Apparatus'", *Spill Technology Newsletter*, 4, pp. 232-241, 1979.

Wolfe, M.F., G.J.B. Schwartz, S. Singaram, E.E. Mielbrecht, R.S. Tjeerdema and M.L. Sowby, "Effects of Salinity and Temperature on the Bioavailability of Dispersed Petroleum Hydrocarbons to the Golden-Brown Algae, *Isochrysis galbana*", *Archives of Environmental Contamination and Toxicology*, 35, pp. 268-273, 1998.

Wolfe, M.F., H.E. Olsen, K.A. Gasuad, R.S. Tjeerdema and M.L. Sowby, "Induction of Heat Shock Protein (hsp)60 in *Isochrysis galbana* Exposed to Sublethal Preparations of Dispersant and Prudhoe Bay Crude Oil", *Marine Environmental Research*, 47, pp. 473-489, 1999.

Wu, J., Y. Xu, T. Dabros and H. Hamza, "Development of a Method for Measurement of Relative Solubility of Nonionic Surfactants", *Colloids and Surfaces A: Physiocochem Engineering Aspects*, 232, pp. 229-237, 2004.

Ysambertt, F., R. Anton and J-L. Salager, "Retrograde Transition in the Phase Behaviour of Surfactant-Oil-Water Systems Produced by an Oil Equivalent Alkane Carbon Number Scan", *Colloids and Surfaces A; Physicochemical and Engineering Aspects*, 125, pp. 131-136, 1997.

Yu, H., B.K. Man, L.L.Chan, M.H. Lam, P.K. Lam, L. Wang, H. Jin and R.S. Wu, "Cloud-point Extraction of Nodularin-R from Natural Waters", *Analytica Chimica Acta*, 509, pp. 63-70, 2004.

Zhang, L., L. Luo, S. Zhao, Z-C. Xu, J-Y An and J-Y Yu, "Effect of Different Acidic Fractions in Crude Oil on Dynamic Interfacial Tensions in Surfactant/alkali/model Oil Systems", *Journal of Petroleum Science and Engineering*, 41, pp. 189-198, 2004.

Available online at www.sciencedirect.com
ScienceDirect
Marine Pollution Bulletin 54 (2007) 983–993
MARINE POLLUTION BULLETIN
www.elsevier.com/locate/marpolbul

Effects of chemical dispersants and mineral fines on crude oil dispersion in a wave tank under breaking waves

Zhengkai Li [a,*], Paul Kepkay [a], Kenneth Lee [a], Thomas King [a], Michel C. Boufadel [b], Albert D. Venosa [c]

[a] Center for Offshore Oil and Gas Environmental Research, Bedford Institute of Oceanography, Fisheries and Oceans Canada, P.O. Box 1006, Dartmouth, Canada NS B2Y 4A2
[b] Civil and Environmental Engineering Department, Temple University, Philadelphia, PA 19122, USA
[c] National Risk Management Research Laboratory, US EPA, Cincinnati, OH 45268, USA

Abstract

The interaction of chemical dispersants and suspended sediments with crude oil influences the fate and transport of oil spills in coastal waters. A wave tank study was conducted to investigate the effects of chemical dispersants and mineral fines on the dispersion of oil and the formation of oil–mineral-aggregates (OMAs) in natural seawater. Results of ultraviolet spectrofluorometry and gas chromatography flame ionized detection analysis indicated that dispersants and mineral fines, alone and in combination, enhanced the dispersion of oil into the water column. Measurements taken with a laser in situ scattering and transmissometer (LISST-100X) showed that the presence of mineral fines increased the total concentration of the suspended particles from 4 to 10 l l l^{ffi1}, whereas the presence of dispersants decreased the particle size (mass mean diameter) of OMAs from 50 to 10 l m. Observation with an epifluorescence microscope indicated that the presence of dispersants, mineral fines, or both in combination significantly increased the number of particles dispersed into the water.
┐ 2007 Elsevier Ltd. All rights reserved.

Keywords: Crude oil; Dispersants; Mineral fines; Oil droplets; OMAs; Breaking waves

1. Introduction

Marine oil spills have become more common as offshore oil production and transport increased over the last few decades (Anderson and LaBelle, 2000; NRC, 2003). The detrimental impacts of crude oil spills in the open ocean are largely mitigated by natural dispersion processes where wave action leads to the formation of micron-sized droplets that are eventually diluted to concentrations below toxic threshold limits (Delvigne and Sweeney, 1988; Lee, 2002; Li and Garrett, 1998; Shaw, 2003; Tkalich and Chan, 2002). In nearshore or estuarine waters, oil droplets are likely to be incorporated into oil–mineral-aggregates (OMAs) as a result of their interaction with the high loads of suspended particulates that are typical in coastal regions (Bragg and Owen, 1995; Le Floch et al., 2002; Owens and Lee, 2003; Owens et al., 2003; Page et al., 2000). Detailed studies of OMA formation have revealed that both mineral fines and organic particles can stabilize oil droplets within the water column (Bragg and Yang, 1995; Delvigne et al., 1987; Lee, 2002; Lee and Stoffyn-Egli, 2001; Lee et al., 1996, 2003; Muschenheim and Lee, 2002; Omotoso et al., 2002). The results of laboratory experiments (Cloutier et al., 2002; Lee et al., 1997; Omotoso et al., 2002; Stoffyn-Egli and Lee, 2002) and shoreline field trials (Lee et al., 1997; Lunel et al., 1997; Owens et al., 1995; Owens and Lee, 2003) have demonstrated that the production of OMAs enhances the natural dispersion of oil spills and reduces their environmental persistence by enhancing bioremediation.

* Corresponding author. Tel.: +1 902 426 3442; fax: +1 902 426 6695.
E-mail address: liz@mar.dfo-mpo.gc.ca (Z. Li).

0025-326X/$ - see front matter ┐ 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2007.02.012

There is a renewed interest to consider the use of chemical oil dispersants to combat oil spills at sea to minimize the impact of oil slicks on seabirds and the transport of oil to coastal and/or shoreline environments. The utilization of dispersants in coastal regions has been approached with caution due to concerns over the potential exposure of both pelagic and benthic organisms to chemically-dispersed oil. Conflicting results have been reported with regard to the effects of chemical dispersants on formation and fate of OMAs in both laboratory and field studies (Gearing and Gearing, 1983; Guyomarch et al., 1999, 2002; MacKay and Hussain, 1982; Page et al., 2000). For example, MacKay and Hussain (1982) and Gearing and Gearing (1983) found that OMA formation was negligible with chemically-dispersed oil in water column, whereas Guyomarch et al. (1999, 2002) reported that significant amounts of oil were incorporated into OMAs when oils and chemical dispersants were mixed with mineral fines.

The application of dispersants may alter the rate and extent of oil–mineral aggregate formation relative to that which occurs between oil and mineral fines alone. On one hand, chemical dispersants could reduce droplet size and lead to the formation of smaller and denser OMAs compared to aggregates formed in the absence of the dispersants. On the other hand, chemical dispersants may change the surface physicochemical properties of oil droplets to impair the binding of oil to mineral fines. To distinguish between these possibilities, we carried out a wave tank experiment to: (1) investigate the aggregation of mineral fines with physically or chemically-dispersed oil and (2) determine the dynamic particle size distributions of the physically- and chemically-dispersed oil and OMAs. Data generated from this study are useful in modeling the fate and transport of the dispersed oil and for conducting risk assessment related to the application of chemical dispersants in nearshore waters and estuarine environments that are rich in suspended particulates.

2. Methods

2.1. Wave tank facility and the generation of breaking waves

The oil–mineral aggregation experiment was conducted at a wave tank facility located at the Bedford Institute of Oceanography (Dartmouth, Nova Scotia, Canada) which was designed and constructed under a Joint Project Agreement between Fisheries and Oceans Canada and the US Environmental Protection Agency. The wave tank facility measures 16 m long, 0.6 m wide, and 2 m high, with an average water depth of 1.25 m (Fig. 1). Different wave patterns are generated by a paddle situated 1 m from the front wall of the tank. The paddle is linked to an adjustable cam that controls stroke length to alter wave height. The wave frequency (and thus the wavelength) is controlled by the rotation speed of the cam. Wave absorbers are made of eight porous stainless-steel screens and were placed opposite the wave maker to minimize wave reflection by dissipation wave energy upon passing of the waves through them. The pore sizes of the screens ranged from 0.2 to 2.5 cm and the area porosity were approximately between 40% and 70%. The order of the screens and their spacing were obtained by trial and error to minimize reflection.

The wave tank simulated the propagation and breaking of deep water waves based on linear wave theory. Breaking waves cause velocity shear and hence result in the mixing of oil and dispersant and the dispersion of oil slick (Nilson et al., 1985; Shaw, 2003; Tkalich and Chan, 2002). Velocity shear with its associated friction causes the dissipation of kinetic energy of the fluid. Of particular interest is the kinetic energy dissipation rate per unit mass (e), a scalable parameter in evaluating the extent of mixing and dispersion of oils at sea (Delvigne and Sweeney, 1988; Kaku et al., 2006) and the interaction kinetics between oil and suspended particulate matters (Payne et al., 2003).

For this study, the paddle stroke of the wave tank was set at 7 cm to generate a train of high frequency waves (1.0 Hz with a wave height of 0.12 m and wave length of 1.56 m) for 20 s, followed by a cycle of low frequency waves (0.4 Hz with a wave height of 0.05 m and wave length of 9.75 m) for 20 s. Because the propagation speed of a wave is proportional to its period, the train of large period waves eventually catches up with the train of short period waves. This design was made to create recurrent breaking waves approximately 7.5 m downstream of the paddle. The energy dissipation rate in the mixing zone of the wave tank was estimated through the water profile measurements using seven wave gauges and a time-series of water velocity measurements at various depths and locations (Venosa et al., 2005). The energy dissipation rate in the mixing zone was computed to decrease from 0.5 W/kg (or m^2/s^3) at the surface to about 0.01 W/kg at the depth of 25 cm. This mixing energy level corresponded to the lower end of the breaking waves of the surface layer at sea (Delvigne and Sweeney, 1988).

2.2. Experimental procedures for oil dispersion study in the wave tank

A two-factor, two-level [dispersant (0, 3 ml) and mineral fines (0, 50 g)] factorial experimental design was used to investigate the effects of chemical dispersants on the formation and fate of OMAs. The test oil was 150 ml of weathered Scotia Shelf Condensate, a light crude oil (with a density of 0.8690 mg l^{ffi1}, dynamic viscosity of 6 mPa s and oil/water interfacial tension of 18.4 mN m^{ffi1}). The dispersant was Corexit 9500 (Nalco Energy Service, L.P. Sugar Land, TX) at a dispersant-to-oil (DOR) ratio of 1:50. The mineral fines were American Petroleum Institute (API No. 9) kaolin (Mesa Alta, New Mexico), with a cation exchange capacity of 6.8 meq/100 g, a median particle size of 0.6 lm, and a density of 2.60 g/cm^3. The wave tank was filled with filtered (5 lm) natural seawater from the Bedford Basin of Halifax harbor (with average water

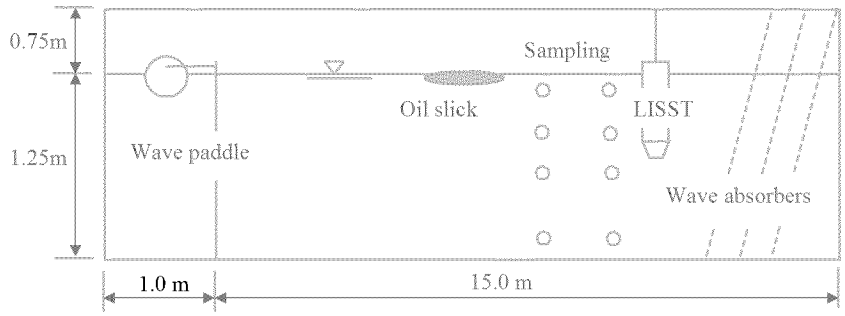


Fig. 1. Schematic representation of the wave tank: LISST is laser in situ scattering and transmission particle counter; sampling ports are stainless-steel tubes connected to a gang of 100 ml syringes.

temperature of 5 °C and salinity 30 PSU). For each experiment, one liter of seawater containing a combination of oil, dispersant, and mineral fines (defined by the experimental design matrix) was premixed in a 2-L flask on a reciprocating shaker at 150 cycles per min and 22 mm stroke length for 2 h; the mixing energy level has been found to be the minimum rate to disrupt the layer of surface oil (Khelifa et al., 2005; Stoffyn-Egli and Lee, 2002). The mixture was then released on the surface in the middle of the tank immediately prior to an incoming breaking wave. Samples (100 ml) were collected simultaneously from two horizontal locations (1.5 and 4 m downstream of oil application) and four depths (5, 20, 60, and 110 cm under water) at five time points (1, 10, 30, 60, and 300 min after oil addition). The side and bottom walls and the wave absorbers were thoroughly cleaned after each experimental run to minimize the residual oil amount that was left in the system and to reduce the influence of the oil input for the following runs. The distribution of oil in the tank was determined by (1) ultraviolet fluorescence (UVF) spectroscopy of samples taken directly from the wave tank and (2) gas chromatography with flame ionization detection (GC-FID) analysis of total petroleum hydrocarbons (TPH) after solvent extraction. The dynamic particle size distribution and total suspended particle concentration were measured using a laser in situ scattering and transmissometer (LISST-100X). The morphology of the formed oil droplets and OMAs were observed by an epifluorescence microscopy. Description of these methods is detailed below.

2.3. Oil dispersion and OMA formation by UVF

The ultraviolet fluorescence spectroscopy technique of Kepkay et al. (2002) was applied to measure the dissolved/dispersed oil in aqueous phase and the oil aggregated into OMAs. Samples collected from the wave tank were vigorously shaken and 3 ml of the suspension were rapidly transferred to an ultraviolet-grade methacrylate disposable cuvette (VWR International Inc., Mississauga, ON). The suspension was then immediately scanned in the dissolved/dispersed fraction and aggregated fraction using a QM-1 spectrofluorometer running FeliX software (PTI, Inc., Birmingham, NJ). The optimal excitation wavelength that produced the highest emission peaks was 320 nm; this wavelength with a slit width of ±2 nm was used in all subsequent emission scans from 340 to 500 nm.

2.4. Oil dispersion and OMA formation by GC-FID

Samples (100 ± 20 ml) collected from the tank were filtered through micro-fiber-glass filters (1.2 lm, Whatman GF/C, Fisher Scientific, Canada) to separate the dissolved/colloidal phase from the aggregated phase. The filtrate was transferred to a separatory funnel for extraction of hydrocarbon with dichloromethane (DCM) using EPA method 3510 C (EPA, 2003). The solid phase was processed using soxhlet extraction with dichloromethane over an 18 h cycle with EPA method 3540 C (EPA, 2003). The extracts from both phases were concentrated to 1 ml and analyzed by GC-FID (NRCC, 1997) to measure total petroleum hydrocarbon (TPH) in the filtrate phase (mg TPH per liter of tank water) and the particulate phase (mg TPH per gram of solids). The GC analysis was performed using high resolution Agilent 6890 gas chromatography coupled to an Agilent 5973N flame ionization detector (Agilent Technologies, Willmington, DE, USA) and an MDN-5S column of 30 m · 0.25 mm id 0.25 lm film thickness (Supelco, Canada); the GC conditions are: helium carrier gas at 1 ml/min, cool on-column injection with oven track mode (track 3 °C higher than the oven temperature program), hold at 50 °C for 2 min, ramp at 30 °C/min to 300 °C and hold 10 min at this temperature; the FID conditions are: 320 °C, hydrogen flow 40 ml/min, air flow 450 ml/min and helium flow at 45 ml/min. These conditions are designed to measure the C10–C35 hydrocarbons, a range representing the major components identified in the sample extracts. A fuel oil standard (Restek Diesel Fuel #2, 50% weathered; Bellefonte, PA, USA), which represents an ideal quantification range from C10 to C35, was used to prepare a 5-point calibration curve based on the average response factors of the total peak area of the standard chromatogram. For quality assurance, a method blank was prepared with each batch of ten

samples, and the method recoveries (89 ± 13% soxhlet and 90 ± 14% dissolve extractions) were monitored to ensure adequate accuracy and precision. These recoveries are comparable to surrogate recoveries using the same extraction procedure and using gas chromatography mass spectrometry (GC-MS).

The ratio of the TPH in the two phases (l g^{ffi1}) is a measurement of the extent of partitioning of TPH between liquid and solid phases. Oil distribution as TPH in the bulk aqueous phase was also calculated as the sum of dissolved/colloidal or aggregate phases inside the tank.

2.5. Particle size distribution using LISST-100X

Oil droplet size distribution inside the wave tank was determined by a Type C laser in situ scattering and transmissometer (LISST-100X; Sequoia, Seattle, WA) situated 4.5 m downstream from oil application in the wave tank with the detection window at a depth of 0.6 m. This location was approximately in the middle of the tank downstream from oil addition both horizontally (between oil addition and the end) and vertically (from the surface to the bottom) to represent overall particle distribution downstream. The particle size distributions were subdivided into 32 particle size intervals located logarithmically from 2.50 to 500 lm in diameter, with the upper size in each bin 1.18 times the lower. Particle size distribution was expressed as the average volumetric concentration of particles falling into each interval of the size range. In general, the particle size distribution from LISST fits a lognormal distribution, and the mass (or volume) mean diameter of the dispersed oil droplets is used to quantitatively compare the average size of the particles. The LISST-100X was operated in real time mode to acquire dynamic particle size distribution and total particle concentrations (from 2.5 to 500 lm) every 3 s.

2.6. Epifluorescence microscopy of dispersed oil and oil–mineral-aggregates (OMAs)

Samples were collected for observation of dispersed oil droplets under transmitted light and ultraviolet-epifluoresence illumination (excitation filter 340–380 nm; reflection short pass filter 400 nm; suppression long pass filter 430 nm) using a Leitz Orthplan microscope (Lee et al., 1985). The microscope is fitted with an Olympus DP70 digital camera, an HBO 50 mercury lamp, and an OptiScan computer-controlled motorized stage (Prior Scientific Inc., Rockland, MA). Photomicrographs of the physically- and chemically-dispersed oil droplets and OMAs were recorded at magnifications of 160· or 400· and quantified using image analysis software Image-Pro5.0 (Media Cybernetics Inc. Silver Spring, MD). Enumeration results were reported as number of particles per unit volume of water samples.

3. Results

3.1. Distribution of oil and oil–mineral aggregates in the wave tank

Distribution of crude oil dispersed in the wave tank was quantified by three indicators: the UVF emission spectra of dispersed and/or aggregated oil, the GC-FID analysis of TPH, and the LISST-100X for total oil-containing particle concentration in the bulk aqueous phase.

The UVF spectra from the wave tank samples (Fig. 2) were similar to those obtained from results of previous flask experiments, where the aggregation of oil with mineral fines results in distinct shifts in the spectra compared to oil dispersed in seawater (Kepkay et al., 2002). The UVF spectra emission peaks at various excitation ranges are correlated to the different dissolved/dispersed fractions of crude oil (ASTM, 2002): most single ring aromatics, such as BTEX, phenol, and cresol, produce strong emission peaks between 290 and 320 nm; lower and medium molecular weight aromatics, such as naphthalene and phenanthrene, generate peaks at 325–370 nm; and higher molecular weight multi-ring compounds, such as resins, form another major broad peak from 400–470 nm. Since the concentration of single ring aromatics have likely been reduced from the weathering of the Scotia Shelf Condensate and the solubility of these compounds are high, the effects of dispersants and mineral fines on their partitioning into the bulk water phase are probably insignificant.

The treatment effects on the vertical dispersion of oil at the 1.5 m downstream location were clear: the natural dispersion of oil was limited in its effectiveness (Fig. 2a); the action of dispersant (Fig. 2b) or mineral fines (Fig. 2c) on their own enhanced oil dispersion; and the action of mineral fines and dispersant in combination was the most effective in dispersing the oil into the bulk aqueous phase (Fig. 2d). Samples taken at 4 m downstream location did not exhibit strong UVF signals over all depths after 1 min.

After 10 min, emission spectra from samples taken at different depths and horizontal locations became similar for the same treatment, indicating that breaking waves resulted in a complete mixing downstream of the breaking waves. Hence, the fluorescence intensity was presented in Fig. 3 as an average value from samples taken at the four different depths and two horizontal locations (8 readings) for each treatment at each time point (from 10 to 300 min). The average fluorescence intensity of oil over 340–370 nm emission bandwidth followed the general order: oil < oil + kaolin < oil + dispersant < oil + dispersant + kaolin (Fig. 3a). After 300 min of dispersion, the chemically-dispersed oil and the oil dispersed by mineral fines exhibited average emissions between 340 and 370 nm that were 2–3.5 times higher than the naturally dispersed oil. The dispersant and kaolin in combination increased the average fluorescence between 340 and 370 nm by 5.6-fold relative to the natural oil. The dispersal of oil fractions with fluorescence between 400 and 480 nm

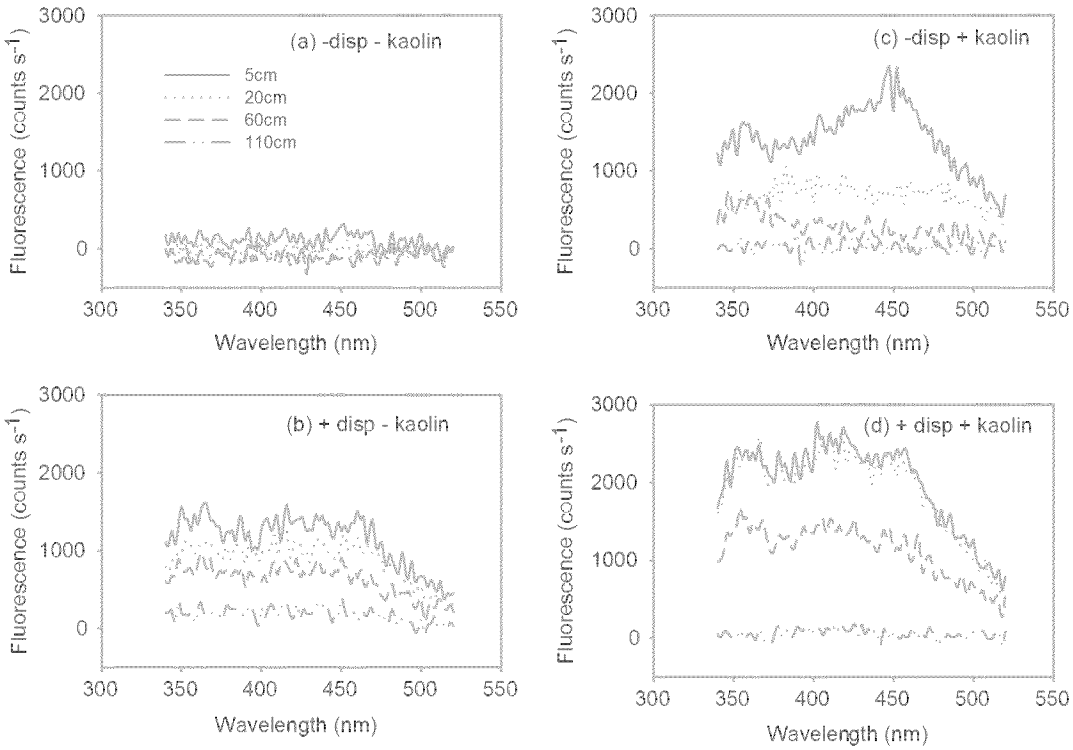


Fig. 2. Ultraviolet Emission spectra of Scotia Light Condensate dispersed at 4 depths (5, 20, 60, and 110 cm) 1.5 m downstream 1 min after addition into seawater in combination with low concentrations of kaolin particles and/or oil dispersant under breaking waves. All the UVF spectra have been corrected for natural fluorescence and light scattering by blanks of seawater used in each wave tank treatment.

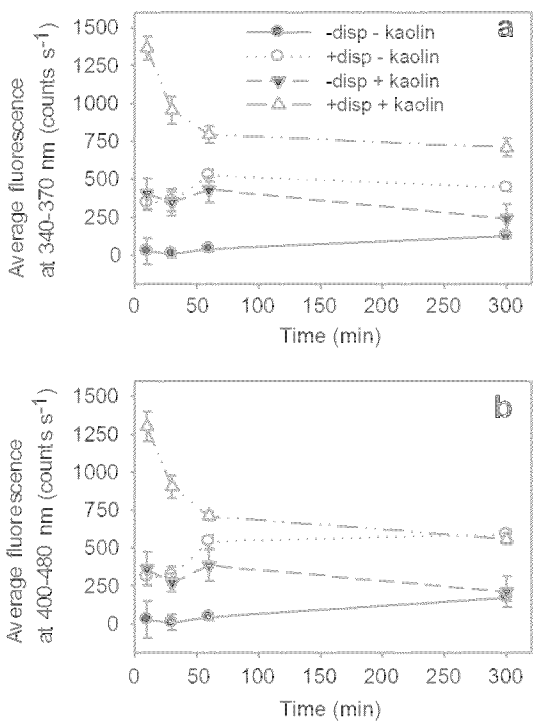


Fig. 3. Ultraviolet fluorescence measurement of (a) dispersed oil fraction with average fluorescence at 340–370 nm and (b) oil fraction with average fluorescence at 400–480 nm over time. Results shown are the tank average value for each time point and error bars represent one standard deviation of eight samples.

followed a similar pattern, except that kaolin on its own did not increase the final fluorescence at 300 min, whereas dispersants or kaolin plus dispersants increased average fluorescence by three times. Together, these results suggest that chemical dispersants, mineral fines, and the two in combination stimulated the dispersion of two-ring and multiple-ring aromatic fractions of oil into the bulk aqueous phase.

The distribution of oil as a result of dispersal by dispersants and mineral fines in the wave tank was further quantified with GC-FID analysis of total petroleum hydrocarbons (TPH) from samples collected at four depths and two locations. Similar to the UVF results, TPH concentrations from both the dissolved/colloidal and aggregated phases exhibited no temporal and spatial difference among samples taken at time points after 10 min due to the mixing effect in the wave tank. Hence the average concentrations of dissolved/colloidal TPH in the seawater, in the aggregated solid phase, and the total TPH from two horizontal locations and four depths (8 readings) were calculated; the results are presented in Fig. 4.

The presence of chemical dispersants significantly ($P = 0.01$) increased the concentration of dissolved/colloidal oil in the wave tank (Fig. 4a); the presence of mineral fines appears to have reduced the amount of soluble/colloidal oil in seawater. Conversely, the presence of chemical dispersants ($P = 0.007$) and the combination of the two ($P = 0.00001$) significantly increased the concentration of

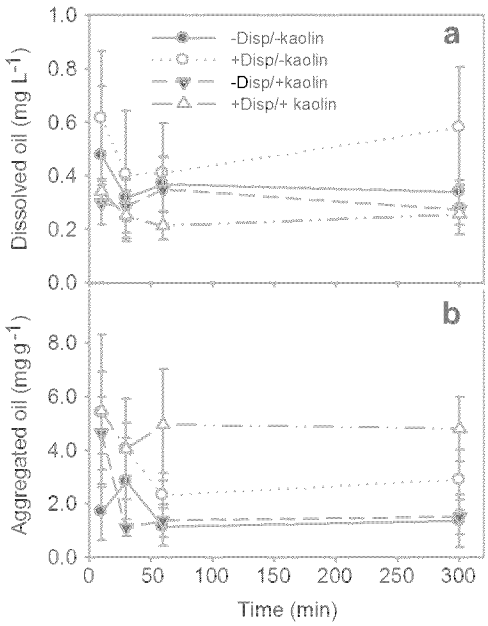


Fig. 4. GC-FID measurement of: (a) dissolved oil (mg per liter of seawater) and (b) aggregated oil (mg per gram of particles). Results shown are the average value for each time point and error bars represent one standard deviation of eight samples.

TPH associated with aggregates within the solid phase (Fig. 4b). The effects of dispersants and mineral fines on oil dispersion in the water column of the wave tank are also revealed by the partitioning ratios of oil between the liquid phase and the solid phase after 300 min dispersion (Fig. 5). Under natural dispersion of oil alone, the liquid to solid ratio was probably due to the ratio of insoluble/aggregated components to the soluble/colloidal fraction of the TPH. The presence of either mineral fines or dispersants alone increased the partitioning ratio, suggesting that either part of the insoluble/aggregated TPH has been dispersed by the presence of chemical dispersants, or part of the TPH was absorbed by mineral fines and hence the overall density was increased to overcome the buoyancy. The presence of both dispersants and mineral fines increased the ratio further, indicating that the combination of the two had synergistic effects, stimulating the entrainment of oil into the bulk aqueous phase.

3.2. Particle size distribution

The dynamic concentrations of suspended particles (oil droplets or OMAs) in the bulk aqueous phase were measured by the LISST-100X and the results are shown in Fig. 6. In the absence of mineral fines, the total dispersed oil concentrations first increased to a maximum at 10 min and then decreased gradually to a relatively constant value. In the presence of mineral fines, however, the peak concentrations were detected earlier and gradually decreased with time. The total particle concentrations in the presence of mineral fines were always two times higher than in their absence, with the final total average concentrations being 10 and 4 l l l[ffi1], respectively. Chemical dispersants did not affect total suspended particle concentrations, probably due to the vigorous mixing caused by breaking waves.

Fig. 7 displays the time-series particle size distribution expressed as mass mean diameter (MMD) of the particles measured by using the LISST-100X. The natural (physical) dispersion of oil in the absence of either dispersant or mineral fines first produced large droplets (with MMDs of 150 lm) that gradually decreased in size to MMDs of approximately 40 lm after continuous dispersion under breaking waves for 200 min. The presence of mineral fines reduced the initial particle size to MMDs of about 70 lm, but the OMAs with these sizes appear to become stable and did not break up further. The presence of dispersant dramatically reduced the sizes of both dispersed oil droplets (+disp/ffikaolin) and OMAs (+disp/+kaolin) to MMDs of 15–25 lm in the beginning, and further reduced the MMDs to nearly 10 lm after 200 min dispersion.

Fig. 8 presents typical distributions of particle size that were recorded by LISST-100X for different treatments over four representative time points; the particle size statistics are listed in Table 1. In the absence of chemical dispersants and mineral fines, naturally dispersed oil has relatively large oil droplets size initially, with the majority of the particles present in a lognormal distribution (Fig. 8a), and

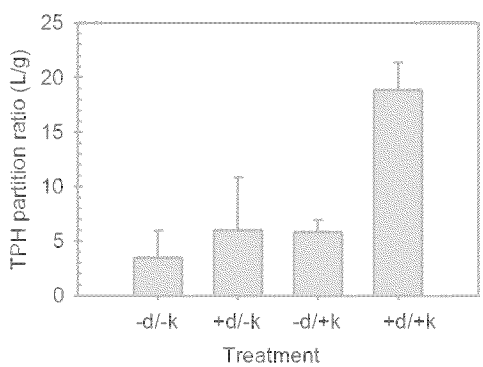


Fig. 5. Treatment effects on the partitioning ratio of oil between dissolved/colloidal phase and solid phase (d: dispersant; k: kaolin).

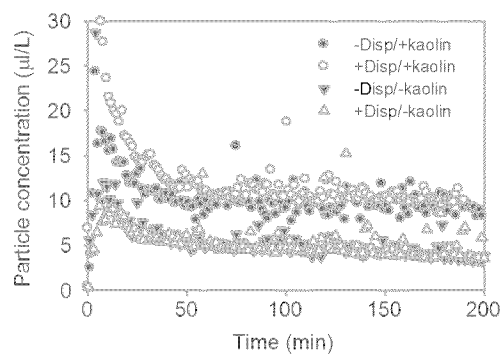


Fig. 6. LISST measurement of total particle concentration of dispersed oil and OMAs. Note that the maximum value occurred at time zero for situations where mineral fines were added.

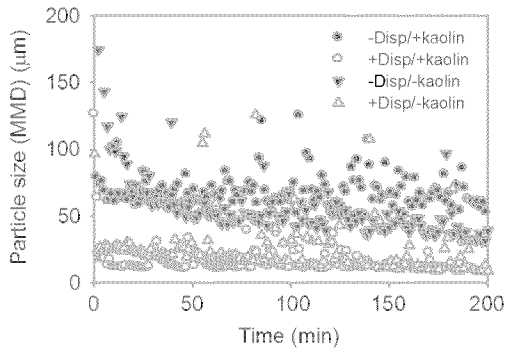


Fig. 7. LISST measurement of mass mean particle diameter of dispersed oil and OMAs.

more than 50% of the dispersed oil volume present as droplets larger than 200 lm (mass medium diameter, or $d_{50\%}$) and a geometric standard deviation (GSD) of 1.90 (Table 1). Further dispersion of the oil by breaking waves gradually reduced the size of oil droplets over time and resulted in a shift of the peak area of lognormal size distribution and a decrease of the peak height (Fig. 8a), so that the $d_{50\%}$ of the droplet size was almost an order of magnitude lower than initial size after 300 min of turbulent mixing by breaking waves (Table 1). The presence of chemical dispersants dramatically decreased the initial droplet size (Fig. 8b), with a $d_{50\%}$ of only 22 lm, an order-of-magnitude lower than in the absence of dispersants. This is because the dispersants dramatically reduced the surface tension of the oil, which was broken into small droplets when it was homogenized on the reciprocating shaker. Further dispersion of oil in the presence of chemical dispersants in wave tank generated large amount of smaller particles so that

Table 1
Statistics of the particle size distribution of the dispersed oil droplets or OMAs

Treatment	Time (min)	$d_{16\%}$ [a]	$d_{50\%}$ [a]	$d_{84\%}$ [a]	GSD [b]
ffiDisp/ffised	1	75	200	270	1.90
	10	54	128	240	2.11
	60	23	50	77	1.83
	300	3.5	30	57	4.04
+ Disp/ffised	1	5	22	39	2.79
	10	7	22	37	2.30
	60	3	20.5	34	3.37
	300	<2.5	2.7	11	NA
ffiDisp/+sed	1	25	70	112	2.12
	10	4	53	102	5.05
	60	4.5	54	95	4.59
	300	6	54	80	3.65
+ Disp/+sed	1	3.5	10.5	23	2.56
	10	3	10	22	2.71
	60	3	10	27	3.00
	300	3	9.5	17	2.38

[a] $d_{16\%}$, $d_{50\%}$, $d_{84\%}$ represent the 16%, 50%, or 84% of total mass of the droplets that are smaller than this diameter (lm); $d_{50\%}$ is the mass medium diameter.
[b] GSD is geometric standard deviation calculated as: $(d_{84\%}/d_{16\%})^{1/2}$; the GSD value reflects the variation of the particle size.

after 300 min 84% of droplets were less than 11 lm in diameter and 50% were less than 3 lm (Table 1). The presence of mineral fines also decreased the initial particle size compared to the natural dispersion of oil (Fig. 8c). However, the formed oil–mineral aggregates appear to be more stable than their counterparts of the dispersed oil droplets. The shift of peak location on the size distribution profiles was not observed over time, only the variation of peak area

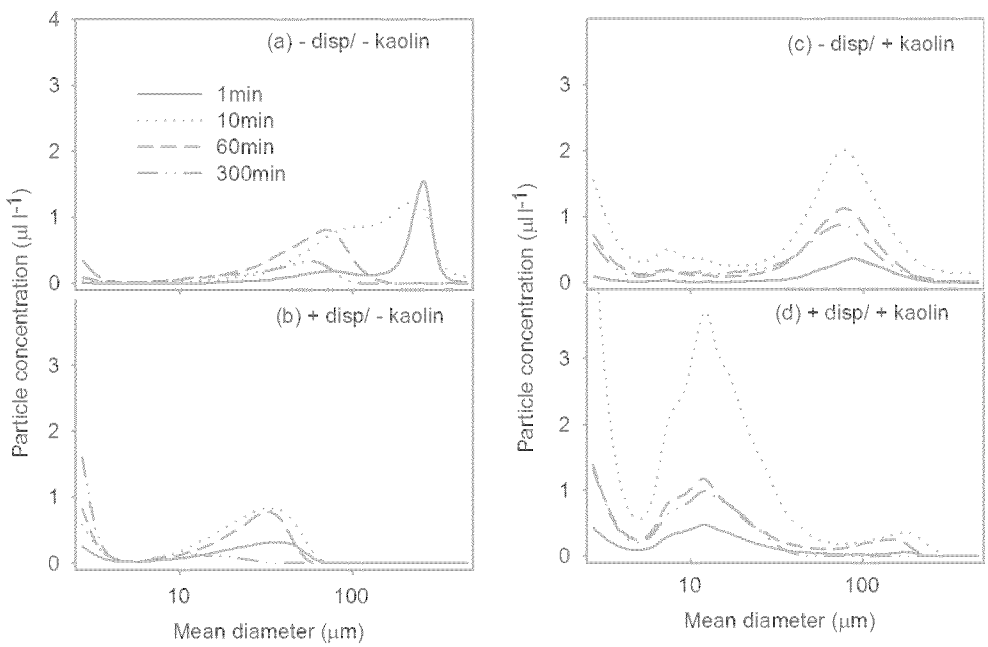


Fig. 8. LISST measurement of time-series particle size distribution in volumetric concentration as a function of particle size interval.

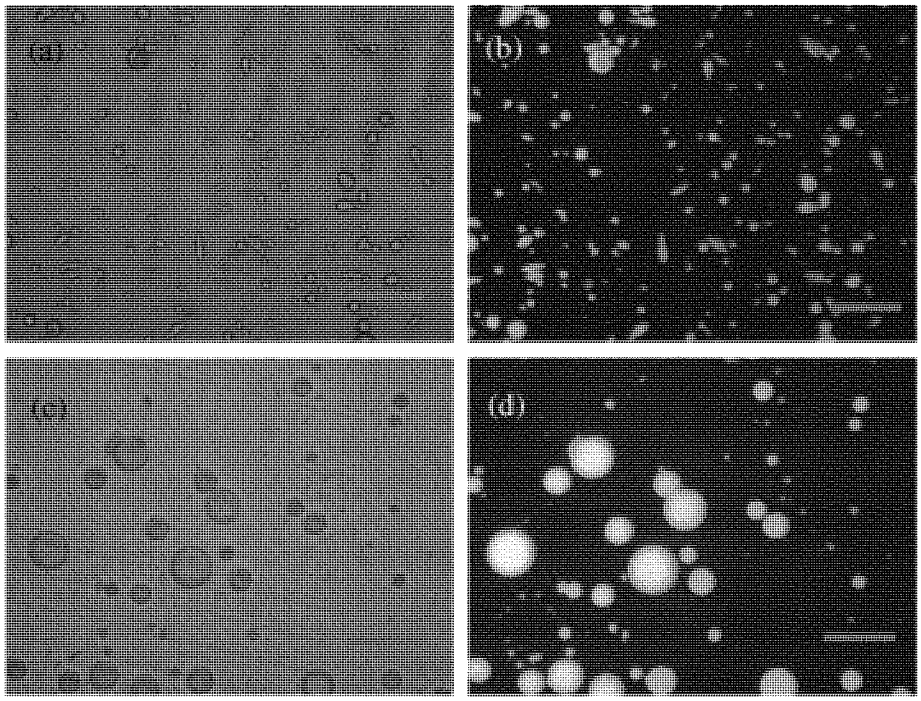


Fig. 9. Images of oil droplet formation under the inspection of epiflorescent microscope: OMAs formed in the presence (a and b) and absence (c and d) of dispersant from the observation under transmitted light (a and c) or epiflurescent (b and d) light illumination. Samples taken for imaging were diluted b y a factor of 20 before microscopy. Bars shown are 100 Im.

and height due to the transport of the OMAs in the tank (Fig. 8c). The presence of both chemical dispersants and mineral fines produced the smallest initial particle size (Fig. 8d), with the $d_{50\%}$ of 10.5 Im and remained small for the whole duration of the experiment (Table 1). The volumetric fraction of OMAs had three peak areas: a small peak centered at size of more than 100 Im, a large peak located at approximately 10 Im, and a third peak situated around 2.5 Im, the optical cutting edge of the LISST-100X (Fig. 8d). The peak of the largest size was leveled off with dispersion, whereas the fraction of small OMAs remained much larger than in the absence of dispersants, suggesting that the small chemically-dispersed OMAs were suspended in the bulk water column through hydrodynamic mixing.

Consistent with the LISST results, the size of the OMAs formed by chemically-dispersed oil were much smaller than the physically dispersed when observed by transmitted and epifluorescent microscopy (Fig. 9). The chemically-dispersed OMAs appeared to have predominantly fractal structures in morphology, whereas the physically dispersed OMAs were largely spherical. Enumeration of the dispersed oil and OMA particles under the microscope indicated that either chemical dispersants or mineral fines alone dramatically increased the particle number per unit volume.

4. Discussion

Observations on the stabilization of oil droplets in the water column by the formation of oil–mineral aggregates with suspended sediment have been well described in the literature (Bragg and Yang, 1995; Delvigne et al., 1987; Lee, 2002; Lee and Stoffyn-Egli, 2001; Lee et al., 1996, 2003; Muschenheim and Lee, 2002; Omotoso et al., 2002). However in the context of oil spill response operations, there is little information on the influence of chemical oil dispersants on the formation and fate of OMAs. Given that both chemical and physical dispersion lead to the generation of micron-sized droplets (Darling et al., 1990; Delvigne and Sweeney, 1988; Lunel, 1995), and that chemical dispersants alter the surface physicochemical properties of the dispersed oil droplets (Al-Sabagh and Atta, 1999; Dalmazzone et al., 2005; Oebius, 1999), the interactions of chemically-dispersed oil with suspended sediment need also to be understood to accurately assess the risks involved during the application of dispersants in particle-rich nearshore and estuarine waters.

The results obtained from our wave tank study indicate that dispersants and mineral fines can have strong and cumulative effects on the formation and transport of oil droplets and OMAs. The interaction of chemical dispersant with oil and mineral fines increased the dissolved and aggregated oil concentration in the bulk aqueous phase (Figs. 3, 4 and 6) and reduced the size of oil droplets and OMAs (Figs. 7 and 8). OMAs formed in the absence of dispersant exhibited stable distributions in suspension, but their particle sizes were generally larger than the naturally dispersed oil droplets subjected to hours of dispersion under breaking waves (Fig. 7; Table 1). The chemical dispersion of the same oil promoted the entrainment of the

oil into the bulk aqueous phase (Figs. 3, 4 and 6) and decreased the size of dispersed oil droplets within a short period of time (Figs. 7 and 8; Table 1). The effects of chemical dispersant and mineral fines on size distribution and morphology of dispersed oil and OMAs were verified by observation via microscope (Fig. 9).

The oil-droplet size distribution observed in this study is consistent with the range reported by previous investigators. Darling et al. (1990) and Fingas et al. (1995) reported mass mean diameters of 20–45 lm in laboratory dispersant effectiveness tests. Lunel (1995) reported that more than 80% of the chemically-dispersed oil droplets in the sea were in the range of 1–70 lm, and that the effect of chemical dispersants increased the number of small droplets by 5–30-fold compared to untreated oil while the number of large droplets did not change appreciably. In our system, the mass mean diameter of the untreated oil droplets was progressively reduced from more than 150 to 40 lm after the prolonged action of the breaking waves. In contrast, the chemically dispersed oil was reduced from a MMD of 25 to 15 lm for the same period of mixing time and energy dissipation rate in the wave tank (Fig. 7).

The reported OMA size distribution also varied from system to system, depending on the types of oil, loading and type of suspended particles, and the mixing energy of the system (Guyomarch et al., 1999, 2002; Khelifa et al., 2002, 2005; Sterling et al., 2004, 2005). Guyomarch et al. (2002) reported an average size of the OMAs in the settled phase in the flask experiment varied from 50 to 1000 lm. Sterling et al. (2004, 2005) reported that the diameters of the suspended oil–mineral aggregates at a sediment-to-oil ratio of 1:3 with were between 10 and 100 lm under a shear rate of 20 s^{ffi1}. In our system, the observed mass mean diameter of the OMAs was nearly 50 lm in the absence of chemical dispersants and 10 lm in the presence of dispersants, which are within the observed range of Sterling et al. (2004, 2005). Suspension of dispersed oil or OMAs at the representative hydrodynamic regime may be critical for enhancing oil dispersion because oil that subsequently becomes entrained within bottom sediments may persist for a long time due to the slow biodegradation rates of polycyclic aromatic hydrocarbon under anaerobic conditions (Quantin et al., 2005).

A synergistic effect by chemical dispersants and mineral fines on the transfer of oil to dissolved/dispersed phases was observed in this study (Figs. 3 and 4) and this effect can be due to a number of contributing factors. First, dispersant reduced water–oil interfacial tension and stimulated the breakup of the oil slick (Lessard and Demarco, 2000). Second, mineral fines reduced the time period for the splitting of oil droplets into small and stable drops (Hill et al., 2002), which is otherwise a rate-limiting step for oil dispersion by microscale turbulence (Lunel, 1995). Third, the interaction of mineral fines and oil reduced the buoyancy of oil droplets and therefore transferred more oil into the bulk aqueous phase (Lee, 2002; Owens and Lee, 2003). In addition, the partitioning of oil from the dissolved to solid was remarkably increased when chemical dispersants and mineral fines were present in combination (Fig. 5). Mineral fines are reported to absorb oil at their surface due to the hydrophobic surface properties (Marczewski and Szymula, 2002; Szymula and Marczewski, 2002). Dispersants, while primarily reducing the oil/water interfacial tension, may also stimulate the interaction between oil and mineral fines (Dantas et al., 2001).

5. Summary and conclusion

Until recently, the use of chemical oil dispersants has been focused on offshore spills due to concerns over toxic effects of dispersants and chemically-dispersed oil on the biota, especially in light of the fact that the extent of dispersion may be limited by the depth of waters in coastal regions. With the development of "low toxicity" dispersant formulations and case studies demonstrating their efficacy and net environmental benefit (Lunel et al., 1997), the application of chemical dispersants in nearshore environments is now being considered.

Since oil spills most frequently occur in nearshore and estuarine waters (NRC, 2005), where the suspended sediment load is usually high, the interaction of physically and chemically-dispersed oil during oil spill response or other offshore oil and gas activities is inevitable. Our results from a unique wave tank facility demonstrate how physical and chemical dispersion under breaking waves may interact with suspended sediments in the nearshore marine environment. The results from this study support the conclusion that oil–mineral aggregation can occur for both physically and chemically-dispersed oil. The effect of dispersants reduces oil and OMA droplet size distribution. The effect of mineral fines increases the suspended particle concentration in the water column and droplet stability; once formed, OMAs neither readily breakup further nor recoalesce after dispersion. The synergistic effect of dispersants and mineral fines enhances the transfer of oil from the surface downward into the water column; and a large number of small particles is produced as a result of interaction of dispersants and mineral fines with crude oil. The small particles tend to be suspended in the water column rather than settle down to the bottom because of the hydrodynamic mixing. The impact of this synergistic effect on overall fate and toxicity of the oil, such as biodegradation rate and the potential impact on pelagic and benthic organisms needs to be evaluated further.

Although this experimental study on the interaction between chemical dispersants and mineral fines during oil dispersion was conducted under breaking waves that are typical of the coastal waters and the open sea with respect to energy dissipation rate (Delvigne and Sweeney, 1988), various mixing energy conditions should be studied to represent a broader range of environmental conditions in the field. For example, although mixing energy has proven to be essential for the formation of OMAs as well as their size distribution and buoyancy (Lee and Stoffyn-Egli, 2001),

and OMA formation has been observed in the field with breaking wave heights at less than 30 cm (Owens et al., 1995; Sergy et al., 2003), a quantitative association of OMA formation and transport with the level of mixing energy at sea does not exist yet. Since this study, the wave tank facility has been upgraded to extend the length from 16 to 32 m for further dispersant effectiveness tests under a variety of non-breaking regular wave and breaking wave conditions (to simulate more violent breakers and create higher energy dissipation rates); additional studies are planned to investigate the interaction of oil, mineral fines and chemical dispersants under a suite of sea mixing energy conditions. Many other factors, such as oil types and weathering state, surface properties of mineral fines, and the ratio of oil to mineral fines, should also be evaluated for their impact on oil dispersion effectiveness by chemical dispersants. The potential application of chemical oil dispersants to "surf washing" operations warrants further study since oil droplet formation has been hypothesized to be a catalyst for the OMA formation on which this shoreline clean-up procedure is based (Lee, 2002). Further research is warranted to model interactions between crude oil, suspended sediments and chemical dispersants, and to investigate the time-scale of chemical oil dispersion and oil–mineral aggregation. The knowledge gained from these systematic studies in the wave tank that can reproduce a variety of natural waves is expected to provide quantitative guidance for risk assessment and decision-making during the selection of nearshore oil-spill countermeasure techniques.

Acknowledgements

This research was supported by the Panel of Energy Research and Development (PERD) Canada, US EPA (research contract No. 68-C-00-159), and NOAA/UNH Coastal Response Research Center (NOAA Grant No. NA04NOS4190063 UNH Agreement No. 06-085). Essential technical and logistical support for this research program was provided by Matt Arsenault, Jay Bugden, Matthew Coady, Susan Cobanli, Jennifer Dixon, Xiaowei Ma, and Peter Thamer. Views expressed by the authors do not necessarily reflect the position of the US Environmental Protection Agency.

References

Al-Sabagh, A.M., Atta, A.M., 1999. Water-based non-ionic polymeric surfactants as oil spill dispersants. Journal of Chemical Technology and Biotechnology 74 (11), 1075–1081.

Anderson, C.M., LaBelle, R.P., 2000. Update of comparative occurence rates for offshore oil spills. Spill Science & Technology Bulletin 6 (5–6), 303–321.

ASTM, 2002. American Society for Testing and Materials. Standard Test Method for Waterborne Petroleum Oils by Fluorescence Analysis. ASTM D3650-90, West Conshohocken, PA.

Bragg, J.R., Owen, E.H., 1995. Shoreline Cleansing by Interactions between Oil and Fine Mineral Particles. American Petroleum Institute, Washington, DC, pp. 219–227.

Bragg, J.R., Yang, S.H., 1995. Clay-oil flocculation and its effects on the rate of natural cleansing in Prince William Sound following the Exxon Valdez oil spill. In: Wells, P.G., Butler, J.N., Hughes, J.S. (Eds.), Exxon Valdez Oil Spill – Fate and Effects in Alaskan Waters. American Society for Testing and Materials, Philadelphia, PA, pp. 178–214.

Cloutier, D., Amos, C.L., Hill, P.R., Lee, K., 2002. Oil erosion in an annular flume by seawater of varying turbidities: a critical bed shear stress approach. Spill Science & Technology Bulletin 8 (1), 83–93.

Dalmazzone, C., Noik, C., Komunjer, L., 2005. Mechanism of crude-oil/water interface destabilization by silicone demulsifiers. SPE Journal 10 (1), 44–53.

Dantas, T.N.D., Neto, A.A.D., Moura, E.F., 2001. Microemulsion systems applied to breakdown petroleum emulsions. Journal of Petroleum Science and Engineering 32 (2–4), 145–149.

Darling, P.S., Mackay, D., Mackay, N., Brandvik, P.J., 1990. Droplet size distributions in chemical dispersion of oil spills: toward a mathematical model. Oil and Chemical Pollution 7 (3), 173–198.

Delvigne, G.A.L., Sweeney, C.E., 1988. Natural dispersion of oil. Oil and Chemical Pollution 4 (4), 281–310.

Delvigne, G.A.L., Van del Stel, J.A., Sweeney, C.E., 1987. Measurements of vertical turbulent dispersion and diffusion of oil droplets and oil-particles. Anchorage, Alaska. US Department of the Interior, Minerals Management Service. Report No. MMS 87-111, p. 501.

EPA, 2003. US Environmental Protection Agency. Test Methods (2003). EPA Methods 3510C, 3540C and 8100.

Fingas, M.F., Kyle, D., Tennyson, E., 1995. Dispersant effectiveness: studies into the causes of effectiveness variations. In: Lane, P. (Ed.), The Use of Chemicals in Oil Spill Response. ASTM STP 1252. American Society for Testing and Materials, Philadelphia, pp. 92–132.

Gearing, J.N., Gearing, P.J., 1983. Suspended load and solubility effect on sedimentation of petroleum hydrocarbons in controlled estuarine ecosystems. Canadian Journal of Fisheries and Aquatic Science 40, 54–62.

Guyomarch, J., Le Floch, S., Merlin, F.X., 2002. Effect of suspended mineral load, water salinity and oil type on the size of oil–mineral aggregates in the presence of chemical dispersant. Spill Science & Technology Bulletin 8 (1), 95–100.

Guyomarch, J., Merlin, F., Bernanose, P., 1999. Oil interaction with mineral fines and chemical dispersion: behaviour of the dispersed oil in coastal or estuarine conditions. Environment Canada's 22nd Arctic and Marine Oilspill (AMOP) Technical Seminar, Calgary, Alberta, Canada, pp. 137–149.

Hill, P.S., Khelifa, A., Lee, K., 2002. Time-scale for oil droplet stabilization by mineral particles in turbulent suspensions. Spill Science & Technology Bulletin 8 (1), 73–81.

Kaku, V.J., Boufadel, M.C., Venosa, A.D., 2006. Evaluation of mixing energy in laboratory flasks used for dispersant effectiveness testing. Journal of Environmental Engineering-ASCE 132 (1), 93–101.

Kepkay, P.E., Bugden, J.B.C., Lee, K., Stoffyn-Egli, P., 2002. Application of ultraviolet fluorescence spectroscopy to monitor oil–mineral aggregate formation. Spill Science & Technology Bulletin 8 (1), 101–108.

Khelifa, A., Stoffyn-Egli, P., Hill, P.S., Lee, K., 2002. Characteristics of oil droplets stabilized by mineral particles: effects of oil type and temperature. Spill Science & Technology Bulletin 8 (1), 19–30.

Khelifa, A., Stoffyn-Egli, P., Hill, P.S., Lee, K., 2005. Effects of salinity and clay type on oil–mineral aggregation. Marine Environmental Research 59 (3), 235–254.

Le Floch, S., Guyomarch, J., Merlin, F.X., Stoffyn-Egli, P., Dixon, J., Lee, K., 2002. The influence of salinity on oil–mineral aggregate formation. Spill Science & Technology Bulletin 8 (1), 65–71.

Lee, K., 2002. Oil-particle interactions in aquatic environments: influence on the transport, fate, effect and remediation of oil spills. Spill Science & Technology Bulletin 8 (1), 3–8.

Lee, K., Stoffyn-Egli, P., 2001. Characterization of oil–mineral aggregates. In: Proceedings of the 2001 International Oil Spill Conference. American Petroleum Institute, Washington, DC, pp. 991–996.

Lee, K., Lunel, T., Wood, P., Swannel, R., Stoffyn-Egli, P., 1997. Shoreline cleanup by acceleration of clay-oil flocculation process.

American Petroleum Institute, Washington, DC, Publication No. 4651, pp. 235–240.

Lee, K., Stoffyn-Egli, P., Tremblay, G.H., Owens, E.H., Sergy, G.A., Guenette, C.C., Prince, R.C., 2003. Oil–mineral aggregate formation on oiled beaches: natural attenuation and sediment relocation. Spill Science & Technology Bulletin 8 (3), 285–296.

Lee, K., Weise, A.M., St-Pierre, S., 1996. Enhanced oil biodegradation with mineral fine interaction. Spill Science & Technology Bulletin 3 (4), 263–267.

Lee, K., Wong, C.S., Cretney, W.J., Whitney, F.A., Parson, T.R., Lalli, C.M., Wu, J., 1985. Microbial response to crude oil and Corexit 9527: SEAFLUXES enclosure study. Microbial Ecology 11, 337–351.

Lessard, R.R., Demarco, G., 2000. The significance of oil spill dispersants. Spill Science & Technology Bulletin 6 (1), 59–68.

Li, M., Garrett, C., 1998. The relationship between oil droplet size and upper ocean turbulence. Marine Pollution Bulletin 36, 961–970.

Lunel, T., 1995. Understanding the mechanism of dispersion through oil droplet size measurements at sea. In: Lane, P. (Ed.), The Use of Chemicals in Oil Spill Response. ASTM STP 1252. American Society for Testing and Materials, Philadelphia, PA, pp. 240–270.

Lunel, T., Swannell, R., Rusin, J., 1997. Monitoring the effectiveness of response operations during the Sea Empress incident: a key component of the successful. Oceanographic Literature Review 44 (12), 1570–1571.

MacKay, D., Hussain, K., 1982. An exploratory study of sedimentation of naturally and chemically-dispersed oil. Environment Canada Report, EE-35, Ottawa, Ontario, Canada, 24p.

Marczewski, A.W., Szymula, M., 2002. Adsorption of asphaltenes from toluene on mineral surface. Colloids and Surfaces A–Physicochemical and Engineering Aspects 208 (1–3), 259–266.

Muschenheim, D.K., Lee, K., 2002. Removal of oil from the sea surface through particulate interactions: review and prospectus. Spill Science & Technology Bulletin 8 (1), 9–18.

Nilson, J., Naess, A., Volent, Z., 1985. Measurements of oil concentrations in the water column under breaking waves. Norwegian Hydrotechnical Laboratory, Report STF 60 A 85079, Trondheim, Norway.

NRC, 2003. National Research Council: Oil in the Sea III: Inputs, Fates and Effects. National Academies Press, Washington, DC.

NRC, 2005. National Research Council: Understanding Oil Spill Dispersants: Efficacy and Effects. National Academies Press, Washington, DC.

NRCC, 1997. National Research Council of Canada, Centre D'Expertise en Analyse Environnementale du Québec, Dosage Des Hyrdocarbons, MA 410-HYD. 1.0.1997.

Oebius, H.U., 1999. Physical properties and processes that influence the clean up of oil spills in the marine environment. Spill Science & Technology Bulletin 5 (3–4), 177–289.

Omotoso, O.E., Munoz, V.A., Mikula, R.J., 2002. Mechanisms of crude oil–mineral interactions. Spill Science & Technology Bulletin 8 (1), 45–54.

Owens, E.H., Davis Jr. R.A, Michel, J., Stritzke, K., 1995. Beach cleaning and the role of technical support in the 1993 Tampa Bay spill. American Petroleum Institute, Washington, DC, Publication No. 4620, pp. 627–634.

Owens, E.H., Lee, K., 2003. Interaction of oil and mineral fines on shorelines: review and assessment. Marine Pollution Bulletin 47 (9–12), 397–405.

Owens, E.H., Sergy, G.A., Guenette, C.C., Prince, R.C., Lee, K., 2003. The reduction of stranded oil by in situ shoreline treatment options. Spill Science & Technology Bulletin 8 (3), 257–272.

Page, C.A., Bonner, J.S., Sumner, P.L., McDonald, T.J., Autenrieth, R.L., Fuller, C.B., 2000. Behavior of a chemically-dispersed oil and a whole oil on a near-shore environment. Water Research 34 (9), 2507–2516.

Payne, J.R., Clayton Jr., J.R., Kirstein, B.E., 2003. Oil/suspended particulate material interactions and sedimentation. Spill Science & Technology Bulletin 8 (2), 201–221.

Quantin, C., Joner, E.J., Portal, J.M., Berthelin, J., 2005. PAH dissipation in a contaminated river sediment under oxic and anoxic conditions. Environmental Pollution 134 (2), 315–322.

Sergy, G.A., Guenette, C.C., Owens, E.H., Prince, R.C., Lee, K., 2003. In situ treatment of oiled sediment shorelines. Spill Science & Technology Bulletin 8 (3), 237–244.

Shaw, J.M., 2003. A microscopic view of oil slick break-up and emulsion formation in breaking waves. Spill Science & Technology Bulletin 8 (5–6), 491–501.

Sterling, M.C., Bonner, J.S., Ernest, A.N.S., Page, C.A., Autenrieth, R.L., 2005. Application of fractal flocculation and vertical transport model to aquatic sol-sediment systems. Water Research 39 (9), 1818–1830.

Sterling, M.C., Bonner, J.S., Page, C.A., Fuller, C.B., Ernest, A.N.S., Autenrieth, R.L., 2004. Modeling crude oil droplet-sediment aggregation in nearshore waters. Environmental Science & Technology 38 (17), 4627–4634.

Stoffyn-Egli, P., Lee, K., 2002. Formation and characterization of oil–mineral aggregates. Spill Science & Technology Bulletin 8 (1), 31–44.

Szymula, M., Marczewski, A.W., 2002. Adsorption of asphaltenes from toluene on typical soils of Lublin region. Applied Surface Science 196 (1–4), 301–311.

Tkalich, P., Chan, E.S., 2002. Vertical mixing of oil droplets by breaking waves. Marine Pollution Bulletin 44 (11), 1219–1229.

Venosa, A.D., Kaku, V.J., Boufadel, M.C., Lee, K., 2005. Measuring energy dissipation rates in a wave tank. In: Proceedings of 2005 International Oil Spill Conference, Miami, FL. American Petroleum Institute, Washington, DC.

Marine Pollution Bulletin 60 (2010) 1550–1559

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Effects of temperature and wave conditions on chemical dispersion efficacy of heavy fuel oil in an experimental flow-through wave tank

Zhengkai Li [a,*], Kenneth Lee [a], Thomas King [a], Michel C. Boufadel [b], Albert D. Venosa [c]

[a] COOGER, Bedford Institute of Oceanography, Fisheries and Oceans (DFO) Canada, Dartmouth, NS, Canada B2Y 4A2
[b] Department of Civil and Environmental Engineering, Temple University, Philadelphia, PA 19122, USA
[c] Land Remediation and Pollution Control Division, U.S. EPA, Cincinnati, OH 45268, USA

a r t i c l e i n f o

Keywords:
Chemical dispersion
Heavy fuel oil
Temperature effect
Wave effect
Dynamic dispersant effectiveness
Droplet size distribution

a b s t r a c t

The effectiveness of chemical dispersants (Corexit 9500 and SPC 1000) on heavy fuel oil (IFO180 as test oil) has been evaluated under different wave conditions in a flow-through wave tank. The dispersant effectiveness was determined by measuring oil concentrations and droplet size distributions. An analysis of covariance (ANCOVA) model indicated that wave type and temperature significantly ($p < 0.05$) affected the dynamic dispersant effectiveness (DDE). At higher temperatures (16 ffC), the test IFO180 was effectively dispersed under breaking waves with a DDE of 90% and 50% for Corexit 9500 and SPC 1000, respectively. The dispersion was ineffective under breaking waves at lower temperature (10 ffC), and under regular wave conditions at all temperatures (10–17 ffC), with DDE < 15%. Effective chemical dispersion was associated with formation of smaller droplets (with volumetric mean diameters or VMD 6 200 l m), whereas ineffective dispersion produced large oil droplets (with VMD P 400 l m).

ffl 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Marine fuel oil spills occur as a result of leaks during commercial shipping operations and many other types of maritime accidents. Heavy fuel oils, which are typically characterized as having elevated polar fractions and diminished evaporative fractions, are usually far more viscous than unrefined crude oils and tend to be more difficult to disperse in the aquatic environment. Therefore, field responses to heavy fuel oil spills are more challenging than responses to light and median crude oil spills. Until two decades ago, oils and emulsions with viscosities greater than 2000 cP were considered impossible to disperse chemically (NRC, 1989). Indeed, the present guidelines on oil spill dispersant application of the International Maritime Organization and United Nations Environmental Program state, "Most oils can be successfully treated with dispersants in the first 4–6 h of a spill; one very important exception is heavy fuel oil, which generally cannot be dispersed" (IMO/UNEP, 1995).

Conventional mechanically-based oil spill response technologies are always constrained by weather conditions and sea state and are inefficient for use in the open sea. With the advent of new generation low toxicity and high efficiency chemical dispersants, interest has been renewed in using chemical dispersants as a cost-effective response to oil spills at sea. Over the years, much effort has been directed toward evaluating the feasibility of using chemical dispersants to break up and dissipate heavy fuel oils and oils with relatively high viscosities under various mixing energies and more realistic hydrodynamic regimes that may be encountered in the field. Several laboratory studies have demonstrated that heavy fuel oils may be dispersed. Fiocco et al. (1999) have shown that dispersion can be enhanced given sufficient mixing energy over an extended period of time, and Guyomarch et al. (1999) have found that dispersion efficacy can be increased through controlled multiple applications of chemical dispersants. By comparing the application of three dispersants on IFO180 and IFO380 using four different bench-scale testing protocols, namely the Swirling Flask Test (SFT), the Baffled Flask Test (BFT), the Exxon Dispersant Effectiveness Test (EXDET), and the Warren Spring Laboratory (WSL) test, Clark et al. (2005) pointed out that mixing energy is a predominant factor affecting dispersant effectiveness of fuel oil dispersion. The high mixing energy of the BFT and WSL produced orders-of-magnitude higher dispersant effectiveness than the low mixing energy SFT, whereas application of the EXDET produced values between the two extremes.

Because mixing energy is a critical variable in the generation of well dispersed l m-size oil droplets, elucidation of the linkage between mixing energies of various wave conditions with dispersant efficacy of heavy fuel oil continues to be of interest. In addition, temperature is a critical environmental variable that must be taken into account. Srinivasan et al. (2007) used the BFT to investigate different variables, including dispersant-to-oil ratio, mixing speed,

* Corresponding author. Tel.: +1 902 426 3442; fax: +1 902 426 1440.
E-mail address: Zhengkai.Li@dfo-mpo.gc.ca (Z. Li).

0025-326X/$ - see front matter ffl 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2010.04.012

and temperature, on the effectiveness of three dispersants on heavy fuel oils. They found that chemical dispersion of IFO180 and IFO380 by three dispersants in the BFT was almost two times more effective at 16 °C than at 5 °C when mixing energy was sufficiently high (Srinivasan et al., 2007).

Lab tests are easy to use, low in cost, and valuable for screening dispersants for product selection. However, extrapolation of lab tests results to the field needs to be done with caution because wave and current effects, which influence the transport and dilution of oil in the open sea, cannot be accommodated in the confines of small-scale testing apparatuses, regardless of how closely flow fields generated in a bench-scale test mimic mixing energy at sea. Caution must be exercised when making decisions on real-world oil spill responses based solely on the results from laboratory dispersant tests. A combination of laboratory, wave tank, and field studies have been recommended to establish the best degree of confidence for understanding the limits of dispersants in treating fuel oil spills (Clark et al., 2005; NRC, 2005). In 2003, a series of sea trials off the coast line of the United Kingdom (conducted at a sea surface temperature of 15 °C and wind speed of 7–14 knots) revealed (from visual observation) that a heavy fuel oil IFO180 could be satisfactorily dispersed under appropriate treatment conditions (Colcomb et al., 2005; Lewis, 2004). Subsequently, large-scale wave tank tests were conducted in the OHMSETT facility to evaluate dispersant performance on IFO180 and IFO380 (Trudel et al., 2005). These experiments were carried out at two distinct wave frequencies (30 and 33 cycles per min) to relate chemical dispersion efficacy to mixing energy, with temperature and salinity maintained at the same level as the 2003 UK sea trials. By taking a mass balance approach (where the amount of oil dispersed in the water was estimated by subtracting residual oil recovered at the surface from the initial oil volume) and a visual observation estimation (using the ranking scale used in the 2003 UK sea trials), Trudel et al. (2005) found the chemical dispersant effectiveness on IFO180 varied from 17% to 84%, depending on the dispersant-to-oil ratio, wave frequency, and dispersant type. In comparison, the dispersion efficiency was <26% in a control experiment with no dispersant at high wave frequency.

To better simulate transport and dilution effects of ocean currents in the field, the flow-through wave tank at the Bedford Institute of Oceanography in Dartmouth, Nova Scotia, Canada was used. The tank is used for the evaluation of dynamic dispersant effectiveness under different simulated wave conditions with underwater currents. Regular and breaking waves are generated in the wave tank to simulate various energy dissipation rates in the field. The hydrodynamics of the various wave types generated in the wave tank facility have been characterized and documented (Venosa et al., 2005, 2008; Wickley-Olsen et al., 2008). The new flow-through system has been used to evaluate dispersant effectiveness of fresh and weathered crude oil through measurement of oil concentrations in the water column and the in situ dispersed oil droplet size distributions (Li et al., 2009).

The objective of this study was to evaluate the dynamic dispersant effectiveness (DDE) on a heavy IFO180 fuel oil in the flow-through wave tank under hydrodynamic conditions similar to those found in the field, including the simulation of mixing energy produced by the combined action of different wave conditions and underwater current flow. Special emphasis was also placed on the correlation of dispersant effectiveness of the test IFO180 oil to the seawater temperature under regular non-breaking wave and breaking wave conditions.

2. Materials and methods

2.1. Wave tank facility and testing wave and current conditions

Fig. 1 shows the schematic representation of the wave tank facility that was used in this research. The dimensions are 32 m long, 0.6 m wide, and 2 m high. The average water depth was 1.50 m. Different regular and breaking waves were generated by a computer-controlled flap-type wave maker situated at one end of the tank. Two wave conditions, namely regular non-breaking waves and plunging breaking waves, were tested in this study. The regular non-breaking waves of wavelength 2.44 m and height of 23 cm were generated with 12 cm stroke, 0.80 Hz frequency settings. The plunging breaking waves were produced with a 12 cm stroke and alternating trains of high-frequency waves (0.85 Hz producing a wave length of 2.16 m and wave height 26 cm with a duration of 20 s) and low-frequency waves (0.5 Hz producing a wave length of 6.24 m and a wave height of 9 cm with a duration of 5 s). A uniform current at a flow rate of 3.8 ± 0.2 L s^{-1} was introduced into the wave tank through a manifold system that allows passing a current through it at the average speed of 0.43 cm s^{-1}. The current flow rate was selected to counteract the previously measured surface Stoke's drift velocity of the high frequency (0.85 Hz) regular wave conditions. The presence of current allows for dilution and transport of dispersed oil away from the slick, which simulates prevailing currents in the sea to achieve more realistic field conditions. The hydrodynamics of the wave tank were characterized by four wave gauges that measured water level at a frequency of 100 Hz, and an Acoustic Doppler Velocimeter that measured three-dimensional velocities at a frequency of 50 Hz. The breaking waves were generated using the frequency sweep tech-

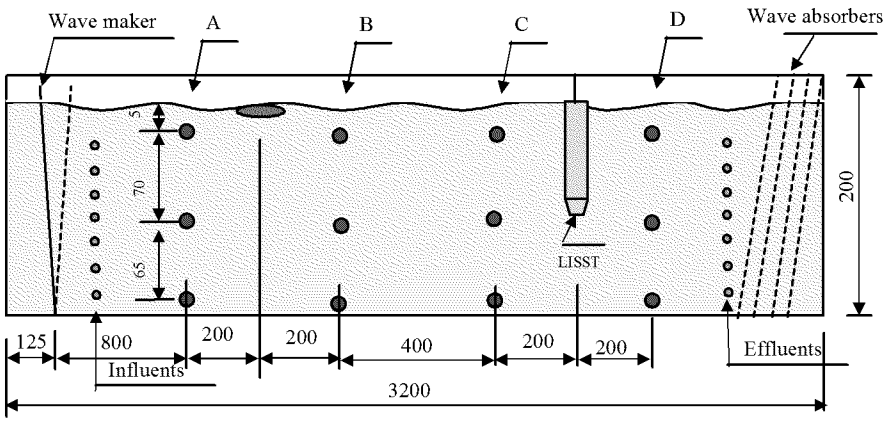


Fig. 1. Schematic representation (all dimensions in cm, not to scale) of the wave tank. Larger circles represent four horizontal sampling locations: (A) 2 m upstream, (B) 2 m downstream, (C) 6 m downstream, and (D) 10 m downstream from the center of the initial oil slick.

nique (Funke and Mansard, 1979), wherein a wave of one frequency is superimposed on another wave of a different frequency, causing the wave to increase in height until it breaks. In this work, during wave breaking, the wave height of the breaking wave was about 26 cm and increased to 33 cm, while the velocity at the surface increased from 0.3 m s^{-1} to 0.5 m s^{-1}. The energy dissipation rate at the breaking point was at least two orders-of-magnitude higher than that of regular waves. The high energy dissipation rate under plunging breaking waves was similar to the breaking wave energy dissipation rate reported in the field (Delvigne and Sweeney, 1988; Drennan et al., 1996; Terray et al., 1996), whereas the values for regular waves were similar to those found on the sea surface layer (Delvigne and Sweeney, 1988).

2.2. Testing dispersants and heavy fuel oil

Two commercial chemical dispersants were tested, Corexit 9500 and SPC 1000; both are listed on EPA's National Contingency Plan Product Schedule, and their precise compositions are proprietary. Corexit 9500 is a hydrocarbon-based reformulation of Corexit 9527 and is meant to be applicable for higher viscosity oils and emulsions. SPC 1000 is a water-based formulation. A heavy IFO180 fuel oil was selected as the test oil in this study, with a specific gravity of 0.960 at 15 °C and an API gravity of 12.5°. The pour point and flash point of the test IFO180 was −9 and 75 °C, respectively. The dynamic viscosity and oil–seawater interfacial tension of IFO180 (at 15 °C) was 2471 mPa s and 21 mN/m, respectively. The test oil was characterized by thin-layer chromatography equipped with a flame-ionization detector (TLC-FID) and gas chromatography (HP 6890 series II) equipped with a mass selective detector (HP 5971A) operating in the selected ion monitoring mode (GC–MS). TLC-FID analysis showed that the tested IFO180 oil contained 27.1% alkanes (saturates), 41.7% aromatics, 27.0% resins, and 4.2% asphaltenes. The GC–MS characterization indicated that the total alkanes, polycyclic aromatic hydrocarbons (PAHs), and alkylated PAHs were 223,067, 14,763, and 190,118 ng mg^{-1}, respectively. The contents of the specific alkanes and aromatic compounds of the test oil are reported in Tables 1 and 2.

Table 1
Alkane components of the test oil IFO180 characterized by GC–MS.

Alkane component	Abbreviation	Content (ng mg^{-1})
n-Decane	C_{10}	1977
Undecane	C_{11}	2838
Dodecane	C_{12}	3607
Tridecane	C_{13}	5161
Tetradecane	C_{14}	7931
Pentadecane	C_{15}	10,127
Hexadecane	C_{16}	10,254
Heptadecane	C_{17}	11,293
2,6,10,14-TMPdecane (pristane)	pri	6327
Octadecane	C_{18}	10,706
2,6,10,14-TMHdecane (phytane)	phy	4284
Nonadecane	C_{19}	10,967
Eicosane	C_{20}	10,332
Heneicosane	C_{21}	12,419
Docosane	C_{22}	14,113
Tricosane	C_{23}	16,716
Tetracosane	C_{24}	16,454
Pentacosane	C_{25}	15,786
Hexacosane	C_{26}	13,098
Heptacosane	C_{27}	11,077
Octacosane	C_{28}	9115
n-Nonacosane	C_{29}	6394
Tricontane	C_{30}	2816
N-heneicontane	C_{31}	2480
Dotriacontane	C_{32}	2487
Tritriacontane	C_{33}	1720
Tetratriacontane	C_{34}	1310
n-Pentatriacontane	C_{35}	1124
17Beta(H), 21 alpha (H)-Hopane		155

2.3. Experimental design and procedure

The effectiveness of the two dispersants on the test IFO180 under the two wave conditions was investigated using a randomized block experimental design. In the first 3 experimental blocks, 6 combinations of the 2 wave conditions and 3 dispersant types (including water as no-dispersant control) were performed once. In the fourth block, only the breaking wave conditions with three dispersant types were conducted for an additional experiment in each treatment. In each block, the treatments were applied in random order to minimize the impacts of confounding factors such as variations in salinity and wind effects.

The experimental and sampling procedures were consistent with the crude oil dispersant efficacy testing in the flow-through wave tank reported previously (Li et al., 2009). Briefly, for each experiment, 300 ml of fuel oil was gently poured onto the filtered seawater surface within a 40 cm ring located 10 m downstream from the wave maker, and 12 ml of dispersant (or seawater for

Table 2
Aromatic components of the test oil IFO180 characterized by GC–MS.

Aromatic component	Abbreviation	Content (ng mg^{-1})
Naphthalene	nap	2005
Methylnaphthalene	C1nap	10,005
Dimethylnaphthalene	C2-nap	18,619
Trimethylnaphthalene	C3-nap	16,072
Tetramethylnaphthalene	C4-nap	8508
Acenaphthene	Acenap	615
Acenaphthylene	Acenapy	<25
Fluorene	Fl	694
Methylfluorene	C1-Fl	2398
Dimethylfluorene	C2-Fl	4013
Trimethylfluorene	C2-Fl	4798
Dibenzothiophene	dibthio	297
Methyldibenzothiophene	C1-dibthio	1037
Dimethyldibenzothiophene	C2-dibthio	1643
Trimethyldibenzothiophene	C3-dibthio	1552
Tetramethyldibenzothiophene	C4-dibthio	849
Phenanthrene	phen	2885
Anthracene	Anthr	435
Methylphenanthrene	C1-phen	2918
Dimethylphenanthrene	C2-phen	15,138
Trimethylphenanthrene	C3-phen	14,324
Tetramethylphenanthrene	C4-phen	8070
Fluoranthene	fluor	278
Pyrene	pyr	1281
Methylpyrene	C1-pyr	6562
Dimethylpyrene	C2-pyr	10,776
Trimethylpyrene	C3-pyr	8452
Tetramethylpyrene	C4-pyr	7038
Naphthobenzothiophene	napbthio	386
Methylnaphthobenzothiophene	C1-napbthio	4150
DimethylNBenzothiophene	C2-napbthio	5452
TrimethylNbenzothiophene	C3-napbthio	1198
TetramethylNbenzothiophene	C4-napbthio	589
Benz[a]anthracene	b[a]anth	1310
Chrysene	Chry	2168
Methylchrysene	C1-Chry	7898
Dimethylchrysene	C2-Chry	16,949
Trimethylchrysene	C3-Chry	7038
Tetramethylchrysene	C4-Chry	4070
Benzo[b]fluoranthene	b[b]fluor	332
Benzo[k]fluoranthene	b[f]flour	81
Benzo[e]pyrene	b[e]pyr	611
Benzo[a]pyrene	b[a]pyr	549
Perylene	peryl	365
Indeno[1,2,3-cd]pyrene	I[1,2,3-cd]pyr	93
Dibenz[a,h]anthracene	dib[a,h]anthr	197
Benzo[ghi]perylene	b[ghi]peryl	180

the control) was sprayed onto the surface of the oil slick through a pressurized nozzle (60 psi, 0.635 mm i.d.). This resulted in a dispersant-to-oil ratio (DOR) of 1:25. The ring was then promptly lifted prior to the arrival of the first wave, and the wave conditions were maintained throughout the 1-hour duration of the experiment.

To collect samples for measuring total dispersed oil concentration, four stainless steel manifolds, each connected to three 100-ml syringes, were located at four horizontal locations (8, 12, 16, and 20 m) downstream from the wave maker. Water samples were collected simultaneously in the water column of the wave tank at three depths (5, 75, and 140 cm) from the average water surface. In addition, water samples were collected from the effluent through the effluent pipe sampling port. Samples were collected at five time points (2, 5, 15, 30, and 60 min after start-up). The samples were extracted in dichloromethane and then analyzed by ultraviolet spectrophotometer adsorption for total petroleum hydrocarbon (TPH) concentrations as described previously (Li et al., 2008; Sorial et al., 2004; Venosa et al., 2002).

The dispersed oil droplet size distribution was measured using a laser in situ scattering and transmissometry (LISST-100X, Type C, Sequoia Scientific, Seattle, WA) following the same procedure used for previous crude oil dispersion experiments (Li et al., 2009). The LISST was suspended vertically in the water column with the detection window submerged about 60 cm beneath the average water surface and approximately 8 m downstream from the center of the initial oil slick. The in situ dispersed oil droplet size distribution was measured continuously by the LISST over the entire experimental duration of 1 h. The LISST-100X recorded 32 particle size intervals logarithmically spaced from 2.5–500 l m in diameter, with the upper size in each bin 1.18 times the lower. The measured particle size distribution is expressed as the average volumetric concentration of oil droplets for each interval of the size range. To compare the dispersed oil droplet size distribution as a function of time at each treatment condition, the volumetric mean diameters (VMD) of the measured droplet size distribution were calculated from:

$$VMD = \frac{\sum V_i \cdot d_i}{V} \quad (1)$$

where V is total volumetric concentration of the particles within the range of 2.5–500 l m, and V_i (i = 1–32) is the volumetric concentration of oil droplets in each size interval with average size d_i, which is the geometric mean of the lower and upper limit of every size range.

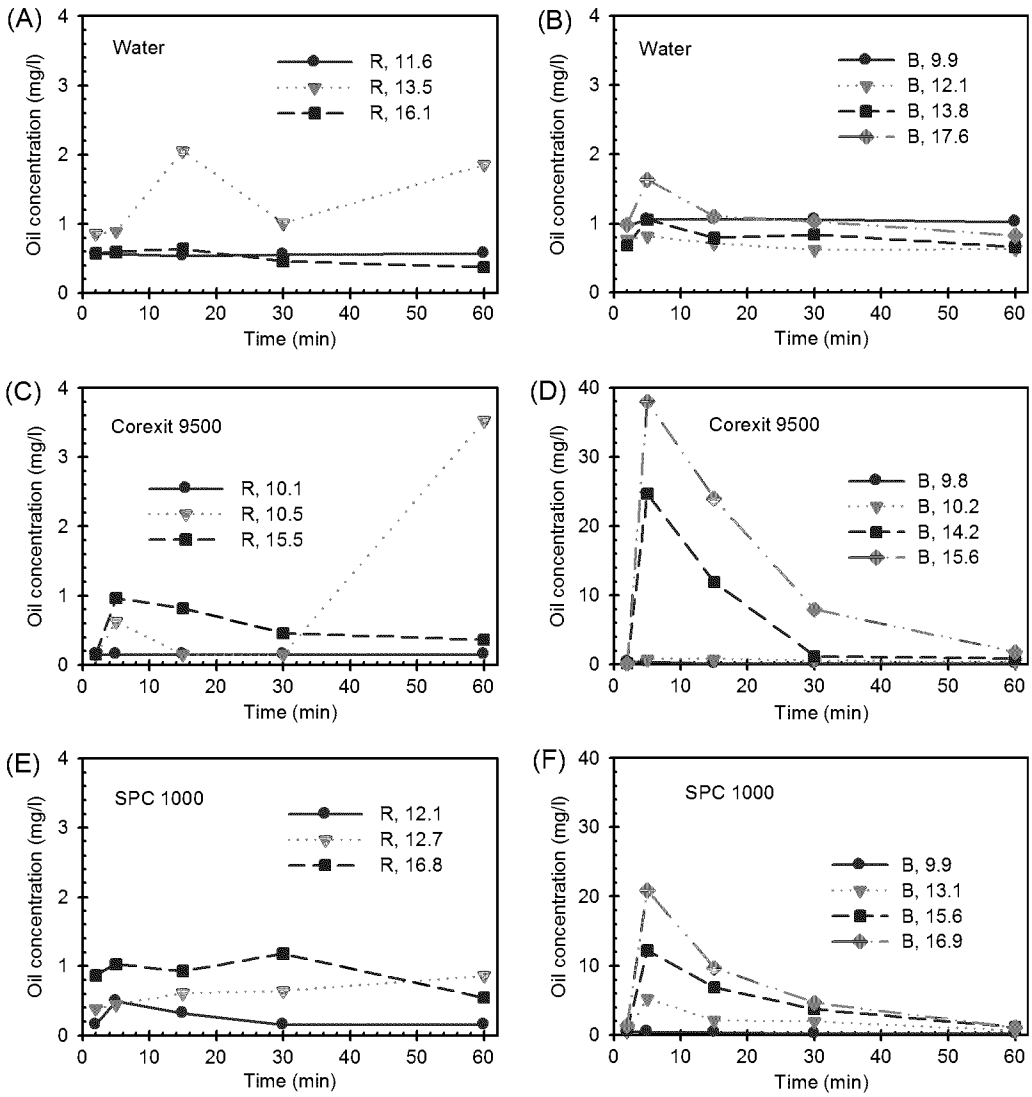


Fig. 2. Effects wave conditions [regular (A, C, and E), breaking (B, D, and F)], and water temperature (numbers in the legend) on dispersed IFO180 oil concentration in the middle of the water column and 10 m downstream from the initial oil slick.

2.4. Data analysis

The effects of the treatment factors on dynamic dispersant effectiveness (DDE) were analyzed by fitting an analysis of covariance (ANCOVA) model, where the response variable was DDE and the explanatory variables were dispersant (categorical, three levels) and wave energy (categorical, two levels), and covariate temperature (continuous). The data analysis was performed using S-Plus 7.0 (Insightful Co., Seattle, WA). A significance level of 0.05 was adopted for statistical tests. A Bonferroni adjustment was performed in analysis of variance (ANOVA) to control for the family-wise alpha rate to reach an adjusted and more strict alpha level at 0.007.

3. Results and discussion

3.1. Effects of temperature and wave conditions on the test IFO180 distribution in the wave tank

The effectiveness of dispersants under different wave conditions with currents was evaluated by monitoring spatial and temporal oil distribution in the flow-through wave tank. Fig. 2 presents the dispersed IFO180 oil concentrations as a function of time at the mid-depth of 75 cm below the surface and located at 10 m downstream from the initial slick. Under regular wave conditions, the measured dispersed IFO180 oil concentrations were consistently low, irrespective of temperature and dispersant (Fig. 2A, C, and E). Under breaking wave conditions, the dispersed oil concentrations were also very low without dispersant (Fig. 2B). In the presence of dispersant (Fig. 2D and F), however, the dispersed IFO180 oil concentrations increased rapidly within the first 5 min and then decreased steadily during the remaining 55 min of the experiment due to dilution by the flow-through current. The effect of temperature (increasing from 9.8 to 16.9 °C) is clearly shown for dispersion of the test IFO180 under breaking wave conditions (Fig. 2D and F) where the peak oil concentrations at higher temperatures were several-fold higher than those at lower temperatures. Similar oil distribution profiles were observed for the other sampling locations and depths (data not shown).

Fig. 3 shows the dispersed oil concentrations in the effluent from the wave tank were similar to those observed within the water column (Fig. 2). Under regular waves, the physically dispersed oil concentrations in the current flow were consistently low regardless of temperature (Fig. 3A), whereas chemically

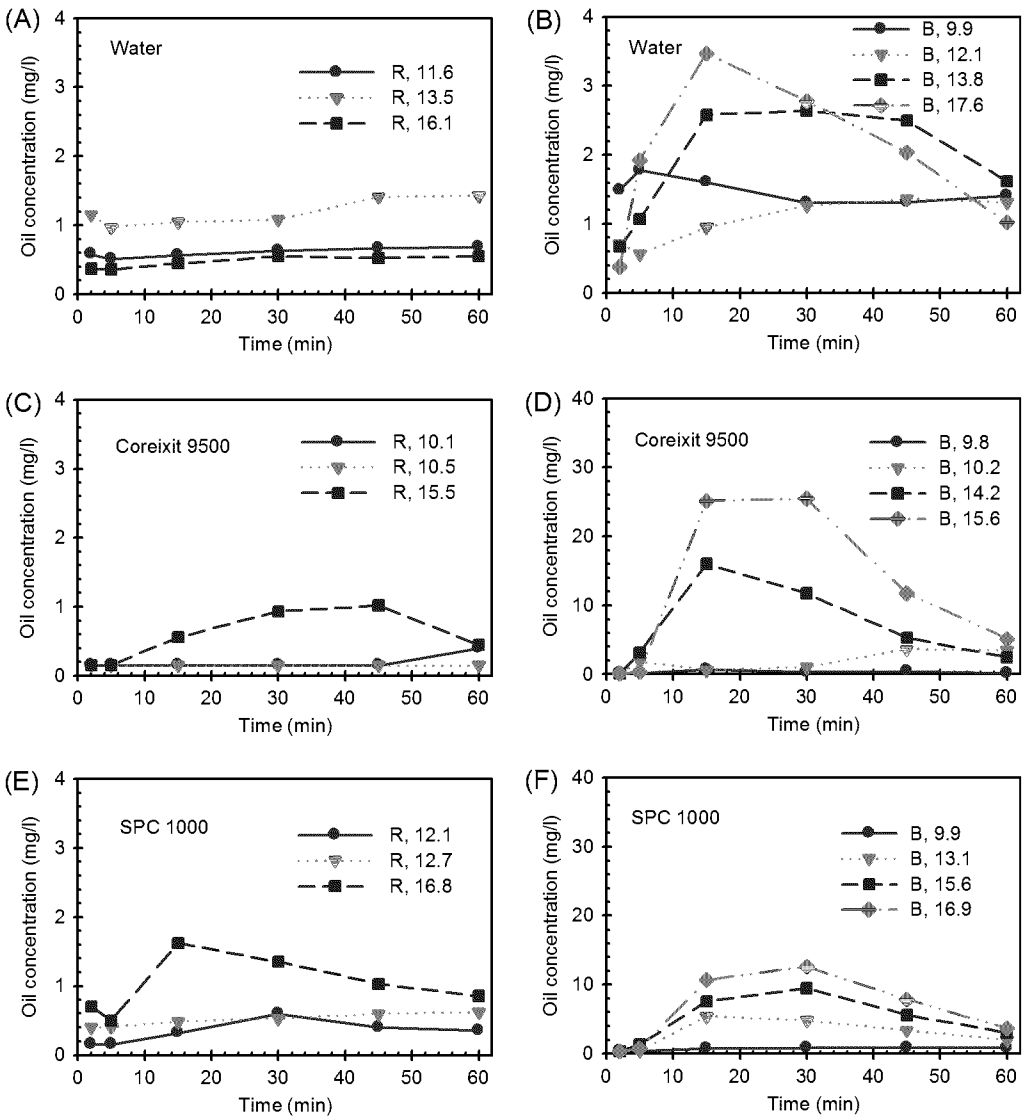


Fig. 3. Effects wave conditions [regular (A, C, and E), breaking (B, D, and F)] and water temperature (numbers in the legend) on dispersed IFO180 oil concentration in the effluent of the wave tank.

dispersed oil concentrations were increased slightly at the highest temperature (Fig. 3C and E). In the absence of dispersants, breaking waves increased the oil concentration in the effluent to some extent, and the values appeared to correlate incrementally with increases in temperature (Fig. 3B). In the presence of dispersants, breaking wave conditions dramatically increased oil concentrations in the effluent, particularly at higher temperature (Fig. 3D and F).

To compare the effects of dispersant type and wave conditions on oil dispersion in this dynamic environment, the time-series oil concentrations at each sampling position (horizontal location and depth) were converted to an equivalent oil concentration (C_{eq}, mg l^{-1}) with the same flux of oil as the time-dependent oil concentrations over the experimental period (1 h):

$$C_{eq} = \frac{\sum_{i=1}^{5} C_i \Delta t_i}{\sum_{i=1}^{5} \Delta t_i} \quad (2)$$

where Δt_i (i = 5) is the time interval between sampling events, and C_i is the measured oil concentration at the specific time point.

Fig. 4 summarizes the time-averaged equivalent oil concentrations at three depths (located at 10 m downstream) in the water column (Fig. 4A–C) and the effluent flow (Fig. 4D) of the wave tank. Under regular waves, high oil concentrations were measured at the surface (Fig. 4A), while a very limited amount of oil was detected in the middle (Fig. 4B) and near the bottom (Fig. 4C) of the wave tank. Applying dispersants did not appear to have reduced the oil concentration at the surface (Fig. 4A), nor did use of dispersants under regular wave condition increase the oil concentrations in the water column (Fig. 4B and C). These data also clearly show that breaking waves reduced the surface oil concentrations (Fig. 4A) and increased the water column oil concentrations (Fig. 4B and C). The effectiveness of dispersants on the test IFO180 dispersion in the water column under breaking waves was dependent on the test temperature. At lower temperatures, the dispersants did not appear to increase the oil concentrations in the middle (Fig. 4B) or near the bottom (Fig. 4C) of the wave tank. At higher temperatures, both Corexit 9500 and SPC 1000 under breaking waves produced markedly higher oil concentrations in the middle (Fig. 4B) and near the bottom (Fig. 4C) of the wave tank than in the absence of dispersant. Correspondingly, use of dispersants reduced the surface oil concentrations compared to those without dispersant under breaking waves (Fig. 4A).

The high sensitivity of the test IFO180 dispersion to seawater temperature was ascertained by the time-averaged oil concentrations of the underwater current flow (Fig. 4D). Under regular wave conditions, the time-averaged dispersed oil concentrations in the effluent were consistently less than 1.5 mg/L. Applying dispersants under regular waves had no distinct effect on the oil concentration in the effluent. Under breaking waves, at lower temperature, oil concentrations were also not affected by dispersants. At higher temperatures, however, the dispersed oil concentration in the effluent was increased by several-fold and was most noticeable under breaking wave conditions.

The effects of dispersants and wave conditions on DDE over the duration of the entire experiment can be evaluated by computing

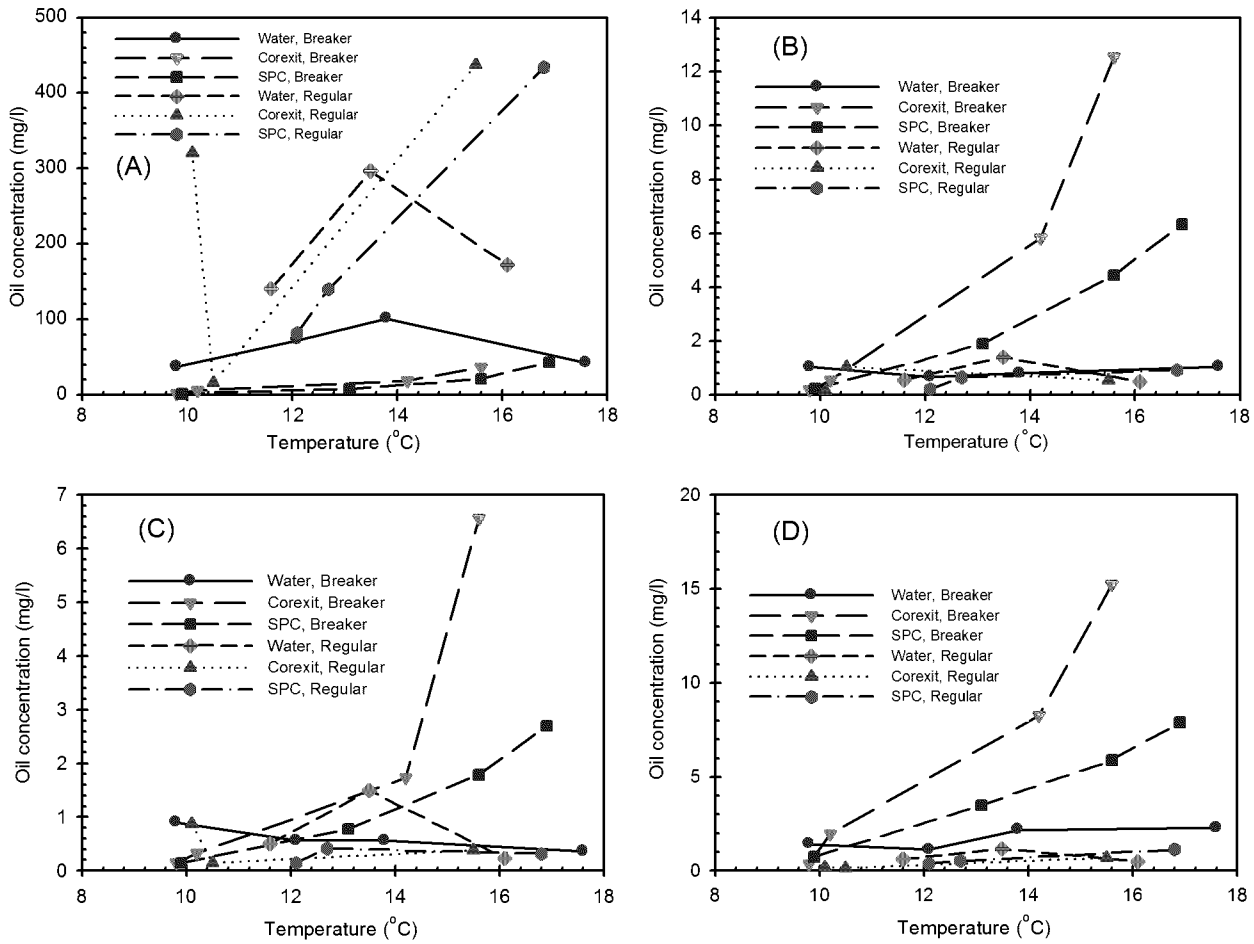


Fig. 4. Effects of temperature and wave conditions on dispersed IFO180 oil concentrations: (A) at the surface, (B) in the middle, (C) at the bottom, and (D) in the effluent, of the wave tank.

the fraction of dispersed oil flowing out of the wave tank with the effluent current plume and the residual dispersed oil in the water column of the wave tank at the end of each experiment:

$$DDE(\%) = \frac{\bar{C}_{effluent} Q_{effluent} T + \bar{C}_{sample} V_{wt}}{q_{Oil} V_{Oil}} \times 100 \quad (3)$$

where $\bar{C}_{effluent}$ is the time-averaged oil concentration in the effluent current carried out of the wave tank (g L^{-1}); $Q_{effluent}$ is the flow rate of the current (L min^{-1}); V_{wt} is total effective water volume of the wave tank (27,000 L); T is the duration of each wave tank experiment (60 min); $\bar{C}_{sample}$ represents the average concentration of oil remaining in the water column after T; q_{oil} is the density of the test oil (g mL^{-1}), and V_{oil} is the volume of oil used in the experiment (300 mL).

Fig. 5 summarizes the estimated DDE of the test IFO180 for the three different dispersant types under the two wave conditions. For physical dispersion, the average DDE in the water column was 7% under regular wave conditions (Fig. 5A), increasing to 14% under breaking waves (Fig. 5B). The application of dispersants under regular wave conditions resulted in DDE of 3% and 6%, respectively, for Corexit 9500 (Fig. 5C) and SPC 1000 (Fig. 5E). Under breaking waves, however, it is clear that dispersant effectiveness was highly dependent on temperature. As shown in Fig. 5D and F, at lower temperature (10 °C) the DDE for Corexit 9500 and SPC 1000 were estimated to be 4% and 6%, respectively, whereas at higher temperature (16 °C), applying dispersants under breaking wave conditions increased the DDE of Corexit 9500 and SPC 1000 to nearly 90% and 50%, respectively.

Fig. 5 also illustrates the analysis of covariance linear regression model predicting the DDE as a function of temperature for different

Table 3
Analysis of variance (ANOVA) of the effects of temperature, dispersant, wave, and their interactions.

Source	D.f.	S.S.	M.S.	F	Pr (F)[a]
Wave (W)	1	0.281	0.281	57.405	0.00003***
Dispersant (D)	2	0.067	0.034	6.880	0.01537*
Temperature (T)	1	0.269	0.269	54.946	0.00004***
W:D	2	0.067	0.033	6.826	0.01571*
W:T	1	0.094	0.094	19.205	0.00176***
D:T	2	0.182	0.091	18.530	0.00064***
W:D:T	2	0.070	0.035	7.111	0.01404*
Residual	9	0.044	0.005		

[a]Single asterisk (*) denotes effect was significant at $p < 0.05$ level; Triple asterisks (***) indicates effect was significant at more strict $p < 0.007$ level (after Bonferroni adjustment).

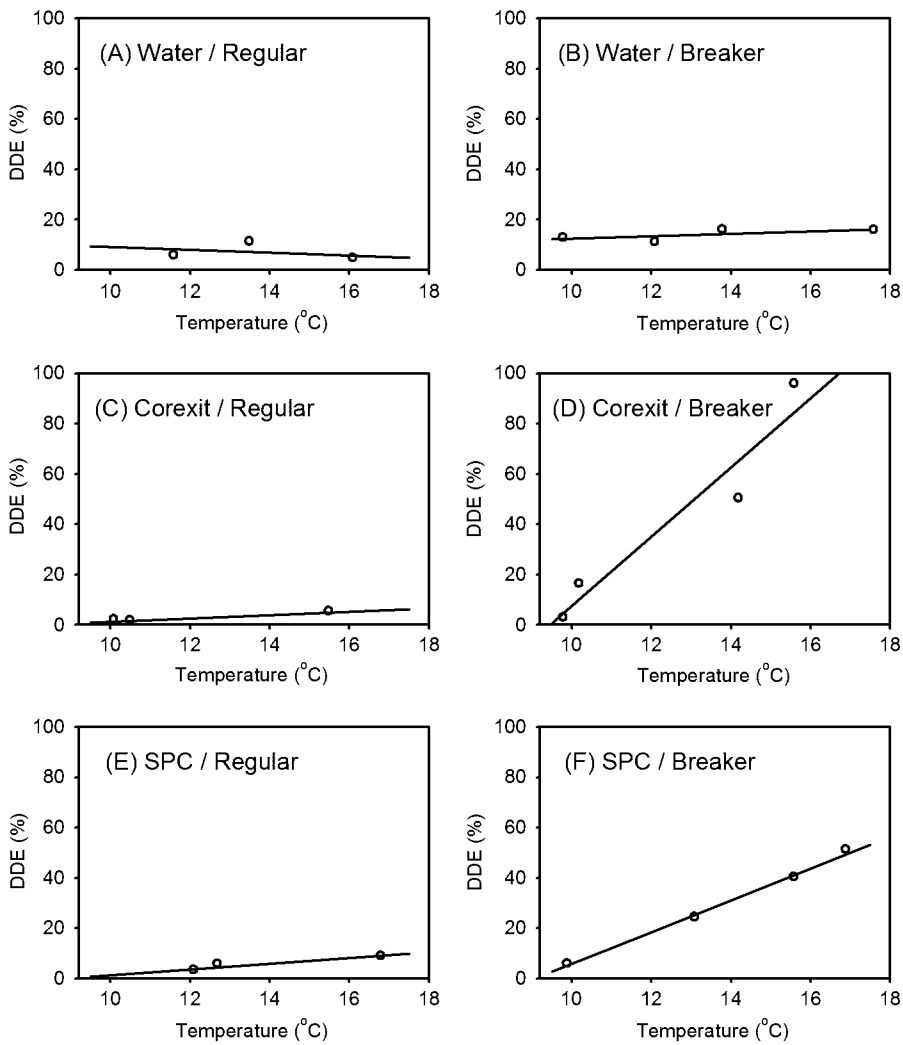


Fig. 5. Dynamic dispersant effectiveness (DDE) of IFO180 as a function of temperature and wave conditions. Dots are experimental data, and lines are the best-fits of the analysis of covariance model.

treatment (dispersant and wave) combinations. An analysis of variance (ANOVA) (Table 3) indicated that all the main factors and the multi-factor interactions had significant effects on DDE ($p < 0.05$). Under more strict criteria with the Bonferroni adjustment ($p < 0.007$), two-way interactions of "wave by temperature" and "dispersant by temperature" and main factors of "temperature" and "wave" were found to affect DDE significantly. Multivariate parameter estimation through linear regression in analysis of covariance (ANCOVA, Table 4) revealed that the treatments of breaking wave conditions ($p = 0.0047$) and Corexit 9500 ($p = 0.0003$) significantly increased the value of the intercepts (i.e., the DDE at the average tested temperature, 12.5 ffC), whereas the treatments with Corexit 9500 ($p = 0.0001$) and SPC 1000 ($p = 0.0101$) under breaking wave conditions significantly increased the slope, meaning that increased seawater temperature under these treatment conditions can significantly improve DDE. The best-fit linear prediction models of the DDE for different treatment combinations are summarized in Table 5 and illustrated in Fig. 5.

3.2. Effects of dispersant and wave conditions on droplet size distribution

Dispersant effectiveness of oil is intrinsically determined by the dispersed oil droplet size distribution (Daling et al., 1990; Lewis et al., 1985), and measurement of the in situ droplet size distribution of the testing system may facilitate comparison of various scales and the droplet sizes observed from the field (NRC, 2005). In this work, the droplet size distribution of the dispersed oil was determined by a laser particle counter (LISST-100X) that was suspended in the water column at the end of the flow-through wave tank, following the same procedure as has been used for crude oil dispersion experiments (Li et al., 2009).

Fig. 6 summarizes the effect of dispersant type and wave energy on the volume mean diameter of the dispersed oil as a function of time in the water column as recorded by the LISST-100X. Under regular waves, regardless of the presence or absence of dispersants, the volume mean diameters of the dispersed oil droplets were consistently high, ranging from 400 to 450 l m throughout the entire experimental period. Under breaking waves, however, even without dispersant (Fig 6A), the volume mean diameters were rapidly reduced from 450 l m to less than 200 l m within 10 min, and remained low thereafter. Application of Corexit 9500 under breaking waves lowered the average dispersed oil droplet sizes from 450 to 100 l m within 5 min, where they remained for the duration of each experiment. Use of SPC 1000 resulted in similar distributions as the application of Corexit 9500, with the initial oil droplet size reduced from 450 l m to less than 200 l m within 5 min, remaining at around 150 l m until the end of the experiments.

The effects of breaking waves and the presence of dispersants on the dispersed oil droplet size distribution exhibited the same pattern for the test IFO180 as those for unweathered MESA crude and unweathered ANS crude oil observed previously (Li et al., 2009). However, the average dispersed droplet sizes of the test

Table 4
Parameter estimates from the analysis of covariance (ANCOVA) model.

Coefficients	Parameter	Std. error	T value	Pr (>|t|)
Intercept				
Water, Breaker	0.1358	0.0364	3.7250	0.0047
Corexit, Breaker	+0.2836	0.0505	5.6129	0.0003
SPC, Breaker	+0.0796	0.0536	1.4836	0.1721
Water, Regular	¬ 0.0586	0.0608	¬ 0.9648	0.3599
Corexit, Regular	¬ 0.1060	0.0550	¬ 1.9288	0.0858
SPC, Regular	¬ 0.0950	0.0605	¬ 1.5705	0.1507
Slope (Temperature)				
Water, Breaker	0.0051	0.0123	0.4189	0.6851
Corexit, Breaker	+0.1328	0.0186	7.1353	0.0001
SPC, Breaker	+0.0582	0.0180	3.2407	0.0101
Water, Regular	¬ 0.0109	0.0251	¬ 0.4350	0.6738
Corexit, Regular	+0.0014	0.0205	0.0701	0.9456
SPC, Regular	+0.0065	0.0229	0.2842	0.7827

Table 5
ANCOVA model predicted estimation of the dynamic dispersant effectiveness (DDE) of different dispersant types under two different wave conditions as a function of temperature (T, ffC).

Treatment	DDE equation
Water, Breaker	0.1358 + 0.0051 * (T ¬ 12.5)
Corexit, Breaker	0.4194 + 0.1379 * (T ¬ 12.5)
SPC, Breaker	0.2154 + 0.0633 * (T ¬ 12.5)
Water, Regular	0.0772–0.0058 * (T ¬ 12.5)
Corexit, Regular	0.0298 + 0.0065 * (T ¬ 12.5)
SPC, Regular	0.0408 + 0.0116 * (T ¬ 12.5)

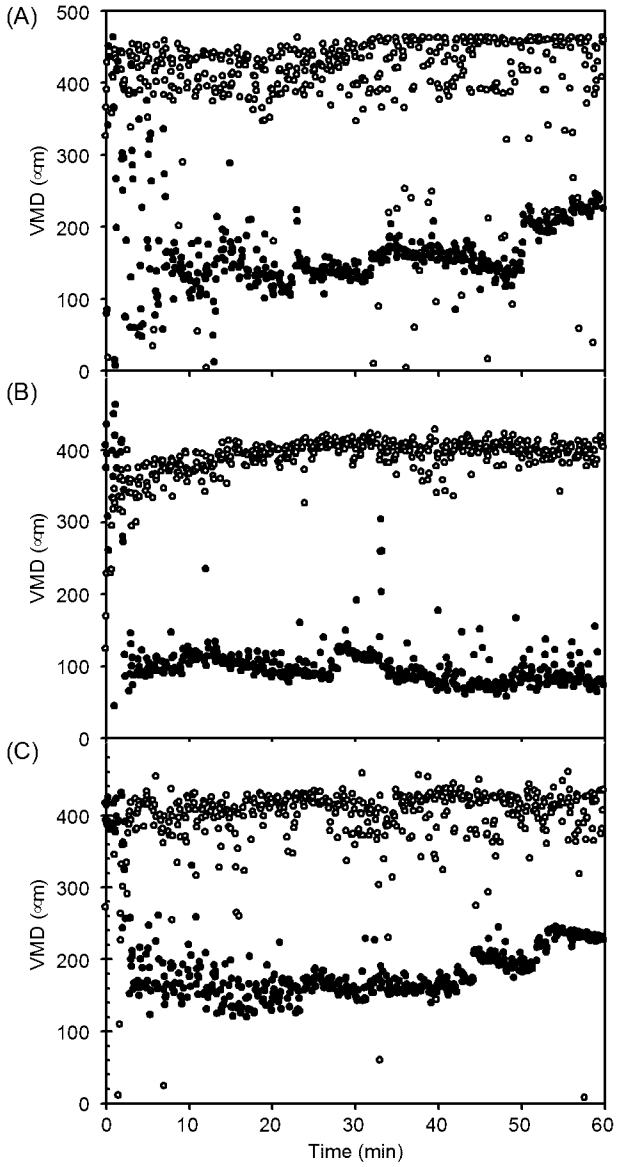


Fig. 6. Effect of dispersant type [(A) water, (B) Corexit 9500, and (C) SPC 1000] and wave conditions [regular (open circles) and breaking (solid dots)] on the volume mean diameter of the dispersed IFO180 oil droplet sizes.

IFO180 under regular waves observed in this study (VMD 400–450 l m) were significantly larger than those of the dispersed crude oil (Li et al., 2009) under the same regular wave conditions (VMD < 300 l m). This can be attributed to the higher viscosity and interfacial tension of the test fuel oil IFO180 than MESA and ANS crude oils, which would also explain the generally poor DDE performance under regular wave conditions (Fig. 5A, C, and E). Physical dispersion of the test IFO180 under breaking waves produced droplet sizes in the same range as those generated by physical dispersion of crude oil, and the DDE's were in good agreement between the test IFO180 (Fig. 5B) and the two crude oils (19% for MESA and 12% for ANS) that were estimated previously (Li et al., 2009). Chemical dispersion of the test IFO180 under breaking waves generated average droplet sizes higher than the crude oil droplet sizes measured in the wave tank under the same breaking wave conditions for the same dispersant (VMD < 50 l m). These sizes were also significantly larger than those suggested as the permanently dispersed droplet sizes, 50–70 l m, in the field (Lunel, 1993; Lunel et al., 1995). This can be due to the relatively higher density of heavy fuel oil (test IFO density is 0.96 g cm^{-3} compared to the weathered MESA, 0.88 g cm^{-3} and fresh ANS, 0.88 g cm^{-3}), hence the higher threshold dispersed oil droplet sizes for the heavier fuel oil.

4. Conclusion

The effects of temperature and wave conditions on the effectiveness of two commercial dispersants in dispersing a heavy fuel oil have been quantitatively studied using a flow-through experimental wave tank. Although heavy fuel oil has been reported to be more difficult to disperse than light and medium crude oils (Chapman et al., 2007; NRC, 2005), we achieved high dispersion efficiencies of heavy fuel oil under breaking wave hydrodynamic conditions at higher temperatures (>14 f°C). Our observations for Corexit 9500 and SPC 1000 demonstrated that the strong effect of seawater temperature dictated the DDE of the test IFO180 within a narrow window ranging from 10 to 17 f°C. The effect of mixing energy from breaking wave conditions agrees with results from field trials (Colcomb et al., 2005) and large-scale tank testing (Trudel et al., 2005) on dispersant effectiveness of heavy fuel oil. The observed temperature and wave effects were also consistent with the laboratory BFT experimental results on heavy fuel oil dispersion (Srinivasan et al., 2007). The results presented here have demonstrated that using chemical dispersants in fuel oil dispersion is feasible at appropriate sea state and environmental conditions and provide key data to update standard operational guidelines (e.g. IMO, USCG) for dispersant use as an oil spill countermeasure at sea.

It is worth noting that the test results reported here are based on one specific IFO180 fuel oil type that was selected as the testing model for heavy fuel oil. These test data should not be generalized to all heavy fuel oils or IFO180 oil types, some of which may have been more or less dispersible under the same testing conditions due to the effects of different physical properties of IFO180 fuel oils (Canevari et al., 2001). Specific chemical compositions (such as resin and asphaltene content) and physical properties (such as pour point and dynamic viscosity) of heavy fuel oils, especially IFO180 oils, could vary substantially from batch to batch.

Acknowledgements

This research was funded by the Program of Energy Research and Development (PERD), US EPA (contract No. 68-C-00-159), and NOAA/UNH Coastal Response Research Center (NOAA Grant No.: NA04NOS4190063 UNH Agreement No.: 06-085). The IFO180 testing oil was kindly provided by Mr. Joseph V. Mullin from the Minerals Management Service of the US Department of the interior. The authors wish to thank D. Bellibeau, J. Niven, B. Robinson and X. Ma for skilful technical assistance. Dr. P.E. Kepkay has provided useful comments on an earlier version to improve the manuscript. The findings, opinions, and recommendations expressed in this report are those of the authors and do not necessarily reflect those of the funding agencies.

References

Canevari, G.P., Calcavecchio, P., Becker, K.W., Lessard, R.R., Fiocco, R.J., 2001. Key parameters affecting the dispersion of viscous oil. In: 2001 International Oil Spill Conference, pp. 4217–4221.

Chapman, H., Purnell, K., Law, R.J., Kirby, M.F., 2007. The use of chemical dispersants to combat oil spills at sea: a review of practice and research needs in Europe. Marine Pollution Bulletin 54 (7), 827–838.

Clark, J., Becker, K., Venosa, A., Lewis, A., 2005. Assessing dispersant effectiveness for heavy fuel oils using small-scale laboratory tests. In: 2005 International Oil Spill Conference, IOSC 2005, pp. 10593–10597.

Colcomb, K., Peddar, M., Salt, D., Lewis, A., 2005. Determination of the limiting oil viscosity for chemical dispersion at sea. In: 2005 International Oil Spill Conference, IOSC 2005, pp. 11506–11511.

Daling, P.S., Mackay, D., Mackay, N., Brandvik, P.J., 1990. Droplet size distributions in chemical dispersion of oil spills – towards a mathematical model. Oil and Chemical Pollution 7 (3), 173–198.

Delvigne, G.A.L., Sweeney, C.E., 1988. Natural dispersion of oil. Oil and Chemical Pollution 4 (4), 281–310.

Drennan, W.M., Donelan, M.A., Terray, E.A., Katsaros, K.B., 1996. Oceanic turbulence dissipation measurements in SWADE. Journal of Physical Oceanography 26 (5), 808–815.

Fiocco, R.J., DeMarco, G., Lessard, R.R., Daling, P.S., Canevari, G.P., 1999. Chemical dispersibility study of heavy bunker fuel oil. In: Environment Canada Arctic and Marine Oil Spill Program Technical Seminar (AMOP) Proceedings, pp. 173–186.

Funke, E.R., Mansard, E.P., 1979. SPLSH A program for the synthesis of episodic waves. Ottawa, Canada, Hydraulics laboratory technical report LTR-HY-65. National Research Council, Ottawa, Canada.

Guyomarch, J., Merlin, F., Colin, S., 1999. Study of the feasibility of chemical dispersion of viscous oils and water-in-oil emulsions. In: Environment Canada's 22nd Arctic and Marine Oilspill (AMOP) Technical Seminar, Calgary, Alberta, Canada, pp. 219–230.

IMO/UNEP, 1995. Guidelines on Oil Spill Dispersant Application, International Maritime Organization, London, UK, 55p.

Lewis, A., 2004. Determination of the limiting oil viscosity for chemical dispersion of oil at sea. Final report for DEFRA, ITOPF, MCA, and OSPL. MCA project 10/9/180. CP & R Branck, Maritime and Coastguard Agency, Southampton, SO 15 1EG, UK.

Lewis, A., Byford, D.C., Laskey, P.R., 1985. The significance of dispersed oil droplet size in determining dispersant effectiveness under various conditions. In: International Oil Spill Conference.

Li, Z., Lee, K., King, T., Boufadel, M.C., Venosa, A.D., 2008. Assessment of chemical dispersant effectiveness in a wave tank under regular non-breaking and breaking wave conditions. Marine Pollution Bulletin 56 (5), 903–912.

Li, Z., Lee, K., King, T., Boufadel, M.C., Venosa, A.D., 2009. Evaluating crude oil chemical dispersion efficacy in a flow-through wave tank under regular non-breaking wave and breaking wave conditions. Marine Pollution Bulletin 58, 735–744.

Lunel, T., 1993. Dispersion: oil droplet size measurement at sea. In: Proceedings of the Sixteenth Arctic and Marine Oil Spill Program (AMOP) Technical Seminar, Calgary, Alberta, Canada. Environment Canada, Ottawa, Ontario, Canada, pp. 1023–1056.

Lunel, T., Davies, L., Brandvik, P.J., 1995. Field trials to determine dispersant effectiveness at sea. In: Proceedings of the 18th Arctic and Marine Oilspill Program (AMOP) Technical Seminar, Edmonton, Alberta, Canada. Environment Canada. Ottawa, Ontario, Canada, pp. 629–651.

NRC, 1989. National Research Council: Using Oil Spill Dispersant on the Sea. National Academy Press, Washington, DC.

NRC, 2005. National Research Council: Understanding Oil Spill Dispersants: Efficacy and Effects. National Academies Press, Washington, DC.

Sorial, G.A., Venosa, A.D., Koran, K.M., Holder, E., King, D.W., 2004. Oil spill dispersant effectiveness protocol. I: Impact of operational variables. Journal of Environmental Engineering – ASCE 130 (10), 1073–1084.

Srinivasan, R., Lu, Q., Sorial, G.A., Venosa, A.D., Mullin, J., 2007. Dispersant effectiveness of heavy fuel oils using baffled flask test. Environmental Engineering Science 24 (9), 1307–1320.

Terray, E.A., Donelan, M.A., Agrawal, Y.C., Drennan, W.M., Kahma, K.K., Williams, A.J., Hwang, P.A., Kitaigorodskii, S.A., 1996. Estimates of kinetic energy dissipation under breaking waves. Journal of Physical Oceanography 26 (5), 792–807.

Trudel, B.K., Belore, R.C., Guarino, A., Lewis, A., Mullin, J., 2005. Determining the viscosity limits for effective chemical dispersion: relating ohmsett results to those from tests at-sea. In: 2005 International Oil Spill Conference, IOSC 2005, pp. 10687–10692.

Venosa, A.D., Kaku, V.J., Boufadel, M.C., Lee, K., 2005. Measuring energy dissipation rates in a wave tank. In: Proceedings of 2005 International Oil Spill Conference, Miami, FL. American Petroleum Institute, Washington, DC.

Venosa, A.D., King, D.W., Sorial, G.A., 2002. The baffled flask test for dispersant effectiveness: a round robin evaluation of reproducibility and repeatability. Spill Science and Technology Bulletin 7 (5–6), 299–308.

Venosa, A.D., Lee, K., Boufadel, M.C., Li, Z., King, T., Wickley-Olsen, E., 2008. Dispersant effectiveness as a function of energy dissipation rate in an experimental wave tank. In: 2008 International Oil Spill Conference, Savannah, GA, pp. 777–784.

Wickley-Olsen, E., Boufadel, M.C., King, T., Li, Z., Lee, K., Venosa, A.D., 2008. Regular and breaking waves in wave tank for dispersion effectiveness testing. In: 2008 International Oil Spill Conference Savannah, GA, pp. 499–506.

Environmental Science & Technology

Critical Review

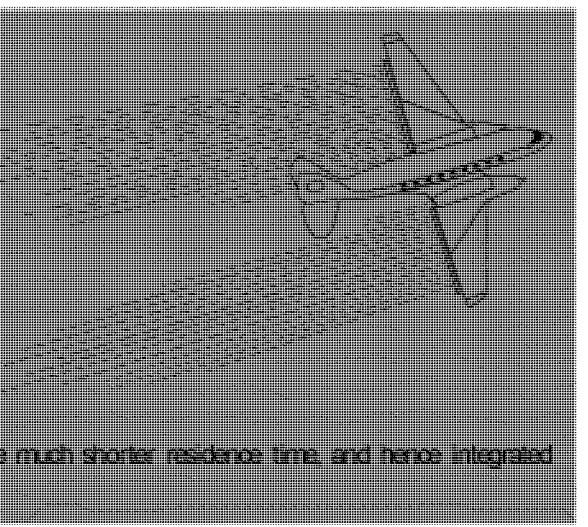
pubs.acs.org/est

Oil Spill Dispersants: Boon or Bane?

Roger C. Prince*

ExxonMobil Biomedical Sciences, Inc., Annandale, New Jersey 08801 United States

Dispersants provide a reliable large-scale response to catastrophic oil spills that can be used when the preferable option of recapturing the oil cannot be achieved. By allowing even mild wave action to disperse floating oil into tiny droplets (<70 μm) in the water column, seabirds, reptiles, and mammals are protected from lethal oiling at the surface, and microbial biodegradation is dramatically increased. Recent work has clarified how dramatic this increase is likely to be: beached oil has an environmental residence of years, whereas dispersed oil has a half-life of weeks. Oil spill response operations endorse the concept of net environmental benefit, that any environmental costs imposed by a response technique must be outweighed by the likely benefits. This critical review discusses the potential environmental debits and credits from dispersant use and concludes that, in most cases, the potential environmental costs of adding these chemicals to a polluted area are likely outweighed by the much shorter residence time, and hence integrated environmental impact, of the spilled oil in the environment.



INTRODUCTION

Oil fuels our modern world, accounting for some 33% of energy consumption in 2013; daily consumption was 87 million barrels per day.[1] Some of this crude oil is produced from wells drilled under the sea, and a large percentage travels by sea between production and consumption. Despite the best efforts of the oil and shipping industries, some gets spilled. Catastrophic spills appropriately garner the public's attention, although, in fact, most spills are rather small. Natural seeps are likely the largest contributor of oil to the world's oceans, followed by nonpoint sources on land.[2] Tanker accidents are becoming less common,[3] but it is still true that a few large spills contribute the most oil released to the sea by ships, and where such spills occur, they release far more oil in a few days than even the most active seeps. The tragic 2010 blowout from the Deepwater Horizon well[4] is a reminder that large releases can also occur from drilling operations.

Crude oil has been part of the biosphere for millions of years,[5] and a large number of microbes, both prokaryotic[6] and eukaryotic,[7] have evolved to consume it. Biodegradation is the eventual fate for all spilled oil that is not collected or burned, and both collection and combustion require that spilled oil be corralled with booms.[8] While skimming can be an effective process if equipment is close to hand and the weather is reasonably calm, and is frequently part of oil spill response plans (e.g., refs 9−12), large spills in remote areas can spread so quickly that skimming becomes extremely difficult. For example, the Deepwater Horizon response, despite enormous efforts, collected only some 3% of the oil released and burned another 5%.[4] Considerable research has been expended, therefore, on trying to enhance the rate of oil biodegradation.

Oil is an unusual substrate for microbial growth for two distinct reasons. On the one hand, most oil molecules have low density and are very sparingly soluble, so oil tends to stay as surface slicks or droplets dispersed by wave action. Biodegradation is then limited by the surface area of the oil−water interface. On the other, whereas hydrocarbons are rich sources of carbon and energy, oil contains no other useful elements for microbial growth. Nitrogen and phosphorus are the most usual limiting nutrients in the sea,[13] followed by iron and other trace requirements.[14] While seawater contains trace levels of these nutrients,[15] the biodegradation of significant concentrations of oil, such as on a shoreline, is likely to exhaust the local supply. Bioremediation, the stimulation of biodegradation, thus aims to overcome these two limitations.

In the case of oil stranded on shorelines in Prince William Sound AK following the spill from the Exxon Valdez, the first response was to remove oil from beaches by washing it back to the sea and collecting it with skimmers.[16] This had the effect of leaving a relatively thin film of oil on the gravel and rocks of the intertidal (and sometimes supra-tidal) zone, and this was bioremediated by the careful addition of oleophilic and slow-release fertilizers to increase the supply of bioavailable nitrogen and phosphorus. This worked quite well, stimulating oil biodegradation between 2- and 5-fold without causing any additional adverse effects,[17−20] but it should be born in mind that the oil had already been on the shoreline for a year before the quantitative experiments reported therein were begun.

Received: February 23, 2015
Revised: April 27, 2015
Accepted: May 4, 2015

 © XXXX American Chemical Society

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001442-00001

Floating oil presents different challenges. Floating oil is a very real hazard to diving birds and mammals (e.g., refs 21 and 22), and oil that beaches is a hazard to shorebirds,[23] invertebrates,[24,25] and mangroves.[26] Ameliorating these hazards was a primary impetus for the initial development of oil spill dispersants in the 1970s.[27] Modern dispersants are complex mixtures of anionic and neutral surfactants in a hydrocarbon solvent (e.g., ref 28) that lower the interfacial tension between oil and water so that minimal wave action and turbulence can disperse the oil into tiny droplets (<70 μm) in the water column. Such droplets are essentially neutrally buoyant, so with minimal turbulence they stay in the water column and diffuse apart. Concentrations of dispersed oil may be ≈1000 ppm in the first minutes after dispersion, but they fall to a few ppm, in a correspondingly larger volume, within hours, and to sub parts per million levels within a day.[29−34] Furthermore, even these concentrations are found only in the top few meters of the sea. Similarly, dispersants can harness the ejection turbulence of oil emanating from an uncontrolled subsea release and allow the formation of tiny oil droplets in the deep sea;[4,34−37] again, the droplets diffuse apart until they are in the sub parts per million concentration range. This diffusive dilution means that, although the levels of nutrients in the sea are relatively low,[13] it does not take long for oil concentrations to fall so that even those low levels are adequate for significant and rapid biodegradation. Hazen et al.[14,35] measured half-lives of n-alkanes of a few days in the dilute (2−442 ppb[34]) dispersed submarine plume from the Deepwater Horizon at 1100−1220 m (and 5 °C), and very similar results were reported for a broad array of individual hydrocarbons at low concentrations in New Jersey seawater at 8 °C,[36,37] in a flume in Trondheim, Norway,[38] at 30−32 °C and in water off the Penang, Malaysia, shore[39] at 27.5 °C. The approximate biodegradation half-life of the total measurable hydrocarbons was 11−14 days, both at low oil concentrations with indigenous nutrients[36,38] (2.5 and 43 ppm oil, respectively) and at slightly higher concentrations (100 ppm oil) with added nutrients.[39] Even the four ring aromatic chrysene and its methyl-, dimethyl-, and ethyl-alkylated forms had half-lives on the order of a month.[36]

DISPERSANTS

Dispersants have been used on a large scale in many responses, notably, the 1993 Braer wreck in the Shetland Islands,[40] the 1996 Sea Empress spill in South Wales,[41] and the 2010 Deepwater Horizon blowout in the Gulf of Mexico.[33] Seven spills in the US portion of the Gulf of Mexico were treated with dispersants between 1995 and 2004.[42] Dispersants are stockpiled, with equipment for their use, in large quantities around the world,[43] and substantial illustrated guidelines for their use are freely available.[44] Nevertheless, their deployment is still controversial, for clearly dispersants are not without potential drawbacks. Most dispersants are not themselves significantly toxic; they have toxicities indistinguishable from common household dish liquids and shampoos,[45] including those used for cleaning oiled seabirds,[46,47] and the majority, including those used in the Deepwater Horizon response, show neither androgen- nor estrogen-receptor activity.[48] Nevertheless, the use of dispersants involves adding more chemicals to an already impacted area, and the water under a recently dispersed oil slick is significantly transiently more toxic to organisms than under the undispersed slick, albeit because of the greater concentrations of oil in the water, not because of any increase in toxicity on an oil weight basis.[47,48,50]

So, how should spill response coordinators decide when and where to use dispersants? Most responders rely on a net environmental benefit analysis, often abbreviated NEBA.[51−55] At first glance, the concept seems oxymoronic: how can anything related to an oil spill have an environmental benefit? However, in actuality, the concept is very useful: everyone involved in a cleanup recognizes that an oil spill is a dreadful environmental insult and is working diligently to minimize adverse impacts and to remove the oil as quickly as possible. The question is whether a response tool will end up doing more harm than good, of whether there will be net environmental improvement despite potential collateral harm done in the short term by the response. Table 1 offers a comparison of the potential hazards and environmental fate of floating slicks and dispersed oils that need to be considered in such analyses.

The first hurdle for dispersants is to demonstrate their fundamental efficacy. In the United States, the U.S. Environmental Protection Agency (USEPA) maintains the National Oil and Hazardous Substances Pollution Contingency Plan Product Schedule[56] as part of the National Contingency Plan and lists chemical dispersants that may be authorized for use. Dispersants on the Product Schedule have demonstrated effectiveness; they can disperse at least 45% of Prudhoe Bay or South Louisiana crude oil in a standard swirling flask test.[57] The swirling flask test is one of several tests designed to discriminate between dispersants with different efficacies on a simple laboratory scale. It does this reasonably well (although the USEPA is considering revising it with a baffled flask test[58]), but unfortunately the passing grade of 45% has often been assumed to indicate expected field performance. In fact, the test dramatically underestimates efficacy in the field, primarily due to the amount of energy it imparts to the floating oil and the volume available for diffusion. Tests in the OHMSETT facility,[59] a wave tank in New Jersey that is 200 m long, 20 m wide, and 2.5 m deep, routinely measure dispersant efficiencies >95%, even at low temperatures with ice in the water.[60]

A second requirement for listing on the USEPA product schedule is that the acute toxicity of the dispersant to two reference species (silverside fish, Menidia beryllina (96 h), and mysid shrimp, Americamysis bahia (48 h)[49,59]) be reported. During the Deepwater Horizon response, the USEPA required that dispersants "have a toxicity value less than or equal to (sic) 23.00 (sic) ppm LC_{50} toxicity value for Menidia or 18.00 ppm LC_{50} for Mysidopsis (Americamysis)";[62] the dispersants being used passed this hurdle.[49]

As mentioned above, the toxicity of modern dispersants is usually so low as to likely have only minimal adverse effects at levels used in response operations (nominal aerial dispersant application rates are 5 gallons of dispersant per acre, 47 L/hectare;[63] diffusion into the top 20 cm of seawater would give concentrations around 23 ppm, and of course further diffusion will continually lower the concentration). However, dispersed oil is significantly more toxic, with acute LC_{50} values more than an order of magnitude lower.[49] There has been some confusion around quantifying the toxicity of dispersed oil;[64,65] acute toxicity arises from a general narcosis caused by dissolved hydrocarbons moving to the lipids of the test organism,[66−68] so if estimates of oil concentrations include small droplets, the toxicity expressed on a per milligram hydrocarbon basis are lower (higher LC_{50}) than that for dissolved components. In any case, dispersants encourage solubility by increasing the surface-

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001442-00002

Table 1

potential hazards and environmental fate of floating slick	potential hazards and environmental fate of dispersants and dispersed oil
	Dispersant application has the potential to create aerosols with potentially adverse effects on humans and wildlife. Such effects are seen only in the laboratory at quite high concentrations and with prolonged exposures.[99-102] They are minimized by protective equipment for applicators and exclusion zones around application. Aerial application is very unlikely to be approved within several miles of a coast.
	The addition of dispersants puts additional chemicals into a polluted area. Nominal application rates of dispersants are 5 gallons per acre (47 L ha^{-1}) for surface spills,[63] equivalent to a dispersant/oil ratio of about 1:20. Subsea injection seems to be effective at much lower doses, perhaps 1:100 or less. Dispersants are composed of chemicals that are generally acceptable for food contact and are biodegradable,[28,93,94] but when they are used at suboptimal doses, they may become incorporated into persistent tarballs.[95]
	Dispersants may persist in the environment. Dispersants are complex mixtures of surfactants and solvents, and very few techniques can measure them all.[104] Much published work has focused on individual chemicals, but since all of the components are widely used in consumer products on a far greater scale than in dispersants, care must be taken in attributing detection to dispersant use.[97]
	Dispersants may not penetrate heavy oils and may "roll-off" before they can be effective.[103,105] Nevertheless, dispersants have been shown to be effective with many heavy oils,[117] and new formulations are being developed to further this use.[106]
	Suboptimal dispersant application may not disperse the oil but may hinder subsequent skimming operations.
	Several studies appear to show that dispersants do not stimulate biodegradation very much.[139-141,36,78] Those experiments were based on a misunderstanding of the role of dispersants,[37] which is simply to disperse oil into tiny droplets that can diffuse apart. Such dilute small droplets, whether generated by dispersants or by low concentrations of oil, are degraded rapidly,[36-38,78] and dispersants show no significant stimulatory effect. Dispersed oils that can recoalesce into larger droplets, as occurs in laboratory experiments with more than a few parts per million oil,[139-141] again show only a small stimulation of biodegradation by dispersants. A clear stimulation of biodegradation by dispersants (many fold) is seen only when dispersed oil at environmentally realistic concentrations[29-36] is compared to an undispersed slick.[37] It is the latter that is the target of dispersants.
	Dispersants have been reported to be toxic to some bacteria.[142] These experiments looked at a few isolated cultivars from beach sand rather than pelagic organisms likely to be exposed to dispersants. Dispersants had no inhibitory effects in experiments with pelagic microbes[36-38,78] and were significantly less toxic than oil alone to Vibrio fischeri (now Aliivibrio fischeri[143]) in the Microtox test.[144]
Floating oil can be lethal to birds or mammals that penetrate it. The oil reduces the insulating properties of feathers and fur and may be toxic if ingested during preening or grooming.[21,22] Mild oiling may be transferred to eggs, chicks, and young.[107] Floating oil also likely kills small invertebrates, algae, fish eggs, and young that are at the sea surface under the slick.[108]	Dispersed oil is potentially toxic to organisms close to the surface when the oil is initially dispersed. This is particularly true for small invertebrates, algae, fish eggs, and young that cannot swim away.[109] Nevertheless, measured dilution suggests that they will not be exposed for very long.[29-36] The remarkably heterogeneous distribution of planktonic organisms, on all scales, probably has more influence on local populations than relatively small-scale mortality due to concentrated dispersions of oil.[145,146] Planktonic microbial species respond remarkably rapidly, even to diel cycles.[147] Fish kills are rarely noted after dispersant use, presumably because fish and other nekton swim away from the dispersed oil.
Floating oil is subject to photooxidation, which polymerizes polycyclic aromatic compounds to polar materials.[110,111] This eventually leads to very persistent tarballs.[112] Beached oil undergoes a similar process, leading to very persistent "pavements".[126,127]	
Transparent planktonic organisms may accumulate polycyclic aromatic compounds leached from slicks and beached oil and may be subject to phototoxicity in the surface layer of the sea.[113]	Dispersed polycyclic aromatic compounds may be accumulated by transparent planktonic organisms, which may then be subject to phototoxicity in the surface layer of the sea.[113]
Floating oil absorbs water; it is not unusual for floating slicks to increase their volume by >50% in 24 h.[114-116] This phenomenon makes dispersion more difficult but not impossible.[117] Over several days, many floating oils emulsify, forming water-in-oil emulsions known as mousse.[118-120] The encapsulated water no longer exchanges with bulk seawater, and biodegradation is likely even more nutrient- and oxygen-limited than in the floating slick. Mousse may be consumed by fish, turtles, etc. and also lead to tarballs.[111,112]	
Small molecules in floating oil evaporate rapidly, becoming volatile organic compounds (VOCs)[119,120] that can reach levels of concern for spill responders.[121] Evaporated molecules may be destroyed by photochemistry[122] or biodegradation.[123]	Dispersion slows evaporation, especially if the oil is dispersed subsea, as some was in the Deepwater Horizon response. The potentially toxic small molecules are retained in the water column until they are biodegraded or escape to the atmosphere over a much greater area due to diffusion at depth.
If it is not physically dispersed by storms or recovered mechanically, then an oil slick will likely beach on a shoreline. If it stays on the shoreline surface, then it may be possible to remove it by physical means, but storms will likely incorporate oil deep into sandy beaches,[124,125] where, if left alone, it will likely remain for decades.[126,127] Beached oil can be a major threat to turtle and shorebird nesting[128] and to mangroves[129] and marshes,[130] and recovery may take years.[131,132]	Dispersants are typically used far from shore in deep water (e.g., >3 miles[148]). Dispersed oil diffuses to sub parts per million levels and is very unlikely to reach a shoreline in noticeable quantities.
If the slick arrives at a shoreline, then biodegradation will be slow because of the high concentration of oil in comparison to the low concentrations of bioavailable nutrients in tidal flows. Biodegradation was too slow to measure directly in the absence of bioremediation in Prince William Sound following the Exxon Valdez spill,[18,19] but even with effective bioremediation, the half-life of total detectable hydrocarbons was of the order of 10 weeks.[18,19] The	Dispersed oil dilutes to such low concentrations that biodegradation is rapid. Measured half-lives are in the days to few weeks time frame.[14,33-37] Even the four ring aromatic chrysene and its methyl-, dimethyl-, and ethyl- forms have half-lives on the order of a month.[36] Nearshore waters from the Chukchi Sea,[78] the North West Atlantic,[36] and deepwater from Trondheim Fjord[38] all contained enough nutrients for substantial biodegradation of a few parts per million oil in 60 days, and it is reasonable to extrapolate that further dilution in more oligotrophic waters would allow similar degradation on similar time scales.

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001442-00003

Table 1. continued

potential hazards and environmental fate of floating slick	potential hazards and environmental fate of dispersants and dispersed oil
biodegradation of bulk oil in the Gulf of Mexico was very slow[74,75] compared to that of dispersed oil.[35,14]	
While oil hydrocarbons are essentially completely biodegradable (eg., ref 36), many of the deeply colored molecules (resins, asphaltenes, polars, etc) are more resistant to biodegradation, although at least some are consumed.[133,134] While these have minimal biological effects, the lifetime of these molecules in a sandy beach may be prolonged, especially if they have been entrained deep in a beach by storm action.[124,132]	While oil hydrocarbons are essentially completely biodegradable (eg., ref 36), many of the deeply colored molecules (resins, asphaltenes, polars, etc) are more resistant to biodegradation, although at least some are consumed.[133,134] Once the hydrocarbons have been degraded, these fractions lack oily characteristics and become essentially indistinguishable from other inert organic matter in the environment, such as humins.[135]
Physical deanup of beached oil can take weeks to years and requires hundreds to thousands of workers with their ancillary vehicles and support services.[16,128,136]	Optimal dispersant application for surface slicks uses planes that can carry 5000 gallons (19000 L) of dispersant, appropriate to treat 1000 acres (405 ha) of slick.[63] Depending on distances from airfields, several sorties can be flown per day, and spills from a tanker can be treated in a few days. Aerial spraying requires daylight (Figure 1). Subsea injection has the advantage of continuing 24 h a day, and the lower application rates mean that dispersant stocks will potentially last longer. If fully successful, oil from a subsea release may never reach the surface before it is biodegraded.[103]

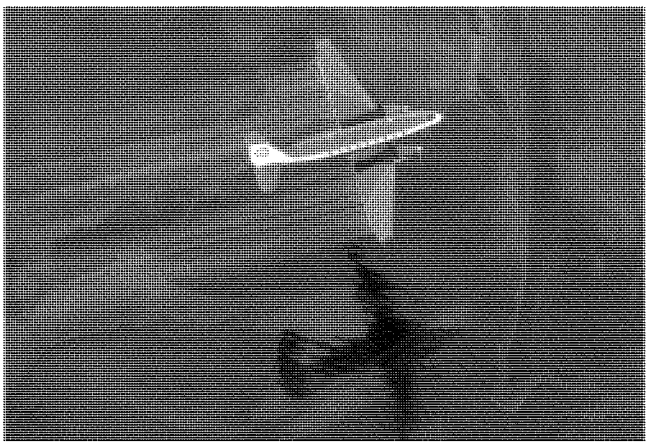


Figure 1. An updated DC3C spraying dispersants on oil from the Deepwater Horizon blowout. The wingspan is 29 m. Reproduced with permission from Airborne Support.

to-volume ratio of the oil, and there is a potential for short-term toxic effects in a dispersed plume. The question is how significant these effects might be in the field. Canonical acute toxicity tests involve constant exposures for 48 or 96 h,[61] but in a response at sea, the concentrations of oil will be dropping rapidly due to dilution by mixing and diffusion. As noted above, concentrations of dispersed oil drop to below 1 ppm within a few hours.[29−33]

Only acute toxicity tests are required for listing under the National Contingency Plan, but of course chronic effects are also a real concern. As expected, the longer exposure required to see chronic effects allows lower concentrations of hydrocarbons to exert an effect, and typical acute-to-chronic ratios are 1:10, that is, it may only take 10% of the acute LC_{50} to have a chronic EC_{50}.[69−72] Again, these concentrations are for prolonged exposures, and it is not clear how these relate to the very low concentrations[29−34] of dispersed oil that are found several days after dispersion.[73]

As noted at the outset, dispersants were initially conceived as a tool for minimizing seabird mortality, and early use weighed that benefit against potential toxicity to planktonic species.[27] However, an additional substantial benefit has now been clearly documented: the biodegradation of dispersed oil is dramatically faster than that of oil in a slick or on a shoreline. Oil on shorelines of Prince William Sound, Alaska, had a half-life of a year or more,[18−20] even with the substantial washing and bioremediation program.[16] Tarballs and mousse associated with the Deepwater Horizon blowout had similar persistence in the environment.[74,75] However, the biodegradation of dispersed oil is rapid and extensive.[14,35−37]

The oil spill response community agrees that the best response to an oil spill would be to collect it from the environment before it reached a shoreline, and many response plans focus on this requirement by staging large amounts of equipment aimed at achieving this goal even in the face of a very large spill (e.g., refs 9−12). However, if oil cannot be collected, particularly if it is unsafe for responders to perform mechanical recovery or because of remote location or hours of daylight, then responders must look to other methods. It is important to recognize that time is of the essence: deciding not to use a response option today may preclude its use tomorrow. This phenomenon, the window-of-opportunity,[76] is particularly relevant to the use of dispersants because as oil weathers by

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001442-00004

evaporation and absorption of underlying water, it becomes progressively more and more difficult to disperse with dispersants. (As an aside, dispersants incorporated into relatively fresh oil will help the dispersion of that oil even after some time if conditions are initially too calm for immediate dispersion,[77] a potential problem for requirements that dispersants be seen to be effective on a trial basis before large-scale application can begin.) Responders must weigh the potential additional short-term toxicity likely incurred immediately under a dispersed surface slick against the benefit of protecting diving birds and animals and having the oil removed from the environment by biodegradation on a time scale of a few weeks rather than years with a vastly smaller human footprint of spill response. The speed of biodegradation is particularly important if the over-riding concern about dispersant use is the potential local increase in toxicity; beached oil and undispersed mousse leach hydrocarbons and have their own potential environmental impacts for a prolonged period. It would also be prudent to bear in mind the environmental impact of large shoreline cleanup operations, which often involve hundreds of workers and the ancillary impacts of their support services. There is also the potential legacy of the final disposal of the oil, which may well involve burial at a secure landfill rather than combustion or biodegradation. Finally, while not an environmental impact, the economic impacts of an oiled marina or shoreline also need to be weighed as responders decide the most appropriate spill-response.

CONCLUDING REMARKS

In the final analysis, some accidents occur so close to shore that oil will undoubtedly reach the shoreline and most likely require some physical cleanup, perhaps followed by bioremediation. However, if the release is in deep water, then it ought to be possible to mobilize dispersants to keep bulk oil from ever reaching a shoreline. If oil is successfully dispersed into the water column, then it is likely that biodegradation will remove the vast majority of it in weeks to months, even in the Arctic and Antarctic.[78] What remains will likely be finely dispersed fragments of nonoily (to the touch) material depleted of hydrocarbons and rich in asphaltic materials and saturated biomarkers such as the hopanes.[36,79,80]

Many questions remain to be answered:

- How does the density of dispersed oil change as biodegradation proceeds? Alkanes are much lighter than water,[81] even at high pressure,[82] as are cyclic alkanes and monoaromatics. However, larger aromatic hydrocarbons, such as chrysene, have densities greater than 1,[83] as do the resins and asphaltenes.[84,85] Since biodegradation preferentially removes alkanes, initially buoyant droplets will become neutral and eventually sink. Is this partially degraded oil further metabolized once deposited on the surface sediment at depth? Reports of substantial amounts of fossil (radiocarbon silent) carbon on the surface sediment at depth[86] could be either unmetabolized oil molecules or the biomass of organisms degrading the oil or, more likely, both. As noted above, some molecules, such as the hopanes, seem very resistant to biodegradation and likely remain with very biodegraded oil (and degrading biomass) as a fingerprint of the initial source.[36,75,79,80]
- What fraction of the oil carbon is mineralized to CO_2 in the initial biodegradation, and what fraction is incorporated into biomass? Classical experiments with aerobes growing on glucose suggest a biomass yield of about 50%,[87] but what is the fate of that biomass? Levy and Lee[88] propose that it is the base for substantial fisheries offshore Atlantic Canada. Chemical analysis of the radiocarbon silent material reported by Chanton et al.[86] will shed light on this question.
- What role does microbial succession play in the biodegradation of oil? Such succession was clearly seen in the Gulf of Mexico following the Deepwater Horizon release,[87−92] but how did it relate to the chemical composition of the residual oil or to the nutrient levels in the water? Do dispersants affect this succession?
- How rapidly are dispersant components degraded in the sea? Early work established the biodegradation of nonionic surfactants in seawater,[93] and dioctylsulfosuccinate is known to be biodegradable[94] and clearly was being degraded close to the Deepwater Horizon spill site where it was being applied in the Corexit dispersant.[95] Nevertheless, traces of dioctylsulfosuccinate have been found far from the Deepwater Horizon accident;[96] was that associated with dispersant application? Traces found close to shore are more likely related to stormwater discharges.[95] Small quantities associated with tarballs[98] are likely the result of suboptimal dispersant application and highlight the need to apply an effective amount of dispersant if its benefits are to be achieved.
- Is biodegradation at depth (>1500 m) fundamentally distinct from biodegradation at the surface? Early work suggested that it is not,[137] but that work was clearly limited in its experimental tools, and there is much to learn. There is no doubt that active hydrocarbon-degrading microbial communities, both aerobic and anaerobic, are present in deep sea sediments.[138]

Already, however, we know enough from laboratory, mesocosm, and field experience to say that dispersed oil is degraded much more rapidly than undispersed oil, likely orders of magnitude more rapidly. This is the key piece of information that seems to be overlooked in most discussions of the potential adverse impacts of using dispersants. Even if there is an adverse local impact of dispersed oil, it will not last long. On the other hand, oil that reaches a shoreline may be there for years.

AUTHOR INFORMATION

Corresponding Author

*E-mail: roger.c.prince@exxonmobil.com. Phone: 908 730 2134. Fax: 908 730 1199.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

I am grateful to Airborne Support (http://airbornesupport.com/basler.html) for the photograph used in Figure 1.

REFERENCES

(1) Statistical Review of World Energy; BP, 2014. http://www.bp.com/en/global/corporate/about-bp/energy-economics/statistical-review-of-world-energy.html.

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001442-00005

(2) National Research Council Oil in the Sea III: Inputs, Fates, and Effects; National Academies Press: Washington, DC, 2003.
(3) Musk, S. Trends in oil spills from tankers and ITOPF non-tanker attended incidents, 2012. http://www.itopf.com/knowledge-resources/documents-guides/document/trends-in-oil-spills-from-tankers-and-itopf-non-tanker-attended-incidents-2012/.
(4) Lubchenco, J.; McNutt, M. K.; Dreyfus, G.; Murawski, S. A.; Kennedy, D. M.; Anastas, P. T.; Chu, S.; Hunter, T. Science in support of the Deepwater Horizon response. Proc. Natl. Acad. Sci. U.S.A. 2012, 109, 20212−20221.
(5) Tissot, B. P.; Welte, D. H. Petroleum Formation and Occurrence; Springer: Berlin, 1984.
(6) Prince, R. C.; Gramain, A.; McGenity, T. J. Prokaryotic hydrocarbon degraders. In Handbook of Hydrocarbon and Lipid Microbiology; Timmis, K. N., Ed.; Springer-Verlag: Berlin, 2010; pp 1672−1692.
(7) Prince, R. C. Eukaryotic hydrocarbon degraders. In Handbook of Hydrocarbon and Lipid Microbiology; Timmis, K. N., Ed.; Springer-Verlag: Berlin, 2010; pp 2066−2078.
(8) Fingas, M. The Basics of Oil Spill Cleanup; CRC Press: Boca Raton, FL, 2012.
(9) Alyeska Oil Spill Prevention, Response and Preparedness, 2014. http://www.alyeska-pipe.com/SafetyEnvironment/PreventionAndResponse.
(10) San Francisco Oil Spill Contingency Plan, 2014. https://www.dfg.ca.gov/ospr/san_francisco_plan.aspx.
(11) Thames Oil Spill Clearance Association, 2014. http://www.pla.co.uk/About-Us/TOSCA.
(12) ITOPF Country Profiles, 2014. http://www.itopf.com/knowledge-resources/countries-regions/.
(13) Atlas, R. M.; Bartha, R. Degradation and mineralization of petroleum in sea water: limitation by nitrogen and phosphorus. Biotechnol. Bioengineer. 1972, 14, 309−318.
(14) Bælum, J.; Borglin, S.; Chakraborty, R.; Fortney, J. L.; Lamendella, R.; Mason, O. U.; Auer, M.; Zemla, M.; Bill, M.; Conrad, M. E.; Malfatti, S. A.; Tringe, S. G.; Holman, H. Y.; Hazen, T. C.; Jansson, J. K. Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. Environ. Microbiol. 2012, 14, 2405−2416.
(15) Garcia, H. E.; Locarnini, R. A.; Boyer, T. P.; Antonov, J. I.; Zweng, M. M.; Baranova, O. K.; Johnson, D. R. World Ocean Atlas 2009: Nutrients (Phosphate, Nitrate, And Silicate), NOAA Atlas NESDIS 71; Levitus, S., Ed.; U.S. Government Printing Office: Washington, DC, 2010; Vol. 4.
(16) Nauman, S. A. Shoreline cleanup: equipment and operations. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1991; pp 141−147.
(17) Lindstrom, J. E.; Prince, R. C.; Clark, J. R.; Grossman, M. J.; Yeager, T. R.; Braddock, J. F.; Brown, E. J. Microbial populations and hydrocarbon biodegradation potentials in fertilized shoreline sediments affected by the T/V Exxon Valdez oil spill. Appl. Environ. Microbiol. 1991, 57, 2514−2522.
(18) Bragg, J. R.; Prince, R. C.; Harner, E. J.; Atlas, R. M. Effectiveness of bioremediation for the Exxon Valdez oil spill. Nature 1994, 368, 413−418.
(19) Prince, R. C.; Clark, J. R.; Lindstrom, J. E.; Butler, E. L.; Brown, E. J.; Winter, G.; Grossman, M. J.; Parrish, R. R.; Bare, R. E.; Braddock, J. F.; Steinhauer, W. G.; Douglas, G. S.; Kennedy, J. M.; Barter, P. J.; Bragg, J. R.; Harner, E. J.; Atlas, R. M. Bioremediation of the Exxon Valdez oil spill: monitoring safety and efficacy. In Hydrocarbon Remediation; Hinchee, R. E., Alleman, B. C., Hoeppel, R. E., Miller, R. N., Eds.; Lewis Publishers: Boca Raton, FL, 1994; pp 107−124.
(20) Prince, R. C.; Bragg, J. R. Shoreline bioremediation following the Exxon Valdez oil spill in Alaska. Biorem. J. 1997, 1, 97−104.
(21) Page, G. W.; Carter, H. R.; Ford, R. G. Numbers of seabirds killed or debilitated in the 1986 Apex Houston oil spill in central California. Studies Avian Biol. 1990, 14, 164−174.
(22) Murphy, S. M.; Day, R. H.; Wiens, J. A.; Parker, K. R. Effects of the Exxon Valdez oil spill on birds: comparisons of pre-and post-spill surveys in Prince William Sound, Alaska. Condor 1997, 99, 299−313.
(23) Henkel, J. R.; Sigel, B. J.; Taylor, C. M. Large-scale impacts of the Deepwater Horizon oil spill: can local disturbance affect distant ecosystems through migratory shorebirds? BioScience 2012, 62, 676−685.
(24) Chasse, C. The ecological impact on and near shores by the Amoco Cadiz oil spill. Mar. Pollut. Bull. 1978, 9, 298−301.
(25) Neuparth, T.; Moreira, S. M.; Santos, M. M.; Reis-Henriques, M. A. Review of oil and HNS accidental spills in Europe: identifying major environmental monitoring gaps and drawing priorities. Mar. Pollut. Bull. 2012, 64, 1085−1095.
(26) Anink, P. J.; Hunt, D. R.; Roberts, D. E.; Jacobs, N. E. Oil spill in Botany Bay: short term effects and long term implications. Wetlands (Australia) 1985, 5, 32−41.
(27) National Research Council Oil Spill Dispersants: Efficacy and Effects; National Academy Press: Washingon, DC, 2005.
(28) COREXIT® Ingredients; Nalco: Sugar Land, TX, 2014. http://www.nalcoesllc.com/nes/1602.htm.
(29) Cormack, D.; Nichols, J. A. The concentrations of oil in sea water resulting from natural and chemically induced dispersion of oil slicks. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1977; pp 381−385.
(30) Lichtenthaler, R. G.; Daling, P. S. Dispersion of chemically treated crude oil in Norwegian offshore waters. In International Oil Spill Conference; American Petroleum Institute: Washington, DC, 1983; pp 7−14.
(31) McAuliffe, C. D.; Johnson, J. C.; Greene, S. H.; Canevari, G. P.; Searl, T. D. Dispersion and weathering of chemically treated crude oils on the ocean. Environ. Sci. Technol. 1980, 14, 1509−1518.
(32) BenKinney, M.; Brown, J.; Mudge, S.; Russell, M.; Nevin, A.; Huber, C. Monitoring effects of aerial dispersant application during the MC252 Deepwater Horizon Incident. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 2011; p 368.
(33) Bejarano, A. C.; Levine, E.; Mearns, A. J. Effectiveness and potential ecological effects of offshore surface dispersant use during the Deepwater Horizon oil spill: a retrospective analysis of monitoring data. Environ. Monit. Assess. 2013, 185, 10281−10295.
(34) Wade, T. L.; Sweet, S. T.; Sericano, J. L.; Guinasso, N. L.; Diercks, A. R.; Highsmith, R. C.; Asper, V. L.; Joung, D. J.; Shiller, A. M.; Lohrenz, S. E.; Joye, S. B. Analyses of water samples from the Deepwater Horizon oil spill: documentation of the subsurface plume. In Monitoring and Modeling the Deepwater Horizon Oil Spill: A Record-Breaking Enterprise; Liu, Y., Macfadyen, A., Ji, Z. J., Weisberg, R. H., Eds; American Geophysical Union: Washington, DC, 2011; pp 77−82.
(35) Hazen, T. C.; Dubinsky, E. A.; DeSantis, T. Z.; Andersen, G. L.; Piceno, Y. M.; Singh, N.; Jansson, J. K.; Probst, A.; Borglin, S. E.; Fortney, J. L.; Stringfellow, W. T.; Bill, M.; Conrad, M. S.; Tom, L. M.; Chavarria, K. L.; Alusi, T. R.; Lamendella, R.; Joyner, D. C.; Spier, C.; Bælum, J.; Auer, M.; Zemla, M. L.; Chakraborty, R.; Sonnenthal, E. L.; D'haeseleer, P.; Holman, H. N.; Osman, S.; Lu, Z.; Van Nostrand, J. D.; Deng, Y.; Zhou, J.; Mason, O. U. Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science 2010, 330, 204−208.
(36) Prince, R. C.; McFarlin, K. M.; Butler, J. D.; Febbo, E. J.; Wang, F. C. Y.; Nedwed, T. J. The primary biodegradation of dispersed crude oil in the sea. Chemosphere 2013, 90, 521−526.
(37) Prince, R. C.; Butler, J. D. A protocol for assessing the effectiveness of oil spill dispersants in stimulating the biodegradation of oil. Environ. Sci. Pollut. Res. 2014, 21, 9506−9510.
(38) Brakstad, O. G.; Nordtug, T.; Throne-Holst, M. Biodegradation of dispersed Macondo oil in seawater at low temperature and different oil droplet sizes. Mar. Pollut. Bull. 2015, 93, 144−152.
(39) Zahed, M. A.; Aziz, H. A.; Isa, M. H.; Mohajeri, L.; Mohajeri, S.; Kutty, S. R. M. Kinetic modeling and half-life study on bioremediation of crude oil dispersed by Corexit 9500. J. Hazard. Mater. 2011, 185, 1027−1031.
(40) Harris, C. The Braer incident: Shetland Islands, January 1993. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1995; pp 813−819.

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001442-00006

(41) Law, R. J.; Kelly, C. The impact of the Sea Empress oil spill. Aquat. Living Resour. 2004, 17, 389−394.
(42) Henry, C. Review of dispersant use in US Gulf of Mexico waters since the Oil Pollution Act of 1990. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 2005; pp 439−442.
(43) Global Dispersant Stockpile; Oil Spill Response Ltd.: London, 2014. http://www.oilspillresponse.com/services-landing/global-dispersant-stockpile.
(44) ExxonMobil Oil Spill Dispersant Guidelines, 2008. crrc.unh.edu/sites/crrc.unh.edu/files/exxonmobil_dispersant_guidelines_2008.pdf.
(45) Word, J. Q.; Clark, J. R.; Word, L. S. Comparison of the acute toxicity of Corexit 9500 and household cleaning products. Hum. Ecol. Risk Assess. 2014, 21, 707−725.
(46) Russell, M.; Holcomb, J.; Berkner, A. 30-years of oiled wildlife response statistics, Proceedings of the 7th International Effects of Oil on Wildlife Conference, Hamburg, Germany, October 14−16, 2003; pp 14−16. pszshhw.bird-rescue.org/pdfs/IBRRC_stats_paper.pdf.
(47) Best Practices for Migratory Bird Care During Oil Spill Response; USWFS: Anchorage, AK, 2003. www.fws.gov/contaminants/fws_oscp_05/fwscontingencyappendices/D-BestPracticesMigBirds/BestPracticesmar04rev.pdf.
(48) Judson, R. S.; Martin, M. T.; Reif, D. M.; Houck, K. A.; Knudsen, T. B.; Rotroff, D. M.; Xia, M.; Sakamuru, S.; Huang, R.; Shinn, P.; Austin, C. P.; Kavlock, R. J.; Dix, D. J. Analysis of eight oil spill dispersants using rapid, in vitro tests for endocrine and other biological activity. Environ. Sci. Technol. 2010, 44, 5979−5985.
(49) Hemmer, M. J.; Barron, M. G.; Greene, R. M. Comparative toxicity of eight oil dispersants, Louisiana sweet crude oil (LSC), and chemically dispersed LSC to two aquatic test species. Environ. Toxicol. Chem. 2011, 30, 2244−2252.
(50) Gardiner, W. W.; Word, J. Q.; Word, J. D.; Perkins, R. A.; McFarlin, K. M.; Hester, B. W.; Word, L. S.; Ray, C. M. The acute toxicity of chemically and physically dispersed crude oil to key arctic species under arctic conditions during the open water season. Environ. Toxicol. Chem. 2013, 32, 2284−2300.
(51) Baker, J. M. Net environmental benefit analysis for oil spill response. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1995; pp 611−614.
(52) Chapman, H.; Purnell, K.; Law, R. J.; Kirby, M. F. The use of chemical dispersants to combat oil spills at sea: a review of practice and research needs in Europe. Mar. Pollut. Bull. 2007, 54, 827−838.
(53) Kirby, M. F.; Law, R. J. Oil spill treatment products approval: the UK approach and potential application to the Gulf region. Mar. Pollut. Bull. 2008, 56, 1243−1247.
(54) API Net Environmental Benefit Analysis for EffEctive Oil Spill Preparedness and Response, 2013. www.api.org/~/media/Files/EHS/Clean_Water/Oil_Spill_Prevention/NEBA/NEBA-Net-Environmental-Benefit-Analysis-July-2013.pdf.
(55) McCay, D. F.; Graham, E. Quantifying tradeoffs-net environmental benefits of dispersant use. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 2014; pp 762−775.
(56) USEPA Alphabetical List of NCP Product Schedule (Products Available for Use During an Oil Spill), 2014 http://www2.epa.gov/emergency-response/alphabetical-list-ncp-product-schedule-products-available-use-during-oil-spill.
(57) USEPA Swirling Flask Dispersant Effectiveness Test, Revised Standard Dispersant Toxicity Test, and Bioremediation Agent Effectiveness Test, 2006. http://www.gpo.gov/fdsys/pkg/CFR-2006-title40-vol27/xml/CFR-2006-title40-vol27-part300-appC.xml.
(58) USEPA National Oil and Hazardous Substances Pollution Contingency Plan: Proposed Rule, 2015. https://www.federalregister.gov/articles/2015/01/22/2015-00544/national-oil-and-hazardous-substances-pollution-contingency-plan.
(59) Ohmsett • National Oil Spill Response Research Facility; BSSE: Washington, DC, 2014. http://www.bsee.gov/Technology-and-Research/Ohmsett/index/.
(60) Belore, R. C.; Trudel, K.; Mullin, J. V.; Guarino, A. Large-scale cold water dispersant effectiveness experiments with Alaskan crude oils and Corexit 9500 and 9527 dispersants. Mar. Pollut. Bull. 2009, 58, 118−128.
(61) Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th ed.; USEPA: Washington, DC, 2002. http://water.epa.gov/scitech/methods/cwa/wet/disk2_index.cfm.
(62) Dispersant Monitoring and Assessment Directive − Addendum; USEPA: Washington, DC, 2010. http://www.epa.gov/bpspill/dispersants/directive-addendum2.pdf.
(63) Dispersant Aerial Application Systems: Airborne Support Incorporated; Clean Gulf Associates: New Orleans, LA, 2014. http://www.cleangulfassoc.com/equipment/all/dispersant-aerial-application.
(64) Coelho, G.; Clark, J.; Aurand, D. Toxicity testing of dispersed oil requires adherence to standardized protocols to assess potential real world effects. Environ. Pollut. 2013, 177, 185−188.
(65) Lewis, M. M.; Pryor, R. Toxicities of oils, dispersants and dispersed oils to algae and aquatic plants: Review and database value to resource sustainability. Environ. Pollut. 2013, 180, 345−367.
(66) McGrath, J. A.; Parkerton, T. F.; Hellweger, F. L.; Di Toro, D. M. Validation of the narcosis target lipid model for petroleum products: gasoline as a case study. Environ. Toxicol. Chem. 2005, 24, 2382−2394.
(67) Di Toro, D. M.; McGrath, J. A.; Stubblefield, W. A. Predicting the toxicity of neat and weathered crude oil: Toxic potential and the toxicity of saturated mixtures. Environ. Toxicol. Chem. 2007, 26, 2 4−36.
(68) McCarty, L. S.; Arnot, J. A.; Mackay, D. Evaluation of critical body residue data for acute narcosis in aquatic organisms. Environ. Toxicol. Chem. 2013, 32, 2301−2314.
(69) Länge, R. R.; Hutchinson, T. H.; Scholz, N.; Solbé, J. Analysis of the ECETOC aquatic toxicity (EAT) database II • comparison of acute to chronic ratios for various aquatic organisms and chemical substances. Chemosphere 1998, 36, 115−127.
(70) Roex, E. W.; Van Gestel, C. A.; Van Wezel, A. P.; Van Straalen, N. M. Ratios between acute aquatic toxicity and effects on population growth rates in relation to toxicant mode of action. Environ. Toxicol. Chem. 2000, 19, 685−693.
(71) Raimondo, S.; Montague, B. J.; Barron, M. G. Determinants of variability in acute to chronic toxicity ratios for aquatic invertebrates and fish. Environ. Toxicol. Chem. 2007, 26, 2019−2023.
(72) Swigert, J. P.; Lee, C.; Wong, D. C. L.; Podhasky, P. Aquatic hazard and biodegradability of light and middle atmospheric distillate petroleum streams. Chemosphere 2014, 108, 1−9.
(73) Landrum, P. F.; Chapman, P. M.; Neff, J.; Page, D. S. Influence of exposure and toxicokinetics on measures of aquatic toxicity for organic contaminants: a case study review. Integr. Environ. Assess. Manage. 2013, 9, 196−210.
(74) Aeppli, C.; Nelson, R. K.; Radovic, J. R.; Carmichael, C. A.; Valentine, D. L.; Reddy, C. M. Recalcitrance and degradation of petroleum biomarkers upon abiotic and biotic natural weathering of Deepwater Horizon oil. Environ. Sci. Technol. 2014, 48, 6726−6734.
(75) Yin, F.; John, G. F.; Hayworth, J. S.; Clement, T. P. Long-term monitoring data to describe the fate of polycyclic aromatic hydrocarbons in Deepwater Horizon oil submerged off Alabama's beaches. Sci. Total Environ. 2015, 508, 46−56.
(76) Nordvik, A. B. The technology windows-of-opportunity for marine oil spill response as related to oil weathering and operations. Spill Sci. Technol. Bull. 1995, 2, 17−46.
(77) Lewis, A. B.; Trudel, K.; Belore, R. C.; Mullin, J. V. Large-scale dispersant leaching and effectiveness experiments with oils on calm water. Mar. Pollut. Bull. 2010, 60, 244−254.
(78) McFarlin, K. M.; Prince, R. C.; Perkins, R.; Leigh, M. B. Biodegradation of dispersed oil in arctic seawater at −1 °C. PLoS One 2014, 9, e84297.
(79) Ourisson, G.; Albrecht, P. Hopanoids. 1. Geohopanoids: the most abundant natural products on Earth? Acc. Chem. Res. 1992, 25, 398−402.

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001442-00007

(80) Valentine, D. L.; Fisher, G. B.; Bagby, S. C.; Nelson, R. K.; Reddy, C. M.; Sylva, S. P.; Woo, M. A. Fallout plume of submerged oil from Deepwater Horizon. Proc. Natl. Acad. Sci. U.S.A. 2014, 111, 15906−15911.
(81) Queimada, A. J.; Quinones-Cisneros, S. E.; Marrucho, I. M.; Coutinho, J. A. P.; Stenby, E. H. Viscosity and liquid density of asymmetric hydrocarbon mixtures. Int. J. Thermophys. 2003, 24, 1221−1239.
(82) Liu, K.; Wu, Y.; McHugh, M. A.; Baled, H.; Enick, R. M.; Morreale, B. D. Equation of state modeling of high-pressure, high-temperature hydrocarbon density data. J. Supercrit. Fluids 2010, 55, 701−711.
(83) Wakeham, W. A.; Cholakov, G. S.; Stateva, R. P. Liquid density and critical properties of hydrocarbons estimated from molecular structure. J. Chem. Eng. Data 2002, 47, 559−570.
(84) Alboudwarej, H.; Beck, J.; Svrcek, W. Y.; Yarranton, H. W.; Akbarzadeh, K. Sensitivity of asphaltene properties to separation techniques. Energy Fuels 2002, 16, 462−469.
(85) Barrera, D. M.; Ortiz, D. P.; Yarranton, H. W. Molecular weight and density distributions of asphaltenes from crude oils. Energy Fuels 2013, 27, 2474−2487.
(86) Chanton, J.; Zhao, T.; Rosenheim, B. E.; Joye, S. B.; Bosman, S.; Brunner, C. A.; Yeager, K. M.; Diercks, A. R.; Hollander, D. Using natural abundance radiocarbon to trace the flux of petrocarbon to the seafloor following the Deepwater Horizon oil spill. Environ. Sci. Technol. 2014, 49, 847−854.
(87) Shiloach, J.; Fass, R. Growing E. coli to high cell density・a historical perspective on method development. Biotechnol. Adv. 2005, 23, 345−57.
(88) Levy, E. M.; Lee, K. Potential contribution of natural hydrocarbon seepage to benthic productivity and the fisheries of Atlantic Canada. Can. J. Fish. Aquat. Sci. 1988, 45, 349−352.
(89) Kostka, J. E.; Prakash, O.; Overholt, W. A.; Green, S. J.; Freyer, G.; Canion, A.; Delgardio, J.; Norton, N.; Hazen, T. C.; Huettel, M. Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the Deepwater Horizon oil spill. Appl. Environ. Microbiol. 2011, 77, 7962−7974.
(90) Dubinsky, E. A.; Conrad, M. E.; Chakraborty, R.; Bill, M.; Borglin, S. E.; Hollibaugh, J. T.; Mason, O. U.; Piceno, Y. M.; Reid, F. C.; Stringfellow, W. T.; Tom, L. M.; Hazen, T. C.; Andersen, G. L. Succession of hydrocarbon-degrading bacteria in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. Environ. Sci. Technol. 2013, 47, 10860−10867.
(91) Lamendella, R.; Strutt, S.; Borglin, S.; Chakraborty, R.; Tas, N.; Mason, O. U.; Hultman, J.; Prestat, E.; Hazen, T. C.; Jansson, J. K. Assessment of the Deepwater Horizon oil spill impact on Gulf coast microbial communities. Front. Microbiol. 2014, 5, 130.
(92) Rodriguez-R, L. M.; Overholt, W. A.; Hagan, C.; Huettel, M.; Kostka, J. E.; Konstantinidis, K. T. Microbial community successional patterns in beach sands impacted by the Deepwater Horizon oil spill. ISME J. 2015, DOI: 10.1038/ismej.2015.5.
(93) Uña, G. V.; García, M. J. N. Biodegradation of non-ionic dispersants in sea-water. Eur. J. Appl. Microbiol. Biotechnol. 1983, 18, 315−319.
(94) Cordon, T. C.; Maurer, E. W.; Stirton, A. J. The course of biodegradation of anionic detergents by analyses for carbon, methylene blue active substance and sulfate ion. J. Am. Oil Chem. Soc. 1970, 47, 203−206.
(95) Gray, J. L.; Kanagy, L. K.; Furlong, E. T.; Kanagy, C. J.; McCoy, J. W.; Mason, A.; Lauenstein, G. Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. Chemosphere 2014, 95, 124−130.
(96) Kujawinski, E. B.; Kido Soule, M. C.; Valentine, D. L.; Boysen, A. K.; Longnecker, K.; Redmond, M. C. Fate of dispersants associated with the Deepwater Horizon oil spill. Environ. Sci. Technol. 2011, 45, 1298−1306.
(97) Hayworth, J. S.; Clement, T. P. Provenance of COREXIT-related chemical constituents found in nearshore and inland Gulf Coast waters. Mar. Pollut. Bull. 2012, 64, 2005−2014.
(98) White, H. K.; Lyons, S. L.; Harrison, S. J.; Findley, D. M.; Liu, Y.; Kujawinski, E. B. Long-term persistence of dispersants following the Deepwater Horizon oil spill. Environ. Sci. Technol. Lett. 2014, 1, 295−299.
(99) Roberts, J. R.; Reynolds, J. S.; Thompson, J. A.; Zaccone, E. J.; Shimko, M. J.; Goldsmith, W. T.; Jackson, M.; McKinney, W.; Frazer, D. G.; Kenyon, A.; Kashon, M. L.; Piedimonte, G.; Castranova, V.; Fedan, J. S. Pulmonary effects after acute inhalation of oil dispersant (COREXIT EC9500A) in rats. J. Toxicol. Environ. Health, Part A 2011, 74, 1381−1396.
(100) Krajnak, K.; Kan, H.; Waugh, S.; Miller, G. R.; Johnson, C.; Roberts, J. R.; Goldsmith, W. T.; Jackson, M.; McKinney, W.; Frazer, D.; Kashon, M. L.; Castranova, V. Acute effects of COREXIT EC9500A on cardiovascular functions in rats. J. Toxicol. Environ. Health, Part A 2011, 74, 1397−1404.
(101) Sriram, K.; Lin, G. X.; Jefferson, A. M.; Goldsmith, W. T.; Jackson, M.; McKinney, W.; Frazer, D. G.; Robinson, V. A.; Castranova, V. Neurotoxicity following acute inhalation exposure to the oil dispersant COREXIT EC9500A. J. Toxicol. Environ. Health, Part A 2011, 74, 1405−1418.
(102) Anderson, S. E.; Franko, J.; Lukomska, E.; Meade, B. J. Potential immunotoxicological health effects following exposure to COREXIT 9500A during cleanup of the Deepwater Horizon oil spill. J. Toxicol. Environ. Health, Part A 2011, 74, 1419−1430.
(103) Johansen, Ø.; Brandvik, P. J.; Farooq, U. Droplet breakup in subsea oil releases−Part 2: Predictions of droplet size distributions with and without injection of chemical dispersants. Mar. Pollut. Bull. 2013, 73, 327−335.
(104) Place, B. J.; Perkins, M. J.; Sinclair, E.; Barsamian, A. L.; Blakemore, P. R.; Field, J. A. Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. Deep-Sea Res. 2014, DOI: 10.1016/j.dsr2.2014.01.015.
(105) Canevari, G. P. The effect of crude oil composition on dispersant performance. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1985; pp 441−444.
(106) Nedwed, T.; Canevari, G. P.; Clark, J. R.; Belore, R. New dispersant delivered as a gel. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 2005; pp 121−125.
(107) Finch, B. E.; Wooten, K. J.; Smith, P. N. Embryotoxicity of weathered crude oil from the Gulf of Mexico in mallard ducks (Anas platyrhynchos). Environ. Toxicol. Chem. 2011, 30, 1885−1891.
(108) Irie, K.; Kawaguchi, M.; Mizuno, K.; Song, J. Y.; Nakayama, K.; Kitamura, S. I.; Murakami, Y. Effect of heavy oil on the development of the nervous system of floating and sinking teleost eggs. Mar. Pollut. Bull. 2011, 63, 297−302.
(109) Almeda, R.; Baca, S.; Hyatt, C.; Buskey, E. J. Ingestion and sublethal effects of physically and chemically dispersed crude oil on marine planktonic copepods. Ecotoxicology 2014, 23, 988−1003.
(110) Garrett, R. M.; Pickering, I. J.; Haith, C. E.; Prince, R. C. Photooxidation of crude oils. Environ. Sci. Technol. 1998, 32, 3719−3723.
(111) Aeppli, C.; Carmichael, C. A.; Nelson, R. K.; Lemkau, K. L.; Graham, W. M.; Redmond, M. C.; Valentine, D. L.; Reddy, C. M. Oil weathering after the Deepwater Horizon disaster led to the formation of oxygenated residues. Environ. Sci. Technol. 2012, 46, 8799−8807.
(112) Goodman, R. Tar balls: the end state. Spill Sci. Technol. Bull. 2003, 8, 117−121.
(113) Barron, M. G.; Carls, M. G.; Short, J. W.; Rice, S. D.; Heintz, R. A.; Rau, M.; Di Giulio, R. Assessment of the phototoxicity of weathered Alaska North Slope crude oil to juvenile pink salmon. Chemosphere 2005, 60, 105−110.
(114) Payne, J. R.; Clayton, J. R.; McNabb, G. D.; Kirstein, B. E. Exxon Valdez oil weathering fate and behavior: model predictions and field observations 1. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1991, pp 641−654.

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001442-00008

(115) Bobra, M. Water-in-oil emulsification: a physicochemical study. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1991; pp 483−488.
(116) Strøm-Kristiansen, T.; Lewis, A.; Daling, P. S.; Hokstad, J. N.; Singsaas, I. Weathering and dispersion of naphthenic, asphaltenic, and waxy crude oils. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1997; pp 631−636.
(117) Lessard, R. R.; DeMarco, G. The significance of oil spill dispersants. Spill Sci. Technol. Bull. 2000, 6, 59−68.
(118) Thingstad, T.; Pengerud, B. The formation of "chocolate mousse" from Statfjord crude oil and seawater. Mar. Pollut. Bull. 1983, 14, 214−216.
(119) Daling, P. S.; Leirvik, F.; Almås, I. K.; Brandvik, P. J.; Hansen, B. H.; Lewis, A.; Reed, M. Surface weathering and dispersibility of MC252 crude oil. Mar. Pollut. Bull. 2014, 87, 300−310.
(120) Gros, J.; Nabi, D.; Würz, B.; Wick, L. Y.; Brussaard, C. P. D.; Huisman, J.; van der Meer, J. R.; Reddy, C. M.; Arey, J. S. First day of an oil spill on the open sea: early mass transfers of hydrocarbons to air and water. Environ. Sci. Technol. 2014, 48, 9400−9411.
(121) Goldstein, B. D.; Osofsky, H. J.; Lichtveld, M. Y. The Gulf oil spill. N. Engl. J. Med. 2011, 364, 1334−1348.
(122) Atkinson, R.; Arey, J. Atmospheric degradation of volatile organic compounds. Chem. Rev. 2003, 103, 4605−4638.
(123) Deguillaume, L.; Leriche, M.; Amato, P.; Ariya, P. A.; Delort, A. M.; Pöschl, U.; Chaumerliac, N.; Bauer, H.; Flossmann, A. I.; Morris, C. E. Microbiology and atmospheric processes: chemical interactions of primary biological aerosols. Biogeosciences 2008, 5, 841−870.
(124) Colcomb, K.; Bedborough, D.; Lunel, T.; Swannell, R.; Wood, P.; Rusin, J.; Bailey, N.; Halliwell, C.; Davies, L.; Sommerville, M.; Dobie, A.; Mitchell, D.; McDonagh, M.; Lee, K.; Shimwell, S.; Davies, B.; Harries, D. Shoreline cleanup and waste disposal issues during the Sea Empress incident. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1997; pp 195−198.
(125) Michel, J.; Owens, E. H.; Zengel, S.; Graham, A.; Nixon, Z.; Allard, T.; Holton, W.; Reimer, P. D.; Lamarche, A.; White, M.; Rutherford, N.; Childs, C.; Mauseth, G.; Challenger, G.; Taylor, E. Extent and degree of shoreline oiling: Deepwater Horizon oil spill, Gulf of Mexico, USA. PLoS One 2013, 8, e65087.
(126) Wang, Z.; Fingas, M.; Owens, E. H.; Sigouin, L.; Brown, C. E. Long-term fate and persistence of the spilled Metula oil in a marine salt marsh environment: degradation of petroleum biomarkers. J. Chromatogr. A 2001, 926, 275−290.
(127) Owens, E. H.; Taylor, E.; Humphrey, B. The persistence and character of stranded oil on coarse-sediment beaches. Mar. Pollut. Bull. 2008, 56, 14−26.
(128) Defeo, O.; McLachlan, A.; Schoeman, D. S.; Schlacher, T. A.; Dugan, J.; Jones, A.; Lastra, M.; Scapini, F. Threats to sandy beach ecosystems: a review. Estuarine, Coastal Shelf Sci.. 2009, 81, 1−12.
(129) Burns, K. A.; Garrity, S. D.; Levings, S. C. How many years until mangrove ecosystems recover from catastrophic oil spills? Mar. Pollut. Bull. 1993, 6, 239−248.
(130) Mendelssohn, I. A.; Andersen, G. L.; Baltz, D. M.; Caffey, R. H.; Carman, K. R.; Fleeger, J. W.; Joye, S. B.; Lin, Q.; Maltby, E.; Overton, E. B.; Rozas, L. P. Oil impacts on coastal wetlands: implications for the Mississippi River Delta ecosystem after the Deepwater Horizon oil spill. BioScience 2012, 62, 562−574.
(131) Michel, J.; Rutherford, N. Impacts, recovery rates, and treatment options for spilled oil in marshes. Mar. Pollut. Bull. 2014, 82, 19−25.
(132) Bernabeu, A. M.; Nuez de la Fuente, M.; Rey, D.; Rubio, B.; Vilas, F.; Medina, R.; González, M. E. Beach morphodynamics forcements in oiled shorelines: coupled physical and chemical processes during and after fuel burial. Mar. Pollut. Bull. 2006, 52, 1156−1168.
(133) Pineda-Flores, G.; Boll-Arguëllo, G.; Lira-Galeana, C.; Mesta-Howard, A. M. A microbial consortium isolated from a crude oil sample that uses asphaltenes as a carbon and energy source. Biodegradation 2004, 15, 145−151.
(134) Tavassoli, T.; Mousavi, S. M.; Shojaosadati, S. A.; Salehizadeh, H. Asphaltene biodegradation using microorganisms isolated from oil samples. Fuel 2012, 93, 142−148.
(135) Hayes, M. H. B. Solvent systems for the isolation of organic components from soils. Soil Sci. Soc. Am. J. 2006, 70, 986−994.
(136) Michaels, D.; Howard, J. Review of the OSHA-NIOSH response to the Deepwater Horizon oil spill: protecting the health and safety of cleanup workers. PLoS Curr. 2012, 4, e4fa83b7576b6e.
(137) Schwarz, J. R.; Walker, J. D.; Colwell, R. R. Deep-sea bacteria: growth and utilization of hydrocarbons at ambient and in situ pressure. Appl. Microbiol. 1974, 28, 982−986.
(138) Kimes, N. E.; Callaghan, A. V.; Aktas, D. F.; Smith, W. L.; Sunner, J.; Golding, B. T.; Drozdowska, M.; Hazen, T. C.; Suflita, J. M.; Morris, P. J. Metagenomic analysis and metabolite profiling of deep-sea sediments from the Gulf of Mexico following the Deepwater Horizon oil spill. Front. Microbiol. 2013, 4, 50.
(139) Van Hamme, J. D.; Ward, O. P. Influence of chemical surfactants on the biodegradation of crude oil by a mixed bacterial culture. Can. J. Microbiol. 1999, 45, 130−137.
(140) Lindstrom, J. E.; Braddock, J. F. Biodegradation of petroleum hydrocarbons at low temperature in the presence of the dispersant COREXIT 9500. Mar. Pollut. Bull. 2002, 44, 739−747.
(141) Venosa, A. D.; Holder, E. L. Biodegradability of dispersed crude oil at two different temperatures. Mar. Pollut. Bull. 2007, 54, 545−553.
(142) Hamdan, L. J.; Fulmer, P. A. Effects of COREXIT® EC9500A on bacteria from a beach oiled by the Deepwater Horizon spill. Aquat. Microb. Ecol. 2011, 63, 101−109.
(143) Urbanczyk, H.; Ast, J. C.; Higgins, M. J.; Carson, J.; Dunlap, P. V. Reclassification of Vibrio fischeri, Vibrio logei, Vibrio salmonicida and Vibrio wodanis as Aliivibrio fischeri gen. nov., comb. nov., Aliivibrio logei comb. nov., Aliivibrio salmonicida comb. nov. and Aliivibrio wodanis comb. nov. Int. J. Syst. Evol. Microbiol. 2007, 57, 2823−2829.
(144) Fuller, C.; Bonner, J.; Page, C.; Ernest, A.; McDonald, T.; McDonald, S. Comparative toxicity of oil, dispersant, and oil plus dispersant to several marine species. Environ. Toxicol. Chem. 2004, 23, 2941−2949.
(145) McManus, M. A.; Woodson, C. B. Plankton distribution and ocean dispersal. J. Exp. Biol. 2015, 215, 1008−1016.
(146) Prairie, J. C.; Sutherland, K. R.; Nickols, K. J.; Kaltenberg, A. M. Biophysical interactions in the plankton: a cross-scale review. Limnol. Oceanogr. 2012, 2, 121−145.
(147) Ottesen, E. A.; Young, C. R.; Eppley, J. M.; Ryan, J. P.; Chavez, F. P.; Scholin, C. A.; DeLong, E. F. Pattern and synchrony of gene expression among sympatric marine microbial populations. Proc. Natl. Acad. Sci. U.S.A. 2013, 110, E488−E497.
(148) Dispersant Tools, Job Aids and Decision Process; Region X Regional Response Team, 2015. www.rrt10nwac.com/Files/NWACP/2015/Section%209406.pdf.

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001442-00009

To: Barron, Mace[Barron.Mace@epa.gov]
From: Conmy, Robyn
Sent: Wed 10/26/2016 2:58:50 PM
Subject: FW: need 200 mL minimum of dispersant for the lab...

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Craig Watts [mailto:craig@hydrosphere.net]
Sent: Wednesday, October 26, 2016 9:50 AM
To: Grosser, Robert <Grosser.Robert@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Sundaravadivelu, Devi <sundaravadivelu.devi@epa.gov>; Peter Meyer <pmeyer@hydrosphere.net>
Subject: RE: need 200 mL minimum of dispersant for the lab...

Bob,

Thank you. We will keep an eye out for it.

Regards,

Craig

Providing Environmental & Product Toxicity Testing since 1986

Craig Watts, Lab Director

Hydrosphere Research

11842 Research Circle

Alachua, FL 32615-6817

T (386) 462-7889

www.hydrosphere.net

CONFIDENTIALITY NOTICE: This message may contain privileged and confidential information from Hydrosphere Research. The information is intended to be for the use of the addressee only. Any disclosure, copying, distribution, or use of the contents by anyone but the addressee is prohibited.

From: Grosser, Robert [mailto:Grosser.Robert@epa.gov]
Sent: Wednesday, October 26, 2016 9:28 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>; Craig Watts <craig@hydrosphere.net>
Cc: Sundaravadivelu, Devi <sundaravadivelu.devi@epa.gov>
Subject: RE: need 200 mL minimum of dispersant for the lab...

Craig:

The Accell dispersant will be shipped today. Since you said you did not need it ASAP, it is coming by ground shipping. It should arrive in 3-4 business days.

Let me know if you need anything else.

Thanks.

Bob

Robert Grosser PhD

Environmental Microbiologist

Pegasus Technical Services, Inc.

On-Site Contractor for the US EPA

26 West Martin Luther King Drive

Mail Location 190

Cincinnati, OH 45268

(513) 569-7529 office

(513) 569-7620 fax

From: Conmy, Robyn
Sent: Tuesday, October 25, 2016 6:24 PM

To: Grosser, Robert <Grosser.Robert@epa.gov>; devi.sundaravadivelu@ptsied.com
Subject: FW: need 200 mL minimum of dispersant for the lab...

As per the email from last week, please arrange for 200 ml of accel to be sent to hydrosphere.
Thanks

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Barron, Mace
Sent: Monday, October 24, 2016 5:06 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>; Holder, Edith <holder.edith@epa.gov>
Subject: need 200 mL minimum of dispersant for the lab...

Just fyi on what they used of corexit and finasol.

Please send new dispersant asap!

Thank you,

Mace

From: Peter Meyer [mailto:pmeyer@hydrosphere.net]
Sent: Monday, October 24, 2016 3:41 PM
To: Barron, Mace <Barron.Mace@epa.gov>
Cc: Craig Watts <craig@hydrosphere.net>
Subject: RE: quick question...

Mace,

We ended up using about 160 mLs of each. So if I had to guess…

1) Corexit: We have about 1,200 mLs left over and…

2) Finasol: Probably about 700 mLs left over.

Neither container was full when we received them.

~Peter

From: Craig Watts
Sent: Saturday, October 22, 2016 7:22 PM
To: Barron, Mace <Barron.Mace@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>
Subject: RE: quick question...

Mace,

I am going to have to hand this one off to Peter.

Craig

From: Barron, Mace [mailto:Barron.Mace@epa.gov]
Sent: Friday, October 21, 2016 11:20 AM
To: Craig Watts <craig@hydrosphere.net>

Subject: quick question...

How much dispersant (e.g., corexit) did you guys use in the tox tests?

Do you recall how much we sent you? And how much you got left over?

We are getting ready to send a new dispersant to you and need to know the amount to send.

Thanks!

Environmental Science & Technology

Article

pubs.acs.org/est

Submersible Optical Sensors Exposed to Chemically Dispersed Crude Oil: Wave Tank Simulations for Improved Oil Spill Monitoring

Robyn N. Conmy,[†,*] Paula G. Coble,[‡] James Farr,[□] A. Michelle Wood,[§,◆] Kenneth Lee,[//,¶] W. Scott Pegau,[⊥] Ian D. Walsh,[#] Corey R. Koch,[#] Mary I. Abercrombie,[‡] M. Scott Miles,[▽] Marlon R. Lewis,[○] Scott A. Ryan,[//] Brian J. Robinson,[//] Thomas L. King,[//] Christopher R. Kelble,[§] and Jordanna Lacoste[○]

[†]National Risk Management Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, 26 West Martin Luther King BoulevardCincinnati, Ohio 45268, United States

[‡]College of Marine Science, University of South Florida, St. Petersburg, Florida 33701, United States

[□]Office of Response and Restoration, National Oceanic and Atmospheric Administration, 1305 East-West Highway, Silver Spring, Maryland 20910, United States

[§]Atlantic Oceanographic & Meteorological Laboratory, National Oceanic and Atmospheric Administration, 4301 Rickenbacker Causeway, Miami, Florida 33149, United States

[//]Department Fisheries and Oceans Canada, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada

[⊥]Oil Spill Recovery Institute, 300 Breakwater Avenue, Cordova, Alaska 99574, United States

[#]WET Labs, Inc., PO Box 518, Philomath, Oregon 97370, United States

[▽]School of Coast and Environment, Louisiana State University, Baton Rouge, Louisiana 70803, United States

[○]Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada

* Supporting Information

In situ fluorometers were deployed during the Deepwater Horizon (DWH) Gulf of Mexico oil spill to track the subsea oil plume. Uncertainties regarding instrument specifications and capabilities necessitated performance testing of sensors exposed to simulated, dispersed oil plumes. Dynamic ranges of the Chelsea Technologies Group AQUAtracka, Turner Designs Cyclops, Satlantic SUNA and WET Labs, Inc. ECO, exposed to fresh and artificially weathered crude oil, were determined. Sensors were standardized against known oil volumes and total petroleum hydrocarbons and benzene-toluene-ethylbenzene-xylene measurements - both collected during spills, providing oil estimates during wave tank dilution experiments. All sensors estimated oil concentrations down to 300 ppb oil, refuting previous reports. Sensor performance results assist interpretation of DWH oil spill data and formulating future protocols.

INTRODUCTION

SMART (special monitoring of applied response technologies) protocols use real-time fluorescence monitoring in decision-making during oil spill dispersant operations[1] to determine efficacy of chemically enhanced dispersion and dispersed oil transport.[2] Chemical dispersants applied to oil slicks (in the presence of mixing energy) reduce the oil−water interfacial tension, forming small oil droplets (<100 um) that are less likely to recoalesce,[3] removing oil from the water surface and suspending neutrally buoyant oil droplets as a subsurface plume. This reduces risk to shorelines and air−water interface biota and also increases microbial biodegradation rates of oil.[4−6] Under SMART, in situ monitoring of surface waters (<2 m) and below (2 - 10 m) evaluates efficacy. Monitoring requires rapid, reliable, easy-to-operate in situ fluorometers. Validation with oil/water samples yields oil mapping with finer temporo-spatial resolution not achieved through coarse discrete measurements alone.

Fluorescence has been an oil spill monitoring tool for more than 30 years.[7,8] Measurements are inherently sensitive, conducted without laborious extraction techniques, time

Received: September 20, 2013
Revised: December 17, 2013
Accepted: December 30, 2013
Published: December 30, 2013

 © 2013 American Chemical Society dx.doi.org/10.1021/es404206y | Environ. Sci. Technol. 2014, 48, 1803−1810

ED_001324_00001447-00001

efficient, chemistry dependent and indicators of hydrocarbon concentration.[9,10] Low viscosity physically dispersed crude oils typically have two broad UV peaks with excitation (Ex) between 240 and 300 nm and emission (Em) centered at ~350 nm (low molecular weight polycyclic aromatic hydrocarbons, LMW PAH, <3 benzene rings) and ~450 nm (higher MW PAH, >3 benzene rings).[11,12] Fluorescence signatures discriminate oils[11−14] but are complicated by varying concentration, degree of chemical and physical dispersion, oil age and state (fresh, weathered, mousses),[11,14,15] and interference of fluorophores with spectro-chemical similarities. Such is the case with colored dissolved organic matter (CDOM) and oil aromatic fractions, which exhibit broad fluorescence peaks and complex spectra. However, crude oils have peak maxima at slightly shorter wavelengths relative to aquatic CDOM (humics, fulvics), facilitating discrimination.

Optical oil detection is a function of matching Ex/Em wavelengths with oil (center wavelength (CWL) and filter bandwidth), power of the light source, and detector sensitivity. Numerous submersible fluorometers are commercially available, each with unique configurations for detecting compounds[16] (and references therein), thus expanding their use in spill response efforts and SMART protocols.[17] Reviews of commercial off-the-shelf (COTS) sensors[17−19] highlight the need for robust calibrations, operational procedures and scalability testing.[20] Reviews have targeted easy-to-conduct, low-cost bench-scale testing. Often tests are conducted in the absence of dispersant, in small flasks with insufficient mixing energy to physically disperse oil into water,[21] at elevated oil concentrations irrelevant to field observations, with limited oil type and weathering states, and without calibration to standards or hydrocarbon concentrations. Thus there are confidence challenges in establishing saturation cutoffs and detection limits relevant for field applications. Effectiveness in quantitative monitoring also remains unclear.[19] Hence, caution must be exercised when scaling up experimental designs to real-world conditions and decision-making. Rather, a combination of laboratory, wave tank, and field studies has been recommended to establish the best degree of confidence for detecting chemically dispersed oil during environmental spills.[22]

SMART provides guidance on surface, not deepwater spill response like the 2010 Deepwater Horizon spill (DWH) where ~800 million liters of light Louisiana sweet crude oil was released from 1500 m ocean depth. Seven million liters[23] of chemical dispersant (Corexit© 9527, 9500A) were applied to reduce environmental impacts of oil, and aid worker safety. The response effort proved challenging partly due to the inability to visually inspect the subsea oil plume. The horizontal, vertical and temporal extent of, and heterogeneity of the plume equated to a "needle-in-the-haystack" situation with grab sampling too coarse to capture its magnitude and extent. Sample number limitations and oil stratification within bottles during the 1−2 h profiler transit time also contributed to challenges in quantifying oil.[24] Thus, responders and scientists were evermore reliant on submersible sensors in lieu of visual observations to provide critical tracking measurements. Initial plume tracking between 1000 and 1400 m was conducted using WET Labs, Inc. ECO fluorometers. Their accessibility and wavelength configuration- wide filter bandwidth and ExEm370/460 nm CWL (Supporting Information (SI) Table S2) to capture broad nature of the peaks- is appropriate for crude oil fluorescence detection. Chelsea Technologies Group AQUAtracka fluorometers were deployed to monitor the transport and fate of oil at large distances from the wellhead and after well capping (latter response, low concentrations).[24] This unit employs filters (ExEm239/360 nm CWL) to capture peak fluorescence from petroleum aromatic fractions with elevated sensitivity (higher signal-to-noise) due to a xenon lamp light source and a photomultiplier tube (PMT) detector; possibly aiding MC252 oil tracking which has a small fraction of highly fluorescing molecules (~20%).[24]

In the wake of the spill many questions remain regarding oil quantification and fate of the subsea oil plume, as well as sensor applicability to detect oil (dynamic range, detection limits, and wavelength configuration).[25] Effects of concentration, dispersant to oil ratio (DOR), dispersant effectiveness,[26] and fluorescence signatures[12] contribute to uncertainties in selecting sensors. Moreover, concerns persist on sensor reliability and applicability of laboratory calibrations using flasks, high oil concentrations and insufficient mixing energies to real-world conditions[19,25] To address uncertainties the performance of select COTS in situ fluorometers deployed during DWH (Chelsea Technologies Group, Turner Designs, WET Labs, Inc.) and a UV spectrophotometer (Satlantic) was evaluated in the Bedford Institute of Oceanography (BIO) wave tank. This work is timely, as findings will assist in the calibration and interpretation of DWH fluorescence measurements. It also coincides with the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) amendments pertaining to fluorometer protocols, reflecting the need for improved confidence in sensors for estimating spilled oil concentrations.

EXPERIMENTAL SECTION

Wave Tank Description. Experiments were conducted at the BIO, Center for Offshore Oil, Gas and Energy Research (COOGER; Dartmouth, Nova Scotia) wave tank, measuring 32 m long, 0.6 m wide, and 2 m high (1.5 m water depth; 28 800 L volume) (SI Figure S1). Water from the Bedford Basin of Halifax Harbor was pumped into the tank through a coarse (25 μm pore size) and fine (5 μm pore size) serial filtration system. Breaking and nonbreaking waves (computer-controlled flat-type wave maker) provided mixing energies to achieve dispersant effectiveness observed in the field. The tank was drained and cleaned with Big Orange detergent and seawater (tank walls, bottom, wave maker and absorbers) after each experiment to remove all oil and surfactants, and sensor optical windows were also cleaned.

Experimental Design. Experiments were conducted using MC252 crude oil (Discoverer Enterprise drillship recovered, May 31−June 7, 2011), and Corexit© 9500 chemical dispersant (1:25 DOR). Artificially weathered oil was generated by bubbling with nitrogen for 36 h (average time responders typically begin dispersant) resulting in mass reduction by 7%. Experimental duplicate and triplicate treatments were run with random design, reducing effects from confounding factors such as wind, rain, temperature, salinity, and seawater quality (SI Table S1). Prior to experiments, oil and dispersant mixtures were prepared in 1 L of seawater by orbital shaker agitation (20 min). Fluorometers (WET Labs, Inc. ECO, Chelsea Technologies Group AQUAtracka, Turner Designs Cyclops) and a UV spectrophotometer (Satlantic SUNA) were deployed at the same cross-sectional position within the tank 20 m from the wave generator (10 m downstream from oil release). Configuration specifications are shown in SI Table S2 and Figure S1. A laser in situ scattering and transmissometry particle size analyzer (Sequoia Scientific LISST-100X, C-Type,), 1 m

ED_001324_00001447-00002

downstream from sensors, collected continuous particle size distribution measurements. Sensors were mounted so that optical windows were positioned 65 cm deep in the tank and warmed up for 15 min prior to oil addition and collection of baseline. A stepwise addition experiment (EXP_{ADD}) of chemically dispersed fresh oil (300 ppb to 12 ppm, tank operated in steady-state mode) was conducted to establish response linearity and dynamic range of sensors to MC252 oil. Each addition of oil/dispersant was allowed to homogenize, with wave mixing, for 1 h prior to subsequent oil additions. Water samples were collected during the final 5 min of each step and sensors were cleaned in between steps using lens wipes and solvent. There was no indication of sensor fouling from oil as sensor response was checked pre- and postcleaning. Continuous optical data were collected during the 9 h experiment and manufacturer calibrations applied.

Dilution experiments (EXP_{DIL}) were conducted with 100 mL of fresh or weathered oil added, with or without dispersant (only chemically dispersed results reported here). Maximum concentration in the tank was less than 3 ppm, when fully dispersed. To simulate field conditions, ocean waves were generated with tank in flow-through mode (flow rate of 3.8 ± 0.2 L s^{-1}) allowing transport of dispersed oil away from the slick and dilution to extinction.[27,28] EXP_{DIL} were conducted for 90 min, where discrete oil concentration samples were collected at 15 time points via tubing through an inlet located next to the sensor deployment frame. Three-dimensional excitation emission matrix fluorescence spectroscopy (EEMS) analyses confirmed the position of fluorescence peaks and degree of chemical dispersion.

Submersible Sensor Data Processing. Sensor specific blanks (2 min average) prior to oil addition were subtracted. Manufacturer calibrations yielded data in recommended units (carbazole and perylene for AQUAtrackas, quinine sulfate dihydrate for ECO, uM nitrate for SUNA weighted index of UV absorption 210−370 nm, and none was provided for the Cyclops). Final 5-min signal averages for each concentration during the EXP_{ADD} was regressed (model 1, linear least-squares) to ppm oil, benzene-toluene-ethylbenzene-xylene (BTEX) or total petroleum hydrocarbons (TPH) values. Regressions were applied to EXP_{DIL} fluorescence and absorption data. Sensor performance and vicarious calibration were validated with laboratory chemistry results. LISST-11X dispersed oil droplet size distribution recorded 32 particle size intervals logarithmically spaced from 2.5−500 μm in diameter, with the upper size in each bin 1.18 times the lower. Distribution is expressed as the average volumetric concentration of droplets within each size range interval. Total particle concentration (TPC) represents the sum of all particle concentrations between 2.5 and 500 μm.[27]

Discrete Samples Analyses. Oil/water samples were collected at time points and analyzed for TPH, BTEX and PAH. TPH values were acquired using a GC-FID (gas chromatograph-flame ionization detector, Agilent 6890 and Supelco column). Extraction methods[29] were used and samples were concentrated under nitrogen to a final volume of 1.0 mL prior to analysis. Peak quantification, using the total area under the curve, yielded hydrocarbon concentrations in seawater. A spike and recovery study with blanks and duplicates verified extraction method efficiency. BTEX (volatile hydrocarbons) were measured via GC-MS (GC-mass spectrometry, Agilent 5973) using a modified version[29] of the standard EPA Method 8240 (purge and trap) for oil applications. A 5 mL sample was transferred into the purge chamber and purged with helium for 11 min. The volatiles were trapped and then desorbed at 225 °C for 2 min. The MS was used in selected ion monitoring mode (SIM) for six ions: 77, 78, 91, 92, 105, and 106 amu. BTEX calibration standards were prepared by diluting a certified standard mix (Supelco). Both standard and samples (blanks and duplicates) were purged, trapped and analyzed in the same manner.

EEM and absorbance spectra were collected with a Horiba Scientific AquaLog spectrofluorometer with a CCD detector. Oil/water samples were analyzed for fluorescence peaks within 10 min of collection. Samples within a 1 cm cuvette were scanned over Ex 220−550 nm and Em 213−610 nm (1 s integration), blank subtracted, inner-filter effect corrected, quinine sulfate dihydrate normalized, and Rayleigh scatter masked.[30,11]

RESULTS AND DISCUSSION

An EEM contour plot of chemically dispersed MC252 oil (4 ppm) collected during an EXP_{DIL} illustrates the UV fluorescent peak maxima centered on ExEm270/325 and ExEm260/450 nm

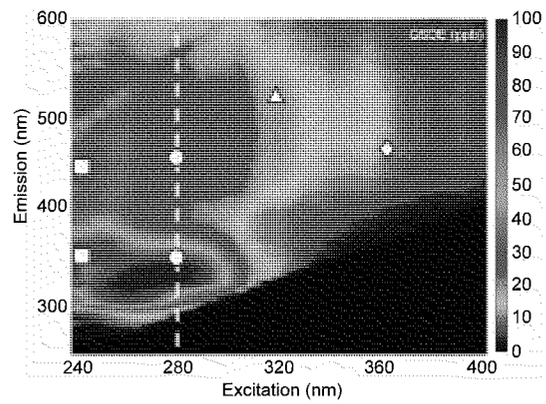


Figure 1. Fluorescence EEM of chemically dispersed fresh MC252 oil (4 ppm) concentration in a EXP_{DIL}. Center Wavelengths (CWL) of Chelsea Technologies Group AQUAtrackas $ExEm_{239/360}$ and $ExEm_{239/440}$ (□), Turner Designs Cyclops $ExEm_{320/510}$ (△), and WET Labs, Inc. ECO $ExEm_{370/460}$ (◊) along with the fluorescence intensity ratio (FIR = $ExEm_{280/340:280/445}$; ○) are overlaid for reference.

(Figure 1). Color map units are ppb QSDE (Quinine Sulfate Dihydrate Equivalents), scaled to peak maximum. CWL of the sensors tested are overlaid illustrating that all sensors were configured to detect some portion of the broad oil signal. Note that the symbols do not take into account wavelength bandwidths, which increases a sensor's ability to detect oil. For reference purposes, white circles on the dotted line represent the fluorescence intensity ratio (FIR) wavelengths used to indicate chemical dispersion of oil.[12]

Manufacturer calibrated sensor response at six cumulative oil concentrations during the EXP_{ADD} is shown in Figure 2 and SI Figure S2. The addition of oil and dispersant formed an oil plume with a sharp leading edge that passed in front of the sensors causing a rapid increase in signal followed by a decrease as the oil mixed/dispersed in the tank. Values at the end of each step (tank homogeneously mixed) are representative of sensor response for a specific oil concentration and established that all sensors detected chemically dispersed oil down to 300 ppb, a critical finding. This is below the laboratory detection limit for GC-FID TPH method, making sensor-estimated oil concentrations evermore valuable for tracking low concentration

ED_001324_00001447-00003

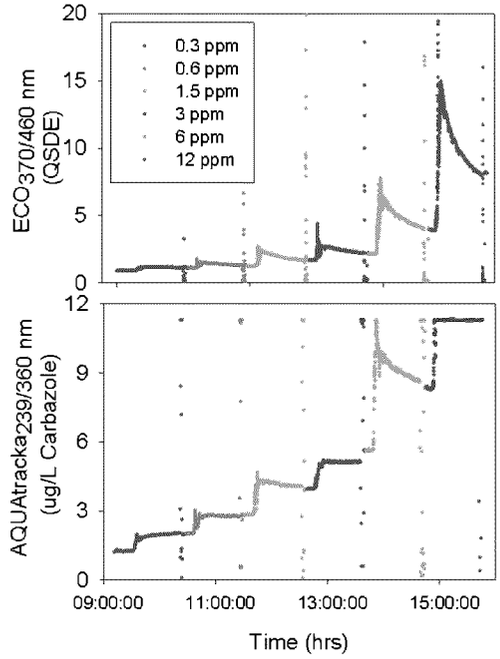


Figure 2. Fluorescence intensity of the ECO and $AQUA_{239/360\ nm}$ sensors exposed to chemically dispersed MC252 crude oil during EXP_{ADD} within the wave tank.

plumes. It also refutes previous notions during the DWH response that certain sensors may not detect oil <1 ppm. This was supported by earlier calibration tests using small flasks which may have been biased by underachieving proper mixing energies negatively impacting dispersant efficiency and solubility of fluorescence molecules into the water phase.[22] Strong linearity of sensor response at the lowest concentrations suggests possible lower detection limits less than 300 ppb (not tested with this experimental design). In any case, sensor response at 300 ppb improves confidence in the instruments deployed for DWH subsea plume monitoring, thereby demonstrating ability to detect low concentrations of oil.

Sensor dynamic range was established during the EXP_{ADD} by regressing fluorescence and absorption averages with oil concentration (Figure 3). Generally, submersible sensors were linearly correlated with TPH, BTEX and oil load, regardless of optical configuration. The ECO was operated in digital mode (no gain control) and the SUNA employed autoadjusting integration time, evidenced by lack of saturation over the entire concentration range. The Cyclops was operated with 100X gain for concentrations ≤6 ppm, but 10X for 12 ppm to avoid saturation. The AQUAtrackas employ BITE (Built in Test Circuitry) to extend calibration intervals. However saturation was exhibited by the $AQUAtracka_{239/360\ nm}$ sensor at 12 ppm oil. This is attributed to higher detector sensitivity, increased power of the light source (flash lamp opposed to LED) and/or wavelength filters closely aligned to the fluorescence maxima of crude oil. The longer wavelength emission AQUA $tracka_{239/440\ nm}$ sensor however did not saturate. Regression equations were employed to calibrate sensors during EXP_{DIL} (Figure 3). This aligns results with response effort measurements: sTPH (speciated TPH; water-insoluble heavy saturate fraction; aromatics > naphthalene; less laborious analytical screening) supplemented with BTEX (more laborious screening) values (Figure 3). All sensors exhibited squared correlation coefficients greater than 0.96. The 12 ppm value for the $AQUAtracka_{239/360\ nm}$ and SUNA (for BTEX) was excluded from the calculation due to saturation. The ECO and BTEX regression excluded values above 1.5 ppm, thereby increasing performance at low concentrations. TPH values for lowest concentration additions are absent because they were below the detection limit of the laboratory-based GC-FID TPH analysis. For all sensors, signal-to-noise ratios permitted detection at low concentrations. Findings demonstrate that all sensors would have been valuable tools during the DWH response given that sTPH and tVOA (total volatile organic analysis; water-soluble fraction; namely BTEX and naphthalene) in field samples were within the linear range of sensors tested in this study.[24] During the spill, sTPH from GC-MS ranged between <1 - 1000 ppb, with subsea plume (900−1300 m) maximum of 485 ppb, 1.2 km from the wellhead and detectable levels extending >10 km from source. In the plume, tVOA concentrations were higher than sTPH, with a maximum of 2112 ppb.

Sensor performance as a function of dispersant presence, oil state, and plume dilution was examined during EXP_{DIL}. TPH and BTEX-calibrated sensor responses for one-fresh and one-weathered, chemically dispersed oil EXP_{DIL} are shown in Figure 4 and SI Figure S3, respectively. Left panels show TPH-calibrated sensor and discrete TPH results (open circles), right panels show BTEX-calibrated sensor and discrete BTEX results (gray circles) over the course of the 90 min experiment. Due to a delayed shipping issue, the $AQUAtracka_{239/440\ nm}$ was operated only during one EXP_{DIL} and appears in the latter figure. EXP_{DIL} particle size distribution (PSD) and concentration of fresh oil droplets corresponding to Figure 4 are shown in Figure 5 (left). Histograms represent a composite of the entire duration of a flow-through experiment (90 min). Presence of particles observed in the <100 um size classes are indicative of chemical dispersion. All experiments using dispersant exhibited oil droplet size ranges consistent with adequate dispersant effectiveness (droplets <100 μm, Figure 5).[26]

For all experiments, MC252 oil state (fresh or weathered) did not appear to influence the sensor response. All sensors responded similarly to both (Figure 4 and SI Figure S3), verified with similar particle size distribution. Like sensor response as a function of oil state lends support for applying regression equations calculated with fresh oil to dilution experiments using weathered oil. Note that oil artificially weathered by 7% may represent oil exposed to physical processes (volatilization due to photo-oxidation, evaporation or dissolution) that occur during a spill of short duration, not necessarily of a prolonged spill with extensive weathering. However, the degradation of light crude oil and the addition of dispersant have been shown to increase the occurrence of longer wavelength fluorescence peaks.[11,15] Hence, sensors may be better capable of detecting extensively weathered oil where wavelength configuration better overlaps with longer fluorescence peaks (Figure 1). This is particularly true for lighter crudes such as MC252 oil that contain a higher proportion of simpler, lower molecular weight hydrocarbons that are more readily physically and biodegraded compared to heavy crudes.[31]

Tank hydrodynamics affected the movement and dispersion of oil within the tank, which varied during dilution experiments. Total particle concentration (TPC) as a function of time was influenced by tank mixing, where experiments exhibited either a gradual or rapid (Figure 5, left and right) decay of oil droplets. Three tests exhibited a gradual increase in signal as oil passed the sensors in the tank followed by gradual decay with dilution (Figure 4). For these cases, maximum values were 4−5 ppm

ED_001324_00001447-00004

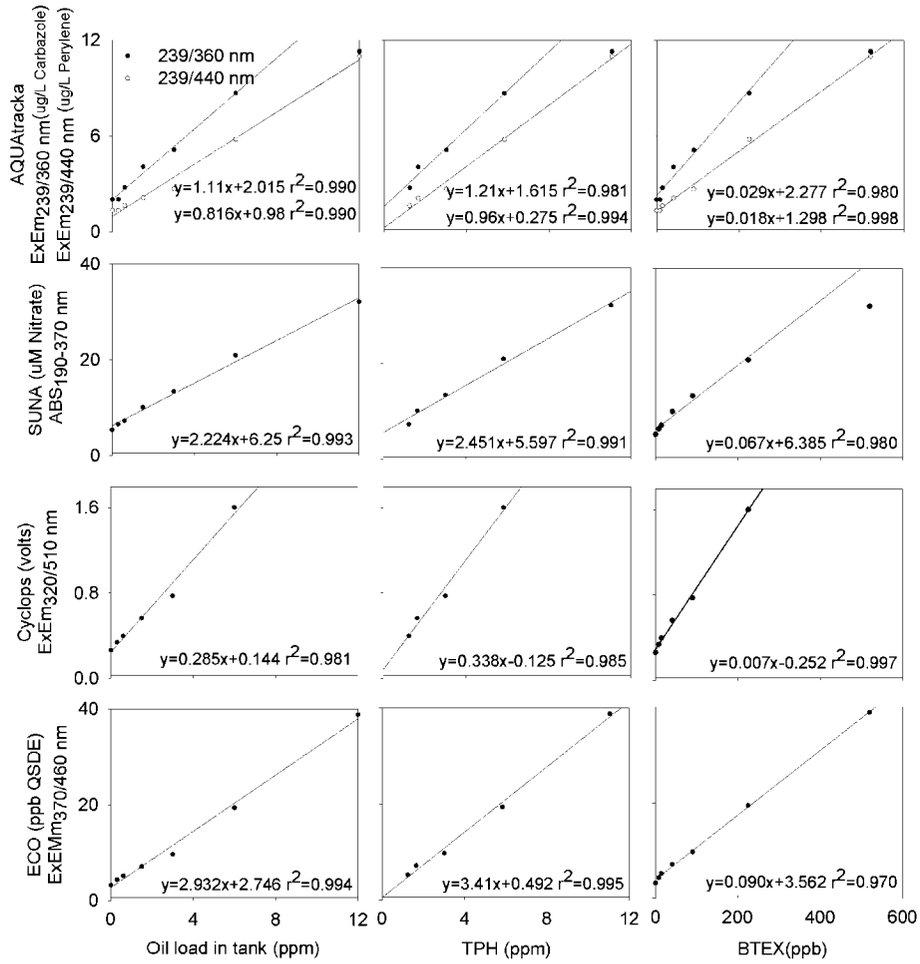


Figure 3. Fluorometric (submersible fluorometers) and spectrophotometric response (SUNA) as a function of oil concentration between 300 ppb and 12 ppm during EXP_{ADD}. Fewer TPH data points due to concentrations below the detection limit of TPH laboratory method.

TPH and 200−250 ppb BTEX, with subsequent plume dilution to 2−3 ppm TPH and 50 ppb BTEX. LISST-100X response (Figure 5, left) indicates like mixing and dispersing conditions in those experiments. Plume dynamics differed in two tests, where steep increases in oil concentration were followed by rapid decay (Figure 6), supported by TPC and droplet size distribution (Figure 5, right) and maximum TPH and BTEX values of 6 ppm and 250 ppb, followed by dilution to concentrations akin to all experiments. TPH and BTEX-calibrated sensor responses follow the chemistry and oil droplet results in all experiments. Agreement among these parameters is critical as they are used in concert during oil spills to track chemically dispersed oil plumes and confirm degree of dispersant effectiveness.

Sensor response during chemically dispersed EXP_{DIL} was validated with chemistry results to verify sensor performance. Percent differences ($\%_{dif}$) and mean absolute error (MAE) between estimated (from sensors) and actual (chemistry results) oil concentrations were calculated for each time point in experiments, providing performance metrics for estimates of TPH and BTEX for sensors exposed to oil. The MAE and mean, median and average $\%_{dif}$ for each sensor for all time points and for time points excluding 0−10 min are listed in Table 1. Time points <10 min were excluded from calculations due to heterogeneity and timing of the plume leading edge in the tank. The percentage of experiments where calibrated sensor responses were within ±25% and +30% of chemistry results are reported.

Based on $\%_{dif}$, the ECO and $AQUAtracka_{239/440\ nm}$ consistently better estimated BTEX, with 91% and 86% of time points within $30\%_{dif}$, respectively. The $AQUAtracka_{239/440\ nm}$ had the lowest median $\%_{dif}$ of <7.96. These values may be biased, however, as the $AQUAtracka_{239/440\ nm}$ was used in only one flow-through EXP_{DIL}. The ECO median $\%_{dif}$ was slightly higher (12%), but had the highest percentage of time points within $\pm30\%_{dif}$ (91%). Accuracy was also high with MAE of ±17.27 and 12.41 ppb for the ECO and $AQUAtracka_{239/440\ nm}$, respectively. The $AQUAtracka_{239/440\ nm}$, Cyclops and SUNA exhibited similar performance for BTEX where 71−79% values were within $\pm30\%_{dif}$. The $AQUAtracka_{239/360\ nm}$ also exhibited higher accuracy (MAE ± 12.43 ppb), but estimates were less consistent (71% of measurements within $30\%_{dif}$). BTEX consists of single ring aromatics, which are highly volatile and moderately soluble (relative to higher ring no. PAHs) in the shallow water of the wave tank, but any loss did not impact the $\%_{dif}$. Extrapolating this to real-world scenarios, surface spills typically have reduced BTEX or tVOA due to evaporation; however subsea oil plumes would likely retain higher dissolved concentrations.[6] Thus the ECO, $AQUAtracka_{239/360\ nm}$ and $AQUAtracka_{239/440\ nm}$ are each well suited for estimating BTEX in the deep sea, but they do vary with accuracy and precision. Results may differ for medium and

ED_001324_00001447-00005

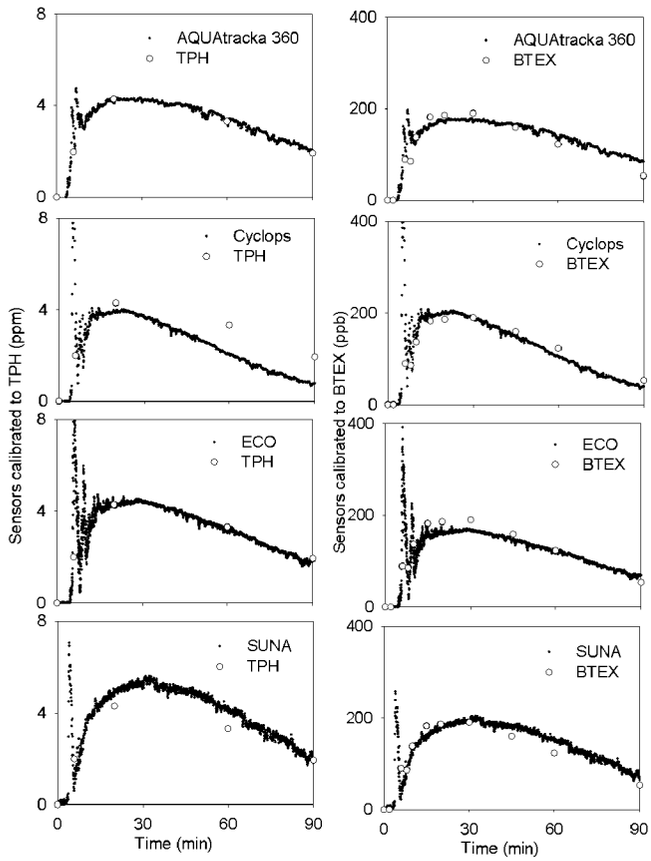


Figure 4. Response of TPH and BTEX-calibrated sensors exposed to chemically dispersed fresh crude oil. TPH and BTEX values from discrete samples are represented by open and gray circles, respectively. Wave tank was operated in flow-through mode (EXP_{DIL}) to observe fluorescence values during the dilution of an oil spill.

heavy oil, however, as BTEX concentrations would vary from the light crude oil tested here.

TPH was better estimated by the SUNA, with respect to both accuracy and precision, followed by the AQUAtracka$_{239/360\ nm}$, with 97% and 84% of time points within 30%$_{dif}$ of actual oil concentrations and MAE of +0.47 and 0.58 ppm, respectively.

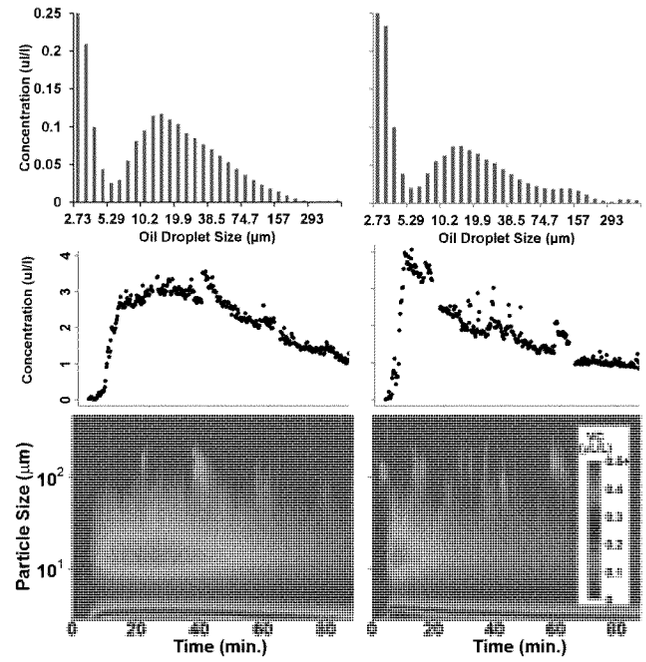


Figure 5. Particle size distribution and concentration within wave tank corresponding to experiments shown in Figures 4 and 6. Histogram plots are composites of entire flow-through experiment. Total particle concentration as a function of time (middle) is shown in scatter plot. Contour plots represent Particle size and volume concentration (VC) as a function of time for chemically dispersed fresh (left) and weathered (right) MC252 crude oil with gradual and rapid decay, respectively.

SUNA had the lowest median %$_{dif}$ of 11% for time points >10 min.

The remaining sensors exhibited median %$_{dif}$ of 18.72−23.87% with fewer values within 30%$_{dif}$ (60−71%) and higher MAE (0.77−0.81 ppm). Compared to BTEX, TPH laboratory analysis captures more compound types which are present at higher concentrations in oil. The SUNA's high performance in estimating TPH may result from absorption, not fluorescence, being measured over a wide wavelength range and oil being comprised of more chromophores than fluorophores. Tank

Table 1. Percent Differences (%$_{dif}$) between Sensors and Chemistry[S]

		TPH (ppm)					BTEX (ppb)				
		ECO 370/460 nm	AQUA 239/360 nm	AQUA 239/440 nm*	Cyclops 320/510 nm	SUNA ABS	ECO 370/460 nm	AQUA 239/360 nm	AQUA 239/440 nm*	Cyclops 320/510 nm	SUNA ABS
all time points	mean %$_{dif}$	31.69	30.24	41.98	40.32	27.92	26.67	30.93	23.14	31.99	30.45
	median %$_{dif}$	23.54	21.15	26.21	25.54	15.68	13.66	20.95	12.34	24.41	23.74
	min %$_{dif}$	0.27	1.96	2.16	1.73	0.76	1.63	3.19	5.29	0.01	0.65
	max %$_{dif}$	183.78	186.85	194.19	199.92	214.04	122.22	128.82	85.95	114.40	140.97
	[MAE]	± 0.91	± 0.67	± 0.78	± 0.90	± 0.68	± 23.53	± 13.46	± 12.41	± 32.22	± 27.68
time points >10 min	mean %$_{dif}$	25.52	19.37	19.22	30.94	13.88	15.54	23.88	14.17	21.11	22.85
	median %$_{dif}$	23.85	18.72	23.87	21.29	11.20	12.00	15.14	7.96	16.67	19.95
	min %$_{dif}$	0.27	1.96	2.16	1.73	0.76	1.63	3.19	5.29	0.01	0.65
	max %$_{dif}$	74.55	53.83	35.22	127.31	46.00	85.57	83.18	31.26	87.71	81.00
	[MAE]	± 0.78	± 0.58	± 0.81	± 0.77	± 0.47	± 17.27	± 12.43	± 12.41	± 29.13	± 24.50
% of >10 min time points	<25%$_{dif}$	56%	72%	57%	56%	90%	86%	68%	86%	64%	63%
	<30%$_{dif}$	69%	84%	71%	60%	97%	91%	71%	86%	79%	74%

[S]Asterisk represents sensor with only one experiment. MAE is mean absolute error.

ED_001324_00001447-00006

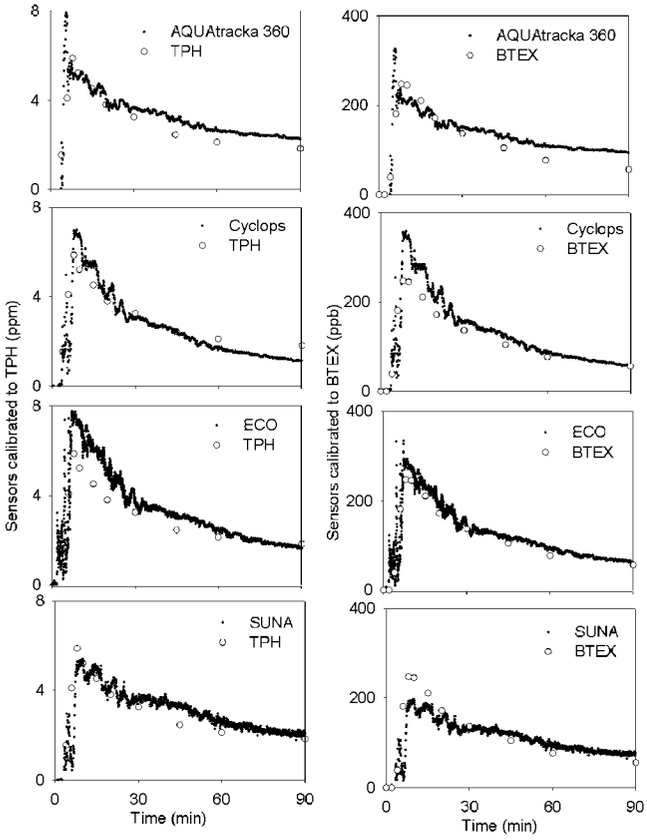


Figure 6. Response of TPH and BTEX-calibrated sensors exposed to chemically dispersed fresh crude oil for dilution experiment with steep increase in particles, followed by rapid decay. TPH and BTEX values from discrete samples are represented by open and gray circles, respectively. Wave tank was operated in flow-through mode (EXP_{DIL}) to observe fluorescence values during the dilution of an oil spill.

results indicate that of the sensors used in the DWH subsea plume, both the $AQUAtracka_{239/360\ nm}$ and ECO would have detected low TPH concentrations. The former may have exhibited better accuracy and precision. However, it could also be speculated that the $AQUAtracka_{239/360\ nm}$ with its narrower dynamic range would have saturated at high concentrations early in the spill and impeded plume tracking. Results are critical and provide evidence that ECO data from early in the spill (high concentrations) can be compared with AQUA data from later in the spill (low concentrations), and that sensors could provide oil concentrations with reasonable accuracy.

Wave tanks simulate natural variability observed in real spills. Thus, vicarious calibration is expected to be more accurate than a laboratory calibration, even though it may suffer from poorer precision. These findings demonstrate the sensitivity of COTS sensors exposed to chemically dispersed MC252 oil and increase confidence using in situ measurements during DWH to fill in the data gaps of coarse discrete samples. During an oil spill, sensor choice should be a function of oil concentration, calibration to TPH or BTEX and trade-offs between accuracy and precision. This work has large implications for plume tracking and understanding fate and transport of spilled oil in the environment.

ASSOCIATED CONTENT

* Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: 513-569-7090; fax: 513-569-7620; e-mail: conmy.robyn@epa.gov.

Present Addresses

◆University of Oregon, Institute of Ecology and Evolution and Institute of Environmental Science, Oregon, USA.

¶Commonwealth Science and Industrial Research Organization, Wealth from Oceans Flagship, Perth, Australia.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank all funding sources for the experiments at COOGER-BIO (Department of Fisheries and Oceans, Canada): IOOS support (Alliance for Coastal Technology), NOAA's supplemental DWH appropriation, salary support from home institutions, DFO Canada and internal travel support by Horiba JY, Oil Spill Recovery Institute, WET Labs, Inc. This document has been reviewed by the U.S. Environmental Protection Agency National Risk Management Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. This is a contribution from the Land Remediation and Pollution Control Division.

REFERENCES

(1) Report, S. P. Special Monitoring of Applied Response Technologies Protocol Report; National Oceanic and Atmospheric Administration, U.S. Coast Guard, U.S. Environmental Protection Agency, 2006; p 43.

(2) Henry, C. B., Roberts, P.O.; Overton, E.B. A primer on in situ fluorometry to monitor dispersed oil. In International Oil Spill Conference, 1999; pp 225-228.

(3) Li, M.; Garrett, C. The relationship between oil droplet size and upper ocean turbulence. Mar. Pollut. Bull. 1998, 36, 961−970.

(4) Lessard, R. R.; Demarco, G., The significance of oil spill dispersants. Spill Sci. Techno. Bull., 2000, 6, (59−68).

(5) Venosa, A. D.; Zhu, X. Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands. Spill Sci. Technol. Bull. 2003, 8, 163−178.

(6) Camilli, R.; Reddy, C. M.; Yoerger, D. R.; Mooy, B. A. S. V.; Jakuba, M. V.; Kinsey, J. C.; McIntyre, C. P.; Sylva, S. P.; Maloney, J. V. Tracking hydrocarbon plume transport and biodegradation at deepwater horizon. Science 2010, 330 (6001), 201−204.

(7) Green, D.; Humphrey, B.; Fowler, B. The use of flow-through fluorometery for tracking dispersed oil. In Internationl Oil Spill Conference, 1983; pp 473−478.

(8) Page, D. S.; Foster, J. C.; Hotham, J. R.; Pendergasst, E.; Herbert, S.; Gonzalez, L.; Gilfillan, E. S.; Hanson, S. A.; Gerber, R. P.; Vallas, D. Long-term fate of dispersed and undispersed crude oil in two nearshore test spills. In International Oil Spill Conference, 1983; pp 479−482.

(9) Keizer, P. D.; D. C., G., Jr. Detection of trace amounts of oil in sea water by fluorescence spectroscopy. J. Fish. Res. Board Can. 1973, 30, 1039−1046.

(10) Østgaard, K.; Jensen, A. Evaluation of direct fluorescence spectroscopy for monitoring aqueous petroleum solutions. Int. J. Environ. Anal. Chem. 1983, 14, 55−72.

ED_001324_00001447-00007

(11) Bugden, J. B. C.; Yeung, C. W.; Kepkay, P. E.; Lee, K. Application of ultraviolet fluorometry and excitation-emission matrix spectroscopy (EEMS) to fingerprint oil and chemically dispersed oil in seawater. Mar. Pollut. Bull. 2008, 56 (4), 677−685.
(12) Kepkay, P.; Young, W.; Bugden, J. B. C.; Li, Z.; Lee, K. Ultraviolet fluorescence spectroscopy (UVFS): A new means of determining the effectiveness of chemical dispersants on oil spills. In International Oil Spill Conference, Savannah, GA, 2008; pp 639−44.
(13) Li, J.; Fuller, S.; Cattle, J.; Way, C. P.; Hibbert, D. B. Matching fluorescence spectra of oil spills with spectra from suspect sources. Anal. Chim. Acta 2004, 514 (1), 51−56.
(14) Christensen, J. H.; Hansen, A. B.; Mortensen, J.; Andersen, O. Characterization and matching of oil samples using fluorescence spectroscopy and parallel factor analysis. Anal. Chem. 2005, 77, 2210−2217.
(15) Zhou, Z.; Liu, Z.; Guo, L. Chemical Evolution of Macondo crude oil during laboratory degradation as characterized by fluorescence EEMs and hydrocarbon composition. Mar. Pollut. Bull. 2012, 66, 164−175.
(16) Conmy, R. N.; Chen, R. F.; Castillo, C. E. D.; Downing, B. Calibrations and quality control of in situ sensors. In Fluorescence Applications in Aquatic Science; Cambridge University Press, 2013.
(17) Report, S. L. R. E. R. Updating the SMART dispersant monitoring protocol: Review of Commercial-Off-The-Shelf instruments; S.L. Ross Environmental Research, 2008; p 19.
(18) Lambert, P. A literature review of portable fluorescence-based oil-in-water monitors. J. Hazard. Mater. 2003, 102, 39−55.
(19) Fuller, C. B.; Bonner, J. S.; Page, C. A.; Arrambide, G.; M.C. Sterling, J.; Ojo, T. Field Instruments for Real Time In-Situ Crude Oil Concentration Measurements; Texas A&M University, 2003; p 10.
(20) Wilde, S. Procedures for monitoring dispersant operations. In 10th Environmental and Scientific Coordinators Workshop , Queenscliff, Australia, 2001, 2001; pp 163−177.
(21) Srinivasan, R.; Lu, Q.; Sorial, G. A.; Venosa, A. D.; Mullin, J. Dispersant effectiveness of heavy fuel oils using baffled flask test. Environ. Eng. Sci. 2007, 24 (9), 1307−1320.
(22) Report, N. R. C. Oil Spill Dispersants: Efficacy and Effects; Washington, DC, 2005; p 400.
(23) Report Oil Budget Calculator: Deepwater Horizon; Federal Interagency Solutions Group, 2010; p 217.
(24) Report, J. A. G. Deepwater Horizon Oil Spill: Review of Subsurface Dispersed Oil and Oxygen Levels Associated with the Deepwater Horizon MC252 Spill of National Significance; National Oceanic and Atmospheric Administration, 2012; p 93.
(25) Report, J. A. G. Review of Data to Examine Subsurface Oil in the Vicinity of DWH MC-252; National Oceanic and Atmospheric Administration, May 19−June 19, 2010; p f29.
(26) Lee, K.; Li, Z.; Boufadel, M. C.; Venosa, A. D.; Miles, M. S. Wave Tank Studies on Dispersant Effectiveness As a Function of Energy Dissipation Rate and Particle Size Distribution; Department of Fisheries and Oceans Canada, 2009; p 67.
(27) Li, Z.; Lee, K.; King, T.; Boufadel, M. C.; Vensosa, A. D. Evaluating crude oil chemical dispersion efficacy in a flow-through wave tank under regular non-breaking wave and breaking wave conditions. Mar. Pollut. Bull. 2009, 58, 735−744.
(28) Li, Z.; Lee, K.; King, T.; Boufadel, M. C.; Vensosa, A. D. Effects of temperature and wave conditions on chemical dispersion efficacy of heavy fuel oil in an experimental flow-through wave tank. Mar. Pollut. Bull. 2010, 60, 1550−59.
(29) Cole, M. G.; King, T. L.; Lee, K. Analytical technique for extracting hydrocarbons from water using sample container as extraction vessel in combination with a roller apparatus. Anal. Chem. 2007, 77, 2210−17.
(30) Coble, P. G. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Mar. Chem. 1996, 51, 325−346.
(31) Atlas, R. M.; Hazen, T. C. Oil biodegradation and bioremediation: A tale of the two worst spills in U.S. history. Environ. Sci. Technol. 2011, 45 (16), 67−09−15.

ED_001324_00001447-00008

Pergamon

Spill Science & Technology Bulletin, Vol. 7, Nos. 5–6, pp. 299–308, 2002
Published by Elsevier Science Ltd.
Printed in Great Britain
1353-2561/02 $ - see front matter

PII: S1353-2561(02)00072-5

RESEARCH

The Baffled Flask Test for Dispersant Effectiveness: A Round Robin Evaluation of Reproducibility and Repeatability

ALBERT D. VENOSA†*, DENNIS W. KING‡,[1] & GEORGE A. SORIAL§,[2]
†*National Risk Management Research Laboratory, US Environmental Protection Agency, 26 W. Martin Luther King Dr., Cincinnati, OH 45268, USA*
‡*Statking Consulting Inc., Fairfield, OH 45014, USA*
§*Department of Civil and Environmental Engineering, University of Cincinnati, Cincinnati, OH 45221-0071, USA*

After two previous investigations demonstrated that the baffled flask test was an effective and reproducible method for screening the effectiveness of dispersant products in the laboratory, the US Environmental Protection Agency decided that before the new protocol could be considered for replacement of the swirling flask test, it would have to be subjected to an interlaboratory round robin investigation. This paper describes how the round robin evaluation was carried out and presents repeatability and reproducibility calculations that demonstrate the superiority of the new method over previous methods. Probabilities for passing various threshold levels of effectiveness values were computed, and the results will be used by the Agency to develop final pass–fail decision rules for dispersant manufacturers wishing to have their products listed on the national contingency plan product schedule.
Published by Elsevier Science Ltd.

Keywords: **Baffled flask test, swirling flask test, dispersants, round robin test, interlaboratory test, dispersion effectiveness, crude oil**

Introduction

In September, 1994, the US Environmental Protection Agency (EPA) officially adopted the swirling flask test (SFT) as its official laboratory screening methodology for testing the effectiveness of dispersants in seawater. The SFT is based on the protocol first developed and adopted by Environment Canada in the 1980s (Fingas *et al.*, 1987, 1989a,b). It essentially consists of a procedure in which a pre-mixed solution of crude oil and dispersant is added to a specially designed glass side-arm flask containing 100 ml artificial seawater and the contents of the flask mixed on an orbital shaker for 10 min followed by a settling time of 10 min. The dispersed oil mixture is then extracted and measured in a spectrophotometer to determine the quantity of oil that had been dispersed into the water column. Listing of a dispersant on the national contingency plan (NCP) product schedule has been contingent on the dispersant being at least 45% effective (50 ± 5%) in dispersing Prudhoe Bay and South Louisiana crude oils in the laboratory test. An important aspect of the test is that a product may be less than 45% effective on one of these oils but

*Corresponding author. Tel.: +1-513-569-7668; fax: +1-513-569-7105.
E-mail addresses: venosa.albert@epa.gov (A.D. Venosa), statking@statkingconsulting.com (D.W. King), george.sorial@uc.edu (G.A. Sorial).
[1] Tel.: +1-513-858-6514; fax: +1-513-858-3022.
[2] Tel.: +1-513-556-2987; fax: +1-513-556-2599.

ED_001324_00001448-00001

be effective enough on the other to still qualify for listing on the schedule. The procedure is simple, inexpensive, and straightforward.

After the SFT was adopted in the final EPA regulation promulgated in September 1994 and described in Appendix C, Subpart J of 40 CFR 300.900 (US EPA, 1996), it was reexamined after its first year of use (IT Corporation, 1995). Reexamination was pursued because of the discovery that unexpectedly large discrepancies had been observed between the data submitted by dispersant manufacturers and those generated by EPA contract laboratories for numerous products on the NCP product schedule. Some of the more important problems identified in the IT report included calibration standards that were outside the detection limits of the analytical instrument, an inappropriate settling time, too stringent a pass/fail criterion, insufficient energy imparted by the orbital shaker in the side-arm flask, and movement in the flask side arm causing a pumping action that contributed to the variable results observed.

Thus, in 1999, EPA decided to rigorously investigate the SFT to understand and resolve its apparent ambiguities and possibly come up with a better, more reproducible protocol. To accomplish the objective, fractional factorial experiments were conducted to determine which factors have the greatest impact on the variance of the method. Such factors as oil type, rotational speed, settling time, dispersant type, and analyst were evaluated systematically. A redesign of the test flask was also considered. Reasons for the poor reproducibility of the SFT were defined and the problems corrected. The results of the research have been submitted (Sorial *et al.*, submitted for publication, submitted for publication), and a new methodology was developed based on a new flask design. The flask is a 150 ml baffled trypsinizing flask used in biological science research and clinical laboratory testing (Fig. 1). The new protocol was named the baffled flask test (BFT). The two previous papers discussed in detail the reproducibility and stability of the new BFT based on carefully conducted experiments by three independent analysts on 18 dispersants and 2 crude oils.

In keeping with the intent for the procedure to be simple, inexpensive, and straightforward, the laboratory test continues to focus on only two oils (a light and a medium crude oil) rather than a broader array of oil types, only one mixing energy level, one settling time, one dispersant-to-water ratio, and one oil-to-water ratio. No attempts have been made to match or calibrate laboratory test conditions with field conditions such as mixing energy or weathering processes (except for the dispersant-to-oil ratio), although research is currently underway to do so. These constraints may limit somewhat broad extrapolation of lab test results to real-world expectations. However, the operating conditions do provide a sufficient rationale to screen candidate dispersants and should provide an overall basis to compare expectations about a dispersant's effectiveness without quantitatively predicting efficiency under real world conditions.

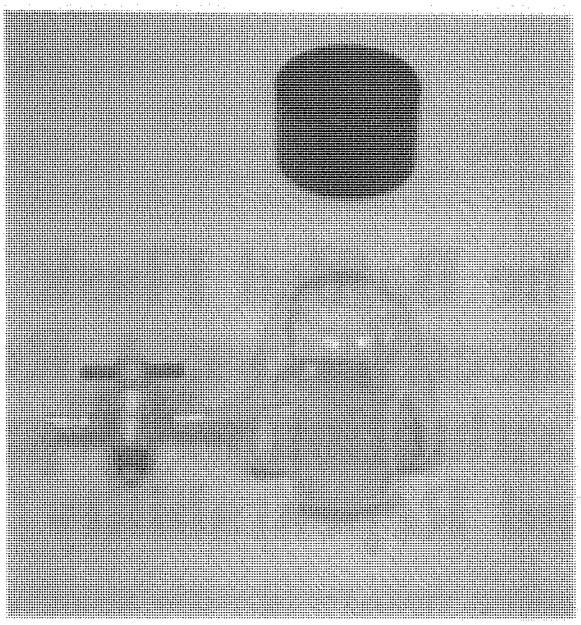


Fig. 1 Photograph of the modified baffled flask showing the stopcock at the bottom.

Although results from the previous research were convincing in their own right, EPA decided that, since Subpart J of the NCP had to be modified to incorporate the new test as a replacement for the SFT, it was appropriate to study the reproducibility and repeatability of the BFT method in even greater detail to fully justify the adoption of a new Agency standard protocol. A round robin collaborative study was initiated in which several independent laboratories were contracted to conduct the new BFT on a selected subset of the 18 dispersants previously tested. Six laboratories agreed to conduct the analysis. To increase the power of the test, the data from the three analysts who conducted the previous research (Sorial *et al.*, submitted for publication) were included in the final database, giving a total of nine independent analysts. The six additional participants in the collaborative investigation were Benchmark Analytics (Center Valley, PA), Bioaquatic Testing (Carollton, TX), Department of Fisheries and Oceans—Canada (Mont-Joli, Quebec), Louisiana State University (Baton Rouge, LA), Payne Environmental (San Diego, CA), and the University of Cincinnati (Cincinnati, OH). The University of Cincinnati analyst in the round robin study was not the same as the previous three. Six of the 18 dispersants were selected for testing

ED_001324_00001448-00002

in the round robin experiments. To determine if the BFT was robust over a wide range of dispersant effectiveness levels, the six dispersants were selected based on high (>80%), medium (30–79%), and low (<30%) effectiveness findings. Two dispersants from each level were chosen. This paper discusses the results from the collaborative study and presents a rationale for developing a decision rule for Agency adoption in screening effective products from non-effective ones for use in oil spills in US navigable marine waters.

Materials and Methods

The baffled flasks, synthetic seawater, crude oils, and dispersants were all provided to each laboratory by EPA so that all analysts used exactly the same materials.

Dispersant products

The 18 original dispersants used in the previous investigation had been randomly assigned letter designations ranging from A through R (Sorial *et al.*, 2001b). To be consistent with the latter paper, the same letter designations for the selected dispersants for this paper will be used. Thus, dispersants A, D, E, F, G, and H were used in the round robin study. The dispersants ranked high (>80% effective) were A and E, the intermediate ranked products (30–80% effective) were D and F, and the low ranked ones (<30% effective) were G and H. The names of the products will remain anonymous relative to the letter designations assigned to them. For a list of the 18 dispersants that were studied in the previous paper, the reader is referred to that paper (Sorial *et al.*, 2001b).

Crude oils and synthetic seawater

Two EPA standard reference oils, Prudhoe Bay crude (PBC) and South Louisiana crude (SLC), were used (US EPA, 1996) as the test oils. These oils were chosen not only because they represent a light to medium range of crude oils, but also because they are standard reference oils that are available on storage in Laramie, Wyoming, free of charge for use by anyone who wants his treatment product tested under the precise conditions specified in the method. The synthetic seawater "Instant Ocean", manufactured by Aquarium Systems of Mentor OH, was prepared by dissolving 34 g of the salt mixture in 1 l of deionized water (i.e., a salinity of 34 ppt). Table 1 provides a list of the ion composition of the sea salt mixture. Following the preparation, the saltwater solution was allowed to equilibrate to the ambient temperature of the laboratory (approximately 23 ± 3 °C).

Table 1 Ingredients of instant ocean synthetic seawater used in the round robin test

Major ion	Total weight (%)	Ionic concentration at 34 ppt salinity (mg/l)
Chloride	47.5	18 700
Sodium	26.3	10 400
Sulfate	6.6	2600
Magnesium	3.2	1200
Calcium	1.0	400
Potassium	1.0	400
Bicarbonate	0.5	200
Boron	0.015	6
Strontium	0.001	8
Solids total	86.1	34 000
Water	13.9	
Total	100.0	

Oil extraction and analysis

The solvent dichloromethane (DCM, pesticide quality) was used for extractions of oil–water samples and all experimental water samples. The test flasks used in the experiments consisted of 150 ml baffled trypsinizing flasks fitted with a glass stopcock (2 mm plug bore) near the bottom of the flask. An orbital shaker (Lab-Line Instruments, Inc., Melrose Park, IL) with variable speed up to 400 rpm and an orbital diameter of 0.75 in. was used to mix the oil/water/dispersant combinations. A Brinkmann Eppendorf repeater pipettor (or equivalent) capable of dispensing 1 µl to 5 ml was used for dispensing the required amounts of the oil and the dispersant. Dispersed oil was measured with UV–VIS spectrophotometers capable of measuring absorbence at 340, 370, and 400 nm (these were the wavelengths used in the original SFT protocol (Fingas *et al.*, 1987)). Standard silica 10-mm path length rectangular cells with PTFE cover were used having a transmittance of >80% over the wavelength range of not greater than 230 nm at the low end of the spectrum to at least 2500 nm at the high end of the spectrum.

Each independent analyst conducted the following procedure separately in his own laboratory. The BFT required four replicate flasks for each product. The test was repeated for each dispersant using two types of crude oil, SLC and PBC.

Oil standards procedure

A stock solution of dispersant–oil mixture in DCM was prepared by adding 800 µl of the dispersant to 2 ml of the oil, and then 18 ml of DCM was added. Determinations of stock solution concentrations were based on the mass measurements after each addition. For generating a six-point calibration curve, a specific volume of the stock standard solution was added

ED_001324_00001448-00003

to 30 ml synthetic seawater in a 125 ml separatory funnel. Liquid/liquid extractions of samples were then performed three times by using 5 ml of DCM for each extraction and adjusting the final extract to 20 ml. The final extract was then transferred to 50 ml crimp style glass vials with aluminum/Teflon seals and stored at 5 °C until the time of analysis. For SLC, the six calibration concentrations were 0.09, 0.2, 0.4, 0.7, 0.9, and 1.3 g/l. For PBC, the six calibration concentrations were 0.05, 0.09, 0.25, 0.5, 0.6, and 0.7 g/l.

Baffled flask test procedure

First, 120 ml of synthetic seawater was added to the baffled flask, followed sequentially by addition of crude oil and finally by the dispersant. A volume of 100 µl of oil was carefully dispensed directly onto the surface of the synthetic seawater using an Eppendorf repeater pipettor with a 5 ml syringe tip attachment. The dispersant was then dispensed onto the center of the oil slick by using a 100-µl syringe tip attachment set to dispense 4 µl, giving a ratio of dispersant-to-oil of 1:25. Care was taken to make certain the dispersant contacted the oil without first touching the water. The flask was then placed on the orbital shaker and mixed for 10 min at a rotation speed of 200 rpm. At the end of the shaking period, the flask was removed from the shaker and allowed to remain stationary on the bench top for 10 min. At the conclusion of the settling time, the first 2 ml of sample was drained from the stopcock and discarded, and then 30 ml of sample was collected in a 50 ml graduated cylinder. The 30 ml sample was then transferred to a 125 ml separatory funnel and extracted three times with 5 ml fresh DCM. The extract was then adjusted to a final volume of 20 ml and transferred to a 50 ml crimp style glass vial with an aluminum/Teflon seal. The vials were stored at 5 °C until the time of analysis, and the holding time never exceeded 10 days. Each of the four replicates was done separately so that shaking and settling times were exactly the same for all. In addition to the four replicate product/oil/seawater mixtures, four replicate oil/seawater mixtures (no dispersant) and four replicate method blanks (seawater alone) were also run for quality control purposes. The average method blank % effectiveness results were similar for all analysts (approximately 3%) in the interlaboratory study; thus, the results were not included in the within-lab and among-lab statistical analysis, since such results would not affect ultimate pass–fail decision-making.

Analysis of extracts

Extracts were analyzed for absorbence at three different wavelengths: 340, 370, and 400 nm. For non-scanning spectrophotometers, the area under the absorbence vs. wavelength curve between wavelengths 340 and 400 nm was determined by applying the trapezoidal rule according to the following equation:

$$\text{Area} = \frac{(\text{Abs}_{340} + \text{Abs}_{370}) \times 30}{2} + \frac{(\text{Abs}_{370} + \text{Abs}_{400}) \times 30}{2} \quad (1)$$

For scanning spectrophotometers, the instrument was programmed to automatically integrate between 340 and 400 nm. The analyst precision objectives were determined by using the relative standard deviation (RSD) for % dispersant effectiveness based on four replicate flasks. The acceptance criterion was based upon the RSD being less than 15%, a level that was considered reasonable based on previous experience and research during the developmental research.:

$$\text{RSD} = \frac{\text{standard deviation of four replicates}}{\text{average concentration}} \times 100 \quad (2)$$

If the RSD was greater than 15%, and one of the four replicates appeared to be an outlier, this replicate was flagged and one more replicate was run for this specific oil/dispersant test. However, if the replicates seemed clustered into two groups and the RSD was greater than 15%, the four replicates for this specific oil/dispersant test were rerun and the original data flagged. If a situation occurred where the acceptance criterion was not met for the third trial, the laboratory was required to stop, report all results, and indicate that the acceptance criterion was never met. Fortunately, this did not occur.

Calculation of reproducibility and repeatability

The purpose of a round robin collaborative evaluation of a method is to enable a regulatory group to formulate a precision statement about a method. The precision of a method is a function of two types of variability: the repeatability variability and the reproducibility variability. The repeatability variability represents the within lab variability, i.e., the variability of the method when carried out on identical samples in the same laboratory with the same operator. However, since 15% was arbitrarily chosen as the basis to identify outliers, the repeatability estimate has a bias because it is based on centralized rather than raw data. Commercial labs that conduct this protocol will be instructed not to eliminate any outliers. EPA will do the outlier tests. The reproducibility variance represents the sum of within-lab and among-lab variabili-

ED_001324_00001448-00004

ties, i.e., the variability of the method when conducted on identical samples in two (or more) labs.

The goal of this collaborative study was to estimate the repeatability and reproducibility standard deviations and the corresponding repeatability and reproducibility coefficients for the laboratory method to be used to obtain dispersant efficiency measurements from various oil dispersant products in various testing labs. To obtain the repeatability and reproducibility parameters, kn, identical samples were prepared for each of six dispersant products and two types of oils ($6 \times 2 = 12$ product/oil combinations). For any particular product/oil sample set, $k = 4$ replicate samples were set up by each of the $n = 9$ labs for analysis.

Let σ_r^2 represent the theoretical repeatability variance and let σ_R^2 represent the theoretical reproducibility variance. Using data obtained in the collaborative study, unbiased estimates of the repeatability and reproducibility variances are given by

$$\hat{\sigma}_r^2 = \mathrm{MSE} \tag{3}$$

$$\hat{\sigma}_R^2 = \frac{(\mathrm{MSLabs} + (k-1)\mathrm{MSE})}{k} \tag{4}$$

where MSE and MSLabs are the mean square error and among-laboratory mean squares, respectively, from a one-way ANOVA on the collaborative study data with labs as the factor effect included in the linear model. The repeatability and reproducibility standard deviation estimates are the square roots of Eqs. (3) and (4), respectively. The right side of Eq. (4) is an estimator of reproducibility given by Satterthwaite (1946) and discussed by Burdick and Sielken (1978). Although this is not the traditional estimator of reproducibility from the expected mean squares in an ANOVA, it is consistent with laboratory testing group definitions and provides an estimator that takes into account both between-lab variability and within-lab variability that affects interlaboratory differences. From Fleiss (1986), a $(1-\alpha) \times 100\%$ confidence interval on the repeatability standard deviation is given by

$$\mathrm{LCL} = \sqrt{\frac{k(n-1)\mathrm{MSE}}{\chi^2_{k(n-1),1-(\alpha/2)}}} \tag{5}$$

$$\mathrm{UCL} = \sqrt{\frac{k(n-1)\mathrm{MSE}}{\chi^2_{k(n-1),\alpha/2}}} \tag{6}$$

where LCL is the lower confidence limit, UCL is the upper confidence limit, and $\chi^2_{(\cdot)}$ is the %age point of a Chi-square distribution with $k(n-1)$ degrees of freedom (df). From Satterthwaite (1946), an approximate $(1-\alpha) \times 100\%$ confidence interval on the reproducibility standard deviation is given by

$$\mathrm{LCL} = \sqrt{\frac{\nu^* \widehat{L}}{\chi^2_{\nu^*,1-(\alpha/2)}}} \tag{7}$$

$$\mathrm{UCL} = \sqrt{\frac{\nu^* \widehat{L}}{\chi^2_{\nu^*,\alpha/2}}} \tag{8}$$

where $\widehat{L} = (\mathrm{MSLabs} + (k-1)\mathrm{MSE})/k$,

$$\nu^* = \frac{(\mathrm{MSLabs} + (k-1)\mathrm{MSE})^2}{\frac{\mathrm{MSLabs}^2}{k-1} + \frac{(k-1)^2\mathrm{MSE}^2}{k(n-1)}} \tag{9}$$

and $\chi^2_{(\cdot)}$ is the %age point of the Chi-square distribution with ν^* degrees of freedom.

The sample size calculations for the collaborative study were based on choosing n and k such that the width W of a $(1-\alpha) \times 100\%$ confidence interval around the true reproducibility standard deviation was below a certain bound d, which was arbitrarily chosen to be 2.5%. The width of the confidence interval, W, defined as UCL–LCL, decreases as a function of n and k.

Repeatability and reproducibility coefficients

Mandel and Lashof (1987) define what are known as repeatability and reproducibility coefficients as

$$r = 2\sqrt{2} \cdot \sigma_r \tag{10}$$

$$R = 2\sqrt{2} \cdot \sigma_R \tag{11}$$

The repeatability coefficient, r, is defined as a quantity such that the probability is 95% that two test results in the same lab (on the same material) will not differ by more than r. The reproducibility coefficient, R, is defined as a quantity such that the probability is 95% that two test results in different labs will not differ by more than R. These coefficients are calculated from the repeatability and reproducibility standard deviation values and give a more directly applicable representation of the results of the collaborative experiment.

Results

Selection of the dispersants for the interlaboratory study

Data from the previous paper (Sorial *et al.*, 2001) showing the average % dispersant effectiveness data pooled with respect to both crude oils are summarized in Fig. 2 for all 18 dispersants tested. Error bars

ED_001324_00001448-00005

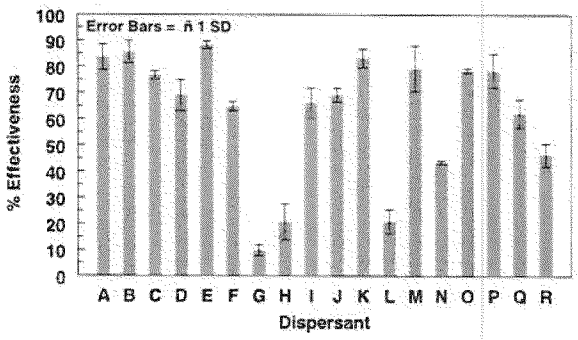


Fig. 2 Effectiveness of 18 dispersants averaged over both oils. Error bars = ±1 SD unit (Sorial et al., 2001b).

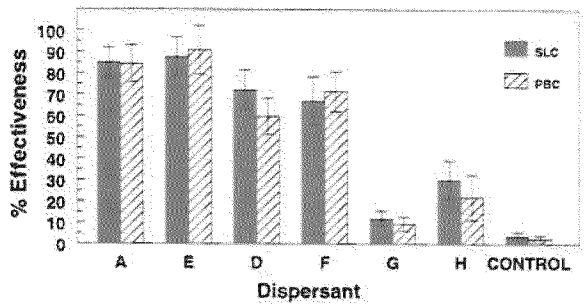


Fig. 3 Comparison of the dispersability of each crude oil by the round robin dispersants averaged over all analysts.

represent ±1 standard deviation unit. To evaluate the robustness of the BFT over a range of dispersant effectiveness, dispersants were selected for the round robin interlaboratory study based upon their ability to disperse oil into the water column. A total of six dispersants were selected: two at % effectiveness levels >80%, two at 30–80%, and two at <30%, representing good, intermediate, and poor dispersants, respectively. The dispersants selected were A and E (good), D and F (intermediate), and G and H (poor).

Differences between crude oils

To determine if the data from both oils could be pooled for purposes of reporting results of the round robin (and also for the final decision rule), it was necessary to compare % dispersant effectiveness for each of the oils for all analysts. Figure 3 summarizes the results of the comparison. Each bar is the mean of 36 data points (nine analysts, four replicates). The error bars represent ±1 standard deviation unit. Results were statistically equivalent ($p > 0.05$) for both oils regardless of the effectiveness of the dispersants. Thus, the data for both crude oils were pooled for all subsequent analyses. The k value originally defined as four replicates per oil type is now redefined as eight for the pooled oil types.

Agreement among analysts

Figure 4 displays how well the nine analysts agreed with respect to overall mean % effectiveness of each of the dispersants. The two highly effective dispersants appear in the upper two sectors of the figure, the intermediate dispersants in the middle sectors, and the poor dispersants in the lower sectors. Each bar in each sector is the mean of 8 points (four replicates per oil type), and error bars represent ±1 standard deviation unit for the eight replicates. The minimum to maximum % effectiveness for Dispersant A ranged from 80 to 90, for Dispersant E from 83 to 96, for Dispersant D from 56 to 75, for Dispersant F from 58 to 82, for Dispersant G from 6 to 13, and for Dispersant H from

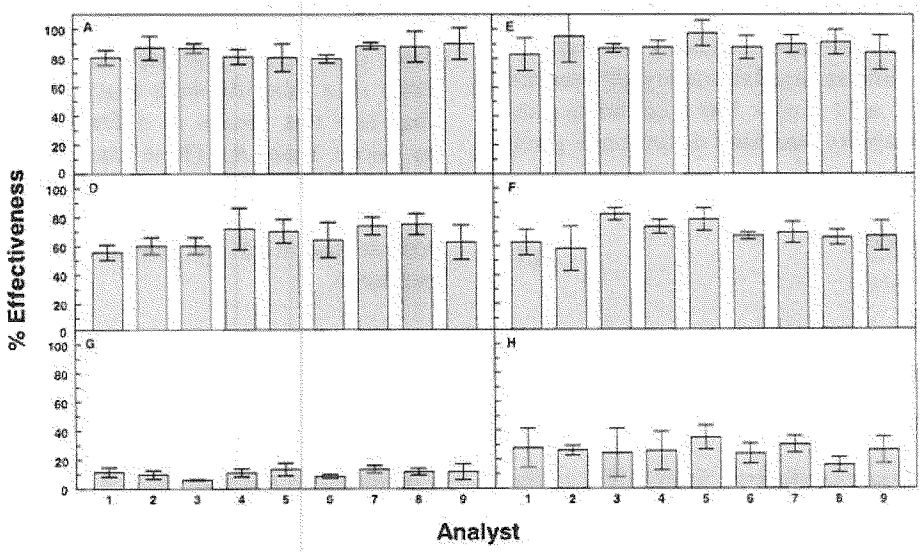


Fig. 4 Effectiveness of dispersants A, E, D, F, G, and H averaged over both crude oils as measured by each analyst.

ED_001324_00001448-00006

16 to 35. Regardless of how effective the dispersant products were, the error bars overlapped among the nine analysts, and there were no significant differences among analysts ($p > 0.05$) regardless of dispersant.

Repeatability and reproducibility

The values for repeatability and reproducibility computed from the interlaboratory study are shown in Fig. 5. Each bar in Fig. 5 is the mean of 72 measurements (nine analysts, eight replicate analyses for each dispersant). Two error bars are shown associated with the means. One is the repeatability standard deviation (SD_r), and the other is the reproducibility standard deviation (SD_R). Normally, the SD_r is lower than the SD_R, but in this case both standard deviations were essentially the same. Thus, all of the error associated with the BFT is inherent in the method itself rather than due to differences among independent analysts. The variability is deemed acceptable for purposes of screening effective from ineffective dispersant products in the laboratory.

The repeatability and reproducibility factors, which represent the maximum expected differences between any two measurements by the same analyst [r] or regardless of analyst [R], are summarized in Table 2. The within-lab variability [r] was close to the between-plus within-lab variability [R], again signifying that all of the error in the protocol is inherent rather than influenced by differences among analysts. A repeatability factor of 18% means that the probability is 95% that the maximum expected difference between any two measurements by one analyst for a particular dispersant is ±9%.

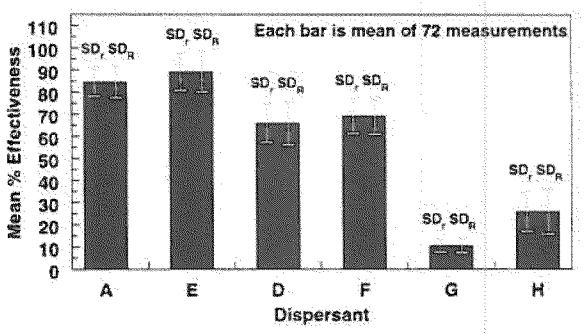


Fig. 5 Repeatability and reproducibility standard deviations from the round robin test.

Table 2 Maximum expected differences in repeatability and reproducibility between measurements

Dispersant	Repeatability factor, r (%)	Reproducibility factor, R (%)
A	18	20
E	25	28
D	24	26
F	23	24
G	8	9
H	25	29
Control	4	3

Establishing a pass–fail decision rule

The BFT has been demonstrated to be a reasonably repeatable and reproducible protocol that establishes mixing conditions that are presumed to be much more reflective of moderately energetic environments where dispersants will be used. Further research is planned to establish a correlation between energy dissipation in the baffled flask vs. energy dissipation at different sea states. It also utilizes more realistic dispersant-to-oil ratios, and the application sequence of dispersant onto the oil slick (rather than premixing the dispersant with the oil) is more typical of that which occurs in an actual open water spill response (Sorial *et al.*, submitted for publication, submitted for publication). It is now important to develop a proper decision rule to enable selection of the most effective dispersant products tested with the BFT. Naturally, the BFT has its limitations since it only tests two types of crude oils and is conducted at only one temperature and one mixing condition, so it cannot serve as the technical basis for making dispersant decisions over a broader range of oil types and environmental conditions. It is merely a laboratory tool to serve as a screening method to test dispersant effectiveness under standardized laboratory conditions, which is only what the NCP product schedule is designed to do.

The decision rule established for the original SFT was that a product must demonstrate an ability to disperse PBC and SLC by an average of 50 ± 5%. Such a low mean % effectiveness value reflects the inadequacy of the SFT itself, and allowing a product to be listed on the NCP product schedule that is no better than 45% effective in dispersing oil in a non-representative lab test is not a prudent way of approving dispersant products for listing on the NCP product schedule. Sorial *et al.* (2001b) reported that the overall mean % effectiveness of the SFT for the 18 dispersants tested was only 19.7%, and the root mean square error (RMSE) was 4.3%, yielding a very high coefficient of variation (CV) of 21.7%. The CV for the BFT was only 7.8% (overall mean = 64.6%, RMSE = 5.04%). A good pass–fail criterion must have a target acceptance value that properly differentiates among effective and mediocre products while still accounting for experimental error. A reasonable approach to establishing a realistic target value while accounting for measurement error is the use of a one-tailed lower 95% confidence level (LCL) to be greater than a given % effectiveness value.

ED_001324_00001448-00007

The measurement error is defined simply to be the standard deviation of the eight replicate measurements made using both crude oils (since we are pooling the data from both crude oils, as concluded earlier). The 95% LCL of the mean of eight replicate measurements is then calculated using the formula:

$$LCL_{95} = \overline{X} - t_{95} \times \frac{(SD)}{\sqrt{k}} \tag{12}$$

where $\overline{X}$ is the average of eight replicate measurements, t_{95} is the 95th percentile t-statistic with 7 df ($t_{0.95,7} = 1.895$), SD is the standard deviation of the eight measurements, and $k = 8$ is the replicate measurements.

When the LCL_{95} is calculated, it is then set higher than a specified threshold % effectiveness level. When a vendor contracts with a laboratory to test its product using the BFT, Eq. (12) is used to determine if the product passed the test. The standard deviation is computed from the pooled oil data, and the LCL_{95} is calculated using $k = 8$. The LCL_{95} is reported to EPA, and the number is compared to the threshold value established for pass–fail to determine if the product gets listed on the NCP product schedule.

Probability of passing based on the interlaboratory study

The overall average reproducibility standard deviation from the six-dispersant, nine-laboratory round robin study was calculated from the individual SD_R for each dispersant (which are graphed as error bars in Fig. 5). The average SD_R was 6.4%. This quantity was used in calculating the probability of a round robin product passing the test for a given mean % effectiveness level. The equation used to compute the probability is (Sorial *et al.*, 2001b):

$$P_\mu\left(\overline{X} - t_{k-1,1-\alpha} \times \frac{s}{\sqrt{k}} > Y\right) \tag{13}$$

where $t_{k-1,1-\alpha}$ is the $100(1-\alpha)$th percentile of the t-distribution with $k-1$ df, $\overline{X}$ is the mean of eight replicate measurements, s is the standard deviation of 6.4%, and Y is the % effectiveness level that must be exceeded to pass the test.

This probability is difficult to compute theoretically, since both $\overline{X}$ and s are random variables, but it can be approximated by simulation. For a given true mean % effectiveness, μ, and the standard deviation derived above, 1000 sets of eight random samples from a normal distribution with true mean μ and standard deviation s were generated. For each set of samples, a mean $\overline{X}$ and standard deviation s was calculated, and the decision rule (Eq. (13)) was checked. The estimated probability of passing for a product with a true mean % effectiveness of μ is

$$P_\mu = \frac{\text{number of sets of samples passed by the decision rule}}{1000} \tag{14}$$

This procedure was repeated for a range of Y values (65%, 70%, and 75%). Probability plots were then generated to determine the probability that a product would pass the protocol for an established rule (such as an LCL_{95} of the mean of k trials being greater than a certain % effectiveness cutoff value). Ideally, such a probability plot should be 0 up to the cutoff value, then rise to 100% at the cutoff value, and stay at 100% beyond that. Anything less than the cutoff value is eliminated, and everything greater is passed. In practice, however, the plot assumes an S-shaped curve. The steeper the curve, the closer it is to ideal. Figure 6 summarizes the simulated probability of the LCL_{95} of a dispersant product effectiveness being above 65%, 70%, or 75% for eight replicates using a standard deviation of 6.4. To achieve a 90% probability of producing an LCL_{95} greater than 65%, 70%, or 75%, a product must actually produce a mean of 74%, 79%, or 84%, respectively. The probability calculations are highly dependent on the variance of the data. If the measured standard deviation by the performing laboratory is higher than 6.4, the probability curve will be flatter, and the true mean of the data will have to be even greater for the product to pass the cutoff effectiveness criterion.

Clearly, the higher the threshold decision value is set, the more difficult it is for a product to pass it since its true mean must be high enough to overcome the effect of variability. However, if a product does pass at the higher decision level, the product will more likely be a truly effective dispersant. Of course, the corollary to this is that the lower the threshold is set, the better

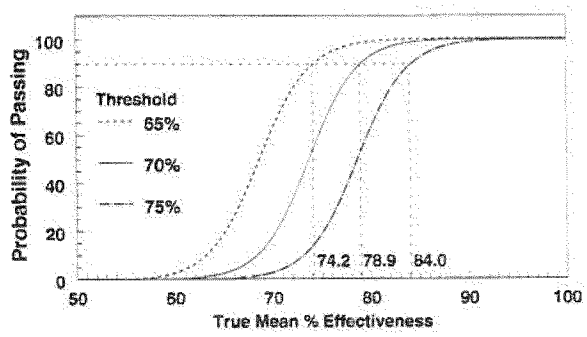


Fig. 6 Probability of a 95% LCL of a dispersant product passing various decision thresholds at various true mean % effectiveness levels.

ED_001324_00001448-00008

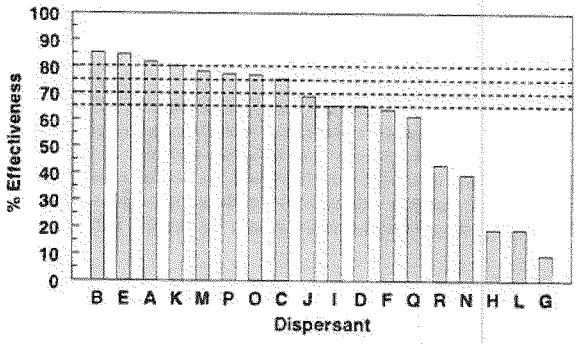


Fig. 7 Ability of the 95% lower confidence level (LCL_{95}) of the original 18 dispersants to achieve various threshold % effectiveness values (each bar is the mean LCL_{95} of the three analysts). The dispersants are arrayed in decreasing order to facilitate viewing.

chance a mediocre or ineffective product will pass. For example, at the same thresholds of 65%, 70%, and 75%, there is a 50% chance that a dispersant would pass such a decision rule if its mean effectiveness level were 69%, 74%, 79%, respectively.

Applying this same reasoning for the 18-dispersant, 2-oil, 3-analyst experiment of Sorial *et al.* (2001b), the LCL_{95} for each dispersant averaged over the three analysts (using the average standard deviation calculated for each of the three dispersant tests) was plotted to determine how many products would pass the various % effectiveness thresholds with those analysts. The data are summarized in Fig. 7. At the 80% threshold, only 3 of the 18 products would pass. At 75%, 7 of the 18 would pass. At 70%, 8 out of 18 would pass, and at 65%, 9 of the 18 would pass. By way of example, using the 70% threshold as the decision rule, the LCL_{95} for Dispersant C, which was the last product in the decreasing array of products that passed the threshold, was 74%. From Fig. 6, the probability that a product with an LCL_{95} of 74% passing a decision rule of 70% is only 45%. However, the reason why it would have passed at such a low probability was that its standard deviation was only 3.7% (data not shown), which was much lower than the 6.4% SD_R for the round robin experiment from which the probabilities were calculated. Thus, if the variability of the eight replicates can be controlled carefully, the chances of a product passing the protocol can be increased.

From the foregoing, setting the pass–fail threshold at 80% appears to be too restrictive, while establishing it at 65% may allow too many products to be listed. Somewhere between 65% and 80% seems to be a prudent threshold level to establish that would allow the listing of effective dispersants while not being overly constraining on the market. It will be the responsibility of EPA Headquarters to make the final decision.

Positive control

In addition to conducting the BFT for purposes of listing a product on the NCP product schedule, it is prudent and appropriate to set up a positive control. The purpose of the positive control is to establish a reproducible standard that must be achieved to qualify a laboratory before its data can be used for passing or failing a product. This will ensure that a laboratory used the method consistent with those participating in the round robin experiment. Either Dispersant A or E could serve equally well for that purpose. When the BFT is conducted on a proposed product, Dispersant A or E would also be run. If the LCL_{95} for the positive control is found to be greater than the threshold EPA decides upon, the laboratory would be qualified, and the data reported for the product proposed to be listed would then be acceptable. If the LCL_{95} for the positive control falls below the required cutoff, the laboratory would have to repeat the test until the correct answer for the positive control is obtained. Again, EPA will make the final decision on what level should be established for the positive control. With respect to the negative control (i.e., oil with no dispersant), since all analysts obtained very similar results (~3% effectiveness), and since the negative control is not used in decision making, running the negative control will be excluded from the BFT for laboratories having to perform a product test.

Discussion

Dispersants are composed of surfactants and solvents. The surfactants reduce the interfacial tension of the water and promote the break-up and dispersion of the slick into fine droplets. Energy in the form of wave action is required to mix the dispersed oil droplets into the water column (Scholz *et al.*, 1999). Without adequate mixing, ineffective dispersion takes place. When a laboratory protocol is designed to test the ability of a dispersant to disperse oil into the water column, it should provide enough energy to allow the dispersant to react properly.

The original SFT was found to give widely varying results in the hands of different testing laboratories (IT Corporation, 1995). Sorial *et al.* (2001a) identified the various factors having an impact on the high variance of the SFT used in screening dispersant products for effectiveness in dispersing oil in water. One of the most important factors was the mixing regime, which was most heavily influenced by the design of the test flask. When a small Erlenmeyer flask is rotated on an orbital shaker at 150 rpm, very little mixing occurs. Since dispersants work best when moderate mixing takes place, it was important to redesign the test flask to

ED_001324_00001448-00009

impart greater mixing energy more characteristic of the over-and-under type of wave action that occurs on the open sea. The simplest way to accomplish that was to use a flask with baffles. The resulting protocol gave quantitatively more realistic mixing conditions and consequently more reproducible results. When the BFT was tested independently by nine different analysts (laboratories) in a round robin interlaboratory experiment, it was found that the BFT gave much better reproducibility and repeatability than the SFT ever before experienced.

The repeatability error (within-lab error) comprised most of the experimental error, and the resulting decision rule has to take into account this source of error. Use of the LCL_{95} when reporting results automatically accounts both for the repeatability error and the inherent error of the method quantifying a product's ability to disperse oil. Thus, when a lab has been contracted to perform a BFT on a product and has to report results in terms of the LCL_{95} being greater than a certain threshold value, the higher the error, the less probable will the product pass the decision rule. It is important to make certain that the protocol is conducted with stringent quality control in order to minimize experimental error. This should not be that difficult, however, since care was exercised in designing the test flask with a stopcock at the bottom to minimize re-suspension of the dispersed oil through manual handling after the settling period. This was one of the problems with the SFT (i.e., manually pouring off the solution often resulted in re-mixing and re-suspending dispersed oil, giving falsely high and uncontrollable readings in the replicates (Sorial *et al.*, 2001a,b)).

Establishing the final decision rule in the manner described in this paper (using the LCL_{95} being greater than a specified % effectiveness value) takes into account inherent error as well as measurement error at the time of the test. Minimizing such error will permit a greater likelihood for a product to be listed on the NCP product schedule. Even with superior quality control, a poor product is unlikely to pass if the decision rule is established high enough to overcome the low probability of passing due to the relative flatness of the probability curve.

Acknowledgements—This project was funded by the US Environmental Protection Agency's National Risk Management Research Laboratory. The findings and conclusions expressed in this publication are solely those of the authors and do not necessarily reflect the views of the Agency. Mention of trade names and commercial products does not constitute endorsement by the US EPA.

References

Burdick, R.K., Sielken, R.L., 1978. Exact confidence intervals for linear combinations of variance components in nested classifications. J. Amer. Stat. Assoc. 73 (363), 632–635.

Fingas, M.F., Hughes, K.A., Schweitzer, M.A., 1987. Dispersant testing at the environmental emergencies technology division. In: Proceedings of the Tenth Arctic Marine Oilspill Program Technical Seminar, 9–11 June, 1987, Edmonton, Alberta, Canada, Conservation and Protection, Environment Canada, pp. 343–356.

Fingas, M.F., Dufort, V.M., Hughes, K.A., Bobra, M.A., Duggan, L.V., 1989a. Laboratory studies on oil spill dispersants. In: Flaherty, L.M. (Ed.), Oil Spill Dispersants: New Ecological Approaches ASTM STP1018, American Society for Testing and Materials, Philadelphia, PA, pp. 207–219.

Fingas, M.F., Munn, D.L., White, B., Stoodley, R.G., Crerar, I.D., 1989b. Laboratory testing of dispersant effectiveness: the importance of oil-to-water ratio and settling time. In: Proceedings of the 1989 Oil Spill Conference, 13–16 February, 1989, San Antonio, TX, American Petroleum Institute, Washington, DC, pp. 365–373.

Fleiss, J.L., 1986. The Design and Analysis of Clinical Experiments, John Wiley & Sons, New York.

IT Corporation, 1995. Investigation of reported problems with swirling flask dispersant effectiveness test—Final Report, Contract no. 68-C2-0108, Work Assignment no. 3-53, JTN 816453, US Environmental Protection Agency, Edison, NJ 08837.

Mandel, J., Lashof, T.W., 1987. The nature of repeatability and reproducibility. J. Quality Technol. 19 (1), 29–36.

Satterthwaite, F.E., 1946. An approximate distribution of estimates of variance components. Biometrics 2, 110–114.

Scholz, D.K., Kucklick, J.H., Pond, R., Walker, A.H., Bostrom, A., Fischbeck, P., 1999. A Decision-Maker's Guide to Dispersants. A Review of the Theory and Operational Requirements. American Petroleum Institute, Washington, DC.

Sorial, G.A., Venosa, A.D., Koran, K.M., Holder, E., King, D.W., submitted for publication. Oil spill dispersant effectiveness protocol: impact of operational variables. J. Environ. Eng., ASCE.

Sorial, G.A., Venosa, A.D., Koran, K.M., Holder, E., King, D.W., submitted for publication. Oil spill dispersant effectiveness protocol: performance of the revised protocol. J. Environ. Eng., ASCE.

US Environmental Protection Agency, 1996. Swirling Flask Dispersant Effectiveness Test. Title 40 Code of Federal Regulations, Pt. 300, Appendix C, 1996 ed., pp. 245–250.

ED_001324_00001448-00010

To: Holder, Edith[holder.edith@epa.gov]
From: Conmy, Robyn
Sent: Fri 4/17/2015 1:44:33 PM
Subject: RE: Accell Clean DWD

Odd that they don't have any in stock. We currently don't have any in the lab correct?

We have 3 birthday parties, soccer, and piano this weekend. Sigh. Weekends are no longer restful….

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

From: Holder, Edith
Sent: Friday, April 17, 2015 9:41 AM
To: Conmy, Robyn
Cc: Bryan, Elisha
Subject: FW: Accell Clean DWD

Do we want this if it is not part of a production lot?

Edith Holder

Pegasus Technical Services, Inc.

On-Site Contractor to the U.S. EPA

ORD/NRMRL/LRPCD

26 W. Martin Luther King Dr.

Cincinnati, OH 45268

Phone: 513-569-7178

Email: holder.edith@epa.gov

From: Bryan, Elisha
Sent: Friday, April 17, 2015 8:23 AM
To: Holder, Edith
Subject: Re: Accell Clean DWD

Hi Edie,

The ZI-400 company contacted me. They were wondering on when you need it by because they will have to make that small of a quantity up in the lab.

Any plans for this weekend? It looks like the weather is going to be really nice. We might go to Earth Day at Sawyer Point.

Thanks,

Elisha Bryan

Pegasus Technical Services, Inc.

On-Site Contractor to the U.S. EPA

ORD/NRMRL/LRPCD

26 W. Martin Luther King Dr.

Cincinnati, OH 45268

Phone: 513-965-4805

Email: bryan.elisha@epa.gov

From: Holder, Edith
Sent: Thursday, April 16, 2015 4:34 PM
To: Bryan, Elisha
Subject: RE: Accell Clean DWD

Thank you for following up on this.

Edith Holder

Pegasus Technical Services, Inc.

On-Site Contractor to the U.S. EPA

ORD/NRMRL/LRPCD

26 W. Martin Luther King Dr.

Cincinnati, OH 45268

Phone: 513-569-7178

Email: holder.edith@epa.gov

From: Bryan, Elisha
Sent: Thursday, April 16, 2015 3:50 PM
To: Holder, Edith
Subject: Re: Accell Clean DWD

Hi Edie,

No I have not heard from the manufacturers. I did try different email addresses for both of those companies last Friday. Maybe if I do not here from them by tomorrow I will give them a call. I just prefer email since it is a lot easier to track.

Elisha Bryan

Pegasus Technical Services, Inc.

On-Site Contractor to the U.S. EPA

ORD/NRMRL/LRPCD

26 W. Martin Luther King Dr.

Cincinnati, OH 45268

Phone: 513-965-4805

Email: bryan.elisha@epa.gov

From: Holder, Edith
Sent: Thursday, April 16, 2015 2:53 PM
To: Bryan, Elisha; Conmy, Robyn
Subject: Accell Clean DWD

Greetings,

The above dispersant was received today.

Elisha, Robyn is hoping that we will be able to obtain Saf-Ron Gold and ZI-400. Have you heard anything from their manufacturers?

Thanks,

Edie

Edith Holder

Pegasus Technical Services, Inc.

On-Site Contractor to the U.S. EPA

ORD/NRMRL/LRPCD

26 W. Martin Luther King Dr.

Cincinnati, OH 45268

Phone: 513-569-7178

Email: holder.edith@epa.gov

To: conmy.robyn@gmail.com[conmy.robyn@gmail.com]
From: Conmy, Robyn
Sent: Wed 11/4/2015 7:39:24 PM
Subject: FW: API Dispersant research Review
API Disp Research Rev Interim Report October2015.docx

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>
Robyn N. Conmy, Ph.D.
Research Ecologist
USEPA/NRMRL/LRPCD
26 West MLK Drive
Cincinnati, Ohio 45268
513-569-7090 (office)
513-431-1970 (EPA mobile)
727-692-5333 (Personal mobile)
conmy.robyn@epa.gov

-----Original Message-----
From: Ken Trudel [mailto:ken@slross.com]
Sent: Tuesday, November 03, 2015 11:37 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: API Dispersant research Review

Dear Robin:

As per my earlier email, I have prepared an interim report on the review of recent dispersant research papers. Could I please impose on you to have a look at it. Please have a glance at the explanatory bits early in the report and then look most closely at the sections titled "Problems"
and give me your thoughts. These "problem" papers are the main focus and challenge of the project; the review of the good papers is simply a review. Please send any comments about the treatment.

We are trying to schedule a one-hour meeting for the middle of next week in New Orleans at Clean Gulf.

Regards,

Ken

--
Ken Trudel
SL Ross Environmental Research Ltd.
Tel: (613) 232-1564; Fax: (613) 232-6660
200 - 1140 Morrison Drive, Ottawa, ON
CANADA, K2H 8S9
email: ken@slross.com
web site: http://www.slross.com

IPIECA, OGP and API Review of Newly Published Oil Spill Dispersant Research Papers 2013-15:

INTERIM REPORT: JANUARY 1, 2013 TO OCTOBER 1, 2015

For

American Petroleum Institute

1220 L Street, NW

Washington, DC 20005-4070

CONFIDENTIAL

By

S.L. Ross Environmental Research Limited

1140 Morrison Drive, Suite 200

Ottawa, ON, Canada

October 1, 2015

[Type text]

Acknowledgements

In Preparation

Executive Summary

In Preparation

CONFIDENTIAL

CONFIDENTIAL

Contents

Preface 2
Introduction 2
Overview of Publications 5
Subsea Dispersant Injection (SSDI) 7
Experimental Studies in SSDI 7
SSDI Studies with Problems 8
Fate and Degradation of Dispersed Oil and Dispersants 9
Field Studies During the Macondo Spill 9
Subsurface plumes of oil and dispersant 9
Monitoring for Corexit Dispersants 10
Laboratory Experimental Studies 11
Problems in Studies of the Fate of Dispersed Oil and Dispersants 11
Microbial Degradation 14
Effects of Dispersants, Dispersed Oil and Chemically Dispersed Spills 18
Effects of the Macondo Spill 18
Experimental Toxicology Studies 19
Composition of the Exposure Media 19
Sensitivity to Dispersed Oil 20
Effects of Variables on Toxicity 20
Sublethal Effects of Dispersant and Dispersed Oil 21
Problems in Studies of Effects of Oil and Dispersants 21
Summary of Problems in Effects Research 23
Health 26
Problems with Human Health and Safety Studies 26
Effectiveness 28
Editorial, Policy Etc 28
References 29
Appendix 1 – Bibliography to August 31, 2015 37

CONFIDENTIAL

ED_001324_00001453-00004

Preface

This document is a detailed outline for the report for the American Petroleum Institute project to review recent dispersant research publications. This outline was requested during one of the panel's earlier conference calls. The objective of preparing the document was to prepare a strawman outline for the project report, showing where we were going with the reviews. As you may recall, the objective of the panel was to prepare a document that tells both the good news and bad news about the new dispersant research. The challenge has been to separate the good papers from the bad papers and to prepare a document that discussed each group in a useful way. The main challenge has been to identify the important scientific flaws in the problem papers that lead the authors to incorrect conclusions. After re-reviewing a selection of the papers it appeared that there were two types of problems: a) dispersant specific problems (e.g. using nominal oil concentrations in toxicity studies) and b) basic scientific problems. The document below has sorted the papers into subjects; classified the papers as either problematic or not problematic; developed a classification scheme for some of the problems; and has applied the system to a selection of papers. The problems are discussed in plain language, so please do not circulate this document. Problems will be discussed in a more constructive way in the final project report.

CONFIDENTIAL

Introduction

The Macondo oil spill (April to July 2010) greatly increased awareness of chemical oil spill dispersants among researchers and the public in general. This heightened awareness of dispersants resulted in a rapid increase in dispersant research and an avalanche of research publications. The present project reviewed these new publications in order to relate the new results to the spill response management questions facing spill response planners and to the research practices and standards developed over the years to help address these management questions. To this end a 12-member panel of experienced dispersant researchers was assembled to review recent research. This interim report is a strawman template for the final report for this project. It considers the 98 papers identified for review from January 1, 2013 to April 30, 2014.

Before embarking on any dispersant research study, it is important for researchers to recognize that chemical oil spill dispersant is a tool whose sole purpose is to manage the behaviour of oil spilled at sea in order to minimize its risks to human and environmental health and to speed environmental recovery. Since dispersants were first developed in the 1960s, they have been

ED_001324_00001453-00005

the subject of considerable research and development, which over the years has come to focus narrowly on three management-oriented questions, namely:

a) What do dispersants do to the oil and how effective are they in dissipating surface oil slicks (and oil discharged from subsea blowouts)?
b) What is the fate of the dispersed oil and dispersant?
c) What are the environmental effects of dispersants and dispersed oil?

Because dispersants are first and foremost a tool used in very specific way, research methods have been refined over the years to simulate the very specific field conditions that result from their use (e.g., Coelho et al 2013). This helps to maximize the applicability of research data to field related questions. For example, in studying the toxic effects of mixtures of oil and dispersants at sea, researchers recognized that organisms exposed to dispersed oil would be exposed to an initial spike of high concentrations of toxicant that would quickly decline to background within a matter of hours or days. To simulate this exposure, researchers developed a "spike exposure protocol" to simulate the real-world exposure, (e.g., Singer et al…). The "spike exposure protocol" not only allows workers to extrapolate from research results to predict effects of dispersant use in the field, but it also provides a standard exposure procedure that enables inter-comparison of results in different studies. Dispersant researchers have developed a number of these research protocols. When standard protocols exist for the area of research being undertaken, using the protocols helps in interpreting and applying the results.

CONFIDENTIAL

Prior to Macondo, dispersant planning focused solely on application to surface oil slicks. However, Macondo was a very unusual spill in that it was a subsea blowout. This allowed for a very novel way to deploy dispersants, namely injecting the dispersant subsurface, directly into the discharging oil jet. This gave rise to a series of new research questions concerning the potential behaviour, movement and persistence of the discharged oil, effectiveness of the dispersant and the effects of the dispersed oil.

Recognizing that there was a large volume of material published in recent years, it was decided to limit the review to papers published in peer-reviewed journals, which had already been subjected to scrutiny for scientific rigour. In addition it was decided to limit the review to papers published since 2013. The following report provides:

a) an overview of the papers;
b) a summary of the findings of each publication organized by subject area; and

c) a brief discussion of the problems encountered in some research that may limit the usefulness of their results.

CONFIDENTIAL

ED_001324_00001453-00007

Overview of Publications

The 98 papers considered for review from January 1, 2013 to April 30, 2014 were considered for inclusion in this interim report (See Literature Cited below). These publications have been organized into categories for purposes of this report (Table 1 below), including:

a) subsea dispersant injection;
b) fate and degradation of oil and dispersants;
c) effects of dispersed oil and dispersants;
d) dispersant phenomenology, effectiveness and products;
e) health effects; and
f) editorials.

The vast majority of new publications have addressed the fate and effects of oil and dispersants, so this report focuses more on these papers.

Literature published from April 2014 to present is listed in Appendix 1 – Bibliography.

CONFIDENTIAL

ED_001324_00001453-00008

Table 1 Papers organized by subject (As of April 2014)

SSDI Research	Fate / Degradation of Dispersed Oil and Dispersants		Effects of Dispersants and Dispersed Oil		Effectiveness, Products, Theory	Health	Editorial
SSDI Research	**Macondo**		**Macondo**		**Theory**	Health	Editorial
Brandvik et al. 2013 Johansen et al. 2013 Nedwed and Coolbaugh 2013	Spier et al. 2013 Mendoza et al. 2013 Zhou et al. 2013 Place et al. 2014 Gray et al. 2013	Perkins et al. 2013 Bejarano et al. 2013 Sammarco et a; 2013	Fitzgerald et al. 2013 Ali et al. 2013 Echols et al. 2013 Montagna et al. 2013	Montagna Moody, Paul Ziervogel Bejarano	King et al. 2013 Wang et al. 2013	D'Andrea and Reddy 2013 Afonne et al. 2013	Adams 2013. Paul, I. 2013. Arnold C.2013 Peavler B. 2013. Perrons, R.K..2013. Schultz, C. 2013 Schrope, M. 2013. Stokstad, E. 2013
	Degradation – Experimental		**Lethality**		**New Products**		
	Lee et al. 2013 Prince and Butler 2013 Prince et al.2013 McFarlin et al.2014 Campo et al.2013	Joo et al. 2013 Batchu et al.2014 Macías-Zamora et al.2013 Vilcaez J et al.2013	Adams et al. 2013 Almeda et al. 2013 Anderson et al 2013 Coelho et al. 2013. Rico-Martínez et al. 2013 Gardiner et al. 2013 Goodbody-Gringley et al. 2013	Kuhl, et al. 2013 Lee KW, et al. 2013 Martin et al. 2013 Radniecki, et al. 2013 Ozhan et al. 2013a Ozhan et al. 2013b Powers et al. 2013	Saha et al. 2013		
	Methods/Equipment		**Sublethal Effects**		**Effectiveness**		
	Conmy et al. 2013 Lavallee et al. 2013 Mendoza,W et al. 2013 Zhou et al. 2013 Van Scoy et al. 2013		**Bioaccumulation** Chase et al. 2013 **Enzyme Induction/Metabolites** Milinkovitch et al. 2013	**Gross Abnormality or Histopatology** Agamy 2013 Agamy 2013 **Growth/Reproduction** Brewton et al. 2013 Zhang et al. 2013 Polli et al. 2013	Venosa et al. 2013 Venkataraman et al. 2013		
	Fate Experimental		**Immunosuppresion** Barron et al. 2013				
	Ehrenhauser et al. 2013 Liyana-Arachchi et al. 2013 Gong, et al. 2013	Kuhl et al. 2013 Zhou et al. 2013	**Performance and Fitness** Claireaux, et al. 2013 Milinkovitch et al. 2013				

CONFIDENTIAL

Subsea Dispersant Injection (SSDI)

Subsea dispersant injection had received very little research attention prior to 2010, but proved to be a key response tool during the 2010 Macondo incident. In Macondo, SSDI played an important role in minimizing the amount of oil from the blowout that reached the sea surface, thus protecting the environment and health and safety of response workers. SSDI will clearly be incorporated into spill-response strategies for future offshore drilling operations where appropriate.

Nedwed and Coolbaugh 2014 (no paper) reported on recent industry research initiatives, which address:

a) Effectiveness of subsea injection - Scaled testing is evaluating injection methods and effects of dispersant-to-oil ratios. The effect on oil droplet size of dispersant-to-oil ratio, low-solvent dispersants and dispersant injection method and location are being studied. Preliminary research findings include:
    a. Dispersant was effective at reducing droplet size even with low DOR's;
    b. Injection wands were effective;
    c. Removing solvent from dispersants improves dispersant effectiveness; amd
    d. Injecting dispersant more than 5 pipe diameters above discharge point was effective.

b) The fate and effects of oil dispersed in deep water;

c) Upgrades to numerical modeling to better predict the fate of dispersed oil in deep water; and

d) Monitoring tools to determine the effectiveness of subsea injection.

CONFIDENTIAL

Experimental Studies in SSDI

Brandvik et al 2013 studied the size distribution of droplets produced in a subsea discharge using a new meso-scale test facility at SINTEF. Brandvik described the facility and presented results from tests with Oseberg Blend crude oil, with and without injection of chemical dispersants (Corexit 9500). Experiments without dispersants examined droplet size distributions generated using exit diameters 0.5 to 3 mm, flow rates 0.2 to 5L/min. with and without injection of air. Effects of dispersants were studied at a single nozzle diameter (1.5 mm) at flow rates of 1.2 to 1.5 L/m, with DORs from 0 to 1:25.

In the experiments without dispersants, with constant nozzle diameter (0.5mm), increasing the flow rate (0.2 to 0.5 L/min.) caused a shift towards smaller droplets. The result was similar when the flow rate was held constant (5 L/min) and the nozzle diameter reduced. Releasing air in combination with the oil (GOR=1:1) gives larger oil droplets. Finally, injecting dispersant into the combined oil and gas release reduced the droplet diameter compared to both a combined release and an oil alone release with the constant flow rate and nozzle diameter. Droplet size declined as proportion of dispersant to oil increases.

The following are in preparation.

ED_001324_00001453-00010

Johansen et al. 2013

SSDI Studies with Problems

None

CONFIDENTIAL

Fate and Degradation of Dispersed Oil and Dispersants

This section considers papers dealing with the environmental fate of dispersants and dispersed oil. Papers have been grouped into:

a) Field studies conducted during the Macondo spill;
b) Experimental studies of oil fate and microbial degradation of dispersed oil and dispersants; and
c) Methods developed to monitor or analyse for dispersants or dispersed oil.

Field Studies During the Macondo Spill

Subsurface plumes of oil and dispersant

One of the key challenges of the Macondo spill was identifying and tracking the subsurface plumes of oil discharged from the seabed. A number of field surveys and modeling studies were completed during the actual event to locate subsurface oil plumes and track the fate of the oil and dispersant they contained. These are summarized in Spier et al. 2013.

Spier et al. 2013 examined analyses of oil in water samples taken offshore at depth from May 9 to June 26 during the spill. They identified the vertical plume of hydrocarbons rising to the surface from the blowout and addressed several research questions, as follows.

a) Concerning the location and direction of subsurface hydrocarbon plumes, Spier et al. identified plumes with mid-depths of 25, 265, 865, and 1175 m and identified the direction of movement of each. Plumes at 25, 265 and 1175 were clearly persistent, but the 865-m-deep plume was not sampled often enough to assess persistence.

b) Spier confirmed the existence of the 1175 m plume that had been predicted in various modeling studies published at the time.

c) Spier tested and confirmed that a plume had formed at 800 m as predicted by models and confirmed it.

d) Spier confirmed that the oil composition of the plumes varied with depth. Their analysis determined that the more soluble compounds were found in the deeper plume. This gave further evidence that the more water soluble compounds were extracted from the oil deeper in the water column leaving the less water soluble ones to dominate in the upper water column.

Mendoza et al. 2013 monitored the subsurface distribution of oil and dispersant within 40 km(?) of the DWH spill site from May 27 to June 4 during the spill. They took water samples using CTD casts and analysed them for HCs using fluorescence techniques (excitation and emission matrix (EEM) fluorescence and parallel factorial analysis (PARAFAC) modeling techniques) (*We need a chemist to assess the usefulness of these techniques*). The benzene and naphthalene-

ED_001324_00001453-00012

like components of crude oil exhibited a maximum at ~1200 m, coinciding with the 1175-m centred

plume identified by others. The maximum fluorescence of the component associated with the dispersant (i.e., Corexit EC9500A) was observed at the same depth. The plume observed at this depth was attributed to the dispersed crude oil from the Deepwater Horizon oil spill[1].

Zhou et al.[2] also studied the distribution of oil in surface and deep waters near the DWH spill site during May and June using fluorescence EEMs techniques coupled with PARAFAC modeling. This group also identified petroleum hydrocarbons to the SW of the DWH site at a depth of 1100 to 1400 m.

The following are in preparation.

Bejarano et al. 2013.

Monitoring for Corexit Dispersants

Prior to Macondo little attention was paid to monitoring the fate of the dispersant during a spill response. During Macondo monitoring the fate and degradation of the dispersant became an issue due to the amount used and concern voiced by the public. During the response itself, attempts to monitor the dispersant focussed on analysing the dioctylsulfosuccinate (DOSS) component of the product. Recently published work involved analysing environmental samples taken during the response and developing methods for analysing components of the dispersants other than DOSS.

CONFIDENTIAL

Place et al. developed an analytical method for detecting all four classes of surfactants discharged in the response to the Macondo spill , as well as the hydrolysis products of DOSS, the enantiomeric mixture ofα-and β-ethylhexyl osuccinate (α -/β-EHSS). The new analytical method was then used to evaluate practices for sample collection, storage, and analysis to identify those that produced high quality data. Place concluded that the method "Large volume, direct injection of seawater followed by liquid chromatography tandem mass spectrometry (LC-MS/MS)" minimized analytical artifacts, analysis time, and both chemical and solid waste. The authors reported the ranges of analyte concentrations observed in Macondo water samples analysed.

Perkins et al. (abstract only) reported on concentrations of the major constituents of Corexit, Span 80, Tween 80, Tween 85, as well as, DOSS and its primary hydrolysis products in depth specific water column samples collected from priority sites in the Gulf of Mexico in 2012. These

[1] Results demonstrate the application of EEM and PARAFAC to simultaneously monitor selected PAH, dispersant-containing and humic-like fluorescence components in the oil spill region in the Gulf of Mexico. Four of the seven derived PARAFAC loadings were associated with the Macondo crude oil components. The other three components were associated with the dispersant, an unresolved component and colored dissolved organic matter (CDOM).
[2] Zhou, LG , A Shiller, S Lohrenz, V Asper, C. Osburn2013 Characterization of oil components from the Deepwater Horizon oil spill in the Gulf of Mexico using fluorescence EEM and PARAFAC techniques Marine Chemistry Volume 148, 20 January 2013, Pages 10–21

values are compared to those for samples collected from similar sites in 2010 and 2011. Additionally, a preliminary method for extracting and quantifying DOSS and degradation products from marine sediment, using LVI-LC-MS/MS, is presented.

Gray et al. 2013 collected water samples at the surface and at depth during two cruises in May/ June 2010 for DOSS analysis. A newly developed LC/MS/MS technique for the determination of dioctylsulfosuccinate (DOSS) in clean and oily sea water was applied to the samples. Although DOSS was present at high concentration in the immediate vicinity of the well where it was being continuously applied, a combination of biodegradation, photolysis, and dilution likely reduced persistence at concentrations exceeding the USEPA aquatic life benchmark beyond this immediate area.

Laboratory Experimental Studies

The following are in preparation.

Ehrenhauser, F –	bubble bursting as fate process
Liyana-Arachchi –	bubble bursting
Gong, -	oil sediment interactions, review
Gong –	effect sed sorption on photodeg and ozonation
Zhou, Z., -	chem evolution by degr inj lab

Problems in Studies of the Fate of Dispersed Oil and Dispersants

The following provides an overview of research issues arising from papers in this subject area. The types of major errors are summarized in Table 2 below. Errors have been divided into dispersant-research-specific errors and scientific errors. The majority of the discussion focuses on the dispersant-specific errors; scientific errors are discussed only when they influence the discussion of dispersant-related processes.

The problems associated with Sammarco et al. 2013 were discussed in Wilson et al 2014. These are scientific problems and are not dispersant-related. They included problems with experimental design, their sampling strategy, chemical and data analysis, and the basis and interpretation for their human health risk conclusions.

Zuijdgeest and Huettel (2012) studied the penetration of chemically treated and untreated oil into intertidal beach sand. Their objective was to gain knowledge about the fate of oil that had been treated with dispersants in offshore areas during the Macondo spill. The authors concluded that "the addition of dispersants permits crude oil components to penetrate faster and deeper into permeable saturated sands, where anaerobic conditions may slow degradation of these compounds, thus extending the persistence of potentially harmful PAHs in the marine environment. Application of dispersants in nearshore oil spills should take into account enhanced penetration depths into saturated sands as this may entail potential threats to the groundwater."

The problem with their study was that they used an incorrect mental model of the contact of spilled dispersant-treated oil with intertidal sand beached in Macondo. In Macondo, although dispersants were being used regularly 40 mi offshore, the only oil that contacted sand beaches

ED_001324_00001453-00014

was patches of heavily weathered, viscous mouse, some of which may have contained trace amounts of some dispersant constituents. This oil would not have penetrated into the sand, even with tidal pumping. On the other hand, Zuijdgeest and Huettel's experiment studied the penetration into sand columns of dispersants, physically-dispersed oil and chemically –dispersed oil in the form of sonically disrupted suspensions of fresh oil in water. These contained micron-sized droplets of fresh oil. This may have simulated suspensions that would form if fresh oil were to be treated with dispersants in the interdidal zone or in shallow nearshore areas. However, it did not simulate Macondo conditions or conditions in any spill where dispersants may be used many miles offshore. In short, the published results and conclusions did not simulate the actual conditions during Macondo, so their published conclusions are misleading.

Kuhl et al 2013. The experiment and paper by Kuhl et al. included a number of dispersant-related and scientific challenges. In short, the authors attempted to use microcosm experiments to study the effects water salinity on the fate and degradation of untreated and dispersant-treated, weathered oil in a shallow nearshore environment. Their specific objectives were:

1) to explore the role of salinity and time on partitioning of PAHs into the aqueous phase from layers of oil, dispersant and chemically dispersed oil applied to the water's surface in microcosms, and to study their subsequent biodegradation and associated toxicity to Fundulus. grandis.

2) to model the role of these variables on mortality; and

3) to determine if a common environmental sampling analysis (aqueous PAH concentrations) accurately reflects acute toxicity of dispersed and non-dispersed crude oil.

To this end the authors layered untreated oil, dispersant and oil-dispersant mix on the surface of aquaria containing sediment and water at three salinities and allowed the aquaria to age under quiescent conditions for up to 16 weeks. PAH concentrations in the water and the toxicity of the water to F. grandis were monitored after 1, 4 and 16 weeks. The authors reported that:

a) Compared with undispersed oil, COREXIT exponentially increased the PAH concentrations in the WAF for up to 16 wk;
b) Hopane-normalized concentrations indicated that biodegradation was slowed for the first 4 wk.
c) Dispersed crude oil and COREXIT were acutely toxic following 1 wk of biodegradation with no correlation between PAH concentrations and crude oil WAF mortality.
d) Both dispersant and dispersant oil mixtures remained toxic for at least 4 wk at the lowest salinity tested, suggesting increased sensitivity or reduced biodegradation of toxic components in low-saline environments.
e) At the lowest salinity, oil dispersed with COREXIT was more toxic than either the COREXIT alone or oil alone, even after 16 wk of biodegradation.

There were several general problems with the design in terms of interpretation and application in the real world as follows.

ED_001324_00001453-00015

a) The authors had an inappropriate or unlikely mental model of dispersant use in that it is unlikely that oil slicks would be treated with dispersants in shallow, nearshore environments.
b) It is unrealistic for oil in this environment to remain undisturbed for 16 weeks, making observations concerning transfer of PAH from the slick to the water difficult to interpret in terms of real world applicability. This invalidates all conclusions. Further, any oil that mixed into the water column would have diluted quickly by mixing. This invalidates all time-dependent conclusions regarding degradation and toxicity.
c) Preparation of the exposure medium is poorly described in that the extent to which the oil was mixed into the aquaria or not is not described. When an oil-dispersant mixture is applied to a water surface, it does not mix spontaneously, but if disturbed it mixes very readily with the water phase with very little mixing energy. There is no mention in the paper of measures taken to prevent mixing during the experimental set up, nor were water samples taken at time 0 to confirm that there was no initial mixing. Unfortunately accidental mixing at the time of setup could account for the anomalous pattern of mixing observed during later sampling times.
d) The apparent non-linear relation between salinity and oil fate are difficult to assess because of the lack of replication. This is a scientific error and it invalidates most of the conclusions about effects of salinity on any oil fate processes.

Table 2 Summary of Challenges in Fate/Degradation Studies

FIDENTIAL

Fate/Degradation	Sammarco	Kuhl	Zuijdgeest and Huettel 2012.	
Scientific errors	X	X		
Dispersant –specific errors				
Incorrect mental model of dispersant use		X	X	
Incorrect model of environmental behaviour		X	X	
Incorrect method of preparing media		X		

Microbial Degradation

During the Macondo spill considerable attention was devoted to the biodegradation of the discharged oil and dispersant, as follows.

Lee et al observed that oil that reached the water column in the Macondo spill biodegraded quickly, but that results of some laboratory degradation studies appeared to tell a contradictory story. Lee suggested that the contradictory results may have resulted from study designs that did not reflect the field conditions encountered in both the GOM spill and most oil spills at sea. According to Lee, lab biodegradation studies have at least two important challenges, namely studies must be run using:

a) Dispersed oil concentrations that are as low as they might be expected to be in the field (generally 100 ppm TPH or less); and

b) The dispersions of droplets be as stable as stable as they might be expected to be in the field (droplets are no larger than 70-100 um diameter and are kept in suspension by mild stirring or agitation).

Lee argued that some biodegradation studies used unrealistically high concentrations of dispersed oil in their tests, which produced unrealistic degradation because:

a) Using concentrations of oil or dispersant much higher than in the field inhibited hydrocarbon-degrading bacteria; and

CONFIDENTIAL

a) Biodegradation at much higher oil concentrations than at sea in closed systems can limit degradation because of depletion of nutrients. Attempts to counteract this by using high initial concentrations of nutrients may lead to an unrepresentative modification of the microbial community.

Prince & Butler 2013 conducted a mesocosm study aimed at showing that the rate of primary biodegradation of an oil slick is dramatically stimulated by effectively dispersing the oil slick into the water column using dispersants. They used methods that adhered to the principles outlined in Lee et al. above. In short, in a lightly stirred environment, an untreated slick remained on the water surface during the entire biodegradation study, while a dispersant-treated slick was dispersed into the water and remained in the water column as small dispersed oil droplets. Clearly both biodegradation and evaporation were occurring in both experiments with and without dispersant, but it is obvious that biodegradation was far more extensive in the dispersed oil system than in the untreated one. Within 7 days, the dispersed oil had lost 50% of its detectable hydrocarbons versus 14 % in the untreated experiment. After 40 days the dispersed experiment had lost 84% versus less than 14% in the untreated one. Equally important, naphthalene was lost through a combination of both evaporation and biodegradation. However, its non-volatile alkyl congeners and the phenanthrenes were eliminated by largely by biodegradation alone. Clearly, in this study, dispersed oil degraded much more rapidly and extensively than undispersed oil.

Prince et al. 2013a conducted a degradation study under conditions described by Lee et al.

2013. Under those conditions, oil was extensively and rapidly degraded. They showed that biodegradation of dispersed oil was prompt and extensive when oil is present at the ppm levels expected from a successful application of dispersants – more than 80% of the hydrocarbons of lightly weathered Alaska North Slope crude oil were degraded in 60 d at 8° C in unamended New Jersey (USA) seawater when the oil was present at 2.5 ppm. Degradation half-lives for small molecules like naphthalene were 2 d, most PAHs were <5 days, and the most recalcitrant were 18 to 32 days. The half-times for total extractable HCs was 11 to 14 days. The presence of the dispersant, Corexit 9500 at realistic concentrations did not slow degradation.

McFarlin et al. measured the degradation of physically and chemically dispersed Alaska North Slope crude oil (ANSCO) by indigenous Arctic microbiota in seawater at -1°C. Primary biodegradation was measured in standard mesocosm and mineralization in respirometer systems using respirometry and gas chromatography-mass spectrometry (GC-MS) analysis for measurement. Arctic microorganisms degraded both fresh and weathered crude oil in the presence and absence of Corexit 9500 at -1°C, with biodegradation oil losses ranging from 46 to 61% and mineralization losses up to 11% over 60 days. Weathered ANS dispersed with Corexit 9500 underwent a 57% loss in Arctic seawater after 60 days in their experiment, but experienced an 88% loss in New Jersey seawater in the same time, suggesting that in the Arctic, ANS crude oil degrades slightly more slowly than oil intemperate regions. The authors concluded that the biodegradation of oil in Arctic seawater is extensive at -1°C, and that the biodegradation of dilute, dispersed oil is not inhibited by the presence of Corexit 9500.

CONFIDENTIAL

Campo et al studied whether the microbial communities of the GOM were capable of degrading Corexit 9500 or dispersed oil at temperatures typical of GOM surface waters (25°C) and bottom waters (5°C), using C9500 (as represented by DOSS), South Louisiana crude oil (SLC) alone, and SLC dispersed by C9500. Exposure media were prepared in artificial seawater and an inoculate prepared using microbial communities harvested from GOM surface and bottom waters (1500 m) grown on media augmented with crude oil at 25 and 5 deg C.. Oil and dispersant concentrations were measured, but were several times higher than at sea.

This study revealed that DOSS, spontaneously and quickly hydrolyzed to MOSS and 2-EH at 25 °C but degraded little at 5 °C during the 42 days of the study. The implication was that DOSS may not persist in warm waters, but may persist for a long time in waters at very cold temperatures. Biodegradation of DOSS at 25 °C was faster in the presence of oil than without oil.

Alkane biodegradation proceeded rapidly and virtually completely at warm temperature. Degradation rates were 5-fold greater in dispersed oil than in the untreated oil, which agreed with previous findings.

The authors concluded that in their experiments crude oil biodegradation in general was readily enhanced by the addition of dispersant, but PAH biodegradation at 5 °C may have been more a function of the inherent solubility of PAHs compared to alkanes rather than due to the influence of the dispersant.

The following are in preparation.

Batchu -	degrad DOSS by photodegr
Joo –	eff disp on oil deg – mesocosm
Macías-Zamora –	eff disp o degrad PAH in sed
Vilcaez J –	model degrad kinetics of oil droplets

CONFIDENTIAL

Table 3 Summary of Fate and Degradation Papers

DWH	**Fate Experimental**	**Degradation – Experimental**	**Methods/Equipment**
Spier –Plume tracking in DWH Mendoza, vert dist oil and disperant in DWH Zhou Character oil components in water Sammarco distr HCs in water in DWH Gray - fate of DSS in GOM Perkins distr Corexit in water seds Bejarano	Kuhl – eff dispersants/salinity oil weathering Ehrenhauser, F – bubble bursting as fate process Liyana-Arachchi – bubble bursting Gong, - oil sediment interactions, review Gong – effect sed sorption on photodeg and ozonation Zhou, Z., - chem evolution by degr inj lab Zuijdgeest A., and M. Huettel. 2012.	Lee,K. – degrad studies must consider dilution Prince RC, Butler JD. lab dispersed oil Prince RC – lab dispersed oil stimulates degrad McFarlin – degr disp oil Arctic stimulates degrad Campo - eff temp degr Corexit and disp oil **Baelum** – eff oil in bacterial community Batchu - degrad DOSS by photodegr Changkyu – eff disp on oil deg – mesocosm Macías-Zamora – eff disp o degrad PAH in sed Vilcaez J – model degrad kinetics of oil droplets	Van Scoy - SPMDs) Conmy, - Submersible optical sensors Lavallee - Fluorescence fingerprinting Mendoza, - fluorescence and PARAFAC Zhou - EEM and PARAFAC techniques. Place – trace analy surfactants in disp/ water

CONFIDENTIAL

CONFIDENTIAL

Effects of Dispersants, Dispersed Oil and Chemically Dispersed Spills

This section includes all studies dealing with the effects of dispersants and dispersed oil. It includes:

a) Studies of dispersant-related effects observed during actual spills (Macondo); and

b) Experimental studies undertaken to understand the cause-effect relationship between oil exposure and effects and the level of exposure needed to cause these effects.

Effects of the Macondo Spill

Montagna et al. reported on monitoring cruises that were deployed in fall of 2010 to measure potential impacts on benthic invertebrate communities. Sediment samples were taken for chemical, physical and biological analyses. Although the benthic distribution of oil and its effects may have resulted from effects of dispersants on the fate of oil, the paper did not address the potential role of dispersants.

The most severe relative reduction of faunal abundance and diversity extended to 3 km from the wellhead in all directions covering an area about 24 km2. Moderate impacts were observed up to 17 km towards the southwest and 8.5 km towards the northeast of the wellhead covering an area 148 km2.

Benthic effects were correlated to total petroleum hydrocarbon, polycyclic aromatic hydrocarbons and barium concentrations, and distance to the wellhead; but not distance to hydrocarbon seeps. Thus, benthic effects are more likely due to the oil spill, and not natural hydrocarbon seepage.

CONFIDENTIAL

The following are in preparation.

Ziervogel	Enhanced protein hydrolysis
Fitzgerald –	Contam in reef fish
Moody,	Lack of Impact by DWH
Echols–	tox tests on DWH water samples
Montagna	PA Benthic Footprint DWH
Paul	Tox Mutagenicity GOM waters

Experimental Toxicology Studies

The objectives of toxicological experimentation related to oil spill dispersants are to understand:

1) effects caused by oil and dispersants at the organism level; and
2) the level of exposure needed to cause these effects.

These subjects are being studied to improve understanding of the risks posed by spills and dispersants to the fish and wildlife exposed to them. The effects of these potential toxicants at the organism level include both mortality and sublethal effects. Sublethal effects range from innocuous effects like bioaccumulation of hydrocarbons to major injuries like gross anatomical abnormalities and reproductive impairment. Just as important as the effects caused are the levels of exposure needed to cause the effects. Planners assess the risks posed by spills and spill response by comparing the expected levels of environmental contamination during the event to the experimentally derived estimates of levels of exposure needed to cause effects.

Historically toxicological research has employed standardized exposure protocols (e.g., 96-hour LC50) to facilitate comparison of new results to the existing knowledge base. Dispersant work adheres to these protocols to a degree. Because of the nature of petroleum and the way dispersants are used to respond to them, spills produce a particular type of exposure conditions. This posed a problem for planners trying to use standard toxicological data to assess risks in the field. In order to overcome these challenges, over the years, researchers have developed exposure protocols (e.g., spike exposure protocol), and standard methods for preparing and characterizing exposure media in order to simplify the task of using the experimental data to estimate risks in the field (Coelho et al. 2013). While it is not essential to adhere to these standards, adherence does facilitate the use and inter-comparability of research data.

Composition of the Exposure Media

Recently, several authors have attempted to understanding of the roles of different components of dispersions to toxicity.

Gardiner et al. 2013 - In a study of the sensitivity of Arctic species, Gardiner investigated the relative contributions of different components of the exposure media to its toxicity. They used three methods to prepare media: standard WAF, more energetic WAF or BWWAF; and standard CEWAF. They used GC to quantify the chemical composition of the exposure media. They observed that varying prep methods changed the relative composition of the stock exposure media, as well as its apparent toxicity. Specifically, the standard low energy WAF contained only the more soluble components of the oil and few of the insoluble components; higher energy BWWAF contained a higher concentration of the soluble components, but also added some insoluble components; and CEWAF increased the amount of oil and increased the insoluble components to the point where oil the composition of the dispersed oil was similar to the parent oil. In toxicity terms when toxicity is related to oil concentrations expressed as TPH, the different media were ranked as follows in terms of toxicity: WAF>BWWAF>CEWAF. Toxicity correlated most strongly with naphthalene concentrations. Gardiner concluded that toxic components were the naphthalene components, which were soluble and were the most abundant components of

ED_001324_00001453-00023

the soluble components in WAF. Altering the prep method increased the relative amounts of less toxic components in the medium, so that the apparent LC 50 declined when expressed as TPH. Gardiner also concluded that the presence of dispersant in the media did not contribute to the toxicity of the exposure medium.

Anderson prepared media using a standard way and measured an extended group of PAHs in exposure media using GC. They recognized that the CEWAF had higher concentrations of PAH in the media than the WAF and this was reflected in higher levels of mortality in the CEWAF than WAF.

Sensitivity to Dispersed Oil

Gardiner used standard spike exposure method to study sensitivity of arctic species to physically and chemically dispersed oil. The effects thresholds as defined by the LC10 were above TPH concentrations predicted to occur in the field within hours of surface application of dispersants. They also showed that the sensitivity of arctic species under arctic conditions were similar to temperate species.

Goodbody-Gringely studied the effects of physically dispersed and chemically dispersed oil on survivorship and setting behaviour of coral larvae. The purpose was to examine the effects of exposure to both fresh and weathered DWH oil and dispersants using short-term assays (96-hr) to monitor larval survivorship, settlement, and behavioral responses. The author the used spike exposure method and measured exposures using TPH and GC-fid. The methods of preparing WAF and CEWAF were not described.

CONFIDENTIAL

The author concluded that the higher concentrations of oil in the CEWAF tests caused mortality in both species tested. The author failed to acknowledge that the dispersant oil mixture provided the higher oil concentrations in the test and the higher exposures caused the greater mortality. The toxicity thresholds expressed in TPH were consistent with those I other studies.
The author did not comment on the possible risks to Florida from the Macondo spill.

The following are in preparation.

Adams J
Lee KW,
Martin
Radniecki,
Ozhan
Ozhan
Powers

Effects of Variables on Toxicity

Effect of Species

Gardiner showed differences in sensitivity among the following Arctic species: copepod (Calanus glacialis), juvenile Arctic cod (Boreogadus saida), and larval sculpin (Myoxocephalus sp.). They also identified a significant difference in sensitivity between copepods of different ages.

Rico-Martinez attempted to study the toxic effects of physically dispersed oil and chemically dispersed oil on the marine rotifer, Brachionus manjavacas. The authors demonstrated that there were large differences in apparent sensitivity among the species studies.

Life stage

Anderson studied effects of WAF and CEWAF dispersed oil on juvenile mud crabs to identify any long-term effects of acute exposure on long term survival. They concluded that if the juvenile crabs survive initial exposure to oil, there were no latent effects that appeared post-exposure.

Temperature

Gardiner showed that Arctic animals tested at Arctic temperatures were no more or less sensitive to dispersed oil than temperate zone species tested at temperate zone temperatures.

Ultraviolet light

Alameda showed that UVB radiation increased the lethal effects of dispersed crude oil in mesozooplankton communities by 35%.

Sublethal Effects of Dispersant and Dispersed Oil

In preparation

Bioaccumulation

Chase

Enzyme Induction/Metabolites

Milinkovitch T,

CONFIDENTIAL

Immunosuppresion

Barron
Performance and Fitness
Claireaux G,
Milinkovitch T,

Gross Abnormality/Histopatology

Agamy
Agamy

Growth/Reproduction

Brewton
Zhang
Zhang
Polli

Problems in Studies of Effects of Oil and Dispersants

The following provides an overview of research issues arising in several papers. The types of major errors are summarized in Table 4 below. Errors have been divided into dispersant-research-specific errors and scientific errors. The most of the discussion will address the dispersant-specific errors; scientific errors will be discussed only when they influence the

discussion of dispersant-related processes.

Anderson studied effects of dispersed oil on juvenile mud crabs assessing the acute effects of non-dispersed and chemically dispersed crude oil. Toxicity tests were done using an EPA standard protocol that involved 96-hr static non-renewal assays. Exposure media were prepared in a standard way. All toxicity results were reported in terms of Nominal oil concentrations, though exposure media were analysed for "extended PAH concentrations". Anderson reported that the chemical dispersion of crude oil significantly increased acute toxicity of the WAF in juvenile mud crabs compared to naturally dispersed oil, but they did acknowledge that the concentrations of PAH in the CEWAF were higher than the WAF and that correlated with higher mortality. By failing to report toxicity results based on measured exposure concentrations makes it difficult to use data to assess risk in the field.

Rico-Martinez attempted to study the toxic effects of physically dispersed oil and chemically dispersed oil on the marine rotifer, *Brachionus manjavacas*. They used the ASTM standard exposure protocol and a standard method for preparing exposure media. They did not attempt to characterize their exposure media in any way and reported toxicity results in NOMINAL. One of their stated objectives was to assess the effects of a crude oil and Corexit 9500A mixture at concentrations that are environmentally relevant. This was not actually attempted in the paper and could not have been done as the authors did not measure the oil concentrations in their exposure media at all.

Although it was not part of their study design the authors did attempt to draw conclusions about the contributions of the components of the oil and dispersant in the exposure media. They reported that synergistic toxic effects of the dispersant and oil increased the toxicity of the oil by a factor of 52 times. This line of discussion was not supported by their experimental design because they did not measure the concentrations of oil in their exposure medium. Their conclusion was completely unfounded, but has become often quoted in the literature.

CONFIDENTIAL

Alameda attempted to determine the effects of short-term crude oil exposure on the survival and bioaccumulation of PAHs in natural mesozooplankton assemblages; and assess the lethal effects of dispersant-treated crude oil and dispersant (CorexitEC9500A) on coastal mesozooplankton communities.

They used non-standard 16- and 48- hour static non-replacement toxicity method. They used an apparently standard method for preparing exposure media and verified that the exposure method contained oil droplets of a size ingestible by the zooplankton. They used NOMINAL oil concentrations, but measured the concentrations of PAHs in the parent oil. They used ratios of zooplankton oil concentrations to nominal oil concentrations in exposure media as indices of bioaccumulation.

Mortality of mesozooplankton increased with increasing NOMINAL oil concentration. Median lethal concentrations were reported but were of little use because of NOMINAL exposure concentrations. Authors concluded that dispersant and dispersant- treated oil were 2.3 and 3.4 times more toxic than crude oil alone mesozooplankton, but this is not valid because of NOMINAL exposure concentrations.

In addition, Alameda attempted to study the accumulation of PAHs from exposure media by mixed species zooplankton assemblages, as well as by a single species, A. tonsa. None of the results from this part of the study is useful because the actual composition of the exposure medium is not known. Even the relative accumulation rates estimated are difficult to interpret because the actual relative concentrations of the compounds in the exposure media are not known. The author calculated and reported bioaccumulation factors, but these are of little use because: a) they are based on the body burdens of individuals of many species that have different uptake mechanisms and different clearance rates; and b) there is no way of knowing how close these body burdens are to steady-state. In short, the results and conclusions from this part of the study are of limited value.

Kuhl – The experiment and paper by Kuhl et al. included a number of dispersant-related and scientific challenges. In short, the authors attempted to use microcosm experiments to study the effects water salinity on the fate, degradation and toxicity of untreated and dispersant-treated, weathered oil in a shallow nearshore environment. The specific objectives were:

1) to explore the role of salinity and time on partitioning of PAHs into the aqueous phase from layers of oil, dispersant and chemically dispersed oil applied to the water's surface in microcosms, and to study their subsequent biodegradation and associated toxicity to Fundulus. grandis.

2) to model the role of these variables on mortality; and

3) to determine if a common environmental sampling analysis (aqueous PAH concentrations) accurately reflects acute toxicity of dispersed and non-dispersed crude oil.

CONFIDENTIAL

Some of the challenges in Kuhl are discussed above in the Fate Section. The toxicity data in this study are difficult to interpret. There was a range of toxic effects but toxicity was not correlated with the concentration of any the PAH. Even if toxicity could be linked to the oil, ther results would be difficult to apply because of the experimental design.

Summary of Problems in Effects Research

To this point in the report writing only problems in the experimental studies have been investigated. The most significant sources of problems are listed in Table 4.

One important problem, seen in Kuhl et al., was an incorrect mental model the behaviour of dispersant-treated oil in a nearshore environment. Their experimental setup involved layering dispersant-treated oil on water in a mesocosm and allowing it to sit, undisturbed for 16 weeks to study the effect of the presence of the dispersant on partitioning of PAH from the oil slick into the water. While interesting, this is a completely unrealistic exposure scenario and the results cannot be related to any real-world situation. In the real-world waves or even tidal action would quickly mix the dispersant-treated oil into the water.

A second problem observed in Kuhl, involved monitoring the degradation of dispersant and dispersant-treated oil in a confined system for 16 weeks and expecting to observe changes reflective of a real-world system. In the real world, the entrained HCs and dispersant would dissipate quickly by dilution making any changes observed in the microcosm artefactual.

A third problem, also observed in Kuhl, was their failure to adequately describe the method used to prepare their exposure media. It appears that in different mesocosms, they layered either oil, dispersant or dispersant-treated oil on the water surface. Dispersant-treated oil on a water surface is very unstable and disperses readily with even a mild disturbance. Great care would be needed to prevent the treated oil from mixing into the water. The authors prepared at least nine of such mesocosms. It is possible/likely(?) that some unintentional dispersion might have taken place during the setup of one or more of these mesocosms. This alone might have accounted for the non-linear effects attributed to effects of salinity or aging time. The authors did not describe precautions taken to prevent mixing or take samples at time 0 to demonstrate that no inadvertent dispersion had taken place duiring setup.

The most common problem in these studies is the failure to measure the oil or HC concentrations in exposure media. In all studies this failure meant that the sensitivity of the test organism to the hydrocarbons is not determined quantitatively and the results cannot be used to assess risk or to be compared with sensitivity values from other studies. In addition, it makes it impossible to relate effects to the different components in the exposure media. For example, in Rico-Martinez, the most frequently cited publication, the authors concluded that elevated mortalities in their oil+dispersant experiment were caused by synergistic toxicity of the oil and dispersant, failing to acknowledge that the true cause was greatly elevated oil concentrations in the dispersant-treated experiment. The failure to measure oil in exposure media invalidated their conclusion. Alameda had the same problem. In Alameda, failure to measure oil concentrations in exposure media invalidated all conclusions in their bioaccumulation study because the actual oil exposure concentrations were completely unknown.

CONFIDENTIAL

Table 4 Summary of Problems in Experimental Effects Papers						
Effects	Anderson	Rico-Martinez	Alameda	Kuhl		
Scientific errors				x		
Dispersant-specific errors						
Incorrect mental model of dispersant use				x		
Incorrect model of environmental behaviour				x		
Inappropriate test protocol						
Inappropriate method for exposure media prep				x		
Inappropriate method for characterizing exposure media	x	x	x			

CONFIDENTIAL

Health

Problems with Human Health and Safety Studies

D'Andrea and Reddy (2013) assessed the adverse health effects of the Gulf oil spill exposure in subjects participating in the clean-up activity along the coast of Louisiana. This retrospective study included subjects that had been exposed and unexposed to the oil spill and dispersant. Using medical charts, clinical data including white blood cell count, platelets count, hemoglobin, hematocrit, blood urea nitrogen, creatinine, alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), and somatic symptom complaints by the subjects were reviewed and analyzed. Authors reported that hematologic analysis showed that platelet counts were significantly decreased in the exposed group compared with those in the group unexposed to the oil spill. Conversely, the hemoglobin (g per dL) and hematocrit (%) levels were significantly increased among oil spill-exposed subjects compared with the unexposed subjects. The authors concluded that clean-up workers exposed to the oil spill and dispersant experienced significantly altered blood profiles, liver enzymes, and somatic symptoms.

This is a poorly designed, executed, analyzed, and interpreted study. Numerous limitations preclude drawing any conclusions about potential health effects in workers associated with the clean-up of the Deepwater Horizon oil spill. The shortcomings include: biased target and control study groups, failure to control for risk factors associated with several outcome measures and which have clearly different occurrence rates in the study groups, absence of quantitative or qualitative exposure data, and consequently, over-interpretation of the data presented. While the authors acknowledge some of these limitations and correctly note that this research does not address possible causal relationship between the spill and worker health outcomes, their conclusion that the study results support the potential for hematological and hepatic toxicity is also without merit.[3]

[3] Many thanks to reviewers: Heidi S. Erickson and Ken Satin, ETC/HES for this review.

ED_001324_00001453-00030

Table 5 Problems with Health and Safety Papers						
Health and Safety	D' Andrea and Redy					
Scientific errors	x					

CONFIDENTIAL

Effectiveness
In preparation

Editorial, Policy Etc
IN Preparation

CONFIDENTIAL

Literature Cited

Adams, E. Eric. 2013. Comment on "Evolution of the Macondo Well Blowout: Simulating the Effects of the Circulation and Synthetic Dispersants on the Subsea Oil Transport" A polemic. This correspondence comments on the droplet size distribution used by the authors to predict the effects of sub-surface dispersants applied to the Macondo well blowout. Environmental Science & Technology (2013), 47(20), 11905 CODEN: ESTHAG; ISSN: 0013-936X[See - Paris CB, Hénaff ML, Aman ZM, Subramaniam A, Helgers J, Wang DP, Kourafalou VH, Srinivasan A.Evolution of the Macondo well blowout: simulating the effects of the circulation and synthetic dispersants on the subsea oil transport. (Environ Sci Technol. 2012 Dec 18;46(24):13293-302. doi: 10.1021/es303197h. Epub 2012 Dec 3]

Adams J, Sweezey M, Hodson PV. Oil and oil dispersant do not cause synergistic toxicity to fish embryos.Environ Toxicol Chem. 2013 Sep 23. doi: 10.1002/etc.2397. [Epub ahead of print]

Afonne OJ, Onyiaorah IV, Orisakwe OE.2013.Toxicity of Chevron Escravos crude oil and chemical dispersant on guinea pig testicular function. J Basic Clin Physiol Pharmacol. 2013 Nov 1;24(4):321-9. doi: 10.1515/jbcpp-2012-0060.

Agamy, E. 2013. Impact of laboratory exposure to light Arabian crude oil, dispersed oil and dispersant on the gills of the juvenile brown spotted grouper (Epinephelus chlorostigma): A histopathological study. Marine Environmental Research, 86:46-55.

Agamy, E. 2013. Sub-chronic exposure to crude oil, dispersed oil and dispersant induces histopathological alterations in the gills of the juvenile rabbit fish (Siganus canaliculatus). Ecotoxicology and Environmental Safety, 92:180-190.

Ali, A.O., C. Hohn, P.J. Allen, L. Ford, M.B. Dail, S. Pruett, L. Petrie-Hanson. 2014. The effects of oil exposure on peripheral blood leukocytes and splenic melano-macrophage centers of Gulf of Mexico fishes. Marine Pollution Bulletin, Available online 6 January 2014

Almeda, R., Z. Wambaugh, Z. Wang, C. Hyatt, Z. Liu, E. Buskey. 2013. Interactions between zooplankton and crude oil: toxic effects and bioaccumulation of polycyclic aromatic hydrocarbons PLoS One (2013), 8(6),

Aman ZM, Paris CB.Response to comment on "evolution of the macondo well blowout: simulating the effects of the circulation and synthetic dispersants on the subsea oil transport".Environ Sci Technol. 2013 Oct 15;47(20):11906-7. doi: 10.1021/es404183y. Epub 2013 Oct 1. No abstract available.

Anderson, S.S., Peterson, C.H., Cherr, G.N., Hampton, S., Blum, M.. 2012. Casual Observations on DWH Dispersant Effects Expose the Lack of Rigorous Science: Response to Rorick and Colleagues. Bioscience 62(12):1010-.

Anderson, JA, Kuhl AJ, Anderson AN. 2014. Toxicity of Oil and Dispersed Oil on Juvenile Mud Crabs, Rhithropanopeus harrisii. Bull Environ Contam Toxicol. 2014 Apr;92(4):375-80.

D'Andrea MA1, Reddy GK.1011. Health consequences among subjects involved in Gulf oil spill clean-up activities. Am J Med. 2013 Nov;126(11):966-74. doi: 10.1016/j.amjmed.2013.05.014. Epub 2013 Sep 17

Arnold C. Studies seek to find answers about Gulf oil spill legacy. Lancet. 2013 Aug 24;382(9893):673-4.

CONFIDENTIAL

Barron, M.G. 2012. Ecological impacts of the deepwater horizon oil spill: implications for immunotoxicity. Toxicol Pathol. 2012;40(2):315-20.

Barron, M.G., M.J. Hemmer, C.R. Jackson. 2013. Development of aquatic toxicity benchmarks for oil products using species sensitivity distributions. Integr Environ Assess Manag. 2013 Apr 2. doi: 10.1002/ieam.1420.

Barsamian, Adam L.; Perkins, Matt J.; Field, Jennifer A.; Blakemore, Paul R. Regioselective syntheses of [13C]4-labelled sodium 1-carboxy-2-(2-ethylhexyloxycarbonyl)ethanesulfonate and sodium 2-carboxy-1-(2-ethylhexyloxycarbonyl)ethanesulfonate from [13C]4-maleic anhydride Journal of Labelled Compounds and Radiopharmaceuticals (2014) Ahead of Print CODEN: JLCRD4; ISSN: 0362-4803

Baelum, J., Borglin, S., Chakraborty, R., Fortney, J.L., Lamendella, R., Mason, O.U., Auer, M., Zemla, M., Bill, M., Conrad, M.E. 2012. Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. Environmental Microbiology 14(9):2405-16.

Batchu Sudha Rani(1,2); Ramirez Cesar E.(1,2); Gardinali Piero R.(1,2) Correspondence(s): Gardinali P.R. Stability of dioctyl sulfosuccinate (DOSS) towards hydrolysis and photodegradation under simulated solar conditions Science of the Total Environment (15 May 2014), Volume 481, Number 1, pp. 260-265, 28 refs. ISSN: 0048-9697 E-ISSN: 1879-1026 DOI: 10.1016/j.scitotenv.2014.02.033 Published by: Elsevier

Bejarano, A.C., Levine, E. and Mearns, A.J. In Press. Effectiveness and potential ecological effects of offshore surface dispersant use during the Deepwater Horizon oil spill: a retrospective analysis of monitoring data. Environmental Monitoring and Assessment: In Press.

Broje V.; Gala W.R.; Nedwed T. 2013. API research on fate and effects of dispersants and dispersed oil in deepwater environment ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013).

Brandvik PJ, Johansen Ø, Leirvik F, Farooq U, Daling PS.Droplet breakup in subsurface oil releases--part 1: experimental study of droplet breakup and effectiveness of dispersant injection. Mar Pollut Bull. 2013 Aug 15;73(1):319-26. doi: 10.1016/j.marpolbul.2013.05.020. Epub 2013 Jun 21.

Brewton RA, Fulford R, Griffitt RJ.Gene Expression and Growth as Indicators of Effects of the BP Deepwater Horizon Oil Spill on Spotted Seatrout (Cynoscion nebulosus). J Toxicol Environ Health A. 2013;76(21):1198-209. doi: 10.1080/15287394.2013.848394.

Campo, P., A. D. Venosa, and M. T. Suidan. 2013.Biodegradability of Corexit 9500 and Dispersed South Louisiana Crude Oil at 5 and 25 °C. Environ. Sci. Technol., 47 (4):1960–1967.

Changkyu Joo, Won Joon Shim, Gi Beum Kim, Sung Yong Ha, Moonkoo Kim, Joon Geon An, Eunsic Kim, Beom Kim, Seung Won Jung, Young-Ok Kim, Un Hyuk Yim. 2013. Mesocosm study on weathering characteristics of Iranian Heavy crude oil with and without dispersants. Journal of Hazardous Materials, 248–249:37-46

Chase, D.A., Edwards, D.S., Qin, G., Wages, M.R., Willming, M.M., Anderson, T.A., Maul, J.D. 2013. Bioaccumulation of petroleum hydrocarbons in fiddler crabs (Uca minax) exposed to weathered MC-252 crude oil alone and in mixture with an oil dispersant. Science of The Total Environment 444:121-127.

Claireaux G, Théron M, Prineau M, Dussauze M, Merlin FX, Le Floch S. Effects of oil exposure

CONFIDENTIAL

and dispersant use upon environmental adaptation performance and fitness in the European sea bass, Dicentrarchus labrax. Aquat Toxicol. 2013 Apr 15;130-131:160-70. doi: 10.1016/j.aquatox.2013.01.004. Epub 2013 Jan 18.

Coelho,G., J.Clark, D.Aurand. 2013.Toxicity testing of dispersed oil requires adherence to standardized protocols to assess potential real world effects. Environmental Pollution, 177:185–188

Conmy, R. N., P.G. Coble , J. Farr , M.Wood , Kenneth Lee , W. Scott Pegau , Ian Walsh , Corey Koch , mary abercrombie , Scott Miles , marlon lewis , scott ryan , Brian Robinson , Thomas A King , Christopher R Kelble , and J. Lacoste.2014. Submersible optical sensors exposed to chemically-dispersed crude oil: wave tank simulations for improved oil spill monitoring Environ. Sci. Technol., Just Accepted Manuscript

Claireaux G., M. Théron, M. Prineau, M. Dussauze, F.-X. Merlin, S. Le Floch. 2013. Effects of oil exposure and dispersant use upon environmental adaptation performance and fitness in the European sea bass, Dicentrarchus labrax. Aquatic Toxicology 130–131:160-170.

Ehrenhauser, Franz S.; Avij, Paria; Shu, Xin; Dugas, Victoria; Woodson, Isaiah; Liyana-Arachchi, Thilanga; Zhang, Zenghui; Hung, Francisco R.; Valsaraj, Kalliat T. [Reprint Author] Bubble bursting as an aerosol generation mechanism during an oil spill in the deep-sea environment: laboratory experimental demonstration of the transport pathway. Environmental Science-Processes & Impacts, (2014) Vol. 16, No. 1, pp. 65-73. http://rsc.li/process-impacts. ISSN: 2050-7887. E-ISSN: 2050-7895.

Field J.A.; Place B.J.; Perkins M.2013. Analytical tools for unraveling the complex mixture of oil dispersants ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013

Fitzgerald Timothy Patrick and Gohlke Julia M.2014. Contaminant levels in Gulf of Mexico reef fish after the Deepwater Horizon oil spill as measured by a fishermen-led testing program Environmental Science & Technology Just Accepted Manuscript

Fuller, C.B., J. S. Bonner, M. S. Islam, C. Page, T. Ojo, W. Kirkey. 2013.Estimating sub-surface dispersed oil concentration using acoustic backscatter response. Marine Pollution Bulletin, Volume 70, Issues 1–2, 15 May 2013, Pages 140-146 (ExxonMobil Biblio Jul10)

Gardinali P.R.; Echols B.; Rand G.M.; Benkinney M; Stubblefield W.; Pelz O.X..2013. Influence of Deepwater Horizon field-collected oil characteristics and exposure conditions on aquatic toxicity test results ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013)

Gardiner., W.,J.Q. Word, J.D. Word, R.A. Perkins, K.M. McFarlin, B.W. Hester, L.S. Word, and C.M. Ray CM.2013. The acute toxicity of chemically and physically dispersed crude oil to key arctic species under arctic conditions during the open water season. Environ Toxicol Chem. 32(10):2284–2300.

Glover C.M.; Parker A.M.; Linden K.G.; Rosario-Ortiz F.L.2013. Organic matter in ocean water as a proxy for indirect photochemical degradation of the dispersants used in the Deepwater Horizon oil spill ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013)

Gong Yanyan(1); Zhao Xiao(1); Qian Tianwei(1,2); Zhao Dongye(1); O'Reilly S.E.(3) Effects of oil dispersant and oil on sorption and desorption of phenanthrene with Gulf Coast marine sedimentsEnviron. Pollut. (2014), Volume 185, pp. 240-249, 39 refs.

Gong, Yanyan; Zhao, Xiao; Cai, Zhengqing; O'Reilly, S. E.; Hao, Xiaodi; Zhao, DongyeA review

CONFIDENTIAL

of oil, dispersed oil and sediment interactions in the aquatic environment: Influence on the fate, transport and remediation of oil spillsMarine Pollution Bulletin (2014) Ahead of Print CODEN: MPNBAZ; ISSN: 0025-326X

Gong Y.; Zhao X.; Zhao D.; O'Reilly S.E.2013. Effects of oil dispersants on sorption capacity and hysteresis of polycyclic aromatic hydrocarbons with Gulf Coast marine sediments ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013),

Gong Y.; Zhao X.; Zhao D.; O'Reilly S.E.2013. Effects of oil dispersants and sediment sorption on photodegradation and ozonation of persistent oil compounds in the Gulf Coast ecosystems. ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013

Goodbody-Gringley G, Wetzel DL, Gillon D, Pulster E, Miller A, et al. 2013. Toxicity of Deepwater Horizon Source Oil and the Chemical Dispersant, Corexit® 9500, to Coral Larvae. PLoS ONE 8(1): e45574. doi:10.1371/journal.pone.0045574

Gray JL, Kanagy LK, Furlong ET, Kanagy CJ, McCoy JW, Mason A, Lauenstein G. 2013.Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. Chemosphere. 2014 Jan;95:124-30. doi: 10.1016/j.chemosphere.2013.08.049. Epub 2013 Sep 17.

Hazen T.C. 2013. Deepwater Horizon oil spill: A systems biology approach to an ecological disaster ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013)

Johansen, Ø. P. Brandvik, U. Farooq. 2013. Droplet breakup in subsea oil releases – Part 2: Predictions of droplet size distributions with and without injection of chemical dispersants Marine Pollution Bulletin, 73(1):327-335.

Joo, Changkyu / Shim, Won Joon / Kim, Gi Beum / Ha, Sung Yong / Kim, Moonkoo / An, Joon Geon / Kim, Eunsic / (...) / Yim, Un Hyuk,2013.Mesocosm study on weathering characteristics of Iranian Heavy crude oil with and without dispersants Journal of Hazardous Materials, 248-249, p.37-46, Mar 2013

King, T., J. Clyburne, K. Lee, B. Robinson. 2013.Interfacial film formation: Influence on oil spreading rates in lab basin tests and dispersant effectiveness testing in a wave tank Marine Pollution Bulletin 71 (1–2):83-91.

Kover S.C.(1); Rosario-Ortiz F.L.(1); Linden K.G.(1) Correspondence(s): Linden K.G.(1) Photochemical fate of solvent constituents of Corexit oil dispersants Water Research (2014), Volume 52, Number 1, pp. 101-111, 6 Tables and spectra, 28 refs. CODEN: WATRAG ISSN: 0043-1354 DOI: 10.1016/j.watres.2013.12.013 Published by: Elsevier Ltd, Langford Lane, Kidlington, OX5 1GB, Oxford, United Kingdom

Kuhl, Adam J.; Nyman, J. Andrew; Kaller, Michael D.; Green, Christopher C. 2013. Dispersant and salinity effects on weathering and acute toxicity of South Louisiana crude oilEnviron Toxicol Chem 2013;32:2611-2620. © 2013 SETAC.

Lavallee, JL; Bugden, JBC; Kepkay, PE; Lee, K Fluorescence fingerprinting of oils dispersed in seawater: application of ultraviolet fluorescence (UVF) and excitation-emission matrix spectroscopy (EEMS) Canadian technical report of fisheries and aquatic sciences/Rapport technique canadien des sciences halieutiques et aquatiques [Can. Tech. Rep. Fish. Aquat. Sci./Rapp. Tech. Can. Sci. Halieut. Aquat.]. no. 2997, 212 pp. 2012. ISSN: 0706-6457 Published by: DFO, Dartmouth, NS (Canada), [mailto:info@dfo-mpo.gc.ca] URL (Document): http://www.dfo-mpo.gc.ca/Library/346586.pdf URL

ED_001324_00001453-00036

(Document): http://www.dfo-mpo.gc.ca/

Lee KW, Shim WJ, Yim UH, Kang JH.Acute and chronic toxicity study of the water accommodated fraction (WAF), chemically enhanced WAF (CEWAF) of crude oil and dispersant in the rock pool copepod Tigriopus japonicus. Chemosphere 92(9):1161-8.

Lee, K., T. Nedwed, D. Palandro, R. Prince. 2013.Lab tests on the biodegradation of chemically dispersed oil should consider the rapid dilution that occurs at sea Mar. Pollut. Bull. 73 (1) 314-318.

Lewis M, Pryor R.2013.Toxicities of oils, dispersants and dispersed oils to algae and aquatic plants: review and database value to resource sustainability. Environ Pollut. 2013 Sep;180:345-67. doi:

Liyana-Arachchi TP, Zhang Z, Ehrenhauser FS, Avij P, Valsaraj KT, Hung FR.Bubble bursting as an aerosol generation mechanism during an oil spill in the deep-sea environment: molecular dynamics simulations of oil alkanes and dispersants in atmospheric air/salt water interfaces. Environ Sci Process Impacts. 2014 Jan;16(1):53-64. doi: 10.1039/c3em00391d.10.1016/j.envpol.2013.05.001. Epub 2013 Jun 14.

Macías-Zamora JV, Meléndez-Sánchez AL, Ramírez-Álvarez N, Gutiérrez-Galindo EA, Orozco-Borbón MV. 2013. On the effects of the dispersant Corexit 9500© during the degradation process of n-alkanes and PAHs in marine sediments. Environ Monit Assess. 2014 Feb;186(2):1051-61. doi: 10.1007/s10661-013-

Martin JD, Adams J, Hollebone B, King T, Brown RS, Hodson PV. Chronic toxicity of heavy fuel oils to fish embryos using multiple exposure scenarios. Environ Toxicol Chem. 2014 Mar;33(3):677-87. doi: 10.1002/etc.2486. Epub 2014 Jan 24.

McFarlin KM, Prince RC, Perkins R, Leigh MB. Biodegradation of dispersed oil in Arctic seawater at -1°C. PLoS One. 2014 Jan 8;9(1):e84297. doi: 10.1371/journal.pone.0084297. eCollection 2014.3438-2. Epub 2013 Oct 27.

Mendoza,W., D. Riemer and R. Zika 2013. Application of fluorescence and PARAFAC to assess vertical distribution of subsurface hydrocarbons and dispersant during the Deepwater Horizon oil spill Environ. Sci.: Processes Impacts, 2013,15, 1017-1030

Milinkovitch T, Imbert N, Sanchez W, Le Floch S, Thomas-Guyon H. Toxicological effects of crude oil and oil dispersant: biomarkers in the heart of the juvenile golden grey mullet (Liza aurata). Ecotoxicol Environ Saf. 2013 Feb;88:1-8. doi: 10.1016/j.ecoenv.2012.10.029. Epub 2012 Dec 4.

Milinkovitch T, Thomas-Guyon H, Lefrançois C, Imbert N.2013. Dispersant use as a response to oil spills: toxicological effects on fish cardiac performance.Fish Physiol Biochem. 2013 Apr;39(2):257-62. doi: 10.1007/s10695-012-9696-z. Epub 2012 Jul 28.

Montagna PA, Baguley JG, Cooksey C, Hartwell I, Hyde LJ, Hyland JL, Kalke RD, Kracker LM, Reuscher M, Rhodes ACE. 2013. Deep-Sea Benthic Footprint of the Deepwater Horizon Blowout. PLoS ONE 8(8):e70540. Find it Online*

Montagna, P.A., J.G. Baguley, C. Cooksey, and J.L. Hyland. 2013. Deepwater Horizon Oil Spill: Assessment of Potential Impacts on the Deep Soft-Bottom Benthos. Interim Data Summary Report. NOAA Technical Memorandum NOS NCCOS 166. NOAA, National Centers for Coastal Ocean Science, Charleston, SC. 32 p.

Moody, R.M., J. Cebrian and K. Heck Jr. 2013. Interannual Recruitment Dynamics for Resident and Transient Marsh Species: Evidence for a Lack of Impact by the Macondo Oil Spill PLoS ONE 8(3):e58376

ED_001324_00001453-00037

Nedwed T.; Coolbaugh T. 2013. Industry-sponsored subsea dispersant injection research ACS National Meeting Book of Abstracts; American Chemical Society - 245th ACS National Meeting and Exposition (2013)

Olsen, G.H., C. Klok, A.J. Hendriks, P. Geraudie, L. De Hoop, F. De Laender, E. Farmen, B.E. Grøsvik, B.H. Hansen, M. Hjorth, C.R. Jansen, T. Nordtug, E. Ravagnan, K. Viaene, J. Carroll.2013. Toxicity data for modeling impacts of oil components in an Arctic ecosystem. Marine Environmental Research 90: 9-17.

Ozhan K, Miles SM, Gao H, Bargu S. Relative Phytoplankton growth responses to physically and chemically dispersed South Louisiana sweet crude oil. Environ Monit Assess. 2014 Feb 12. [Epub ahead of print]

Ozhan K, Bargu S. Distinct responses of Gulf of Mexico phytoplankton communities to crude oil and the dispersant corexit(®) Ec9500A under different nutrient regimes. Ecotoxicology. 2014 Apr;23(3):370-84. doi: 10.1007/s10646-014-1195-9. Epub 2014 Jan 28.

Paris CB, Hénaff ML, Aman ZM, Subramaniam A, Helgers J, Wang DP, Kourafalou VH, Srinivasan A. Evolution of the Macondo well blowout: simulating the effects of the circulation and synthetic dispersants on the subsea oil transport. Environ Sci Technol. 2012 Dec 18;46(24):13293-302. doi: 10.1021/es303197h. Epub 2012 Dec 3. (NOTE A 2012 PAPER included because of published comments by AMMAN (2013) and ADAMS (2013))

Paul, I. 2013. Australian revised National Plan policy and process for the recognition, application and monitoring of oil spill control agents, including dispersants. Proceedings of the 36th AMOP Technical Seminar on Environmental Contamination and Response (2013), pp. 797-808.

Paul, J.H., D. Hollander, P. Coble, K. Daly, S. Murasko, D. English, J. Basso, J. Delaney, L. McDaniel, and C. Kovach. 2013. Toxicity and Mutagenicity of Gulf of Mexico Waters During and After The Deepwater Horizon Oil Spill Environmental Science & Technology 47(17):9651-9.

Peavler B. 2013. Environment Arctic: Advancing Arctic oil spill response. Petroleum Review. 67(798): 24-25.

Perkins, M., B. Place, J. Field. 2013. LVI-LC-MS/MS analysis of Corexit in GOM water column and sediments. Abstracts, 68th Northwest Regional Meeting of the American Chemical Society, Corvallis, OR, United States, July 21-24 (2013), NORM-18. American Chemical Society: Washington.

Perrons, R.K..2013. Assessing the damage caused by Deepwater Horizon: Not just another Exxon Valdez Marine Pollution Bulletin 71(1-2):20-2.

Place, Benjamin J.; Perkins, Matt J.; Sinclair, Ewan; Barsamian, Adam L.; Blakemore, Paul R.; Field, Jennifer A. Trace analysis of surfactants in Corexit oil dispersant formulations and seawater Deep Sea Research, Part II: Topical Studies in Oceanography (2014) Ahead of Print CODEN: DSROEK; ISSN: 0967-0645

Polli, J.R.; Zhang, Y; Pan, X. 2013. Investigating crude oil-dispersant mixture-induced reproductive toxicity and its mechanism in C. elegans Abstracts of Papers, 246th ACS National Meeting & Exposition, Indianapolis, IN, ENVR-174.

Powers, Sean P.; Hernandez, Frank J.; Condon, Robert H.; Marcus Drymon, J.; Free, Christopher M. 2013. Novel pathways for injury from offshore oil spills: direct, sublethal and indirect effects of the Deepwater Horizon oil spill on pelagic Sargassum communities. PLoS One (2013), 8(9), e74802 CODEN: POLNCL; ISSN: 1932-6203

CONFIDENTIAL

Prince RC, Butler JD. 2013. A protocol for assessing the effectiveness of oil spill dispersants in stimulating the biodegradation of oil. Environ Sci Pollut Res Int. 2013 Aug 13. [Epub ahead of print]

Prince RC, McFarlin KM, Butler JD, Febbo EJ, Wang FC, Nedwed TJ.The primary biodegradation of dispersed crude oil in the sea. Chemosphere 90(2):521-6.

Radniecki, T.S., M. Schneider, L. Semprini. 2013. The influence of Corexit 9500A and weathering on Alaska North Slope crude oil toxicity to the ammonia oxidizing bacterium, Nitrosomonas europaea Marine Pollution Bulletin 68(1–2): 64-70.

Ramirez, C., S.R. Batchu, P.R. Gardinali. 2013. High sensitivity liquid chromatography tandem mass spectrometric methods for the analysis of dioctyl sulfosuccinate in different stages of an oil spill response monitoring effort. Analytical and Bioanalytical Chemistry 405(12):4167-4175.

Reichert, M.D., L.M. Walker LM. 2013. Interfacial Tension Dynamics, Interfacial Mechanics, and Response to Rapid Dilution of Bulk Surfactant of a Model Oil-Water-Dispersant System. Langmuir 29(6):1857-1867.

Rico-Martínez R, T.W. Snell, T.L. Shearer. 2013. Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A® to the Brachionus plicatilis species complex (Rotifera). Environmental Pollution 173:5-10.

Saha, A., A. Nikova, P. Venkataraman, V.T. John, A. Bose A. 2013.Oil emulsification using surface-tunable carbon black particles. ACS Appl Mater Interfaces5(8):3094-100.

Sammarco, P.W., S.R. Kolian, R.A. Warby, J.L. Bouldin, W.A. Subra, S.A. Porter SA. 2013. Distribution and concentrations of petroleum hydrocarbons associated with the BP/Deepwater Horizon Oil Spill, Gulf of Mexico. Marine Pollution Bulletin: In Press.

Schultz, C. 2013 Monitoring and Modeling the Deepwater Horizon Oil Spill: A Record-Breaking Enterprise.Eos, Transactions American Geophysical Union 94(20):185–186.

Schrope, M. 2013. Researchers debate oil-spill remedy. Nature 493(7433):461.

Spier, C., W. Stringfellow, T.C. Hazen, M. Conrad. 2013.Distribution of hydrocarbons released during the 2010 MC252 oil spill in deep offshore waters Environmental Pollution 173: 224-230.

Stokstad, E. 2013. BP Research Dollars Yield Signs of Cautious Hope. Science 339(6120):636-637.

Venosa, A., E.L. Holder. 2013. Determining the dispersibility of South Louisiana crude oil by eight oil dispersant products listed on the NCP Product Schedule. Marine Pollution Bulletin 66(1–2): 73-77.

Van Scoy AR, Voorhees J, Anderson BS, Philips BM, Tjeerdema RS. 2013.Use of semipermeable membrane devices (SPMDs) to characterize dissolved hydrocarbon fractions of both dispersed and undispersed oil. Environ Sci Process Impacts. 2013 Oct 23;15(11):2016-22. doi: 10.1039/c3em00275f.

Venkataraman P, J. Tang, E. Frenkel, G.L. McPherson, J. He, S.R. Raghavan, V. Kolesnichenko, A. Bose, V.T. John. 2013. Attachment of a hydrophobically modified biopolymer at the oil-water interface in the treatment of oil spills. ACS Appl Mater Interfaces. 2013 May;5(9):3572-80.

Vilcaez J, Li L, Hubbard S. 2013. A new model for the biodegradation kinetics of oil droplets: application to the Deepwater Horizon oil spill in the Gulf of Mexico. Geochemical

CONFIDENTIAL

Transactions 14(1):4. Find it Online*

Wang, W., Y. Zheng, K. Lee2013. Chemical dispersion of oil with mineral fines in a low temperature environment Marine Pollution Bulletin 72(1): 205-12.

Zhang, Y.Q., D.L. Chen, A.C. Ennis, J.R. Polli, P. Xiao, B.H. Zhang, E.J. Stellwag, A. Overton, X.P. Pan. 2013. Chemical dispersant potentiates crude oil impacts on growth, reproduction, and gene expression in Caenorhabditis elegans. Archives of Toxicology 87(2):371-382.

Zhang, Y., Polli, J. Pan, X. 2013. Oil/dispersant induced the aberrant expression of miRNAs in C. elegans: Implications in reproductive toxicity. Abstracts of Papers, 246th ACS National Meeting & Exposition, Indianapolis, IN

Zhang, Y., Pan, X. 2013.Chemical dispersant synergy the Deepwater Horizon oil spill's toxicity on the growth, reproduction, and gene expression of Caenorhabditis elegans. Abstracts of Papers American Chemical Society, (AUG 19 2012) Vol. 244, pp. 37-ENVR. Meeting Info.: 244th National Fall Meeting of the American-Chemical-Society (ACS). Philadelphia, PA, USA. August 19 -23, 2012. Amer Chem Soc, Div Hist Chem; Amer Chem Soc. CODEN: ACSRAL. ISSN: 0065-7727.

Ziervogel, K., and Arnosti, C., 2013. Enhanced protein and carbohydrate hydrolysis in plume-associated deep waters initially sampled during the early stages of the Deepwater Horizon oil spill. Deep Sea Research Part II: Topical Studies in Oceanography: In Press.

Zhou, Z., Z. Liu, L. Guo. 2013. Chemical evolution of Macondo crude oil during laboratory degradation as characterized by fluorescence EEMs and hydrocarbon composition Marine Pollution Bulletin 66(1–2):164-175.

Zhou, Z., G. Laodong, A.M. Shiller, S.E. Lohrenz, V.L. Asper, C.L. Osburn. 2013. Characterization of oil components from the Deepwater Horizon oil spill in the Gulf of Mexico using fluorescence EEM and PARAFAC techniques. Marine Chemistry Volume 148:10–21.

Zuijdgeest A., and M. Huettel. 2012. Dispersants as Used in Response to the MC252-Spill Lead to Higher Mobility of Polycyclic Aromatic Hydrocarbons in Oil-Contaminated Gulf of Mexico Sand. PLoS ONE 7(11): e50549. doi:10.1371/journal.pone.0050549

CONFIDENTIAL

Appendix 1 – Bibliography to August 31, 2015.

Almeda R, Hyatt C, Buskey EJ. 2014. Toxicity of dispersant Corexit 9500A and crude oil to marine microzooplankton. Ecotoxicol. Environ. Saf. 106C:76-85

Almeda R, Baca S, Hyatt C, Buskey EJ. Ingestion and sublethal effects of physically and chemically dispersed crude oil on marine planktonic copepods.Ecotoxicology. 2014 Aug;23(6):988-1003. doi: 10.1007/s10646-014-1242-6. Epub 2014 Apr 23.Free PMC Article

Almeda R, Bona S, Foster CR, Buskey EJ. Dispersant Corexit 9500A and chemically dispersed crude oil decreases the growth rates of meroplanktonic barnacle nauplii (Amphibalanus improvisus) and tornaria larvae (Schizocardium sp.).Mar Environ Res. 2014 Aug;99:212-7. doi: 10.1016/j.marenvres.2014.06.007. Epub 2014 Jun 24.

Almeda R, Connelly TL, Buskey EJ. Novel insight into the role of heterotrophic dinoflagellates in the fate of crude oil in the sea.Sci Rep. 2014 Dec 19;4:7560. doi: 10.1038/srep07560.Free PMC Article

Almeda R, Hyatt C, Buskey EJ. Toxicity of dispersant Corexit 9500A and crude oil to marine microzooplankton.Ecotoxicol Environ Saf. 2014 Aug;106:76-85. doi: 10.1016/j.ecoenv.2014.04.028. Epub 2014 May 14.

Anderson Lively JA, McKenzie J. Toxicity of the dispersant Corexit 9500 to early life stages of blue crab, Callinectes sapidus.Bull Environ Contam Toxicol. 2014 Dec;93(6):649-53. doi: 10.1007/s00128-014-1370-y. Epub 2014 Aug 31.

Almeda R, Wambaugh Z, Chai C, Wang Z, Liu Z, Buskey EJ. Effects of crude oil exposure on bioaccumulation of polycyclic aromatic hydrocarbons and survival of adult and larval stages of gelatinous zooplankton.PLoS One. 2013 Oct 7;8(10):e74476. doi: 10.1371/journal.pone.0074476. eCollection 2013.Free PMC Article

Almeda R, Wambaugh Z, Wang Z, Hyatt C, Liu Z, Buskey EJ. Interactions between zooplankton and crude oil: toxic effects and bioaccumulation of polycyclic aromatic hydrocarbons.PLoS One. 2013 Jun 28;8(6):e67212. doi: 10.1371/journal.pone.0067212. Print 2013.Free PMC Article

Awkerman, JA, Raimondo S, Jackson CR, Barron MG. 2014. Augmenting aquatic species sensitivity distributions with interspecies toxicity estimation models. Environ. Toxicol. Chem. 33(3):688-95.

Bacosa HP, Erdner DL, Liu Z. Differentiating the roles of photooxidation and biodegradation in the weathering of Light Louisiana Sweet crude oil in surface water from the Deepwater Horizon site.Mar Pollut Bull. 2015 Jun 15;95(1):265-72. doi: 10.1016/j.marpolbul.2015.04.005. Epub 2015 Apr 18.

Barsamian AL, Perkins MJ, Field JA, Blakemore PR. Regioselective syntheses of [13C]4-labelled sodium 1-carboxy-2-(2-ethylhexyloxycarbonyl)ethanesulfonate and sodium 2-carboxy-1-(2-ethylhexyloxycarbonyl)ethanesulfonate from [13C]4-maleic anhydride.J Labelled Comp Radiopharm. 2014 May 15;57(5):397-401. doi: 10.1002/jlcr.3189. Epub 2014 Feb 12. Erratum in: J Labelled Comp Radiopharm. 2014 Aug;57(10):625.Free PMC Article

Batchu SR, Ramirez CE, Gardinali PR. Stability of dioctyl sulfosuccinate (DOSS) towards hydrolysis and photodegradation under simulated solar conditions.Sci Total Environ.

ED_001324_00001453-00041

2014 May 15;481:260-5. doi: 10.1016/j.scitotenv.2014.02.033. Epub 2014 Mar 4.

Benner SW, John VT, Hall CK. Intercomparison of oil spill prediction models for accidental blowout scenarios with and without subsea chemical dispersant injection.J Phys Chem B. 2015 Jun 11;119(23):6979-90. doi: 10.1021/acs.jpcb.5b01092. Epub 2015 Jun 1.

Bejarano AC, Clark JR, Coelho GM. Issues and challenges with oil toxicity data and implications for their use in decision making: a quantitative review.Environ Toxicol Chem. 2014 Apr;33(4):732-42. doi: 10.1002/etc.2501. Epub 2014 Feb 25. Review.

Bejarano AC, Barron MG. Development and practical application of petroleum and dispersant interspecies correlation models for aquatic species.Environ Sci Technol. 2014 Apr 15;48(8):4564-72. doi: 10.1021/es500649v. Epub 2014 Mar 28.

Bejarano AC, Barron MG. 2014. Development and Practical Application of Petroleum and Dispersant Interspecies Correlation Models for Aquatic Species. Environ Sci Technol. 2014 Mar 28. [Epub ahead of print]

Bejarano AC, Clark JR, Coelho GM. 2014 Issues and challenges with oil toxicity data and implications for their use in decision making: A quantitative review. Environ Toxicol Chem. 2014 Apr;33(4):732-42. doi: 10.1002/etc.2501. Epub 2014 Feb 25.

Bookstaver M, Bose A, Tripathi A. Interaction of Alcanivorax borkumensis with a Surfactant Decorated Oil-Water InterfaceLangmuir. 2015 Jun 2;31(21):5875-81. doi: 10.1021/acs.langmuir.5b00688. Epub 2015 May 21.

Brewton RA, Fulford R, Griffitt RJ. Gene expression and growth as indicators of effects of the BP Deepwater Horizon oil spill on spotted seatrout (Cynoscion nebulosus). J Toxicol Environ Health A. 2013;76(21):1198-209. doi: 10.1080/15287394.2013.848394.

Cohen JH, McCormick LR, Burkhardt SM. Effects of dispersant and oil on survival and swimming activity in a marine copepod.Bull Environ Contam Toxicol. 2014 Apr;92(4):381-7. doi: 10.1007/s00128-013-1191-4. Epub 2014 Jan 9.

COOLBAUGH, T; DEMICCO, E; KENNEDY, E DISPERSANT-RELATED OIL SPILL RESPONSE COMMUNICATION TOOLS: TOWARD AN ENHANCED APPROACH TO CONVEYING COMPLEX TOPICS IN AN APPROACHABLE MANNER INTERNATIONAL OIL SPILL CONFERENCE [IOSC] (Savannah, GA, 5/5-8/2014) PROCEEDINGS v.2014, no.1, pp.1163-1171, May 2014. (ISSN 2169-3358; Available at http://iosc.org as of 11/7/2014) Laursen April 21 2015

Cornwall W. Critics question plans to spray dispersant in future deep spillsScience. 2015 Apr 3;348(6230):27. doi: 10.1126/science.348.6230.27. Epub 2015 Apr 2. No abstract available.

Daling PS, Leirvik F, Almås IK, Brandvik PJ, Hansen BH, Lewis A, Reed M. Surface weathering and dispersibility of MC252 crude oil.Mar Pollut Bull. 2014 Oct 15;87(1-2):300-10. doi: 10.1016/j.marpolbul.2014.07.005. Epub 2014 Aug 22

Dussauze M, Pichavant-Rafini K, Le Floch S, Lemaire P, Theron M. Acute toxicity of chemically and mechanically dispersed crude oil to juvenile sea bass (Dicentrarchus labrax): Absence of synergistic effects between oil and dispersants.Environ Toxicol Chem. 2015 Jul;34(7):1543-51. doi: 10.1002/etc.2931. Epub 2015 May 7.

Elarbaoui S, Richard M, Boufahja F, Mahmoudi E, Thomas-Guyon H. Effect of crude oil exposure and dispersant application on meiofauna: an intertidal mesocosm

ED_001324_00001453-00042

experiment.Environ Sci Process Impacts. 2015 May;17(5):997-1004. doi: 10.1039/c5em00051c. Epub 2015 May 7.

Fu J, Gong Y, Zhao X, O'Reilly SE, Zhao D. Effects of oil and dispersant on formation of marine oil snow and transport of oil hydrocarbons.Environ Sci Technol. 2014 Dec 16;48(24):14392-9. doi: 10.1021/es5042157. Epub 2014 Dec 4.

Garcia, Tzintzuni I.; Shen, Yingjia; Crawford, Douglas; Oleksiak, Marjorie F.; Whitehead, Andrew; Walter, Ronald B. 2012. RNA-Seq reveals complex genetic response to Deepwater Horizon oil release in Fundulus grandis. BMC Genomics (2012), 13, 474 CODEN: BGMEET; ISSN: 1471-2164

Garr AL, Laramore S, Krebs W. Toxic effects of oil and dispersant on marine microalgae.Bull Environ Contam Toxicol. 2014 Dec;93(6):654-9. doi: 10.1007/s00128-014-1395-2. Epub 2014 Oct 5.

Kleindienst S, Paul JH, Joye SB. Using dispersants after oil spills: impacts on the composition and activity of microbial communities.Nat Rev Microbiol. 2015 Jun;13(6):388-96. doi: 10.1038/nrmicro3452. Epub 2015 May 6. Review.

Kleindienst S, Grim S, Sogin M, Bracco A, Crespo-Medina M, Joye SB. Diverse, rare microbial taxa responded to the Deepwater Horizon deep-sea hydrocarbon plume.ISME J. 2015 Jul 31. doi: 10.1038/ismej.2015.121. [Epub ahead of print]

Koyama, J., Chie Imakado, Seiichi Uno, Takako Kuroda, Shouichi Hara, Takahiro Majima, Hideyuki Shirota, Nathaniel C. Añasco. 2014. Simulated distribution and ecotoxicity-based assessment of chemically-dispersed oil in Tokyo Bay. Marine Pollution Bulletin

Lindo-Atichati, C.B. Paris, M. Le Hénaff, M. Schedler, A.G. Valladares Juárez, R. Müller. 2014. Simulating the effects of droplet size, high-pressure biodegradation, and variable flow rate on the subsea evolution of deep plumes from the Macondo blowout. Deep Sea Research Part II: Topical Studies in Oceanography, In Press, Corrected Proof, Available online 30 January 2014

Marti, M. E.; Colonna, W. J.; Patra, P.; Zhang, H.; Green, C.; Reznik, G.; Pynn, M.; Jarrell, K.; Nyman, J. A.; Somasundaran, P.; Glatz, C. E.; Lamsal, B. P. 2014. Production and characterization of microbial biosurfactants for potential use in oil-spill remediation Enzyme and Microbial Technology (2014), 55, 31-39 CODEN: EMTED2; ISSN: 0141-0229

McDaniel LD, Basso J, Pulster E, Paul JH. Sand patties provide evidence for the presence of Deepwater Horizon oil on the beaches of the West Florida Shelf.Mar Pollut Bull. 2015 Aug 15;97(1-2):67-77. doi: 10.1016/j.marpolbul.2015.06.032. Epub 2015 Jun 20.

Mu J, Jin F, Ma X, Lin Z, Wang J. Comparative effects of biological and chemical dispersants on the bioavailability and toxicity of crude oil to early life stages of marine medaka (Oryzias melastigma).Environ Toxicol Chem. 2014 Nov;33(11):2576-83. doi: 10.1002/etc.2721. Epub 2014 Sep 16.

Not Known ?? Simulation Study of Hydrophobically Modified Chitosan as an Oil Dispersant Additive

Nørregaard RD, Gustavson K, Møller EF, Strand J, Tairova Z, Mosbech A. Ecotoxicological

CONFIDENTIAL

investigation of the effect of accumulation of PAH and possible impact of dispersant in resting high arctic copepod Calanus hyperboreus.Aquat Toxicol. 2015 Jul 19;167:1-11. doi: 10.1016/j.aquatox.2015.07.006. [Epub ahead of print]

Pham PH, Huang YJ, Chen C, Bols NC. 2013. Corexit 9500 inactivates two enveloped viruses of aquatic animals but enhances the infectivity of a non-enveloped fish virus. Appl Environ Microbiol. 2013 Nov 22. [Epub ahead of print]

Pie HV, Mitchelmore CL. Acute toxicity of current and alternative oil spill chemical dispersants to early life stage blue crabs (Callinectes sapidus).Chemosphere. 2015 Jun;128:14-20. doi: 10.1016/j.chemosphere.2015.01.008.

Pietroski JP, White JR, DeLaune RD.Effects of dispersant used for oil spill remediation on nitrogen cycling in Louisiana coastal salt marsh soil.Chemosphere. 2014 Aug 11;119C:562-567. doi: 10.1016/j.chemosphere.2014.07.050. [Epub ahead of print]

Polli, Joseph Ryan; Zhang, Yanqiong; Pan, Xiaoping 2014 Dispersed crude oil amplifies germ cell apoptosis in Caenorhabditis elegans, followed a CEP-1-dependent pathway Archives of Toxicology (2014), 88(3), 543-551 CODEN: ARTODN; ISSN: 0340-5761

Prince RC. Oil spill dispersants: boon or bane?Environ Sci Technol. 2015 Jun 2;49(11):6376-84. doi: 10.1021/acs.est.5b00961. Epub 2015 May 15.

Socolofsky SA, Adams EE, Boufadel MC, Aman ZM, Johansen Ø, Konkel WJ, Lindo D, Madsen MN, North EW, Paris CB, Rasmussen D, Reed M, Rønningen P, Sim LH, Uhrenholdt T, Anderson KG, Cooper C, Nedwed TJ.Mar Pollut Bull. 2015 Jul 15;96(1-2):110-26. doi: 10.1016/j.marpolbul.2015.05.039. Epub 2015 May 26.

Studivan MS, Hatch WI, Mitchelmore CL. Responses of the soft coral Xenia elongata following acute exposure to a chemical dispersant.Springerplus. 2015 Feb 13;4:80. doi: 10.1186/s40064-015-0844-7. eCollection 2015. Free PMC Article

Ramachandran G, Huynh T, Banerjee S, Groth C, Stenzel M, Blair A, Engel L, Sandler D, Kwok R, Stewart P. 0304 The NIEHS GuLF STUDY: Estimate of workers' exposures through the inhalation route on seven response vessels near the well-site during the Deepwater Horizon oil spill.Occup Environ Med. 2014 Jun;71 Suppl 1:A105. doi: 10.1136/oemed-2014-102362.329.

Rial D, Vázquez JA, Murado MA. 2013 Toxicity of spill-treating agents and oil to sea urchin embryos. Sci Total Environ. 2013 Nov 29;472C:302-308. doi: 10.1016/j.scitotenv.2013.11.030. [Epub ahead of print]

Riehm DA, McCormick AV. The role of dispersants' dynamic interfacial tension in effective crude oil spill dispersion.Mar Pollut Bull. 2014 Jul 15;84(1-2):155-63. doi: 10.1016/j.marpolbul.2014.05.018. Epub

Rooker, Jay R. [Reprint Author]; Kitchens, Larissa L.; Dance, Michael A.; Wells, R. J. David; Falterman, Brett; Cornic, Maelle 2013. Spatial, Temporal, and Habitat-Related Variation in Abundance of Pelagic Fishes in the Gulf of Mexico: Potential Implications of the Deepwater Horizon Oil Spill. PLoS One, (OCT 10 2013) Vol. 8, No. 10, pp. Article No.: e76080. http://ukpmc.ac.uk/journals/1217/. ISSN: 1932-6203. E-ISSN: 1932-6203.

Shi, Rujie; Yu, Kewei 2014. Impact of exposure of crude oil and dispersant (COREXIT EC 9500A) on denitrification and organic matter mineralization in a Louisiana salt marsh sediment Chemosphere (2014) Ahead of Print CODEN: CMSHAF; ISSN: 0045-6535

CONFIDENTIAL

Sørensen L, Melbye AG, Booth AM. 2013. Oil droplet interaction with suspended sediment in the seawater column: Influence of physical parameters and chemical dispersants. Mar Pollut Bull. 2013 Nov 18. pii: S0025-326X(13)00670-X. doi: 10.1016/j.marpolbul.2013.10.049. [Epub ahead of print]

Steffy, David A. [Reprint Author]; Nichols, Alfred C.; Morgan, L. Joe; Gibbs, Rachael 2013. Evidence that the Deepwater Horizon Oil Spill Caused a Change in the Nickel, Chromium, and Lead Average Seasonal Concentrations Occurring in Sea Bottom Sediment Collected from the Eastern Gulf of Mexico Continental Shelf Between the Years 2009 and 2011. Water Air and Soil Pollution, (NOV 2013) Vol. 224, No. 11, pp. Article No.: 1756. http://www.springerlink.com/content/100344/. CODEN: WAPLAC. ISSN: 0049-6979. E-ISSN: 1573-2932.

Tansel,B., Mengshan Lee, Jillian Berbakov, Derya Z. Tansel, Urpiana Koklonis 2014. Dispersion of Louisiana crude oil in salt water environment by Corexit 9500A in the presence of natural coastal materials Estuarine, Coastal and Shelf Science, Volume 143, 20 April 2014, Pages 58-64

Temkin AM, Bowers RR, Magaletta ME, Holshouser S, Maggi A, Ciana P, Guillette LJ, Bowden JA, Kucklick JR, Baatz JE, Spyropoulos DD. Effects of Crude Oil/Dispersant Mixture and Dispersant Components on PPARγ Activity in Vitro and in Vivo: Identification of Dioctyl Sodium Sulfosuccinate (DOSS; CAS #577-11-7) as a Probable Obesogen.Environ Health Perspect. 2015 Jul 2. [Epub ahead of print]Free Article

Vikebø FB, Rønningen P, Meier S, Grøsvik BE, Lien VS. Dispersants have limited effects on exposure rates of oil spills on fish eggs and larvae in shelf seas.Environ Sci Technol. 2015 May 19;49(10):6061-9. doi: 10.1021/acs.est.5b00016. Epub 2015 Apr 30.

Xu Y, Duncan FE, Xu M, Woodruff TK. Use of an organotypic mammalian in vitro follicle growth assay to facilitate female reproductive toxicity screening.Reprod Fertil Dev. 2015 Feb 18. doi: 10.1071/RD14375. [Epub ahead of print]

Zhao X, Gong Y, O'Reilly SE, Zhao D. Effects of oil dispersant on solubilization, sorption and desorption of polycyclic aromatic hydrocarbons in sediment-seawater systems.Mar Pollut Bull. 2015 Mar 15;92(1-2):160-9. doi: 10.1016/j.marpolbul.2014.12.042. Epub 2015 Jan 20.

Ortmann AC, Lu Y. Initial community and environment determine the response of bacterial communities to dispersant and oil contamination.Mar Pollut Bull. 2015 Jan 15;90(1-2):106-14. doi: 10.1016/j.marpolbul.2014.11.013. Epub 2014 Dec 6.

Singkran N. Contingency plan improvement for managing oil spills in the coastal waters of Thailand.Mar Pollut Bull. 2014 Dec 15;89(1-2):149-59. doi: 10.1016/j.marpolbul.2014.10.013. Epub 2014 Oct 28.

Pietroski JP, White JR, DeLaune RD. Effects of dispersant used for oil spill remediation on nitrogen cycling in Louisiana coastal salt marsh soil.Chemosphere. 2015 Jan;119:562-7. doi: 10.1016/j.chemosphere.2014.07.050. Epub 2014 Aug 12.

Riehm DA, McCormick AV. The role of dispersants' dynamic interfacial tension in effective crude oil spill dispersion.Mar Pollut Bull. 2014 Jul 15;84(1-2):155-63. doi: 10.1016/j.marpolbul.2014.05.018. Epub 2014 Jun 2.

Wise CF, Wise JT, Wise SS, Thompson WD, Wise JP Jr, Wise JP Sr. Chemical dispersants used in the Gulf of Mexico oil crisis are cytotoxic and genotoxic to sperm whale skin cells.Aquat Toxicol. 2014 Jul;152:335-40. doi: 10.1016/j.aquatox.2014.04.020. Epub 2014 Apr 25.

ED_001324_00001453-00045

Zhao, L., J. Torlapati, M. C. Boufadel*, T. King, B. Robinson, and K. Lee. 2014. VDROP: A Comprehensive model for generating the droplet size distribution of oils in water. Incorporation of the interfacial tension and oil viscosity. Chemical Engineering Journal, Accepted, 2014.

Zheng M, Ahuja M, Bhattacharya D, Clement TP, Hayworth JS, Dhanasekaran M. 2013 Evaluation of differential cytotoxic effects of the oil spill dispersant Corexit 9500. Life Sci. 2013 Dec 17. pii: S0024-3205(13)00757-1. doi: 10.1016/j.lfs.2013.12.010. [Epub ahead of print]

Zhao L, Boufadel MC, Socolofsky SA, Adams E, King T, Lee K. Evolution of droplets in subsea oil and gas blowouts: development and validation of the numerical model VDROP-Jar Pollut Bull. 2014 Jun 15;83(1):58-69. doi: 10.1016/j.marpolbul.2014.04.020. Epub 2014 Apr 26.

Koyama J, Imakado C, Uno S, Kuroda T, Hara S, Majima T, Shirota H, Añasco NC. Simulated distribution and ecotoxicity-based assessment of chemically-dispersed oil in Tokyo Bay.Mar Pollut Bull. 2014 Aug 30;85(2):487-93. doi: 10.1016/j.marpolbul.2014.04.001. Epub 2014 Apr 20.

Almeda R, Baca S, Hyatt C, Buskey EJ. Ingestion and sublethal effects of physically and chemically dispersed crude oil on marine planktonic copepods.Ecotoxicology. 2014 Aug;23(6):988-1003. doi: 10.1007/s10646-014-1242-6. Epub 2014 Apr 23.Free PMC Article

Shi R, Yu K. Impact of exposure of crude oil and dispersant (COREXIT® EC 9500A) on denitrification and organic matter mineralization in a Louisiana salt marsh sediment.Chemosphere. 2014 Aug;108:300-5. doi: 10.1016/j.chemosphere.2014.01.055. Epub 2014 Feb 26.

Polli JR, Zhang Y, Pan X. Dispersed crude oil amplifies germ cell apoptosis in Caenorhabditis elegans, followed a CEP-1-dependent pathway.Arch Toxicol. 2014 Mar;88(3):543-51. doi: 10.1007/s00204-014-1198-6. Epub 2014 Feb 5.

Ozhan K, Bargu S. Distinct responses of Gulf of Mexico phytoplankton communities to crude oil and the dispersant corexit(®) Ec9500A under different nutrient regimes.Ecotoxicology. 2014 Apr;23(3):370-84. doi: 10.1007/s10646-014-1195-9. Epub 2014 Jan 28.

Marti ME, Colonna WJ, Patra P, Zhang H, Green C, Reznik G, Pynn M, Jarrell K, Nyman JA, Somasundaran P, Glatz CE, Lamsal BP. Production and characterization of microbial biosurfactants for potential use in oil-spill remediation. Enzyme Microb Technol. 2014 Feb 5;55:31-9. doi: 10.1016/j.enzmictec.2013.12.001. Epub 2013 Dec 12. Erratum in: Enzyme Microb Technol. 2014 Jun 10;60:81.

Fitzgerald TP, Gohlke JM. Contaminant levels in Gulf of Mexico reef fish after the Deepwater Horizon oil spill as measured by a fishermen-led testing program.Environ Sci Technol. 2014 Feb 4;48(3):1993-2000. doi: 10.1021/es4051555. Epub 2014 Jan 16.

Gong Y, Zhao X, O'Reilly SE, Qian T, Zhao D. Effects of oil dispersant and oil on sorption and desorption of phenanthrene with Gulf Coast marine sediments.Environ Pollut. 2014 Feb;185:240-9. doi: 10.1016/j.envpol.2013.10.031. Epub 2013 Nov 27.

Rial D, Vázquez JA, Murado MA. Toxicity of spill-treating agents and oil to sea urchin embryos.Sci Total Environ. 2014 Feb 15;472:302-8. doi: 10.1016/j.scitotenv.2013.11.030. Epub 2013 Nov 30.

Roberts JR, Anderson SE, Kan H, Krajnak K, Thompson JA, Kenyon A, Goldsmith WT,

CONFIDENTIAL

McKinney W, Frazer DG, Jackson M, Fedan JS. Evaluation of Pulmonary and Systemic Toxicity of Oil Dispersant (COREXIT EC9500A(®)) Following Acute Repeated Inhalation Exposure.Environ Health Insights. 2015 Feb 9;8(Suppl 1):63-74. doi: 10.4137/EHI.S15262. eCollection 2014.

Zheng M, Ahuja M, Bhattacharya D, Clement TP, Hayworth JS, Dhanasekaran M. Evaluation of differential cytotoxic effects of the oil spill dispersant Corexit 9500Life Sci. 2014 Jan 30;95(2):108-17. doi: 10.1016/j.lfs.2013.12.010. Epub 2013 Dec 17.

CONFIDENTIAL

To: Grosser, Robert[Grosser.Robert@epa.gov]; devi.sundaravadivelu@ptsied.com[devi.sundaravadivelu@ptsied.com]
From: Conmy, Robyn
Sent: Tue 10/25/2016 10:24:21 PM
Subject: FW: need 200 mL minimum of dispersant for the lab...

As per the email from last week, please arrange for 200 ml of accel to be sent to hydrosphere. Thanks

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Barron, Mace
Sent: Monday, October 24, 2016 5:06 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>; Holder, Edith <holder.edith@epa.gov>
Subject: need 200 mL minimum of dispersant for the lab...

Just fyi on what they used of corexit and finasol.

Please send new dispersant asap!

Thank you,

Mace

From: Peter Meyer [mailto:pmeyer@hydrosphere.net]
Sent: Monday, October 24, 2016 3:41 PM
To: Barron, Mace <Barron.Mace@epa.gov>
Cc: Craig Watts <craig@hydrosphere.net>
Subject: RE: quick question...

Mace,

We ended up using about 160 mLs of each. So if I had to guess…

1) Corexit: We have about 1,200 mLs left over and…

2) Finasol: Probably about 700 mLs left over.

Neither container was full when we received them.

~Peter

From: Craig Watts
Sent: Saturday, October 22, 2016 7:22 PM
To: Barron, Mace <Barron.Mace@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>
Subject: RE: quick question...

Mace,

I am going to have to hand this one off to Peter.

Craig

From: Barron, Mace [mailto:Barron.Mace@epa.gov]
Sent: Friday, October 21, 2016 11:20 AM
To: Craig Watts <craig@hydrosphere.net>

Subject: quick question...

How much dispersant (e.g., corexit) did you guys use in the tox tests?

Do you recall how much we sent you? And how much you got left over?

We are getting ready to send a new dispersant to you and need to know the amount to send.

Thanks!

To: Andrew Remsen[drewremsen@gmail.com]
From: Conmy, Robyn
Sent: Mon 5/18/2015 6:55:05 PM
Subject: if you haven't seen yet....
2015Prince_EST.PDF

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

Environmental Science & Technology

Critical Review

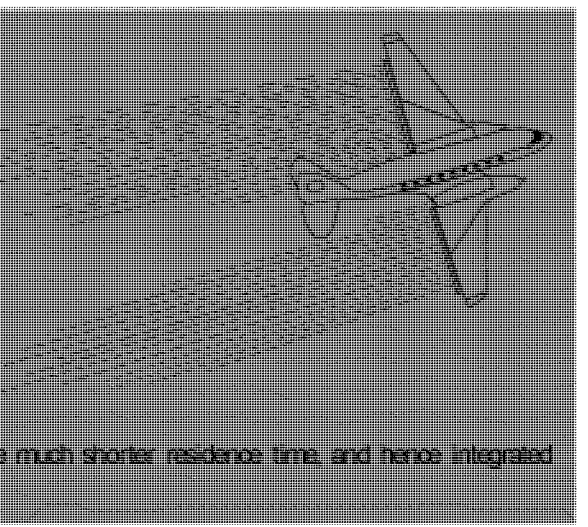
pubs.acs.org/est

Oil Spill Dispersants: Boon or Bane?

Roger C. Prince*

ExxonMobil Biomedical Sciences, Inc., Annandale, New Jersey 08801 United States

Dispersants provide a reliable large-scale response to catastrophic oil spills that can be used when the preferable option of recapturing the oil cannot be achieved. By allowing even mild wave action to disperse floating oil into tiny droplets (<70 μm) in the water column, seabirds, reptiles, and mammals are protected from lethal oiling at the surface, and microbial biodegradation is dramatically increased. Recent work has clarified how dramatic this increase is likely to be: beached oil has an environmental residence of years, whereas dispersed oil has a half-life of weeks. Oil spill response operations endorse the concept of net environmental benefit, that any environmental costs imposed by a response technique must be outweighed by the likely benefits. This critical review discusses the potential environmental debits and credits from dispersant use and concludes that, in most cases, the potential environmental costs of adding these chemicals to a polluted area are likely outweighed by the much shorter residence time, and hence integrated environmental impact, of the spilled oil in the environment.



INTRODUCTION

Oil fuels our modern world, accounting for some 33% of energy consumption in 2013; daily consumption was 87 million barrels per day.[1] Some of this crude oil is produced from wells drilled under the sea, and a large percentage travels by sea between production and consumption. Despite the best efforts of the oil and shipping industries, some gets spilled. Catastrophic spills appropriately garner the public's attention, although, in fact, most spills are rather small. Natural seeps are likely the largest contributor of oil to the world's oceans, followed by nonpoint sources on land.[2] Tanker accidents are becoming less common,[3] but it is still true that a few large spills contribute the most oil released to the sea by ships, and where such spills occur, they release far more oil in a few days than even the most active seeps. The tragic 2010 blowout from the Deepwater Horizon well[4] is a reminder that large releases can also occur from drilling operations.

Crude oil has been part of the biosphere for millions of years,[5] and a large number of microbes, both prokaryotic[6] and eukaryotic,[7] have evolved to consume it. Biodegradation is the eventual fate for all spilled oil that is not collected or burned, and both collection and combustion require that spilled oil be corralled with booms.[8] While skimming can be an effective process if equipment is close to hand and the weather is reasonably calm, and is frequently part of oil spill response plans (e.g., refs 9−12), large spills in remote areas can spread so quickly that skimming becomes extremely difficult. For example, the Deepwater Horizon response, despite enormous efforts, collected only some 3% of the oil released and burned another 5%.[4] Considerable research has been expended, therefore, on trying to enhance the rate of oil biodegradation.

Oil is an unusual substrate for microbial growth for two distinct reasons. On the one hand, most oil molecules have low density and are very sparingly soluble, so oil tends to stay as surface slicks or droplets dispersed by wave action. Biodegradation is then limited by the surface area of the oil−water interface. On the other, whereas hydrocarbons are rich sources of carbon and energy, oil contains no other useful elements for microbial growth. Nitrogen and phosphorus are the most usual limiting nutrients in the sea,[13] followed by iron and other trace requirements.[14] While seawater contains trace levels of these nutrients,[15] the biodegradation of significant concentrations of oil, such as on a shoreline, is likely to exhaust the local supply. Bioremediation, the stimulation of biodegradation, thus aims to overcome these two limitations.

In the case of oil stranded on shorelines in Prince William Sound AK following the spill from the Exxon Valdez, the first response was to remove oil from beaches by washing it back to the sea and collecting it with skimmers.[16] This had the effect of leaving a relatively thin film of oil on the gravel and rocks of the intertidal (and sometimes supra-tidal) zone, and this was bioremediated by the careful addition of oleophilic and slow-release fertilizers to increase the supply of bioavailable nitrogen and phosphorus. This worked quite well, stimulating oil biodegradation between 2- and 5-fold without causing any additional adverse effects,[17−20] but it should be born in mind that the oil had already been on the shoreline for a year before the quantitative experiments reported therein were begun.

Received: February 23, 2015
Revised: April 27, 2015
Accepted: May 4, 2015

 © XXXX American Chemical Society

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001457-00001

Floating oil presents different challenges. Floating oil is a very real hazard to diving birds and mammals (e.g., refs 21 and 22), and oil that beaches is a hazard to shorebirds,[23] invertebrates,[24,25] and mangroves.[26] Ameliorating these hazards was a primary impetus for the initial development of oil spill dispersants in the 1970s.[27] Modern dispersants are complex mixtures of anionic and neutral surfactants in a hydrocarbon solvent (e.g., ref 28) that lower the interfacial tension between oil and water so that minimal wave action and turbulence can disperse the oil into tiny droplets (<70 μm) in the water column. Such droplets are essentially neutrally buoyant, so with minimal turbulence they stay in the water column and diffuse apart. Concentrations of dispersed oil may be ≈1000 ppm in the first minutes after dispersion, but they fall to a few ppm, in a correspondingly larger volume, within hours, and to sub parts per million levels within a day.[29−34] Furthermore, even these concentrations are found only in the top few meters of the sea. Similarly, dispersants can harness the ejection turbulence of oil emanating from an uncontrolled subsea release and allow the formation of tiny oil droplets in the deep sea;[4,34−37] again, the droplets diffuse apart until they are in the sub parts per million concentration range. This diffusive dilution means that, although the levels of nutrients in the sea are relatively low,[13] it does not take long for oil concentrations to fall so that even those low levels are adequate for significant and rapid biodegradation. Hazen et al.[14,35] measured half-lives of n-alkanes of a few days in the dilute (2−442 ppb[34]) dispersed submarine plume from the Deepwater Horizon at 1100−1220 m (and 5 °C), and very similar results were reported for a broad array of individual hydrocarbons at low concentrations in New Jersey seawater at 8 °C,[36,37] in a flume in Trondheim, Norway,[38] at 30−32 °C and in water off the Penang, Malaysia, shore[39] at 27.5 °C. The approximate biodegradation half-life of the total measurable hydrocarbons was 11−14 days, both at low oil concentrations with indigenous nutrients[36,38] (2.5 and 43 ppm oil, respectively) and at slightly higher concentrations (100 ppm oil) with added nutrients.[39] Even the four ring aromatic chrysene and its methyl-, dimethyl-, and ethyl-alkylated forms had half-lives on the order of a month.[36]

DISPERSANTS

Dispersants have been used on a large scale in many responses, notably, the 1993 Braer wreck in the Shetland Islands,[40] the 1996 Sea Empress spill in South Wales,[41] and the 2010 Deepwater Horizon blowout in the Gulf of Mexico.[33] Seven spills in the US portion of the Gulf of Mexico were treated with dispersants between 1995 and 2004.[42] Dispersants are stockpiled, with equipment for their use, in large quantities around the world,[43] and substantial illustrated guidelines for their use are freely available.[44] Nevertheless, their deployment is still controversial, for clearly dispersants are not without potential drawbacks. Most dispersants are not themselves significantly toxic; they have toxicities indistinguishable from common household dish liquids and shampoos,[45] including those used for cleaning oiled seabirds,[46,47] and the majority, including those used in the Deepwater Horizon response, show neither androgen- nor estrogen-receptor activity.[48] Nevertheless, the use of dispersants involves adding more chemicals to an already impacted area, and the water under a recently dispersed oil slick is significantly transiently more toxic to organisms than under the undispersed slick, albeit because of the greater concentrations of oil in the water, not because of any increase in toxicity on an oil weight basis.[47,48,50]

So, how should spill response coordinators decide when and where to use dispersants? Most responders rely on a net environmental benefit analysis, often abbreviated NEBA.[51−55] At first glance, the concept seems oxymoronic: how can anything related to an oil spill have an environmental benefit? However, in actuality, the concept is very useful: everyone involved in a cleanup recognizes that an oil spill is a dreadful environmental insult and is working diligently to minimize adverse impacts and to remove the oil as quickly as possible. The question is whether a response tool will end up doing more harm than good, of whether there will be net environmental improvement despite potential collateral harm done in the short term by the response. Table 1 offers a comparison of the potential hazards and environmental fate of floating slicks and dispersed oils that need to be considered in such analyses.

The first hurdle for dispersants is to demonstrate their fundamental efficacy. In the United States, the U.S. Environmental Protection Agency (USEPA) maintains the National Oil and Hazardous Substances Pollution Contingency Plan Product Schedule[56] as part of the National Contingency Plan and lists chemical dispersants that may be authorized for use. Dispersants on the Product Schedule have demonstrated effectiveness; they can disperse at least 45% of Prudhoe Bay or South Louisiana crude oil in a standard swirling flask test.[57] The swirling flask test is one of several tests designed to discriminate between dispersants with different efficacies on a simple laboratory scale. It does this reasonably well (although the USEPA is considering revising it with a baffled flask test[58]), but unfortunately the passing grade of 45% has often been assumed to indicate expected field performance. In fact, the test dramatically underestimates efficacy in the field, primarily due to the amount of energy it imparts to the floating oil and the volume available for diffusion. Tests in the OHMSETT facility,[59] a wave tank in New Jersey that is 200 m long, 20 m wide, and 2.5 m deep, routinely measure dispersant efficiencies >95%, even at low temperatures with ice in the water.[60]

A second requirement for listing on the USEPA product schedule is that the acute toxicity of the dispersant to two reference species (silverside fish, Menidia beryllina (96 h), and mysid shrimp, Americamysis bahia (48 h)[49,59]) be reported. During the Deepwater Horizon response, the USEPA required that dispersants "have a toxicity value less than or equal to (sic) 23.00 (sic) ppm LC_{50} toxicity value for Menidia or 18.00 ppm LC_{50} for Mysidopsis (Americamysis)";[62] the dispersants being used passed this hurdle.[49]

As mentioned above, the toxicity of modern dispersants is usually so low as to likely have only minimal adverse effects at levels used in response operations (nominal aerial dispersant application rates are 5 gallons of dispersant per acre, 47 L/hectare;[63] diffusion into the top 20 cm of seawater would give concentrations around 23 ppm, and of course further diffusion will continually lower the concentration). However, dispersed oil is significantly more toxic, with acute LC_{50} values more than an order of magnitude lower.[49] There has been some confusion around quantifying the toxicity of dispersed oil;[64,65] acute toxicity arises from a general narcosis caused by dissolved hydrocarbons moving to the lipids of the test organism,[66−68] so if estimates of oil concentrations include small droplets, the toxicity expressed on a per milligram hydrocarbon basis are lower (higher LC_{50}) than that for dissolved components. In any case, dispersants encourage solubility by increasing the surface-

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001457-00002

Table 1

potential hazards and environmental fate of floating slick	potential hazards and environmental fate of dispersants and dispersed oil
	Dispersant application has the potential to create aerosols with potentially adverse effects on humans and wildlife. Such effects are seen only in the laboratory at quite high concentrations and with prolonged exposures.[99-102] They are minimized by protective equipment for applicators and exclusion zones around application. Aerial application is very unlikely to be approved within several miles of a coast.
	The addition of dispersants puts additional chemicals into a polluted area. Nominal application rates of dispersants are 5 gallons per acre (47 L ha^{-1}) for surface spills,[63] equivalent to a dispersant/oil ratio of about 1:20. Subsea injection seems to be effective at much lower doses, perhaps 1:100 or less. Dispersants are composed of chemicals that are generally acceptable for food contact and are biodegradable,[28,93,94] but when they are used at suboptimal doses, they may become incorporated into persistent tarballs.[95]
	Dispersants may persist in the environment. Dispersants are complex mixtures of surfactants and solvents, and very few techniques can measure them all.[104] Much published work has focused on individual chemicals, but since all of the components are widely used in consumer products on a far greater scale than in dispersants, care must be taken in attributing detection to dispersant use.[97]
	Dispersants may not penetrate heavy oils and may "roll-off" before they can be effective.[103,105] Nevertheless, dispersants have been shown to be effective with many heavy oils,[117] and new formulations are being developed to further this use.[106]
	Suboptimal dispersant application may not disperse the oil but may hinder subsequent skimming operations.
	Several studies appear to show that dispersants do not stimulate biodegradation very much.[139-141,36,78] Those experiments were based on a misunderstanding of the role of dispersants,[37] which is simply to disperse oil into tiny droplets that can diffuse apart. Such dilute small droplets, whether generated by dispersants or by low concentrations of oil, are degraded rapidly,[36-38,78] and dispersants show no significant stimulatory effect. Dispersed oils that can recoalesce into larger droplets, as occurs in laboratory experiments with more than a few parts per million oil,[139-141] again show only a small stimulation of biodegradation by dispersants. A clear stimulation of biodegradation by dispersants (many fold) is seen only when dispersed oil at environmentally realistic concentrations[29-36] is compared to an undispersed slick.[37] It is the latter that is the target of dispersants.
	Dispersants have been reported to be toxic to some bacteria.[142] These experiments looked at a few isolated cultivars from beach sand rather than pelagic organisms likely to be exposed to dispersants. Dispersants had no inhibitory effects in experiments with pelagic microbes[36-38,78] and were significantly less toxic than oil alone to Vibrio fischeri (now Aliivibrio fischeri[143]) in the Microtox test.[144]
Floating oil can be lethal to birds or mammals that penetrate it. The oil reduces the insulating properties of feathers and fur and may be toxic if ingested during preening or grooming.[21,22] Mild oiling may be transferred to eggs, chicks, and young.[107] Floating oil also likely kills small invertebrates, algae, fish eggs, and young that are at the sea surface under the slick.[108]	Dispersed oil is potentially toxic to organisms close to the surface when the oil is initially dispersed. This is particularly true for small invertebrates, algae, fish eggs, and young that cannot swim away.[109] Nevertheless, measured dilution suggests that they will not be exposed for very long.[29-36] The remarkably heterogeneous distribution of planktonic organisms, on all scales, probably has more influence on local populations than relatively small-scale mortality due to concentrated dispersions of oil.[145,146] Planktonic microbial species respond remarkably rapidly, even to diel cycles.[147] Fish kills are rarely noted after dispersant use, presumably because fish and other nekton swim away from the dispersed oil.
Floating oil is subject to photooxidation, which polymerizes polycyclic aromatic compounds to polar materials.[110,111] This eventually leads to very persistent tarballs.[112] Beached oil undergoes a similar process, leading to very persistent "pavements".[126,127]	
Transparent planktonic organisms may accumulate polycyclic aromatic compounds leached from slicks and beached oil and may be subject to phototoxicity in the surface layer of the sea.[113]	Dispersed polycyclic aromatic compounds may be accumulated by transparent planktonic organisms, which may then be subject to phototoxicity in the surface layer of the sea.[113]
Floating oil absorbs water; it is not unusual for floating slicks to increase their volume by >50% in 24 h.[114-116] This phenomenon makes dispersion more difficult but not impossible.[117] Over several days, many floating oils emulsify, forming water-in-oil emulsions known as mousse.[118-120] The encapsulated water no longer exchanges with bulk seawater, and biodegradation is likely even more nutrient- and oxygen-limited than in the floating slick. Mousse may be consumed by fish, turtles, etc. and also lead to tarballs.[111,112]	
Small molecules in floating oil evaporate rapidly, becoming volatile organic compounds (VOCs)[119,120] that can reach levels of concern for spill responders.[121] Evaporated molecules may be destroyed by photochemistry[122] or biodegradation.[123]	Dispersion slows evaporation, especially if the oil is dispersed subsea, as some was in the Deepwater Horizon response. The potentially toxic small molecules are retained in the water column until they are biodegraded or escape to the atmosphere over a much greater area due to diffusion at depth.
If it is not physically dispersed by storms or recovered mechanically, then an oil slick will likely beach on a shoreline. If it stays on the shoreline surface, then it may be possible to remove it by physical means, but storms will likely incorporate oil deep into sandy beaches,[124,125] where, if left alone, it will likely remain for decades.[126,127] Beached oil can be a major threat to turtle and shorebird nesting[128] and to mangroves[129] and marshes,[130] and recovery may take years.[131,132]	Dispersants are typically used far from shore in deep water (e.g., >3 miles[148]). Dispersed oil diffuses to sub parts per million levels and is very unlikely to reach a shoreline in noticeable quantities.
If the slick arrives at a shoreline, then biodegradation will be slow because of the high concentration of oil in comparison to the low concentrations of bioavailable nutrients in tidal flows. Biodegradation was too slow to measure directly in the absence of bioremediation in Prince William Sound following the Exxon Valdez spill,[18,19] but even with effective bioremediation, the half-life of total detectable hydrocarbons was of the order of 10 weeks.[18,19] The	Dispersed oil dilutes to such low concentrations that biodegradation is rapid. Measured half-lives are in the days to few weeks time frame.[14,33-37] Even the four ring aromatic chrysene and its methyl-, dimethyl-, and ethyl- forms have half-lives on the order of a month.[36] Nearshore waters from the Chukchi Sea,[78] the North West Atlantic,[36] and deepwater from Trondheim Fjord[38] all contained enough nutrients for substantial biodegradation of a few parts per million oil in 60 days, and it is reasonable to extrapolate that further dilution in more oligotrophic waters would allow similar degradation on similar time scales.

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001457-00003

Table 1. continued

potential hazards and environmental fate of floating slick	potential hazards and environmental fate of dispersants and dispersed oil
biodegradation of bulk oil in the Gulf of Mexico was very slow[74,75] compared to that of dispersed oil.[35,14]	
While oil hydrocarbons are essentially completely biodegradable (eg., ref 36), many of the deeply colored molecules (resins, asphaltenes, polars, etc) are more resistant to biodegradation, although at least some are consumed.[133,134] While these have minimal biological effects, the lifetime of these molecules in a sandy beach may be prolonged, especially if they have been entrained deep in a beach by storm action.[124,132]	While oil hydrocarbons are essentially completely biodegradable (eg., ref 36), many of the deeply colored molecules (resins, asphaltenes, polars, etc) are more resistant to biodegradation, although at least some are consumed.[133,134] Once the hydrocarbons have been degraded, these fractions lack oily characteristics and become essentially indistinguishable from other inert organic matter in the environment, such as humins.[135]
Physical deanup of beached oil can take weeks to years and requires hundreds to thousands of workers with their ancillary vehicles and support services.[16,128,136]	Optimal dispersant application for surface slicks uses planes that can carry 5000 gallons (19000 L) of dispersant, appropriate to treat 1000 acres (405 ha) of slick.[63] Depending on distances from airfields, several sorties can be flown per day, and spills from a tanker can be treated in a few days. Aerial spraying requires daylight (Figure 1). Subsea injection has the advantage of continuing 24 h a day, and the lower application rates mean that dispersant stocks will potentially last longer. If fully successful, oil from a subsea release may never reach the surface before it is biodegraded.[103]

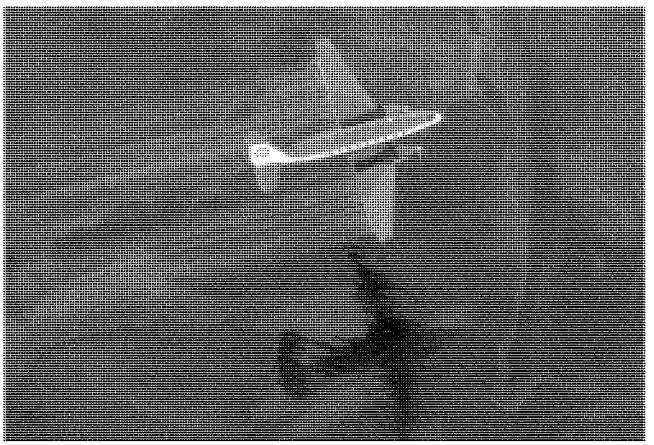


Figure 1. An updated DC3C spraying dispersants on oil from the Deepwater Horizon blowout. The wingspan is 29 m. Reproduced with permission from Airborne Support.

to-volume ratio of the oil, and there is a potential for short-term toxic effects in a dispersed plume. The question is how significant these effects might be in the field. Canonical acute toxicity tests involve constant exposures for 48 or 96 h,[61] but in a response at sea, the concentrations of oil will be dropping rapidly due to dilution by mixing and diffusion. As noted above, concentrations of dispersed oil drop to below 1 ppm within a few hours.[29−33]

Only acute toxicity tests are required for listing under the National Contingency Plan, but of course chronic effects are also a real concern. As expected, the longer exposure required to see chronic effects allows lower concentrations of hydrocarbons to exert an effect, and typical acute-to-chronic ratios are 1:10, that is, it may only take 10% of the acute LC_{50} to have a chronic EC_{50}.[69−72] Again, these concentrations are for prolonged exposures, and it is not clear how these relate to the very low concentrations[29−34] of dispersed oil that are found several days after dispersion.[73]

As noted at the outset, dispersants were initially conceived as a tool for minimizing seabird mortality, and early use weighed that benefit against potential toxicity to planktonic species.[27] However, an additional substantial benefit has now been clearly documented: the biodegradation of dispersed oil is dramatically faster than that of oil in a slick or on a shoreline. Oil on shorelines of Prince William Sound, Alaska, had a half-life of a year or more,[18−20] even with the substantial washing and bioremediation program.[16] Tarballs and mousse associated with the Deepwater Horizon blowout had similar persistence in the environment.[74,75] However, the biodegradation of dispersed oil is rapid and extensive.[14,35−37]

The oil spill response community agrees that the best response to an oil spill would be to collect it from the environment before it reached a shoreline, and many response plans focus on this requirement by staging large amounts of equipment aimed at achieving this goal even in the face of a very large spill (e.g., refs 9−12). However, if oil cannot be collected, particularly if it is unsafe for responders to perform mechanical recovery or because of remote location or hours of daylight, then responders must look to other methods. It is important to recognize that time is of the essence: deciding not to use a response option today may preclude its use tomorrow. This phenomenon, the window-of-opportunity,[76] is particularly relevant to the use of dispersants because as oil weathers by

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001457-00004

evaporation and absorption of underlying water, it becomes progressively more and more difficult to disperse with dispersants. (As an aside, dispersants incorporated into relatively fresh oil will help the dispersion of that oil even after some time if conditions are initially too calm for immediate dispersion,[77] a potential problem for requirements that dispersants be seen to be effective on a trial basis before large-scale application can begin.) Responders must weigh the potential additional short-term toxicity likely incurred immediately under a dispersed surface slick against the benefit of protecting diving birds and animals and having the oil removed from the environment by biodegradation on a time scale of a few weeks rather than years with a vastly smaller human footprint of spill response. The speed of biodegradation is particularly important if the over-riding concern about dispersant use is the potential local increase in toxicity; beached oil and undispersed mousse leach hydrocarbons and have their own potential environmental impacts for a prolonged period. It would also be prudent to bear in mind the environmental impact of large shoreline cleanup operations, which often involve hundreds of workers and the ancillary impacts of their support services. There is also the potential legacy of the final disposal of the oil, which may well involve burial at a secure landfill rather than combustion or biodegradation. Finally, while not an environmental impact, the economic impacts of an oiled marina or shoreline also need to be weighed as responders decide the most appropriate spill-response.

CONCLUDING REMARKS

In the final analysis, some accidents occur so close to shore that oil will undoubtedly reach the shoreline and most likely require some physical cleanup, perhaps followed by bioremediation. However, if the release is in deep water, then it ought to be possible to mobilize dispersants to keep bulk oil from ever reaching a shoreline. If oil is successfully dispersed into the water column, then it is likely that biodegradation will remove the vast majority of it in weeks to months, even in the Arctic and Antarctic.[78] What remains will likely be finely dispersed fragments of nonoily (to the touch) material depleted of hydrocarbons and rich in asphaltic materials and saturated biomarkers such as the hopanes.[36,79,80]

Many questions remain to be answered:

- How does the density of dispersed oil change as biodegradation proceeds? Alkanes are much lighter than water,[81] even at high pressure,[82] as are cyclic alkanes and monoaromatics. However, larger aromatic hydrocarbons, such as chrysene, have densities greater than 1,[83] as do the resins and asphaltenes.[84,85] Since biodegradation preferentially removes alkanes, initially buoyant droplets will become neutral and eventually sink. Is this partially degraded oil further metabolized once deposited on the surface sediment at depth? Reports of substantial amounts of fossil (radiocarbon silent) carbon on the surface sediment at depth[86] could be either unmetabolized oil molecules or the biomass of organisms degrading the oil or, more likely, both. As noted above, some molecules, such as the hopanes, seem very resistant to biodegradation and likely remain with very biodegraded oil (and degrading biomass) as a fingerprint of the initial source.[36,75,79,80]
- What fraction of the oil carbon is mineralized to CO_2 in the initial biodegradation, and what fraction is incorporated into biomass? Classical experiments with aerobes growing on glucose suggest a biomass yield of about 50%,[87] but what is the fate of that biomass? Levy and Lee[88] propose that it is the base for substantial fisheries offshore Atlantic Canada. Chemical analysis of the radiocarbon silent material reported by Chanton et al.[86] will shed light on this question.
- What role does microbial succession play in the biodegradation of oil? Such succession was clearly seen in the Gulf of Mexico following the Deepwater Horizon release,[87−92] but how did it relate to the chemical composition of the residual oil or to the nutrient levels in the water? Do dispersants affect this succession?
- How rapidly are dispersant components degraded in the sea? Early work established the biodegradation of nonionic surfactants in seawater,[93] and dioctylsulfosuccinate is known to be biodegradable[94] and clearly was being degraded close to the Deepwater Horizon spill site where it was being applied in the Corexit dispersant.[95] Nevertheless, traces of dioctylsulfosuccinate have been found far from the Deepwater Horizon accident;[96] was that associated with dispersant application? Traces found close to shore are more likely related to stormwater discharges.[95] Small quantities associated with tarballs[98] are likely the result of suboptimal dispersant application and highlight the need to apply an effective amount of dispersant if its benefits are to be achieved.
- Is biodegradation at depth (>1500 m) fundamentally distinct from biodegradation at the surface? Early work suggested that it is not,[137] but that work was clearly limited in its experimental tools, and there is much to learn. There is no doubt that active hydrocarbon-degrading microbial communities, both aerobic and anaerobic, are present in deep sea sediments.[138]

Already, however, we know enough from laboratory, mesocosm, and field experience to say that dispersed oil is degraded much more rapidly than undispersed oil, likely orders of magnitude more rapidly. This is the key piece of information that seems to be overlooked in most discussions of the potential adverse impacts of using dispersants. Even if there is an adverse local impact of dispersed oil, it will not last long. On the other hand, oil that reaches a shoreline may be there for years.

AUTHOR INFORMATION

Corresponding Author

*E-mail: roger.c.prince@exxonmobil.com. Phone: 908 730 2134. Fax: 908 730 1199.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

I am grateful to Airborne Support (http://airbornesupport.com/basler.html) for the photograph used in Figure 1.

REFERENCES

(1) Statistical Review of World Energy; BP, 2014. http://www.bp.com/en/global/corporate/about-bp/energy-economics/statistical-review-of-world-energy.html.

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001457-00005

(2) National Research Council Oil in the Sea III: Inputs, Fates, and Effects; National Academies Press: Washington, DC, 2003.
(3) Musk, S. Trends in oil spills from tankers and ITOPF non-tanker attended incidents, 2012. http://www.itopf.com/knowledge-resources/documents-guides/document/trends-in-oil-spills-from-tankers-and-itopf-non-tanker-attended-incidents-2012/.
(4) Lubchenco, J.; McNutt, M. K.; Dreyfus, G.; Murawski, S. A.; Kennedy, D. M.; Anastas, P. T.; Chu, S.; Hunter, T. Science in support of the Deepwater Horizon response. Proc. Natl. Acad. Sci. U.S.A. 2012, 109, 20212−20221.
(5) Tissot, B. P.; Welte, D. H. Petroleum Formation and Occurrence; Springer: Berlin, 1984.
(6) Prince, R. C.; Gramain, A.; McGenity, T. J. Prokaryotic hydrocarbon degraders. In Handbook of Hydrocarbon and Lipid Microbiology; Timmis, K. N., Ed.; Springer-Verlag: Berlin, 2010; pp 1672−1692.
(7) Prince, R. C. Eukaryotic hydrocarbon degraders. In Handbook of Hydrocarbon and Lipid Microbiology; Timmis, K. N., Ed.; Springer-Verlag: Berlin, 2010; pp 2066−2078.
(8) Fingas, M. The Basics of Oil Spill Cleanup; CRC Press: Boca Raton, FL, 2012.
(9) Alyeska Oil Spill Prevention, Response and Preparedness, 2014. http://www.alyeska-pipe.com/SafetyEnvironment/PreventionAndResponse.
(10) San Francisco Oil Spill Contingency Plan, 2014. https://www.dfg.ca.gov/ospr/san_francisco_plan.aspx.
(11) Thames Oil Spill Clearance Association, 2014. http://www.pla.co.uk/About-Us/TOSCA.
(12) ITOPF Country Profiles, 2014. http://www.itopf.com/knowledge-resources/countries-regions/.
(13) Atlas, R. M.; Bartha, R. Degradation and mineralization of petroleum in sea water: limitation by nitrogen and phosphorus. Biotechnol. Bioengineer. 1972, 14, 309−318.
(14) Bælum, J.; Borglin, S.; Chakraborty, R.; Fortney, J. L.; Lamendella, R.; Mason, O. U.; Auer, M.; Zemla, M.; Bill, M.; Conrad, M. E.; Malfatti, S. A.; Tringe, S. G.; Holman, H. Y.; Hazen, T. C.; Jansson, J. K. Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. Environ. Microbiol. 2012, 14, 2405−2416.
(15) Garcia, H. E.; Locarnini, R. A.; Boyer, T. P.; Antonov, J. I.; Zweng, M. M.; Baranova, O. K.; Johnson, D. R. World Ocean Atlas 2009: Nutrients (Phosphate, Nitrate, And Silicate), NOAA Atlas NESDIS 71; Levitus, S., Ed.; U.S. Government Printing Office: Washington, DC, 2010; Vol. 4.
(16) Nauman, S. A. Shoreline cleanup: equipment and operations. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1991; pp 141−147.
(17) Lindstrom, J. E.; Prince, R. C.; Clark, J. R.; Grossman, M. J.; Yeager, T. R.; Braddock, J. F.; Brown, E. J. Microbial populations and hydrocarbon biodegradation potentials in fertilized shoreline sediments affected by the T/V Exxon Valdez oil spill. Appl. Environ. Microbiol. 1991, 57, 2514−2522.
(18) Bragg, J. R.; Prince, R. C.; Harner, E. J.; Atlas, R. M. Effectiveness of bioremediation for the Exxon Valdez oil spill. Nature 1994, 368, 413−418.
(19) Prince, R. C.; Clark, J. R.; Lindstrom, J. E.; Butler, E. L.; Brown, E. J.; Winter, G.; Grossman, M. J.; Parrish, R. R.; Bare, R. E.; Braddock, J. F.; Steinhauer, W. G.; Douglas, G. S.; Kennedy, J. M.; Barter, P. J.; Bragg, J. R.; Harner, E. J.; Atlas, R. M. Bioremediation of the Exxon Valdez oil spill: monitoring safety and efficacy. In Hydrocarbon Remediation; Hinchee, R. E., Alleman, B. C., Hoeppel, R. E., Miller, R. N., Eds.; Lewis Publishers: Boca Raton, FL, 1994; pp 107−124.
(20) Prince, R. C.; Bragg, J. R. Shoreline bioremediation following the Exxon Valdez oil spill in Alaska. Biorem. J. 1997, 1, 97−104.
(21) Page, G. W.; Carter, H. R.; Ford, R. G. Numbers of seabirds killed or debilitated in the 1986 Apex Houston oil spill in central California. Studies Avian Biol. 1990, 14, 164−174.
(22) Murphy, S. M.; Day, R. H.; Wiens, J. A.; Parker, K. R. Effects of the Exxon Valdez oil spill on birds: comparisons of pre-and post-spill surveys in Prince William Sound, Alaska. Condor 1997, 99, 299−313.
(23) Henkel, J. R.; Sigel, B. J.; Taylor, C. M. Large-scale impacts of the Deepwater Horizon oil spill: can local disturbance affect distant ecosystems through migratory shorebirds? BioScience 2012, 62, 676−685.
(24) Chasse, C. The ecological impact on and near shores by the Amoco Cadiz oil spill. Mar. Pollut. Bull. 1978, 9, 298−301.
(25) Neuparth, T.; Moreira, S. M.; Santos, M. M.; Reis-Henriques, M. A. Review of oil and HNS accidental spills in Europe: identifying major environmental monitoring gaps and drawing priorities. Mar. Pollut. Bull. 2012, 64, 1085−1095.
(26) Anink, P. J.; Hunt, D. R.; Roberts, D. E.; Jacobs, N. E. Oil spill in Botany Bay: short term effects and long term implications. Wetlands (Australia) 1985, 5, 32−41.
(27) National Research Council Oil Spill Dispersants: Efficacy and Effects; National Academy Press: Washingon, DC, 2005.
(28) COREXIT® Ingredients; Nalco: Sugar Land, TX, 2014. http://www.nalcoesllc.com/nes/1602.htm.
(29) Cormack, D.; Nichols, J. A. The concentrations of oil in sea water resulting from natural and chemically induced dispersion of oil slicks. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1977; pp 381−385.
(30) Lichtenthaler, R. G.; Daling, P. S. Dispersion of chemically treated crude oil in Norwegian offshore waters. In International Oil Spill Conference; American Petroleum Institute: Washington, DC, 1983; pp 7−14.
(31) McAuliffe, C. D.; Johnson, J. C.; Greene, S. H.; Canevari, G. P.; Searl, T. D. Dispersion and weathering of chemically treated crude oils on the ocean. Environ. Sci. Technol. 1980, 14, 1509−1518.
(32) BenKinney, M.; Brown, J.; Mudge, S.; Russell, M.; Nevin, A.; Huber, C. Monitoring effects of aerial dispersant application during the MC252 Deepwater Horizon Incident. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 2011; p 368.
(33) Bejarano, A. C.; Levine, E.; Mearns, A. J. Effectiveness and potential ecological effects of offshore surface dispersant use during the Deepwater Horizon oil spill: a retrospective analysis of monitoring data. Environ. Monit. Assess. 2013, 185, 10281−10295.
(34) Wade, T. L.; Sweet, S. T.; Sericano, J. L.; Guinasso, N. L.; Diercks, A. R.; Highsmith, R. C.; Asper, V. L.; Joung, D. J.; Shiller, A. M.; Lohrenz, S. E.; Joye, S. B. Analyses of water samples from the Deepwater Horizon oil spill: documentation of the subsurface plume. In Monitoring and Modeling the Deepwater Horizon Oil Spill: A Record-Breaking Enterprise; Liu, Y., Macfadyen, A., Ji, Z. J., Weisberg, R. H., Eds; American Geophysical Union: Washington, DC, 2011; pp 77−82.
(35) Hazen, T. C.; Dubinsky, E. A.; DeSantis, T. Z.; Andersen, G. L.; Piceno, Y. M.; Singh, N.; Jansson, J. K.; Probst, A.; Borglin, S. E.; Fortney, J. L.; Stringfellow, W. T.; Bill, M.; Conrad, M. S.; Tom, L. M.; Chavarria, K. L.; Alusi, T. R.; Lamendella, R.; Joyner, D. C.; Spier, C.; Bælum, J.; Auer, M.; Zemla, M. L.; Chakraborty, R.; Sonnenthal, E. L.; D'haeseleer, P.; Holman, H. N.; Osman, S.; Lu, Z.; Van Nostrand, J. D.; Deng, Y.; Zhou, J.; Mason, O. U. Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science 2010, 330, 204−208.
(36) Prince, R. C.; McFarlin, K. M.; Butler, J. D.; Febbo, E. J.; Wang, F. C. Y.; Nedwed, T. J. The primary biodegradation of dispersed crude oil in the sea. Chemosphere 2013, 90, 521−526.
(37) Prince, R. C.; Butler, J. D. A protocol for assessing the effectiveness of oil spill dispersants in stimulating the biodegradation of oil. Environ. Sci. Pollut. Res. 2014, 21, 9506−9510.
(38) Brakstad, O. G.; Nordtug, T.; Throne-Holst, M. Biodegradation of dispersed Macondo oil in seawater at low temperature and different oil droplet sizes. Mar. Pollut. Bull. 2015, 93, 144−152.
(39) Zahed, M. A.; Aziz, H. A.; Isa, M. H.; Mohajeri, L.; Mohajeri, S.; Kutty, S. R. M. Kinetic modeling and half-life study on bioremediation of crude oil dispersed by Corexit 9500. J. Hazard. Mater. 2011, 185, 1027−1031.
(40) Harris, C. The Braer incident: Shetland Islands, January 1993. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1995; pp 813−819.

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001457-00006

(41) Law, R. J.; Kelly, C. The impact of the Sea Empress oil spill. Aquat. Living Resour. 2004, 17, 389−394.
(42) Henry, C. Review of dispersant use in US Gulf of Mexico waters since the Oil Pollution Act of 1990. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 2005; pp 439−442.
(43) Global Dispersant Stockpile; Oil Spill Response Ltd.: London, 2014. http://www.oilspillresponse.com/services-landing/global-dispersant-stockpile.
(44) ExxonMobil Oil Spill Dispersant Guidelines, 2008. crrc.unh.edu/sites/crrc.unh.edu/files/exxonmobil_dispersant_guidelines_2008.pdf.
(45) Word, J. Q.; Clark, J. R.; Word, L. S. Comparison of the acute toxicity of Corexit 9500 and household cleaning products. Hum. Ecol. Risk Assess. 2014, 21, 707−725.
(46) Russell, M.; Holcomb, J.; Berkner, A. 30-years of oiled wildlife response statistics, Proceedings of the 7th International Effects of Oil on Wildlife Conference, Hamburg, Germany, October 14−16, 2003; pp 14−16. pszshhw.bird-rescue.org/pdfs/IBRRC_stats_paper.pdf.
(47) Best Practices for Migratory Bird Care During Oil Spill Response; USWFS: Anchorage, AK, 2003. www.fws.gov/contaminants/fws_oscp_05/fwscontingencyappendices/D-BestPracticesMigBirds/BestPracticesmar04rev.pdf.
(48) Judson, R. S.; Martin, M. T.; Reif, D. M.; Houck, K. A.; Knudsen, T. B.; Rotroff, D. M.; Xia, M.; Sakamuru, S.; Huang, R.; Shinn, P.; Austin, C. P.; Kavlock, R. J.; Dix, D. J. Analysis of eight oil spill dispersants using rapid, in vitro tests for endocrine and other biological activity. Environ. Sci. Technol. 2010, 44, 5979−5985.
(49) Hemmer, M. J.; Barron, M. G.; Greene, R. M. Comparative toxicity of eight oil dispersants, Louisiana sweet crude oil (LSC), and chemically dispersed LSC to two aquatic test species. Environ. Toxicol. Chem. 2011, 30, 2244−2252.
(50) Gardiner, W. W.; Word, J. Q.; Word, J. D.; Perkins, R. A.; McFarlin, K. M.; Hester, B. W.; Word, L. S.; Ray, C. M. The acute toxicity of chemically and physically dispersed crude oil to key arctic species under arctic conditions during the open water season. Environ. Toxicol. Chem. 2013, 32, 2284−2300.
(51) Baker, J. M. Net environmental benefit analysis for oil spill response. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1995; pp 611−614.
(52) Chapman, H.; Purnell, K.; Law, R. J.; Kirby, M. F. The use of chemical dispersants to combat oil spills at sea: a review of practice and research needs in Europe. Mar. Pollut. Bull. 2007, 54, 827−838.
(53) Kirby, M. F.; Law, R. J. Oil spill treatment products approval: the UK approach and potential application to the Gulf region. Mar. Pollut. Bull. 2008, 56, 1243−1247.
(54) API Net Environmental Benefit Analysis for EffEctive Oil Spill Preparedness and Response, 2013. www.api.org/~/media/Files/EHS/Clean_Water/Oil_Spill_Prevention/NEBA/NEBA-Net-Environmental-Benefit-Analysis-July-2013.pdf.
(55) McCay, D. F.; Graham, E. Quantifying tradeoffs-net environmental benefits of dispersant use. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 2014; pp 762−775.
(56) USEPA Alphabetical List of NCP Product Schedule (Products Available for Use During an Oil Spill), 2014 http://www2.epa.gov/emergency-response/alphabetical-list-ncp-product-schedule-products-available-use-during-oil-spill.
(57) USEPA Swirling Flask Dispersant Effectiveness Test, Revised Standard Dispersant Toxicity Test, and Bioremediation Agent Effectiveness Test, 2006. http://www.gpo.gov/fdsys/pkg/CFR-2006-title40-vol27/xml/CFR-2006-title40-vol27-part300-appC.xml.
(58) USEPA National Oil and Hazardous Substances Pollution Contingency Plan: Proposed Rule, 2015. https://www.federalregister.gov/articles/2015/01/22/2015-00544/national-oil-and-hazardous-substances-pollution-contingency-plan.
(59) Ohmsett • National Oil Spill Response Research Facility; BSSE: Washington, DC, 2014. http://www.bsee.gov/Technology-and-Research/Ohmsett/index/.
(60) Belore, R. C.; Trudel, K.; Mullin, J. V.; Guarino, A. Large-scale cold water dispersant effectiveness experiments with Alaskan crude oils and Corexit 9500 and 9527 dispersants. Mar. Pollut. Bull. 2009, 58, 118−128.
(61) Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th ed.; USEPA: Washington, DC, 2002. http://water.epa.gov/scitech/methods/cwa/wet/disk2_index.cfm.
(62) Dispersant Monitoring and Assessment Directive − Addendum; USEPA: Washington, DC, 2010. http://www.epa.gov/bpspill/dispersants/directive-addendum2.pdf.
(63) Dispersant Aerial Application Systems: Airborne Support Incorporated; Clean Gulf Associates: New Orleans, LA, 2014. http://www.cleangulfassoc.com/equipment/all/dispersant-aerial-application.
(64) Coelho, G.; Clark, J.; Aurand, D. Toxicity testing of dispersed oil requires adherence to standardized protocols to assess potential real world effects. Environ. Pollut. 2013, 177, 185−188.
(65) Lewis, M. M.; Pryor, R. Toxicities of oils, dispersants and dispersed oils to algae and aquatic plants: Review and database value to resource sustainability. Environ. Pollut. 2013, 180, 345−367.
(66) McGrath, J. A.; Parkerton, T. F.; Hellweger, F. L.; Di Toro, D. M. Validation of the narcosis target lipid model for petroleum products: gasoline as a case study. Environ. Toxicol. Chem. 2005, 24, 2382−2394.
(67) Di Toro, D. M.; McGrath, J. A.; Stubblefield, W. A. Predicting the toxicity of neat and weathered crude oil: Toxic potential and the toxicity of saturated mixtures. Environ. Toxicol. Chem. 2007, 26, 2 4−36.
(68) McCarty, L. S.; Arnot, J. A.; Mackay, D. Evaluation of critical body residue data for acute narcosis in aquatic organisms. Environ. Toxicol. Chem. 2013, 32, 2301−2314.
(69) Länge, R. R.; Hutchinson, T. H.; Scholz, N.; Solbé, J. Analysis of the ECETOC aquatic toxicity (EAT) database II • comparison of acute to chronic ratios for various aquatic organisms and chemical substances. Chemosphere 1998, 36, 115−127.
(70) Roex, E. W.; Van Gestel, C. A.; Van Wezel, A. P.; Van Straalen, N. M. Ratios between acute aquatic toxicity and effects on population growth rates in relation to toxicant mode of action. Environ. Toxicol. Chem. 2000, 19, 685−693.
(71) Raimondo, S.; Montague, B. J.; Barron, M. G. Determinants of variability in acute to chronic toxicity ratios for aquatic invertebrates and fish. Environ. Toxicol. Chem. 2007, 26, 2019−2023.
(72) Swigert, J. P.; Lee, C.; Wong, D. C. L.; Podhasky, P. Aquatic hazard and biodegradability of light and middle atmospheric distillate petroleum streams. Chemosphere 2014, 108, 1−9.
(73) Landrum, P. F.; Chapman, P. M.; Neff, J.; Page, D. S. Influence of exposure and toxicokinetics on measures of aquatic toxicity for organic contaminants: a case study review. Integr. Environ. Assess. Manage. 2013, 9, 196−210.
(74) Aeppli, C.; Nelson, R. K.; Radovic, J. R.; Carmichael, C. A.; Valentine, D. L.; Reddy, C. M. Recalcitrance and degradation of petroleum biomarkers upon abiotic and biotic natural weathering of Deepwater Horizon oil. Environ. Sci. Technol. 2014, 48, 6726−6734.
(75) Yin, F.; John, G. F.; Hayworth, J. S.; Clement, T. P. Long-term monitoring data to describe the fate of polycyclic aromatic hydrocarbons in Deepwater Horizon oil submerged off Alabama's beaches. Sci. Total Environ. 2015, 508, 46−56.
(76) Nordvik, A. B. The technology windows-of-opportunity for marine oil spill response as related to oil weathering and operations. Spill Sci. Technol. Bull. 1995, 2, 17−46.
(77) Lewis, A. B.; Trudel, K.; Belore, R. C.; Mullin, J. V. Large-scale dispersant leaching and effectiveness experiments with oils on calm water. Mar. Pollut. Bull. 2010, 60, 244−254.
(78) McFarlin, K. M.; Prince, R. C.; Perkins, R.; Leigh, M. B. Biodegradation of dispersed oil in arctic seawater at −1 °C. PLoS One 2014, 9, e84297.
(79) Ourisson, G.; Albrecht, P. Hopanoids. 1. Geohopanoids: the most abundant natural products on Earth? Acc. Chem. Res. 1992, 25, 398−402.

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001457-00007

(80) Valentine, D. L.; Fisher, G. B.; Bagby, S. C.; Nelson, R. K.; Reddy, C. M.; Sylva, S. P.; Woo, M. A. Fallout plume of submerged oil from Deepwater Horizon. Proc. Natl. Acad. Sci. U.S.A. 2014, 111, 15906−15911.
(81) Queimada, A. J.; Quinones-Cisneros, S. E.; Marrucho, I. M.; Coutinho, J. A. P.; Stenby, E. H. Viscosity and liquid density of asymmetric hydrocarbon mixtures. Int. J. Thermophys. 2003, 24, 1221−1239.
(82) Liu, K.; Wu, Y.; McHugh, M. A.; Baled, H.; Enick, R. M.; Morreale, B. D. Equation of state modeling of high-pressure, high-temperature hydrocarbon density data. J. Supercrit. Fluids 2010, 55, 701−711.
(83) Wakeham, W. A.; Cholakov, G. S.; Stateva, R. P. Liquid density and critical properties of hydrocarbons estimated from molecular structure. J. Chem. Eng. Data 2002, 47, 559−570.
(84) Alboudwarej, H.; Beck, J.; Svrcek, W. Y.; Yarranton, H. W.; Akbarzadeh, K. Sensitivity of asphaltene properties to separation techniques. Energy Fuels 2002, 16, 462−469.
(85) Barrera, D. M.; Ortiz, D. P.; Yarranton, H. W. Molecular weight and density distributions of asphaltenes from crude oils. Energy Fuels 2013, 27, 2474−2487.
(86) Chanton, J.; Zhao, T.; Rosenheim, B. E.; Joye, S. B.; Bosman, S.; Brunner, C. A.; Yeager, K. M.; Diercks, A. R.; Hollander, D. Using natural abundance radiocarbon to trace the flux of petrocarbon to the seafloor following the Deepwater Horizon oil spill. Environ. Sci. Technol. 2014, 49, 847−854.
(87) Shiloach, J.; Fass, R. Growing E. coli to high cell density・a historical perspective on method development. Biotechnol. Adv. 2005, 23, 345−57.
(88) Levy, E. M.; Lee, K. Potential contribution of natural hydrocarbon seepage to benthic productivity and the fisheries of Atlantic Canada. Can. J. Fish. Aquat. Sci. 1988, 45, 349−352.
(89) Kostka, J. E.; Prakash, O.; Overholt, W. A.; Green, S. J.; Freyer, G.; Canion, A.; Delgardio, J.; Norton, N.; Hazen, T. C.; Huettel, M. Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the Deepwater Horizon oil spill. Appl. Environ. Microbiol. 2011, 77, 7962−7974.
(90) Dubinsky, E. A.; Conrad, M. E.; Chakraborty, R.; Bill, M.; Borglin, S. E.; Hollibaugh, J. T.; Mason, O. U.; Piceno, Y. M.; Reid, F. C.; Stringfellow, W. T.; Tom, L. M.; Hazen, T. C.; Andersen, G. L. Succession of hydrocarbon-degrading bacteria in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. Environ. Sci. Technol. 2013, 47, 10860−10867.
(91) Lamendella, R.; Strutt, S.; Borglin, S.; Chakraborty, R.; Tas, N.; Mason, O. U.; Hultman, J.; Prestat, E.; Hazen, T. C.; Jansson, J. K. Assessment of the Deepwater Horizon oil spill impact on Gulf coast microbial communities. Front. Microbiol. 2014, 5, 130.
(92) Rodriguez-R, L. M.; Overholt, W. A.; Hagan, C.; Huettel, M.; Kostka, J. E.; Konstantinidis, K. T. Microbial community successional patterns in beach sands impacted by the Deepwater Horizon oil spill. ISME J. 2015, DOI: 10.1038/ismej.2015.5.
(93) Uña, G. V.; García, M. J. N. Biodegradation of non-ionic dispersants in sea-water. Eur. J. Appl. Microbiol. Biotechnol. 1983, 18, 315−319.
(94) Cordon, T. C.; Maurer, E. W.; Stirton, A. J. The course of biodegradation of anionic detergents by analyses for carbon, methylene blue active substance and sulfate ion. J. Am. Oil Chem. Soc. 1970, 47, 203−206.
(95) Gray, J. L.; Kanagy, L. K.; Furlong, E. T.; Kanagy, C. J.; McCoy, J. W.; Mason, A.; Lauenstein, G. Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. Chemosphere 2014, 95, 124−130.
(96) Kujawinski, E. B.; Kido Soule, M. C.; Valentine, D. L.; Boysen, A. K.; Longnecker, K.; Redmond, M. C. Fate of dispersants associated with the Deepwater Horizon oil spill. Environ. Sci. Technol. 2011, 45, 1298−1306.
(97) Hayworth, J. S.; Clement, T. P. Provenance of COREXIT-related chemical constituents found in nearshore and inland Gulf Coast waters. Mar. Pollut. Bull. 2012, 64, 2005−2014.
(98) White, H. K.; Lyons, S. L.; Harrison, S. J.; Findley, D. M.; Liu, Y.; Kujawinski, E. B. Long-term persistence of dispersants following the Deepwater Horizon oil spill. Environ. Sci. Technol. Lett. 2014, 1, 295−299.
(99) Roberts, J. R.; Reynolds, J. S.; Thompson, J. A.; Zaccone, E. J.; Shimko, M. J.; Goldsmith, W. T.; Jackson, M.; McKinney, W.; Frazer, D. G.; Kenyon, A.; Kashon, M. L.; Piedimonte, G.; Castranova, V.; Fedan, J. S. Pulmonary effects after acute inhalation of oil dispersant (COREXIT EC9500A) in rats. J. Toxicol. Environ. Health, Part A 2011, 74, 1381−1396.
(100) Krajnak, K.; Kan, H.; Waugh, S.; Miller, G. R.; Johnson, C.; Roberts, J. R.; Goldsmith, W. T.; Jackson, M.; McKinney, W.; Frazer, D.; Kashon, M. L.; Castranova, V. Acute effects of COREXIT EC9500A on cardiovascular functions in rats. J. Toxicol. Environ. Health, Part A 2011, 74, 1397−1404.
(101) Sriram, K.; Lin, G. X.; Jefferson, A. M.; Goldsmith, W. T.; Jackson, M.; McKinney, W.; Frazer, D. G.; Robinson, V. A.; Castranova, V. Neurotoxicity following acute inhalation exposure to the oil dispersant COREXIT EC9500A. J. Toxicol. Environ. Health, Part A 2011, 74, 1405−1418.
(102) Anderson, S. E.; Franko, J.; Lukomska, E.; Meade, B. J. Potential immunotoxicological health effects following exposure to COREXIT 9500A during cleanup of the Deepwater Horizon oil spill. J. Toxicol. Environ. Health, Part A 2011, 74, 1419−1430.
(103) Johansen, Ø.; Brandvik, P. J.; Farooq, U. Droplet breakup in subsea oil releases−Part 2: Predictions of droplet size distributions with and without injection of chemical dispersants. Mar. Pollut. Bull. 2013, 73, 327−335.
(104) Place, B. J.; Perkins, M. J.; Sinclair, E.; Barsamian, A. L.; Blakemore, P. R.; Field, J. A. Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. Deep-Sea Res. 2014, DOI: 10.1016/j.dsr2.2014.01.015.
(105) Canevari, G. P. The effect of crude oil composition on dispersant performance. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1985; pp 441−444.
(106) Nedwed, T.; Canevari, G. P.; Clark, J. R.; Belore, R. New dispersant delivered as a gel. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 2005; pp 121−125.
(107) Finch, B. E.; Wooten, K. J.; Smith, P. N. Embryotoxicity of weathered crude oil from the Gulf of Mexico in mallard ducks (Anas platyrhynchos). Environ. Toxicol. Chem. 2011, 30, 1885−1891.
(108) Irie, K.; Kawaguchi, M.; Mizuno, K.; Song, J. Y.; Nakayama, K.; Kitamura, S. I.; Murakami, Y. Effect of heavy oil on the development of the nervous system of floating and sinking teleost eggs. Mar. Pollut. Bull. 2011, 63, 297−302.
(109) Almeda, R.; Baca, S.; Hyatt, C.; Buskey, E. J. Ingestion and sublethal effects of physically and chemically dispersed crude oil on marine planktonic copepods. Ecotoxicology 2014, 23, 988−1003.
(110) Garrett, R. M.; Pickering, I. J.; Haith, C. E.; Prince, R. C. Photooxidation of crude oils. Environ. Sci. Technol. 1998, 32, 3719−3723.
(111) Aeppli, C.; Carmichael, C. A.; Nelson, R. K.; Lemkau, K. L.; Graham, W. M.; Redmond, M. C.; Valentine, D. L.; Reddy, C. M. Oil weathering after the Deepwater Horizon disaster led to the formation of oxygenated residues. Environ. Sci. Technol. 2012, 46, 8799−8807.
(112) Goodman, R. Tar balls: the end state. Spill Sci. Technol. Bull. 2003, 8, 117−121.
(113) Barron, M. G.; Carls, M. G.; Short, J. W.; Rice, S. D.; Heintz, R. A.; Rau, M.; Di Giulio, R. Assessment of the phototoxicity of weathered Alaska North Slope crude oil to juvenile pink salmon. Chemosphere 2005, 60, 105−110.
(114) Payne, J. R.; Clayton, J. R.; McNabb, G. D.; Kirstein, B. E. Exxon Valdez oil weathering fate and behavior: model predictions and field observations 1. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1991, pp 641−654.

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001457-00008

(115) Bobra, M. Water-in-oil emulsification: a physicochemical study. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1991; pp 483−488.
(116) Strøm-Kristiansen, T.; Lewis, A.; Daling, P. S.; Hokstad, J. N.; Singsaas, I. Weathering and dispersion of naphthenic, asphaltenic, and waxy crude oils. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1997; pp 631−636.
(117) Lessard, R. R.; DeMarco, G. The significance of oil spill dispersants. Spill Sci. Technol. Bull. 2000, 6, 59−68.
(118) Thingstad, T.; Pengerud, B. The formation of "chocolate mousse" from Statfjord crude oil and seawater. Mar. Pollut. Bull. 1983, 14, 214−216.
(119) Daling, P. S.; Leirvik, F.; Almås, I. K.; Brandvik, P. J.; Hansen, B. H.; Lewis, A.; Reed, M. Surface weathering and dispersibility of MC252 crude oil. Mar. Pollut. Bull. 2014, 87, 300−310.
(120) Gros, J.; Nabi, D.; Würz, B.; Wick, L. Y.; Brussaard, C. P. D.; Huisman, J.; van der Meer, J. R.; Reddy, C. M.; Arey, J. S. First day of an oil spill on the open sea: early mass transfers of hydrocarbons to air and water. Environ. Sci. Technol. 2014, 48, 9400−9411.
(121) Goldstein, B. D.; Osofsky, H. J.; Lichtveld, M. Y. The Gulf oil spill. N. Engl. J. Med. 2011, 364, 1334−1348.
(122) Atkinson, R.; Arey, J. Atmospheric degradation of volatile organic compounds. Chem. Rev. 2003, 103, 4605−4638.
(123) Deguillaume, L.; Leriche, M.; Amato, P.; Ariya, P. A.; Delort, A. M.; Pöschl, U.; Chaumerliac, N.; Bauer, H.; Flossmann, A. I.; Morris, C. E. Microbiology and atmospheric processes: chemical interactions of primary biological aerosols. Biogeosciences 2008, 5, 841−870.
(124) Colcomb, K.; Bedborough, D.; Lunel, T.; Swannell, R.; Wood, P.; Rusin, J.; Bailey, N.; Halliwell, C.; Davies, L.; Sommerville, M.; Dobie, A.; Mitchell, D.; McDonagh, M.; Lee, K.; Shimwell, S.; Davies, B.; Harries, D. Shoreline cleanup and waste disposal issues during the Sea Empress incident. In International Oil Spill Conference Proceedings; American Petroleum Institute: Washington, DC, 1997; pp 195−198.
(125) Michel, J.; Owens, E. H.; Zengel, S.; Graham, A.; Nixon, Z.; Allard, T.; Holton, W.; Reimer, P. D.; Lamarche, A.; White, M.; Rutherford, N.; Childs, C.; Mauseth, G.; Challenger, G.; Taylor, E. Extent and degree of shoreline oiling: Deepwater Horizon oil spill, Gulf of Mexico, USA. PLoS One 2013, 8, e65087.
(126) Wang, Z.; Fingas, M.; Owens, E. H.; Sigouin, L.; Brown, C. E. Long-term fate and persistence of the spilled Metula oil in a marine salt marsh environment: degradation of petroleum biomarkers. J. Chromatogr. A 2001, 926, 275−290.
(127) Owens, E. H.; Taylor, E.; Humphrey, B. The persistence and character of stranded oil on coarse-sediment beaches. Mar. Pollut. Bull. 2008, 56, 14−26.
(128) Defeo, O.; McLachlan, A.; Schoeman, D. S.; Schlacher, T. A.; Dugan, J.; Jones, A.; Lastra, M.; Scapini, F. Threats to sandy beach ecosystems: a review. Estuarine, Coastal Shelf Sci.. 2009, 81, 1−12.
(129) Burns, K. A.; Garrity, S. D.; Levings, S. C. How many years until mangrove ecosystems recover from catastrophic oil spills? Mar. Pollut. Bull. 1993, 6, 239−248.
(130) Mendelssohn, I. A.; Andersen, G. L.; Baltz, D. M.; Caffey, R. H.; Carman, K. R.; Fleeger, J. W.; Joye, S. B.; Lin, Q.; Maltby, E.; Overton, E. B.; Rozas, L. P. Oil impacts on coastal wetlands: implications for the Mississippi River Delta ecosystem after the Deepwater Horizon oil spill. BioScience 2012, 62, 562−574.
(131) Michel, J.; Rutherford, N. Impacts, recovery rates, and treatment options for spilled oil in marshes. Mar. Pollut. Bull. 2014, 82, 19−25.
(132) Bernabeu, A. M.; Nuez de la Fuente, M.; Rey, D.; Rubio, B.; Vilas, F.; Medina, R.; González, M. E. Beach morphodynamics forcements in oiled shorelines: coupled physical and chemical processes during and after fuel burial. Mar. Pollut. Bull. 2006, 52, 1156−1168.
(133) Pineda-Flores, G.; Boll-Arguëllo, G.; Lira-Galeana, C.; Mesta-Howard, A. M. A microbial consortium isolated from a crude oil sample that uses asphaltenes as a carbon and energy source. Biodegradation 2004, 15, 145−151.
(134) Tavassoli, T.; Mousavi, S. M.; Shojaosadati, S. A.; Salehizadeh, H. Asphaltene biodegradation using microorganisms isolated from oil samples. Fuel 2012, 93, 142−148.
(135) Hayes, M. H. B. Solvent systems for the isolation of organic components from soils. Soil Sci. Soc. Am. J. 2006, 70, 986−994.
(136) Michaels, D.; Howard, J. Review of the OSHA-NIOSH response to the Deepwater Horizon oil spill: protecting the health and safety of cleanup workers. PLoS Curr. 2012, 4, e4fa83b7576b6e.
(137) Schwarz, J. R.; Walker, J. D.; Colwell, R. R. Deep-sea bacteria: growth and utilization of hydrocarbons at ambient and in situ pressure. Appl. Microbiol. 1974, 28, 982−986.
(138) Kimes, N. E.; Callaghan, A. V.; Aktas, D. F.; Smith, W. L.; Sunner, J.; Golding, B. T.; Drozdowska, M.; Hazen, T. C.; Suflita, J. M.; Morris, P. J. Metagenomic analysis and metabolite profiling of deep-sea sediments from the Gulf of Mexico following the Deepwater Horizon oil spill. Front. Microbiol. 2013, 4, 50.
(139) Van Hamme, J. D.; Ward, O. P. Influence of chemical surfactants on the biodegradation of crude oil by a mixed bacterial culture. Can. J. Microbiol. 1999, 45, 130−137.
(140) Lindstrom, J. E.; Braddock, J. F. Biodegradation of petroleum hydrocarbons at low temperature in the presence of the dispersant COREXIT 9500. Mar. Pollut. Bull. 2002, 44, 739−747.
(141) Venosa, A. D.; Holder, E. L. Biodegradability of dispersed crude oil at two different temperatures. Mar. Pollut. Bull. 2007, 54, 545−553.
(142) Hamdan, L. J.; Fulmer, P. A. Effects of COREXIT® EC9500A on bacteria from a beach oiled by the Deepwater Horizon spill. Aquat. Microb. Ecol. 2011, 63, 101−109.
(143) Urbanczyk, H.; Ast, J. C.; Higgins, M. J.; Carson, J.; Dunlap, P. V. Reclassification of Vibrio fischeri, Vibrio logei, Vibrio salmonicida and Vibrio wodanis as Aliivibrio fischeri gen. nov., comb. nov., Aliivibrio logei comb. nov., Aliivibrio salmonicida comb. nov. and Aliivibrio wodanis comb. nov. Int. J. Syst. Evol. Microbiol. 2007, 57, 2823−2829.
(144) Fuller, C.; Bonner, J.; Page, C.; Ernest, A.; McDonald, T.; McDonald, S. Comparative toxicity of oil, dispersant, and oil plus dispersant to several marine species. Environ. Toxicol. Chem. 2004, 23, 2941−2949.
(145) McManus, M. A.; Woodson, C. B. Plankton distribution and ocean dispersal. J. Exp. Biol. 2015, 215, 1008−1016.
(146) Prairie, J. C.; Sutherland, K. R.; Nickols, K. J.; Kaltenberg, A. M. Biophysical interactions in the plankton: a cross-scale review. Limnol. Oceanogr. 2012, 2, 121−145.
(147) Ottesen, E. A.; Young, C. R.; Eppley, J. M.; Ryan, J. P.; Chavez, F. P.; Scholin, C. A.; DeLong, E. F. Pattern and synchrony of gene expression among sympatric marine microbial populations. Proc. Natl. Acad. Sci. U.S.A. 2013, 110, E488−E497.
(148) Dispersant Tools, Job Aids and Decision Process; Region X Regional Response Team, 2015. www.rrt10nwac.com/Files/NWACP/2015/Section%209406.pdf.

DOI: 10.1021/acs.est.5b00961
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

ED_001324_00001457-00009

To: Holder, Edith[holder.edith@epa.gov]
Cc: Bryan, Elisha[Bryan.Elisha@epa.gov]
From: Conmy, Robyn
Sent: Wed 3/4/2015 6:55:42 PM
Subject: RE: List on shared drive
dispersant sample letter_RC edits.docx

I made a slight change to the first paragraph. Otherwise, it looks great.

Thanks,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

From: Holder, Edith
Sent: Tuesday, March 03, 2015 3:29 PM
To: Conmy, Robyn
Cc: Bryan, Elisha
Subject: FW: List on shared drive

Robyn and Elisha,

Thank you Elisha for writing this. It looks pretty good. One question is the first line

The U.S. Environmental Protection Agency is developing

Should that be past tense "has developed" since the protocol has been released for comments. Or is it correct in the present tense as the reason we want to obtain the dispersants is to set the decision rules?

Edie

Edith Holder

Pegasus Technical Services, Inc.

On-Site Contractor to the U.S. EPA

ORD/NRMRL/LRPCD

26 W. Martin Luther King Dr.

Cincinnati, OH 45268

Phone: 513-569-7178

Email: holder.edith@epa.gov

From: Bryan, Elisha
Sent: Tuesday, March 03, 2015 9:10 AM
To: Holder, Edith
Subject: Re: List on shared drive

Hi Edie,

Here is the sample letter, any suggestions before Robyn looks it over?

I tried to make it as easy to understand as possible. I think it will still raise concerns with some of the manufacturers like with what happened with the SWAs.

Elisha

From: Holder, Edith
Sent: Monday, March 2, 2015 2:41 PM
To: Bryan, Elisha
Cc: Conmy, Robyn
Subject: RE: List on shared drive

Elisha,

We received a liter of Finasol OSR 52 within the last year, so we probably don't need that one.

We have close to a L of Dispersit SPC 1000, but it is approaching 10 years old. We have Corexit 9500 from 2010 (approx. 50 mL). There is approx. 20 mL of old JD2000. Then there are small amounts (<2 mL) of Sea Brat, ZI400, Nokomis 3F4, and Saf-ron Gold.

I would say contact everyone except the manufacturers of Finasol, but Robyn can weigh in with her opinion. Robyn should have the opportunity to edit the product request email prior to sending.

Linda Whiteley (MARINE D-BLUE CLEAN™) called me a couple of weeks ago asking about our SWA results. After telling her that we had no results ready for release, I told her that we would be doing more dispersant testing and that I would like to include her product in our testing. So perhaps the note to her could mention that.

Edie

Edith Holder

Pegasus Technical Services, Inc.

On-Site Contractor to the U.S. EPA

ORD/NRMRL/LRPCD

26 W. Martin Luther King Dr.

Cincinnati, OH 45268

Phone: 513-569-7178

Email: holder.edith@epa.gov

From: Bryan, Elisha
Sent: Friday, February 27, 2015 4:51 PM
To: Holder, Edith
Subject: List on shared drive

Hi Edie,

I made the list and put it on the shared drive:

L:\Public\NRMRL-PUB\Holder\OilSpill

6 of the 19 listed are companies that we have previously tried to contact about SWA and either wanted us to sign an agreement or never responded. Another one has not gotten updated contact information and the company is in Japan. I highlighted these, maybe we already have them in the lab. Most of these do not seem to have a shelf life, do we still want to get new stuff if we already have it?

I can work on the email draft next week.

Have a nice weekend!

Elisha

Dear Dispersant Manufacturer,

The U.S. Environmental Protection Agency will be conducting a series of Dispersant Efficacy experiments using the newly proposed Baffled Flask Test for inclusion in the 40 CFR Appendix C to Part 300 Subpart J. I am a contractor with EPA's Office of Research and Development, National Risk Management Research Laboratory (NRMRL), Land Remediation and Pollution Control Division (LRPCD), working on this research.

As your product is listed on the U.S. EPA National Contingency Plan Product Schedule, we would like to include your product in our research and are interested in procuring a small quantity. I am contacting all manufacturers listed so that we can take into consideration the different characteristics of the dispersants.

This research will not change the current status of your product on the Product Schedule. More information on the proposed revisions and who to contact for comments can be found here: https://www.federalregister.gov/articles/2015/01/22/2015-00544/national-oil-and-hazardous-substances-pollution-contingency-plan#h-44.

Could you please send me a price quote for one liter / quart of *DISPERSANT*?

Thank you,

Elisha Bryan

Pegasus Technical Services, Inc.
On-Site Contractor to the U.S. EPA
ORD/NRMRL/LRPCD
26 W. Martin Luther King Dr.
Cincinnati, OH 45268
Phone: 513-569-7047
Email: bryan.elisha@epa.gov

Supporting Information material to accompany ES&T Research Article

"Submersible optical sensors exposed to chemically-dispersed crude oil: wave tank simulations for improved oil spill monitoring"

Robyn N. Conmy[†*], Paula G. Coble[‡], James Farr[∞], A. Michelle Wood[¶], Kenneth Lee[§], W. Scott Pegau[Ω], Ian Walsh[#], Corey R. Koch[#], Mary I. Abercrombie[‡], M. Scott Miles[€], Marlon R. Lewis[£], Scott A. Ryan[§], Brian J. Robinson[§], Thomas King[§], Christopher R. Kelble[¶], Jordanna Lacoste[£]

[†]U.S. Environmental Protection Agency, Office of Research and Development, NRMRL
[‡]University of South Florida, College of Marine Science
[∞]National Oceanic and Atmospheric Administration, Office of Response and Restoration
[¶] National Oceanic and Atmospheric Administration, Atlantic Oceanographic & Meteorological Laboratory[1]
[§]Bedford Institute of Oceanography, Department Fisheries and Oceans Canada[2]
[Ω] Oil Spill Recovery Institute
[#]WET Labs, Inc
[€]Louisiana State University, School of Coast and Environment
[£] Dalhousie University, Department of Oceanography

[*]**Correspondence to 26 West MLK Drive, Cincinnati, Ohio; conmy.robyn@epa.gov; ph: 513-569-7090; fax: 513-569-7620**

Pages S1-S6

[1]University of Oregon, Institute of Ecology and Evolution

[2] Commonwealth Science and Industrial Research Organization, Wealth from Oceans Flagship

Table S1. Experimental design for Dilution and Stepwise Addition treatments.

CORE EXPERIMENTS				STEPWISE ADDITION EXPERIMENTS			
Oil State	Dispersant Treatment	Oil Volume	Experiments Conducted	Oil State	Dispersant Treatment	Oil Volume	[Oil] in fully mixed tank
fresh	no dispersant	100 ml	duplicate	fresh	1:25 DOR	10 ml	300 ppb
fresh	1:25 DOR	100 ml	duplicate	fresh	1:25 DOR	20 ml	600 ppb
weathered	no dispersant	100 ml	duplicate	fresh	1:25 DOR	50 ml	1.5 ppm
weathered	1:25 DOR	100 ml	triplicate	fresh	1:25 DOR	100 ml	3 ppm
				fresh	1:25 DOR	200 ml	6 ppm
				fresh	1:25 DOR	400 ml	12 ppm

Table S2. Submersible sensor configuration and dynamic range provided by manufacturers. FWHM represents Full Width at Half Max.

Manufacturer	Instrument	Light source	Excitation λ (nm)	Emission λ (nm)	Detector	Dynamic Range
Chelsea Technologies Group	UV AQUAtracka (Refined oil)	Xenon lamp	239, 26 FWHM	360, 70 FWHM	PMT	0.001- 10 µg/L Carbazole
	UV AQUAtracka (Crude oil)	Xenon lamp	239, 26 FWHM	440, 110 FWHM	PMT	0.001 - 10 µg/L Perylene
Turner Designs	Cyclops (Crude oil)	LED	320, 130 BP	510, 180 BP	Photodiode	0 - 2700 ppb PTSA Salt
WET Labs Inc	ECO	LED	370, 10 FWHM	460, 120 FWHM	Photodiode	0.01 - 500 ppb Quinine Sulfate
Satlantic	SUNA	Deuterium	210-370 abs	n/a	Spectrometer	0.07 - 28 uM Nitrate

ED_001324_00001464-00003

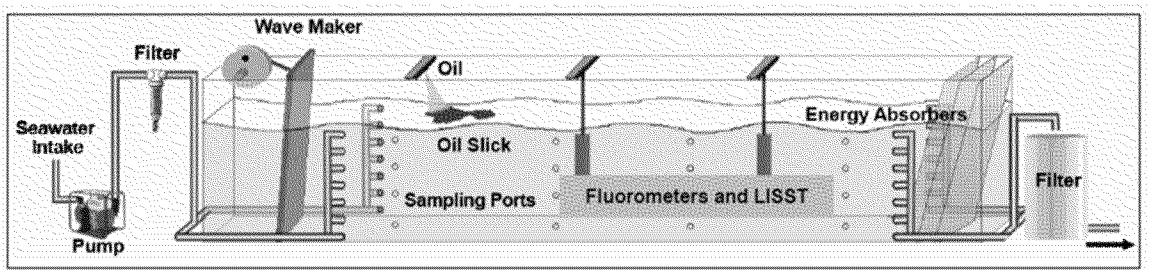


Figure S1. Schematic representation of the wave tank facility. Sampling location options are represented by open circles. Discrete samples were collected 10 m downstream of addition of oil to tank, coincident with sensor location. Plunging breaking waves were produced with a 12 cm stroke and alternating trains of high-frequency waves (0.85 Hz producing a wave length of 2.16 m and wave height 26 cm with a duration of 20 s) and low-frequency waves (0.5 Hz producing a wave length of 6.24 m and a wave height of 9 cm with a duration of 5 s) (Li et al., 2010).

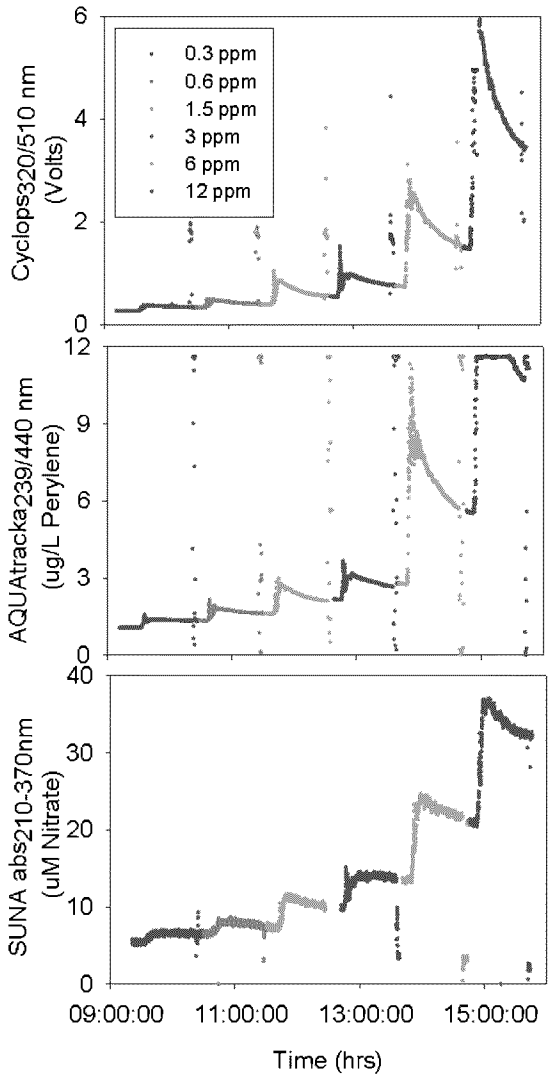


Figure S2. Fluorescence intensity (Cyclops and $AQUA_{239/440\ nm}$) and absorption (SUNA) of sensors exposed to chemically-dispersed MC252 crude oil during EXP_{ADD} within the wave tank.

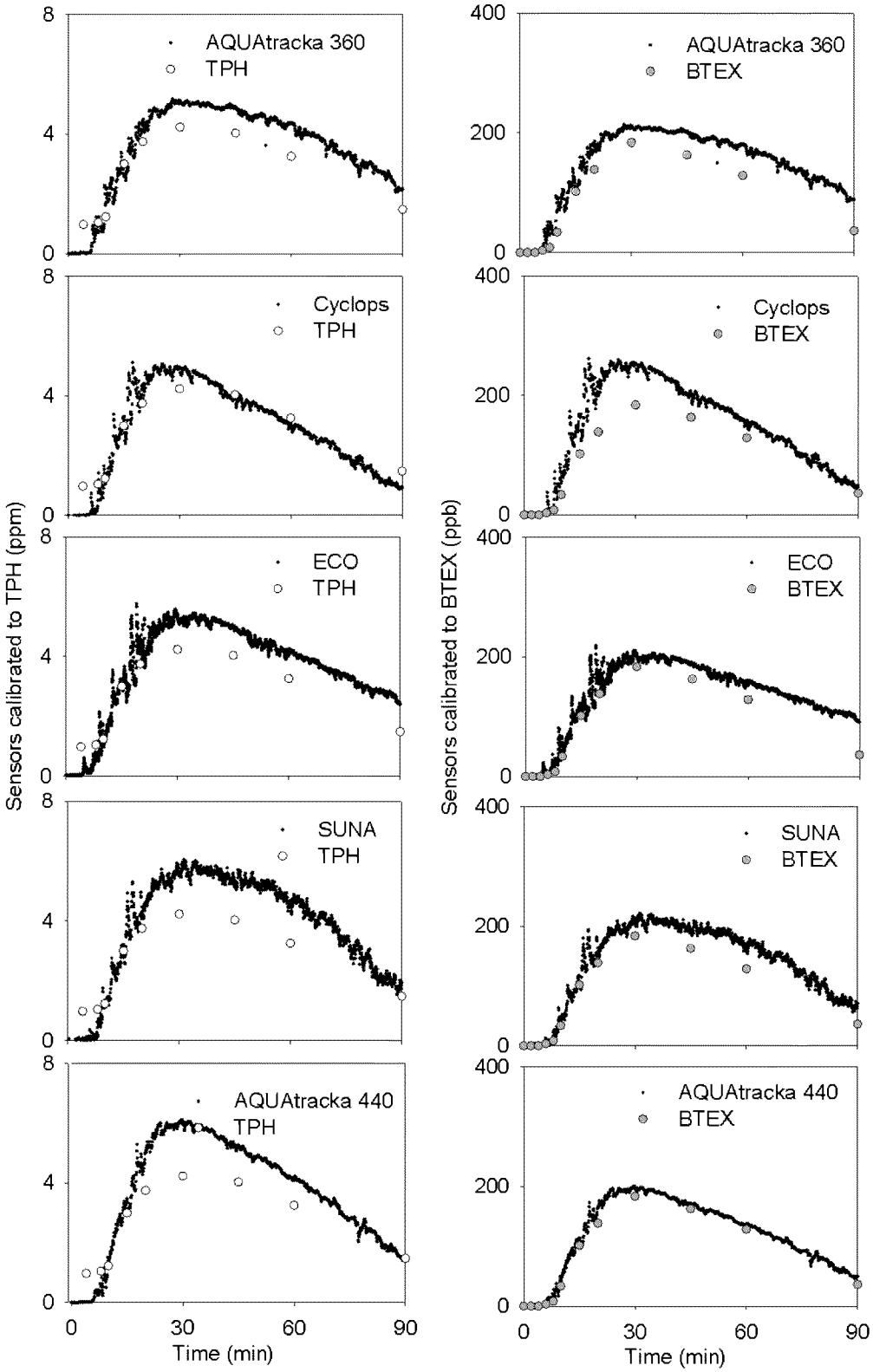


Figure S3. Response of TPH and BTEX-calibrated sensors exposed to chemically-dispersed weathered crude oil. TPH and BTEX values from discrete samples are represented by open and gray circles, respectively. Wave tank was operated in flow-through mode (EXP_{DIL}) to observe fluorescence values during the dilution of an oil spill.

To: Robinson, Brian[Brian.Robinson@dfo-mpo.gc.ca]; King, Thomas L[Tom.King@dfo-mpo.gc.ca]
Cc: McIntyre, Claire E[Claire.McIntyre@dfo-mpo.gc.ca]
From: Conmy, Robyn
Sent: Tue 8/11/2015 7:00:28 PM
Subject: RE: update

Sounds great!

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Robinson, Brian [mailto:Brian.Robinson@dfo-mpo.gc.ca]
Sent: Tuesday, August 11, 2015 2:55 PM
To: Conmy, Robyn; King, Thomas L
Cc: McIntyre, Claire E
Subject: RE: update

How about 11am Atlantic Time? We can call you at your office number if that works.

From: Conmy, Robyn [mailto:Conmy.Robyn@epa.gov]
Sent: August-11-15 3:47 PM
To: Robinson, Brian; King, Thomas L
Subject: RE: update

I'm available all day tomorrow. Just let me know what works for you.

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Robinson, Brian [mailto:Brian.Robinson@dfo-mpo.gc.ca]
Sent: Tuesday, August 11, 2015 2:31 PM
To: Conmy, Robyn; King, Thomas L
Subject: RE: update

Hi Robyn,

I've been meaning to send you a message. All the experiments were completed and everything went really well. We even had time to throw in a few bonus runs with IFO-120 and Finasol. MC252 was interesting…it gave a really strong response on the fluorometers.

Would tomorrow morning work for a call? I'll bring Claire in too so we can talk about the Horiba data.

Brian

From: Conmy, Robyn [mailto:Conmy.Robyn@epa.gov]
Sent: August-11-15 2:57 PM
To: Robinson, Brian; King, Thomas L
Subject: update

Hey guys! Sooo….. how did the final experiments go? Anxious to talk with you about closing up this work and the final report. Do you have time this week to talk?

Thanks,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

To: Holder, Edith[holder.edith@epa.gov]
Cc: Bryan, Elisha[Bryan.Elisha@epa.gov]
From: Conmy, Robyn
Sent: Tue 3/3/2015 6:57:46 PM
Subject: RE: List on shared drive

Thanks Edie.

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

From: Holder, Edith
Sent: Monday, March 02, 2015 2:42 PM
To: Bryan, Elisha
Cc: Conmy, Robyn
Subject: RE: List on shared drive

Elisha,

We received a liter of Finasol OSR 52 within the last year, so we probably don't need that one.

We have close to a L of Dispersit SPC 1000, but it is approaching 10 years old. We have Corexit 9500 from 2010 (approx. 50 mL). There is approx. 20 mL of old JD2000. Then there are small amounts (<2 mL) of Sea Brat, ZI400, Nokomis 3F4, and Saf-ron Gold.

I would say contact everyone except the manufacturers of Finasol, but Robyn can weigh in with her opinion. Robyn should have the opportunity to edit the product request email prior to sending.

Linda Whiteley (MARINE D-BLUE CLEAN™) called me a couple of weeks ago asking about our SWA results. After telling her that we had no results ready for release, I told her that we would be doing more dispersant testing and that I would like to include her product in our testing. So perhaps the note to her could mention that.

Edie

Edith Holder

Pegasus Technical Services, Inc.

On-Site Contractor to the U.S. EPA

ORD/NRMRL/LRPCD

26 W. Martin Luther King Dr.

Cincinnati, OH 45268

Phone: 513-569-7178

Email: holder.edith@epa.gov

From: Bryan, Elisha
Sent: Friday, February 27, 2015 4:51 PM
To: Holder, Edith
Subject: List on shared drive

Hi Edie,

I made the list and put it on the shared drive:

L:\Public\NRMRL-PUB\Holder\OilSpill

6 of the 19 listed are companies that we have previously tried to contact about SWA and either wanted us to sign an agreement or never responded. Another one has not gotten updated contact information and the company is in Japan. I highlighted these, maybe we already have them in the lab. Most of these do not seem to have a shelf life, do we still want to get new stuff if we already have it?

I can work on the email draft next week.

Have a nice weekend!

Elisha

To: Robinson, Brian[Brian.Robinson@dfo-mpo.gc.ca]; King, Thomas L[Tom.King@dfo-mpo.gc.ca]
From: Conmy, Robyn
Sent: Tue 8/11/2015 6:47:00 PM
Subject: RE: update

I'm available all day tomorrow. Just let me know what works for you.

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Robinson, Brian [mailto:Brian.Robinson@dfo-mpo.gc.ca]
Sent: Tuesday, August 11, 2015 2:31 PM
To: Conmy, Robyn; King, Thomas L
Subject: RE: update

Hi Robyn,

I've been meaning to send you a message. All the experiments were completed and everything went really well. We even had time to throw in a few bonus runs with IFO-120 and Finasol. MC252 was interesting…it gave a really strong response on the fluorometers.

Would tomorrow morning work for a call? I'll bring Claire in too so we can talk about the Horiba data.

Brian

From: Conmy, Robyn [mailto:Conmy.Robyn@epa.gov]
Sent: August-11-15 2:57 PM
To: Robinson, Brian; King, Thomas L
Subject: update

Hey guys! Sooo….. how did the final experiments go? Anxious to talk with you about closing up this work and the final report. Do you have time this week to talk?

Thanks,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

To: Campo-Moreno, Pablo (campomp)[campomp@UCMAIL.UC.EDU]
From: Conmy, Robyn
Sent: Thur 7/30/2015 3:21:14 PM
Subject: RE: CHEM36329 Editor decision - revise

That is good news. Can you send the final citation when you have it? How are the biodegradation experiments coming along?

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Campo-Moreno, Pablo (campomp) [mailto:campomp@UCMAIL.UC.EDU]
Sent: Thursday, July 30, 2015 10:44 AM
To: Conmy, Robyn
Subject: Re: CHEM36329 Editor decision - revise

Robyn,

Finally good news about the JD-2000 paper. Chemosphere has accept it with a minor revision: we need to add a conclusion at the end of the paper (see below)

Regards,

Pablo Campo-Moreno, Ph.D.
Research Assistant Professor
University of Cincinnati
Department of Biomedical, Chemical and Environmental Engineering
766 ERC
Cincinnati, OH 45221-0012
PH:(513)-556-3637
Fax: (513)-556-4162
campomp@ucmail.uc.edu

It is a capital mistake to theorize before one has data. Insensibly one begins to twist facts to suit theories, instead of theories to suit facts.
The adventures for Sherlock Holmes

On Jul 30, 2015, at 5:51 AM, Suidan, Makram (suidanmt) <SUIDANMT@UCMAIL.UC.EDU> wrote:

On 7/30/15, 12:36 PM, "Chemosphere" <chem-eo@elsevier.com> wrote:

Dear Dr. Suidan,

I can now inform you that the reviewers and editor have evaluated the manuscript "Effect of Dispersants on the Biodegradation of South Louisiana Crude Oil at 5 and 25 °C" (Dr. Makram T Suidan). I am pleased to say that it has been favourably received and publication with minor revision is recommended (see below and on http://ees.elsevier.com/chem/).

I look forward with interest to receipt of the modified version. You should explain how and where each point of the reviewers' comments has been incorporated. For this, use submission item "Revision Notes" when uploading your revision. Also, indicate the changes in marked changes version of the revised manuscript (submission item "Revision, changes marked"). Should you disagree with any part of the reviews, please explain why.

To facilitate further review, lines in this manuscript should be consecutively numbered.

Please strictly follow the formatting requirements as presented in the Guide for Authors.

As it is in the interest of authors to have a short turnaround time, we kindly ask you to submit the revised manuscript as soon as possible but within 21 DAYS. If the revised manuscript is not submitted before the deadline, the manuscript will be considered withdrawn.

To submit a revision, go to http://ees.elsevier.com/chem/ and log in as an author. You will find your submission record under Submission(s) Needing Revision.

For guidelines on how to submit your revised manuscript please go the following address:
http://help.elsevier.com/app/answers/detail/p/7923/a_id/91

When submitting your revised manuscript, please ensure that you upload the source files (e.g. Word). Uploading a PDF file at this stage will create delays should your manuscript be finally accepted for publication. If your revised submission does not include the source files, we will contact you to request them.

When resubmitting, please present any figures, tables etc. as separate files. See the Artwork Guidelines on the home page right menu for further file naming conventions, referencing and format issues.

I hope that you will find the comments to be of use to you and am looking forward with interest to receiving your revision.

NOTE: Upon submitting your revised manuscript, please upload the source files for your article. For additional details regarding acceptable file formats, please refer to the Guide for Authors at:
http://www.elsevier.com/journals/chemosphere/0045-6535/guide-for-authors

When submitting your revised paper, we ask that you include the following items:

Response to Reviewers (mandatory)

This should be a separate file labeled "Response to Reviewers" that carefully addresses, point-by-point, the issues raised in the comments appended below. You should also include a suitable rebuttal to any

specific request for change that you have not made. Mention the page, paragraph, and line number of any revisions that are made.

Manuscript and Figure Source Files (mandatory)

We cannot accommodate PDF manuscript files for production purposes. We also ask that when submitting your revision you follow the journal formatting guidelines. Figures and tables may be embedded within the source file for the submission as long as they are of sufficient resolution for Production. For any figure that cannot be embedded within the source file (such as *.PSD Photoshop files), the original figure needs to be uploaded separately. Refer to the Guide for Authors for additional information.
http://www.elsevier.com/journals/chemosphere/0045-6535/guide-for-authors

Highlights (mandatory)

Highlights consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). See the following website for more information
http://www.elsevier.com/highlights

Graphical Abstract (optional)

Graphical Abstracts should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Refer to the following website for more information:
http://www.elsevier.com/graphicalabstracts

Please note that this journal offers a new, free service called AudioSlides: brief, webcast-style presentations that are shown next to published articles on ScienceDirect (see also http://www.elsevier.com/audioslides). If your paper is accepted for publication, you will automatically receive an invitation to create an AudioSlides presentation.

Please note that we allow 60 days for the first author revision and 45 days for any additional author revisions that are required.

Chemosphere features the Interactive Plot Viewer, see: http://www.elsevier.com/interactiveplots. Interactive Plots provide easy access to the data behind plots. To include one with your article, please prepare a .csv file with your plot data and test it online at http://authortools.elsevier.com/interactiveplots/verification before

submission as supplementary material.

Thank you for submitting your work to this journal.

Kind regards,
Prof. Yu Liu
Editor
Chemosphere

.....
Important note: If a reviewer has provided a review or other materials as attachments, those items will not be in included in this letter. Please ensure therefore that you log on to the journal site and check if any attachments have been provided.

COMMENTS FROM EDITORS AND REVIEWERS

Reviewer #1: In this research paper, authors have reported quantitative biodegradability of a primary component in the oil spill dispersant JD-2000, in the presence and absence of crude oil.
The findings are unique and significant enough for publication. The manuscript is well prepared.
However, it would be good to provide a conclusion at the end of the paper which is somehow missing from the manuscript.

.....
Author's Checklist

Please follow this checklist to avoid another round of review. The best way is to refer to Chemosphere website for author's guidelines.

MS Number
Add Chemosphere code number in all your correspondence.

Abstract/Text
The page limitation has to be followed. Unless specified differently by the editor the number of tables plus figures should not exceed seven.

Text
Follow the format with respect to unit, section headings, line numbers,

etc.
Delete all well known equations (e.g., 1st-order reaction, isotherms, etc.).

Units
Use SI units; follow the correct format (e.g., mg kg-1).
Use M for mol/L; d for days, h for hours, min for minutes, etc.
Be consistent! Use uppercase L for l (litre).

Syntax
Please always leave a space between number and SI unit (e.g. 4.6 mm, 21 °C, 24 h etc.),
however no space before the "%", "/" and ":" signs. Moreover, provide an adequate space in
between words.
Use periods for decimal points.
Don't use italics to highlight text or reference literature. Italics should be used for
Latin/Greek expressions only.

Acronyms
Define acronyms when they first appear; thereafter directly use them.
Again: be consistent!
Do not create new acronyms.
Don't use improper names for chemicals, e.g., "dioxins" for PCDD
No need to define if they are no longer used.
No need to define simple chemical formulas.

Math
Slash equations in Text/Tables [e.g., (a)/c), but stack numerators over denominators e.g.,
a
--- in equations.
C
Use numerical numbers 1, 2, etc, for equations.

Citation
Use author-date format. For one author, use author's name; for 2 authors, spell out the
names of both authors; for 3 or more than 3 authors, use et al. All cited references must be listed.
Follow the chronological order.

References
Use abbreviated journals (see the ISI list under

(http://library.caltech.edu/reference/abbreviations/, which is freely accessible).
Delete all issue numbers for consistency.
Capitalize the 1st letter of each word for books/reports/thesis; use lowercase letters for paper titles.

Tables/Figures
Follow the format; numbered sequentially.
Give a list of the figure captions on a separate page.

Significant figures
Consider the reproducibility of your results (errors in sampling, extraction and
measurements) when presenting data.

****
For further assistance, please visit our customer support site at http://help.elsevier.com/app/answers/list/p/7923. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

To: advenosa@cinci.rr.com[advenosa@cinci.rr.com]
From: Conmy, Robyn
Sent: Tue 10/27/2015 6:03:38 PM
Subject: fluorometry citation
conmy et al 2014 ES&T_published.pdf
Conmy et al 2013 ES&T_supporting information_FINAL dec2013.docx

Here is the paper published last year along with the supplemental material. This paper and the references therein should be of help.

Cheers,

Robyn

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

Environmental Science & Technology

Article

pubs.acs.org/est

Submersible Optical Sensors Exposed to Chemically Dispersed Crude Oil: Wave Tank Simulations for Improved Oil Spill Monitoring

Robyn N. Conmy,[†,*] Paula G. Coble,[‡] James Farr,[□] A. Michelle Wood,[§,◆] Kenneth Lee,[//,¶] W. Scott Pegau,[⊥] Ian D. Walsh,[#] Corey R. Koch,[#] Mary I. Abercrombie,[‡] M. Scott Miles,[▽] Marlon R. Lewis,[○] Scott A. Ryan,[//] Brian J. Robinson,[//] Thomas L. King,[//] Christopher R. Kelble,[§] and Jordanna Lacoste[○]

[†]National Risk Management Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, 26 West Martin Luther King BoulevardCincinnati, Ohio 45268, United States

[‡]College of Marine Science, University of South Florida, St. Petersburg, Florida 33701, United States

[□]Office of Response and Restoration, National Oceanic and Atmospheric Administration, 1305 East-West Highway, Silver Spring, Maryland 20910, United States

[§]Atlantic Oceanographic & Meteorological Laboratory, National Oceanic and Atmospheric Administration, 4301 Rickenbacker Causeway, Miami, Florida 33149, United States

[//]Department Fisheries and Oceans Canada, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada

[⊥]Oil Spill Recovery Institute, 300 Breakwater Avenue, Cordova, Alaska 99574, United States

[#]WET Labs, Inc., PO Box 518, Philomath, Oregon 97370, United States

[▽]School of Coast and Environment, Louisiana State University, Baton Rouge, Louisiana 70803, United States

[○]Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada

* Supporting Information

In situ fluorometers were deployed during the Deepwater Horizon (DWH) Gulf of Mexico oil spill to track the subsea oil plume. Uncertainties regarding instrument specifications and capabilities necessitated performance testing of sensors exposed to simulated, dispersed oil plumes. Dynamic ranges of the Chelsea Technologies Group AQUAtracka, Turner Designs Cyclops, Satlantic SUNA and WET Labs, Inc. ECO exposed to fresh and artificially weathered crude oil, were determined. Sensors were standardized against known oil volumes and total petroleum hydrocarbons and benzene-toluene-ethylbenzene-xylene measurements - both collected during spills, providing oil estimates during wave tank dilution experiments. All sensors estimated oil concentrations down to 300 ppb oil, refuting previous reports. Sensor performance results assist interpretation of DWH oil spill data and formulating future protocols.

INTRODUCTION

SMART (special monitoring of applied response technologies) protocols use real-time fluorescence monitoring in decision-making during oil spill dispersant operations[1] to determine efficacy of chemically enhanced dispersion and dispersed oil transport.[2] Chemical dispersants applied to oil slicks (in the presence of mixing energy) reduce the oil−water interfacial tension, forming small oil droplets (<100 um) that are less likely to recoalesce,[3] removing oil from the water surface and suspending neutrally buoyant oil droplets as a subsurface plume. This reduces risk to shorelines and air−water interface biota and also increases microbial biodegradation rates of oil.[4−6] Under SMART, in situ monitoring of surface waters (<2 m) and below (2 - 10 m) evaluates efficacy. Monitoring requires rapid, reliable, easy-to-operate in situ fluorometers. Validation with oil/water samples yields oil mapping with finer temporo-spatial resolution not achieved through coarse discrete measurements alone.

Fluorescence has been an oil spill monitoring tool for more than 30 years.[7,8] Measurements are inherently sensitive, conducted without laborious extraction techniques, time

Received: September 20, 2013
Revised: December 17, 2013
Accepted: December 30, 2013

 © XXXX American Chemical Society

ED_001324_00001490-00001

efficient, chemistry dependent and indicators of hydrocarbon concentration.[9,10] Low viscosity physically dispersed crude oils typically have two broad UV peaks with excitation (Ex) between 240 and 300 nm and emission (Em) centered at ~350 nm (low molecular weight polycyclic aromatic hydrocarbons, LMW PAH, <3 benzene rings) and ~450 nm (higher MW PAH, >3 benzene rings).[11,12] Fluorescence signatures discriminate oils[11−14] but are complicated by varying concentration, degree of chemical and physical dispersion, oil age and state (fresh, weathered, mousses),[11,14,15] and interference of fluorophores with spectro-chemical similarities. Such is the case with colored dissolved organic matter (CDOM) and oil aromatic fractions, which exhibit broad fluorescence peaks and complex spectra. However, crude oils have peak maxima at slightly shorter wavelengths relative to aquatic CDOM (humics, fulvics), facilitating discrimination.

Optical oil detection is a function of matching Ex/Em wavelengths with oil (center wavelength (CWL) and filter bandwidth), power of the light source, and detector sensitivity. Numerous submersible fluorometers are commercially available, each with unique configurations for detecting compounds[16] (and references therein), thus expanding their use in spill response efforts and SMART protocols.[17] Reviews of commercial off-the-shelf (COTS) sensors[17−19] highlight the need for robust calibrations, operational procedures and scalability testing.[20] Reviews have targeted easy-to-conduct, low-cost bench-scale testing. Often tests are conducted in the absence of dispersant, in small flasks with insufficient mixing energy to physically disperse oil into water,[21] at elevated oil concentrations irrelevant to field observations, with limited oil type and weathering states, and without calibration to standards or hydrocarbon concentrations. Thus there are confidence challenges in establishing saturation cutoffs and detection limits relevant for field applications. Effectiveness in quantitative monitoring also remains unclear.[19] Hence, caution must be exercised when scaling up experimental designs to real-world conditions and decision-making. Rather, a combination of laboratory, wave tank, and field studies has been recommended to establish the best degree of confidence for detecting chemically dispersed oil during environmental spills.[22]

SMART provides guidance on surface, not deepwater spill response like the 2010 Deepwater Horizon spill (DWH) where ~800 million liters of light Louisiana sweet crude oil was released from 1500 m ocean depth. Seven million liters[23] of chemical dispersant (Corexit© 9527, 9500A) were applied to reduce environmental impacts of oil, and aid worker safety. The response effort proved challenging partly due to the inability to visually inspect the subsea oil plume. The horizontal, vertical and temporal extent of, and heterogeneity of the plume equated to a "needle-in-the-haystack" situation with grab sampling too coarse to capture its magnitude and extent. Sample number limitations and oil stratification within bottles during the 1−2 h profiler transit time also contributed to challenges in quantifying oil.[24] Thus, responders and scientists were evermore reliant on submersible sensors in lieu of visual observations to provide critical tracking measurements. Initial plume tracking between 1000 and 1400 m was conducted using WET Labs, Inc. ECO fluorometers. Their accessibility and wavelength configuration- wide filter bandwidth and ExEm370/460 nm CWL (Supporting Information (SI) Table S2) to capture broad nature of the peaks- is appropriate for crude oil fluorescence detection. Chelsea Technologies Group AQUAtracka fluorometers were deployed to monitor the transport and fate of oil at large distances from the wellhead and after well capping (latter response, low concentrations).[24] This unit employs filters (ExEm239/360 nm CWL) to capture peak fluorescence from petroleum aromatic fractions with elevated sensitivity (higher signal-to-noise) due to a xenon lamp light source and a photomultiplier tube (PMT) detector; possibly aiding MC252 oil tracking which has a small fraction of highly fluorescing molecules (~20%).[24]

In the wake of the spill many questions remain regarding oil quantification and fate of the subsea oil plume, as well as sensor applicability to detect oil (dynamic range, detection limits, and wavelength configuration).[25] Effects of concentration, dispersant to oil ratio (DOR), dispersant effectiveness,[26] and fluorescence signatures[12] contribute to uncertainties in selecting sensors. Moreover, concerns persist on sensor reliability and applicability of laboratory calibrations using flasks, high oil concentrations and insufficient mixing energies to real-world conditions[19,25] To address uncertainties the performance of select COTS in situ fluorometers deployed during DWH (Chelsea Technologies Group, Turner Designs, WET Labs, Inc.) and a UV spectrophotometer (Satlantic) was evaluated in the Bedford Institute of Oceanography (BIO) wave tank. This work is timely, as findings will assist in the calibration and interpretation of DWH fluorescence measurements. It also coincides with the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) amendments pertaining to fluorometer protocols, reflecting the need for improved confidence in sensors for estimating spilled oil concentrations.

EXPERIMENTAL SECTION

Wave Tank Description. Experiments were conducted at the BIO, Center for Offshore Oil, Gas and Energy Research (COOGER; Dartmouth, Nova Scotia) wave tank, measuring 32 m long, 0.6 m wide, and 2 m high (1.5 m water depth; 28 800 L volume) (SI Figure S1). Water from the Bedford Basin of Halifax Harbor was pumped into the tank through a coarse (25 μm pore size) and fine (5 μm pore size) serial filtration system. Breaking and nonbreaking waves (computer-controlled flat-type wave maker) provided mixing energies to achieve dispersant effectiveness observed in the field. The tank was drained and cleaned with Big Orange detergent and seawater (tank walls, bottom, wave maker and absorbers) after each experiment to remove all oil and surfactants, and sensor optical windows were also cleaned.

Experimental Design. Experiments were conducted using MC252 crude oil (Discoverer Enterprise drillship recovered, May 31−June 7, 2011), and Corexit© 9500 chemical dispersant (1:25 DOR). Artificially weathered oil was generated by bubbling with nitrogen for 36 h (average time responders typically begin dispersant) resulting in mass reduction by 7%. Experimental duplicate and triplicate treatments were run with random design, reducing effects from confounding factors such as wind, rain, temperature, salinity, and seawater quality (SI Table S1). Prior to experiments, oil and dispersant mixtures were prepared in 1 L of seawater by orbital shaker agitation (20 min). Fluorometers (WET Labs, Inc. ECO, Chelsea Technologies Group AQUAtracka, Turner Designs Cyclops) and a UV spectrophotometer (Satlantic SUNA) were deployed at the same cross-sectional position within the tank 20 m from the wave generator (10 m downstream from oil release). Configuration specifications are shown in SI Table S2 and Figure S1. A laser in situ scatteringand transmissometryparticle size analyzer (Sequoia Scientific LISST-100X, C-Type,), 1 m

ED_001324_00001490-00002

downstream from sensors, collected continuous particle size distribution measurements. Sensors were mounted so that optical windows were positioned 65 cm deep in the tank and warmed up for 15 min prior to oil addition and collection of baseline. A stepwise addition experiment (EXP_{ADD}) of chemically dispersed fresh oil (300 ppb to 12 ppm, tank operated in steady-state mode) was conducted to establish response linearity and dynamic range of sensors to MC252 oil. Each addition of oil/dispersant was allowed to homogenize, with wave mixing, for 1 h prior to subsequent oil additions. Water samples were collected during the final 5 min of each step and sensors were cleaned in between steps using lens wipes and solvent. There was no indication of sensor fouling from oil as sensor response was checked pre- and postcleaning. Continuous optical data were collected during the 9 h experiment and manufacturer calibrations applied.

Dilution experiments (EXP_{DIL}) were conducted with 100 mL of fresh or weathered oil added, with or without dispersant (only chemically dispersed results reported here). Maximum concentration in the tank was less than 3 ppm, when fully dispersed. To simulate field conditions, ocean waves were generated with tank in flow-through mode (flow rate of 3.8 ± 0.2 L s^{-1}) allowing transport of dispersed oil away from the slick and dilution to extinction.[27,28] EXP_{DIL} were conducted for 90 min, where discrete oil concentration samples were collected at 15 time points via tubing through an inlet located next to the sensor deployment frame. Three-dimensional excitation emission matrix fluorescence spectroscopy (EEMS) analyses confirmed the position of fluorescence peaks and degree of chemical dispersion.

Submersible Sensor Data Processing. Sensor specific blanks (2 min average) prior to oil addition were subtracted. Manufacturer calibrations yielded data in recommended units (carbazole and perylene for AQUAtrackas, quinine sulfate dihydrate for ECO, uM nitrate for SUNA weighted index of UV absorption 210−370 nm, and none was provided for the Cyclops). Final 5-min signal averages for each concentration during the EXP_{ADD} was regressed (model 1, linear least-squares) to ppm oil, benzene-toluene-ethylbenzene-xylene (BTEX) or total petroleum hydrocarbons (TPH) values. Regressions were applied to EXP_{DIL} fluorescence and absorption data. Sensor performance and vicarious calibration were validated with laboratory chemistry results. LISST-11X dispersed oil droplet size distribution recorded 32 particle size intervals logarithmically spaced from 2.5−500 μm in diameter, with the upper size in each bin 1.18 times the lower. Distribution is expressed as the average volumetric concentration of droplets within each size range interval. Total particle concentration (TPC) represents the sum of all particle concentrations between 2.5 and 500 μm.[27]

Discrete Samples Analyses. Oil/water samples were collected at time points and analyzed for TPH, BTEX and PAH. TPH values were acquired using a GC-FID (gas chromatograph-flame ionization detector, Agilent 6890 and Supelco column). Extraction methods[29] were used and samples were concentrated under nitrogen to a final volume of 1.0 mL prior to analysis. Peak quantification, using the total area under the curve, yielded hydrocarbon concentrations in seawater. A spike and recovery study with blanks and duplicates verified extraction method efficiency. BTEX (volatile hydrocarbons) were measured via GC-MS (GC-mass spectrometry, Agilent 5973) using a modified version[29] of the standard EPA Method 8240 (purge and trap) for oil applications. A 5 mL sample was transferred into the purge chamber and purged with helium for 11 min. The volatiles were trapped and then desorbed at 225 °C for 2 min. The MS was used in selected ion monitoring mode (SIM) for six ions: 77, 78, 91, 92, 105, and 106 amu. BTEX calibration standards were prepared by diluting a certified standard mix (Supelco). Both standard and samples (blanks and duplicates) were purged, trapped and analyzed in the same manner.

EEM and absorbance spectra were collected with a Horiba Scientific AquaLog spectrofluorometer with a CCD detector. Oil/water samples were analyzed for fluorescence peaks within 10 min of collection. Samples within a 1 cm cuvette were scanned over Ex 220−550 nm and Em 213−610 nm (1 s integration), blank subtracted, inner-filter effect corrected, quinine sulfate dihydrate normalized, and Rayleigh scatter masked.[30,11]

RESULTS AND DISCUSSION

An EEM contour plot of chemically dispersed MC252 oil (4 ppm) collected during an EXP_{DIL} illustrates the UV fluorescent peak maxima centered on ExEm270/325 and ExEm260/450 nm

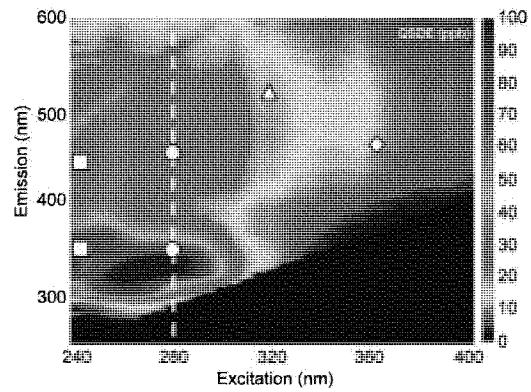


Figure 1. Fluorescence EEM of chemically dispersed fresh MC252 oil (4 ppm) concentration in a EXP_{DIL}. Center Wavelengths (CWL) of Chelsea Technologies Group AQUAtrackas $ExEm_{239/360}$ and $ExEm_{239/440}$ (□), Turner Designs Cyclops $ExEm_{320/510}$ (△), and WET Labs, Inc. ECO $ExEm_{370/460}$ (◊) along with the fluorescence intensity ratio (FIR = $ExEm_{280/340:\ 280/445}$; ○) are overlaid for reference.

(Figure 1). Color map units are ppb QSDE (Quinine Sulfate Dihydrate Equivalents), scaled to peak maximum. CWL of the sensors tested are overlaid illustrating that all sensors were configured to detect some portion of the broad oil signal. Note that the symbols do not take into account wavelength bandwidths, which increases a sensor's ability to detect oil. For reference purposes, white circles on the dotted line represent the fluorescence intensity ratio (FIR) wavelengths used to indicate chemical dispersion of oil.[12]

Manufacturer calibrated sensor response at six cumulative oil concentrations during the EXP_{ADD} is shown in Figure 2 and SI Figure S2. The addition of oil and dispersant formed an oil plume with a sharp leading edge that passed in front of the sensors causing a rapid increase in signal followed by a decrease as the oil mixed/dispersed in the tank. Values at the end of each step (tank homogeneously mixed) are representative of sensor response for a specific oil concentration and established that all sensors detected chemically dispersed oil down to 300 ppb, a critical finding. This is below the laboratory detection limit for GC-FID TPH method, making sensor-estimated oil concentrations evermore valuable for tracking low concentration

ED_001324_00001490-00003

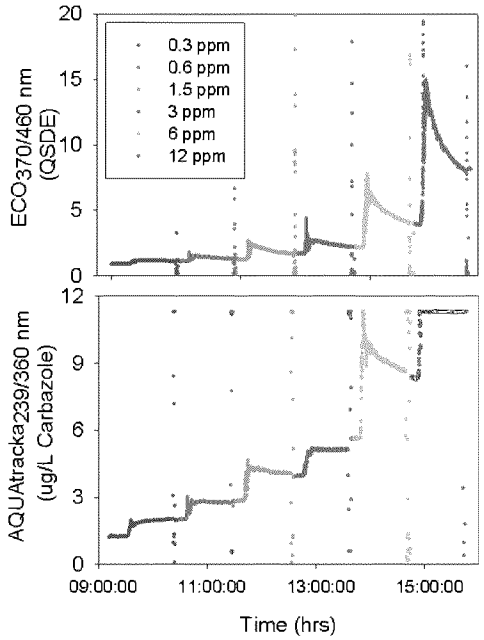


Figure 2. Fluorescence intensity of the ECO and $AQUA_{239/360\ nm}$ sensors exposed to chemically dispersed MC252 crude oil during EXP_{ADD} within the wave tank.

plumes. It also refutes previous notions during the DWH response that certain sensors may not detect oil <1 ppm. This was supported by earlier calibration tests using small flasks which may have been biased by underachieving proper mixing energies negatively impacting dispersant efficiency and solubility of fluorescence molecules into the water phase.[22] Strong linearity of sensor response at the lowest concentrations suggests possible lower detection limits less than 300 ppb (not tested with this experimental design). In any case, sensor response at 300 ppb improves confidence in the instruments deployed for DWH subsea plume monitoring, thereby demonstrating ability to detect low concentrations of oil.

Sensor dynamic range was established during the EXP_{ADD} by regressing fluorescence and absorption averages with oil concentration (Figure 3). Generally, submersible sensors were linearly correlated with TPH, BTEX and oil load, regardless of optical configuration. The ECO was operated in digital mode (no gain control) and the SUNA employed autoadjusting integration time, evidenced by lack of saturation over the entire concentration range. The Cyclops was operated with 100X gain for concentrations ≤6 ppm, but 10X for 12 ppm to avoid saturation. The AQUAtrackas employ BITE (Built in Test Circuitry) to extend calibration intervals. However saturation was exhibited by the $AQUAtracka_{239/360\ nm}$ sensor at 12 ppm oil. This is attributed to higher detector sensitivity, increased power of the light source (flash lamp opposed to LED) and/or wavelength filters closely aligned to the fluorescence maxima of crude oil. The longer wavelength emission AQUA $tracka_{239/440\ nm}$ sensor however did not saturate. Regression equations were employed to calibrate sensors during EXP_{DIL} (Figure 3). This aligns results with response effort measurements: sTPH (speciated TPH; water-insoluble heavy saturate fraction; aromatics > naphthalene; less laborious analytical screening) supplemented with BTEX (more laborious screening) values (Figure 3). All sensors exhibited squared correlation coefficients greater than 0.96. The 12 ppm value for the $AQUAtracka_{239/360\ nm}$ and SUNA (for BTEX) was excluded from the calculation due to saturation. The ECO and BTEX regression excluded values above 1.5 ppm, thereby increasing performance at low concentrations. TPH values for lowest concentration additions are absent because they were below the detection limit of the laboratory-based GC-FID TPH analysis. For all sensors, signal-to-noise ratios permitted detection at low concentrations. Findings demonstrate that all sensors would have been valuable tools during the DWH response given that sTPH and tVOA (total volatile organic analysis; water-soluble fraction; namely BTEX and naphthalene) in field samples were within the linear range of sensors tested in this study.[24] During the spill, sTPH from GC-MS ranged between <1 - 1000 ppb, with subsea plume (900−1300 m) maximum of 485 ppb, 1.2 km from the wellhead and detectable levels extending >10 km from source. In the plume, tVOA concentrations were higher than sTPH, with a maximum of 2112 ppb.

Sensor performance as a function of dispersant presence, oil state, and plume dilution was examined during EXP_{DIL}. TPH and BTEX-calibrated sensor responses for one-fresh and one-weathered, chemically dispersed oil EXP_{DIL} are shown in Figure 4 and SI Figure S3, respectively. Left panels show TPH-calibrated sensor and discrete TPH results (open circles), right panels show BTEX-calibrated sensor and discrete BTEX results (gray circles) over the course of the 90 min experiment. Due to a delayed shipping issue, the $AQUAtracka_{239/440\ nm}$ was operated only during one EXP_{DIL} and appears in the latter figure. EXP_{DIL} particle size distribution (PSD) and concentration of fresh oil droplets corresponding to Figure 4 are shown in Figure 5 (left). Histograms represent a composite of the entire duration of a flow-through experiment (90 min). Presence of particles observed in the <100 um size classes are indicative of chemical dispersion. All experiments using dispersant exhibited oil droplet size ranges consistent with adequate dispersant effectiveness (droplets <100 μm, Figure 5).[26]

For all experiments, MC252 oil state (fresh or weathered) did not appear to influence the sensor response. All sensors responded similarly to both (Figure 4 and SI Figure S3), verified with similar particle size distribution. Like sensor response as a function of oil state lends support for applying regression equations calculated with fresh oil to dilution experiments using weathered oil. Note that oil artificially weathered by 7% may represent oil exposed to physical processes (volatilization due to photo-oxidation, evaporation or dissolution) that occur during a spill of short duration, not necessarily of a prolonged spill with extensive weathering. However, the degradation of light crude oil and the addition of dispersant have been shown to increase the occurrence of longer wavelength fluorescence peaks.[11,15] Hence, sensors may be better capable of detecting extensively weathered oil where wavelength configuration better overlaps with longer fluorescence peaks (Figure 1). This is particularly true for lighter crudes such as MC252 oil that contain a higher proportion of simpler, lower molecular weight hydrocarbons that are more readily physically and biodegraded compared to heavy crudes.[31]

Tank hydrodynamics affected the movement and dispersion of oil within the tank, which varied during dilution experiments. Total particle concentration (TPC) as a function of time was influenced by tank mixing, where experiments exhibited either a gradual or rapid (Figure 5, left and right) decay of oil droplets. Three tests exhibited a gradual increase in signal as oil passed the sensors in the tank followed by gradual decay with dilution (Figure 4). For these cases, maximum values were 4−5 ppm

ED_001324_00001490-00004

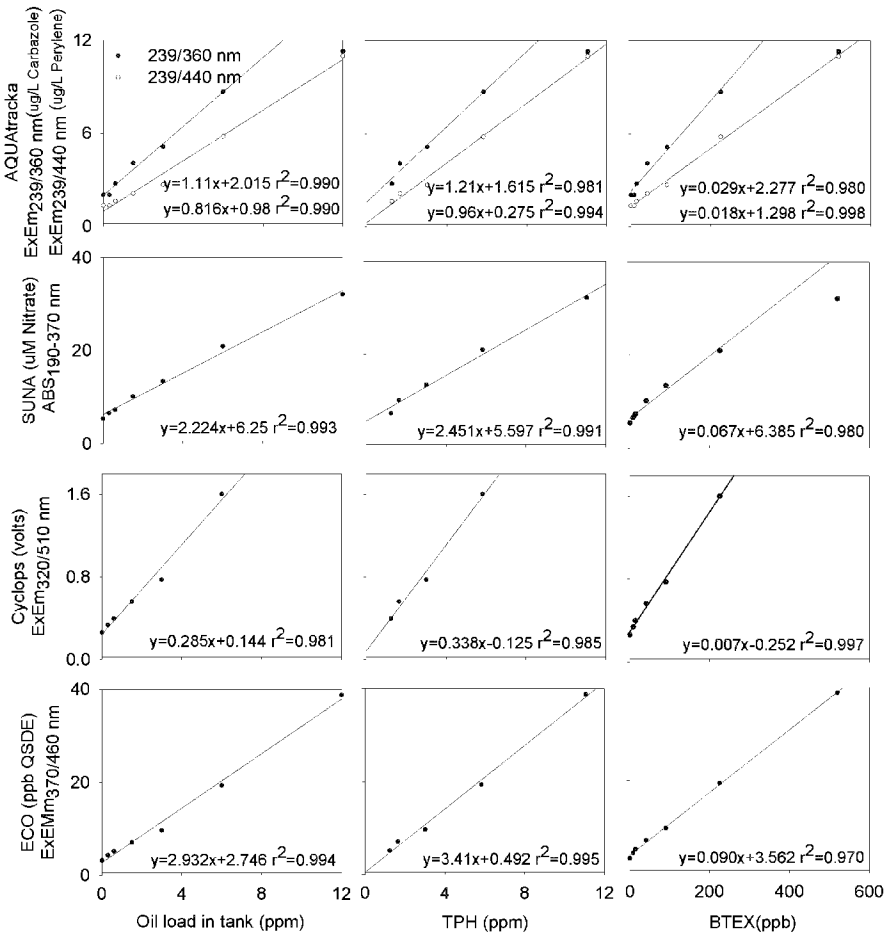


Figure 3. Fluorometric (submersible fluorometers) and spectrophotometric response (SUNA) as a function of oil concentration between 300 ppb and 12 ppm during EXP_{ADD}. Fewer TPH data points due to concentrations below the detection limit of TPH laboratory method.

TPH and 200−250 ppb BTEX, with subsequent plume dilution to 2−3 ppm TPH and 50 ppb BTEX. LISST-100X response (Figure 5, left) indicates like mixing and dispersing conditions in those experiments. Plume dynamics differed in two tests, where steep increases in oil concentration were followed by rapid decay (Figure 6), supported by TPC and droplet size distribution (Figure 5, right) and maximum TPH and BTEX values of 6 ppm and 250 ppb, followed by dilution to concentrationsakin to all experiments. TPH and BTEX-calibrated sensor responses follow the chemistry and oil droplet results in all experiments. Agreement among these parameters is critical as they are used in concert during oil spills to track chemically dispersed oil plumes and confirm degree of dispersant effectiveness.

Sensor response during chemically dispersed EXP_{DIL} was validated with chemistry results to verify sensor performance. Percent differences ($\%_{dif}$) and mean absolute error (MAE) between estimated (from sensors) and actual (chemistry results) oil concentrations were calculated for each time point in experiments, providing performance metrics for estimates of TPH and BTEX for sensors exposed to oil. The MAE and mean, median and average $\%_{dif}$ for each sensor for all time points and for time points excluding 0−10 min are listed in Table 1. Time points <10 min were excluded from calculations due to heterogeneity and timing of the plume leading edge in the tank. The percentage of experiments where calibrated sensor responses were within ±25% and +30% of chemistry results are reported.

Based on $\%_{dif}$, the ECO and AQUAtracka $_{239/440\ nm}$ consistently better estimated BTEX, with 91% and 86% of time points within $30\%_{dif}$, respectively. The AQUAtracka$_{239/440\ nm}$ had the lowest median $\%_{dif}$ of <7.96. These values may be biased, however, as the AQUAtracka$_{239/440\ nm}$ was used in only one flow-through EXP_{DIL}. The ECO median $\%_{dif}$ was slightly higher (12%), but had the highest percentage of time points within $\pm30\%_{dif}$ (91%). Accuracy was also high with MAE of ±17.27 and 12.41 ppb for the ECO and AQUAtracka$_{239/440\ nm}$, respectively. The AQUAtracka$_{239/440\ nm}$, Cyclops and SUNA exhibited similar performance for BTEX where 71−79% values were within $\pm30\%_{dif}$. The AQUAtracka$_{239/360\ nm}$ also exhibited higher accuracy (MAE ± 12.43 ppb), but estimates were less consistent (71% of measurements within $30\%_{dif}$). BTEX consists of single ring aromatics, which are highly volatile and moderately soluble (relative to higher ring no. PAHs) in the shallow water of the wave tank, but any loss did not impact the $\%_{dif}$. Extrapolating this to real-world scenarios, surface spills typically have reduced BTEX or tVOA due to evaporation; however subsea oil plumes would likely retain higher dissolved concentrations.[6] Thus the ECO, AQUAtracka$_{239/360\ nm}$ and AQUAtracka$_{239/440\ nm}$ are each well suited for estimating BTEX in the deep sea, but they do vary with accuracy and precision. Results may differ for medium and

ED_001324_00001490-00005

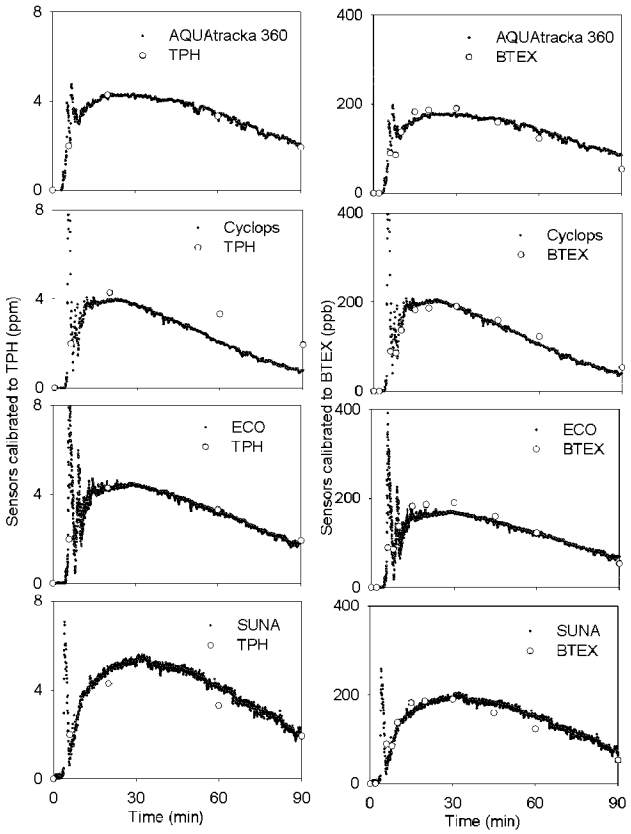


Figure 4. Response of TPH and BTEX-calibrated sensors exposed to chemically dispersed fresh crude oil. TPH and BTEX values from discrete samples are represented by open and gray circles, respectively. Wave tank was operated in flow-through mode (EXP_{DIL}) to observe fluorescence values during the dilution of an oil spill.

heavy oil, however, as BTEX concentrations would vary from the light crude oil tested here.

TPH was better estimated by the SUNA, with respect to both accuracy and precision, followed by the $AQUAtracka_{239/360\ nm}$, with 97% and 84% of time points within 30%$_{dif}$ of actual oil concentrations and MAE of +0.47 and 0.58 ppm, respectively.

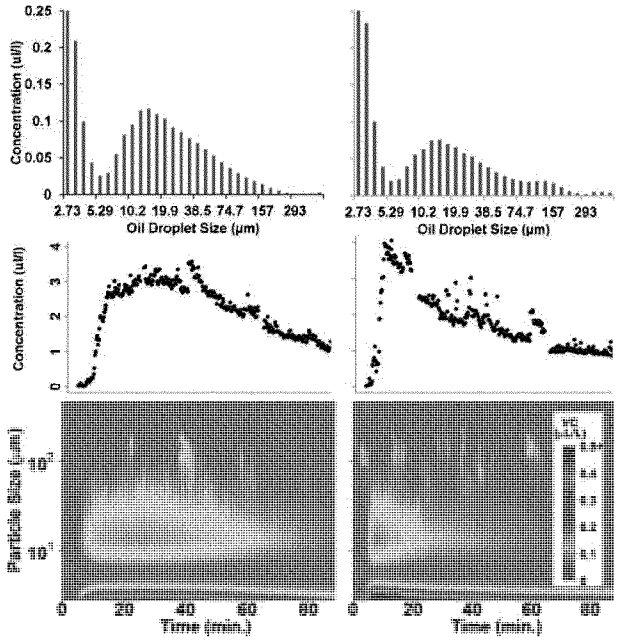


Figure 5. Particle size distribution and concentration within wave tank corresponding to experiments shown in Figures 4 and 6. Histogram plots are composites of entire flow-through experiment. Total particle concentration as a function of time (middle) is shown in scatter plot. Contour plots represent Particle size and volume concentration (VC) as a function of time for chemically dispersed fresh (left) and weathered (right) MC252 crude oil with gradual and rapid decay, respectively.

SUNA had the lowest median %$_{dif}$ of 11% for time points >10 min.

The remaining sensors exhibited median %$_{dif}$ of 18.72−23.87% with fewer values within 30%$_{dif}$ (60−71%) and higher MAE (0.77−0.81 ppm). Compared to BTEX, TPH laboratory analysis captures more compound types which are present at higher concentrations in oil. The SUNA's high performance in estimating TPH may result from absorption, not fluorescence, being measured over a wide wavelength range and oil being comprised of more chromophores than fluorophores. Tank

Table 1. Percent Differences (%$_{dif}$) between Sensors and Chemistry[S]

		TPH (ppm)					BTEX (ppb)				
		ECO 370/460 nm	AQUA 239/360 nm	AQUA 239/440 nm*	Cyclops 320/510 nm	SUNA ABS	ECO 370/460 nm	AQUA 239/360 nm	AQUA 239/440 nm*	Cyclops 320/510 nm	SUNA ABS
all time points	mean %$_{dif}$	31.69	30.24	41.98	40.32	27.92	26.67	30.93	23.14	31.99	30.45
	median %$_{dif}$	23.54	21.15	26.21	25.54	15.68	13.66	20.95	12.34	24.41	23.74
	min %$_{dif}$	0.27	1.96	2.16	1.73	0.76	1.63	3.19	5.29	0.01	0.65
	max %$_{dif}$	183.78	186.85	194.19	199.92	214.04	122.22	128.82	85.95	114.40	140.97
	[MAE]	± 0.91	± 0.67	± 0.78	± 0.90	± 0.68	± 23.53	± 13.46	± 12.41	± 32.22	± 27.68
time points >10 min	mean %$_{dif}$	25.52	19.37	19.22	30.94	13.88	15.54	23.88	14.17	21.11	22.85
	median %$_{dif}$	23.85	18.72	23.87	21.29	11.20	12.00	15.14	7.96	16.67	19.95
	min %$_{dif}$	0.27	1.96	2.16	1.73	0.76	1.63	3.19	5.29	0.01	0.65
	max %$_{dif}$	74.55	53.83	35.22	127.31	46.00	85.57	83.18	31.26	87.71	81.00
	[MAE]	± 0.78	± 0.58	± 0.81	± 0.77	± 0.47	± 17.27	± 12.43	± 12.41	± 29.13	± 24.50
% of >10 min time points	<25%$_{dif}$	56%	72%	57%	56%	90%	86%	68%	86%	64%	63%
	<30%$_{dif}$	69%	84%	71%	60%	97%	91%	71%	86%	79%	74%

[S]Asterisk represents sensor with only one experiment. MAE is mean absolute error.

ED_001324_00001490-00006

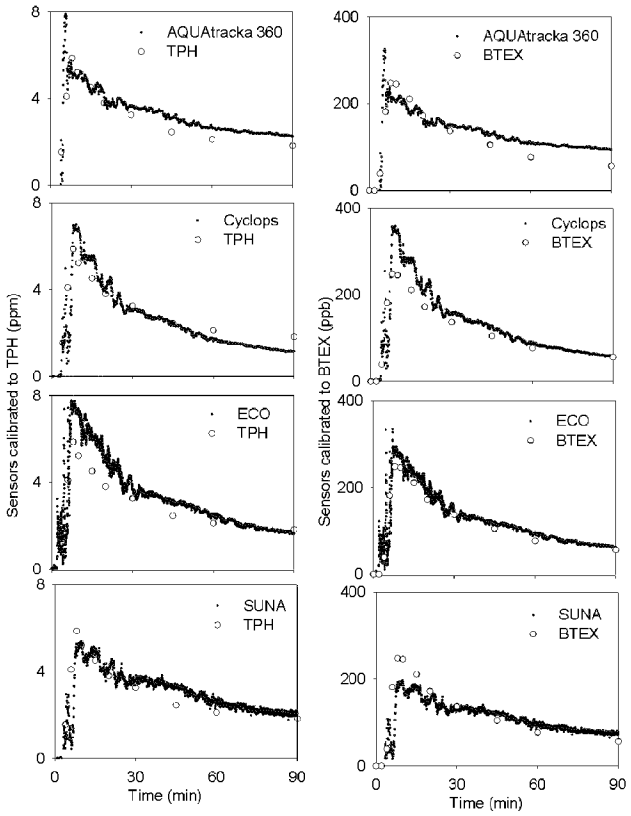


Figure 6. Response of TPH and BTEX-calibrated sensors exposed to chemically dispersed fresh crude oil for dilution experiment with steep increase in particles, followed by rapid decay. TPH and BTEX values from discrete samples are represented by open and gray circles, respectively. Wave tank was operated in flow-through mode (EXP_{DIL}) to observe fluorescence values during the dilution of an oil spill.

results indicate that of the sensors used in the DWH subsea plume, both the $AQUAtracka_{239/360\ nm}$ and ECO would have detected low TPH concentrations. The former may have exhibited better accuracy and precision. However, it could also be speculated that the $AQUAtracka_{239/360\ nm}$ with its narrower dynamic range would have saturated at high concentrations early in the spill and impeded plume tracking. Results are critical and provide evidence that ECO data from early in the spill (high concentrations) can be compared with AQUA data from later in the spill (low concentrations), and that sensors could provide oil concentrations with reasonable accuracy.

Wave tanks simulate natural variability observed in real spills. Thus, vicarious calibration is expected to be more accurate than a laboratory calibration, even though it may suffer from poorer precision. These findings demonstrate the sensitivity of COTS sensors exposed to chemically dispersed MC252 oil and increase confidence using in situ measurements during DWH to fill in the data gaps of coarse discrete samples. During an oil spill, sensor choice should be a function of oil concentration, calibration to TPH or BTEX and trade-offs between accuracy and precision. This work has large implications for plume tracking and understanding fate and transport of spilled oil in the environment.

ASSOCIATED CONTENT

* Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: 513-569-7090; fax: 513-569-7620; e-mail: conmy.rdbyn@epa.gov.

Present Addresses

◆ University of Oregon, Institute of Ecology and Evolution and Institute of Environmental Science, Oregon, USA.

¶ Commonwealth Science and Industrial Research Organization, Wealth from Oceans Flagship, Perth, Australia.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank all funding sources for the experiments at COOGER-BIO (Department of Fisheries and Oceans, Canada): IOOS support (Alliance for Coastal Technology), NOAA's supplemental DWH appropriation, salary support from home institutions, DFO Canada and internal travel support by Horiba JY, Oil Spill Recovery Institute, WET Labs, Inc. This document has been reviewed by the U.S. Environmental Protection Agency National Risk Management Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. This is a contribution from the Land Remediation and Pollution Control Division.

REFERENCES

(1) Report, S. P. *Special Monitoring of Applied Response Technologies Protocol Report*; National Oceanic and Atmospheric Administration, U.S. Coast Guard, U.S. Environmental Protection Agency, 2006; p 43.

(2) Henry, C. B., Roberts, P.O.; Overton, E.B. A primer on in situ fluorometry to monitor dispersed oil. In *International Oil Spill Conference*, 1999; pp 225-228.

(3) Li, M.; Garrett, C. The relationship between oil droplet size and upper ocean turbulence. *Mar. Pollut. Bull.* 1998, *36*, 961−970.

(4) Lessard, R. R.; Demarco, G., The significance of oil spill dispersants. *Spill Sci. Techno. Bull.*, 2000, *6*, (59−68).

(5) Venosa, A. D.; Zhu, X. Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands. *Spill Sci. Technol. Bull.* 2003, *8*, 163−178.

(6) Camilli, R.; Reddy, C. M.; Yoerger, D. R.; Mooy, B. A. S. V.; Jakuba, M. V.; Kinsey, J. C.; McIntyre, C. P.; Sylva, S. P.; Maloney, J. V. Tracking hydrocarbon plume transport and biodegradation at deepwater horizon. *Science* 2010, *330* (6001), 201−204.

(7) Green, D.; Humphrey, B.; Fowler, B. The use of flow-through fluorometery for tracking dispersed oil. In *Internationl Oil Spill Conference*, 1983; pp 473−478.

(8) Page, D. S.; Foster, J. C.; Hotham, J. R.; Pendergasst, E.; Herbert, S.; Gonzalez, L.; Gilfillan, E. S.; Hanson, S. A.; Gerber, R. P.; Vallas, D. Long-term fate of dispersed and undispersed crude oil in two nearshore test spills. In *International Oil Spill Conference*, 1983; pp 479−482.

(9) Keizer, P. D.; D. C., G., Jr. Detection of trace amounts of oil in sea water by fluorescence spectroscopy. *J. Fish. Res. Board Can.* 1973, *30*, 1039−1046.

(10) Østgaard, K.; Jensen, A. Evaluation of direct fluorescence spectroscopy for monitoring aqueous petroleum solutions. *Int. J. Environ. Anal. Chem.* 1983, *14*, 55−72.

ED_001324_00001490-00007

(11) Bugden, J. B. C.; Yeung, C. W.; Kepkay, P. E.; Lee, K. Application of ultraviolet fluorometry and excitation-emission matrix spectroscopy (EEMS) to fingerprint oil and chemically dispersed oil in seawater. *Mar. Pollut. Bull.* 2008, *56* (4), 677−685.

(12) Kepkay, P.; Young, W.; Bugden, J. B. C.; Li, Z.; Lee, K. Ultraviolet fluorescence spectroscopy (UVFS): A new means of determining the effectiveness of chemical dispersants on oil spills. In *International Oil Spill Conference*, Savannah, GA, 2008; pp 639−44.

(13) Li, J.; Fuller, S.; Cattle, J.; Way, C. P.; Hibbert, D. B. Matching fluorescence spectra of oil spills with spectra from suspect sources. *Anal. Chim. Acta* 2004, *514* (1), 51−56.

(14) Christensen, J. H.; Hansen, A. B.; Mortensen, J.; Andersen, O. Characterization and matching of oil samples using fluorescence spectroscopy and parallel factor analysis. *Anal. Chem.* 2005, *77*, 2210−2217.

(15) Zhou, Z.; Liu, Z.; Guo, L. Chemical Evolution of Macondo crude oil during laboratory degradation as characterized by fluorescence EEMs and hydrocarbon composition. *Mar. Pollut. Bull.* 2012, *66*, 164−175.

(16) Conmy, R. N.; Chen, R. F.; Castillo, C. E. D.; Downing, B. Calibrations and quality control of in situ sensors. In *Fluorescence Applications in Aquatic Science*; Cambridge University Press, 2013.

(17) Report, S. L. R. E. R. *Updating the SMART dispersant monitoring protocol: Review of Commercial-Off-The-Shelf instruments*; S.L. Ross Environmental Research, 2008; p 19.

(18) Lambert, P. A literature review of portable fluorescence-based oil-in-water monitors. *J. Hazard. Mater.* 2003, *102*, 39−55.

(19) Fuller, C. B.; Bonner, J. S.; Page, C. A.; Arrambide, G.; M.C. Sterling, J.; Ojo, T. *Field Instruments for Real Time In-Situ Crude Oil Concentration Measurements*; Texas A&M University, 2003; p 10.

(20) Wilde, S. Procedures for monitoring dispersant operations. In *10th Environmental and Scientific Coordinators Workshop* , Queenscliff, Australia, 2001, 2001; pp 163−177.

(21) Srinivasan, R.; Lu, Q.; Sorial, G. A.; Venosa, A. D.; Mullin, J. Dispersant effectiveness of heavy fuel oils using baffled flask test. *Environ. Eng. Sci.* 2007, *24* (9), 1307−1320.

(22) Report, N. R. C. *Oil Spill Dispersants: Efficacy and Effects*; Washington, DC, 2005; p 400.

(23) *Report Oil Budget Calculator: Deepwater Horizon*; Federal Interagency Solutions Group, 2010; p 217.

(24) Report, J. A. G. *Deepwater Horizon Oil Spill: Review of Subsurface Dispersed Oil and Oxygen Levels Associated with the Deepwater Horizon MC252 Spill of National Significance*; National Oceanic and Atmospheric Administration, 2012; p 93.

(25) Report, J. A. G. *Review of Data to Examine Subsurface Oil in the Vicinity of DWH MC-252*; National Oceanic and Atmospheric Administration, May 19−June 19, 2010; p f29.

(26) Lee, K.; Li, Z.; Boufadel, M. C.; Venosa, A. D.; Miles, M. S. *Wave Tank Studies on Dispersant Effectiveness As a Function of Energy Dissipation Rate and Particle Size Distribution*; Department of Fisheries and Oceans Canada, 2009; p 67.

(27) Li, Z.; Lee, K.; King, T.; Boufadel, M. C.; Vensosa, A. D. Evaluating crude oil chemical dispersion efficacy in a flow-through wave tank under regular non-breaking wave and breaking wave conditions. *Mar. Pollut. Bull.* 2009, *58*, 735−744.

(28) Li, Z.; Lee, K.; King, T.; Boufadel, M. C.; Vensosa, A. D. Effects of temperature and wave conditions on chemical dispersion efficacy of heavy fuel oil in an experimental flow-through wave tank. *Mar. Pollut. Bull.* 2010, *60*, 1550−59.

(29) Cole, M. G.; King, T. L.; Lee, K. Analytical technique for extracting hydrocarbons from water using sample container as extraction vessel in combination with a roller apparatus. *Anal. Chem.* 2007, *77*, 2210−17.

(30) Coble, P. G. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Mar. Chem.* 1996, *51*, 325−346.

(31) Atlas, R. M.; Hazen, T. C. Oil biodegradation and bioremediation: A tale of the two worst spills in U.S. history. *Environ. Sci. Technol.* 2011, *45* (16), 67−09−15.

ED_001324_00001490-00008

Characterizing Dispersant Effectiveness of Crude Oils at High Salinities: Implications for Subsea Spill Preparedness
(BAA-BSSE Oil Spill Response Research - Solicitation # E15PS00027)

Robyn N. Conmy*, Ph.D., U.S. Environmental Protection Agency
National Risk Management Research Laboratory, 26 W. MLK Drive, Cincinnati, OH 45268
Tel: 513-569-7090, Fax: 513-569-7620, Email: conmy.robyn@epa.gov *Correspondence

Michel Boufadel, Ph.D., New Jersey Institute of Technology
Department of Civil and Environmental Engineering, University Heights Newark, NJ 07102
Tel: 973-596-6079, Fax: 973-596-5790, Email: boufadel@gmail.com

Thomas King, M.Sc., Department of Fisheries and Oceans Canada
Bedford Institute of Oceanography, 1 Challenger Driver, Dartmouth, Nova Scotia B2Y 4A2
Tel: 902-426-4172, Fax: 902-426-1440, Email: Tom.King@dfo-mpo.gc.ca

Executive Summary
Potential oil exploration and production activities in close proximity to hypersaline water bodies, such as brine channels in the Arctic or brine pools in the Gulf of Mexico (GoM), necessitates characterization of Dispersant Effectiveness (DE) at high salinities. The 2010 GoM Deepwater Horizon (DWH) oil spill was the largest and deepest oil release ever to be treated with chemical dispersants as the primary mitigation tool, demonstrating that dispersants could be injected directly into gushing oil at 1500 m deep. Yet, if such a release were to occur into hypersaline waters, it is currently unknown whether dispersants would be an effective response tool, particularly during extreme mixing of brine with overlying seawater. Needed are approaches to evaluate the performance and behavior of dispersants at high salinities. In response to the Bureau of Safety and Environmental Enforcement's (BSEE's) Broad Agency Announcement (BAA) # E15PS00027, Topic #7, this white paper addresses critical knowledge gaps by proposing: (1) to evaluate DE at high salinities using the U.S. Environmental Protection Agency's (EPA) Baffled-Flask Effectiveness Test (BFT) and (2) to characterize DE during wave tank simulations of high pressure, subsea releases of oil, injected with dispersant at high salinities and subsequent mixing with average ocean salinity waters. The proposed work will significantly improve the response community's ability to predict DE in high salinity environments and improve preparedness for mitigating spills in remote locations in the Arctic and deep GoM.

Introduction
In the U.S., chemical dispersants must be listed on the National Contingency Plan (NCP) Product Schedule (PS) to be considered as oil spill treating agents. Listing requires dispersants be > 45% effective in dispersing Prudhoe Bay and South Louisiana crude oils using the U.S. EPA Swirling Flask Dispersant Effectiveness Test (SFT) (Appendix C of Subpart J of 40 CFR 300.900). Due to large discrepancies in DE values reported by manufacturers and contract laboratories, an improved method was developed, the Baffled-Flask Test (BFT)[1,2], now required in the proposed NCP decision rule, (Federal Register 2015), providing better reproducibility and higher DE criteria[3] with new reference oils (Table 1). Both the SFT and BFT are conducted at 34 psu ocean salinity.

Surfactants, the active ingredient in chemical dispersants, reduce the oil-water interfacial tension,

ED_001324_00001496-00001

forming small oil droplets (< 100 μm) that are less likely to re-coalesce[4], suspending oil droplets as a plume with increased surface area to volume ratio. Most surfactants are more lipophilic (oil-loving) in freshwater and increase in hydrophilicity (water-loving) as the salinity rises[4]. The stability of oil droplets is salinity dependent, increasing with ionic strength. However, above a certain point (surfactant dependent), this increased ionic strength results in more surfactant molecules leaving the oil droplet. Previous studies have examined this effect, whereby maximum DE is exhibited between 40 to 60 psu[5] (and references therein), which is above average ocean salinities (35 psu, with range of 32-37 psu). Above this, DE is substantially lower at higher salinities. Figure 1 captures this effect. It is important to note that many previous studies were conducted using DE test methods at varying dispersant-oil ratios (DOR), with insufficient mixing energy for dispersion compared to the BFT. Additionally, given that dispersant application would typically be considered for brackish or typical ocean water (34 psu), most studies target the reduced DE observed at low to average ocean salinity. Only three SFT studies evaluated DE at a maximum salinity of 90 psu[5]. Hence, there is a need to better characterize DE at higher salinities, as hypersaline waters can be found naturally in regions of oil exploration and production activities.

Table 1. Dispersant Effectiveness at 34 psu salinity, 5 and 25 °C, and 250 rpm with Alaskan North Slope Crude and IFO 120 using the US EPA Baffled-Flask Test. (Modified from table in the NCP proposed decision rule, Federal Register).

Oil	Temperature	Dispersant	Average	Std. Dev.	Variance	Coef. of Variation	LCL95
ANS	5	Disperit SPC1000	90.12	2.88	8.31	3.20	87.75
	5	Corexit 9500	79.57	5.37	28.85	6.75	75.15
	5	JD2000	77.39	3.54	12.51	4.57	74.48
	5	SafRonGold	59.35	3.44	11.82	5.79	56.52
	5	ZI400	56.78	3.02	9.09	5.31	54.30
	25	Disperit SPC1000	93.24	2.37	5.62	2.54	91.29
	25	Corexit 9500	95.04	0.91	0.83	0.96	94.29
	25	JD2000	81.01	3.17	10.05	3.91	78.40
	25	SafRonGold	66.52	1.94	3.76	2.91	64.93
	25	ZI400	61.64	2.10	4.41	3.41	59.91
IFO 120	5	JD2000	72.46	4.23	17.87	5.83	68.98
	5	Disperit SPC1000	76.22	13.88	192.68	18.21	64.80
	5	Corexit 9500	65.95	7.20	51.82	10.92	60.02
	5	Nokomis 3AA	33.47	6.11	37.34	18.26	28.45
	5	SafRonGold	23.57	2.86	8.20	12.15	21.21
	5	ZI400	20.71	3.91	15.25	18.86	17.49
	25	JD2000	84.10	6.62	43.86	7.88	78.65
	25	Disperit SPC1000	81.37	8.84	78.18	10.87	74.09
	25	Corexit 9500	77.39	6.80	46.22	8.79	71.79
	25	Nokomis 3AA	66.76	6.87	47.17	10.29	61.11
	25	SafRonGold	44.43	4.16	17.30	9.36	41.00
	25	ZI400	43.88	5.27	27.74	12.00	39.55

ANS at 5 °C: $DE_{LCL95} \geq 70\%$
ANS at 25 °C: $DE_{LCL95} \geq 75\%$
IFO 120 at 5 °C: $DE_{LCL95} \geq 35\%$
IFO 120 at 25 °C: $DE_{LCL95} \geq 65\%$

Hypersaline waters are operationally defined as waters with higher salt content than otherwise expected, forming naturally primarily via three mechanisms: (1) evaporation, (2) ice formation and (3) salt deposit dissolution. Evaporation-induced hypersalinity occurs in warm shallow waters where salinities are <50 psu (Everglades Florida Bay). Hypersalinity also arises from ice formation at high latitudes via salt extrusion from ice matrices, where brine channels (up to 200 psu) at the bottom of ice, sink, even to the sea floor. Hypersaline waters are also found on the GoM seafloor where sediments overlie Jurassic-age salt deposits (Louann and Campeche Salt Layers), resulting in salt-tectonics (movement of salt bodies), where conduits transfer salt through sea floor, forming brine pools or lakes with salinity upward of 150 psu.

Decision-making regarding dispersant use typically entails questions on whether salinity is high

ED_001324_00001496-00002

enough for sufficient DE. Given the increase in leasing, exploration and production activities in regions where hypersaline waters form (Arctic and Northern GoM), the response community is forced to ask whether dispersants are a viable response option for subsea releases in hypersaline waters. Uncertainties exist regarding the magnitude of DE at high salinities in part because of insufficient mixing energy of the tests in previous studies, wall effects, as well as lack of testing at salinities found in brine pools. Furthermore, in spite of the advantages of the BFT whose mixing is well characterized[3,7], the BFT does not account for the dilution of oil and dispersants that would occur at sea. Wave tanks, however, provide a remedy for this either by being sufficiently wide (Ohmsett tank) or by having currents to flush the water, as with the DFO Canada wavetank[8,9].

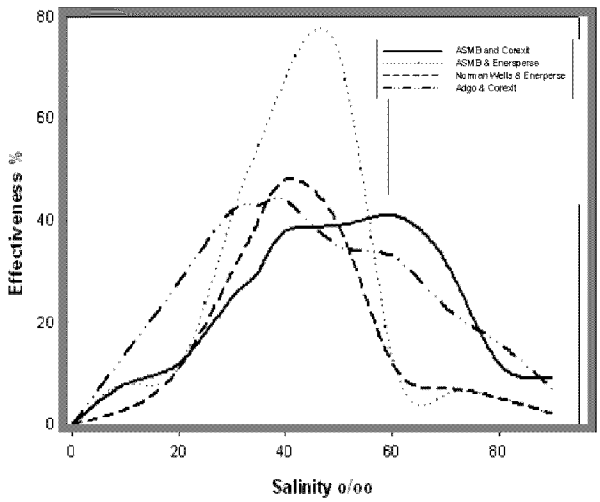


Figure 1. Dispersant Effectiveness as a function of Salinity using the SWT. Figure modified from Fingas, 1991[6].

To address knowledge gaps, and gather a more comprehensive picture pertaining to the influence of salinity on dispersant effectiveness, the **objectives** of the proposed research, therefore, are to:

1) Evaluate the influence of salinity on dispersant effectiveness for select oils and dispersants, from 10-150 psu salinity, using the BFT which better provides sufficient mixing energy. Reduction in mixing energy will also be explored to mimic Reduction in mixing away from subsea blowouts.

2) Characterize DE during wave tank simulations of high pressure, subsea releases of oil injected with dispersant at high salinities (up to 150 psu) and subsequent mixing with average ocean salinity waters to capture relative changes in DE.

Experimental Methodology

The probability of success for this project is high and the risk is low, as it builds upon previous work conducted with the BFT and subsea oil releases in wave tanks. To address objective #1, bench scale tests will be conducted to evaluate DE over a wide salinity range

using the BFT which evaluates dispersants using two EPA standard reference oils (ANS and IFO 120) with DOR = 1:25 at 250 rpm mixing speed at 5 and 25°C, and 34 psu salinity. Dispersant effectiveness is determined using a modified trypsinizing flask, artificial seawater and a standard orbital shaker to provide turbulent mixing. An oil-water sample is removed, extracted with dichloromethane (DCM), and analyzed for oil content by UV-visible spectrophotometry at wavelengths ranging between 340 and 400 nm. Oil concentrations are then calculated and DE determined. Here we propose to conduct experiments using the BFT procedure, but supplemented with additional salinities and oils. Additional oils allow comparison to previous SFT and BFT studies and also include new EPA standard reference oils for FY16. This enhanced testing matrix (below) will provide a comprehensive evaluation of the influence of salinity on dispersant effectiveness, where six replicates for each treatment will be required plus two method blank replicates. To generate the salinities, Artificial Seawater GP2 formulation will be used, with careful consideration given to mimic not only the magnitude of the total salt content, but also the relative concentrations of specific salt ions. For example, Gulf of Mexico brines are elevated in Mg compared to the Arctic.

Dispersants Type	Oil Type	Temp. °C	Salinity (psu)	
JD2000	South Louisiana Crude	5	10	80
Corexit 9500	Prudhoe Bay Crude	25	20	90
Dispersit SPC1000	IFO 120 reference oil		30	100
Finasol OSR52	Alaskan North Slope reference oil		34	110
	TBD proposed reference oil		40	120
	TBD proposed reference oil		50	130
			60	140
			70	150

To address objective #2, Oil dispersion experiments will be conducted in a flow-through wave tank, measuring 32 m long, that is equipped with an underwater oil release system to simulate a subsea oil release. Water from the Bedford Basin of Halifax Harbor is pumped into the tank through a coarse and fine (25; 5 μm pore size) serial filtration system. Breaking and non-breaking waves (flat-type wave maker) provide mixing energies to achieve dispersant effectiveness observed in the field. The tank is drained and cleaned after each experiment to remove all oil and surfactants. Salinity of the tank water will be adjusted using Artificial Seawater to obtain salinity of up to 150 psu. Released oil will have a DOR = 1:25 and simulate reservoir temperatures.

Tank experiments will be conducted in flow-through mode for physically-dispersed and chemically-dispersed oil. DE will be evaluated by measurement of dispersed oil concentrations from (1) grab samples taken from different stations, (2) *in-situ* droplet size distribution analysis and (3) *in-situ* fluorescence signal. The produced droplet size distribution (DSD) will be determined by using a Laser *In-Situ* Particle Counter (LISST-100X; Sequoia Scientific Inc.) to track dispersed oil droplets. Fluorometers, akin to ones deployed during spills, will also monitor the DE (WET Labs ECO, Chelsea Technologies Group AQUAtrackas, Turner Designs Group Cyclops and GmBH Trios). Sensors will be positioned downstream of the release at fixed positions to measure dispersed droplets. Discrete grab samples will be analyzed for (1) TPH (Total Petroleum Hydrocarbons) using a using GC-Flame Ionization Detector, (2) BTEX (benzene-toluene-ethylene-xylene, volatile hydrocarbons) using GC-Mass Spectrometry and (3)

ED_001324_00001496-00004

optical properties using UV-visible spectroscopy. The combined analyses will provide for quantifying dispersed oil concentration, partitioning of hydrocarbon compounds in seawater, and DSD as a function of oil type, dispersant type, water temperature and salinity. Salinity mixing experiments will be conducted (via pumping low salinity water into the tank downstream of the injection point) to characterize relative changes in DE when chemically-dispersed oil in hypersaline waters is subsequently mixed with lower salinities. Plume spatial distribution will be modeled using the computational fluid dynamics (CFD) model Fluent (www.ansys.com), and oil droplet distribution will be modeled using the model VDROP[10]. Oil viscosity and the oil-water interfacial tension of dispersed oil will be measured using rheometers and the spinning-drop technique, respectively. These will be used as input to models and to interpret the experimental results.

Results will be compared with existing knowledge in the published literature on behavior of oil dispersion at high salinities in laboratory and wave tank settings as well as our previous tank studies. Upon request for a full proposal to this BAA, the proposers shall coordinate with BSEE to ensure that the testing parameters meet the objectives.

Impact and Products

Project results will be reported in tabular and/or graphical format and included in a report suitable for publication in the peer-reviewed literature. Product deliverables will include results of (1) the influence of high salinity on dispersant effectiveness using Baffled-Flask Test, and (2) DE at high salinities within wave tank simulated sub-sea release plumes, (3) the effect of dilution on dispersant effectiveness when in high to low salinity waters. Recommendations will be made on the effects of salinity on dispersant effectiveness to inform the behavior of spilled oil in the environment and improve the formulation of Best Practices for rapid decision making during spill response.

Level of Effort

This project will be conducted over a period of one year. Bench scale DE tesing will be conducted at the U.S. EPA under the direction of Robyn Conmy, Ph.D., whom has focused expertise in oil spill monitoring and countermeasure technology. Wave tank subsea oil release simulations will be led by Michel Boufadel, Ph.D. of NJIT and Tom King of DFO Canada. Dr. Boufadel has extensive expertise in physico-chemical processes affecting oil. Mr. King has over 23 years' experience with focus on the fate and behavior of various oils spilled in aquatic environments.

Facilities

Bench scale dispersant effectiveness tesing will be conducted at the U.S. Environmental Protection Agency, EPA Andrew W. Breidenbach Environmental Research Center (AWBERC), in Cincinnati, OH. This research facility developed the new Baffled-Flask Dispersant Effectiveness Test and is equipped with necessary in-situ and bench spectroscopic instrumentation to conduct this work. Subsea oil release simulations at high salinities will be conducted at the Bedford Institute of Oceanography wave tank. This facility is equipped to perform all analyses to meet objective #2.

References

[1]Venosa et al., (2002) The BFT for dispersant effectiveness: Round Robin evaluation of reproducibility and

ED_001324_00001496-00005

repeatability, Spill Sci. Technol. Bull. 7, 299-308.
[2]Kaku et al., (2006) Evaluation of mixing energy in laboratory flasks used for dispersant effectiveness testing, J. Environ. Eng. 132: 93-101.
[3]Sorial et al., (2004) Oil spill dispersant effectiveness protocol I: Impact of operational variables, J. Environ. Eng., 130 (10), 1073-1084.
[4]Li and Garrett (1998) The relationship between oil droplet size and upper ocean turbulence. Mar. Pollut. Bull. 36, 961–970.
[5]Fingas (2004) Dispersant, Salinity and Prince William Sound. Report for the Prince William Sound Regional Citizens' Advisory Council. 57 pp.
[6]Fingas (1991) Studies on the Physical and Chemical Behaviour of Oil and Dispersant Mixtures, in *Proceedings of the International Oil Spill Conference,* 419-426.
[7]Kaku et al., (2006) Flow dynamics in eccentrically rotating flasks used for dispersant effectiveness testing, Environ. Fluid Mechanics 6(4): 385-406.
[8]Li et al., (2008) Assessment of chemical dispersant effectiveness in a wave tank under regular non-breaking and breaking wave conditions, Mar. Pollut. Bul. 56, 903-912.
[9]King et al., (2013) Interfacial film formation: Influence on Oil Spreading Rates in Lab Basin Tests and Dispersant Effectiveness Testing in a Wave Tank, Mar. Pollut. Bul. 71: 83-91.
[10]Zhao et al., (2014) VDROP: A comprehensive model for droplet formation of oils and gases in liquids - Incorporation
of the interfacial tension and droplet viscosity, Chemical Engineering Science. 253, 93-106.

ED_001324_00001496-00006

POTENTIAL EFFECTIVENESS OF DISPERSANT USE IN THE U.S. BEAUFORT AND CHUKCHI SEAS

for:

Bureau of Safety and Environmental Enforcement
U.S. Department of the Interior
381 Elden Street
Herndon, VA 20170

by:

S.L. Ross Environmental Research Ltd.
200-1140 Morrison Drive
Ottawa, ON
K2H 8S9

December, 2014

This study was funded by the Bureau of Safety and Environmental Enforcement (BSEE), U.S. Department of the Interior, Washington, D.C., under Contract E13PC00026.

SL ROSS

Acknowledgements

This study was funded by the Bureau of Safety and Environmental Enforcement (BSEE), U.S. Department of the Interior, Washington, D.C., under Contract E13PC00026.

Disclaimer

This draft final report has not been reviewed by the Bureau of Safety and Environmental Enforcement (BSEE), nor has it been approved for publication. Approval, when given, does not signify that the contents necessarily reflect the views and policies of the BSEE, nor does mention of the trade names or commercial products constitute endorsement or recommendation for use.

ED_001324_00001497-00002

Executive Summary

The objective of the study has been to provide information to support dispersant use decision-making with respect to oil spill chemical dispersant effectiveness under the environmental conditions likely to be encountered in the U.S. Beaufort and Chukchi Seas.

The goals of the goals research were to:

1. Identify the prevailing environmental conditions in the U.S. Beaufort and Chukchi Seas that would affect dispersant performance (see Section 4 of this report);
2. Identify existing dispersant effectiveness tests that have been completed on Alaskan oils under conditions similar to those that exist in these two regions and identify gaps in the knowledge of dispersant effectiveness (see Section 5 of this report);
3. Conduct dispersant effectiveness tests at medium scale to address the knowledge gaps (see Section 7 of this report);

The primary environmental factors that have been shown to influence dispersant effectiveness include: wave energy; water salinity; water temperature; and presence and concentration of ice.

With the exception of isolated near shore fresh water outlets there is minimal variation (between 28 to 34 ppt (parts per thousand)), both temporally and spatially, in surface water salinity throughout the U.S. Chukchi and Beaufort Sea regions, at least from a chemical dispersant effectiveness standpoint. The performance of most chemical dispersants is generally not affected by salinity in the range of 25 to 35 ppt. Water salinities in the nearshore area of the Beaufort and Chukchi Seas can be considerably lower than the offshore areas for short periods of the year due to the influence of fresh water river outflows and ice melt. Fresh water from river outflow can form a 1 to 2 m deep lens under the landfast ice that may extend up to 25 km offshore from the major river mouths. The surface water salinities in these areas approach that of fresh water during the early ice breakup period and prior to the mixing of the upper and lower water layers by strong winds and waves. The presence of this near shore fresh water during this period could hinder the effectiveness of chemical dispersants.

ED_001324_00001497-00003

The surface water temperatures in the Chukchi and Beaufort Seas in the open water seasons can vary from close to freezing (-2 °C) to upwards of 10 °C. The chemical dispersibility of crude oil is affected by the temperature only as it affects the viscosity of the oil. The oil's viscosity is affected by the water temperature on which it sits and during sunny days by the heating of the surface of the oil by the sun. Different crude oils can have very different viscosities at the same temperature and many can be quite fluid at temperatures near freezing. There is really no water temperature that will completely restrict the use of chemical dispersant. The oil viscosity – temperature relationship must be considered for each oil before an evaluation of the potential for dispersant effectiveness can be made.

The presence of greater than 9/10ths ice cover eliminates the possibility of using conventional surface applied dispersant operations. The presence of the ice either encapsulates the oil making it impossible to target with aerial applied dispersant or dampens the surface energy such that there is insufficient energy to break the oil into droplets once treated. In ice conditions with less than 3/10ths cover chemical dispersants will perform much the same as in open water conditions since the surface mixing energy is not overly dampened by the presence of the ice. The performance of dispersants in 3/10ths to 8/10ths ice cover without additional mixing energy applied is somewhat uncertain. Research suggests that performance may be enhanced in these conditions by the energy generated at the ice edge as the ice pieces jostle even under low wave energy. In the Chukchi Sea region open water periods can exist in the months from June through November depending on the year. Open water is most common in the months of July through October in this region but the shoulder months of June and November may present dispersant application opportunities depending on the ice year. In the Beaufort Sea region the maximum likely extent for open water dispersant use is from July to October with the more likely season being from August to October.

Long term historical wave height data is not available in the U.S. Chukchi and Beaufort Sea regions for statistical assessment of the potential for successful dispersant use on a broad geographic scale. Long term wind data are, however, available for the region in the form of offshore hindcast wind data sets. Wave heights based on the Beaufort wind scale have been derived from hindcast wind speed data for this project. The percentage of time that wave heights

ED_001324_00001497-00004

favorable for chemical dispersant use exist in the US Beaufort and Chukchi Seas has been determined based on an assessment of 30 years of hindcast wind data from 5 stations located across the study area. For the open water months of July through October wave conditions favourable for chemical dispersant use exist across both regions from 71 to 91% of the time. These assessments do not take into account other variables such as visibility or daylight.

A review of scientific literature on the results of chemical dispersant effectiveness tests specifically conducted on Alaskan crude oils has been undertaken. Research that investigated chemical dispersant effectiveness under arctic conditions and in the presence of ice using other oil types has also been included in the review due to paucity of research in this area. The review has focused on the parameters that could affect dispersant performance that are of specific concern to the arctic environment. These parameters include the test temperature, the water salinity, the mixing energy and the presence of ice. Oil type, dispersant type and dispersant dosage have also been considered in this review. With a few exceptions, only tests that have used Alaskan oil have been reviewed. Only 10 of the 48 test programs reviewed studied dispersion in the presence of ice and only 7 of these varied the ice concentration in the test matrix and only two used Alaskan oil in the testing. Of the 48 studies reviewed, temperature was varied in 15, salinity was varied in 16, and mixing energy was varied in 29.

At the present time the only oils being produced and transported (by pipeline to shore) in the Beaufort and Chukchi Seas are Northstar and Endicott crude oils and are the only crude oils that could be spilled directly into Alaskan offshore waters from present production fields. No oils are presently being produced in Chukchi Sea waters. Alaska North Slope Crude (ANS), or Prudhoe Bay crude as it was named in early test programs, has been the Alaskan oil of choice for most of the dispersant effectiveness tests conducted on Alaskan oils. This oil is the pipeline blend that is transported to Valdez via the Trans-Alaska Pipeline. Only five of the test programs reviewed evaluated dispersant effectiveness on specific North Slope production crudes (Endicott, Northstar and Pt. McIntyre). These oils were used in projects that were conducted for the US Department of the Interior with oils sourced by them specifically for cold water dispersant effectiveness testing. These oils are possibly more representative of the type of crude that might be spilled in the Beaufort and Chukchi Seas. As new oils come into production in the waters of

ED_001324_00001497-00005

the Beaufort and Chukchi Seas they should be evaluated for their potential for chemical dispersion under the range of environmental conditions they might be spilled in.

Most of the past dispersant effectiveness tests on Alaskan oils used one or both of Corexit 9500 and Corexit 9527 in their testing.

There is concern in temperate or subarctic climates that temperature is a problem in dispersant effectiveness and that dispersants should not or cannot be used in cold climates. This concern is generally unfounded based on the research results, except in the case of so-called "high pour point oils", some of which can become semi-solid at temperatures well above freezing. Reduced temperature simply increases the viscosity of the spilled oil. The viscosity of the oil at the water temperature in which it is spilled is the key factor in deciding dispersant effectiveness and not the temperature per se. This points to the need to fully understand the properties of the specific oils that are likely to spilled in cold waters.

Numerous laboratory-scale, meso-scale and field studies, dating back to the late seventies, have been conducted to study the effect of water salinity on the effectiveness of oil spill chemical dispersants on a range of oils. The consistent significant finding of all of these tests is that dispersant designed for use in marine environments (30 to 35 ppt water salinity) are considerably less effective when the water salinity falls below about 20 ppt or above 40 ppt. Of the dispersants most commonly used in US waters, Corexit 9500 has been shown to be more effective than Corexit 9527 in lower salinity waters. Dispersants have been formulated for use in fresh water and these have also been tested for effectiveness over a range of water salinities, although not as extensively as the marine dispersants. The effectiveness of the freshwater dispersants have been shown to generally be much better than the marine products in freshwater but often achieve their best results in waters between 10 and 20 ppt salinity. The effectiveness of dispersants on the Alaskan oil under varying water salinities generally is consistent with the findings for other crude oils in these historical studies.

ED_001324_00001497-00006

Tests conducted in the past few years using the more energetic US EPA baffled flask test have indicated that the effect of salinity on dispersant effectiveness may not be as significant as identified in the earlier work. This may be due to the higher mixing energy imparted in this test.

After considering the above factors and discussion with a technical representative from the Bureau of Safety and Environmental Enforcement (BSEE), a suite of dispersant effectiveness tests using the SL Ross meso-scale wind-wave tank on four Alaskan crude oils with four water salinities were selected for additional study to shed more light on the effectiveness of marine dispersants over a range of water salinities under high energy breaking wave conditions.

Alaska North Slope, Endicott, Northstar and Kuparuk crude oil were used in the testing. Tests were conducted on fresh, evaporated and evaporated plus emulsified crude oils (3 weathered states in total). Northstar does not form a stable emulsion so tests could not be conducted on emulsions of this oil. Tests were conducted in water with salinities of 5, 10, 20 and 30 ppt. All tests were conducted with a water temperature of 10 °C. Corexit 9500 was applied at a dispersant to oil ratio of 1:20 in all tests.

The oil collected from the surface containment zone at the end of each test was compared to the volume of oil initially released to determine the quantity of oil removed from the surface during the test or the raw dispersant effectiveness (DE_{raw}).The DE_{raw} estimates have been adjusted by multiplying them by the volume fraction of oil present in drops smaller than 70 microns to arrive at a final dispersant effectiveness estimate that is referred to as DE_{70} throughout the report. The 70 micron drop size value has been reported as being the maximum oil drop size that will be permanently dispersed based on a series of field tests.

The fresh oils were more effectively dispersed than the weathered oils that were more effectively dispersed than the weathered and emulsified oils. The most complete data sets collected (due to limitations of the LISST in measuring large oil drops) were for the fresh oil tests. The results for the fresh oils indicate that the DE_{70} values are highest for the 30 ppt water and in all cases drop linearly as the test water salinity decreased to 5 ppt. The Northstar crude was most easily dispersed with complete dispersion (> 95% DE_{70} in 20 ppt salt water), followed by fresh ANS

ED_001324_00001497-00007

(95% DE_{70} in 30 ppt salt water), fresh Kuparuk (85% DE_{70} in 30 ppt salt water) and Endicott (40% DE_{70} in 30 ppt salt water). The DE_{70} dropped linearly to a value between 15 to 25 % for the oils tested as the water salinity decreased to 5 ppt. Similar linear trends in DE_{70} reduction with decreasing water salinity were identified in the weathered oil tests.

The highest DE_{70} recorded for any of the emulsified oils was 20% in 30 ppt salt water.

Even under the high energy breaking wave conditions used in this test program it would appear that water salinity had a significant effect on the ability of Corexit 9500 dispersant to assist in the generation of small oil drops that would likely be permanently dispersed in an ocean setting. The effect noted was the linear decrease in the volume of oil drops generated with small enough size to be considered permanently dispersed as the water salinity decreased from 30 ppt to 5 ppt.

The oil drops in some of the tests were visibly larger than the upper measurement limit of the LISST device used to measure oil drop size distributions and reliable drop size data could not be gathered for these tests. Development of an in-situ oil drop measurement system capable of cost-effectively measuring drop sizes in the 10 to 3000 micron range would improve the state-of-the art in oil dispersion monitoring both in large tank and field test conditions.

ED_001324_00001497-00008

Table of Contents

1 Objectives 3

2 Goals 3

3 Background 3

4 Typical Environmental Conditions in the U.S. Beaufort and Chukchi Seas that Affect Dispersant Effectiveness 4

4.1 Water Salinity 4
4.2 Water Temperature 13
4.3 Ice Cover 14
4.4 Wave Energy 17

5 Historical Dispersant Effectiveness Testing on Alaskan Oils and Identification of Knowledge Gaps 24

5.1 Spill Related Properties of Alaskan Oils 24
5.2 Background - Mechanisms of Chemical Dispersion 27
5.3 Dispersant Effectiveness Test Results on Alaskan Oils 29
5.4 Oil Type 30
5.5 Dispersant Type 31
5.6 Water Temperature 31
5.7 Water Salinity 33
5.8 Mixing Energy 34
5.9 Effect of Ice on Dispersant Effectiveness 35

6 Identification of Knowledge Gaps 43

6.1 Water temperature 43
6.2 Water Salinity 44
6.3 Presence of Ice 45
6.4 Wave Energy 46
6.5 Alaskan Oil Types 47
6.6 Dispersant Types Tested 47

7 Meso-Scale Tank Tests 47

7.1 Oils and Dispersant Used in Test Program 48
7.2 Test Methods and Equipment 48
7.3 Test Variables and Test Matrix 51
7.4 Results 51
7.4.1 Dispersant Effectiveness (DE) Estimates 51
7.4.2 Dispersed Oil Drop Size Distributions 55
7.4.3 Drop Size Adjusted Dispersant Effectiveness Estimates 62
7.4.4 SL Ross Tank Test Results Discussion 69

8 References 71

9 Appendix A : Detailed Dispersant Effectiveness Tables 79

10 Appendix B : Annotated Bibliography: Past Research Relevant to Dispersion of Alaskan Oils in the US Beaufort and Chukchi Seas 81

Chemical Dispersant Research: Dispersant Effectiveness Testing at Ohmsett Using Aircraft Application Dosages

1 Objectives

The objective of the study has been to provide information to support dispersant use decision-making with respect to dispersant effectiveness under the environmental conditions likely to be encountered in the U.S. Beaufort and Chukchi Seas.

2 Goals

The goals of the proposed research were to:

4. Identify the prevailing environmental conditions in the U.S. Beaufort and Chukchi Seas that would affect dispersant performance (see Section 4 of this report);
5. Identify existing dispersant effectiveness tests that have been completed on Alaskan oils under conditions similar to those that exist in these two regions and identify gaps in the knowledge of dispersant effectiveness (see Section 5 of this report);
6. Conduct dispersant effectiveness tests at medium scale to address the knowledge gaps (see Section 6 of this report);
7. Write a technical report on the study and a technical paper for presentation at a suitable oil spill conference or technical seminar (delivered under separate cover).

3 Background

In the event of an offshore oil spill rapid decisions must be made with regard to the best course of action to mitigate potential effects of the spillage. Chemical dispersants are one of the tools to be considered. The initial question to be answered when deciding if dispersant application is an appropriate action is "Will the dispersant be effective in removing a significant percentage of the oil from the surface?" If not, then the dispersant operation would not be considered. If chemical dispersants are deemed to be potentially effective other issues such as a) the logistical feasibility of getting the dispersant to the spill site; b) applying it at the appropriate dosage; and c) the net environmental benefit of using the dispersant must also be considered in the decision making

process prior to the final approval for dispersant use. This report addresses only the issue of dispersant effectiveness.

The potential effectiveness of a chemical dispersant will depend on the type of dispersant used, the type and weathered state of the oil to be treated, and the prevailing environmental conditions at the time of application (water temperature, water salinity, wave energy present and presence of ice). A number of dispersant effectiveness studies have been completed using Alaskan oils, in warm and cold waters, in different water salinities, and in the presence of ice, including several studies funded by the US Department of the Interior. This report provides a focused review of this research, a review of the range of environmental conditions that could be encountered in the two regions during a dispersant application operation and the identification of additional research requirements regarding the use of dispersants specific to the U.S. Beaufort and Chukchi Sea regions. Meso-scale tank tests of dispersant effectiveness on four Alaskan crude oils have also been conducted in this study to help fill in some of the knowledge gaps on dispersant use under conditions that could be encountered in these regions.

4 Typical Environmental Conditions in the U.S. Beaufort and Chukchi Seas that Affect Dispersant Effectiveness

The primary environmental factors that have been shown to influence dispersant effectiveness include: wave energy present at the time of dispersant application or shortly after the application; water salinity; water temperature (primarily the effect of temperature on the oil's properties of viscosity and pour point); and presence and concentration of ice (and the influence of the ice on the surface and sub-surface mixing energy). The range of conditions that exist for these four parameters in the U.S. Beaufort and Chukchi Sea regions are documented along with their variability on both a seasonal and spatial basis on a scale appropriate for decision purposes.

4.1 Water Salinity

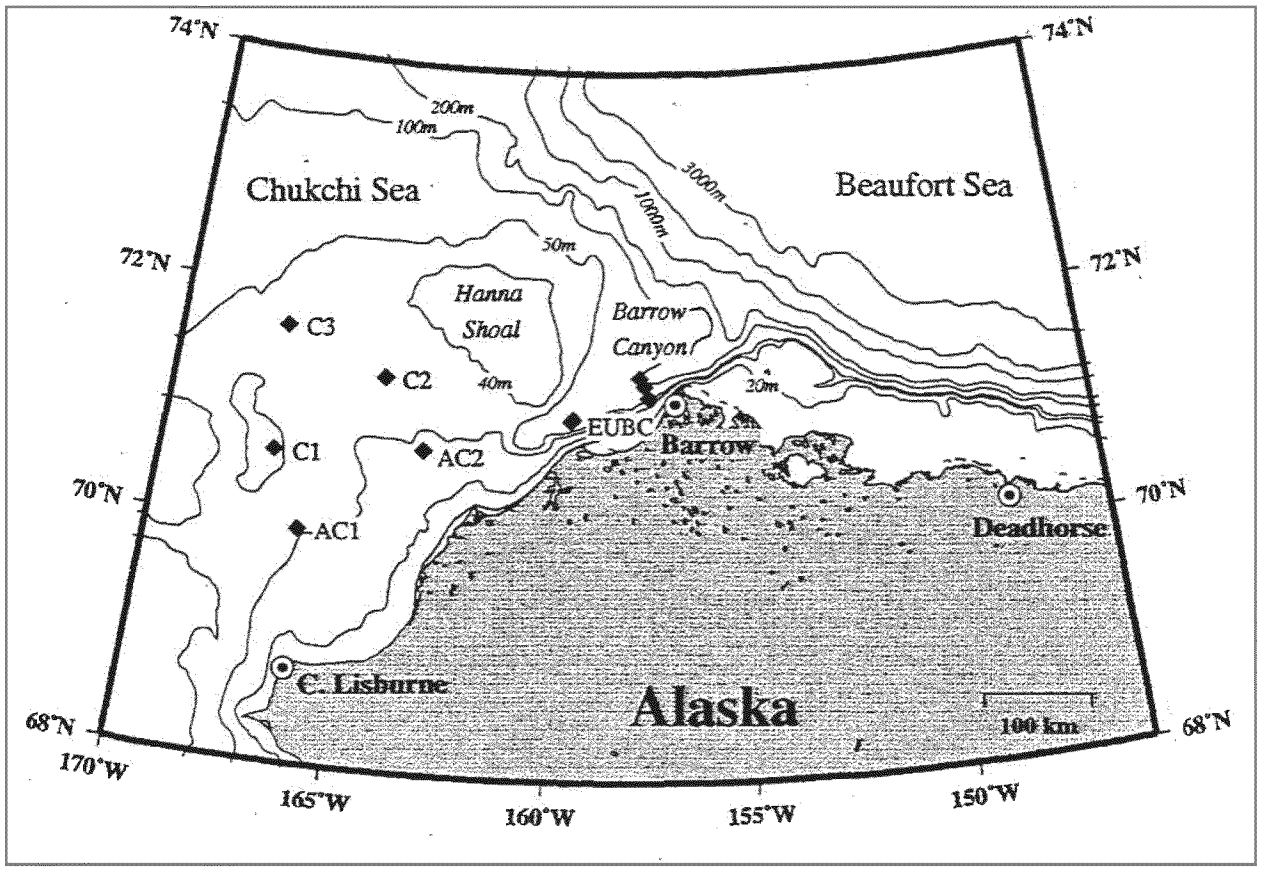
With the exception of isolated near shore fresh water outlets there is minimal variation, both temporally and spatially, in surface water salinity throughout the U.S. Chukchi and Beaufort Sea regions, at least from a chemical dispersant effectiveness standpoint. The performance of most chemical dispersants is generally not affected by salinity in the range of 25 to 35 ppt (SL Ross

2010). Studies at specific offshore sites have identified surface water salinity variation between 28 and 34 ppt (Weingartner and Danielson 2010, Weingartner et al. 2005). Weingartner (1998) also measured surface water salinity at various locations in the Chukchi Basin (see Figure 4-1). Seasonal surface water salinities were shown to vary between about 30 to 34 ppt (Figure 4-2), well within the range for effective chemical dispersant use. Figure 4-3 shows salinity measurements, also made by Weingartner, along an east-west transect from Barrow AK to Wrangel Island in the west. Measured salinities in all of these cases ranged from about 29 to 32 ppt.

Measured surface water salinity in the Beaufort Sea offshore region ranges between 27 and 34 ppt (Aagaard et al,1988, Proshutinsky et al, 2003, Weingartner et al. 2006). Figure 4-7 and Figure 4-8 show salinity profiles measured by Aagaard for the western and eastern extents of the Beaufort Sea (transects W and D shown in Figure 4-6). The salinities are somewhat lower 28 to 29 ppt in the east versus 28 to 31 in the west. Weingartner's salinity measurements were made at the sites along the outer shelf identified in Figure 4-4. Figure 4-5 shows the measured surface water salinities that were taken along the shelf. The salinities vary from about 32 ppt in the west to about 29 in the east. The salinities in all reported cases for the offshore Beaufort Sea region are sufficiently high that chemical dispersant effectiveness would not be significantly affected. Proshutinsky also concluded that "In ice-free regions, seasonal temperature fluctuations reach up to several degrees, whereas salinity fluctuations are small."

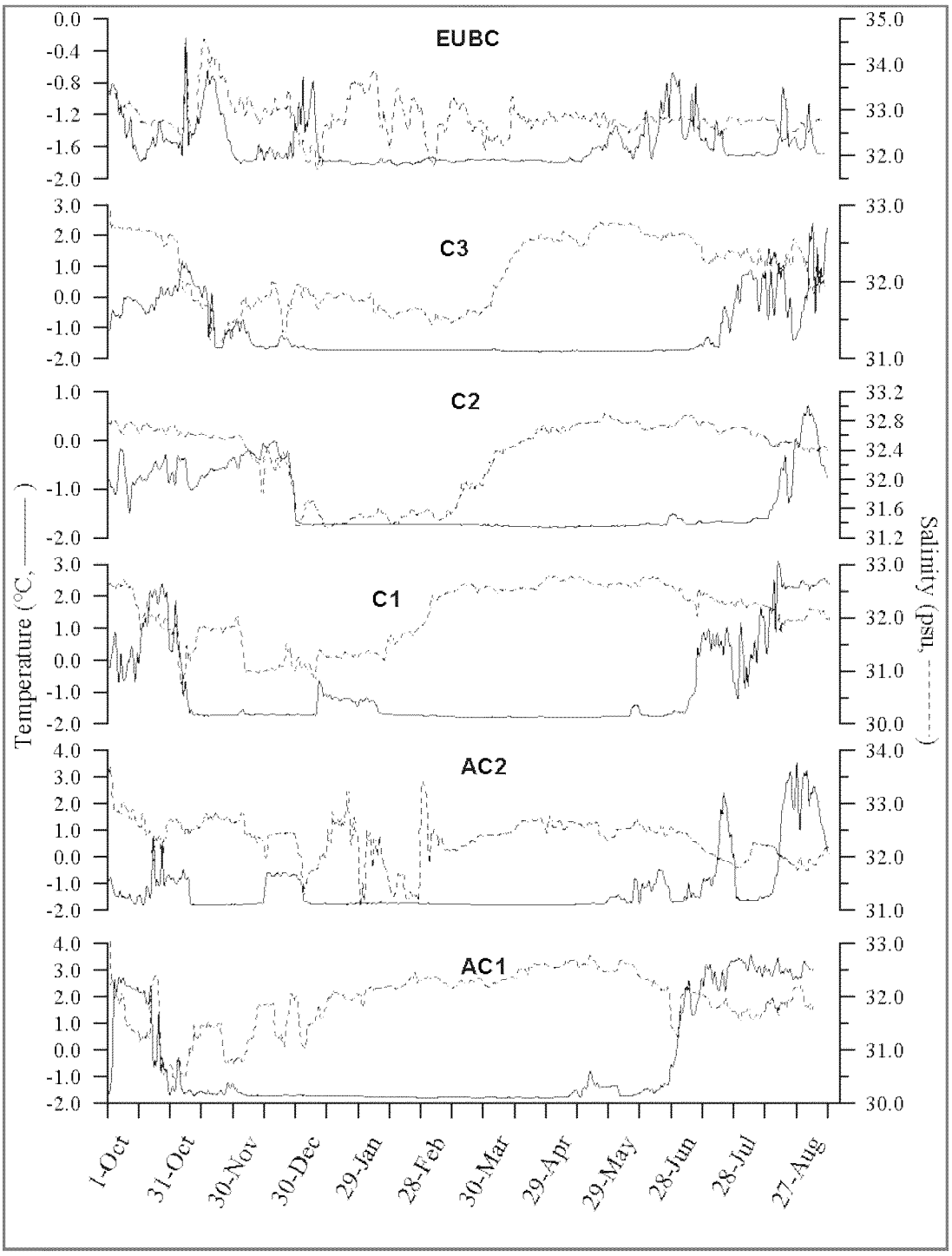
Water salinities in the nearshore area of the Beaufort Sea can be considerably lower than the offshore areas for short periods of the year due to the influence of fresh water river outflows and ice melt. Fresh water from river outflow can form a 1 to 2 m deep lens under the landfast ice that may extend up to 25 km offshore from the major river mouths (Weingartner et al. 2005). The surface water salinities in these areas approach that of fresh water during the early ice breakup period and prior to the mixing of the upper and lower water layers by strong winds and waves. The presence of this near shore fresh water during this period could hinder the effectiveness of chemical dispersants. However, dispersants are generally not approved for use in waters less than 10 m, so this may not be an issue depending on the extent of the surface freshwater plume offshore.

Nearshore, bottom, salinity measurements in about 10 m water depths offshore from the Sagavanirktok River (see Figure 4-9 for mooring locations) reveal bottom water salinities as low as 14 ppt early in the spring melt and freshet period (Weingartner et al. 2005). During breakup the bottom water salinity drops from greater than 30 ppt over a period of about 1 week at the beginning of August to as low as 15 ppt and then climbs back up to 30 by the end of August as the surface lens of fresh water is mixed down into the water layer. Figure 4-10 shows the measured bottom water salinity in about 7 m of water at the Dinkum mooring. These measurements confirm the presence of the surface fresh water lens and its eventual influence on water salinity at depth.



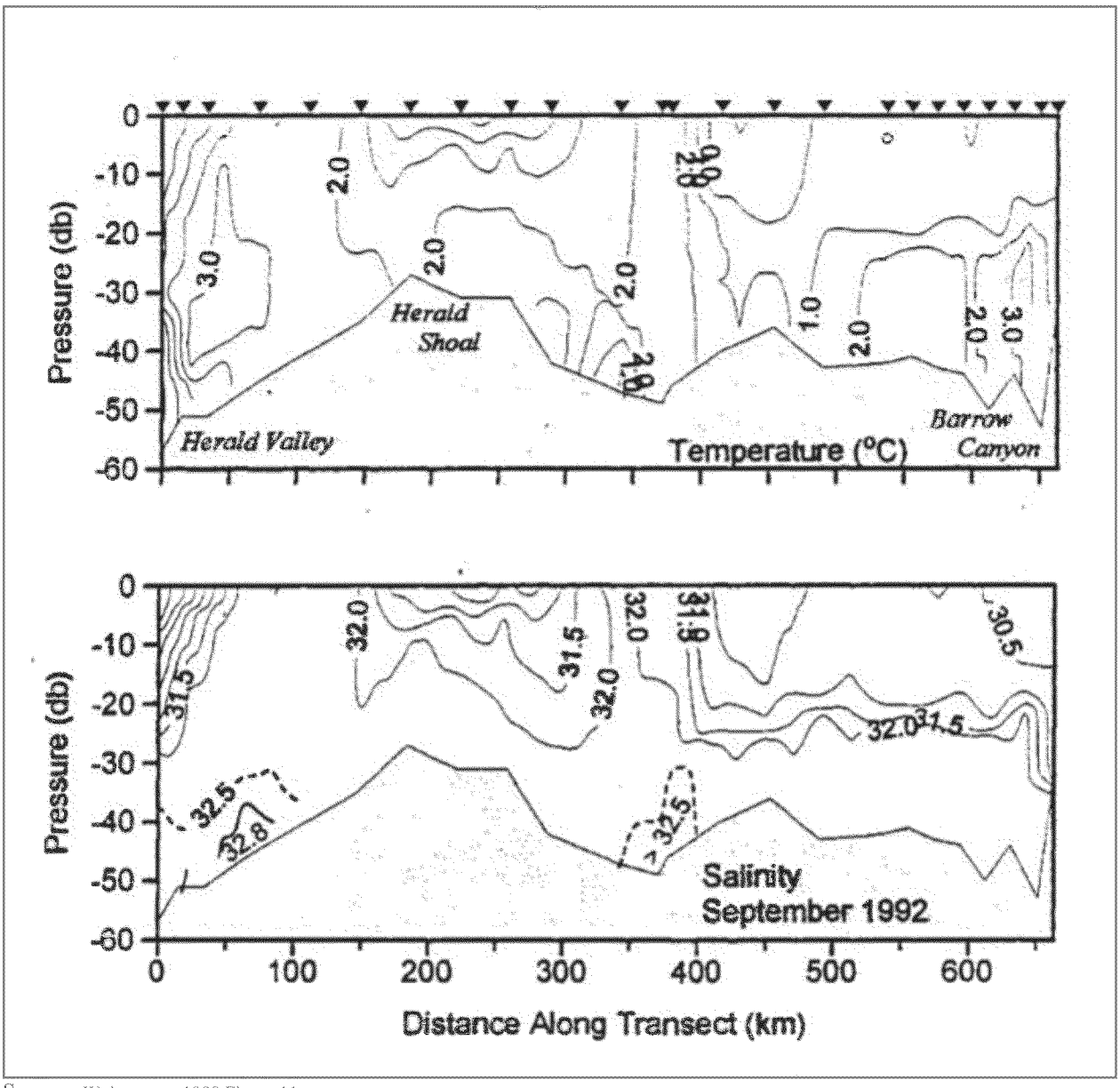
Source: Weingartner 1998 Figure 2

Figure 4-1 Location of Temperature and Salinity Measurements Reported in Figure 1-2



Source: Weingartner 1998 Figure 8

Figure 4-2 Seasonal Temperature and Salinity Variation on the Chukchi Shelf



Source: Weingartner 1998 Figure 11

Figure 4-3 Salinities and Temperatures Along an E-W Transect in the Chukchi Sea (Wrangel Is to Barrow)

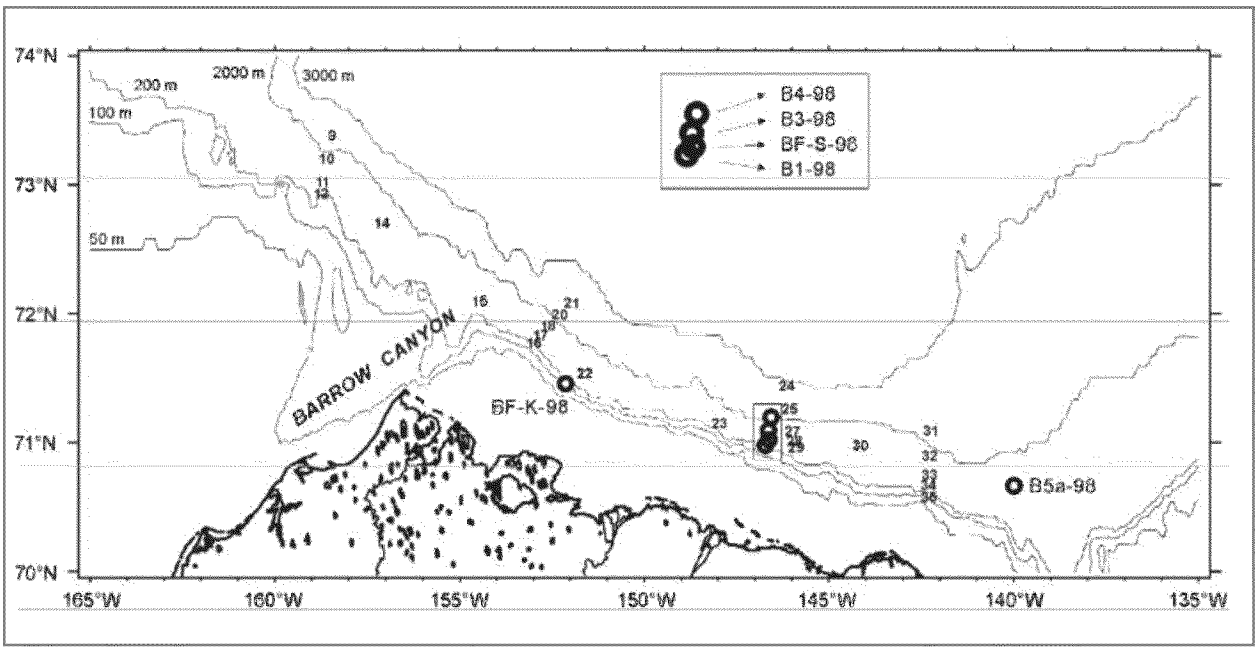


Figure 4-4 Mooring and Hydrographic Station Locations (source: Weingartner 2006 Figure 7)

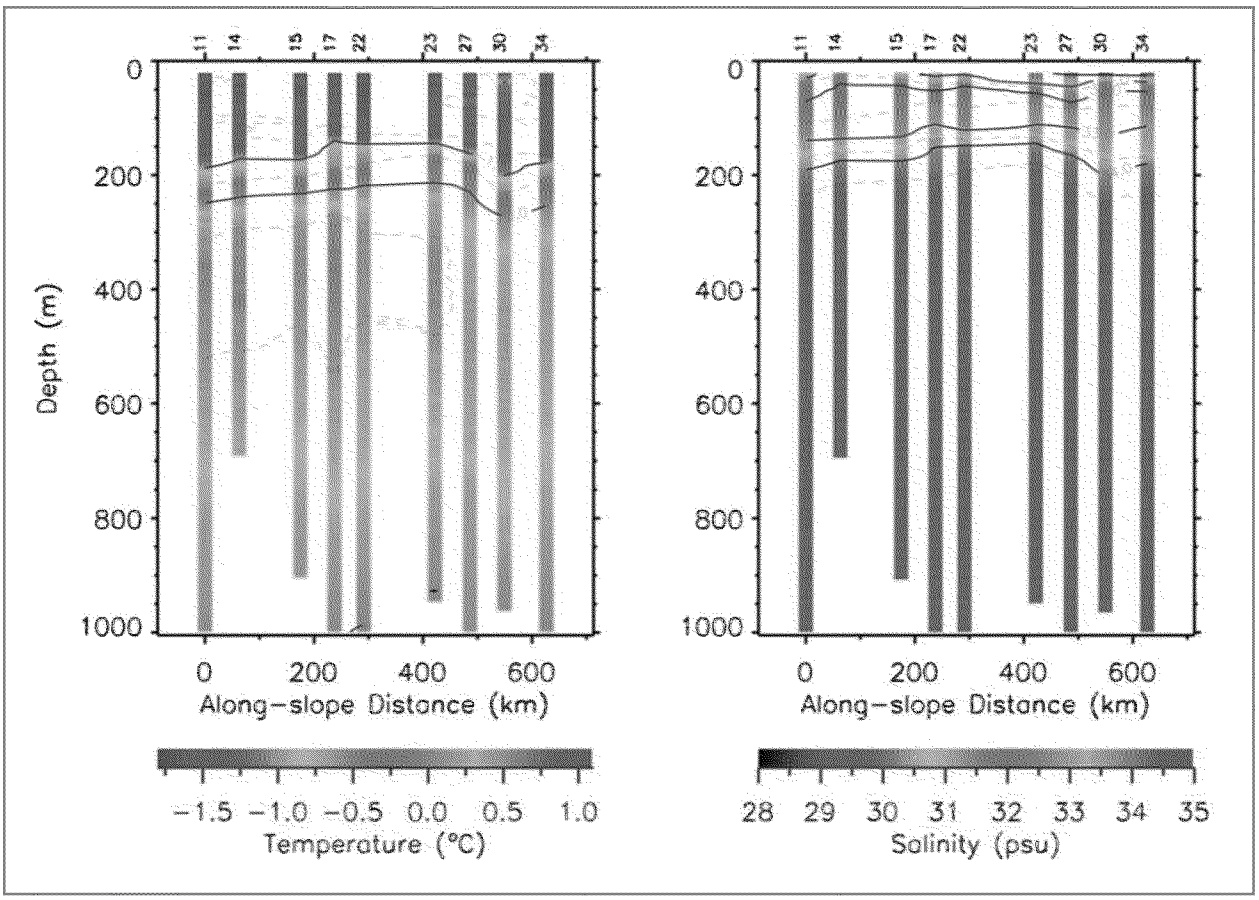


Figure 4-5 Salinity and Temperature Measurements Along Alaskan BS Slope (source: Weingartner 2006 Figure 36)

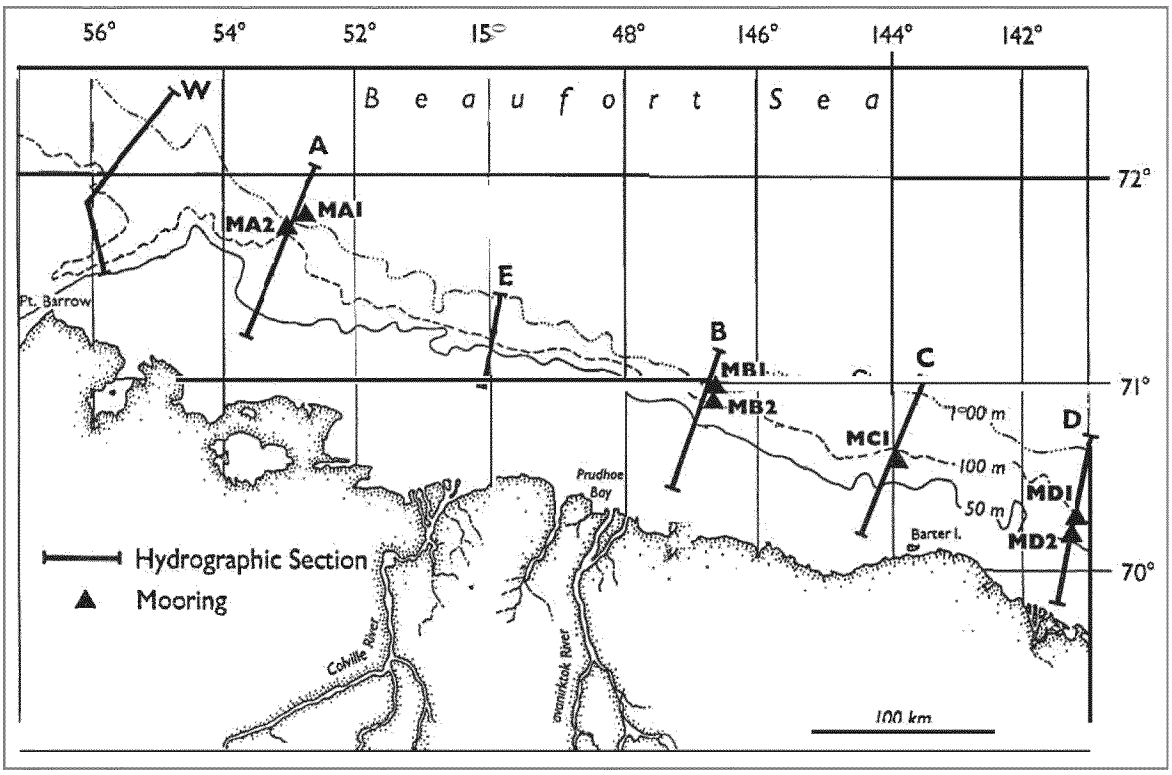


Figure 4-6 October Measurement Transects and Mooring Locations (source: Aagaard 1988 **Figure 1)**

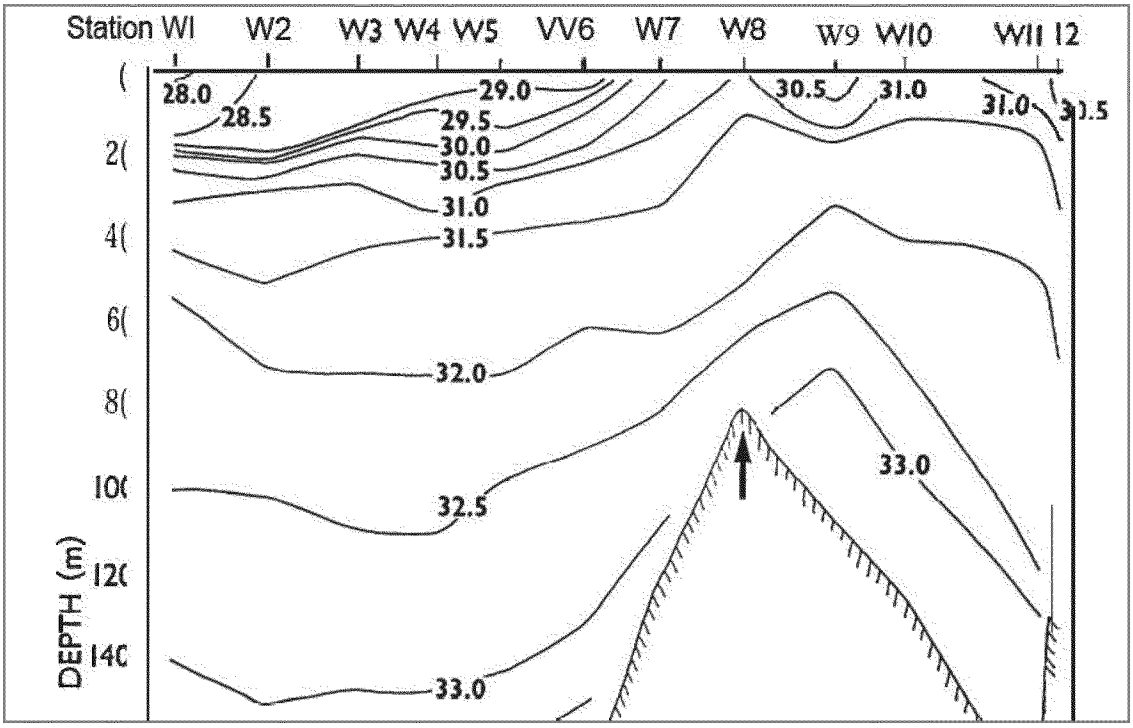


Figure 4-7 October 1988 Water Salinities at Transect W (source: Aagaard 1988 **Figure 8)**

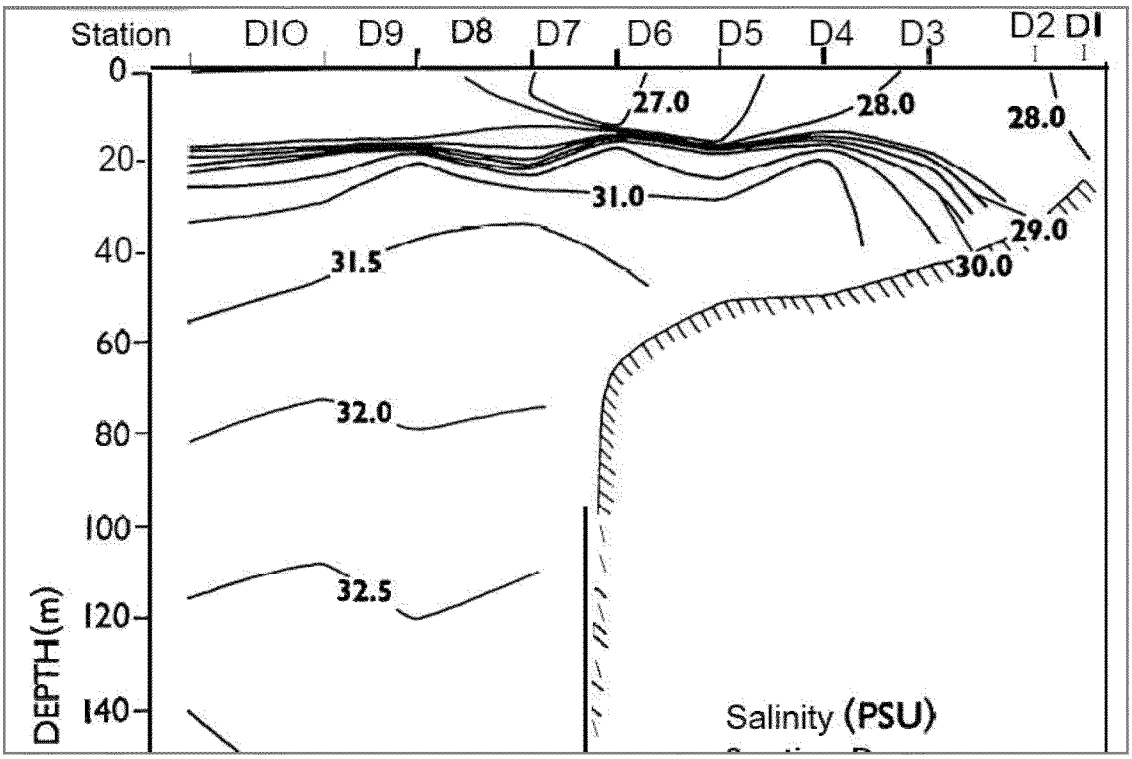


Figure 4-8 October 1988 Water Salinities at Transect D (source: Aagaard 1988 **Figure 53)**

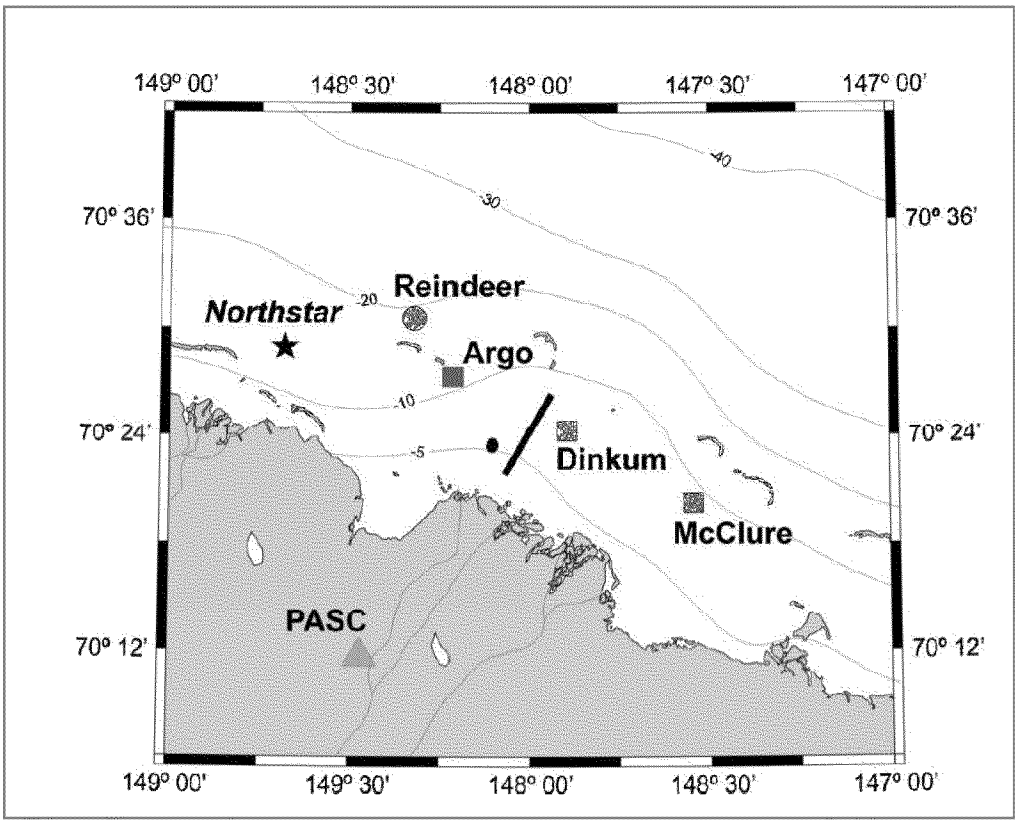


Figure 4-9 Nearshore Instrument Locations (source Weingartner et al. 2005)

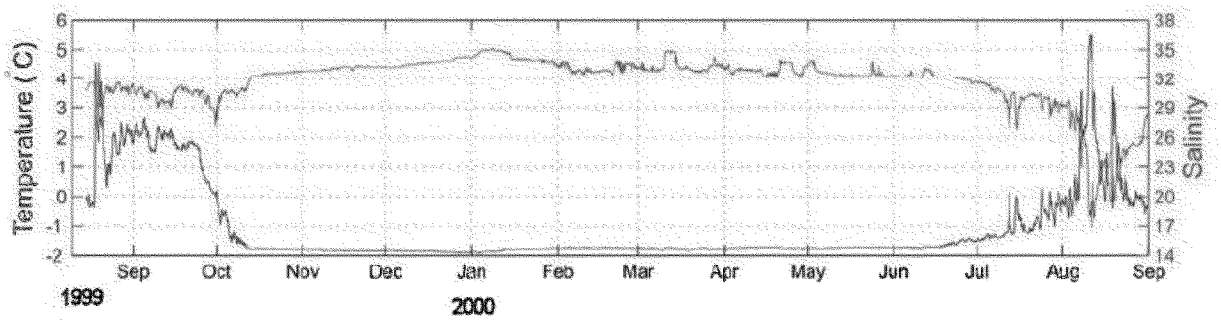


Figure 4-10 Nearshore Water Salinity in Beaufort Sea at Dinkum Deployment (source: Figure 45 Weingartner et al. 2005)

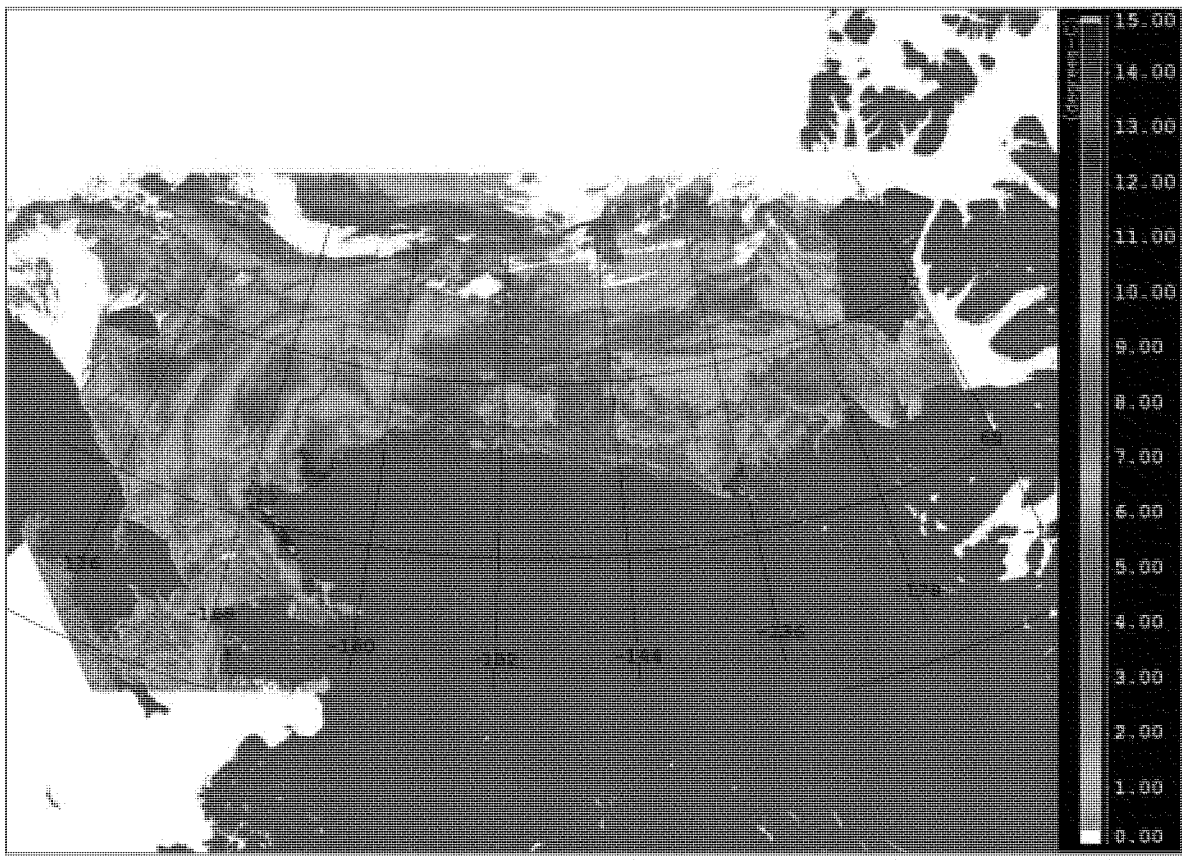
4.2 Water Temperature

The dispersibility of a crude oil is a strong function of its viscosity, which in turn is often very temperature dependent. As crude oils cool their viscosities increase and with increasing viscosities there is a potential for the reduction of dispersant effectiveness. If the water temperature is below the pour point of the oil (and the oil's temperature has reached the water temperature) the oil may not be fluid and is very viscous. Under these conditions the dispersant does not easily mix with the oil when applied. As a result of the combination of the poor mixing of the dispersant into the oil and the high viscosity of the oil dispersants are not effective at these temperatures. The chemical dispersibility of the crude oil is affected by the temperature only as it affects the viscosity of the oil. The oil's viscosity is affected by the water temperature on which it sits and during sunny days by the heating of the surface of the oil by the sun. Different crude oils can have very different viscosities at the same temperature and many can be quite fluid at temperatures near freezing. There is really no water temperature that will completely restrict the use of chemical dispersant. The oil viscosity – temperature relationship must be considered for each candidate oil before an evaluation of the potential for dispersant effectiveness can be made.

The surface water temperatures in the Chukchi and Beaufort Seas in the open water seasons can vary from close to freezing (-2 °C) to upwards of 10 °C. Figure 4-2, Figure 4-3, Figure 4-5, and Figure 4-10 all show surface water temperatures in the Chukchi and Beaufort Seas that fall with this range. The satellite image in Figure 4-11 shows August surface water temperatures in the US Beaufort Sea ranging from 2 to 9 °C and in the Chukchi Sea from 4 to 14 °C with considerable

spatial variability in both regions. The wide spatial variation in surface water temperatures in both the Chukchi and Beaufort Seas can be seen in the extensive imagery catalogued at http://mather.sfos.uaf.edu/~mschmidt/ims_summary_new.html.

The decision to use dispersants from a potential effectiveness standpoint in these regions will require a case-by-case evaluation of the prevailing water temperature and the oil viscosity-temperature relationship.



Source: http://mather.sfos.uaf.edu/~mschmidt/ak_beaufort_sea_2007/ims_beaufort_aug_2007.html.

Figure 4-11 August 22, 2007 MODIS sea surface temperature image of the Chukchi Sea and Beaufort Sea

4.3 Ice Cover

The presence of greater than 9/10ths ice cover eliminates the possibility of using conventional surface applied dispersant operations. The presence of the ice either encapsulates the oil making it impossible to target with aerial applied dispersant or dampens the surface energy such that there is insufficient energy to break the oil into droplets once treated. In ice conditions with less

than 3/10ths cover chemical dispersants will perform much the same as in open water conditions since the surface mixing energy is not overly dampened by the presence of the ice. The performance of dispersants in 3/10ths to 8/10ths ice cover without additional mixing energy applied is somewhat uncertain. Research suggests that performance may be enhanced in these conditions by the energy generated at the ice edge as the ice pieces jostle even under low wave energy (SL Ross 2002, 2006a). Additional research on the potential for dispersant use under partial ice cover conditions is currently being considered (OGP 2014). Regardless, it is instructive to identify the potential periods of open water when traditional surface dispersant application operations could be successful. In the Chukchi Sea region open water periods can exist in the months from June through November depending on the year based on an assessment of the US National Snow and Ice Data Center's archived ice cover data located at the following URL: (http://nsidc.org/data/seaice_index/archives/image_select.html). Open water is most common in the months of July through October in this region but the shoulder months of June and November may present dispersant application opportunities depending on the ice year. In the Beaufort Sea region the maximum likely extent for open water dispersant use is from July to October with the more likely season being from August to October. Table 4-1 shows the months when the two regions were primarily in open water or low ice concentration conditions for the years from 1998 to 2013.

Table 4-1 Open Water period in US Chukchi and Beaufort Sea Regions

Year	Chukchi Sea Open Water Months	Beaufort Sea Open Water Months
1998	July, Aug., Sept., Oct., Nov	~~July~~, Aug., Sept., Oct.
1999	July, Aug., Sept., Oct.	Aug., Sept., ~~Oct.~~
2000	July, Aug., Sept., Oct., ~~Nov~~[1]	Aug., Sept., Oct.
2001	July, Aug., Sept., Oct.	~~Aug.~~, Sept.
2002	~~June~~, July, Aug., Sept., Oct., Nov	Aug., Sept., Oct.
2003	June, July, Aug., Sept., Oct., Nov	Aug., Sept., Oct.
2004	July, Aug., Sept., Oct., ~~Nov~~	Aug., Sept., Oct.
2005	~~June~~, July, Aug., Sept., Oct., ~~Nov~~	Aug., Sept., Oct.
2006	July, Aug., Sept., Oct., Nov	Aug., Sept., Oct.
2007	~~June~~, July, Aug., Sept., Oct., ~~Nov~~	Aug., Sept., Oct.
2008	July, Aug., Sept., Oct.	~~July~~, Aug., Sept., Oct.
2009	July, Aug., Sept., Oct.	July, Aug., Sept., Oct.
2010	July, Aug., Sept., Oct., ~~Nov~~	Aug., Sept., Oct.
2011	~~June~~, July, Aug., Sept., Oct.	Aug., Sept., Oct.
2012	July, Aug., Sept., Oct.	Aug., Sept., Oct.
2013	July, Aug., Sept., Oct.	Aug., Sept., Oct.
[1]Crossed out months had partial open water in the region		
Source: imagery at http://nsidc.org/data/seaice_index/archives/image_select.html		

An additional source of information on ice cover for the central Chukchi Sea area is Figure 4-12. This shows the open water and ice cover conditions for the Burger drilling prospect from 1995 to 2011. The results are similar to the general trends identified in Table 4-1.

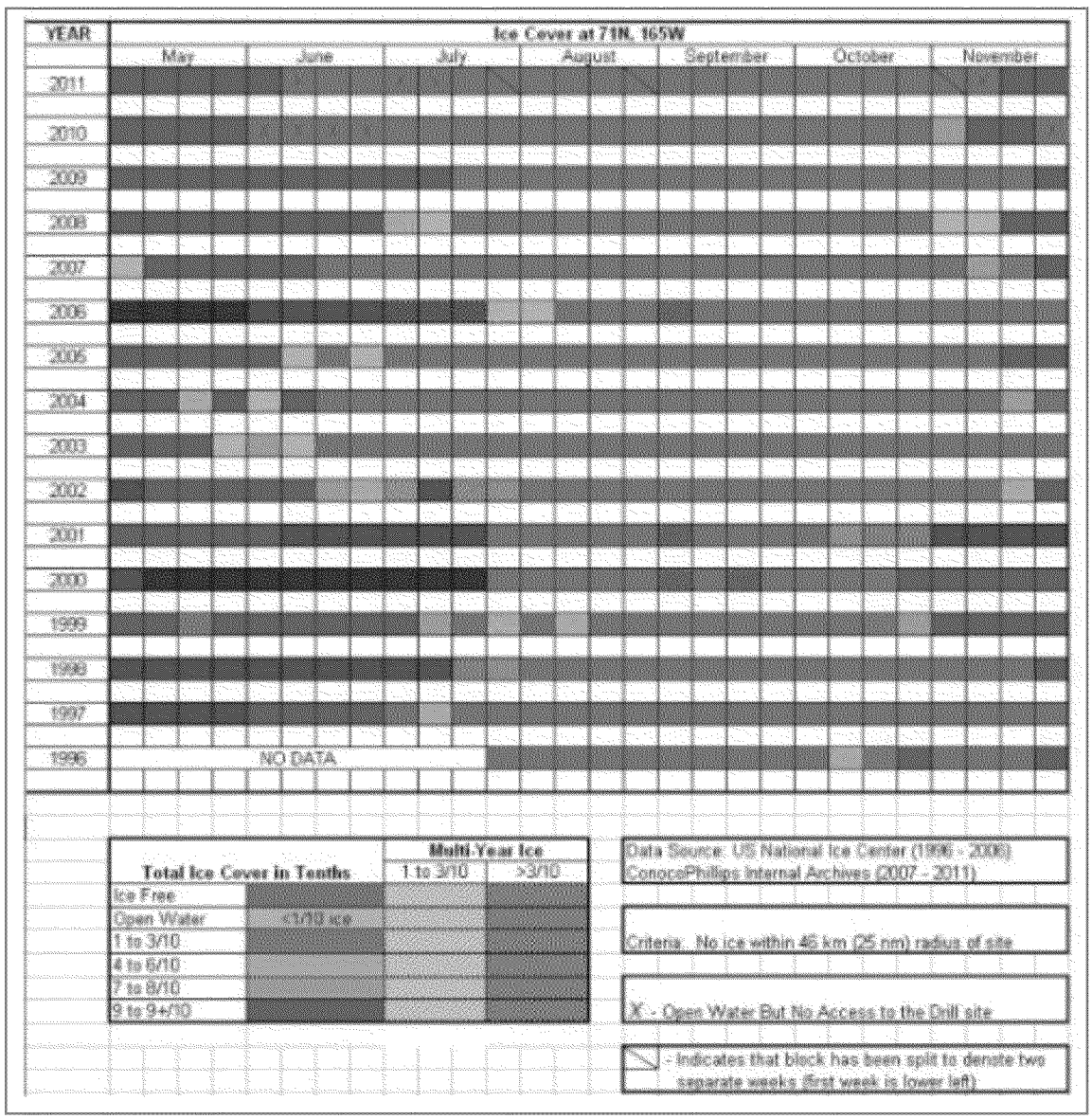


Figure 4-12 Ice Cover at Burger prospect in Central Chukchi Sea (source: Wang et al. 2012)

An additional source of information on ice cover for the US Beaufort Sea north of Point Thompson around Shell's Sivulliq drilling prospect is Figure 4-13. This shows the typical open water and ice cover conditions for the Shell's Sivulliq drilling prospect. The results are similar to the general ice cover trends for the Beaufort Sea Region identified in Table 4-1.

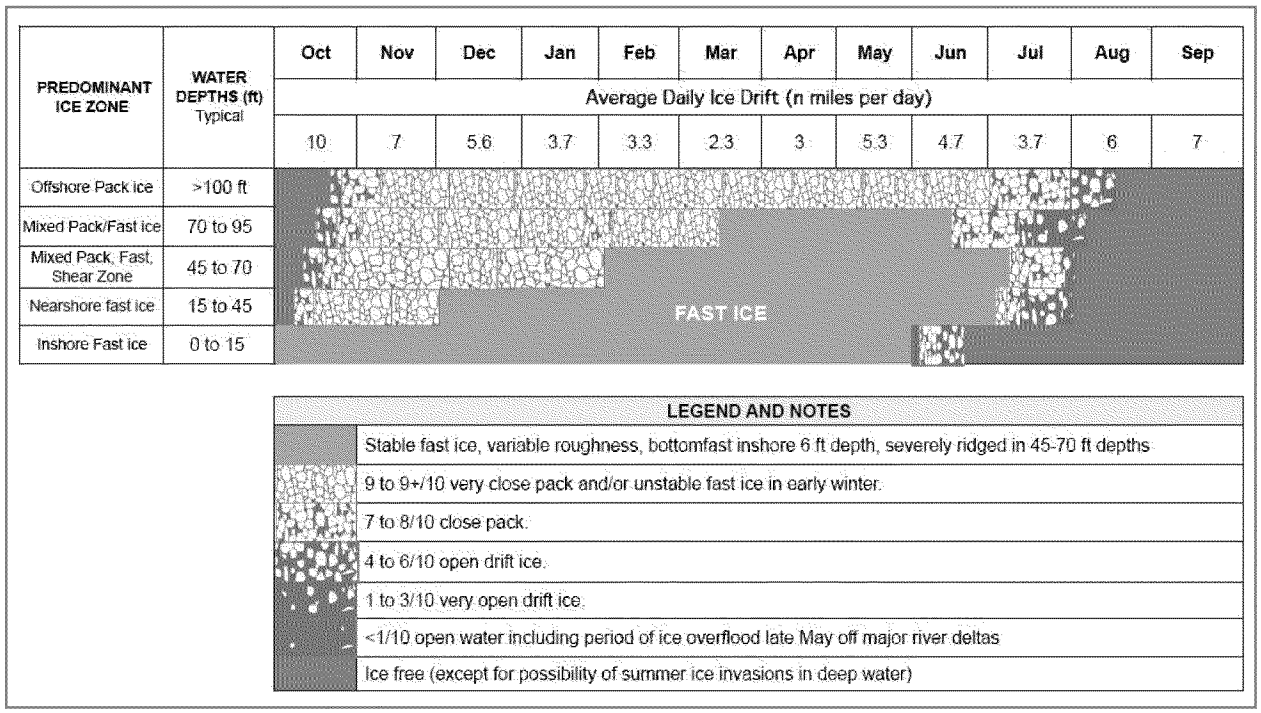


Figure 4-13 Ice Cover at Sivulliq prospect north of Point Thompson in the Beaufort Sea (source: Dickins 2007)

In periods of complete ice cover dispersants could be used in conjunction with ice-breaker support and the application of additional mixing energy through the azimuthal stern drive propeller systems deployed by modern icebreakers (SL Ross 2005, 2006a, 2006c). Subsea dispersant injection during a subsurface well blowout is another type of dispersant operation that could be conducted in a complete ice cover environment. Dispersant effectiveness and net environmental benefit considerations would have to be evaluated on a case- by-case basis before implementing these types of operations.

4.4 Wave Energy

Long term historical wave height data is not available in the U.S. Chukchi and Beaufort Sea regions. A few recent years of wave data at specific locations are available from summer buoy deployments (see http://www.aoos.org/aoos-data-resources) but this data is insufficient both temporally and spatially to quantitatively assess the potential for dispersant effectiveness in the region. Long term wind data are, however, available for the region through shore-based weather stations and offshore hindcast analyses. Wave heights can be derived from wind speed data

through the Beaufort wind scale in the absence of actual measured wave height data and that approach has been taken to estimate wave heights for this project. The Beaufort scale is provided in Table 4-2. The wave height and wind speed data from this table have been plotted in Figure 4-14 to develop a polynomial prediction of wave height as a function of wind speed.

Table 4-2 Beaufort Wind Scale

Force	Wind (Knots)	WMO Classification	Appearance of Wind Effects	
			On the Water	On Land
0	Less than 1	Calm	Sea surface smooth and mirror-like	Calm, smoke rises vertically
1	1-3	Light Air	Scaly ripples, no foam crests	Smoke drift indicates wind direction, still wind vanes
2	4-6	Light Breeze	Small wavelets, crests glassy, no breaking	Wind felt on face, leaves rustle, vanes begin to move
3	7-10	Gentle Breeze	Large wavelets, crests begin to break, scattered whitecaps	Leaves and small twigs constantly moving, light flags extended
4	11-16	Moderate Breeze	Small waves 1-4 ft. becoming longer, numerous whitecaps	Dust, leaves, and loose paper lifted, small tree branches move
5	17-21	Fresh Breeze	Moderate waves 4-8 ft taking longer form, many whitecaps, some spray	Small trees in leaf begin to sway
6	22-27	Strong Breeze	Larger waves 8-13 ft, whitecaps common, more spray	Larger tree branches moving, whistling in wires
7	28-33	Near Gale	Sea heaps up, waves 13-19 ft, white foam streaks off breakers	Whole trees moving, resistance felt walking against wind
8	34-40	Gale	Moderately high (18-25 ft) waves of greater length, edges of crests begin to break into spindrift, foam blown in streaks	Twigs breaking off trees, generally impedes progress
9	41-47	StrongGale	High waves (23-32 ft), sea begins to roll, dense streaks of foam, spray may reduce visibility	Slight structural damage occurs, slate blows off roofs
10	48-55	Storm	Very high waves (29-41 ft) with overhanging crests, sea white with densely blown foam, heavy rolling, lowered visibility	Seldom experienced on land, trees broken or uprooted, "considerable structural damage"
11	56-63	Violent Storm	Exceptionally high (37-52 ft) waves, foam patches cover sea, visibility more reduced	
12	64+	Hurricane	Air filled with foam, waves over 45 ft, sea completely white with driving spray, visibility greatly reduced	

Source: http://www.spc.noaa.gov/faq/tornado/beaufort.html

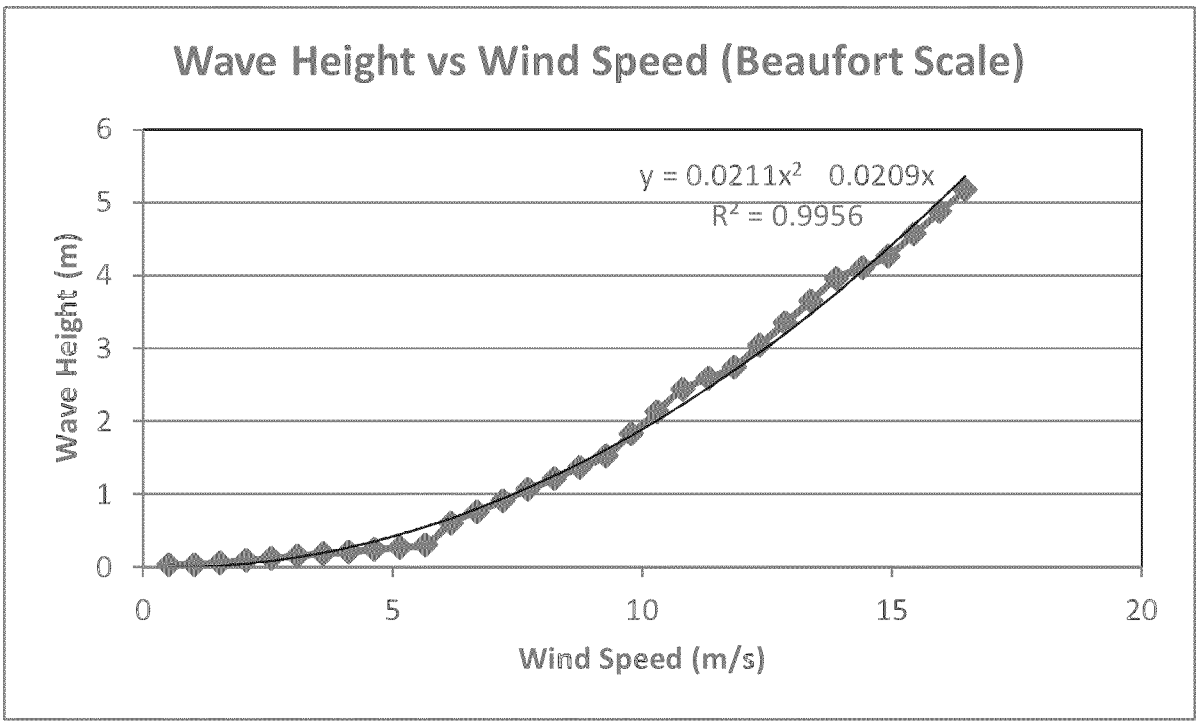


Figure 4-14 Beaufort Wind Scale Wave height Wind Speed Correlation

Land based weather station data were acquired from NOAA for a number of locations along the Chukchi and Beaufort coast lines and these data sets were evaluated for use in the project. Unfortunately, the weather station data had significant data gaps over the past 10 years of data and was not of sufficient quality for use in this project. Fortunately, the Bureau of Ocean Energy Management (BOEM) funded a project to develop a hindcast wind data set for the U.S. Chukchi and Beaufort Sea regions that spans the 31 year period from 1979 to 2009. The data set is known as CBHAR (Chukchi/Beaufort High-resolution Atmosphere Reanalysis). Hourly, ten meter data from five grid points from the BOEM data set have been acquired for this project for use in estimating wave heights in the region. Locations of the offshore grid points selected for analysis are provided in Table 4-3. These locations are mapped in Figure 4-15.

Table 4-3 BOEM Hindcast Wind Data Grid Point Locations

Station Identifier	Longitude	Latitude	Nearby Land Weather Station
Station1	-167.5	69.0	Cape Lisbourne
Station2	-166.0	71.0	Wainwright
Station3	-156.0	72.0	Barrow
Station4	-148.0	71.0	Prudhoe Bay
Station 5	-140.0	70.0	Barter island

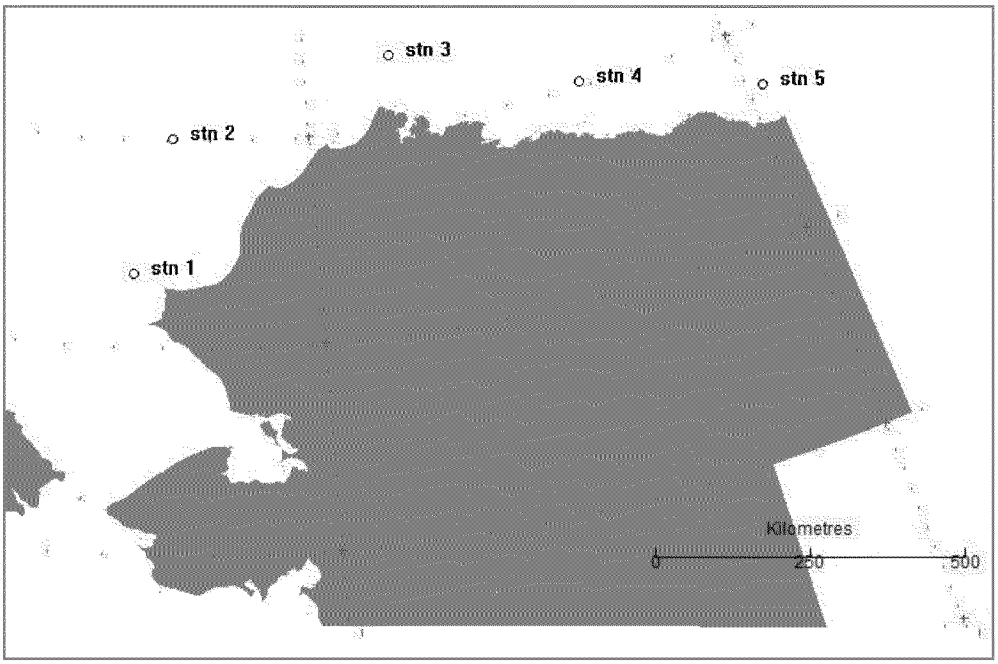


Figure 4-15 Hindcast Wind Grid Point Locations

The Beaufort wind-wave height correlation from Figure 4-14 has been applied to the BOEM hindcast wind data to provide wave height estimates for use in dispersant effectiveness evaluation across the region. The criteria in Table 4-4 have been used to define the times when aerial chemical dispersant application by large aircraft would be possible based on prevailing wind speeds and likely effective based on wave conditions and ice cover. These criteria were established for use in a dispersant use GAP analysis (SL Ross 2011). The rationale for the selection of these criteria is as follows.

Dispersants can be applied by large aircraft until wind speeds are high enough to impair the dispersant spraying operation. At speeds greater than 15 m/s large aircraft have difficulty maintaining their spray path and the high winds affect the dispersant fallout (Exxon 2000). Dispersants are most effective when slicks of fresh or lightly weathered oils can be sprayed with an adequate dose of effective dispersant product in the presence of breaking waves. In offshore environments breaking waves develop when wind speeds exceed 3.5 to 5.4 m/s and waves are 0.5 to 1 m in height. Dispersants can be applied in non-breaking wave conditions, where dispersion might not occur immediately, if breaking waves are likely to occur within a

reasonable time after dispersant application. Research has shown that dispersants applied to slicks on calm seas will cause effective dispersion if the treated slicks are exposed to breaking waves within 48 hours (Lewis, et al. 2010). Dispersant effectiveness begins to be impaired at wave heights of 3 m and above, as at this point waves begin to entrain a considerable proportion of the slick into the water and hold it in suspension temporarily, making it difficult to hit the oil with dispersant spray.

Table 4-4 Operating Limits for Large Aircraft Dispersant Operations

Aerial Dispersant Application (large aircraft)	Favorable	Marginal	Not Possible
Wind Speed (m/s)	<13	>=13 to <=15	>15
Wave Height (m)	>0.6 and <3.0 OR >0.0 and <3.0 if >=0.6 in following 48 h	>=3.0 and <=4.6	> 4.6 OR <0.6 if <0.6 in following 48 h
Ice Cover (10^{ths})	<5/10	5/10 to 9/10	>9/10

The conditions identified in Table 4-4 were applied to the hourly hindcast wind data (and wave heights estimated using the correlation in Figure 4-14) over the full 31 years of data and for the past 10 years to determine the percentage of time that aerial dispersant applications could be effective. Table 4-5 summarizes the results of this assessment for wind station 1. The results for the assessment over the past 10 years (columns 5, 6 and 7) are very similar to the full 31 year analysis (columns 2, 3 and 4) with a maximum of a few percent difference in any given month. Based on this assessment chemical dispersant use would be favorable for 80 to 90% of the time at this location. This does not account for other operational limitations such as visibility and daylight hours as this was out of the scope of the study. Detailed tables for the remaining wind stations are provided in Appendix A.

Table 4-5 Percentage of Time Dispersants Would Be Effective at Wind Station 1 (Chukchi Sea Offshore Cape Lisbourne)

Percent of Time Dispersants Effective (48 hour Calm Wind Grace Period)						
	31 year Data Analysis (1979 to 2009)			10 year Data Analysis (2000 to 2009)		
Month	Favorable	Impaired	Not Favorable	Favorable	Impaired	Not Favorable
1	0	0	100 (ice covered)	0	0	100 (ice covered)
2	0	0	100 (ice covered)	0	0	100 (ice covered)
3	0	0	100 (ice covered)	0	0	100 (ice covered)
4	0	0	100 (ice covered)	0	0	100 (ice covered)
5	0	0	100 (ice covered)	0	0	100 (ice covered)
6	80	3	17	78	3	19
7	87	3	10	87	3	10
8	90	4	5	87	4	9
9	86	8	6	91	5	4
10	82	13	5	82	13	6
11	78	13	9	81	12	7
12	0	0	100 (ice covered)	0	0	100 (ice covered)

Table 4-6 shows the percentage of time that dispersants would be favorable for all 5 of the wind stations based on the most recent 10 years of data (column 5 from the detailed table results). For the months of July through October, where data exists for all wind stations, the least variability in the results occurs in September where favorable conditions across both regions range from 87 to 91%. The highest variability for these months is in July where favorable conditions range from 71 to 87%, with the Beaufort Sea region having the lowest percent of time with favorable conditions.

Table 4-6 Percentage of Time Dispersants Would Be Favorable Based on 10 Year Data Analysis: All Wind Stations

Month	Percent of Time Dispersant Effective (48 hr Calm Wind Grace Period)						
	10 Year Data Analysis (2000 to 2009): Favorable						
	stn 1	stn 2	stn 3	stn 4	stn 5	Average	Std. Dev.
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	78	77	0	0	0	77	1
7	87	83	76	71	81	80	6
8	87	87	79	75	78	81	5
9	91	90	87	87	87	88	2
10	82	88	86	84	79	84	4
11	81	82	0	0	0	81	1
12	0	0	0	0	0	0	0

5 Historical Dispersant Effectiveness Testing on Alaskan Oils and Identification of Knowledge Gaps

This section summarizes the results of past chemical dispersant effectiveness studies that have been conducted on Alaskan oils for use in dispersant use decision-making. The extensive libraries of Environment Canada and SL Ross Environmental research Ltd., the Arctic and Marine Oil Spill Technical Seminar (AMOP), the International Oil Spill Conference Proceedings (IOSC), the Louisiana Universities Marine Consortium (LUMCON) library and other scientific Journals have been searched for this purpose.

5.1 Spill Related Properties of Alaskan Oils

The physical properties of a number of Alaskan production and pipeline oils are provided in Table 5-1 to show the range of oils that have been found in Alaska to date. A study of the change in the properties of the ANS blend that has been transported via the Alyeska pipeline over the past 30 years has also been recently conducted (SL Ross 2013). This study shows that the pipeline oil has progressively become less dense, less viscous and less likely to form stable emulsions. See Figure 5-1 and Figure 5-2. These property changes result in oil that is likely more amenable to chemical dispersion. The change in property is due to a change in the percentage of each production field's contribution to the total flow and possibly due to a change in the properties of the individual field oils themselves. Not enough data has been collected on the properties of the individual field crude oils to track their property changes over time. This information is provided to demonstrate that results from dispersant effectiveness tests conducted on a Prudhoe Bay pipeline crude in the 80's may not be comparable to effectiveness tests completed on Alaska North Slope crude in the 2000's due to different oil composition.

Table 5-1 Alaskan Oil Physical Properties

Oil Name	Density (g/cc) @ 15 °C			Viscosity (cP) @15 °C			Pour Point (°C)		
	fresh	2-day	2-week	fresh	2-day	2-week	fresh	2-day	2-week
ANS Blend (2004)[1]	0.870	0.920	0.930	10.2 (0%)	164 (30.8%)	374 (38.2%)	-21	3	9
ANS Blend (2012)	0.874	0.923	0.937	14 (0%)	156 (27%)	505 (35%)	<-18	-6	0
Endicott (2004)[1]	0.921	0.924	0.925	205 (0%)	247 (0.8 %)	342 (5.3%)	12	15	15
Kuparuk (2004)[1]	0.920	0.943	0.956	81 (0%)	351 (9.5%)	1391 (20.3 %)	<-21	<-21	-9
Lisbourne (1994)[2]	0.875	0.906	0.923	15.9 (0%)	187 (21.1%)	327 (30.6%)	-3	7	15
Northstar(2004)[1]	0.821	0.868	0.877	2.1 (0%)	14.4 (43.7%)	24.4 (52%)	<-21	0	3
Pt. McIntyre (1994)[2]	0.895	0.911	0.920	44 (0%)	125 (9.8%)	441 (18%)	-4	3	11

[1]OCS Study MMS 2008-033 Empirical Weathering Properties of Oil in Ice and Snow, Project Number 1435-01-04-RP-34501 (Mar Inc. 2008)

[2] Spill Related Properties of Fresh and weathered Alaskan Crude Oils (SL Ross 1994)

ED_001324_00001497-00033

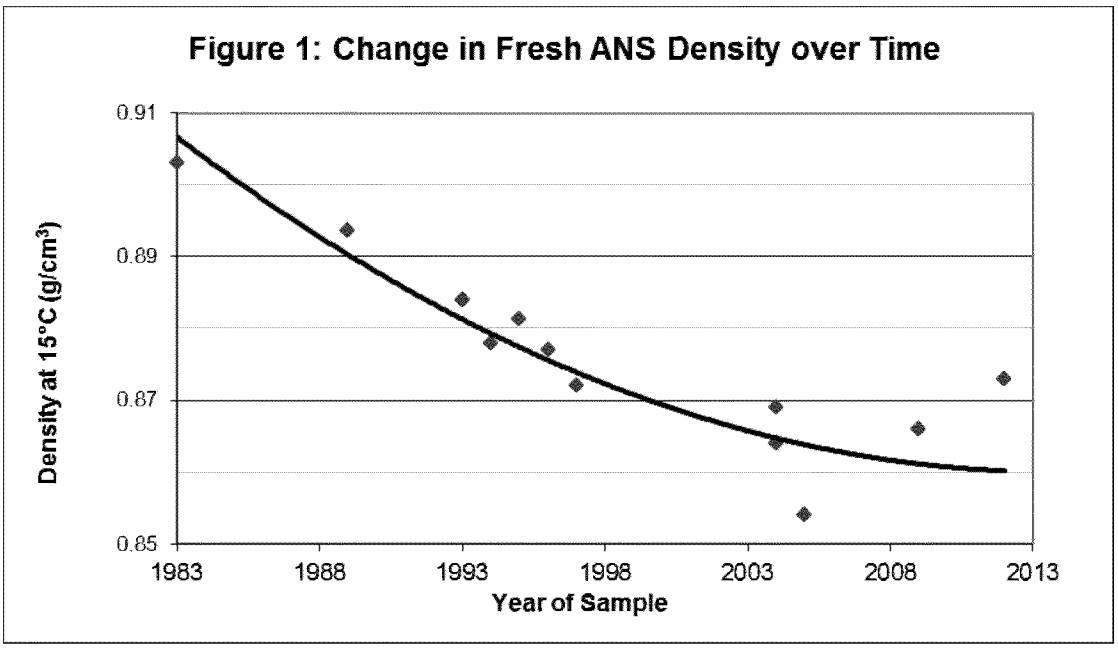


Figure 5-1 Change in Fresh ANS Density over Time

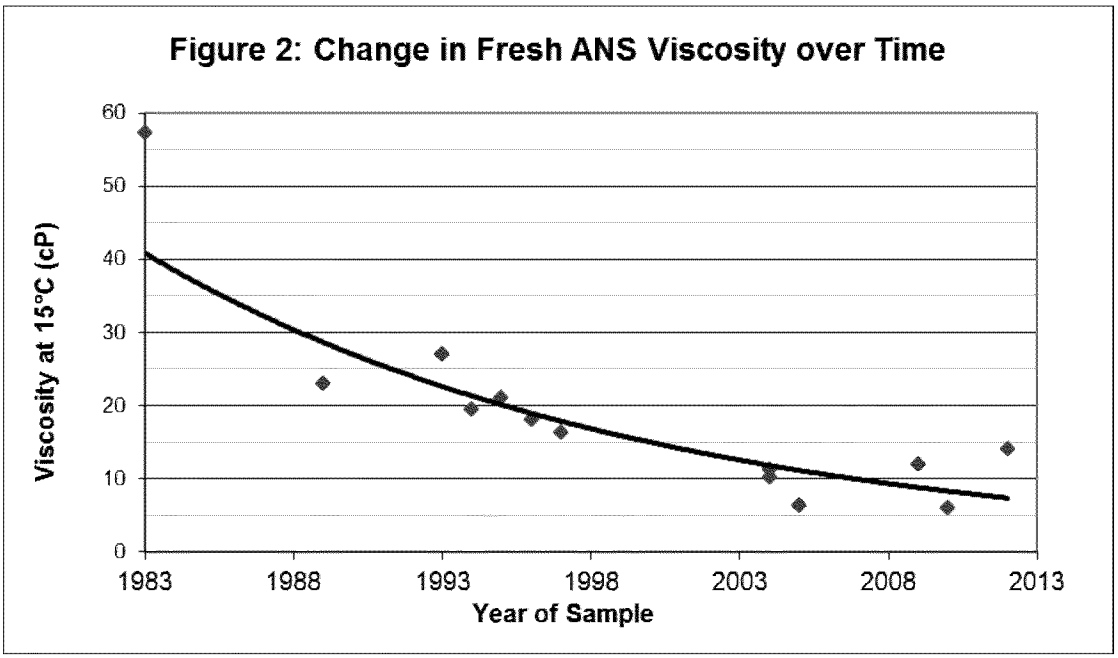


Figure 5-2 Change in Fresh ANS Viscosity over Time

5.2 Background - Mechanisms of Chemical Dispersion

When spilled on water, oil exhibits a cohesiveness or resistance to break up. This cohesive strength is due to the interfacial tension or contractile skin between the oil and water. A chemical dispersant sprayed onto an oil slick acts at the oil-water interface to reduce this interfacial tension. This action promotes the break-up of the oil film into droplets that disperse into the water phase. If the droplets are small enough they will have little buoyancy and will be carried away and diluted by normal ocean current and movement.

Surface active agents (surfactants) are the key components of a chemical dispersant. These compounds contain both a water compatible and an oil compatible group. Because of this molecular structure, the surfactant locates at the oil-water interface, reduces the interfacial tension, and thereby enables the oil slick to break up into finely dispersed oil droplets with the addition of significantly less energy than in the absence of the dispersant. Mackay and Hossain (1982) estimated that a concentration at an oil/water interface of 1 volume of dispersant per 500 volumes of oil will cause a 20-fold reduction in interfacial tension, say, from 20 dynes/cm to 1 dyne/cm. Since dispersants are normally applied at a ratio of 1 volume of dispersant to 20 volumes of oil, the implication is that only a few percent of the dispersant is being effective at any time, most being present in the bulk of the oil and thus remote from the interface.

Despite the great decrease in interfacial tension, some mixing energy is needed to promote movement and dispersion of the fine oil droplets into the water column. This energy can be supplied either by the natural motion and currents of the sea or by mechanical means such as work boats. The greater the available energy, the less dispersant or less effective dispersant is required.

A dispersant formulation also contains a solvent. Since many of the surface agents used in oil spill dispersant formulations are viscous, some form of solvent is necessary to reduce viscosity so that the mixture may be properly applied by conventional spray equipment. In addition, the solvent may act to depress the freezing point for low temperature usage and to enhance the mixing/penetration of the surfactant(s) into more viscous oils. In general, present day surfactants have demonstrated very low toxicity. In addition, these current formulations have substituted de-

aromatized hydrocarbons or aqueous solvents, resulting in very low toxicity dispersant formulations as compared with early formulations.

By their very nature, present-day dispersants include active ingredients that are more soluble in water than in oil. So the dispersant must be applied directly to the oil; otherwise the chemical will be lost to the water phase. Even when applied directly to the oil the chemicals will leach into the water, but the rate at which this happens is not well understood. Most products contain so-called "anionic" surfactants, like sulphosuccinates, in combination with "non-ionic" surfactants, like sorbitan ester surfactants (the SPANS® family of surfactants) and polyethoxylated sorbitan ester surfactants (the TWEEN® family). Studies on the subject (Knudsen et al. 1994, Hokstad et al. 1996) indicate that anionic surfactant compounds will rapidly leach into water, but that the rate of leaching of the non-ionic compounds is uncertain and dependent on a number of factors. Clearly, the leaching process is a complicated one, and it can be assumed that certain components of modern dispersant products will gradually leach from a layer of crude oil into the underlying water column and negatively affect the dispersibility of the oil. Tests conducted at Ohmsett demonstrated that under calm conditions enough dispersant remained with a treated oil slick to still be effective 2 to 6 days of exposure depending on the drift speed of the slick over the water surface and the exposure of the underside of the slick to surfactant free water (SL Ross 2007, 2008). This suggests that an oil spill can be dosed in relatively calm conditions with the expectation that the dispersant will remain with the oil and become effective when sea states and mixing energies increase after a few days.

5.3 Dispersant Effectiveness Test Results on Alaskan Oils

A review of scientific literature on the results of chemical dispersant effectiveness tests, specifically conducted on Alaskan crude oils, has been undertaken. Research that investigated chemical dispersant effectiveness under arctic conditions and in the presence of ice using other oil types has also been included in the review due to paucity of research in this area. Table 5-2 identifies the literature that has been reviewed and identifies the key variables that were studied in each test series. The review has focused on the parameters that could affect dispersant performance that are of specific concern to the arctic environment. These parameters include the test temperature, the water salinity, the mixing energy and the presence of ice. Oil type, dispersant type and dispersant dosage have also been considered in this review. With a few exceptions, only tests that have used Alaskan oil have been reviewed.

Only 10 of the 48 test programs reviewed studied dispersion in the presence of ice and only 7 of these varied the ice concentration in the test matrix and only two used Alaskan oil in the testing. Of the 48 studies reviewed, temperature was varied in 15, salinity was varied in 16, and mixing energy was varied in 29.

Most of the unique aspects of chemical dispersant use and effectiveness in the Arctic are due to the potential presence of ice in various forms and concentrations throughout the year. Mixing energy in the arctic environment may be reduced or eliminated due to the presence of ice. Melting ice may result in reduced surface water salinity. Ice may dampen overall surface water mixing or add localized mixing energy to the system. Water temperature is another important factor as cold water temperatures will reduce the dispersant and oil temperature, increase the oil viscosity and potentially reduce the effectiveness of dispersants due to the increased viscosity that may inhibit both the mixing of the dispersant into the oil and the breakup of the oil into small droplets. The impact on dispersant effectiveness of these factors is discussed in more detail in the following sections with specific reference to tests conducted on Alaskan crude oils.

5.4 Oil Type

At the present time the only oils being produced and transported (by pipeline to shore) in the Beaufort and Chukchi Seas are Northstar and Endicott crude oils and are the only crude oils that could be spilled directly into Alaskan offshore waters from present production fields. The Northstar oilfield is located six miles off the Beaufort Sea coast 11 miles northwest of Prudhoe Bay. Oil from this field is shipped to the Trans-Alaska Pipeline System via a trenched 10 inch pipeline to shore. The Endicott field is approximately four miles off the Beaufort Sea coast and 10 miles southeast of Prudhoe Bay. Oil from this field is shipped to the Trans-Alaska Pipeline System via a 16 inch pipeline to shore. No oils are presently being produced in Chukchi Sea waters.

Alaska North Slope Crude (ANS), or Prudhoe Bay crude as it was named in early test programs, has been the Alaskan oil of choice for most of the dispersant effectiveness tests conducted on Alaskan oils. This is evident in Table 5-2 where testing on Alaskan crudes other than the ANS blend crude occurred in only 5 of the research programs reviewed. The ANS or Prudhoe Bay crudes are both oils that were sampled from the Trans-Alaska Pipeline system that delivers Alaskan oil to Valdez. This oil is a blend of the oils produced from the various fields on the North Slope. Because this oil moves to the south by pipeline it is not likely to be spilled in the Beaufort or Chukchi Seas. As shown in Section 1 the physical properties of this Alaskan pipeline blend also have changed over the years and care must be taken when comparing dispersant effectiveness tests conducted on these oils that span many years. Only five of the test programs evaluated dispersant effectiveness on specific North Slope production crudes (Endicott, Northstar and Pt. McIntyre). These oils were used in projects that were conducted for BSEE's predecessor, the Minerals Management Service (MMS), with oils sourced by them specifically for cold water dispersant effectiveness testing. These oils are possibly more representative of the type of crude that might be spilled in the Beaufort and Chukchi Seas. As new oils come into production in the waters of the Beaufort and Chukchi Seas they should be evaluated for their potential for chemical dispersion under the range of environmental conditions they might be spilled in.

5.5 Dispersant Type

Most of the dispersant effectiveness tests on Alaskan oils identified in Table 5-2 used one or both of Corexit 9500 and Corexit 9527 in their testing. This is not surprising since these brands have been the most stockpiled products in North America and have been on the market for many years. Other dispersants tested on ANS crude under a variety of conditions include Archochem D609, Atlantol AT-7, BP 1100WD, Citrikleen, Corexit 9550, Dispersit SPC1000, Dispolene 34S, Dasic Fresh Water, Dasic Slickgone NS, Enersperse 700, Enersoerse 1037, Finasol OSR 5, Finasol OSR 7, Finasol OSR 51, Finasol OSR 52, Inipol IP90, Inipol IPF, Inipol PC, OFC D-609, Slickgone NS, and Superdispersant 25. Many of these products are no longer commercially available or on the US EPA NCP list of approved products for US waters. The significance of dispersant type will be discussed more fully when addressing the other variables that are specific to Arctic dispersant use (cold temperatures, salinity, mixing energy and ice). If new chemical formulations are developed or 'green' dispersants are developed their effectiveness on the crude oils currently being produced on the North Slope should be tested using bench and large scale test methods.

5.6 Water Temperature

There is concern in temperate or subarctic climates that temperature, per se, is a problem in dispersant effectiveness, and that dispersants should not or cannot be used in cold climates. This concern is generally unfounded, except in the case of so-called "high pour point oils", some of which can become semi-solid at temperatures well above freezing. Reduced temperature simply increases the viscosity of the dispersant product and the spilled oil. Dispersant products can be formulated to be non-viscous in cold temperatures, so this is not a problem. The viscosity of the spilled oil will become higher at low temperatures, but perhaps not too high for effective chemical dispersion. In early work by Mackay (1979), that used the Mackay Nadeau and Steelman (MNS) test and a hoop tank, it was concluded that the primary effect of water temperature was to lower the oil temperature and increase the oil viscosity which in turn reduced the dispersant effectiveness. In later work where Prudhoe Bay crude oil was used, Mackay (1986) concluded that "There is no inherent reason to suggest that cold climate dispersion is significantly less feasible than temperate dispersion" and that "dispersion is likely to require more dispersant under these cold conditions but the extra amount is unlikely to be large except when the oils are unusually viscous, close to their pour point or waxy". Tests conducted using

the ExxonMobil dispersant effectiveness test (EXDET) by Mackay (1995) found a slight **decrease** in effectiveness of C 9527 on ANS crude as the temperature **increased** from 4.4 °C to 15.5 °C. Byford et al (1983) found in Warren Spring Laboratory tests (WSL) and tank tests, that included North Slope Crude oil conducted at 0 and 10 °C, that in all cases the dispersant performance was not reduced in the lower temperatures and in some cases was enhanced. Moles et al (2001, 2002) found that dispersant effectiveness, as measured by the swirling flask test (SFT), on fresh ANS was below the detectable limits when the water temperature was 3 °C. They also found that the effectiveness of Corexit 9500 was similar at 10 and 22 °C in 32 ppt salt water but lower effectiveness was measured in the cold condition for Corexit 9527. Chandrasekar et al (2005, 2006) found that the best dispersion of ANS was achieved in the baffled flask test (BFT) at 22 °C with a reduction in effectiveness at both 5°C and 35 °C. Fingas et al (2006) found that Corexit 9500 was equally effective in the SFT test on ANS crude at temperatures between 5 to 10 °C and **decreased** when the temperature **increased** to 25 °C. In a study using Alberta Sweet Mixed Blend crude oil and the SFT test Fingas et al (2005b) found a similar trend with similar effectiveness at 5 to 20 °C and a reduction in effectiveness at 25 °C. Earlier work with the SFT test by Fingas et al (1991) found a reduction in dispersant effectiveness when temperatures were lowered from 50 °C to 0 °C. Large scale tests conducted in cold water conditions (-2.8 to 3.3°C) at the Ohmsett facility (SL Ross 2002, 2003, 2006, 2006b) resulted in near complete dispersion (74 to 99%) of fresh ANS, Endicott, Northstar and Pt. McIntyre crude oils. The weathered and emulsified oils were not as easily dispersed in a number of the tests with measured effectiveness ranging from 3 to 35%.

Crude oils that are relatively non-viscous and whose viscosities do not increase to very high values when temperatures are lowered to 0° C are likely to be chemically dispersible over the full range of possible water temperatures. On the other hand, fresh or weathered oils that are relatively viscous to begin with, but yet dispersible at high temperatures, may become excessively viscous and un-dispersible when lowered to freezing temperatures. The main lesson here is that it is the viscosity of the oil at the water temperature in which it is spilled that is the key factor in deciding dispersant effectiveness and not the temperature per se. This points to the need to fully understand the fresh and weathered oil viscosities of the specific oils that are likely to spilled in cold waters. This is accomplished through laboratory testing and modelling. Once such analysis is done, it should become evident if a specific oil is a good candidate for dispersant

use or not. From the past testing that has been conducted on Alaskan oils it appears that the Alaskan oils produced to date are generally dispersible at cold temperatures when fresh and slightly weathered but may not be readily dispersed when heavily weathered and emulsified. Additional cold water testing of the chemical dispersibility of specific Alaskan oils when weathered and emulsified may be useful to further define the time window of opportunity for the successful dispersion of these oils under typical conditions that could be encountered in the study region.

5.7 Water Salinity

An extensive review of worldwide scientific and technical journals was undertaken for the US Department of Interior (SL Ross 2010) to identify relevant literature on the use of chemical treating agents for oil spill response in fresh and brackish water. This review found that numerous laboratory-scale, meso-scale and field studies, dating back to the late seventies, have been conducted to study the effect of water salinity on the effectiveness of oil spill chemical dispersants on a range of oils. The consistent significant finding of all of these tests is that dispersant designed for use in marine environments (30 to 35 ppt salinity) are considerably less effective when the salinity falls below about 20 ppt or above 40 ppt. Of the dispersants most commonly used in US waters, Corexit 9500 has been shown to be more effective than Corexit 9527 in lower salinity waters.

Dispersants have been formulated for use in fresh water and these have also been tested for effectiveness over a range of water salinities, although not as extensively as the marine dispersants. The effectiveness of the freshwater dispersants have been shown to generally be much better than the marine products in freshwater but often achieve their best results in waters between 10 and 20 ppt salinity.

Most of the research identified in Table 5-2 where salinity was varied in the test matrix form part of the previous review described above (SL Ross 2010). Table 5-2 references identify research that was specific to Alaskan crude oils (exclusively Alaska North Slope blend or Prudhoe Bay crude). The effectiveness of dispersants on the Alaskan oil under varying water salinities generally is consistent with the findings for other crude oils. The work by Chandrasekar et al (2006) and Nagaraja et al (2008) were not part of the 2010 review. It is interesting to note that in the work reported by Chandrasekar and Nagaraja that the effectiveness of dispersant A

(assumed to be Corexit 9500 based on previous reports by this author) on ANS crude in the BFT varied by less than 15% over the salinity range (from 10 to 34 ppt) and temperature range tested (5 to 35 °C). These tests showed greater effectiveness of a marine dispersant (Corexit 9500) on ANS crude at low water salinity than has been demonstrated by many other test programs. This improvement may be due to the higher energy level imparted in the BFT when compared to the SFT which was used in many of the other tests that investigated the dispersibility of ANS crude at different temperatures and salinities. The work conducted by Abbasova (2005) also show high dispersibility of a Caspian crude oil in low salinity water (12ppt) using the BFT. In early work by Cox and Shultz (1981) using the MNS test apparatus, no difference in dispersant effectiveness was identified over salinities ranging from 10 to 32 ppt. Unfortunately the dispersants used in these tests were not identified by brand name.

The water salinities in the US Beaufort and Chukchi Seas are generally greater than 20 ppt, even during the spring ice melt season, with the exception of fresh water outflows inside the barrier islands of the US Beaufort Sea and isolated near shore regions of the Chukchi Sea (see Task 1 Report). Additional testing of Alaskan oils in low salinity water may be a low priority since chemical dispersants designed for marine use have been shown to generally be effective on most oils and specifically on ANS crude oil in waters with salinities 20 ppt and greater. If new 'green dispersants' are developed and proposed for Arctic use their effectiveness in water salinities ranging from 15 to 35 ppt should be evaluated on Alaskan oils.

5.8 Mixing Energy

Mixing energy is of obvious importance to the dispersion of marine oil spills: simply put, the more mixing the better. Oil slicks can be broken up into small droplets and disperse naturally under high energy conditions but much more rapid dispersion and dispersion under much less vigorous wave energies can be accomplished by applying chemical dispersants as described in Section 2 above. The bench-scale, and meso-scale laboratory tests and outdoor wave tanks all impart a slightly different mixing energy to the oil-water-dispersant system and thus can result in different dispersant effectiveness estimates for a given oil and dispersant combination. A comparison of dispersant effectiveness estimates using identical oils and dispersants was made for the swirling flask test (SFT), baffled flask test (BFT), ExxonMobil dispersant effectiveness test (EXDET), the Warren Spring Laboratory test (WSL), and Ohmsett test tank (SL Ross 2011).

Based on the results of this study it can be assumed that the mixing energy rankings for these tests fall into the following pattern: SFT<BFT<EXDET<Ohmsett. Clark et al (2005) found a similar relationship in their comparison of small-scale DE test results: SFT< EXDET <BFT=WSL. Numerous other studies have compared small scale test results from different test apparatus and have directly measured the energy present in the various test apparatus. The issue of wave energy and dispersant effectiveness is universal to both warm and cold climate conditions and a complete discussion of this issue is beyond the scope of this review. The issue is mentioned only to highlight the fact that results from one set of tests using one apparatus and test method may not give the same results or trends as those from another method. This may be one of the reasons for the somewhat inconsistent results that have been reported for dispersant effectiveness as a function of temperature and salinity discussed above. Of more importance to this document is the effect that the presence of ice has on the level of energy available for the chemical dispersion of oil. The attenuation of waves and surface energy by floating ice is a well-known phenomenon and the higher the ice concentration the more the waves are dampened. The influence of ice on the dispersion process is discussed under a separate heading below.

5.9 Effect of Ice on Dispersant Effectiveness

There have been a limited number of dispersant effectiveness studies where ice has been present during the test and even fewer where Alaskan oils have been tested. Dispersant effectiveness in ice was studied by Mackay et al. 1979; Cox and Shultz 1981; Byford et al. 1983; Brown et al. 1985; and Brown and Goodman 1996, SL Ross 2002, Owens 2004, Spring et al 2006, Sorstrom et al 2010. The work here ranged from experiments in small containers, to medium sized-tank experiments, to experiments at a large wave basin in Calgary, at the Ohmsett facility and in one field trial. Of these studies only those by Owens and SL Ross used Alaskan oils.

In the small-scale tests conducted by Cox and Shultz and Byford the presence of ice with a surface coverage of between 20 and 50% generally increased the chemical dispersion of the oil. This was speculated to be due to the result of the pumping action of the broken ice pieces. Brown et al 1985 also measured higher dispersion rates than achieved in similar open water conditions in tests where both slush and broken ice were present. Tests in the Ohmsett facility (SL Ross 2002) with fresh and weathered (10% evaporative loss) ANS crude conducted in 80% ice cover resulted in greater than 95% dispersion. The measured dispersant effectiveness (DE) values for ANS weathered to 20% loss were 54% in 8/10th ice, 30% in 4/10th ice and 22% in open water.

All of these tests were conducted in non-breaking wave, low energy swells. Chemical dispersion was enhanced by the presence of ice in these tests. The increased dispersion over open water conditions was attributed to the pumping action or turbulence caused by the movement of the ice pieces in the swell. Owens et al (2004) reported similar improvement over open water conditions in the chemical dispersion of ANS crude oil when brash ice was present in 4/10 and 8/10 coverage.

The presence of ice has been shown to improve the chemical dispersion of oil in low energy conditions by a number of studies. Successful dispersion of fresh and evaporated ANS in ice appears to be possible in lower wave energies than would normally disperse oil in open water conditions due to the additional turbulence caused by the moving ice. Some movement of the broken ice is necessary to generate the dispersion.

Ship propeller wash and water jets have been shown to be effective in dispersing oil that has been treated with dispersant in a broken ice system in the absence of any natural mixing. This was demonstrated in meso-scale tests and in a wave basin (Nedwed et al 2007, Spring et al 2006) and in the field (Sorstrom et al 2010). Oil treated by dispersant in a solid or 80+% ice cover was effectively dispersed in all of these tests using ice-breaking, ship propeller wash and water jets. In all cases the droplet sizes generated were very small and the droplets remained suspended in the water for long periods.

Table 5-2 **Chemical Dispersant Research on Alaskan Oil: Summary Table**

Research Paper	Oil	Dispersant	Test Method[1]	Parameters Varied in Tests[2]			
				Temp	Salinity	Energy	Ice
Abbasova, A. et al. 2005	Chirag crude oil (Caspian Sea)	Finasol OSR51, Superdispersant 25, Corexit EC 9527a, Corexit EC 9500a, Slickgone NS, Inipol IP90	BF	X	X	X	O
Anderson, J.W., D.L. McQuerry, S.L. Kiesser. 1985.	Alaska North Slope	14 dispersants	MNS	X	X	X	O
Belore, R. 2003.	fresh and weathered Hibernia and ANS crude oils	Corexit 9500 and 9527	Ohmsett wave tank	X	X	X	O
Belore, R., S. Ross. 2000	Alaskan North Slope crude	Corexit 9500 and 9527	SLR wave tank	X	X	X	O
Blondina, G., M Singer, I. Lee, M. Ouano, M. Hodgins, R. Tjeerdema. 1999.	Prudhoe bay crude plus various others	9500 & 9527	SF	X	√	X	O
Brandvik, P.J., 0.0. Knudsen, M.O. Moldestad and P.S. Dating, 1995.	Norwegian Oils Only	Inipol IPF, Inipol PC, Enersperse 700, Finasol OSR-52, Dasic Fresh Water, 9500 & 9527 + others	IFP	X	√	X	O
Brown, H.M., D.K.Weiss, R.H. Goodman. 1990	North Slope & Drift River crude	Corexit 9550 or 9527	Esso Wave Tank	X	X	X	O
Brown et al. 1985	Federated crude (ASMB)	Corexit 9527 & CRX-8	Esso Wave Tank	X	X	X	X

ED_001324_00001497-00045

Research Paper	Oil	Dispersant	Test Method[1]	Parameters Varied in Tests[2]			
				Temp	Salinity	Energy	Ice
Brown, H.M., R.H. Goodman. 1987	ANS	9527, 9550, Enersperse 700	Esso Wave Tank	X	X	√	O
Brown and Goodman 1996.	Federated crude (ASMB)	Corexit 9500 and Corexit 9527	Esso Wave Tank	X	X	√	√
Byford, D., P. Green, A. Lewis. 1983	Lago Medio, North Slope Crude, Medium Fuel Oil	Corexit 9527, Corexit 9550 BP1100WD, Fiansol OSR5, Dispolene 34S, Arcochem D609	WSL, BP Wave Tank	√	√	X	X
Chandrasekar, S. G.A., Sorial, J.W. Weaver. 2003.	Prudhoe Bay South Louisiana crude and #2 Fuel oil	Corexit 9500 and Dispersit-SPC 1000	BF	√	X	√	O
Chandrasekar, S.; Sorial, G.A.; Weaver, J.W. 2006.	Alaskan Prudhoe Bay, South Louisiana crude oil, No. 2 Fuel Oil (fresh & weathered)	Corexit 9500 and Dispersit-SPC 1000	BF	√	√	√	O
Cox, J., L. Shultz 1981	Sadlerochit crude fresh, 10 and 30% weatehred, 10% weathered Arctic diesel, 10% weathered Kuparuk crude	X, Y, Z	MNS, Hoop Tank	X	X	√	√
Fingas, M., B. Fieldhouse, Z. Wang. 2006.	ANS	Corexit 9500 and 9527	SF	√	√	X	O
Fingas, M., B.Fieldhouse, Z.Wang. 2005b	ASMB	Corexit 9500	SF	√	√	X	O

ED_001324_00001497-00046

Research Paper	Oil	Dispersant	Test Method[1]	Parameters Varied in Tests[2]			
				Temp	Salinity	Energy	Ice
Fingas, M., L. Ka'aihue. 2005a	Various including ANS	various	various	√	√	√	O
Fingas, M.F., I. Bier, M. Bobra and S. Callaghan. 1991	ASMB, Norman Wells, Adgo	Corexit 9527, Enersperse 700 and Citrikleen	SF	√	√	X	O
Fiocco, R.J., P.S. Daling, G. DeMarco, R.R. Lessard, 1999.	Alaska North Slope	Corexit 9500	MNS, WSL	X	X	√	O
George-Ares, A., R.R. Lessard, K.W. Becker, G.P. Canevari and R.J. Fiocco,	ANS + 3 other non-Alaskan oils	Corexit 9500, calcium chloride modified 9500, Dasic freshwater, Enersperse 1037, Inipol IPF	EXDET	X	√	X	O
Lewis, A.; Crosbie, A.; Davies, L.; Lunel, T. 1998.	Forties Blend, Alaskan North Slope crude, and IFO-180 fuel oil	Corexit 9500 and Dasic Slickgone NS	Field test	X	X	√	O
Li, Z., K. Lee, T. King, M. Boufadel, A. Venosa. 2009.	ANS and Mesa crude	Corexit 9500 & Dispersit-SPC 1000	EPA / DFO wave tank	X	X	√	O
Mackay, D. , R. Mascarenhas, K. Hossain, T. McGee. 1979	Alberta Mixed Blend (ASMB), Lago Medio	9527	MNS, Hoop, CSSA	√	X	√	X
Mackay, D. 1995.	Alaskan North Slope crude	Corexit 9527	MNS, WSL, Exdet,Esso Wave Tank	√	√	√	O
Mackay, D., Chau, Y. Poon. 1986	Prudhoe Bay Crude and various others	9527, 9550, BPMA700	MNS, Hoop, RF,Bobra	√	√	√	O

ED_001324_00001497-00047

Research Paper	Oil	Dispersant	Test Method[1]	Parameters Varied in Tests[2]			
				Temp	Salinity	Energy	Ice
McAuliffe, C.D. et al. 1981.	Alaskan Prudhoe Bay crude oil	Code Named H & J	Field	X	X	√	O
Moles, A., L. Holland, J. Short. 2002	ANS	9500 & 9527	SF	√	√	X	O
Moles, A.L. Holland, J. Short, 2001	ANS	9500 & 9527	SF	√	√	X	O
Nagarajan, K., N. Deshpande, G. Sorial, J. Weaver. 2008.	Alaskan Prudhoe Bay, South Louisiana crude oil, No. 2 Fuel Oil (fresh & weathered)	Corexit 9500 (A) & Dispersit-SPC 1000 (B)	BF	√	√	√	O
Nedwed, T. , R. Belore, W. Spring, D. Blanchet. 2007	Chayvo Z6	Corexit 9527	SL Ross Wave Tank, AKER Ice Basin (prop wash)	X	X	√	√
Owens, C.K.R. Belore, 2004.	Alaska North Slope, Hibernia, Chayvo	Corexit 9527	Ohmsett	X	X	√	√
Payne, J.R. et al. 1985	Alaskan Prudhoe Bay crude oil	Corexit 9550, Finasol OSR-7, EC. O ATLANT'TOL AT-7, and OFC D-609	Old EPA protocol	√	√	X	O
SL Ross, MAR Inc. 2003	ANS, Endicott, Northstar, Middle Ground Shoal & Pt. McIntyre (fresh and weathered)	Corexit 9527	SLR Wave Tank & Ohmsett	X	X	√	O
SL Ross, MAR Inc. 2006	ANS, Endicott, Northstar & Pt. McIntyre (fresh and weathered)	Corexit 9527	SLR Wave Tank & Ohmsett	X	X	√	O

ED_001324_00001497-00048

Research Paper	Oil	Dispersant	Test Method[1]	Parameters Varied in Tests[2]			
				Temp	Salinity	Energy	Ice
SL Ross, MAR Inc. 2009	ANS, Endicott, Oseberg, Rock, Ewing Bank, IFO 30	Corexit 9500	SLR Wave Tank & Ohmsett	X	X	√	O
SL Ross. 2002.	Alaska North Slope and Hibernia (fresh and weathered)	Corexit 9500 & 9527	Ohmsett	X	X	√	√
SL Ross. 2006.	fresh, air sparged and emulsions of Endicott, IFO 30, IFO 120 and Sockeye crude	Corexit 9500 & 9527	SLR Wave Tank & Ohmsett	X	X	√	O
SL Ross. 2010. (lit review)	Various including ANS	various	various	X	√	X	O
SL Ross. 2011	Endicott, ANS plus 12 other crude oils	Corexit 9500	Ohmsett, WSL, BF,SF,Exdet	X	X	√	O
Smith, D., G. Holliday. 1979	ANS	H & J	Field tests	X	X	√	O
Spring, W, T. Nedwed, R. Belore.2006	Chayvo Z6	Corexit 9527	SL Ross wave tank & AKER wave basin (prop wash)	X	X	√	√
Sorial, G.A.K.M. Karen, H. Edith, A.D. Venosa, D.W. King. 2001.	Alaskan Prudhoe Bay and South Louisiana crude	18 Different Products	SFT & BF	X	X	√	O
Sørstrøm, S.E., Per Johan Brandvik, I. Buist, P. Daling, D. Dickins, L. Faksness, S.Potter, J. Rasmussen and I. Singsaas.2010.	Troll B crude	Corexit 9500	Field test	X	X	√	√

ED_001324_00001497-00049

Research Paper	Oil	Dispersant	Test Method[1]	Parameters Varied in Tests[2]			
				Temp	Salinity	Energy	Ice
Strom-Kristiansen, T., P.S. Daling, A. Lewis, A.B. Nordvik. 1994.	ANS plus Brent Blend, Murban and Bonny Light	9527, 9554, Enersperse 700,Several others	MNS, IFP, WSL	X	X	√	O
Venosa, A.D.G.A. Sorial, D.W. King. 2001	ANS and South Louisiana Crude	Six – test results not tied to dispersant brand	BFT	X	X	X	O
Venosa, A., K. Lee, M. Boufadel, Z. Li, E. Wickley-Olsen, T. King, 2008	ANS & Mesa light	Corexit 9500 and Dispersit SPC 1000	EPA/DFO wave tank	X	X	√	O
White, D.M.; Ask, I.; Behr-Andres, C. 1999.	Alaska North Slope	Corexit 9500	Modified SFT & EXDET	X	X	√	O
Wrenn, B., A. Virkus, B. Mukherjee, A. Venosa. 2009.	Mars crude	Various experimental formulations	BF	X	X	X	O

[1]RF- Rotating Flask, MNS- Mackay Nadeau Steelman test, Bobra- double tube test, SFT- Swirling Flask, BFT- EPA Baffled Flask Test, IFP- French dilution tests, WSL-Warren Spring Laboratory rotating flask, EXDET- ExxonMobil Dispersant Effectiveness Test

[2]O – parameter not tested, √ parameter varied in test, X parameter not varied.

ED_001324_00001497-00050

6 Identification of Knowledge Gaps

The results of the review of past dispersant effectiveness testing on Alaskan oils (section 5) and the environmental conditions in the US Chukchi and Beaufort Seas relevant to chemical dispersion (chapter 4) are discussed in this section with the goal of identifying gaps in our understanding of the potential effectiveness of dispersants in these regions.

6.1 Water temperature

The dispersibility of a crude oil is a strong function of its viscosity, which in turn is often very temperature dependent. The surface water temperatures in the Chukchi and Beaufort Seas in the open water seasons can vary from close to freezing (-2 °C) to upwards of 14 °C with considerable spatial variability in both regions. As crude oils cool their viscosities increase and with increasing viscosities there is a potential for the reduction of dispersant effectiveness. If the water temperature is below the pour point of the oil and the oil is no longer fluid the oil will also not be amenable to chemical dispersion because the dispersant cannot easily mix with the oil. There is really no water temperature that will completely restrict the use of chemical dispersant. The main lesson here is that it is the viscosity of the oil at the water temperature in which it is spilled that is the key factor in deciding dispersant effectiveness and not the temperature per se. The oil viscosity – temperature relationship must be considered for each candidate oil before an evaluation of the potential for dispersant effectiveness can be made. From the past testing that has been conducted on Alaskan oils it appears that the Alaskan oils produced to date are generally dispersible at cold temperatures when fresh and slightly weathered but may not be readily dispersed when heavily weathered and emulsified. Additional cold water testing of the chemical dispersibility of specific Alaskan oils when weathered and emulsified may be useful to further define the time window of opportunity for the successful dispersion of these oils under typical conditions that could be encountered in the study region.

ED_001324_00001497-00051

6.2 Water Salinity

Most of the past DE tests on crude oils in general and Alaskan oils specifically have shown that dispersant designed for use in marine environments (30 to 35 ppt salinity) are considerably less effective when the water salinity falls below about 20 ppt or above 40 ppt. However, the effectiveness of a dispersant on both ANS crude and a Caspian crude oil in the baffled flask test (BFT) showed considerably higher dispersibility at low water salinities (10 to 15 ppt) than has been demonstrated in many other test programs. This improvement may be due to the higher energy level imparted in the BFT when compared to the SFT which was used in many of the other tests that investigated the dispersibility of ANS crude at different temperatures and salinities.

With the exception of isolated near shore fresh water outlets there is minimal variation (between 28 and 34 ppt), both temporally and spatially, in surface water salinity throughout the U.S. Chukchi and Beaufort Sea regions. This offshore salinity variation is well within the optimal effectiveness range for marine chemical dispersants.

Water salinities in the nearshore area of the Beaufort Sea can be considerably lower than the offshore areas for short periods of the year due to the influence of fresh water river outflows and ice melt. Fresh water from river outflow can form a 1 to 2 m deep lens under the landfast ice that may extend up to 25 km offshore from the major river mouths. The surface water salinities in these areas approach that of fresh water during the early ice breakup period and prior to the mixing of the upper and lower water layers by strong winds and waves. The presence of this near shore fresh water during this period could hinder the effectiveness of chemical dispersants. Additional work on the effectiveness of dispersants over a range of salinities under realistic breaking wave conditions may be warranted to further investigate their potential for these near shore fresh water outflow regions.

ED_001324_00001497-00052

6.3 Presence of Ice

There have been a limited number of dispersant effectiveness studies where ice has been present during the test and even fewer where Alaskan oils have been tested. The presence of ice has been shown to improve the chemical dispersion of oil in low energy conditions. Successful dispersion of fresh and evaporated ANS in ice appears to be possible in lower wave energies than would normally disperse oil in open water conditions due to the additional turbulence caused by the moving ice. Ship propeller wash and water jets have been shown to be effective in dispersing oil that has been treated with dispersant in a solid or 80+% ice cover in the absence of any natural mixing. In these cases the drop sizes generated were very small and the drops remained suspended in the water for long periods.

In the Chukchi Sea region open water periods where conventional aerial application methods could be used generally exist only in the months from June through November. Open water is most common in the months of July through October in this region but the shoulder months of June and November may present dispersant application opportunities depending on the ice year. In the Beaufort Sea region the maximum likely extent for open water dispersant use is from July to October with the more likely season being from August to October. The remainder of the year will see short transition periods from open water to complete (90+ %) ice cover which will affect conventional dispersant use. In ice conditions with less than 3/10ths cover chemical dispersants will perform much the same as in open water conditions since the surface mixing energy is not overly dampened by the presence of the ice. The presence of greater than 9/10ths ice either encapsulates the oil making it impossible to target with aerial applied dispersant or dampens the surface energy such that there is insufficient energy to break the oil into droplets once treated The performance of dispersants in 3/10ths to 8/10ths ice cover without additional mixing energy applied is somewhat uncertain and requires additional study. Additional research on the potential for dispersant use under partial ice cover conditions is currently underway (OGP 2014, BSEE 2014).

ED_001324_00001497-00053

6.4 Wave Energy

An assessment of hindcast historical wind data and a correlation between wind speeds and wave energy was used to identify the percentage of time that aerial dispersant operations could be viable in section 5, both from a spray application efficiency standpoint and the presence of sufficient wave energy for oil dispersion. Based on this assessment chemical dispersant use would be favorable for 80 to 90% of the time in the two regions. This does not account for other operational limitations such as visibility and daylight hours as this was out of the scope of the study. Based on this assessment wind speeds and wave heights in the region should not generally be an impediment to chemical dispersant use.

Mixing energy is of obvious importance to the dispersion of marine oil spills with more mixing being better. Oil slicks can be broken up into small droplets and disperse naturally under high energy conditions but much more rapid dispersion and dispersion under much less vigorous wave energies can be accomplished by applying chemical dispersants. The bench-scale, and meso-scale laboratory tests and outdoor wave tanks all impart a slightly different mixing energy to the oil-water-dispersant system and thus can result in different dispersant effectiveness estimates for a given oil, dispersant water salinity, temperature combination. A comparison of dispersant effectiveness estimates for the swirling flask test (SFT), baffled flask test (BFT), ExxonMobil dispersant effectiveness test (EXDET), the Warren Spring Laboratory test (WSL), and Ohmsett test tank ranks the energy for these tests into the following pattern: SFT<BFT=WSL<EXDET<Ohmsett. The issue is mentioned only to highlight the fact that results from one set of tests using one apparatus and test method may not give the same results or trends as those from another method. This may be one of the reasons for the somewhat inconsistent results that have been reported for dispersant effectiveness as a function of temperature and salinity discussed above. It is always preferable to test dispersants under the most realistic conditions possible but this is not always possible due to permitting requirements for field trials or costs associated with large tank tests. Additional DE testing on Alaskan oils using realistic breaking wave conditions and varying environmental conditions would be useful to solidify our understanding of the potential for chemical dispersants.

ED_001324_00001497-00054

6.5 Alaskan Oil Types

At the present time the only oils being produced and transported (by pipeline to shore) in the Beaufort and Chukchi Seas are Northstar and Endicott crude oils and are the only crude oils that could be spilled directly into Alaskan offshore waters from present production fields. No oils are presently being produced in Chukchi Sea waters. Alaska North Slope Crude (ANS), or Prudhoe Bay crude as it was named in early test programs, has been the Alaskan oil of choice for most of the dispersant effectiveness tests conducted on Alaskan oils. Only five of the test programs evaluated dispersant effectiveness on specific North Slope production crudes (Endicott, Northstar and Pt. McIntyre). These oils were used in projects that were conducted for MMS with oils sourced by them specifically for cold water dispersant effectiveness testing. These oils are possibly more representative of the type of crude that might be spilled in the Beaufort and Chukchi Seas. As new oils come into production in the waters of the Beaufort and Chukchi Seas they should be evaluated for their potential for chemical dispersion under the range of environmental conditions they might be spilled in.

6.6 Dispersant Types Tested

Most of the dispersant effectiveness tests on Alaskan oils identified in Table 5-2 used one or both of Corexit 9500 and Corexit 9527 in their testing. This is not surprising since these brands have been the most stockpiled products in North America and have been on the market for many years. More wide spread testing of additional National Contingency Plan Listed dispersants on Alaskan oils under realistic conditions would provide spill responders with important information concerning the potential use of other products in this environment.

7 Meso-Scale Tank Tests

After discussion with the a technical representative from BSEE, a suite of DE tests using the SL Ross wind-wave tank on four Alaskan crude oils with four water salinities were selected for additional study to shed more light on the effectiveness of marine dispersants over a range of water salinities under high energy breaking wave conditions.

ED_001324_00001497-00055

7.1 Oils and Dispersant Used in Test Program

Four Alaskan crude oils were used in the test program. The oils and their basic fresh and weathered oil physical properties are shown in Table 7-1. The second column in Table 7-1 shows the degree of weathering that each of the oils received to achieve the weathered oil properties shown. The densities and viscosities reported in Table 7-1 were measured by SL Ross during the course of the current project. The modeling constants used to estimate volume percent evaporation as a function of oil density shown in the last four columns of the table are from previous oil analyses conducted on the test oils (SL Ross, MAR, DF Dickins, 2008).

Table 7-1 Test Oil Properties

Oil Name	Degree of Weathering (% loss by mass)	Density (kg/m^3 @ 10 °C)		Viscosity (cP @ 10 °C & 100 s^{-1})			Evaporation Modeling Constants			
		Fresh	Weathered	Fresh	Weathered	Weathered & Emulsified (50% water content)	Standard density (kg/m^3)	Standard density temperature (°C)	Density Constant #1	Density Constant #2
Endicott	6.0	0.924	0.935	340	1030	2370 to 3380	920.8	288.7	59.0	0.7736
Kuparuk	13.1	0.923	0.951	124	970	2050 to 4400	920.5	288.7	169.9	0.585
Northstar	43.1	0.823	0.869	1	20	not applicable	820.7	288.7	104.3	0.804
ANS Blend	21.4	0.879	0.940	19	1230	4000 to 5000	869.3	288.7	163.2	0.635

7.2 Test Methods and Equipment

All tests were conducted in the SL Ross wave tank shown in Figure 7-1. This tank is 10 m long by 1.3 m wide by 1.2 m deep and is fitted with a computer controlled wave paddle and energy dissipating beach. The tank is fitted with a cooling coil that permits the maintenance of the water at temperatures down to near freezing conditions. The tank was operated with a 20 cm breaking wave (trough to crest) that was programmed to break in the oil slick every 30 seconds. Tests were run for 15 minutes. Oil was held within the wave breaking zone using an air bubble barrier. Dispersant effectiveness was quantified by collecting the surface oil remaining at the end of each

ED_001324_00001497-00056

test to determine the percentage of oil dispersed and by measuring the dispersed oil drop size distributions using a LISST particle size analyzer placed 40 cm below the water surface in the center of the bubble barrier containment zone.

Figure 7-1 SL Ross Wave Tank

For the fresh and weathered oil tests approximately 750 ml of oil was placed into a container, weighed, emptied onto the water surface inside the containment area and the empty container re-weighed to determine the mass of oil discharged. Dispersant was applied using an air pressure powered sprayer fitted with a Spraying Systems Company flat fan nozzle (800015) operated at 50 psi. The dispersant sprayer is shown in Figure 7-2. The quantity of dispersant needed to achieve a 1:20 dose rate (by mass) was loaded into the sprayer prior to application and the full quantity of dispersant was applied to the surface of the oil. This dose rate was used as it is the manufacturer's recommended dosage for general use. The dose rate was not changed in the study so comparisons of dispersant effectiveness with varying water salinities could be made with all other conditions kept constant. For the weathered and emulsified oil tests 500 ml of weathered oil was mixed with 500 ml of salt water of the same salinity as the water of the test being

ED_001324_00001497-00057

conducted. The oil and water were mixed for 8 minutes using the paint stirrer shown in Figure 7-3. This method for realistic emulsion generation was developed in a previous study for the US Department of the Interior (SL Ross 2005). The dispersant dose used in the emulsion tests was again 1:20 based on the total mass of the oil and water mixture spilled.

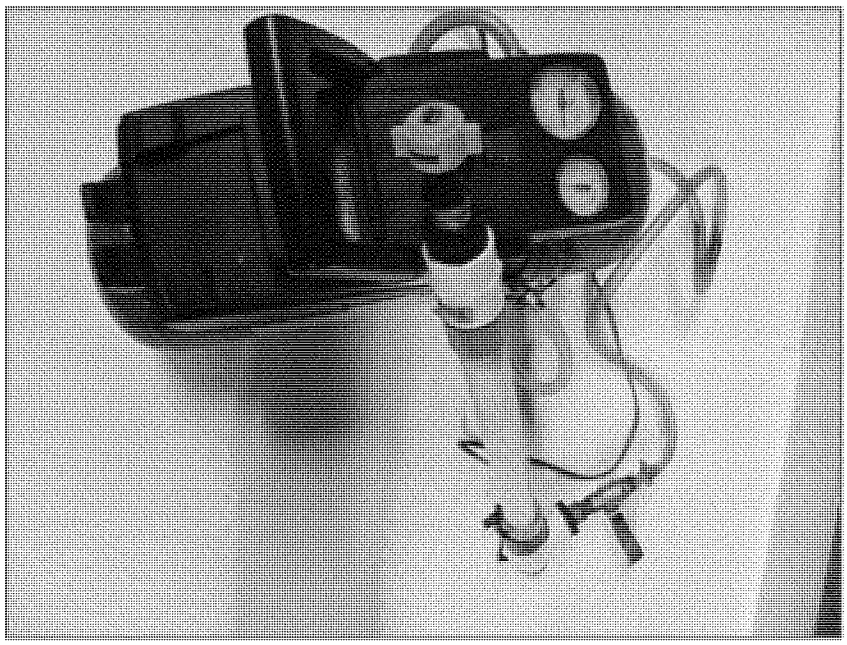


Figure 7-2 Dispersant Applicator

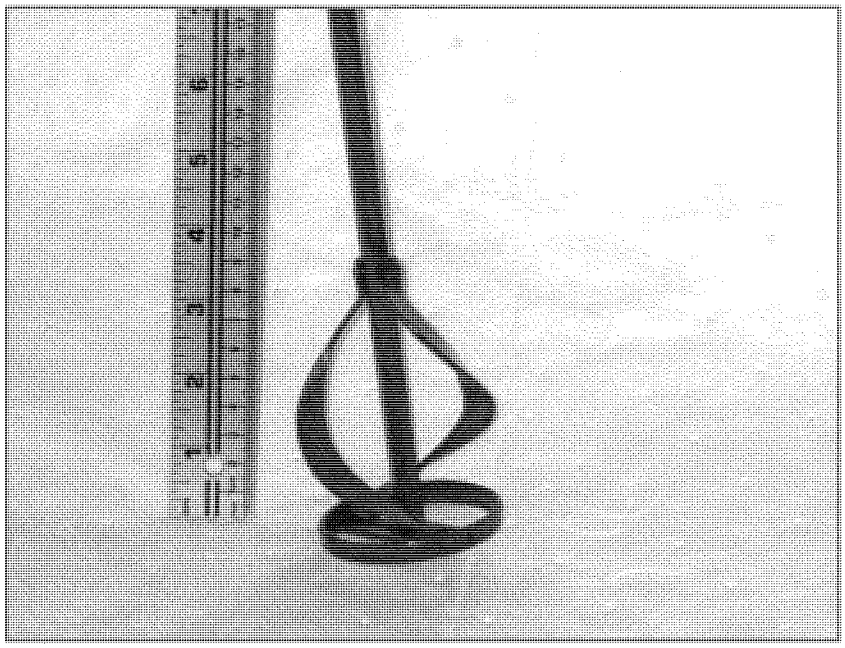


Figure 7-3 Emulsion Mixer

ED_001324_00001497-00058

7.3 Test Variables and Test Matrix

Corexit 9500 was used in all tests. To keep the test matrix at a manageable level only one dispersant to oil ratio was investigated for each type/weathered state of oil. A 1:20 DOR was used in all tests so a comparison of dispersant effectiveness as a function of water salinity could be made.

Alaska North Slope crude (provided by BSEE), Endicott, Northstar and Kuparuk crude oil (from SL Ross supply) were used in the testing. Tests were conducted on fresh, evaporated and evaporated plus emulsified crude oils (3 weathered states in total). Northstar does not form a stable emulsion so tests could not be conducted on emulsions of this oil. Emulsions were generated using the experience and techniques developed during earlier projects funded by the US Mineral Management Service that identified realistic water-in-oil emulsion making methods (SL Ross & A. Lewis, 2005). Tests were conducted in water with salinities of 5, 10, 20 and 30 ppt. All tests were conducted with a water temperature of 10 °C.

7.4 Results

7.4.1 Dispersant Effectiveness (DE) Estimates

The oil collected from within the containment zone at the end of each 15 minute SL Ross wave tank test was treated with a small amount of emulsion breaker (Alcopol) and placed in a heated bath and for several hours to facilitate removal of any water. The density of the oil was then measured to allow the estimation of oil loss through evaporation using the modeling constants provided in Table 7-1. This was important for the fresh oils and the lighter Northstar crude in particular. After accounting for evaporative losses the percent of oil lost to the water column, or a raw estimate of percent dispersed, without any adjustment for oil drop size, was calculated for each test. The dispersant effectiveness estimates presented in this section do not take into account the quality of the dispersion as measured by the oil drop size distribution in the dispersion. The results for the unadjusted dispersant effectiveness (DE_{raw}) estimates are presented in Figure 7-4 (Endicott), Figure 7-5 (Kuparuk), Figure 7-6 (Northstar) and Figure 7-7 (ANS). The DE_{raw} values reported in these figures reflect the oil lost from the surface of the containment area over the duration of the test. The complicated beaches in this test tank make it

ED_001324_00001497-00059

impractical to collect any oil that resurfaces outside of the containment zone at the end of the test. It is likely that some portion of the oil entrained by breaking waves during the test is in the form of large droplets that escape the bubble barrier system at depth, are only temporarily dispersed or entrained and then rise to the surface outside of the containment zone. This will not occur in all tests and the extent of this will be a direct function of the dispersed oil drop size distributions and depth of the dispersions generated. The DE_{raw} values reported in this section reflect the maximum possible DE that can be expected for the oil and water salinity combinations tested. A second dispersant effectiveness that accounts for the oil drop size distribution measured, and is a more realistic indication of dispersant effectiveness in an offshore setting, is presented in the following section.

No tests were completed with fresh water (zero water salinity). The DE values plotted for the zero water salinity values on these figures are the 'no dispersant applied' control test results for the oils. These control results are plotted to enable a rapid comparison of the untreated natural dispersion case to the dispersant applied tests over the range of salinities tested (5 to 30 ppt).

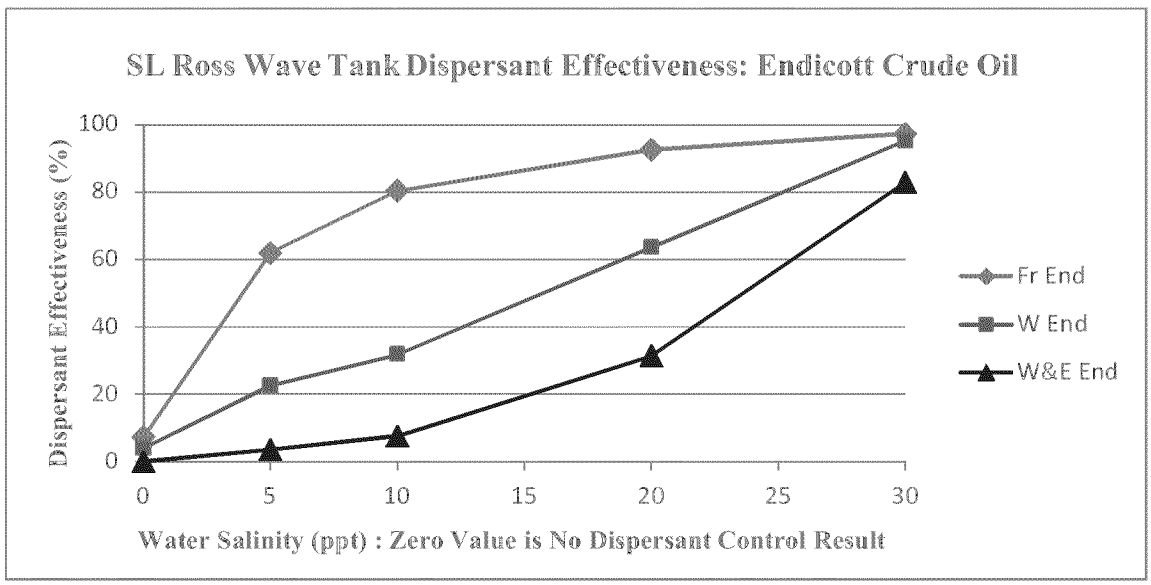


Figure 7-4 Dispersant Effectiveness versus Water Salinity: Endicott Crude Oil

ED_001324_00001497-00060

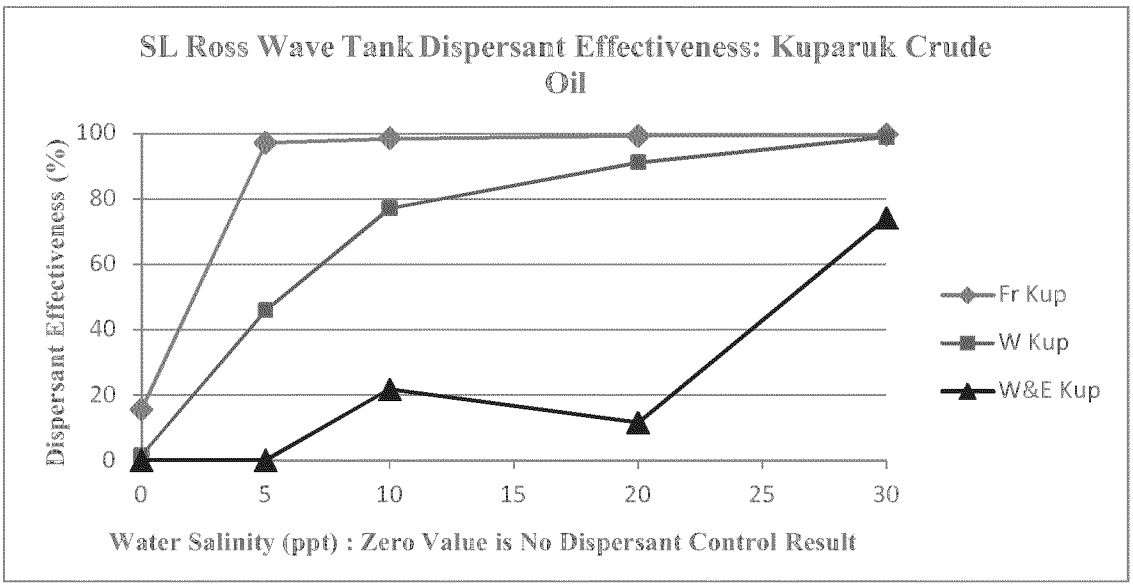


Figure 7-5 Dispersant Effectiveness versus Water Salinity: Kuparuk Crude Oil

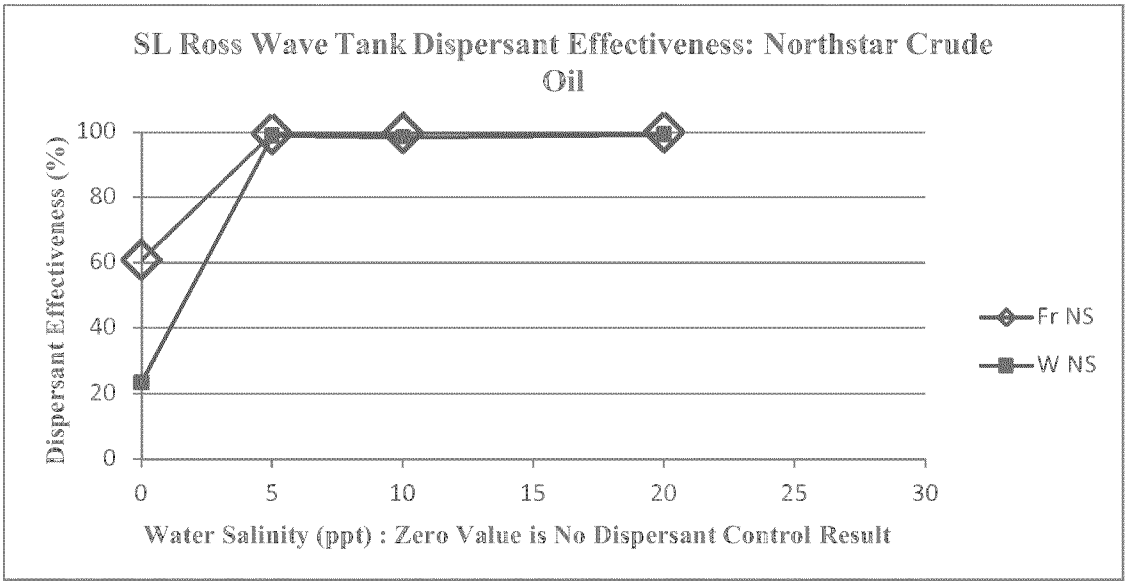


Figure 7-6 Dispersant Effectiveness versus Water Salinity: Northstar Crude Oil

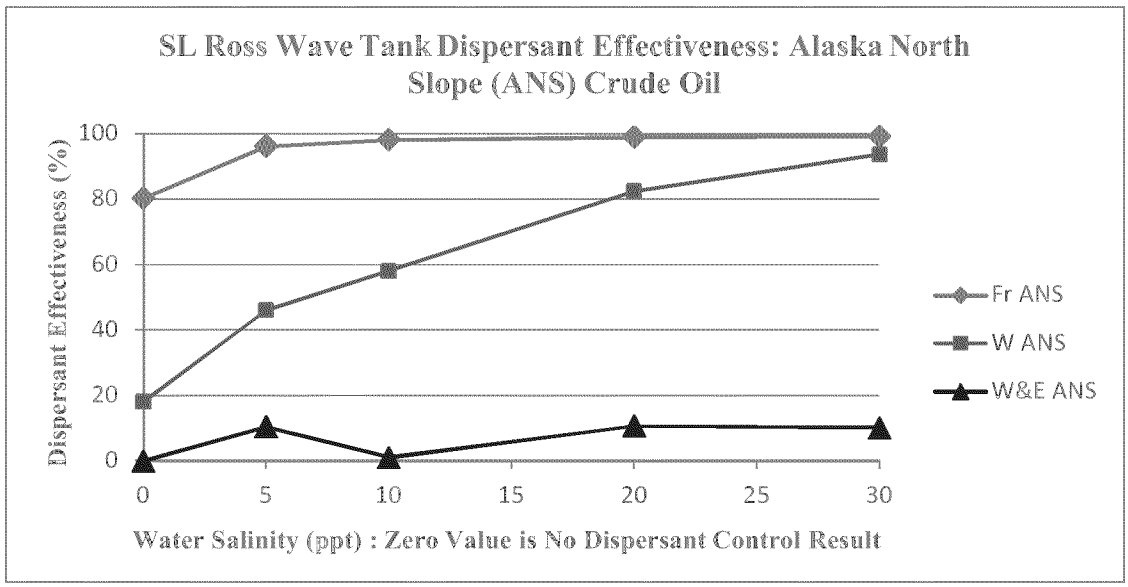


Figure 7-7 Dispersant Effectiveness versus Water Salinity: Alaska North Slope Crude Oil

The DE_{raw} values measured for the various oils tested showed consistent results. The fresh oils were more effectively dispersed than the weathered oils that were more effectively dispersed than the weathered and emulsified oils. The fresh and weathered Northstar crude oils were both completely dispersed at all water salinities (see Figure 7-6). With the exception of the Endicott crude oil, which has the highest fresh oil viscosity of those tested, the fresh oils registered high DE_{raw} (greater than 80%) at all water salinities tested. However, it is important to emphasize that the quality of the dispersions, as measured by the oil drop size distributions, generated varied over the range of water salinities tested and this issue is discussed in the next section. For three of the weathered oils the effectiveness of the dispersant increased as the water salinity increased. Significant dispersion (greater than 60%) of emulsified oil was achieved only for the highest water salinity (30 ppt) tests for the Endicott (Figure 7-4) and Kuparuk (Figure 7-5) oils. The emulsified ANS (Figure 7-7) did not disperse at any of the water salinities tested. Northstar crude did not form a stable water-in-oil emulsion.

About 80% of the fresh ANS (Figure 7-7) was lost to the water column in the control test (no dispersant applied); more than even the much lighter Northstar crude (Figure 7-6) where 60% was lost in the control test. The oil-water interfacial tension (o-w IFT) of the fresh ANS was measured to determine if the oil possibly contains production chemicals that might be affecting

the dispersion process. Chemicals are routinely added to crude oil streams during the production process and pipeline transportation to minimize emulsion formation, break water-in-oil emulsions and to improve the flow properties of the crude oil. The o-w IFT of the fresh ANS was measured to be approximately 13 dynes/cm^2. This is considerably lower than the o-w IFTs of the other Alaskan oils tested in this study which were 19.6 dynes/cm^2 for Kuparuk, 22.1 for Northstar and 27 for Endicott, and may explain the high natural dispersion of this oil when fresh. The less dense and much less viscous Northstar crude used in the testing has an o-w IFT of 22 dynes/cm^2. One would normally expect the less viscous Northstar crude oil to have a higher natural dispersion than the more viscous ANS but the natural dispersion for this oil was less than the ANS. This lower natural dispersion of the Northstar crude relative to the ANS can be attributed to Northstar's higher o-w IFT.

7.4.2 Dispersed Oil Drop Size Distributions

A LISST (Laser In-Situ Scattering and Transmissometry) device was deployed at 40 cm below the water surface during all of the tank tests. Figure 7-8 through Figure 7-15 show the results from these measurements. Two figures are provided for each of the test oils. All of the graphs show averaged data during elevated oil concentrations over the full test run. Only those data points where the oil concentration in the water column was 1.5 times the background oil concentration measured in the tank prior to the test are used in generating these averages. This ensures that only data during elevated oil concentrations are used in the analysis. The first figure in each set shows the average volume median diameter (VMD) of the oil drop size distributions. The second figure shows the average volume percent of the oil present in the water column in drops smaller than 70 microns in diameter. This value has been chosen because past research has shown that only oil in drops 70 microns and smaller are likely to be permanently dispersed in an ocean setting and those greater than 70 microns are likely to be temporarily entrained (Lunel, 1993). This study is the only comprehensive data set that we are aware of that documents the oil drop size distributions for chemically dispersed crude oils in an ocean setting.

The LISST device used in the testing can measure oil drops in the 5 to 500 micron range. Drops larger than this are not registered by the device. If a significant amount of oil is present in the

ED_001324_00001497-00063

water in drops larger than 500 microns then the oil drop distributions and oil concentrations measured by the LISST are not accurate. During each test the dispersed oil cloud was observed through the viewing window of the test tank and those tests where large oil drops were present (0.5 mm and larger) were identified. **Solid lines** and **enlarged data markers** in the following graphs denote where **reliable LISST data** was recorded, **dashed lines** indicate that the LISST data are **not reliable** due to the presence of large oil drops in the dispersion.

The dispersant effectiveness results presented in section 7.4.1 provide an indication of the percentage of oil removed from the water surface in the oil containment zone by breaking waves in the SL Ross dispersant effectiveness test tank. The results in this section provide additional information on the quality of the oil dispersions through the drop size distribution data. As discussed above it has been shown that drops greater than 70 mi6crons may only be temporarily entrained in certain ocean conditions so it is important to characterize the oil drop size distributions in the dispersions to determine how much of the dispersed oil is likely to remain dispersed. In Figure 7-8 and Figure 7-9 the oil drop size distributions for the fresh Endicott dispersions were reliable for all but the control (no dispersant applied) test. The oil VMD decreased linearly from about 190 microns at 5 ppt to about 110 microns at 30 ppt (Figure 7-8). The volume of drops with diameters less than 70 microns increased in a linear fashion from about 20 % at 5 ppt to 40 % at 30 ppt as seen in Figure 7-9. The LISST data for the weathered and weathered & emulsified tests with Endicott were valid only for the 30 ppt water tests. The trends in drop sizes from fresh to weathered to weathered & emulsified (W&E) oil are as would be expected. The fresh oil test had a smaller VMD and higher percentage of oil in drops less than 70 microns than were present in the weathered oil test and the weathered oil test had smaller drops and a higher percentage less than 70 microns than the W&E test. The oil drops in the Endicott fresh oil dispersion in 30 ppt salt water were the largest of the four fresh oils tested. How the drop size distributions reported in this section affect the estimates of ultimate dispersant effectiveness is presented in the following section 7.4.3.

ED_001324_00001497-00064

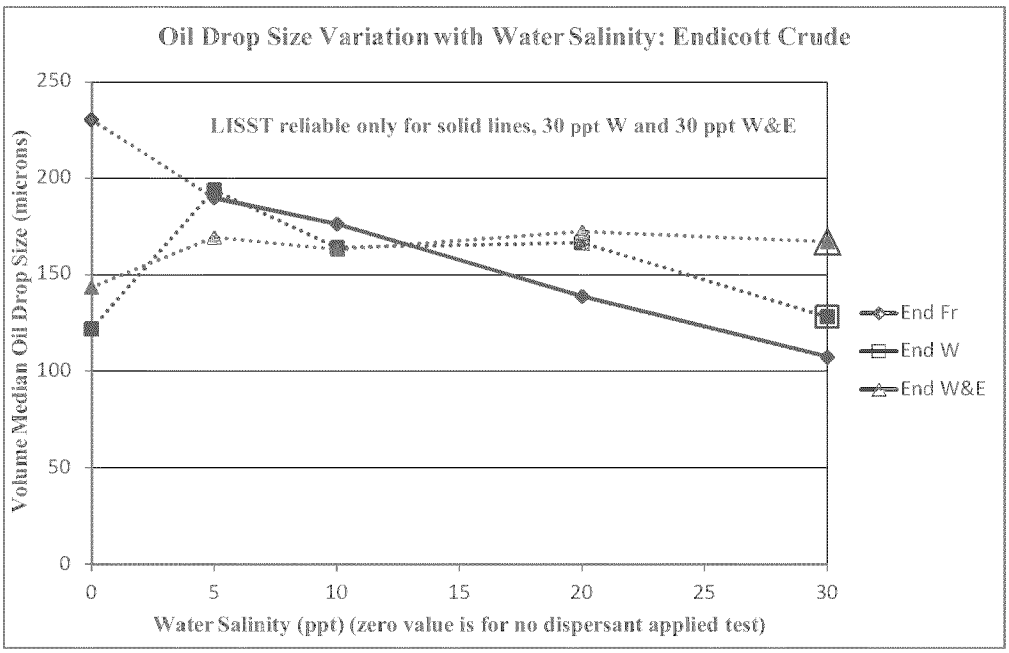


Figure 7-8 Oil Drop Size Variation Versus Water Salinity: Endicott Crude

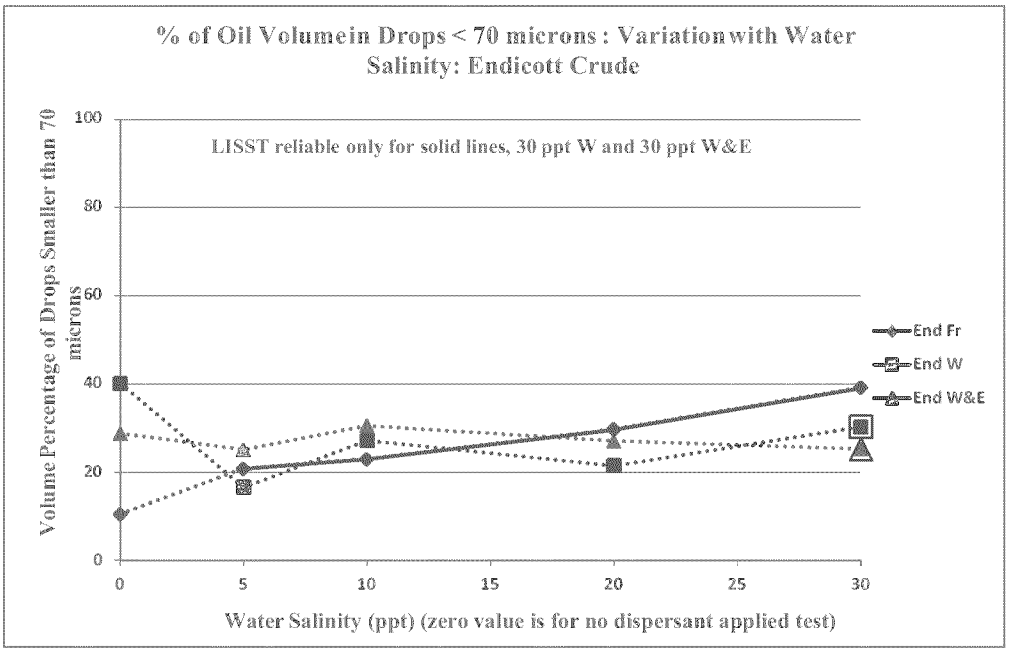


Figure 7-9 Volume Percent of Oil in Drops Less than 70 Microns: Endicott Crude

The LISST results for Kuparuk oil are summarized in Figure 7-10 and Figure 7-11. For both fresh and weathered Kuparuk crude the LISST data are reliable for both the 20 and 30 ppt water salinity tests. The oil drop VMDs for both the fresh and weathered Kuparuk were smaller than 70 microns for the 30 ppt salinity water as seen in Figure 7-10 and a high percentage of oil was

present in oil drops less than 70 microns in both cases (85% for the fresh oil and 68% for weathered oil, as seen in Figure 7-11). The VMD oil drop sizes were about 125 microns for both the fresh and weathered oil in the 20 ppt salinity water tests, considerably larger than in the 30 ppt tests (Figure 7-10). The W&E dispersion in 30 ppt salt water had a VMD of about 180 microns, and only about 25% of the dispersed oil was in drops less than 70 microns.

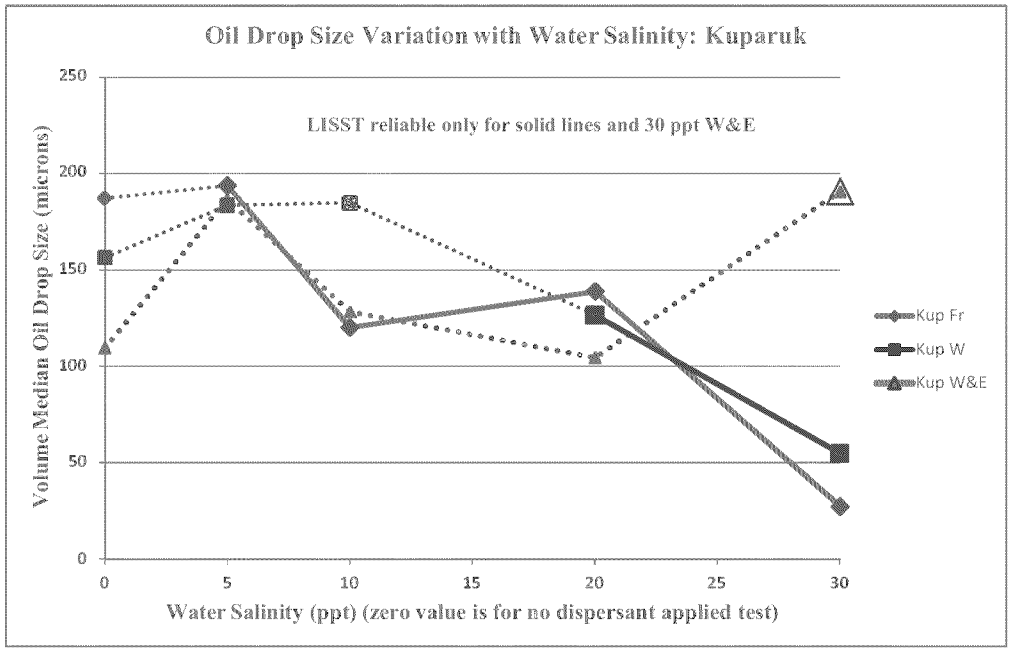


Figure 7-10 Oil Drop Size Variation Versus Water Salinity: Kuparuk Crude

ED_001324_00001497-00066

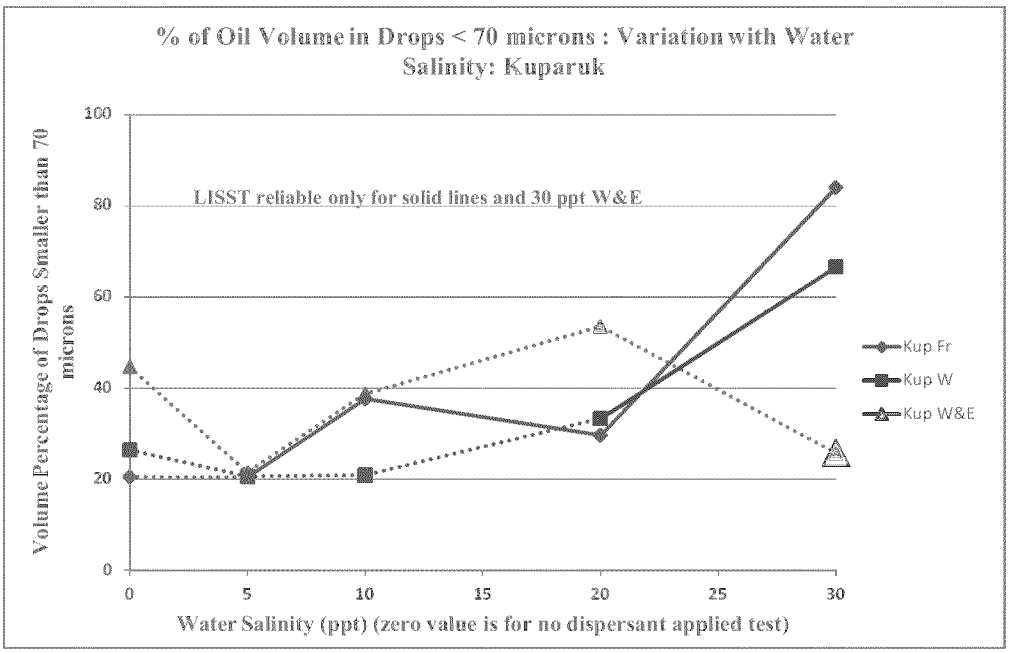


Figure 7-11 Volume Percent of Oil in Drops Less than 70 Microns: Kuparuk Crude

The LISST results for Northstar oil are summarized in Figure 7-12 and Figure 7-13. For Northstar crude the LISST data was reliable for all but the no dispersant applied control case. Tests were not conducted with Northstar crude oil at 30 ppt water salinity because the dispersant was 100% effective in dispersing the oil at 20 ppt and the dispersion generated in the 20 ppt water was a coffee colored dispersion which indicates a dispersion with a small oil drop size distribution. The oil drop VMDs for both the fresh and weathered Northstar were 70 microns or smaller for the 20 ppt salinity water as seen in Figure 7-12. About 95% of oil was present in oil drops less than 70 microns for the fresh oil and about 58% for weathered oil, as seen in Figure 7-13. The VMD oil drop sizes for the fresh oil test at 5 ppt and the weathered oil tests at 5 and 10 ppt were considerably larger (ranged between 150 and 180 microns as seen in Figure 7-12).

ED_001324_00001497-00067

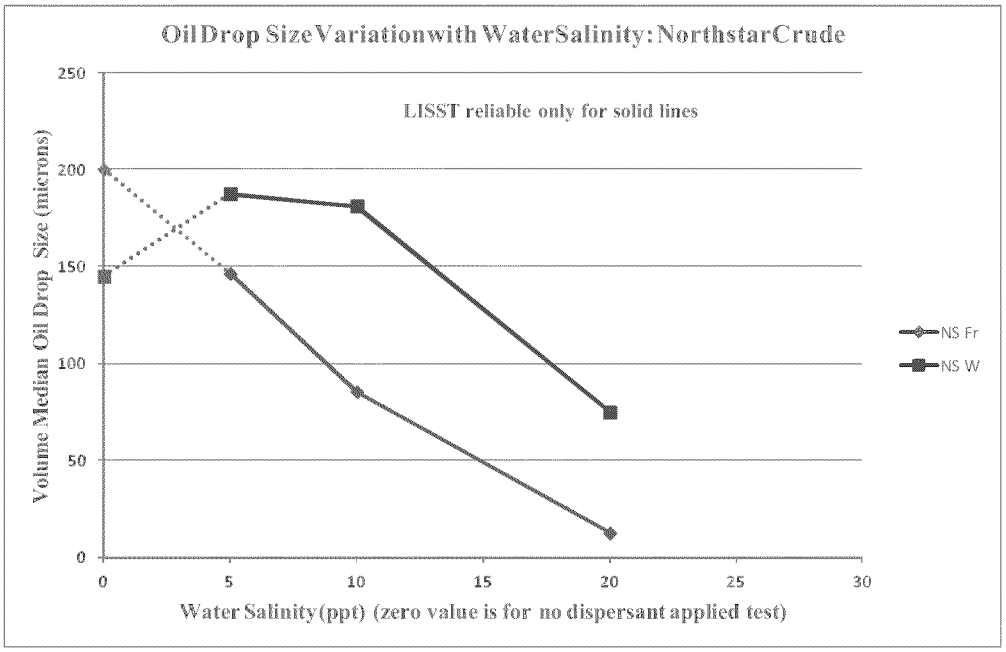


Figure 7-12 Oil Drop Size Variation Versus Water Salinity: Northstar Crude

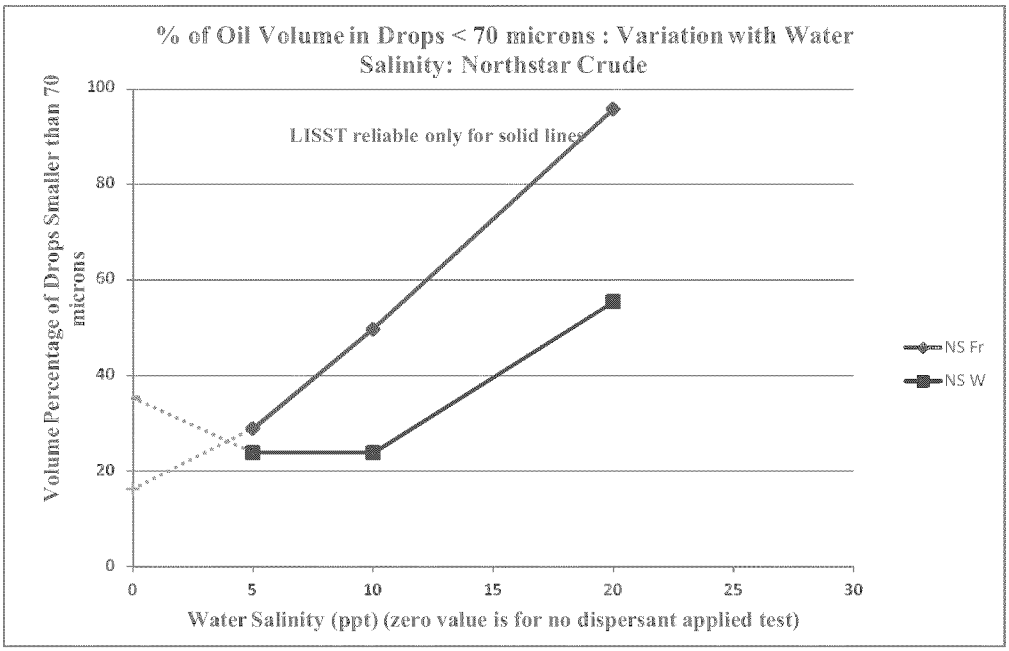


Figure 7-13 Volume Percent of Oil in Drops Less than 70 Microns: Northstar Crude

The LISST results for ANS oil are summarized in Figure 7-14 and Figure 7-15. For ANS crude the LISST data was reliable for the fresh oil tests at all salinities and at both the 20 and 30 ppt water salinity for the weathered oil. The LISST data was not reliable for any of the control tests (no dispersant applied) or for any of the W&E tests. The oil drop VMDs for the fresh oil were

about 25 microns and for the weathered ANS were about 125 microns for the 30 ppt salinity water as seen in Figure 7-14. About 90% of the oil was present in oil drops less than 70 microns for the fresh ANS test at 30 ppt but only 30 % for the weathered oil test (see Figure 7-15). In the 20 ppt salinity water tests the VMD oil drop sizes were about 60 microns in the fresh oil test and 160 microns with the weathered oil (Figure 7-14). In the 10 ppt water salinity tests for ANS the oil drop VMD increased to about 160 microns, similar to the weathered oil value at 20 ppt. The oil drop VMD increased to nearly 200 microns in the 5 ppt water salinity test.

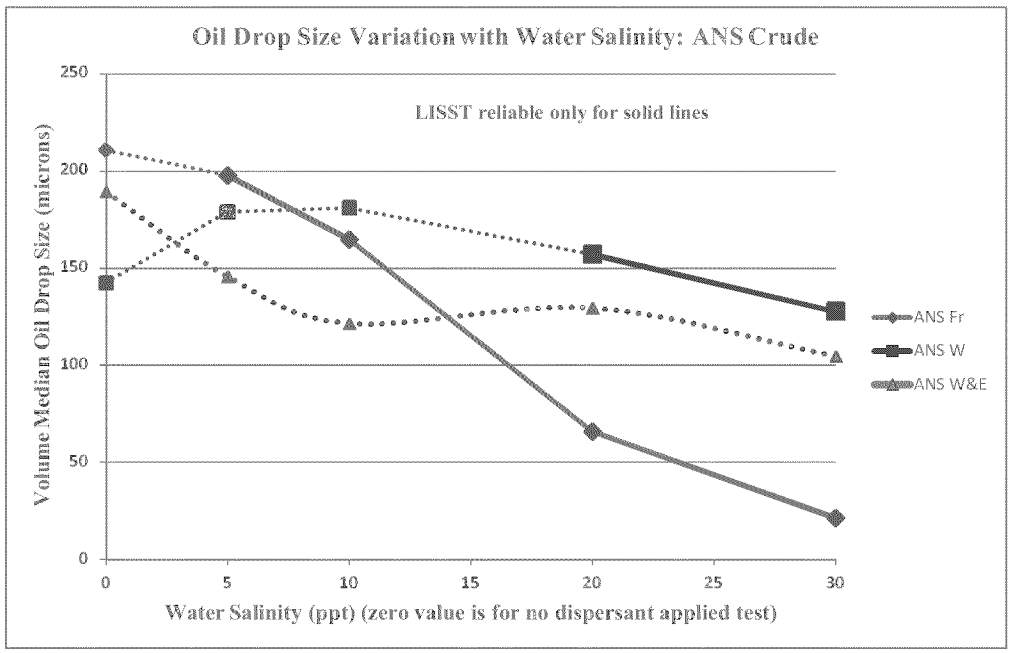


Figure 7-14 Oil Drop Size Variation Versus Water Salinity: ANS Crude

ED_001324_00001497-00069

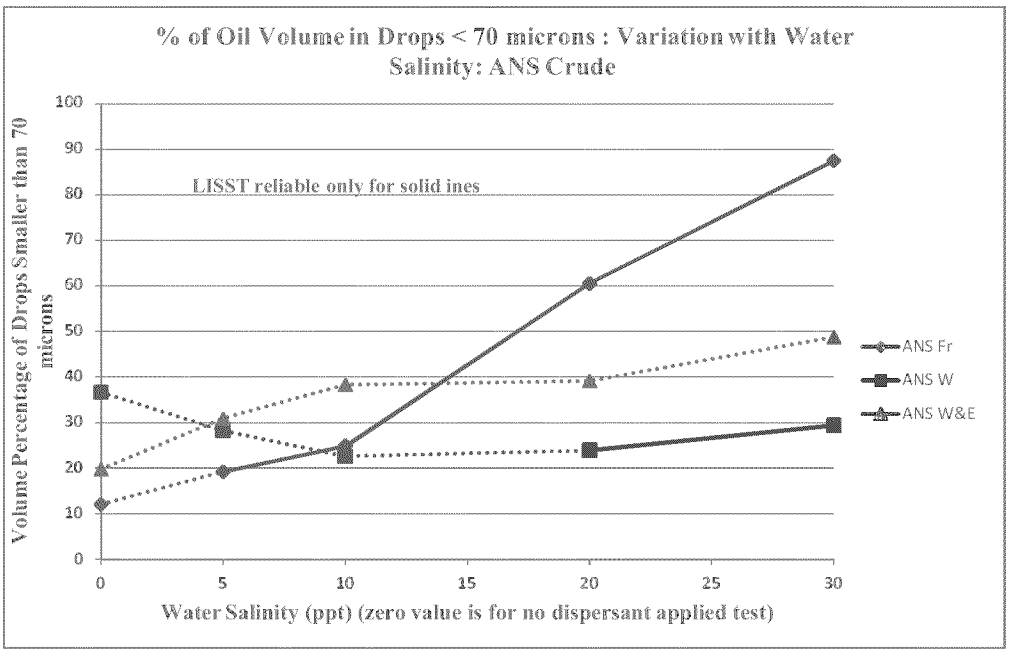


Figure 7-15 Volume Percent of Oil in Drops Less than 70 Microns: ANS Crude

7.4.3 Drop Size Adjusted Dispersant Effectiveness Estimates

The raw dispersant effectiveness (DE_{raw}) estimates presented in section 7.4.1 have been adjusted by multiplying the DE_{raw} values by the volume fraction of oil present in drops smaller than 70 microns. The 70 micron drop size value has been reported as being the maximum oil drop size that will remain dispersed based on field tests that utilized 7 different pre-mixed oil and dispersant combinations and wind conditions varying from 1.5 m/s to 7 m/s (Lunel, 1993). A second set of adjusted dispersant effectiveness results has been prepared using a 125 micron oil diameter cutoff to demonstrate the sensitivity of the estimate to the cutoff drop diameter. The 125 micron cutoff has been used based on additional accounts that have indicated that oil drops larger than 70 microns (100 microns) can remain dispersed (Neff 1990, Forrester 1971). It is possible that in heavier seas larger oil drops would be dispersed for long enough periods that even if they re-surfaced they would form sheens and not reform into thick slicks. The drop size data used to generate these adjusted DE values are strictly reliable only for the solid line and large open symbol data shown on these figures due to the upper limit of drop size detectability of the LISST device used to measure the drops, as was previously discussed. For those cases where the LISST data are not reliable (dashed lines) the adjusted DE estimates are likely higher than the

actual adjusted effectiveness would be because the LISST is not registering the presence of large oils drops which would result in a lower volume fraction of the oil being present in the small drop size categories.

The un-adjusted or raw DE graphs presented in 7.4.1 are duplicated in this section and shown on the same page as the adjusted DE graphs for easy comparison of the results. The 70 micron adjusted dispersant effectiveness (DE_{70}) values are generally lower than the raw DE values. The lower water salinity tests also generally had a greater reduction in DE when compared to the 30 ppt tests. Since many of the low salinity tests also had drop sizes outside of the range of the LISST device the adjusted DE values reported are also likely exaggerated. This would indicate that significantly larger oil drops than 70 microns are being removed from the containment zone in the SL Ross wave tank and that larger oil drops were generally formed as the water salinity decreased. When the oil drop size diameter cutoff for successful dispersion is relaxed to 125 microns the DE_{125} estimates generally increase by about 15 to 20% across all oils and water salinities but there was still a significant reduction in dispersant effectiveness compared to the unadjusted values.

The Endicott crude was the most difficult to disperse of the 4 oils. The adjusted DE_{70} of the fresh and weathered Endicott were only 40 and 30 %, respectively (see Figure 7-17). For the fresh Endicott there was a linear reduction in DE_{70} as the water salinity decreased. The DE_{70} in 5 ppt salt water was 18%.

For the Kuparuk crude the fresh and weathered DE_{70} values were about 85% and 65%, respectively (see Figure 7-20). The reduction in DE_{70} as the salinity decreased was again linear if the 20 ppt fresh oil value is treated as an outlier. The rate of decrease in effectiveness for Kuparuk was higher than for the Endicott. The DE_{70} in 5 ppt salt water was 20ppt, similar to the Endicott value. The weathered Kuparuk DE_{70} is slightly less than the fresh DE_{70} and reduced with declining salinity at a similar or slightly higher rate.

For the Northstar crude oil, the lightest product tested, the fresh oil DE_{70} at 20 ppt (the highest water salinity tested with this oil) was about 95%. The DE_{70} again dropped linearly, as the

ED_001324_00001497-00071

salinity decreased, to a value of about 30% in 5 ppt salt water (see Figure 7-23). The weathered Northstar DE_{70} was about 75% in 20 ppt water and dropped to 40% in both 10 and 5 ppt salt water.

The ANS DE_{70} for fresh oil in 30 ppt salt water was over 90% and again decreased linearly to about 20% in 5 ppt water (see Figure 7-26). The weathered ANS DE_{70} was only 50% in 30 ppt salt water and dropped to below 30% in 20 ppt water.

The highest DE_{70} recorded for any of the emulsified oils was 20% in 30 ppt salt water.

When the oil drop size diameter cutoff for successful dispersion is relaxed to 125 microns the DE_{125} estimates generally increase by about 15 to 20% across all oils and water salinities when compared to the DE_{70} values. This is strictly a result of an additional 15 to 20% of the oil in the dispersion being present in drops with sizes between 70 and 125 microns.

ED_001324_00001497-00072

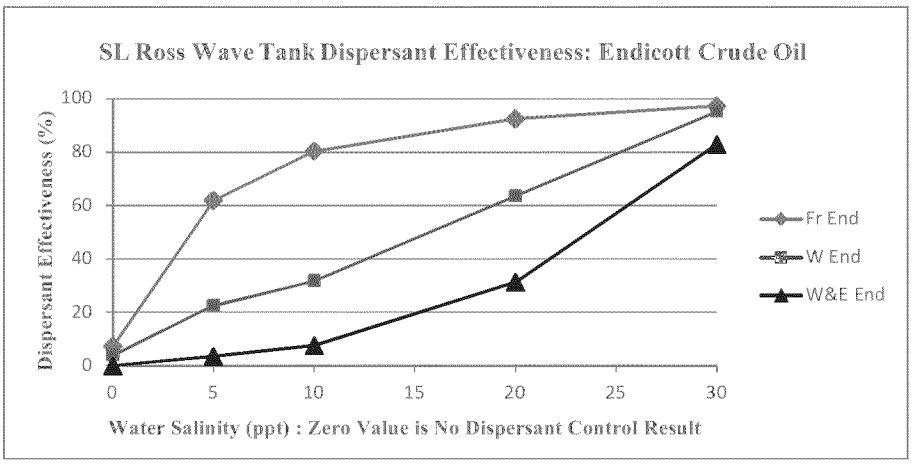


Figure 7-16 Dispersant Effectiveness versus Water Salinity: Endicott Crude Oil (copy of Figure 7-4)

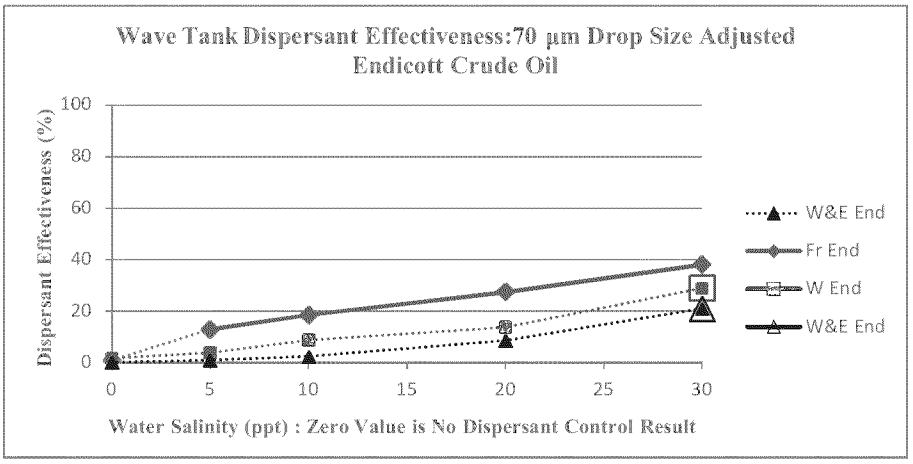


Figure 7-17 Drop Size Adjusted (70µm) Dispersant Effectiveness versus Water Salinity: Endicott Crude Oil

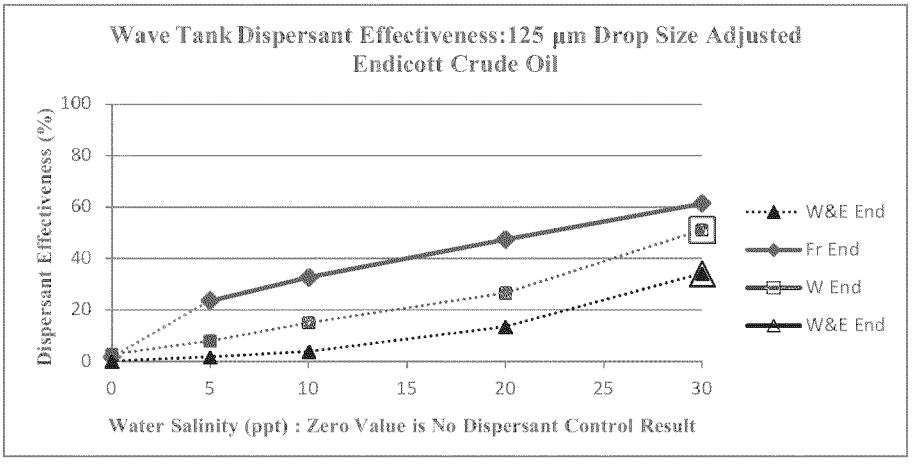


Figure 7-18 Drop Size Adjusted (125µm) Dispersant Effectiveness versus Water Salinity: Endicott Crude Oil

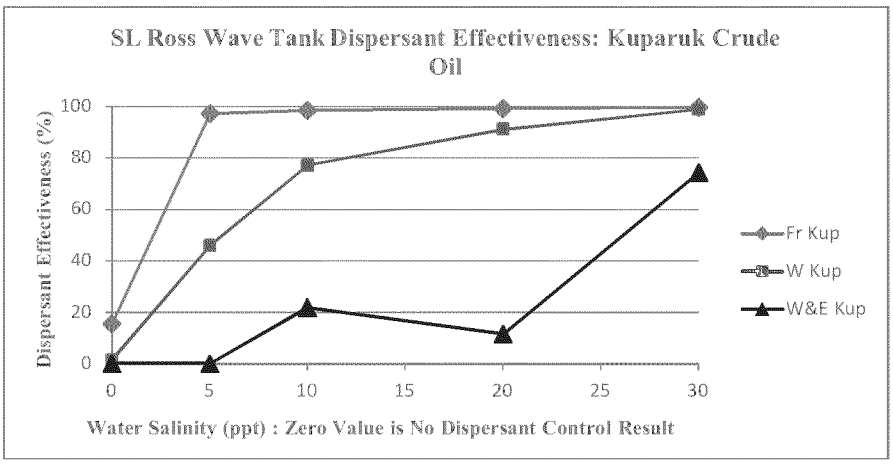


Figure 7-19 Dispersant Effectiveness versus Water Salinity: Kuparuk Crude Oil (copy of Figure 7-5)

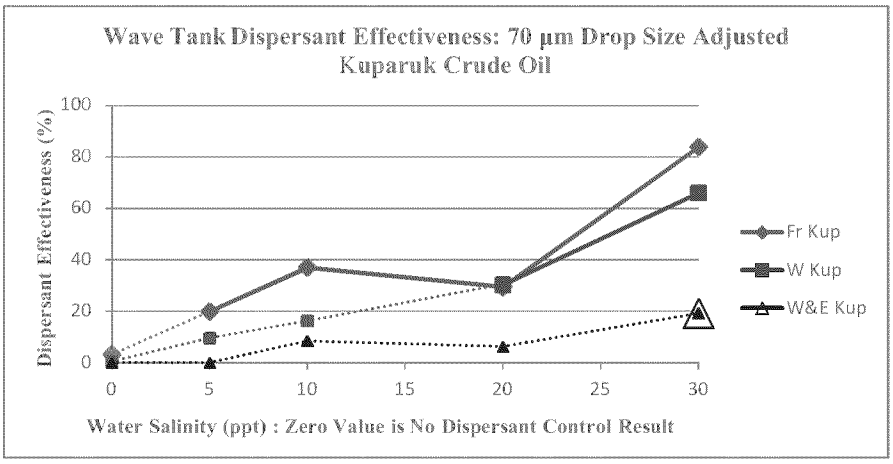


Figure 7-20 Drop Size Adjusted (70µm) Dispersant Effectiveness versus Water Salinity: Kuparuk Crude Oil

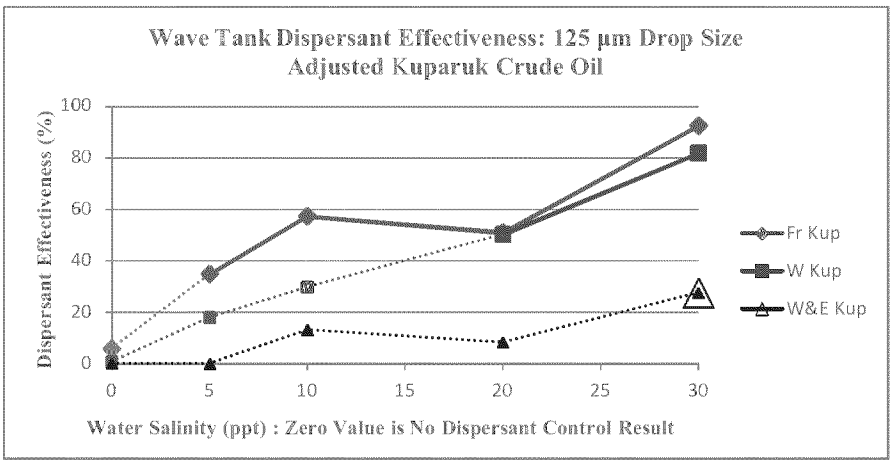


Figure 7-21 Drop Size Adjusted (125µm) Dispersant Effectiveness versus Water Salinity: Kuparuk Crude Oil

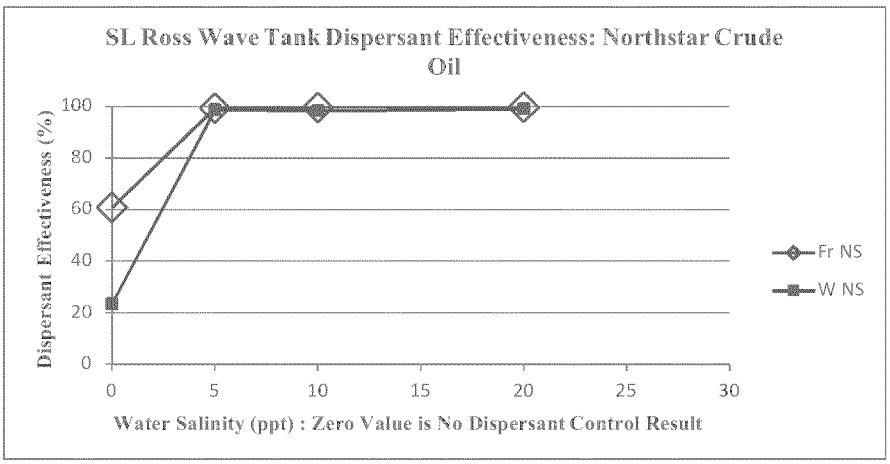


Figure 7-22 Dispersant Effectiveness versus Water Salinity: Northstar Crude Oil (copy of Figure 7-6)

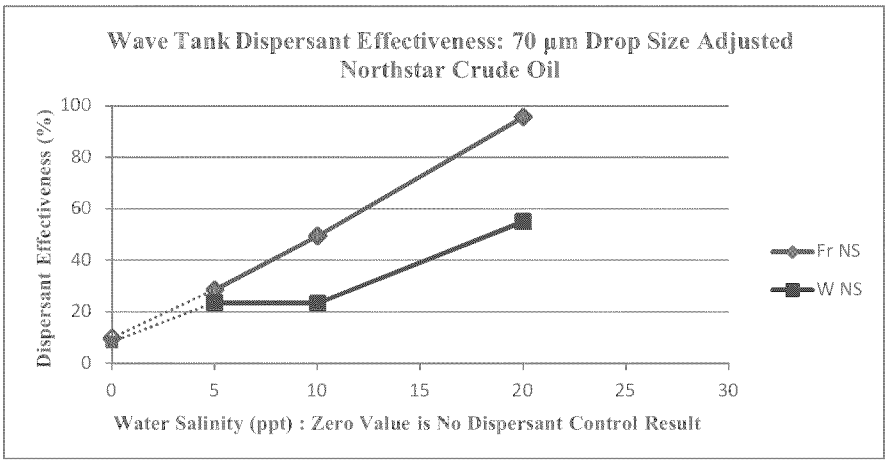


Figure 7-23 Drop Size Adjusted (70µm) Dispersant Effectiveness versus Water Salinity: Northstar Crude Oil

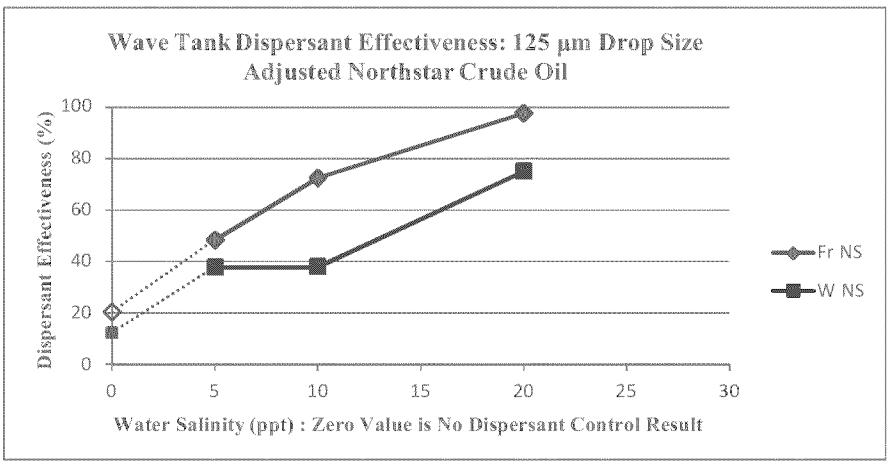


Figure 7-24 Drop Size Adjusted (125µm) Dispersant Effectiveness versus Water Salinity: Northstar Crude Oil

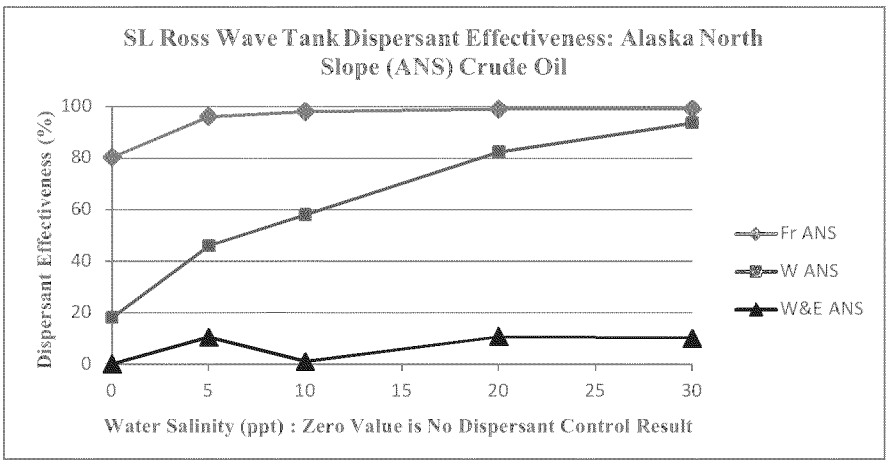


Figure 7-25 Dispersant Effectiveness versus Water Salinity: ANS Crude Oil (copy of Figure 7-7)

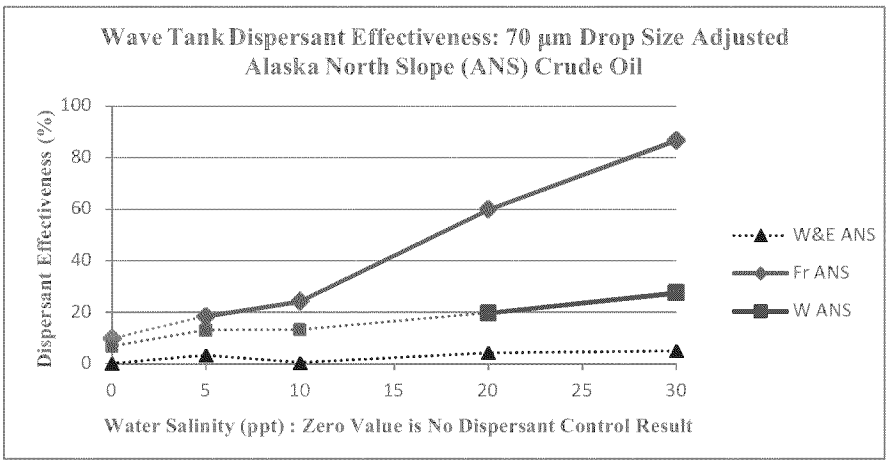


Figure 7-26 Drop Size Adjusted (70µm) Dispersant Effectiveness versus Water Salinity: ANS Crude Oil

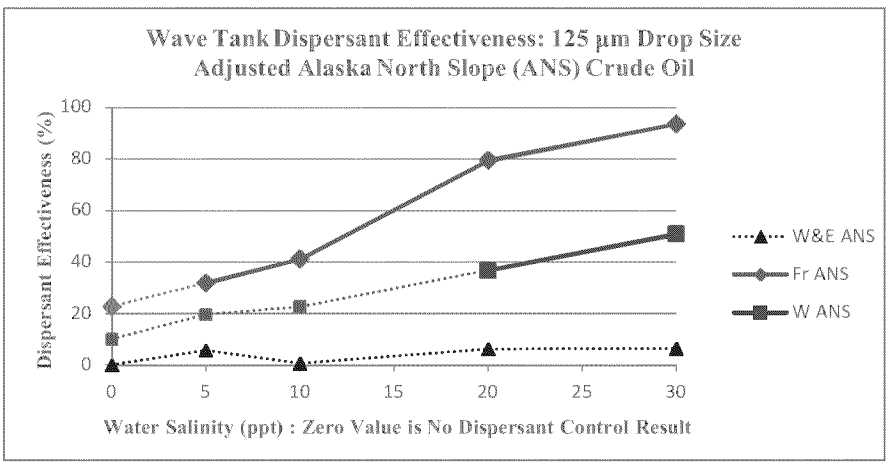


Figure 7-27 Drop Size Adjusted (125µm) Dispersant Effectiveness versus Water Salinity: ANS Crude Oil

7.4.4 SL Ross Tank Test Results Discussion

The dispersant had an effect on all of the oils at all water salinities (5 through 30 ppt) when compared to the no dispersant applied control case. The oil drops sizes in the dispersions increased as the salinity decreased demonstrating the reduced effect of the Corexit 9500 dispersant on the oils at lower water salinities.

Final dispersant effectiveness estimates (DE_{70}) were made by adjusting the quantity removed from the surface by the volume percent of oil present in drops less than 70 microns.

The most complete data sets collected (due to limitations of the LISST in measuring large oil drops) were for the fresh oil tests. The results for the fresh oils indicate that the DE_{70} values are highest for the 30 ppt water and in all cases drop linearly as the test water salinity decreased to 5 ppt. The Northstar crude was most easily dispersed with complete dispersion (> 95% DE_{70} in 20 ppt salt water), followed by fresh ANS (95% DE_{70}), fresh Kuparuk (85% DE_{70}) and Endicott (40% DE_{70}). The DE_{70} dropped linearly to between 15 to 25 % for the oils tested as the water salinity decreased to 5 ppt.

An alternate DE_{125} was also estimated using a 125 micron cutoff to demonstrate the sensitivity of the drop size cutoff for permanent dispersion on the final DE estimate. The 125 micron value was arbitrarily chosen as a significantly larger drop diameter to investigate the effect on the estimated DE. The DE_{125} estimates generally increase only by about 15 to 20% across all oils and water salinities when compared to the DE_{70} values. The same trends in DE with water salinity and oil type are present in the DE_{125} results.

The oil drops in some of the tests were visibly larger than the upper measurement limit of the LISST device and reliable drop size data could not be gathered for these tests. Development of an in-situ oil drop measurement system capable of cost-effectively measuring drop sizes in the 10 to 3000 micron range would improve the state-of-the art in oil dispersion monitoring both in large tank and field test conditions.

ED_001324_00001497-00077

The SL Ross wind wave tank was outfitted with a new computer controlled paddle and wave dampening beaches in 2013. This upgrade now permits the creation of breaking waves at a specific location and with a specific frequency and wave height. In the tank's previous configuration the wave generation was limited to a confused sea type of motion with no significant plunging wave activity. Dispersant effectiveness test results in the old wind-wave facility have correlated well with results collected in the Ohmsett wave tank (SL Ross 2003). This is the first extensive series of tests conducted in the new facility with the use of the plunging or breaking waves that have been described in Section 7.2. There was no visible loss of oil from the containment zone at the surface of the tank during the tests. The breaking waves did propel significant quantities of small and large oil drops into the upper 2/3 of the water depth when the oil was fresh and relatively non-viscous. In tests conducted with the old tank configuration large oil drops were not driven as deep into the water column and then re-surfaced more quickly than in the breaking wave environment. This provided less time for the sub-surface movement of the larger oil drops out of the containment zone. This is the likely reason for the high unadjusted dispersant effectiveness results in the tests where the oil drop sizes were significantly greater than 70 microns. Due to the presence of multiple beach elements in the tank it is not possible to collect oil surfacing outside of the bubble containment zone. Based on the experience gained in this test program we recommend that smaller breaking waves be used in future tests to minimize the depth of penetration of oil in each breaking event and minimize the loss of large dispersed oil drops from the containment region.

ED_001324_00001497-00078

8 References

Aagaard, K., C. H. Pease, S.A. Salo. 1988. Appendix A. Beaufort Sea Mesoscale Circulation Study– Preliminary Results. National U.S. Department of Commerce Oceanic and Atmospheric Administration Environmental Research Laboratories Pacific Marine Environmental Laboratory. Reprint of NOAA Technical Memorandum ERL PMEL-82.

Abbasova, A. et al. 2005. Evaluation of dispersants for use in the Azerbaijan region of the Caspian Sea. In 2005 International Oil Spill Conference; Prevention, Preparedness, Response, and Restoration: May 15-19, 2005, Miami Beach Convention Center, Miami Beach, Florida. Washington, D.C.: American Petroleum Institute. pp. 247-252.

Anderson, J.W., D.L. McQuerry, S.L. Kiesser. 1985. Laboratory evaluation of chemical dispersants for use on oil spills at sea. Environmental Science and Technology, 19:5, 454-457

Belore, R., S. Ross. 2000. Laboratory study to compare the effectiveness of chemical dispersants when applied dilute versus neat. In Proceedings of the Twenty-Third Arctic and Marine Oilspill Program Technical Seminar, June 14 to 16, 2000, Coast Plaza Suite Hotel, Vancouver, British Columbia, Canada. Ottawa, Ont.: Environment Canada. pp. 733-748.

Belore, R. 2003. Large wave tank dispersant effectiveness testing in cold water. In IOSC 2003 Prevention, Preparedness, Response and Restoration, Perspectives for a Cleaner Environment: April 6-11, 2003, Vancouver, British Columbia, Canada. Washington, D.C.: American Petroleum Institute. pp. 381-385. URL

Blondina, G., M Singer, I. Lee, M. Ouano, M. Hodgins, R. Tjeerdema. 1999. Influence of Salinity on Petroleum Accommodation by Dispersants. Spill Science and Technology Bulletin. Vol. 5 No. 2 pp 127-134. 1999.

Brandvik, P.J., 0.0. Knudsen, M.O. Moldestad and P.S. Dating, 1995. "Laboratory Testing of Dispersants Under Arctic Conditions", in The Use of Chemicals in Oil Spill Response, STP 1252, American Society for Testing and Materials, Philadelphia, PA, pp. 191-206, 1995.

Brown, H., R. Goodman. 1985. Dispersant Effectiveness in Cold Water. In Proceedings: Eighth Arctic and Marine Oilspill Program Technical Seminar, June 18-20, 1985, Edmonton, Alberta. Environment Canada. pp. 245-259.

Brown, H., R. Goodman. 1987. The Dispersion of Alaska North Slope oil in Wave basin tests. Report to Alaska Clean Seas. July , 1987 (ERCL.RS.87.29).

ED_001324_00001497-00079

Brown, H.M., D.K.Weiss, R.H. Goodman. 1990. Emulsion formation in dispersant-treated crude oil. In Proceedings: Thirteenth Arctic and Marine Oilspill Program Technical Seminar, June 6-8, 1990, Chateau Lacombe, Edmonton, Alberta. Environment Canada. pp. 255-264.

Brown, H., R. Goodman. 1996. The Use of Dispersants in Broken Ice. In Proceedings: Nineteenth Arctic and Marine Oilspill Program Technical Seminar, June 12-14, 1996, Calgary, Alberta. Environment Canada. pp. 453-460.

BSEE, 2014. Bureau of Safety and Environmental Enforcement. Effectiveness of Dispersants in Slush and Broken Ice. Contract E14PC00047. In progress.

Byford, D., P. Green, A. Lewis. 1983. Factors Influencing the Performance and Selection of Low-Temperature Dispersants. Proceeding of the 1983 Arctic and Marine Oil spill Conference. pp 140 -150.

Chandrasekar, S. G.A., Sorial, J.W. Weaver. 2003. Determining dispersant effectiveness data for a suite of environmental conditions. In IOSC 2003 Prevention, Preparedness, Response and Restoration, Perspectives for a Cleaner Environment: April 6-11, 2003, Vancouver, British Columbia, Canada. Washington, D.C.: American Petroleum Institute. pp. 331-334.

Chandrasekar, S., G.A. Sorial, J.W. Weaver. 2005. Dispersant effectiveness on three oils under various simulated environmental conditions. Environmental Engineering Science, 22:3, 324-336.

Chandrasekar, S., G.A. Sorial, J.W. Weaver. 2006. Dispersant effectiveness on oil spills – impact of salinity. ICES Journal of Marine Science, 63:8, 1418-1430. ISSN:1054-3139. DOI:10.1016/j.icesjms.2006.04.019

Clark, J., K. Becker, A. venosa, A. Lewis. 2005. Assessing Dispersant Effectiveness for heavy Fuel Oils Using Small-scale Laboratory Tests. Proceedings of the 2005 International Oil Spill Conference, Miami Beach, pp. 59-63, 2005

Cox, J., L. Schultz. 1981. Dispersant Effectiveness Under Arctic Conditions, Including Ice. In Proceedings: Fourth Arctic and Marine Oilspill Program Technical Seminar, June 16-18, 1981, Edmonton, Alberta. Environment Canada. pp. 373-400.

Dickins, D.F., A.A. Allen. 2007. Shell's Beaufort Sea Exploratory Drilling Program - Oil Spill Response in Ice. Prepared for Shell Exploration and Production Co., Anchorage AK.

Exxon, 2000. Spill Oil Spill Response Field Manual. ExxonMobil Upstream Research Company, Houston.

ED_001324_00001497-00080

Fiocco, R.J., P.S. Daling, G. DeMarco, R.R. Lessard, 1999. Advancing laboratory/field dispersant effectiveness testing. In Beyond 2000, Balancing Perspectives: Proceedings: 1999 International Oil Spill Conference: March 8-11, 1999, Seattle, Washington. Washington, D.C.: American Petroleum Institute. pp. 177-185.

Fingas, M.F., I. Bier, M. Bobra and S. Callaghan. 1991."Studies on the Physical and Chemical Behaviour of Oil and Dispersant Mixtures", Proceedings of the 1991 International Oil Spill Conference, American Petroleum Institute, Washington, DC, pp. 419-426, 1991.

Fingas, M., L. Ka'aihue. 2005a. A literature Review of the Variation of Dispersant Effectiveness with Salinity. ",Proceedings of the 28th Arctic Marine Oilspill Program Technical Seminar, Environment Canada, Ottawa, ON, pp. 1043-1083, 2005.

Fingas, M., B.Fieldhouse, Z.Wang. 2005b. The Effectiveness of Dispersants under Various Temperature and Salinity Regimes. ",Proceedings of the 28th Arctic Marine Oilspill Program Technical Seminar, Environment Canada, Ottawa, ON, , pp. 377-389, 2005b.

Fingas, M., B. Fieldhouse, Z. Wang. 2006. The Effectiveness of Dispersants on Alaska North Slope Crude Oil under Various Temperature and Salinity Regimes. ",Proceedings of the 29th Arctic Marine Oilspill Program Technical Seminar, Environment Canada, Ottawa, ON, pp. 821-827, 2006.

George-Ares, A., R.R. Lessard, K.W. Becker, G.P. Canevari and R.J. Fiocco. 2001. "Modification of the Dispersant Corexit 9500 for Use in Freshwater", in Proceedings of the 2001 International Oil Spill Conference, American Petroleum Institute, Washington, DC, pp. 1209-1211, 2001.

Hokstad, J.N., B. Knudsen and Per Daling. 1996. Oil-surfactant interaction and mechanism studies- Part1: Leaching of surfactants from oil to water. Chemical composition of dispersed oil. IKU Sintef Group report to ESSO Norge a.s., ESCOST report No. 21.

Knudsen, O.O., P.J. Brandvik and A. Lewis. 1994. Testing oil spills with W/O emulsion inhibitors- a laboratory study of surfactant leaching from oil to the water phase. Proceedings of the Seventeenth Arctic and Marine Oilspill Program Technical Seminar. June 8-10, Vancouver, BC. Pp 1023-1034.

Lewis, A., K. Trudel, R. Belore, J. Mullin. 2010. Large-scale dispersant leaching and effectiveness experiments with oils on calm water. Marine Pollution Bulletin, 60(2):244-254.

Lewis, A.; Crosbie, A.; Davies, L.; Lunel, T. 1998. Large scale field experiments into oil weathering at sea and aerial application of dispersants. In Proceedings: Twenty-First Arctic and Marine Oilspill Program Technical Seminar, June 10 to 12, 1998, West Edmonton Mall Hotel, Edmonton, Alberta, Canada. Ottawa, Ont.: Environment Canada. pp. 319-344.

ED_001324_00001497-00081

Lunel, T. 1993. Oil droplet size measurement at sea. Proceedings of the Arctic and Marine Oilspill Program (AMOP) Technical Seminar, 1993.

Li, Z., K. Lee, T. King, M. Boufadel, A. Venosa. 2009. Evaluating Crude Oil Chemical Dispersion Efficacy in a Flow-Through Wave Tank Under Regular Non-Breaking Wave and Breaking Wave Conditions. Marine pollution Bulletin. 58 (2009) 735-744.

Mackay, D., Chau, Y. Poon. 1986 A study of the Mechanism of Chemical Dispersion of Oil Spills. Report EE-76 to Environmental Emergencies Technology Division, Environment Canada. March, 1986.

Mackay, D. and K. Hossain. 1982. Oil-water interfacial tensions in chemical dispersant systems. Report to Environment Canada, Ottawa.

Mackay, D. , R. Mascarenhas, K. Hossain, T. McGee. 1979. The Effectiveness of Chemical Dispersants at Low Temperatures and in the Presence of Ice. Report prepared for ESSO Resources Canada Limited. September, 1979.

Mackay, D. 1995. Effectiveness of chemical dispersants under breaking wave conditions. In Lane, P. (ed.). The Use of Chemicals in Oil Spill Response. Philadelphia, Pa.: American Society for Testing and Materials. pp. 310-340.

Mar Inc., SL Ross, DF Dickins, ESTD. 2008. Empirical Weathering Properties of Oil in Ice and Snow. Project Number 1435-01-04-RP-34501. Prepared for U..S. Department of the Interior Minerals Management Service, Alaska Outer Continental Shelf Region. OCS Study MMS 2008-033.

McAuliffe, C.D. et al. 1981. 1979 Southern California dispersant treated research oil spills. In Proceedings: 1981 Oil Spill Conference (Prevention, Behavior, Control, Cleanup), March 2-5, 1981, Atlanta, Georgia. Washington, D.C.: American Petroleum Institute. pp. 269-282.

Moles, A.L. Holland, J. Short, 2001. Effectiveness of Corexit 9527 and 9500 in Dispersing Fresh, Weathered and Emulsion of Alaska North Slope Crude Oil Under Subarctic Conditions. Anchorage, Ak.: Prince William Sound Regional Citizens' Advisory Council. 24p.

Moles, A., L. Holland, J. Short. 2002. Effectiveness in the Laboratory of Corexit 9527 and 9500 in Dispersing Fresh, Weathered and Emulsion of Alaska North Slope Crude Oil Under Subarctic Conditions. Spill Science and Technology Bulletin, Vol. 7. N0s. 5-6, pp. 241-247, 2002.

Nagarajan, K., N. Deshpande, G. Sorial, J. Weaver. 2008. Dispersant Effectiveness on Oil Spills-Empirical Correlations. In Proceedings: 2008 International Oil Spill Conference. Savannah, Ga.. pp. 801-804.

ED_001324_00001497-00082

Nedwed, T. , R. Belore, W. Spring, D. Blanchet. 2007. Basin scle testing of ASD icebreaker enhanced chemical dispersion of oil spills. In Proceedings of the Thirtieth Arctic and Marine Oilspill Program (AMOP) Technical Seminar: June 5-7, 2004, Edmonton (Alberta) Canada. Ottawa, Ont.: Environment Canada. pp. 151-160.

Neff, J.M. 1990. Composition and fate of petroleum and spill treating agents in the marine environment. Sea Mammals and Oil: Confronting the Risks. J.R. Geraci and D.J. St Aubin (eds.). Academic Press: New York. Pp1-33.

OGP. 2014. http://www.arcticresponsetechnology.org/research-projects/dispersant-testing-under-realistic-conditions/scope-of-work.

Owens, C.K.R. Belore, 2004. Dispersant effectiveness testing in cold water and brash ice. In Proceedings of the Twenty-Seventh Arctic and Marine Oilspill Program (AMOP) Technical Seminar: June 8-10, 2004, Edmonton (Alberta) Canada. Ottawa, Ont.: Environment Canada. pp. 819-841.

Payne, J.R. et al. 1985. Estimating dispersant effectiveness under low temperature-low salinity conditions. In Proceedings: 1985 Oil Spill Conference, (Prevention, Behavior, Control, Cleanup), February 25-28, 1985, Los Angeles, California. Washington, D.C.: American Petroleum Institute. pp. 638.

Proshutinsky, Andrey Y., Mark A. Johnson, Tatiana O. Proshutinsky, James A. Maslanik. 2003. Beaufort and Chukchi Sea Seasonal Variability for Two Arctic Climate States. University of Alaska Coastal Marine Institute. OCS Study MMS 2003-024.

SL Ross. 2013. Spill Related Properties of 2012 ANS Crude Oil Report prepared for Prince William Sound Shippers Association. March 2013.

SL Ross. 2011. Comparison of Large-Scale (Ohmsett) and Small-Scale Dispersant Effectiveness Test Results. Report prepared for U.S. Department of the Interior, Minerals Management Service, Herndon, VA, June 2011.

SL Ross. 2011b. Spill Response Gap Study for the Canadian Beaufort Sea and the Canadian Davis Strait Contract No. 110027 Submitted to:National Energy Board. July 12, 2011.

SL Ross, MAR, 2011c. Dispersant Effectiveness Testing On Viscous, U.S. Outer Continental Shelf Crude Oils: Phase III. Report Prepared for U.S. Department of the Interior Minerals Management Service, Herndon, VA. February 2011.

SL Ross, 2010. Literature Review of Chemical Oil Spill Dispersants and Herders in Fresh and Brackish Waters. Report Prepared for U.S. Department of the Interior Minerals Management Service, Herndon, VA. January 2010

SL Ross. 2009. Low-Dose Repeat-Application Dispersant Testing. Report prepared for U.S. Department of the Interior, Minerals Management Service, Herndon, VA, August 2009.

ED_001324_00001497-00083

SL Ross, A. Lewis 2008. Calm Seas Application and Dispersant Wash-Out. Report prepared for U.S. Department of the Interior, Minerals Management Service, Herndon, VA, December 2008.

SL Ross, MAR Inc. & DF Dickins, 2008b. Empirical Weathering Properties of Oil in Ice and Snow. Report prepared for U.S. Department of the Interior, Minerals Management Service, Alaska Outer Continental Shelf Region, Anchorage, AK. OCS Study MMS AK-04-06.

SL Ross, MAR, 2008c. Dispersant Effectiveness Testing On Viscous, U.S. Outer Continental Shelf Crude Oils: Phase II. Report Prepared for U.S. Department of the Interior Minerals Management Service, Herndon, VA. November 2008.

SL Ross, A. Lewis 2007. Changes In Dispersant Effectiveness with Extended Exposure in Calm Seas. Report prepared for U.S. Department of the Interior, Minerals Management Service, Herndon, VA, December 2007.

SL Ross. 2006. Dispersant Effectiveness Testing On Water-In-Oil Emulsions At Ohmsett. Report prepared for U.S. Department of the Interior, Minerals Management Service, Herndon, VA, September 2006.

SL Ross. 2006a. Dispersant Effectiveness Testing in Ice (SL Ross Tank Tests)- Project Report to ExxonMobil Upstream Research Upstream Research Company, July 2006

SL Ross, MAR. 2006b. Dispersant Effectiveness Testing in Cold Water on Four Alaskan Crude Oils. Report prepared for U.S. Department of the Interior, Minerals Management Service, Herndon, VA, August 2006b.

SL Ross. 2006c. Dispersant Effectiveness Testing on Chayvo Z-6 Crude Oil in Cold Water and Ice at Ohmsett, Report to ExxonMobil Upstream Research Company, July 2006

SL Ross 2006d. Vessel Assisted Dispersion in an Ice Field Using Scale Model Ice-Breakers at the AKER Helsinki Test Basin. Report to BP Exploration, Sakhalin Developments, February 2006

SL Ross. 2005. Tank Tests to Evaluate the Effectiveness of Vessel-Assisted Chemical Dispersion in Ice. Report to ExxonMobil Upstream Research Company, April 2005

SL Ross, A. Lewis, 2005b. Development of a Method to Produce Large Quantities of Realistic Water-In-Oil Emulsions for Use in Evaluating Oil Spill Response Equipment and Methods. Report prepared for U.S. Department of the Interior, Minerals Management Service, March 2005.

ED_001324_00001497-00084

SL Ross. 2003. Dispersant Effectiveness Testing in Cold Water on Four Alaskan Crude Oils. Report prepared for U.S. Department of the Interior, Minerals Management Service, Herndon, VA, August 2003.

SL Ross. 2002. Dispersant Effectiveness Testing in Cold Water and Broken Ice. Report prepared for U.S. Department of the Interior, Minerals Management Service, Herndon, VA, May 2002.

SL Ross. 2002b. Dispersant Effectiveness Testing in Cold Water and Broken Ice at Ohmsett. Report to ExxonMobil Upstream Research Company, August 2002.

SL Ross. 1994. Spill Related Properties of Fresh and Weathered Alaskan Crude Oils. Report prepared for Alaska Clean Seas.

Spring, W, T. Nedwed, R. Belore.2006. Icebreaker Enhanced Chemical Dispersion of Oil Spills. In Proceedings of the Twenty-Ninth Arctic and Marine Oilspill Program (AMOP) Technical Seminar: June 5-7, 2004, Vancouver (British Columbia) Canada. Ottawa, Ont.: Environment Canada. pp. 711-727.

Smith, D., G. Holliday. 1979. API/SC-PCO Southern Californian1978 Oil Spill Test Program. Proceedings 1979 Oil Spill Conference, Los Angeles, California, March, 1979. pp 475-482.

Sorial, G.A.K.M. Karen, H. Edith, A.D. Venosa, D.W. King. 2001. Development of a rational oil spill dispersant effectiveness protocol. In 2001 International Oil Spill Conference: Global Strategies for Prevention, Preparedness, Response, and Restoration: March 26-29, 2001, Tampa Convention Center, Tampa, Florida. Washington, D.C.: American Petroleum Institute. pp. 471-478.

Sørstrøm, S.E., Per Johan Brandvik, I. Buist, P. Daling, D. Dickins, L. Faksness, S.Potter, J. Rasmussen and I. Singsaas.2010. Joint industry program on oil spill contingency for Arctic and icecovered waters: SUMMARY REPORT. SINTEF Materials and Chemistry. Report A14181.

Strom-Kristiansen, T., P.S. Daling, A. Lewis, A.B. Nordvik. 1994.Weathering properties and Chemical Dispersibility of Crude Oils Transported in U.S. Waters. Marine Spill Response Corporation, Washington, D.C. MSRC Technical Report Series 93-032, 210 p.

Sullivan, D, J. Farlow, K.A. Sahatjian. 1993. Evaluation of three oil spill laboratory dispersant effectiveness tests. In Proceedings: 1993 International Oil Spill Conference (Prevention, Preparedness, Response): March 29-April 1, 1993, Tampa, Florida. Washington, D.C.: American Petroleum Institute. pp. 515-520.

ED_001324_00001497-00085

Venosa, A.D.G.A. Sorial, D.W. King. 2001. Round-robin testing of a new EPA dispersant effectiveness protocol. In 2001 International Oil Spill Conference: Global Strategies for Prevention, Preparedness, Response, and Restoration: March 26-29, 2001, Tampa Convention Center, Tampa, Florida. Washington, D.C.: American Petroleum Institute. pp. 467-470.

Venosa, A., K. Lee, M. Boufadel, Z. Li, E. Wickley-Olsen, T. King, 2008. Dispersant Effectiveness as a Function of Energy Dissipation Rate in an Experimental Wave Tank. In Proceedings: 2008 International Oil Spill Conference. Savannah, Ga.. pp. 777-784.

Wang, C, M. Quah, P. G. Noble, R.l Shafer, K. A. Soofi, T. Brassfield. 2012. Use of Jack-up Drilling Units in Arctic Seas with Potential Ice Incursions during Open Water Season, Paper OTC 23745, Arctic Offshore Technology Conference, Houston, Texas, USA, 3-5 December 2012.

Weingartner,T. 1998. Circulation on the North Central Chukchi Sea Shelf. Report Prepared for U.S. Department of the Interior Minerals Management Service. OCS Study MMS 98-0026.

Weingartner, Thomas J., Stephen R. Okkonen, Seth L. Danielson. 2005. Circulation and Water Property Variations in the Nearshore Alaskan Beaufort Sea. Institute of Marine Science, University of Alaska. MMS Contract 1435-01-00-CA-31083

Weingartner, Thomas J. 2006. Circulation, thermohaline structure, and cross-shelf transport in the Alaskan Beaufort Sea. Prepared for US Department of Interior. OCS Study MMS 2006-031

Weingartner,T., S. Danielson, 2010. Physical Oceanographic Measurements in the Klondike and Burger Survey Areas of the Chukchi Sea: 2008 And 2009. Prepared for ConocoPhillips Inc., and Shell Exploration & Production Company. Institute of Marine Scienc, University of Alaska.

Weingartner, Thomas, Elizabeth Dobbins,Seth Danielson ,Peter Winsor, Rachel Potter, Hank Statscewich. 2013. Hydrographic variability over the northeastern Chukchi Sea shelf in summer-fall 2008–2010. Continental Shelf Research 67 (2013) 5–22.

White, D.M.,I. Ask, C. Behr-Andres. 1999. Final Report: Effectiveness Testing for Corexit 9500 on Alaska North Slope Crude Oil in Prince William Sound Seawater at 8°C. Fairbanks, Ak: Alaska Department of Environmental Conservation. 47p.

Wrenn, B., A. Virkus, B. Mukherjee, A. Venosa. 2009. Dispersibility of crude oil in fresh water. Environmental Pollution 157 (2009) 1807-1814.

ED_001324_00001497-00086

9 Appendix A : Detailed Dispersant Effectiveness Tables

Table 9-1 Percentage of Time Dispersants Would Be Effective at Wind Station 2 (Chukchi Sea Offshore Wainwright)

Percent of Time Dispersants Effective (48 hour Calm Wind Grace Period)						
	31 year Data Analysis (1979 to 2009)			10 year Data Analysis (2000 to 2009)		
Month	Favorable	Impaired	Not Favorable	Favorable	Impaired	Not Favorable
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	77	2	21	77	2	21
7	83	2	15	83	2	15
8	90	2	7	87	3	10
9	89	6	5	90	5	4
10	86	9	5	88	9	3
11	80	9	11	82	9	9
12	0	0	0	0	0	0

Table 9-2 Percentage of Time Dispersants Would Be Effective at Wind Station 3 (Beaufort Sea Offshore Barrow)

Percent of Time Dispersants Effective (48 hour Calm Wind Grace Period)						
	31 year Data Analysis (1979 to 2009)			10 year Data Analysis (2000 to 2009)		
Month	Favorable	Impaired	Not Favorable	Favorable	Impaired	Not Favorable
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	73	2	25	76	2	22
8	82	2	15	79	3	19
9	87	3	10	87	4	9
10	81	5	14	86	6	8
11	0	0	0	0	0	0
12	0	0	0	0	0	0

ED_001324_00001497-00087

Table 9-3 Percentage of Time Dispersants Would Be Effective at Wind Station 4 (Beaufort Sea Offshore Prudhoe Bay)

Percent of Time Dispersants Effective (48 hour Calm Wind Grace Period)						
	31 year Data Analysis (1979 to 2009)			10 year Data Analysis (2000 to 2009)		
Month	Favorable	Impaired	Not Favorable	Favorable	Impaired	Not Favorable
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	69	3	28	71	3	26
8	79	3	18	75	3	22
9	86	5	10	87	4	10
10	80	7	13	84	7	9
11	0	0	0	0	0	0
12	0	0	0	0	0	0

Table 9-4 Percentage of Time Dispersants Would Be Effective at Wind Station 4 (Beaufort Sea Offshore East of Barter Island)

Percent of Time Dispersants Effective (48 hour Calm Wind Grace Period)						
	31 year Data Analysis (1979 to 2009)			10 year Data Analysis (2000 to 2009)		
Month	Favorable	Impaired	Not Favorable	Favorable	Impaired	Not Favorable
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	80	2	17	81	2	17
8	82	4	14	78	4	17
9	87	4	9	87	4	10
10	82	6	13	79	6	15
11	0	0	0	0	0	0
12	0	0	0	0	0	0

ED_001324_00001497-00088

10 Appendix B : Annotated Bibliography: Past Research Relevant to Dispersion of Alaskan Oils in the US Beaufort and Chukchi Seas

Abbasova, A. et al. 2005. Evaluation of dispersants for use in the Azerbaijan region of the Caspian Sea.
Oil Type: Chirag crude oil (Caspian Sea)
Dispersant Type: Finasol OSR51, Superdispersant 25, Corexit EC 9527a, Corexit EC 9500a, Slickgone NS, Inipol IP90
Water Temperature: assumed to be EPA standard temp of 23 ± 3 °C
Water Salinity: 12 ppt (Caspian Sea Water) Six dispersants were tested on Chirag crude oil in 12 ppt water. All dispersants were effective in dispersing this crude oil at this low salinity. Average effectiveness values ranged from 72 to 84%.
Presence of Ice: Not studied.
Energy Level: EPA Baffled Flask.

Anderson, J.W., D.L. McQuerry, S.L. Kiesser. 1985. Laboratory evaluation of chemical dispersants for use on oil spills at sea
Oil Type: Alaska North Slope crude
Dispersant Type: 14 different dispersants. Dose set to achieve 90 % dispersion of ANS in MNS apparatus. Primary purpose to investigate toxicity of chemically dispersed oil.
Water Temperature: 15 °C.
Water Salinity: 30 ppt
Presence of Ice: Not studied.
Energy Level: MNS standard mixing (1.0 inch of water pressure)

Belore, R., S. Ross. 2000. Laboratory study to compare the effectiveness of chemical dispersants when applied dilute versus neat
Oil Type: Alaska North Slope crude
Dispersant Type: Corexit 9500 and Corexit 9527. Dispersant was applied neat and diluted with salt water. Corexit 9527 performed as well when diluted as when applied neat. Corexit 9500's performance was reduced when applied diluted with sea water. Application of Corexit 9500 in a neat form was recommended.
Water Temperature: 17 °C
Water Salinity: 32 ppt
Presence of Ice: Not studied
Energy Level: SL Ross wave tank, energy not varied.

Belore, R. 2003. Large wave tank dispersant effectiveness testing in cold water
Oil Type: fresh and weathered Alaska North Slope and Hibernia crude
Dispersant Type: Corexit 9500 and Corexit 9527.
Water Temperature: -0.5 to 2.4 °C. Both oils were readily dispersed by both dispersants at the cold test temperatures.
Water Salinity: 32 ppt
Presence of Ice: Not studied
Energy Level: Ohmsett breaking wave conditions. The average wave amplitude for the tests ranged between 16.5 and 22.5 cm and the average wave period was between 1.7 and 1.9 seconds.

ED_001324_00001497-00089

Blondina, G., M Singer, I. Lee, M. Ouano, M. Hodgins, R. Tjeerdema. 1999. Influence of Salinity on Petroleum Accommodation by Dispersants.
Oil Type: various including Prudhoe Bay crude
Dispersant Type: Corexit 9500 and Corexit 9527
Water Temperature: 15 °C
Water Salinity: 0 to 35 ppt. For Prudhoe Bay crude oil 9500 was equally effective from 20 to 35 ppt and effectiveness dropped off below 20 ppt. 9527 effectiveness dropped off below 30 ppt.
Presence of Ice: Not studied
Energy Level: modified Swirling Flask test.

Brandvik, P.J., 0.0. Knudsen, M.O. Moldestad and P.S. Dating, 1995. Laboratory Testing of Dispersants Under Arctic Conditions
Oil Type: Norwegian Crude Oils
Dispersant Type: Corexit 9500 & 9527 plus 12 others, 5 main dispersants tested were Inipol IPF, Inipol IPC, Enersperse 700, Finasol OSR-52 and Dasic Fresh Water.
Water Temperature: 0°C
Water Salinity: 5 to 35 ppt. Dispersants formulated for low salinity performed well at low salinities but not at high salinities. Traditional dispersants formulated for high salinity water did not perform well at low salinities. Many dispersants performed well at the low test temperature on most of the oils tested but effectiveness was a function of the oil type.
Presence of Ice: Not studied
Energy Level: IFP test, energy not varied

Brown, H.M., R.H. Goodman. 1985. Dispersant Effectiveness in Cold Water
Oil Type: Federated crude (Alberta Sweet Mixed Blend)
Dispersant Type: Corexit 9527, and CRX-8.
Water Temperature: -2 °C.
Water Salinity: Tests were conducted at 28 ppt water salinity
Presence of Ice: Slush ice present in test #6 only
Energy Level: Low energy non-breaking waves were used. In 20 cm waves 24 to 33% of the spilled oil was dispersed in the water column even 20 hours after the wave basin energy had been stopped. The single test conducted with slush ice resulted in more oil dispersion than in open water. This was attributed to the shear imparted by the moving slush ice

Brown, H.M., R.H. Goodman. 1987. Outdoor Wave Tank Tests
Oil Type: Alaska North Slope Crude (ANS) weathered 12 to 24 hours on tank prior to dispersant application.
Dispersant Type: Corexit 9527, 9550 and Enersperse 700 were tested. Enersperse was least effective 38% and Corexit 9527 and 9550 were similar with 53 to 63% effectiveness at higher wave energy.
Water Temperature: Water temperature was not varied but tests were conducted at 0 to 5 °C.
Water Salinity: Tests were conducted at 32 ppt water salinity
Presence of Ice: Not studied
Energy Level: The single test conducted with lower wave energy had 19% less effectiveness than the higher energy test (42% versus 61%).

ED_001324_00001497-00090

Brown, H.M., D.K.Weiss, R.H. Goodman. 1990. Emulsion formation in dispersant-treated crude oil.
Oil Type: Alaska North Slope and Drift River crude
Dispersant Type: Corexit 9500 and Corexit 9527. For the non-dispersed portions of slicks of Drift River crude, the application of dispersants enhanced the rate of water incorporation and also increased the viscosity, while the opposite effect was observed for North Slope crude oil: the treated slicks did not emulsify as fast or achieve as high viscosities.
Water Temperature: -not reported
Water Salinity: 30 ppt
Presence of Ice: Not studied
Energy Level: Esso wave basin. The average wave amplitude for the tests was 20 cm and the average wave period was between 1.6 seconds.

Brown and Goodman 1996. The Use of Dispersants in Broken Ice
Oil Type: Federated crude oil
Dispersant Type: Corexit 9527, 9500
Water Temperature: -1.2 °C
Water Salinity: Tests were conducted at 30 ppt water salinity
Presence of Ice: Condition 1: Five-metre diameter booms were positioned and frozen into the ice. Before oil was spilled into the boomed area, low amplitude waves were generated to break the ice. Several such experiments were conducted involving different ice thicknesses and ice coverages. In all cases oil was effectively dispersed (>90% dispersion) even in 95% ice cover and low wave levels
Condition 2: Ice slot was cut into fully formed ice sheet. Oil was placed in the slot. No measureable natural dispersion occurred under low energy with no ice present. With slush ice present some natural dispersion occurred. When dispersant was added to oil in slush ice almost all oil dispersed
Energy Level: Low energy swells by wave board in ESSO outdoor wave basin.

Byford, D., P. Green, A. Lewis. 1983. Factors Influencing the Performance and Selection of Low-Temperature Dispersants.
Oil Type: Lago Medio, North Slope Crude, Medium Fuel Oil
Dispersant Type: Corexit 9527, Corexit 9550 BP1100WD, Fiansol OSR5, Dispolene 34S, Arcochem D609
Water Temperature: 0 and 10 °C. In all cases the dispersant performance was not reduced in the lower temperatures and in some cases was enhanced.
Water Salinity: 0 to 33 ppt. Water salinity was found to affect dispersant performance to varying degrees. One product worked equally as well at all salinities but was a poor performer compared to other products. Product names were not attached to results.
Presence of Ice: 100% broken ice pieces (5 mm x10mmx10mm) in BP wave tank tests. Results suggest that dispersant performance can be enhanced due to the enhanced mixing caused by the pumping action between ice pieces. Dispersion was at least as good in ice conditions as in open water in all tests.
Energy Level: standard WSL test and BP wave tank with 40 mm wave amplitude

ED_001324_00001497-00091

Chandrasekar, S. G.A., Sorial, J.W. Weaver. 2003. Determining dispersant effectiveness data for a suite of environmental conditions.
Oil Type: Prudhoe Bay (PBC), South Louisiana (SLC) crude and #2 Fuel oil. Slight reduction in effectiveness was identified with weathered (10 to 20% loss) versus fresh oil. For PBC the reduction was less than 5%.
Dispersant Type: Corexit 9500 and Dispersit-SPC 1000
Water Temperature: 5, 22 and 35 °C. Increased dispersion with increased temperature for SLC, maximum dispersion at 22°C for both PBC and #2 Fuel oil.
Water Salinity: standard BF 34 ppt assumed
Presence of Ice: Not studied
Energy Level: 150, 200 and 250 rpm on shaker table. Higher effectiveness at higher energies in all tests.

Chandrasekar, S.; Sorial, G.A.; Weaver, J.W. 2006. Dispersant effectiveness on oil spills – impact of salinity.
Oil Type: Alaskan Prudhoe Bay (PBC) fresh, 10% and 20% evaporated, South Louisiana crude oil (SLC) fresh, 10% and 20% evaporated, No. 2 Fuel Oil (2FO) fresh, 3.8% and 7.6% evaporated. Weathered PBC dispersed as well as fresh at 200 rpm and over the full range of temperatures.
Dispersant Type: Dispersants identified as A & B only
Water Temperature: 5, 22 and 35 °C. Highest effectiveness at 250 rpm for the most weathered PBC was achieved at 22 °C.
Water Salinity: 10, 20, 34 ppt. Salinity did not significantly (less than 15% difference over salinity range) impact the effectiveness of dispersant A for the fresh or weathered PBC at any of the temperatures tested.
Presence of Ice: Not studied.
Energy Level: EPA Baffled Flask operated at 150, 200 and 250 rpm. Higher energy resulted in higher dispersion (all other factors constant)

Cox, J., L. Shultz 1981. Dispersant Effectiveness Under Arctic Conditions, Including Ice
Oil Type: Sadlerochit crude fresh, 10 and 30% weathered, 10% weathered Arctic diesel, 10% weathered Kuparuk crude
Dispersant Type: Identified as X,Y and Z only
Water Temperature: -0.6 °C
Water Salinity: 10, 20 and 32 ppt. results showed no difference in dispersant effectiveness over the range of salinities tested
Presence of Ice: 20 and 50% coverage of 1.5 cm thick and 5.7 cm diameter ice
Energy Level: MNS apparatus with ice present. Slight increase in natural dispersion with ice present versus open water. When dispersant was added dispersion rose substantially. No difference in results between 20% and 50% ice coverage

Fingas, M.F., I. Bier, M. Bobra and S. Callaghan. 1991. Studies on the Physical and Chemical Behaviour of Oil and Dispersant Mixtures
Oil Type: Alberta Sweet Mixed Blend, Norman Wells, Adgo
Dispersant Type: Corexit 9527, Enersperse 700 and Citrikleen

ED_001324_00001497-00092

Water Temperature: 0 to 50 °C on a subset of tests with Corexit 9527 and ASMB oil only. Dispersant effectiveness reduced with decrease in temperature.
Water Salinity: Maximum effectiveness was achieved at 40 to 45 ppt and fell sharply with either a decrease or increase in salinity for all dispersant and oil combinations tested.
Presence of Ice: Not studied
Energy Level: SF test with no variation in energy level

Fingas, M., B.Fieldhouse, Z.Wang. 2005b. The Effectiveness of Dispersants under Various Temperature and Salinity Regimesingas
Oil Type: Alberta Sweet Mixed Blend (ASMB)
Dispersant Type: Corexit 9500
Water Temperature: 0 to 25 °C on ASMB oil only. Dispersant effectiveness reduced with temperature increase above 20 °C and was similar over 5 to 20 °C contrary to most other research.
Water Salinity: Maximum effectiveness was achieved at 25 ppt and fell sharply with a decrease below 20 °C.
Presence of Ice: Not studied
Energy Level: SF test with no variation in energy level

Fingas, M., B. Fieldhouse, Z. Wang. 2006. The Effectiveness of Dispersants on Alaska North Slope Crude Oil under Various Temperature and Salinity Regimes
Oil Type: Alaska North Slope Crude (ANS)
Dispersant Type: Corexit 9500
Water Temperature: 5 to 25 °C Dispersant effectiveness reduced with temperature increase above 10 °C and was similar over 5 to 10 °C contrary to most other research where higher temperatures usually result in higher dispersant effectiveness.
Water Salinity: Maximum effectiveness was achieved at 25 ppt at all temperatures and fell sharply with water salinity below 20 ppt.
Presence of Ice: Not studied
Energy Level: SF test with no variation in energy level

Fiocco, R.J., P.S. Daling, G. DeMarco, R.R. Lessard, 1999. Advancing laboratory/field dispersant effectiveness testing
Oil Type: Alaska North Slope weathered oil and emulsions
Dispersant Type: Corexit 9500
Water Temperature: 15 °C
Water Salinity: 32 ppt assumed
Presence of Ice: Not studied
Energy Level: MNS test was run for longer duration (60 minutes from 5) to test Corexit 9500's ability to break emulsions. Successfully dispersion of emulsions measured with viscosities up to 15,000 cP and 65% water content. These data were compared to the 1997 North Sea field trial data where similar results were measured in the field for dispersion of emulsions by Corexit 9500.

George-Ares, A., R.R. Lessard, K.W. Becker, G.P. Canevari and R.J. Fiocco. 2001. Modification of the Dispersant Corexit 9500 for Use in Freshwater

ED_001324_00001497-00093

Oil Type: Alaska North Slope Crude (ANS) plus 3 other South American crudes
Dispersant Type: Corexit 9500, calcium chloride modified 9500, Dasic freshwater, Enersperse 1037, Inipol IPF
Water Temperature: not reported, assumed room temperature and not varied
Water Salinity: All tests conducted using river (0 ppt) or deionized water. Corexit 9500 was modified by blending in calcium chloride solution at various concentrations (concentrations not reported) and tested with ANS crude oil. Conventional Corexit 9500 had an efficiency of 22% in fresh water. The modified Corexit 9500 had measured efficiencies of 29 to 63% depending on the quantity of calcium chloride added. Dasic fresh water was the most effective dispersant on all of the oils tested in the fresh water
Presence of Ice: Not studied
Energy Level: EXDET test with no variation in energy level

Lewis, A., A. Crosbie, L. Davies, T. Lunel. 1998. Large scale field experiments into oil weathering at sea and aerial application of dispersants.
Oil Type: Forties Blend, Alaskan North Slope crude, and IFO-180 fuel oil. ANS was emulsified to 35% water content prior to spraying
Dispersant Type: Corexit 9500 and Dasic Slickgone NS
Water Temperature: 18 °C
Water Salinity: full ocean salinity North Sea
Presence of Ice: Not studied
Energy Level: offshore conditions in 3 to 5 m/s winds with gusts 7 to 8 m/s. 30 m^3 of ANS crude, weathered for 55 h, was completely dispersed with Corexit 9500 applied from the air in two stages with a total DOR of approximately 1:12.

Li, Z., K. Lee, T. King, M. Boufadel, A. Venosa. 2009. Evaluating Crude Oil Chemical Dispersion Efficacy in a Flow-Through Wave Tank Under Regular Non-Breaking Wave and Breaking Wave Conditions
Oil Type: fresh ANS and weathered (14%) Mesa crude oil
Dispersant Type: Corexit 9500 & Dispersit SPC 1000
Water Temperature: not reported
Water Salinity: not reported
Presence of Ice: Not studied.
Energy Level: EPA /DFO wave tank operated with regular non-breaking waves, and plunging breakers. 21 to 36% of oil was dispersed and diluted with dispersants and non-breaking waves. 42 to 62% of the oil was dispersed and diluted with dispersants and plunging breakers. With no dispersants and both wave conditions only 8 to 19% of the oil was dispersed and diluted in the flow through wave tank. Drop sizes were large with regular waves and no dispersant (VMD > 300 um) and small with dispersants and breaking waves (VMD < 50 um).

Mackay, D. , R. Mascarenhas, K. Hossain, T. McGee. 1979. The Effectiveness of Chemical Dispersants at Low Temperatures and in the Presence of Ice.
Oil Type: Alberta Sour Mixed Blend and Lago Medio crude
Dispersant Type: Corexit 9527

ED_001324_00001497-00094

Water Temperature: 0 to 15°C. It was concluded that the primary effect of water temperature was to lower the oil temperature and thus increase the oil viscosity. The increased oil viscosity reduced the dispersant effectiveness.
Water Salinity: 35 ppt
Presence of Ice: Ice was introduced into the hoop tank test apparatus. The effect of the ice was to dampen the waves and generally reduce effectiveness but no other significant conclusions could be drawn about the effects of the ice.
Energy Level: MNS, Hoop tank , and Calm Sea Simulation Apparatus: Hoop tank used in ice studies.

Mackay, D., Chau, Y. Poon. 1986. MNS, Rotating Flask and Hoop Tank Test Results
Oil Type: The effectiveness of was tested on Prudhoe Bay crude oil along with 11 other oils using both the MNS and a rotating flask (RF) test. The Prudhoe Bay crude dispersibility was generally higher than the average of the 12 oils tested indicating that this Alaskan oil was amenable to chemical dispersant use.
Dispersant Type: Corexit 9527, Corexit 9550 and BP MA700
MNS test results of the three dispersants tested on the Prudhoe Bay crude oil Corexit 9550 had the best performance at 71% followed by Corexit 9527 at 45.9% and BP MA 700 at 43.1%.
Water Temperature: 5 to 25° C
Study conclusions: 'There is no inherent reason to suggest that cold climate dispersion is significantly less feasible than temperate dispersion'.
'dispersion is likely to require more dispersant under these cold conditions but the extra amount is unlikely to be large except when the oils are unusually viscous, close to their pour point or waxy'.
Water Salinity: Study conclusions: 'when assessing the suitability of dispersants for use in variable salinity waters there is presently no alternative but to measure the magnitude of the salinity dependence of dispersion on a case by case basis'. The effect of salinity was most notable for Corexit 9527 in this work when compared to Corset 9550 and BP MA700. Since Corexit 9527 (and its more modern sister Corexit 9500) are the primary dispersants in use in Alaska this would suggest that water salinity may be a concern with this particular dispersant formulation.
Presence of Ice: Not investigated
Energy Level: Acknowledged the need to measure both mixing level that generates oil drops as well as the water column suspension energy. Ranked the mixing energies of the various test methods used but only speculated how these would compare to oceanic mixing levels

Mackay, D. 1995. Effectiveness of chemical dispersants under breaking wave conditions
Oil Type: Alaska North Slope weathered 5 to 20%
Dispersant Type: Corexit 9527
Water Temperature: 4.4 to 15.5 °C Increasing temperature resulted in a slight loss of effectiveness
Water Salinity: 0 to 32 ppt. The water salinity did not affect the dispersibility of the weathered ANS in the 5 to 32 ppt range in the EXDET test (this is contrary to other test results). 30 ppt in Esso wave basin.
Presence of Ice: Not studied

ED_001324_00001497-00095

Energy Level: MNS, WSL, EXDET and ESSO wave basin. EXDET was small scale test chosen as being representative, quick, more reproducible and with less waste water. EXDET energy level between 10 to 16 mm amplitude did not affect results. In the turbulent wave conditions of the wave tank (15 cm amplitude 2.5 s period) effectiveness was 90 to 100% at DORs of 1:100 with significant dispersion at DORs of 1:200 and 1:300
Other Results: The author calculated how much of the Exxon Valdez oil spill might have been dispersed if the dispersant available at the time of the spill had been applied. Determined that 38% of the oil could have been dispersed.

McAuliffe, C., B. Steelman, W. Leek, D. Fitzgerald, J. Ray, C. Barker. 1981. 1979 Southern California dispersant treated research oil spills.
Oil Type: Alaska Prudhoe Bay crude
Dispersant Type: Dispersant products identified as H & J (no brand names provided)
Water Temperature: not reported. Offshore Long Beach California in September (warm water)
Water Salinity: full ocean salinity (32 ppt)
Presence of Ice: Not studied
Energy Level: Wind speed or wave heights not reported. Extensive water sampling and analysis was undertaken and significant oil was identified in the water column up to 9 m depth. Dispersant was visually very effective as well.
Results Summary: ANS Oil was dispersible by both products but dispersant H was more effective, as measured by chemical analysis of water samples under the slick. Between 5 to 78% dispersion was measured. When dispersant was applied to the thick oil rather than entire slick dispersant effectiveness values of 60 to 78% were measured. Two hour weathered crude was less dispersible than freshly treated oil.

Moles, A.L. Holland, J. Short, 2001. Effectiveness of Corexit 9527 and 9500 in Dispersing Fresh, Weathered and Emulsion of Alaska North Slope Crude Oil Under Subarctic Conditions
Oil Type: Studied Alaska North Slope Crude (ANS) fresh, weathered and emulsified. Neither dispersant was effective on the weathered oil under any conditions tested.
Dispersant Type: Corexit 9527 and 9500. Comparable results were achieved with the two dispersants. Dispersant was pre-mixed with the oil and emulsions. This may have been one of the reasons for the high effectiveness values recorded for the emulsions.
Water Temperature & Water Salinity: 3, 10 and 22°C and 22 and 32 ppt
In tests using pre-mixed Corexit 9500 and 9527 dispersants and fresh ANS crude oil in the swirling flask test dispersion was not affected by salinity (22 ppt versus 32 ppt) at high temperatures (22°C) but was at low temperatures (10°C). Dispersion levels for fresh ANS were below the detectable limits of the SF test at 3°C and both salinities for both dispersants. Authors concluded that temperature, salinity and weathering are important factors to consider when evaluating the effectiveness of dispersants with weathering having the most profound effect. The effectiveness of 9500 in 32 ppt salinity water was similar at 10 °C and 22 °C. The colder water tests with 9527 and 32 ppt salinity had lower effectiveness.
Presence of Ice: Not studied
Energy Level: Author states that the swirling flask test is a conservative test that applies relatively low mixing energy.

ED_001324_00001497-00096

Moles, A., L. Holland, J. Short. 2002. Swirling Flask Test Results
Oil Type: Alaska North Slope Crude (ANS) fresh, weathered and emulsified.
Dispersant Type: Corexit 9527 and 9500. Similar results were achieve with the two dispersants. Dispersant was pre-mixed with the oil and emulsions. This may have been one of the reasons for the high effectiveness values recorded for the emulsions.
Water Temperature & Water Salinity:
In tests using pre-mixed Corexit 9500 and 9527 dispersants and ANS crude oil in the swirling flask test dispersion was not affected by salinity (22 ppt versus 32 ppt) at high temperatures (22°C) but was at low temperatures (10°C). He concluded that temperature, salinity and weathering are important factors to consider when evaluating the effectiveness of dispersants with weathering having the most profound effect. The effectiveness of 9500 in 32 ppt salinity water was similar at 10 °C and 22 °C. The colder water tests with 9527 and 32 ppt salinity had lower effectiveness.
Presence of Ice: Not studied
Energy Level: Author acknowledges that the low energy level in the swirling flask test may not be a good representation of environmental conditions

Nagarajan, K., N. Deshpande, G. Sorial, J. Weaver. 2008. Dispersant Effectiveness on Oil Spills- Empirical Correlations.
Oil Type: Alaskan Prudhoe Bay (PBC) fresh, 10% and 20% evaporated, South Louisiana crude oil (SLC) fresh, 10% and 20% evaporated, No. 2 Fuel Oil (2FO) fresh, 3.8% and 7.6% evaporated. A linear, empirical correlation between effectiveness in BF test and all parameters tested (salinity, viscosity (as a combined measure of weathering and temperature effect) and rotational speed). Final correlation parameters not reported in paper.
Dispersant Type: Dispersants identified as A (Corexit 9500) & B (Dispersit SPC 1000)
Water Temperature: 5, 10, 16, 22, 27 and 35 °C. Highest effectiveness at 250 rpm for the most weathered PBC was achieved at 22 °C.
Water Salinity: 10, 20, 34 ppt. Salinity did not significantly (less than 15% difference over salinity range) impact the effectiveness of dispersant A for the fresh or weathered PBC at any of the temperatures tested.
Presence of Ice: Not studied.
Energy Level: EPA Baffled Flask operated at 150, 200 and 250 rpm. Higher energy resulted in higher dispersion (all other factors constant), 10% evaporated PBC was shown to be effectively 80+%) dispersed at the medium and high energy levels.

Nedwed, T. , R. Belore, W. Spring, D. Blanchet. 2007. Basin scale testing of ASD icebreaker enhanced chemical dispersion of oil spills
Oil Type:. Chayvo Z6, 3 to 6% weathered by weight
Dispersant Type: Corexit 9527. Dispersant applied to oil either prior to energy addition in broken ice or oil on ice tests or after ice breaker had broken ice and exposed the oil for treatment.
Water Temperature: Air temp 0 to -10 °C
Water Salinity: 32 ppt
Presence of Ice: Ice present in 25% to 100% ice sheet. Oil spilled in broken ice, on top of a full ice sheet and under a full ice sheet.
Energy Level: SL Ross wave tank with trolling motor prop wash. Greater than 90% effectiveness in ice cover ranging from 25% to 90%. Low dose (1:110 also effective. Prop wash

was effective on oil weathered for 98 hours. In AKER ice basin tests all energy was provided by the prop wash of a scale model azimuthal stern drive icebreaker. Dispersant effectiveness values of between 70% and 97% were measured in the tests. Oil drop VMD (d50) of 10 to 75 μm were measured in the tests indicating effective long term dispersion. Oil did not clear the tank after many weeks of sitting with no energy addition indicating long term dispersion even under quiescent conditions.

Owens, C.K.R. Belore, 2004. Dispersant effectiveness testing in cold water and brash ice.
Oil Type: Alaska North Slope, Hibernia, Chayvo crudes fresh and evaporated
Dispersant Type: Corexit 9527
Water Temperature: -0.6 to 0.9 °C
Water Salinity: 32 ppt
Presence of Ice: Yes, brash ice in 4/10 and 8/10 coverage. Higher dispersion was evident in the tests with ice cover when compared to open water at the non-breaking wave, low energy conditions tested for both fresh and weathered ANS and Hibernia crude.
Energy Level: Two wave settings used. Low: 17 cm average wave height with a 5.5 second period. High: 33 cm average wave height with a 4 second period. In low ice concentrations the higher mixing energy was needed to achieve the same dispersion as in the high ice cover, low energy tests.

Payne, J.R. et al. 1985. Estimating dispersant effectiveness under low temperature-low salinity conditions
Oil Type: Alaskan Prudhoe Bay crude oil
Dispersant Type: Corexit 9550, Finasol OSR-7, EC. O ATLANT'TOL AT-7, and OFC D-609 (AT-7 and OSR-7 were significantly less effective than others under all test conditions)
Water Temperature: 1 and 10 °C
Water Salinity: 0, 18 and 33 ppt. Corexit 9550 had best performance (>50% at 1°C, 42 % at 10 °C) at 0 ppt. D-609 outperformed Corexit 9550 at 18 ppt. Corexit 9550 and D-609 were equally effective at 33 ppt.
Presence of Ice: Not studied.
Energy Level: Used an old EPA protocol test (pre- Swirling Flask)

SL Ross. 2002. Dispersant Effectiveness Testing in Cold Water and Broken Ice
Oil Type: fresh and air sparged (evaporated) Alaska North Slope and Hibernia crude oil
Dispersant Type: Corexit 9500 & 9527.
Water Temperature: -0.5 to 2.4°C in open water -0.6 to 0.9°C in ice tests.
Water Salinity: 30 to 32 ppt
Presence of Ice: Broken ice with 50 % and 80% coverage. In open water tests DE of ANS fresh and weathered to 20% loss ranged from 96 to 99%. In ice cover tests DE of fresh and 10% weathered ANS in 8/10th ice >95%, for 20% weathered ANS DE was 22% with no ice, 30% with 4/10ths ice and 54% with 8.10ths ice. Presence of larger amounts of ice increased dispersion. DOR's ranged from 1:81 to 1:31.
Energy Level: Low energy swells (7.5 inch stroke and 10, 12 and 16 cycles /min) in ice tests, standard Ohmsett breaking waves (3.0 inch wave paddle stroke and 35 cycles per minute) in open water tests. DE in open water tests on fresh, 10% and 20% weathered ANS was >96% in all cases.

ED_001324_00001497-00098

SL Ross, MAR Inc. 2003. Dispersant Effectiveness Testing on Alaskan Crude Oils in Cold Water

Oil Type: fresh and air sparged ANS, Endicott, Northstar, Cook Inlet Middle Ground Shoals and Pt. McIntyre crude oils

Dispersant Type: Corexit 9500 & 9527. Ohmsett tests used only Corexit 9527.

Water Temperature: -1.1 to -0.4 °C. In Ohmsett tests Corexit 9527 was effective on all but two fresh and weathered oils at the low temperatures (74 to 86% DE). The 29% weathered Northstar and 11% weathered Endicott had DE of only 8% and 3%, respectively.

Water Salinity: 30 ppt

Presence of Ice: Not studied.

Energy Level: SL Ross wave tank tests and Ohmsett standard dispersant effectiveness test energy level with 3.5 inch paddle stroke and 34 cycles per minute. The SL Ross wave tank tests resulted in lower DE measurements for the fresh Endicott crude oil than the Ohmsett tests (8 to 33% versus 74% at Ohmsett) likely due to energy level differences.

SL Ross. 2006. Dispersant Effectiveness Testing On Water-In-Oil Emulsions At Ohmsett.

Oil Type: fresh, air sparged and emulsions of Endicott, IFO 30, IFO 120 and Sockeye crude

Dispersant Type: Corexit 9527 and 9500.

Water Temperature: 0.6 to 3.3°C at Ohmsett, 9°C in SLR tank. Fresh Endicott dispersed 66% in SLR tank: 6% weathered and emulsion 3 and 3.3% DE. On-tank formed Endicott emulsion (~4000 cP) dispersed 10 to 35%. Mechanically formed emulsion (~6000cP) 0 to 20% DE.

Water Salinity: 30 to 32 ppt

Presence of Ice: Not studied.

Energy Level: Ohmsett standard dispersant effectiveness test energy level with 3.5 inch paddle stroke and 34 cycles per minute. SL Ross wave tank standard dispersant effectiveness test wave energy.

SL Ross & MAR Inc. 2006b. Dispersant Effectiveness Testing in Cold Water on Four Alaskan Crude Oils

Oil Type: fresh, air sparged and on tank weathered ANS, Endicott, Northstar and Pt. McIntyre crude oils

Dispersant Type: Corexit 9527.

Water Temperature: 2.8 to -2.8 °C. Dispersant was effective on all fresh and weathered oils at the low temperatures (85 to 99% DE). Weathered oil effectiveness was much higher in the 2006 vs 2003 test series possibly due to oil property differences (pour point issue?)

Water Salinity: 30 ppt

Presence of Ice: Not studied.

Energy Level: Ohmsett standard dispersant effectiveness test energy level with 3.5 inch paddle stroke and 34 cycles per minute

SL Ross, MAR Inc. 2009. Low-Dose Repeat-Application Dispersant Testing

Oil Type: ANS, Endicott, Oseberg, Rock, Ewing Bank crude oils and IFO 30 fuel oil

Dispersant Type: Corexit 9500 applied in repeat (up to 5 passes) applications of small doses (aircraft application rate) similar cumulative dispersion with multiple small doses compared to one single high dose. DE for the two Alaskan oils ranged from 88 to 95%.

ED_001324_00001497-00099

Water Temperature: 16 to 18°C.
Water Salinity: 30 ppt
Presence of Ice: Not studied.
Energy Level: SL Ross Wave tank and Ohmsett standard effectiveness tests, 3.5 inch stroke, 34 cycles/minute.

SL Ross. 2010. Literature Review of Chemical Oil Spill Dispersants and Herders in Fresh and Brackish Waters
Oil Type: various including Alaska North Slope crude
Dispersant Type : various
Water Temperature: various
Water Salinity: 0 to 40 ppt. Review concluded that dispersants designed for use in marine waters are less effective when salinity falls below 20 ppt or rises above 40 ppt. Fresh water formulations work better in fresh water conditions but provide best results in water with salinities between 10 and 20 ppt
Presence of Ice: Not studied
Energy Level: Not studied

SL Ross, MAR Inc. 2011. Comparison of Large-Scale (Ohmsett) and Small-Scale Dispersant Effectiveness Test Results
Oil Type: Endicott, ANS plus 12 other crude oils
Dispersant Type: Corexit 9500
Water Temperature: 14 to 16°C. Endicott was 95% dispersed with 1:22 DOR at Ohmsett
Water Salinity: 30 to 32 ppt
Presence of Ice: Not studied.
Energy Level: Ohmsett standard dispersant effectiveness test energy level with 3.5 inch paddle stroke and 34 cycles per minute. WSL, Exdet, SF and BF
Fresh Endicott: Ohmsett DE 95%,WSL 27%, BF 80%, SF 62%, Exdet 86%
Endicott 19% evap. : Ohmsett DE 94%,WSL 18%, BF 72%, SF 42%, Exdet 90%
ANS fresh: Ohmsett DE 98%,WSL 28%, BF 76%, SF 67%, Exdet 92%
ANS 0% evap.: Ohmsett DE 97%,WSL 27%, BF 82%, SF 49%, Exdet 88%

Smith, D., G. Holliday. 1979. API/SC-PCO Southern Californian1978 Oil Spill Test Program
Oil Type: Alaska North Slope Crude
Dispersant Type: code named H & J
Water Temperature: warm water
Water Salinity: full ocean salinity
Presence of Ice: Not studied
Energy Level: natural offshore conditions in 4 to 18 knots
Results Summary: ANS Oil was dispersible by both products.

Sorial, G.A.K.M. Karen, H. Edith, A.D. Venosa, D.W. King. 2001. Development of a rational oil spill dispersant effectiveness protocol
Oil Type: Alaskan Prudhoe Bay and South Louisiana crude oil
Dispersant Type: 18 dispersants tested, results not tied to specific dispersant

ED_001324_00001497-00100

Water Temperature: assumed to be EPA standard temp of 23 ± 3 °C
Water Salinity: synthetic sea water (salinity not reported but assumed to be BF standard of 34 ppt)
Presence of Ice: Not studied.
Energy Level: Swirling Flask and EPA Baffled Flask. Baffled Flask results were more reproducible than SFT and resulted in a significantly higher estimate of dispersant effectiveness for a given oil and dispersant combination. Higher effectiveness is attributed to a higher mixing energy in the BF test. The fresh Prudhoe Bay crude oil tested was highly dispersible in the BF tests (>80%) by 10 of the 18 dispersants tested

Sørstrøm, S.E., Per Johan Brandvik, I. Buist, P. Daling, D. Dickins, L. Faksness, S.Potter, J. Rasmussen and I. Singsaas.2010. Joint industry program on oil spill contingency for Arctic and ice covered waters: Summary Report
Oil Type: Troll B, 30 min weathered on water prior to dispersant application, 6 hour weathered, 6 and 7 day weathered
Dispersant Type: Corexit 9500 , Dasic Slickgone NS
Water Temperature: Not reported but in the presence of an ice field: ~ -1°C.
Water Salinity: not reported
Presence of Ice: Yes. Field ice coverage of 70-80%, 80-90%.
Energy Level: Low natural energy situation at pack ice edge. Energy applied by boat thrusters and high pressure water jets after dispersant application. Dispersant efficiency was estimated to be > 90% in all three tests. Oil drop sixes measured were in the 5 to 30 um range.

Strom-Kristiansen, T., P.S. Daling, A. Lewis, A.B. Nordvik. 1994. *IFP, WSL and MNS Testing*
Oil Type: Alaska North Slope, Brent Blend, Murban, Bonny Light
Dispersant Type: Corexit 9527 and 9554 and Enersperse 700 on a full range of oil properties and emulsions. Other dispersants were screened using IFP test on 200°C weathered oil
Water Temperature: All tests conducted on ANS at 13 °C
Water Salinity: All tests on ANS at 35 ppt
Presence of Ice: Not studied
Energy Level: Both IFP and MNS tests used on a range of weathered ANS oils. WSL was used on oil residues collected from meso-scale weathering tank. The ANS crude was dispersible until weathered to a viscosity of about 2 to 3000 cP. The MNS test resulted in higher DE estimates than the IFP test. Fifty percent water content emulsions of weathered ANS were dispersed with similar DE as the weathered ANS. Meso-scale (recirculating flume) weathering and dispersant effectiveness tests (using WSL and IFP tests on oil collected from the flume) were also conducted on ANS. After 4 hours of weathering the IFP test estimated 63% effectiveness after 48 hours the WSL test estimated 24% DE on oil that had lost 42% by weight and was a 69% water content emulsion.

Spring, W, T. Nedwed, R. Belore.2006. Icebreaker Enhanced Chemical Dispersion of Oil Spills.
Oil Type: Chayvo Z6, 3 to 12% weathered by weight
Dispersant Type: Corexit 9527. Dispersant applied to oil either prior to energy addition in broken ice or oil on ice tests or after ice breaker had broken ice and exposed the oil for treatment.

ED_001324_00001497-00101

Water Temperature: Air temp 0 to -10 °C
Water Salinity: 32 ppt
Presence of Ice: Ice present in 0% to 100% ice sheet. Oil spilled in broken ice, on top of a full ice sheet and under a full ice sheet.
Energy Level: SL Ross wave tank with trolling motor prop wash tests resulted in greater than 90% effectiveness in ice cover ranging from 25% to 90%. With prop wash. Waves only tests with dispersant generated 40 to 56% effectiveness. Low dose (1:110 also effective. Prop wash was effective on oil weathered for 98 hours. In AKER ice basin tests all energy was provided by the prop wash of a scale model azimuthal stern drive icebreaker. Dispersant effectiveness values of between 94% and 97% were measured in the tests. A control test with no dispersant applied resulted in DE of <20 %.Oil drop VMD (d50) of 17 to 45 μm were measured in the tests indicating effective long term dispersion. Oil did not clear the tank after many weeks of sitting with no energy addition indicating long term dispersion even under quiescent conditions.

Venosa, A.D.G.A. Sorial, D.W. King. 2001. Round-robin testing of a new EPA dispersant effectiveness protocol.
Oil Type: Alaskan Prudhoe Bay and South Louisiana crude oil
Dispersant Type: Six dispersants tested, results not tied to specific dispersant
Water Temperature: assumed to be EPA standard temp of 23 ± 3 °C
Water Salinity: synthetic sea water (salinity not reported but assumed to be BF standard of 34 ppt)
Presence of Ice: Not studied.
Energy Level: EPA Baffled Flask. Eight different labs used the Baffled Flask test to determine inter-laboratory variability in testing with this method. It was found that the BF test was much more reproducible and repeatable than the SF test. The effectiveness of the 6 dispersants on fresh Prudhoe Bay crude varied widely from a low of 10% to a high of 89%

Venosa, A., K. Lee, M. Boufadel, Z. Li, E. Wickley-Olsen, T. King, 2008. Dispersant Effectiveness as a Function of Energy Dissipation Rate in an Experimental Wave Tank.
Oil Type: fresh ANS and weathered Mesa
Dispersant Type: Corexit 9500 & Dispersit SPC 1000
Water Temperature: not reported
Water Salinity: not reported
Presence of Ice: Not studied.
Energy Level: EPA /DFO wave tank operated with regular waves, spilling and plunging breakers. Measured energy dissipation rate was similar to field measurements. Both dispersants were effective on the ANS but the Dispersit product required higher energy to achieve same dispersion as Corexit 9500.

White, D.M.; Ask, I.; Behr-Andres, C. 1999. Final Report: Effectiveness Testing for Corexit 9500 on Alaska North Slope Crude Oil in Prince William Sound Seawater at 8°C.
Oil Type: Alaska North Slope crude
Dispersant Type: Corexit 9500. Higher dose rate higher dispersant effectiveness.
Water Temperature: 8 °C.
Water Salinity: PWS sea water (31 to 32 ppt).
Presence of Ice: Not studied.

ED_001324_00001497-00102

Energy Level: modified SFT and modified EXDET (no sorbent and same extraction analysis as in SF test). Energy added 0, 12, 24, and 48 hrs after dispersant was applied. Oil was also allowed to 'weather' in the test flask between 0, 12, 24, 48, or 72 hours before dispersant application. Highest dispersion occurred with zero weathering and dispersant contact greater than 0. Lowest dispersion occurred with 0 contact time across all weathering. If oil weathers it is less dispersible. If dispersant does not have a chance to mix with oil before energy is applied, poor dispersion.

Wrenn, B., A. Virkus, B. Mukherjee, A. Venosa. 2009. Dispersibility of crude oil in fresh water. Environmental Pollution 157 (2009) 1807-1814.
Oil Type: weathered Mars crude
Dispersant Type: various experimental formulations
Water Temperature: not reported, assumed room temperature and not varied
Water Salinity: All tests conducted using fresh (0 ppt) water. Objective was to develop a dispersant formulation that would provide good effectiveness in fresh water. Objective was met for the single oil tested but authors indicate many other oils would need to be tested to ensure that the dispersant formulation worked over a range of oils
Presence of Ice: Not studied
Energy Level: EXDET test with no variation in energy level.

ED_001324_00001497-00103