UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY AND POLLUTION
PREVENTION

MEMORANDUM

DATE: 6/09/14

SUBJECT: Fluensulfone Toxicity Studies Data Evaluation Records.

PC Code: 050410
Decision No.: 464527
Petition No.: 2F8019
Risk Assessment Type: N/A
TXR No.: 0056984
MRID No.: See Table

DP Barcode: D420475
Registration Nos.: 11678-TG, 66222-EUG
Regulatory Action: Section 3
Case No.: N/A
CAS No.: 318290-98-1
40 CFR: New Active Ingredient

FROM: Jaime D'Agostino, Toxicologist
Risk Assessment Branch II
Health Effects Division (7509 P)

THRU: Christina Swartz, Chief
Risk Assessment Branch II
Health Effects Division (7509 P)

TO: Jennifer Gaines/Reuben Baris (Team 7)
Insecticide/Rodenticide Branch
Registration Division (7505P)

I. CONCLUSION: Risk Assessment Branch II (RAB II) prepared final data evaluation records reflecting the global partners' current evaluation (or in the case of a disagreement, HED's current evaluation) of the toxicity studies submitted to satisfy data requirements associated with the Section 3 registration for the technical active ingredient fluensulfone (please see table below).

II. ACTION REQUESTED: Risk Assessment Branch II (RABII) is currently evaluating a petition to register the nematicide fluensulfone for use on cucurbit vegetables (Crop Group 9) and fruiting vegetables (Crop Group 8-10) and to establish permanent tolerances for residues resulting from those uses. A risk assessment is being prepared as part of that action. As part of

the process, the Health Effects Division (HED) was asked to provide secondary review and prepare final data evaluation records reflecting the global partners' current evaluation (or in the case of disagreement, HED's current evaluation) of the toxicity studies submitted to satisfy data requirements associated with the section 3 registration for the technical ingredient fluensulfone (please see table below).

III. BACKGROUND: Fluensulfone {5-Chloro-2-[(3,4,4-trifluoro-3-buten-1-yl)sulfonyl]thiazole} is the first nematicide in the fluoroalkenyl class. It is currently undergoing global joint review [review partners are the United States Environmental Protection Agency (U.S. EPA), the Pest Management Regulatory Agency (PMRA) of Canada and the Australian Pesticides and Veterinary Medicines Authority (APVMA)].

The registrant, Makhteshim-Agan of North America, submitted numerous toxicity studies to fulfill the requirements for registration. The toxicity studies were reviewed by PMRA with secondary review from EPA and APVMA. After reviewing the studies, RAB II prepared final data evaluation records reflecting the global partners' conclusions with respect to the overall findings as well as the acceptability of the studies; in the case of disagreement regarding study conclusions, the attached DERs reflect HED's evaluation of the toxicity studies.

Study Type	MRID #	Classification
870.1100: Acute Oral Toxicity Technical (2009)	48574746	Acceptable/guideline
870.1100: Acute Oral Toxicity Technical (2010)	48574745	Acceptable/guideline
870.1100: Acute Oral Toxicity 480 EC (2009)	48574918	Acceptable/guideline
870.1100: Acute Oral Toxicity 480 EC (2011)	48574924	Acceptable/guideline
870.1100: Acute Oral Toxicity Thiazole Sulfonic Acid	48574783	Acceptable/guideline
870.1100: Acute Oral Toxicity Methyl Sulfone	48574787	Acceptable/guideline
870.1100: Acute Oral Toxicity Butene Sulfonic Acid	48574792	Acceptable/guideline
870.1200: Acute Dermal Toxicity Technical	48574747	Acceptable/guideline
870.1200: Acute Dermal Toxicity 480 EC	48574919	Acceptable/guideline
870.1300: Acute Inhalation Toxicity Technical	48574748	Acceptable/guideline
870.1300: Acute Inhalation Toxicity EC 480	48574920	Acceptable/guideline
870.2400: Primary Eye Irritation Technical	48574750	Acceptable/guideline
870.2400: Primary Eye Irritation EC 480 (2009)	48574922	Acceptable/guideline
870.2400: Primary Eye Irritation EC 480 (2011)	48574926	Acceptable/guideline
870.2500: Primary Dermal Irritation Technical	48574749	Acceptable/guideline
870.2500: Primary Dermal Irritation EC 480 (2009)	48574921	Acceptable/guideline
870.2500: Primary Dermal Irritation EC 480 (2011)	48574925	Acceptable/guideline
870.2600: Dermal Sensitization Technical	48574751	Acceptable/guideline
870.2600: Dermal Sensitization EC 480	48574923	Acceptable/guideline
870.3050: 28-day Oral Mouse	48575024	Acceptable/guideline
870.3050: 28-day Oral Rat	48575023	Acceptable/guideline
870.3100: 90-day Oral Mouse	48574753	Acceptable/guideline
870.3100: 90-day Oral Rat	48574754	Acceptable/guideline
870.3150: 90-day Oral Dog	48574755	Acceptable/guideline
870.3200: 21/28-day Dermal	48574780	Acceptable/guideline
870.3465: 90-day Inhalation	49067003	Acceptable/guideline

Study Type	MRID #	Classification
870.3700: Prenatal Developmental Toxicity Rabbit	48574770	Acceptable/guideline
870.3700: Prenatal Developmental Toxicity Rat	48574771	Acceptable/guideline
870.3800: Reproduction and Fertility Effects	48574769	Acceptable/guideline
870.4100: Chronic Toxicity Dog	48574756	Acceptable/guideline
870.4100: Chronic Toxicity Rat	48574765	Acceptable/guideline
870.4200: Carcinogenicity Mouse	48574766	Acceptable/guideline
870.5100: Bacterial Reverse Mutation Technical	48574759	Acceptable/guideline
870.5100: Bacterial Reverse Mutation Technical	48574760	Acceptable/guideline
870.5100: Bacterial Reverse Mutation Technical	48574761	Acceptable/guideline
870.5100: Bacterial Reverse Mutation Thiazole Sulfonic Acid	48574784	Acceptable/guideline
870.5100: Bacterial Reverse Mutation Methyl Sulfone	48574788	Acceptable/guideline
870.5100: Bacterial Reverse Mutation Butene Sulfonic Acid	48574793	Acceptable/guideline
870.5300: Mammalian Gene Mutation Technical	48574763	Acceptable/guideline
870.5300: Mammalian Gene Mutation Methyl Sulfone	48574789	Acceptable/guideline
870.5375: Chromosomal Aberration Technical	48574762	Acceptable/guideline
870.5375: Chromosomal Aberration Thiazole Sulfonic Acid	48574785	Acceptable/guideline
870.5375: Chromosomal Aberration Butene Sulfonic Acid	48574794	Acceptable/guideline
870.5395: In vivo Micronucleus Assay Technical	48574764	Acceptable/guideline
870.5395: In vivo Micronucleus Assay Thiazole Sulfonic Acid	48574786	Acceptable/guideline
870.5395: In vivo Micronucleus Assay Methyl Sulfone	48574791	Acceptable/guideline
870.5395: In vivo Micronucleus Assay Butene Sulfonic Acid	48574795	Acceptable/guideline
870.5555: Unscheduled DNA Synthesis Methyl Sulfone	48574790	Acceptable/guideline
870.6200: Acute Neurotoxicity	48574773	Acceptable/guideline
870.6200: 90-day Neurotoxicity	48574774	Acceptable/guideline
870.7485: Metabolism Rat Single Oral Dose	48574742	Acceptable/guideline
870.7485: Metabolism Rat Repeat Oral Dose	48574744	Acceptable/guideline
870.7485: Metabolism Rat Pharmacokinetics	48574741	Acceptable/guideline
870.7485: Metabolism Rat Tissue Distribution	48574743	Acceptable/guideline
870.7600: Dermal Absorption Rat	48574949	Acceptable/guideline
870.7800: Imunnotoxicity	48574779	Acceptable/guideline
Non-guideline: 28-day Oral Dog	48574752	Acceptable/non-guideline
Non-guideline: 14-day Effect on ALAT Dog	48574776	Acceptable/non-guideline
Non-guideline: Determination of ALAT in Untreated Dogs	48574778	Acceptable/non-guideline
Non-guideline: In Vitro Mode of Action for Decrease of ALAT in Dog Microsomes	48574777	Acceptable/non-guideline
Non-guideline: Mode of Action for Decrease of ALAT in Dogs	48574775	Acceptable/non-guideline

Study Type	MRID #	Classification
Non-guideline: Mechanistic Lung Toxicity in Mice	48574767	Acceptable/non-guideline
Non-guideline: Comparative Biotransformation in Human and Mice Microsomes	48574768	Acceptable/non-guideline
Non-guideline: Comparative Dermal Absorption	48574950	Acceptable/non-guideline
Non-guideline: Acute Neurotoxicity Time to Peak of Effect	48574772	Acceptable/non-guideline
Non-guideline: 28-day Oral Rat Thiazole Sulfonic Acid	49387601	Acceptable/non-guideline
Non-guideline: 28-day Oral Rat Butene Sulfonic Acid	49387602	Acceptable/non-guideline

Tier II Summary (PMRA #2181195, MRID 48574746) revised by the regulatory authorities July, 2013

Study Type: Acute Oral Toxicity - Rat

IIA 5.2.1 – MRID 48574746, PMRA 2181195

Report:	Simon C. (2009) MCW-2 Tech. Acute Oral Toxicity Study in Rats Harlan Laboratories Ltd., Switzerland; unpublished report No. C14933, dated 25 June 2009. Sponsor reference No. R-23417 Dates of experimental work: 25 September to 22 October 2008
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 423, EC method B.1 tris and OPPTS 870.1100 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary:

In an acute oral toxicity study performed according to the Acute Toxic Class Method, three groups, each of 3 fasted female HanRcc:WIST (SPF) rats, were treated with fluensulfone (MCW-2 tech.; 96.78% a.i.) by oral gavage at a dose of 2000 mg/kg bw or 300 mg/kg bw. PEG 300 was used as the vehicle and dosing volume was 10 mL/kg bw.

Two animals treated at 2000 mg/kg bw were found dead on test day 2; all other animals survived until the end of the study period. Slightly ruffled fur, slight sedation, slightly poor coordination, hunched posture and almost to completely closed eyes were the clinical signs associated with administration of the test item, present in the surviving animal of the 2000 mg/kg bw dose group up to day 5, and in animals dosed with 300 mg/kg bw only on the day of treatment.

The only surviving female among those treated at 2000 mg/kg bw lost 4% of body weight during the first week after treatment but recovered by the end of the study and its body weight at study termination was considered within the range commonly recorded for rats of this strain and age. Distended stomach with liquid contents was noted at necropsy of decedent animals; otherwise, no macroscopic findings were recorded in the surviving animals at necropsy.

The median lethal oral dose of MCW-2 tech. was calculated to be 300 mg/kg bw < LD_{50} (female rat) < 2000 mg/kg bw (LD_{50} cut-off value: 1000 mg/kg bw). According to the acute toxicity classification criteria for PMRA, MCW-2 tech. is of slight toxicity via the oral route. This hazard classification warrants the signal word and hazard statement “Caution – Poison” on the Canadian

label. Based on the EPA classification criteria, MCW-2 tech. falls under Category II for acute oral toxicity. Under OCS criteria for acute toxicity endpoints, the test material is considered to be of low acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 tech. (fluensulfone)

Description:	Solid
Lot/Batch:	36372130-291-PF1
Purity:	96.78%
CAS#:	318290-98-1
Stability:	Stable until 31 May 2009

2. Vehicle: Polyethylene glycol 300 (PEG 300)

3. Test animals:

Species:	Rat
Strain:	HanRcc: WIST(SPF) – females
Age:	At dosing: 11 weeks
Weight at dosing:	179.6 – 202.7 g
Source:	Harlan Laboratories Ltd., Laboratory Animal Services, Switzerland
Acclimation period:	1 week
Diet:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum* (except for the overnight fasting period prior to test substance administration and approximately 3-4 hours post dose)
Water:	Community tap water from Füllinsdorf *ad libitum*
Housing:	In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding (‘Lignocel’ Schill AG, Switzerland)

4. Environmental conditions:

Temperature:	22 ± 3 °C
Humidity:	30-70%
Air changes:	10-15 air changes per hour

Photoperiod: Light cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS:

1. In life dates: 25 September to 22 October 2008

2. Animal assignment and treatment

Animals were selected by hand at time of delivery. Animals 1-3 were assigned to group 1 and treated at 2000 mg/kg bw; animals 4-6 were assigned to group 2 and treated at 300 mg/kg bw; animals 7-9 were assigned to group 3 and treated at 300 mg/kg bw. Animals received a single oral dose by gavage following a 16 to 18 hour fast. Animals were provided with food approximately 3 hours post dose.

3. Observations

Animals were observed for mortality, viability, and clinical signs daily during the acclimation period, during the first 30 minutes and at 1, 2, 3 and 5 hours post-dose, and then twice daily during days 2-15. Body weights were measured on test days 1 (prior to dosing), 8 and 15.

4. Preparation of the test substance

The test substance was pulverized into a fine powder, weighed, and then mixed with the vehicle. Administered test material was prepared shortly prior to dosing. Homogeneity was maintained using a magnetic stirrer.

5. Statistics

No statistical method was used.

II. RESULTS AND DISCUSSION

A. MORTALITY

Two animals in the 2000 mg/kg bw dose group were found dead on test day 2. All other animals survived until the end of the study.

Dose (mg/kg bw)	Females	
	Mortality	Time of death
300	0 / 6	NA
2000	2 / 3	Day 2

No. of dead / No. of treated; NA: not applicable
Data obtained from pages 23-25 of the study report.

B. CLINICAL OBSERVATIONS

Slightly ruffled fur, slight sedation, hunched posture and almost to completely closed eyes were noted in all 3 animals dosed at 2000 mg/kg bw on the day of dosing. The only surviving animal continued to show ruffled fur until day 5. In addition, slightly poor coordination was noted for this animal 5 hours after dosing on day 1 and on day 2 of the study. No clinical signs were noted from day 6.

Similar signs were noted in the 6 animals treated at 300 mg/kg bw: almost to completely closed eyes were observed approximately 20 minutes after dosing and persisted up to the 3- or 5-hour observation; slight sedation was recorded approximately 20 minutes post-dose and persisted up to the 5-hour observation; slightly poor coordination was visible in five females 1 or 2 hours post-dosing and persisted up to the 5-hour observation; slight to moderate salivation was noted in 3 females from approximately 20 minutes, 1 or 2 hours after dosing up to the 3-hour observation. Additionally, slightly ruffled fur was observed approximately 20 minutes after dosing up to the 5-hour observation in one female and at the 5-hour observation in 3 females. All animals were free of clinical signs by day 2.

C. BODY WEIGHT

Treatment affected body weight of the only surviving female among those treated at 2000 mg/kg bw, which lost 4% of its body weight during the first week after treatment but recovered by the end of the study. The body weight of this animal at study termination was considered within the range commonly recorded for rats of this strain and age. Body weights of all animals at 300 mg/kg bw were within the range commonly recorded for this age and strain of animal.

D. NECROPSY

Distended stomach with liquid contents was noted at necropsy of decedent animals. No abnormalities were detected at necropsy of animals surviving to termination.

III. APPLICANT'S CONCLUSIONS

The median lethal dose of MCW-2 tech. was calculated to be 300 mg/kg bw < LD50 (female rat) < 2000 mg/kg bw (LD_{50} cut-off value: 1000 mg/kg bw).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments**: This acute oral toxicity study in the rat is classified as acceptable (fully reliable) and satisfies the guideline requirement for an acute oral toxicity study (OPPTS 870.1100; OECD 423) in rats. Another acute toxicity study in rats provided a more definitive LD_{50} value (study report 24555, PMRA #2181196).

C. **Conclusions**: The median lethal dose of MCW-2 tech. (fluensulfone) was calculated to be 300 mg/kg bw < LD_{50} (female rat) < 2000 mg/kg bw (LD_{50} cut-off value: 1000 mg/kg bw). According to the acute toxicity classification criteria for PMRA, MCW-2 tech. is of slight toxicity (based on the oral LD_{50} cut-off value in female rats), and based on the EPA classification criteria, MCW-2 tech. falls under Category II for acute oral toxicity. Under OCS criteria for acute toxicity endpoints, the test material is considered to be of low acute oral toxicity.

D. Deficiencies: None.

Tier II Summary (PMRA #2181196, MRID 48574745) revised by the regulatory authorities July, 2013

Study Type: Acute oral toxicity

IIA 5.2.1 - MRID 48574745, PMRA 2181196

Report:	Haferkorn J. (2010) Acute oral toxicity study of MCW-2 technical in rats Laboratory of Pharmacology and Toxicology GmbH & Co. KG (LPT), Germany; unpublished report No. 24555, dated 3 June 2010. Sponsor reference No. R-23373 Dates of experimental work: 29 July to 1 October 2009
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Isreal
Guidelines:	OECD 423, EC method B.1 tris, OPPTS 870.1100 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary:

In an acute oral toxicity study performed according to the Acute Toxic Class Method, groups of 3 fasted female Wistar rats, were treated with fluensulfone (MCW-2 tech., 96.5% a.i.) by oral gavage at doses of 2000, 1500 or 300 mg/kg bw. 0.8% aqueous hydroxypropylmethylcellulose was used as the vehicle and dosing volume was 10 mL/kg bw. In total, 3 animals were treated with 2000 mg/kg bw of fluensulfone, and 6 animals each were treated with 300 or 1500 mg/kg bw.

Death occurred in 2 animals treated at 2000 mg/kg bw and in all 6 animals treated at 1500 mg/kg bw. One animal treated at 2000 mg/kg bw as well as all 6 animals treated at 300 mg/kg bw survived until the end of the study period. Ataxia, reduced mobility and reduced muscle tone were the most commonly recorded clinical signs. All surviving animals gained the expected weight throughout the study period. No abnormality was recorded at necropsy of either decedent or surviving rats.

Using the Probit analysis according to Finney, the oral LD_{50} of fluensulfone was calculated to be 671 mg/kg bw.

According to the acute toxicity classification criteria for the PMRA, MCW-2 tech. is moderately acutely toxic via the oral route based on the LD_{50} value of 671 mg/kg bw in female rats. This hazard classification warrants the signal word and hazard statement “Warning – Poison” on the Canadian label. Based on the EPA classification criteria, MCW-2 tech. falls under Category III for acute oral toxicity (signal word “Caution”). Under OCS criteria for acute toxicity endpoints, the test material is considered to be of low acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 technical (Fluensulfone)

Description: Yellow crystalline solid as identified by the sponsor; brown solid as identified by the laboratory

Lot/Batch: 36372130-291-PF1

Purity: 96.5%

CAS#: 318290-98-1

Stability: Stable until January 2010

2. Vehicle: 0.8% aqueous hydroxypropylmethylcellulose (Methocel)

3. Test animals:

Species: Rat

Strain: Wistar Crl:WI - females

Age: Approx. 7 weeks at beginning of acclimation

Weight at dosing: 167 - 189 g

Source: Charles River Laboratories, Germany

Acclimation period: At least 5 days

Diet: ssniff® R/M-H V1534 commercial diet (ssniff Spezialdiäten, Germany) *ad libitum* (except for approx 16 hrs fasting period prior to test substance administration and approximately 2-4 hours post dose)

Water: Drinking water *ad libitum*

Housing: In groups of three in Makrolon type-3 plus cages with granulated texture wood as bedding (Granulat 2, J. Brandenburg, Germany)

4. Environmental conditions:

Temperature: 22 ± 3 °C

Humidity: 55 ± 15%

Air changes: Not reported

Photoperiod: Light cycle of 12 hours light and 12 hours dark

B. STUDY DESIGN AND METHODS:

1. In life dates: 29 July to 1 October 2009

2. Preparation of the test substance

Fluensulfone was suspended in the vehicle, 0.8% aqueous hydroxypropylmethylcellulose (Methocel).

3. Animal assignment and treatment

The report does not indicate how animals were assigned to groups. Female animals were administered a single oral dose of test material by gavage. Testing was started at 2000 mg/kg bw. As two animals died, 300 mg/kg bw was tested in two subsequent steps. As no animals died, two further steps at 1500 mg/kg bw were conducted in order to allow a more accurate calculation of the LD_{50}.

4. Observations

Animals were observed once daily for morbidity and mortality. Observations of clinical signs took place before dosing, and immediately, 5, 15, 30 and 60 minutes, post dose as well as at 3, 6 and 24 hours. All surviving animals were observed for a period of 14 days. Body weights were measured prior to test substance administration and weekly thereafter.

5. Statistics

Probit method of Finney. Results from the 2000 mg/kg bw (mortality 2/3) were not included in the calculation.

II. RESULTS AND DISCUSSION

A. MORTALITY

The two deaths of animals in the 2000 mg/kg bw dose group occurred within the first 24 hours post dose. Five of the six deaths which occurred at 1500 mg/kg bw also occurred within 24 hours following administration of test material. The sixth animal to die on study from this group did so on test day 6. All animals in the 300 mg/kg bw dose group survived to study termination.

Dose (mg/kg bw)	Females	
	Mortality	Time of death
300	0 / 6	NA
1500	6 / 6	5, 24 hr post dose 1 found dead on day 6
2000	2 / 3	24 hr post dose

No. of dead / No. of treated; NA: not applicable; hr: hours

Data obtained from page 15 of the study report

B. CLINICAL OBSERVATIONS

Slight ataxia and slightly reduced motility were observed in 3 of the 6 animals treated at 300 mg/kg bw on the day of dosing (6 hr post dose); animals at this dose level appeared normal by the day following the treatment.

Slight to moderate ataxia, slightly to moderately reduced motility and slightly reduced muscle tone were noted in all 6 animals dosed at 1500 mg/kg; 5 of these animals died within 24 hours post dose and the remaining animal was found dead on day 6.

Slight ataxia, slightly reduced motility and slightly reduced muscle tone were observed in all 3 animals treated at 2000 mg/kg bw; 2 of these animals died within 24 hours post dose while the remaining one appeared normal.

C. BODY WEIGHT

Treatment had no effect on body weight of surviving animals

D. NECROPSY

No abnormality was recorded at necropsy of either decedent or surviving rats.

III. APPLICANT'S CONCLUSIONS

The median lethal oral dose of fluensulfone was calculated to be 671 mg/kg bw.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments:** This acute oral toxicity study in the rat is classified as acceptable (fully reliable) and satisfies the guideline requirement for an acute oral toxicity study (OPPTS 870.1100; OECD 423) in rats. Another acute toxicity study in rats was also provided (study report C14933, PMRA #2181195).

C. **Conclusions:** The median lethal oral dose of MCW-2 tech. was calculated to be 671 mg/kg bw. According to the acute toxicity classification criteria for PMRA, MCW-2 tech. (fluensulfone) is of moderate toxicity via the oral route (based on the calculated oral LD_{50} value in female rats), and based on the EPA classification criteria falls under Category III for acute oral toxicity. Under OCS criteria for acute toxicity endpoints, the

test material is considered to be of low acute oral toxicity.

D. **Deficiencies:** The method of animal group selection (i.e. randomization) was not specified in the study report; however, this is not expected to have influenced study results.

Note: The Tier II Summary did not include a full review of this study. This review was prepared by the Pest Management Regulatory Agency, December 18, 2012.

Study Type: Acute Oral Toxicity - Rat OPPTS 870.1100; OECD 423.

Test Material (purity): MCW-2 480 EC (497 g a.i. /L)

Citation: Arcelin, G. (2009). Acute Oral Toxicity Study in Rats. Harlan Laboratories Ltd, Switzerland. Laboratory report number: C45364. Study report date: 13-Oct-2009. Applicant Report Number R23425. DACO 4.6.1. PMRA 2181350.

Sponsor: Makhteshim Chemical Works Ltd., Israel

MRID: 48574918

Executive Summary: In an acute oral toxic class study, 11 week old fasted RccHan:WIST (SPF) rats (6♀/dose) were given a single oral dose of MCW-2 480 EC (497 g a.i. /L) in purified water by gavage at doses of 300 or 2000 mg/kg bw and observed for 14 days.

Oral LD_{50} Females: 300 mg/kg bw < LD_{50} < 2000 mg/kg bw (LD_{50} cut off = 1000 mg/kg bw)

According to the acute toxicity classification criteria for the PMRA, MCW-2 480 EC is of **Slight Toxicity** via the oral route of exposure based on the LD_{50} cut off of 1000 mg/kg bw. This hazard classification warrants the signal word and hazard statement "Caution – Poison" on the Canadian label. According to the US EPA criteria, MCW-2 EC would be assigned to category III, signal word "Caution." Under OCS criteria for acute toxicity endpoints, the test material is considered to be of low acute oral toxicity.

Three out of six animals treated with 2000 mg/kg bw of the test material were found dead twenty-four hours post dose, and one animal had to be killed in extremis 28 hours following dosing. All other animals in this dose group survived to study termination. Clinical signs of toxicity observed in some or all of the animals in the high dose group not surviving to study termination include: poor coordination, ventral recumbancy, colourless urine, cloudy eyes, hypothermia, yellowish discoloured ears, cyanosis, and no reflexes. Clinical signs of toxicity observed in all animals in the high dose group, from 0.5 to 5 hours post dose and persisting until Day 5 or 6, include: slight to moderate sedation, ruffled fur, hunched posture and eyes almost to completely closed. Clinical signs of toxicity were observed in animals in the 300 mg/kg bw dose group on the day of dosing (Day 1) and Day 2, including: ruffled fur, sedation, hunched posture, and almost to completely closed eyes. No clinical signs of toxicity were noted in any animals in this dose group from Day 3 to study termination.

There were no treatment related effects noted on body weight and no treatment related macroscopic observations.

This acute oral study is classified acceptable (fully reliable). This study satisfies the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 423) in the rat.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. Materials and Methods

A. Materials

Test material:	MCW-2 480 EC	
Description:	Clear, orange liquid; stable under storage conditions	
Lot/Batch #:	286-010209-01	
Percent active:	497 g a.i./L	
Vehicle:	Purified, deionized water prepared by Harlan Laboratories Ltd.	
Test species:	Rat	
Strain:	RccHan:WIST (SPF)	
Age at dosing:	11 weeks	
Weight at dosing:	177.9 – 214.1 g females	
Source:	Harlan Laboratories B.V., The Netherlands	
Housing:	In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ("Lignocel" J.Rettenmaier & Söhne GmgH&CoKG, Switzerland)	
Diet:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum* (except for the overnight fasting period prior to intubation and approximately 3-4 hours post dose)	
Water:	Community tap water from Füllinsdorf *ad libitum*	
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	22 ± 3 degrees C 30 - 70% 10-15 / hr 12 hours dark/12 hours light
Acclimation:	6 – 8 days	

B. Study design and methods

1. Study experimentation dates -	Start:	Group 1: 27- May-2009 Group 2: 29-May-2009 Group 3: 17-Jun-2009 Group 4: 23-Jun-2009	End:	Group 1: 10-Jun-2009 Group 2: 12-Jun-2009 Group 3: 1-Jul-2009 Group 4: 07-Jul-2009

2. Animal assignment and treatment - Animals were selected by hand at the time of delivery and assigned to the test groups noted in Table 1. Animals were dosed using a stepwise procedure in which groups of three animals were dosed at one time (4 groups total), beginning with the 2000 mg/kg bw dose level. Following a fast of 17 to 19 hours (access to water was permitted), animals were given a single dose of MCW-2 480 EC by gavage at 300 or 2000 mg/kg bw. The test material was mixed with purified water at a concentration of 0.2 g/ml or 0.03 g/ml and the dosing volume was 10 mL/kg bw. Animals were observed for clinical signs of toxicity and

mortality/viability daily during the acclimation period, during the first 30 minutes, and at 1, 2, 3, and 5 hours post dose on day 1. From days 2-15, animals were observed twice daily for mortality/viability and once daily for clinical signs of toxicity. Animals were weighed on test days 1 (prior to dosing), 8 and 15. Macrocsopic examinations were performed on all animals.

Table 1. Mortality

Dose (mg/kg bw)	Group	Mortality/animals treated
2000	1	1/3
2000	2	3/3
300	3	0/3
300	4	0/3

Data obtained from pages 24 – 27 of study report.

3. Statistics – No statistical analyses were conducted.

II. Results and Discussion

A. Mortality is given in Table 1. Three out of six animals treated with 2000 mg/kg bw of the test material were found dead one day after dosing, and one had to be killed in extremis 28 hours following dosing. The remaining two animals in the high dose group and all animals dosed with 300 mg/kg bw of the test material survived to study termination.

B. Clinical observations – All animals in the 2000 mg/kg bw dose group showed slight to moderate sedation, ruffled fur, hunched posture and eyes almost to completely closed during day 1 (from 0.5 hours to 5 hours post dose). The two animals in this dose group that survived to study termination continued to have ruffled fur up to days 4 or 5 post dose, and hunched posture until day 2 or 3. These two animals showed no other clinical signs of toxicity from days 5 or 6 onward. The animals in the high dose group that died on study had poor coordination anywhere from 2 to 5 hours post dose and ventral recumbancy from 2 to 28 hours post dose. Animal 6, who was killed in extremis 28 hours post dose, also had colourless urine, cloudy eyes, hypothermia, yellowish discoloured ears, cyanosis and no reflexes.

Clinical signs of toxicity observed in animals in the 300 mg/kg bw dose group included sedation (day1, between 0.5 to 5 hours post dose), ruffled fur (days 1 and 2), hunched posture (day 1), and almost to completely closed eyes (day 1). No clinical signs of toxicity were noted in any of the animals in this dose group from Day 3 to study termination.

C. Body Weight – No treatment related effects on body weight were noted. All animals surviving to study termination gained weight during the study.

D. Necropsy – No macroscopic findings were noted in any animal surviving to study

termination. Some observations were noted in decedents: animal 1 had light red discolouration of the liver; animal 4 had a yellowish coloured duodenum which was distended with gas and filled with yellowish content, and animal 5 had a yellowish duodenum distended with gas and yellowish coloured contents, as well as a yellowish coloured ileum and jejunum. The urinary bladder of animal 5 was also distended with urine and red coloured contents. Animal 6 had a stomach distended with yellow coloured contents, and a urinary bladder distended with urine and reddish contents.

E. <u>Investigator's Conclusions</u> – "The median lethal dose of MCW-2 480 EC after single oral administration to female rats, observed over a period of 14 days is:
300 mg/kg bw < LD_{50} (female rat)< 2000 mg/kg bw."

F. <u>Reviewer's Conclusions</u> –

Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

The reviewer is in agreement with the conclusions of the study report. According to the classification scheme outlined in OECD Test Guideline 423, the results of this study indicate an LD_{50} cut-off value of 2000 mg/kg bw (based on the flow chart in Annex 2d of OECD Test Guideline 423 and the death of 1/3 animals at the first step at the 2000 mg/kg bw dose level). However, based on the overall mortality rate of 4/6 animals at 2000 mg/kg bw, the LD_{50} cut-off is considered to be 1000 mg/kg bw.

Based on the acute toxicity classification system of the PMRA, MCW-2 480 EC is of **SlightToxicity** via the oral route based on the LD_{50} cut off of 1000 mg/kg bw. According to the US EPA criteria, MCW-2 EC would be assigned to category III, signal word "Caution." Under OCS criteria for acute toxicity endpoints, the test material is considered to be of low acute oral toxicity.

G. <u>Deficiencies</u> – Necropsy results in the study report state the following: "In the two surviving animals treated with 2000 mg/kg bw the recording of possible macroscopic abnormalities was omitted (Animal 3), and for the other (Animal 2), no macroscopic findings were recorded" (page 21 of study report). This statement seems to indicate that any abnormalities which may have been noted for Animal # 3 were simply excluded or left out of the report. This issue is not deemed to have affected the conclusions drawn in the study.

Tier II Summary (PMRA #2181380, MRID 48574924) revised by the regulatory authorities July, 2013

Study Type Acute oral toxicity

IIIA 7.1.1 – MRID 48574924, PMRA 2181380

Report: Mallaun, M. (2011)
MCW-2 480 EC: Acute Oral Toxicity Study in Rats
Harlan Laboratories Ltd., Switzerland; unpublished report No. D23934, Sponsor No. R-27473 dated 18 May 2011
Dates of experimental work: 15 to 31 March 2011

Sponsor: Makhteshim Chemical Works Ltd., Beer-Sheva, Israel

Guidelines: OECD 423, EEC method B1.tris and OPPTS 870.1100

Deviations: None

GLP/Compliance: Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary: In an acute oral toxicity study performed according to the Acute Toxic Class method, one group of three, followed by another group of three female RccHan: WIST(SPF) rats, were treated with MCW-2 480 EC (473.6 g/L) by oral gavage at a dose of 2000 mg/kg bw. The test substance was formulated in purified water at a concentration of 0.2 g/mL and administered at a dosing volume of 10 mL/kg bw. Treatment was followed by an observation period of 14 days.

No deaths occurred during the study.

All animals showed slight to severe clinical signs on test day 1 including swaying gait, dragging of front and/or rear limbs, decreased activity, ruffled fur and a hunched posture. All signs reverted by test day 2 and were no longer present up to test day 15, the end of the observation period.

Body weight gain was within the range commonly recorded for rats of the strain and age used in this study.

No macroscopic findings were noted at necropsy.

The LD_{50} of MCW-2 480 EC after single oral administration to female rats was greater than 2000 mg/kg bw.

MCW-2 480 EC is not classified under GHS CLP.

According to US EPA criteria, MCW-2 480 EC is assigned to category III, signal word 'Caution'.

According to the acute toxicity classification criteria for the PMRA, MCW-2 480 EC is of low acute toxicity via the oral route.

Under OCS criteria for acute toxicity endpoints, the test material is considered to be of low acute oral toxicity.

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	MCW-2 480 EC
	Description:	Clear orange liquid
	Batch No.:	371-210110-02
	Nominal content:	480 g/L
	Measured content:	473.6 g/L
	Expiry date:	24 January 2012
	Stability:	Stable under storage conditions
2.	**Vehicle:**	Purified water prepared at Harlan Laboratories Ltd. (deionised water which was processed and treated by the PURELAB Option-R unit. This latter links four purification technologies: reverse osmosis, adsorption, ion-exchange and photo oxidation).
3.	**Dose formulation**	The test material was formulated in purified water at a concentration of 20% (weight:weight), which resulted in a white, milky suspension that was considered as suitable for oral application.
4.	**Test animals**	
	Species:	Rat
	Strain:	Wistar [RccHan: WIST(SPF)]
	Age:	10 weeks
	Weight at dosing:	Females: 172.1 g – 192.4 g
	Source:	Harlan Laboratories B.V., The Netherlands
	Acclimation period:	At least 5 days
	Diet:	Pelleted Teklad Rat-Mouse Diet 2914C (Provimi Kliba AG, Switzerland) *ad libitum* (except for the overnight fasting period prior to intubation and approximately 3-4 hours post dose)
	Water:	Community tap water from Itingen *ad libitum*

Housing: In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' J. Rettenmaier & Söhne GmbH & CoKG, Germany, imported by Provimi Kliba AG, Switzerland), including paper enrichment (Enviro-dri from Lillico Biotechnology, UK)

5. Environmental conditions

Temperature: 22 ± 3°C

Humidity: 30-70%

Air changes: 10-15 per hour

Photoperiod: Cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS

1. In life dates: Group 1: 15 to 29 March 2011

Group 2: 17 to 31 March 2011

2. Animal assignment and treatment:

All animals were selected by hand at time of delivery. One group of three animals was dosed, followed by the second group of three. Animals received a single dose of the test substance by oral gavage at 2000 mg/kg bw after being fasted for approximately 18 to 19 hours (access to water was permitted). Food was provided again approximately 3 hours after dosing. The dosing volume was 10 mL/kg bw.

3. Observations:

Animals were checked daily for viability/mortality and clinical signs during the acclimation period, once prior to treatment, within 30 minutes of dosing, and at approximately 1, 2, 3 and 5 hours after test substance administration on test day 1. During days 2-15, animals were examined twice daily for morbidity and once daily for clinical signs. Body weights were taken on test day 1 (prior to dosing), 8 and 15. At study termination, all animals were sacrificed by carbon dioxide asphyxiation and macroscopic examinations were performed.

4. Statistics

No statistical analysis was used.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred during the course of the study.

2000 mg/kg bw
0 / 6

No. of mortalities / No. of treated animals
Data obtained from pages 19 and 20 of the study report.

B. CLINICAL OBSERVATIONS

Results for clinical observations are summarized in Table 1. Slightly swaying gait was observed in 3 animals within 30 minutes and in all animals 1 hour after treatment, and persisted until 3 hours to 5 hours after treatment. Dragging of front and/or rear limbs was observed in all 6 animals 1 hour after treatment and persisted until 3 hours to 5 hours after treatment. Slightly to severely decreased activity was observed in 3 animals within 30 minutes and in all animals 1 hour after treatment and persisted until 5 hours after treatment. Slight to marked ruffled fur was observed in 1 animal 2 hours post treatment and in 2 animals 3 hours after treatment, which persisted until 5 hours after treatment. Hunched posture was observed in 4 animals 2 hours after treatment and persisted until 5 hours after treatment.

Appearance of all animals was normal by day 2 of the study.

Table 1: Clinical signs

	Number of animals (n=6)				
Hours post dose	**0.5**	**1**	**2**	**3**	**5**
Swaying gait	3	6	6	6	2
Dragging of front/rear limbs	0	6	6	6	3
Decreased activity	3	6	6	6	6
Ruffled fur	0	0	1	2	2
Hunched posture	0	0	4	4	4

Data obtained from pages 21 – 24 of the study report

C. BODY WEIGHT

Body weight gain was within the range commonly recorded for rats of the strain and age used in this study.

D. NECROPSY

No macroscopic findings were noted at necropsy.

III. APPLICANT'S CONCLUSIONS

The LD_{50} of MCW-2 480 EC after single oral administration to female rats was greater than 2000 mg/kg bw.

MCW-2 480 EC is not classified under GHS CLP.

According to US EPA criteria, MCW-2 480 EC is assigned to category III, signal word 'Caution'.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments**: This acute oral toxicity study in the rat is classified as acceptable (fully reliable) and satisfies the guideline requirement for an acute oral toxicity study (OPPTS 870.1100; OECD 423) in rats.

C. **Conclusions**: The LD_{50} of the test material was estimated to be greater than 2000 mg/kg bw. According to the acute toxicity classification criteria for PMRA, MCW-2 480 EC is of low acute toxicity via the oral route.

Under OCS criteria for acute toxicity endpoints, the test material is considered to be of low acute oral toxicity.

D. **Deficiencies**: None.

Tier II Summary (PMRA #2181232, MRID 48574783) revised by the regulatory authorities, April 2013

Study Type: Acute oral toxicity - Thiazole sulfonic acid (Metabolite M-3625)

IIA 5.8 – MRID 48574783, PMRA 2181232

Report:	Arcelin G. (2010) MCW-2 METABOLITE #3625: Acute Oral Toxicity Study in Rats Harlan Laboratories Ltd., Switzerland; unpublished report No. C81241, dated 28 June 2010. Sponsor reference No. R-23470 Dates of experimental work: 8 to 30 April 2010
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 423, EC method B.1 tris and OPPTS 870.1100 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary:

In an acute oral toxicity study performed according to the Acute Toxic Class Method, two groups, each of 3 fasted female HanRcc:WIST (SPF) rats, were treated with fluensulfone metabolite thiazole sulfonic acid (MCW-2 metabolite #3625) by oral gavage at a dosage of 2000 mg/kg bw. Purified water was used as the vehicle and dosing volume was 10 mL/kg bw.

All animals survived until the end of the study period. No clinical signs were observed during the course of the study with the exception of 3 animals, in which slightly ruffled fur was observed 2 hours after administration, persisting up to the 3-hour observation in 2 animals.

The body weight of the animals was within the range commonly recorded for rats of this strain and age (all animals gained weight over the study period) and no macroscopic findings were recorded at necropsy.

The oral LD_{50} of thiazole sulfonic acid was greater than 2000 mg/kg bw. Based on PMRA's acute toxicity classification system, thiazole sulfonic acid (metabolite #3625) is considered to be of low acute toxicity via the oral route. Based on the EPA classification criteria, thiazole sulfonic acid falls under Category III for acute oral toxicity. Under OCS criteria for acute toxicity endpoints, thiazole sulfonic acid is considered to be of low acute oral toxicity.

The acute oral toxicity of fluensulfone metabolite #3625 is lower than that of fluensulfone.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 metabolite #3625

Chemical name: 5-chloro-thiazole-2-sulfonic acid sodium salt

Description:	White solid
Lot/Batch:	231PAL071
Purity:	93.6%
CAS#:	Not available
Stability:	Stable until 11 March 2011

2. Vehicle: Purified water

3. Test animals:

Species:	Rat
Strain:	HanRcc: WIST(SPF) - females
Age:	At dosing: 10 weeks
Weight at dosing:	169.8 - 186.0 g
Source:	Harlan Laboratories B.V., The Netherlands
Acclimation period:	6-8 days
Diet:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum* (except for the overnight fasting period prior to intubation and approximately 3-4 hours post dose)
Water:	Community tap water from Füllinsdorf *ad libitum*
Housing:	In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' J. Rettenmaier & Söhne GmbH & CoKG, Germany, imported by Provimi Kliba AG, Switzerland)

4. Environmental conditions:

Temperature:	22 ± 3 °C
Humidity:	30-70%
Air changes:	10-15 air changes per hour
Photoperiod:	Light cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS:

1. In life dates: 8 to 30 April 2010

2. Animals assignment and treatment

Animals were selected by hand at time of delivery. Animals Nos. 1-3 were assigned to group 1 and Nos. 4-6 to group 2; both groups were treated at 2000 mg/kg bw. The animals received a single oral dose of the test item by oral gavage administration after being fasted for approximately 18-19 hours (access to water was permitted). Food was provided again

approximately 3-4 hours after dosing. The dosing volume was 10 mL/kg bw.

3. Preparation of the test substance

The dose formulations were prepared shortly before each dosing occasion using a magnetic stirrer as homogenizer.

The test item was weighed into a tared glass beaker on a suitable precision balance and the vehicle added.

Homogeneity of the test item in the vehicle was maintained during administration using a magnetic stirrer.

4. Statistics

No statistical method was used.

C. OBSERVATIONS:

1. Viability and clinical signs

Animals were examined for viability, mortality and clinical signs daily during the acclimatization period, before treatment, within the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1. Observations for viability were then conducted twice daily during days 2-15 while observations for clinical signs were made once daily during days 2-15.

2. Body weights

Body weights were measured on test days 1 (prior to dosing), 8 and 15.

3. Pathology

All surviving animals were killed at the end of the observation period by carbon dioxide asphyxiation and discarded after macroscopic examinations were performed. An external examination and opening of the abdominal and thoracic cavities for examinations of major organs was performed. The appearance of any macroscopic abnormalities was recorded. No organs or tissues were retained.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred during the study.

B. CLINICAL OBSERVATIONS

No clinical signs were observed during the course of the study with the exception of 3 animals, in which slightly ruffled fur was observed 2 hours after administration, persisting up to the 3-hour observation in 2 animals.

C. BODY WEIGHT

The body weight of the animals was within the range commonly recorded for rats of this strain and age. All animals gained weight over the 14-day observation period.

D. NECROPSY

No macroscopic findings were recorded at necropsy.

III. CONCLUSIONS

The oral LD_{50} of thiazole sulfonic acid was greater than 2000 mg/kg bw.

The acute oral toxicity of fluensulfone metabolite #3625 is lower than that of fluensulfone.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments:** This acute oral toxicity study in the rat is classified as acceptable (fully reliable) and satisfies the guideline requirement for an acute oral toxicity study (OPPTS 870.1100; OECD 423) in rats.

C. **Conclusions:** According to the acute toxicity classification criteria for PMRA, thiazole sulfonic acid (metabolite #3625) is of low toxicity via the oral route (based on the oral LD_{50} value in female rats). Based on the EPA classification criteria, thiazole sulfonic acid falls under Category III for acute oral toxicity. Under OCS criteria for acute toxicity endpoints, thiazole sulfonic acid is considered to be of low acute oral toxicity.

D. **Deficiencies:** No deficiencies were identified.

Tier II Summary (PMRA #2181233, MRID 48574787) revised by the regulatory authorities, April 2013

Study Type: Acute oral toxicity - Methyl sulfone (Metabolite M-3626)

IIA 5.8 – MRID 48574787, PMRA 2181233

Report:	Mallaun M. (2010) MCW-2 METABOLITE #3626: Acute Oral Toxicity Study in Rats Harlan Laboratories Ltd., Switzerland; unpublished report No. C81252, dated 7 July 2010. Sponsor reference No. R-23474 Dates of experimental work: 23 February to 23 March 2010
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 423, EC method B.1 tris and OPPTS 870.1100 Deviations: none
GLP/Compliance:	Yes

Executive Summary:

In an acute oral toxicity study performed according to the Acute Toxic Class Method, three groups, each of 3 fasted female HanRcc:WIST (SPF) rats, were treated with fluensulfone metabolite methyl sulfone (MCW-2 metabolite #3626) by oral gavage at a dosage of 2000 mg/kg bw or 300 mg/kg bw. Corn oil was used as the vehicle and dosing volume was 10 mL/kg bw.

The 3 animals dosed at 2000 mg/kg died or were sacrificed because of overt toxicity within 2 hours of dosing. All 6 animals dosed at 300 mg/kg survived until the end of the observation period.

Clinical signs were recorded during the course of the study and included swaying gait, sedation, hunched posture, labored breathing, tightly shut eyes and/or ruffled fur. All clinical signs completely reverted by test day 3 for all surviving animals.

The body weight of the animals was within the range commonly recorded for rats of this strain and age. All surviving animals gained weight over the study period.

Congested lungs were recorded at necropsy for the 3 animals dosed at 2000 mg/kg bw, while no macroscopic findings were noted for the 6 animals dosed at 300 mg/kg bw.

The acute median lethal dose of methyl sulfone was 300 mg/kg bw < LD_{50} < 2000 mg/kg bw (LD_{50} cut-off value: 500 mg/kg bw). Based on PMRA's acute toxicity classification system, methyl sulfone (metabolite #3626) is considered to be of moderate toxicity via the oral route. Based on the EPA classification criteria, methyl sulfone falls under Category II for acute oral toxicity. Under OCS criteria for acute toxicity endpoints, methyl sulfone is considered to be of low to moderate acute oral toxicity.

The acute oral toxicity of fluensulfone metabolite #3626 is similar to that of fluensulfone.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 metabolite #3626

Chemical name: 5-chloro-2-methylsulfonyl thiazole

Description: Brown, waxy solid

Lot/Batch: 231PAL021

Purity: 98.1%

CAS#: Not available

Stability: Stable until 2 January 2011

2. Vehicle: Corn oil

3. Test animals:

Species: Rat

Strain: HanRcc: WIST(SPF) – females

Age: At dosing: 10 weeks

Weight at dosing: 165.7 – 199.8 g

Source: Harlan Laboratories B.V., The Netherlands

Acclimation period: 6-10 days

Diet: Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum* (except for the overnight fasting period prior to intubation and approximately 3-4 hours post dose)

Water: Community tap water from Füllinsdorf *ad libitum*

Housing: In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding (‘Lignocel’ J. Rettenmaier & Söhne GmbH & CoKG, Germany, imported by Provimi Kliba AG, Switzerland)

4. Environmental conditions:

Temperature: 22 ± 3 °C

Humidity: 30-70%

Air changes: 10-15 air changes per hour

Photoperiod: Light cycle of 12 hours light and 12 hours dark, music during the

daytime light period

B. STUDY DESIGN AND METHODS:

1. In life dates: 23 February to 23 March 2010

2. Animals assignment and treatment

Animals were selected by hand at time of delivery. Animals Nos. 1-3 were assigned to group 1 and treated at 2000 mg/kg bw; animals Nos. 4-6 were assigned to group 2 and treated at 300 mg/kg bw; animals Nos. 7-9 were assigned to group 3 and treated at 300 mg/kg bw. The animals received a single dose of the test item by oral gavage administration after being fasted for approximately 18 to 20 hours (access to water was permitted). Food was provided again approximately 3 hours after dosing. The dosing volume was 10 mL/kg bw.

3. Preparation of the test substance

The dose formulations were prepared shortly before each dosing occasion using a magnetic stirrer, a spatula and an Ultra-Turrax as homogenizers.

MCW-2 metabolite #3626 was ground with a pestle and a mortar and weighed into a tared glass beaker on a suitable precision balance. The vehicle was added.

Homogeneity of the test item in the vehicle was maintained during administration using a magnetic stirrer.

4. Statistics

No statistical method was used.

C. OBSERVATIONS:

1. Viability and clinical signs

Animals were examined for viability, mortality and clinical signs daily during the acclimatization period, before treatment, within the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1. Observations for viability were then conducted twice daily during days 2-15 while observations for clinical signs were made once daily during days 2-15.

2. Body weights

Body weights were measured on test days 1 (prior to dosing), 8 and 15.

3. Pathology

The animal dosed with 2000 mg/kg bw of test material that died spontaneously during the observation period was necropsied as soon as it was found dead. The other two animals from the 2000 mg/kg bw dose group were killed by carbon dioxide asphyxiation for ethical reasons. Necropsy was performed immediately after their sacrifice.

All surviving animals were killed at the end of the observation period by carbon dioxide asphyxiation and discarded after macroscopic examinations were performed. An external examination and opening of the abdominal and thoracic cavities for examinations of major organs was performed. The appearance of any macroscopic abnormalities was recorded. No organs or tissues were retained.

II. RESULTS AND DISCUSSION

A. MORTALITY

One animal dosed at 2000 mg/kg bw spontaneously died soon within 2 hours after oral administration. The other two animals treated at the same dose level had to be killed 2 hours post-dosing for ethical reasons.

All 6 animals dosed at 300 mg/kg bw survived until the end of the observation period.

Dose (mg/kg bw)	Females	
	Mortality	Time of death
300	0 / 6	NA
2000	3 / 3	Day 1 (one found dead, 2 sacrificed *in extremis*)

No. of dead / No. of treated; NA: not applicable

B. CLINICAL OBSERVATIONS

The animals treated at 2000 mg/kg bw were noted with swaying gait, sedation, ruffled fur and tightly shut eyes of slight to moderate degree 1 and 2 hours after dosing.

The animals treated at 300 mg/kg bw were observed with swaying gait, sedation, hunched posture, ruffled fur, tightly shut eyes and labored breathing of slight to moderate degree. These signs were recorded from the 1- or 2 hour-observation time points until the 3-hour observation, the 5-hour observation, or test day 2 (the day after dosing). All clinical signs completely reverted by test day 3.

C. BODY WEIGHT

The body weight of the animals was within the range commonly recorded for rats of this strain and age. All surviving animals gained weight over the 14-day observation period.

D. NECROPSY

Congested lungs were recorded at necropsy for the 3 animals dosed at 2000 mg/kg bw. No macroscopic findings were noted for the 6 animals dosed at 300 mg/kg bw.

III. CONCLUSIONS

The acute median lethal dose of methyl sulfone was 300 mg/kg bw < LD_{50} < 2000 mg/kg bw (LD_{50} cut-off value: 500 mg/kg bw).

The acute oral toxicity of fluensulfone metabolite #3626 is similar to that of fluensulfone.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments:** This acute oral toxicity study in the rat is classified as acceptable (fully reliable) and satisfies the guideline requirement for an acute oral toxicity study (OPPTS 870.1100; OECD 423) in rats.

C. **Conclusions:** According to the acute toxicity classification criteria for PMRA, methyl sulfone (metabolite #3626) is of moderate toxicity via the oral route (based on the oral LD_{50} cut-off value of 500 mg/kg bw in female rats). Based on the EPA classification criteria, methyl sulfone falls under Category II for acute oral toxicity. Under OCS criteria for acute toxicity endpoints, methyl sulfone is considered to be of low to moderate acute oral toxicity.

D. **Deficiencies:** No deficiencies were identified.

Tier II Summary (PMRA #2181234, MRID 48574792) revised by the regulatory authorities April, 2013

Study Type: Acute oral toxicity - Butene sulfonic acid (metabolite M-3627)

IIA 5.8 – MRID 48574792, PMRA 2181234

Report:	Arcelin G. (2010) MCW-2 METABOLITE #3627: Acute Oral Toxicity Study in Rats Harlan Laboratories Ltd., Switzerland; unpublished report No. C81230, dated 28 June 2010. Sponsor reference No. R-23466 Dates of experimental work: 26 January to 18 February 2010
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 423, EC method B.1 tris and OPPTS 870.1100 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary:

In an acute oral toxicity study performed according to the Acute Toxic Class Method, two groups, each of 3 fasted female HanRcc:WIST (SPF) rats, were treated with fluensulfone metabolite butene sulfonic acid (MCW-2 metabolite #3627) by oral gavage at a dosage of 2000 mg/kg bw. Purified water was used as the vehicle and dosing volume was 10 mL/kg bw.

All animals survived until the end of the study period. No clinical signs were observed during the course of the study. The body weight of the animals was within the range commonly recorded for rats of this strain and age (all animals gained weight over the course of the study) and no macroscopic findings were recorded at necropsy.

The LD_{50} of butene sulfonic acid was greater than 2000 mg/kg bw. Based on PMRA's acute toxicity classification system, butene sulfonic acid (metabolite #3627) is considered to be of low acute toxicity via the oral route. Based on the EPA classification criteria, butene sulfonic acid falls under Category III for acute oral toxicity. Under OCS criteria for acute toxicity endpoints, metabolite butane sulfonic acid is considered to be of low acute oral toxicity.

The acute oral toxicity of fluensulfone metabolite #3627 is lower than that of fluensulfone.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material:	MCW-2 metabolite #3627
Chemical name:	3,4,4-trifluorobutene-1-sulfonic acid, sodium salt
Description:	White solid
Lot/Batch:	215PAL44

Purity: 99.5%

CAS#: Not available

Stability: Stable until 21 December 2010

2. Vehicle: Purified water

3. Test animals:

Species: Rat

Strain: HanRcc: WIST(SPF) – females

Age: At dosing: 10 weeks

Weight at dosing: 171.4 – 195.5 g

Source: Harlan Laboratories B.V., The Netherlands

Acclimation period: 7-9 days

Diet: Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum* (except for the overnight fasting period prior to intubation and approximately 3-4 hours post dose)

Water: Community tap water from Füllinsdorf *ad libitum*

Housing: In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' J. Rettenmaier & Söhne GmbH & CoKG, Germany, imported by Provimi Kliba AG, Switzerland)

4. Environmental conditions:

Temperature: 22 ± 3 °C

Humidity: 30-70%

Air changes: 10-15 air changes per hour

Photoperiod: Light cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS:

1. In life dates: 26 January to 18 February 2010

2. Animals assignment and treatment

Animals were selected by hand at time of delivery. Animals Nos. 1-3 were assigned to group 1 and Nos. 4-6 to group 2; both groups were treated at 2000 mg/kg bw. The animals received a single oral dose by oral gavage administration after being fasted for approximately 17 to 19 hours (access to water was permitted). Food was provided again approximately 3 hours after dosing. The dosing volume was 10 mL/kg bw.

3. Preparation of the test substance

The dose formulations were prepared shortly before each dosing occasion using a magnetic

stirrer as homogenizer.

Butene sulfonic acid was weighed into a tared glass beaker on a suitable precision balance and the vehicle added.

Homogeneity of butene sulfonic acid in the vehicle was maintained during administration using a magnetic stirrer.

4. Statistics

No statistical method was used.

C. OBSERVATIONS:

1. Viability and clinical signs

Animals were examined for viability, mortality and clinical signs daily during the acclimatization period, before treatment, within the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1. Observations for viability were then conducted twice daily during days 2-15 while observations for clinical signs were made once daily during days 2-15.

2. Body weights

Body weights were measured on test days 1 (prior to dosing), 8 and 15.

3. Pathology

All surviving animals were killed at the end of the observation period by carbon dioxide asphyxiation and discarded after macroscopic examinations were performed. An external examination and opening of the abdominal and thoracic cavities for examinations of major organs was performed. The appearance of any macroscopic abnormalities was recorded. No organs or tissues were retained.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred during the study.

B. CLINICAL OBSERVATIONS

No clinical signs were observed during the course of the study.

C. BODY WEIGHT

The body weight of the animals was within the range commonly recorded for rats of this strain and age. All animals gained weight over the 14-day observation period.

D. NECROPSY

No macroscopic findings were recorded at necropsy.

III. CONCLUSIONS

The LD_{50} of butene sulfonic acid was greater than 2000 mg/kg bw.

The acute oral toxicity of fluensulfone metabolite #3627 is lower than that of fluensulfone.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

B. Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority.

C. **Reviewer's Comments:** This acute oral toxicity study in the rat is classified as acceptable (fully reliable) and satisfies the guideline requirement for an acute oral toxicity study (OPPTS 870.1100; OECD 423) in rats.

D. **Conclusions:** According to the acute toxicity classification criteria for PMRA, butene sulfonic acid (metabolite #3627) is of low toxicity via the oral route (based on the oral LD_{50} value in female rats). Based on the EPA classification criteria, butene sulfonic acid falls under Category III for acute oral toxicity. Under OCS criteria for acute toxicity endpoints, butane sulfonic acid is considered to be of low acute oral toxicity.

E. **Deficiencies:** No deficiencies were identified.

Tier II Summary (PMRA #2181197, MRID 48574747) revised by the regulatory authorities July, 2013

Study Type Acute percutaneous toxicity

IIA 5.2.2 – MRID 48574747, PMRA 2181197

Report:	Simon C. (2009) MCW-2 Tech. Acute Dermal Toxicity Study in Rats Harlan Laboratories Ltd., Switzerland; unpublished report No. C14944, dated 25 June 2009. Sponsor reference No. R-23418 Dates of experimental work: 25 September to 9 October 2008
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 402, EC method B.3 and OPPTS 870.1200 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Confidentiality statements were provided.

Executive Summary:

In an acute dermal toxicity study, a group of 5 male and 5 female HanRcc:WIST (SPF) rats were treated with fluensulfone (MCW-2 tech., 96.78% a.i.) at 2000 mg/kg bw by dermal application. The test substance was formulated in PEG 300 at the concentration of 0.5 g/mL and applied to the shaved backs of the animals (approximately 10% of the total body surface) at the dosage volume of 4 mL/kg bw. The exposure period was 24 hours. Treatment was followed by an observation period of 14 days.

No deaths occurred during the study, and no clinical signs, either systemic or local, were noted. Between test days 8 and 15 one male rat gained 0.1 g and between test days 1 and 8 one female rat did not gain weight while another gained only 0.5 g. Otherwise, the body weights of the animals were within the range commonly recorded for rats of this strain and age. No macroscopic findings were observed at necropsy.

The acute dermal median lethal dose of fluensulfone was greater than 2000 mg/kg bw.

According to the acute toxicity classification criteria for PMRA, MCW-2 tech. (fluensulfone) is of low acute toxicity via the dermal route (based on the dermal LD_{50} value for males and females). Based on the EPA classification criteria, MCW-2 tech. falls under Category III for acute dermal toxicity (signal word “Caution”). Under OCS criteria for acute toxicity endpoints, the test material is considered to be of low acute dermal toxicity.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 tech. (Fluensulfone)

Description: Solid

Lot/Batch: 36372130-291-PF1

Purity: 96.78%

CAS#: 318290-98-1

Stability: Stable until May 2009

2. Vehicle: Polyethylene glycol 300 (PEG 300)

3. Test animals:

Species: Rat

Strain: HanRcc: WIST(SPF)

Age: At dosing: males: 9 weeks; females: 11 weeks

Weight at dosing: 195.0 g – 251.3 g

Source: Harlan Laboratories Ltd., Laboratory Animal Services, Switzerland

Acclimation period: 1 week

Diet: Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum*

Water: Community tap water from Füllinsdorf *ad libitum*

Housing: During acclimation in groups of 5 per sex in Makrolon type-4 cages with standard softwood bedding. Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel" Schill AG, Switzerland) during treatment and observation period.

4. Environmental conditions:

Temperature: 22 ± 3 °C

Humidity: 30-70%

Air changes: 10-15 air changes per hour

Photoperiod: Light cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS:

1. In life dates: 25 September to 9 October 2008

2. Animal assignment and treatment

Animals were selected by hand at time of delivery. One day before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10% of the total body surface. Only those animals without injury or irritation on the skin were used in the test.

The test item was ground into a fine powder using a mortar and a pestle. Thereafter, it was weighed into a tared glass beaker on a suitable precision balance and the vehicle added. The formulation was prepared shortly before the application using a magnetic stirrer, a spatula and an Ultra-Turrax as homogenizers. Homogeneity was maintained during application using a magnetic stirrer. MCW-2 tech. was applied at a dose of 2000 mg/kg bw (4 mL/kg bw) evenly on the intact skin with a syringe and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and fixed with an elastic adhesive bandage. Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dabbed off with disposable paper towels. Thereafter, the application sites were assessed for dermal reactions. All animals were re-shaved on test days 7 and 13 to facilitate the reading of the local reactions.

3. Observations

Animals were observed for morbidity and clinical signs daily during the acclimation period, and during the first 30 minutes, and at 1, 2, 3, 5 hours post-dose. During days 2 - 15, animals were observed once or twice daily for clinical signs or morbidity, respectively. Body weights were measured prior to exposure to test material on day 1 and then on days 8 and 15.

4. Statistics

No statistical method was used.

II. RESULTS AND DISCUSSION

A. MORTALITY No deaths occurred during the study period.

Dose (mg/kg bw)	Males		Females	
	Mortality	Time of death	Mortality	Time of death
2000	0 / 5	NA	0 / 5	NA

No. of dead / No. of treated; NA: not applicable

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were observed.

C. BODY WEIGHT

Between test days 8 and 15 one male rat gained 0.1 g and between test days 1 and 8 one

female rat did not gain weight while another gained only 0.5 g. Otherwise, the body weights of the animals were within the range commonly recorded for rats of this strain and age.

D. NECROPSY

No macroscopic findings were observed at necropsy.

E. DERMAL IRRITATION

No local reactions were observed during the study.

III. APPLICANT'S CONCLUSIONS

The acute dermal LD_{50} of fluensulfone was greater than 2000 mg/kg bw.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments:** This acute dermal toxicity study in the rat is classified as acceptable (fully reliable) and satisfies the guideline requirement for an acute dermal toxicity study (OPPTS 870.1200; OECD 402) in rats.

C. **Conclusions:** The acute dermal LD_{50} of fluensulfone was greater than 2000 mg/kg bw. According to the acute toxicity classification criteria for PMRA, MCW-2 tech. (fluensulfone) is of low acute toxicity via the dermal route (based on the dermal LD_{50} value for males and females), and based on the EPA classification criteria, MCW-2 tech. falls under Category III for acute dermal toxicity. Under OCS criteria for acute toxicity endpoints, the test material is considered to be of low acute dermal toxicity.

D. **Deficiencies:** None.

Tier II Summary (PMRA #2181351, MRID 48574919) revised by the regulatory authorities July, 2013

Study Type: Acute percutaneous (dermal) toxicity

IIIA 7.1.2 – MRID 48574919, PMRA 2181351

Report: Arcelin G. (2009)
MCW-2 480 EC Acute Dermal Toxicity Study in Rats
Harlan Laboratories Ltd., Switzerland; unpublished report No. C45342, Sponsor No. R-23426, dated 12 October 2009
Dates of experimental work: 27 May to 10 June 2009

Sponsor: Makhteshim Chemical Works Ltd., Beer-Sheva, Israel

Guidelines: OECD 402, EEC method B.3 and OPPTS 870.1200

Deviations: None

GLP/Compliance: Yes. Signed and dated GLP, Quality Assurance and Confidentiality statements were provided

Executive summary:

In an acute dermal limit test, five male and five female RccHan:WIST (SPF) rats were treated with MCW-2 480 EC at 2000 mg/kg bw by dermal application. The test substance was formulated in purified water at the concentration of 0.5 g/mL and applied to the shaved backs of the animals (approximately 10% of the total body surface) at the dosage volume of 4 mL/kg bw. The exposure period was 24 hours. Treatment was followed by an observation period of 14 days.

There were no deaths, and no clinical signs were observed. Very slight to well-defined erythema was observed at the application site of all animals from removal of the dressing (test day 2) to test day 3, 4 or 5. Additionally, scaling was noted in all animals from test day 3 to 5 or to 11. Slight body weight loss (1.4 to 7.3 g, 0.67% to 3.57% bw) was noted in three of five females from days 1 to 8 but overall, body weight gain was within the range commonly recorded for rats of the strain and age used in the study. No abnormality was detected at necropsy.

The dermal LD_{50} of MCW-2 480 EC was greater than 2000 mg/kg bw.

MCW-2 480 EC is assigned category 5 and does not require any labeling under GHS CLP.

According to US EPA criteria, MCW-2 480 EC is assigned to category III, signal word 'Caution'. According to the acute toxicity classification criteria for PMRA, MCW-2 480 EC is of low acute toxicity via the dermal route (based on the dermal LD_{50} value for males and females). Under OCS criteria for acute toxicity endpoints, the test material is considered to be of low acute dermal toxicity.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: MCW-2 480 EC

Description: Orange clear liquid

Batch No.: 286-010209-01

Purity: 497 g/L

Expiry date: 1 February 2011

Stability of Test Item: Stable under storage conditions

2. Vehicle: Purified water prepared at Harlan Laboratories Ltd. (deionised water which was processed and treated by the PURELAB Option-R unit. This latter links four purification technologies: reverse osmosis, adsorption, ion-exchange and photo oxidation).

3. Test animals

Species: Rat

Strain: Wistar [RccHan: WIST(SPF)]

Age: Males: 9 weeks; females: 11 weeks

Weight at dosing: Males: 252.4 g – 258.2 g; females: 197.8 g – 209.7 g

Source: Harlan Laboratories B.V., The Netherlands

Acclimation period: 7 days

Diet: Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum*

Water: Community tap water from Füllinsdorf *ad libitum*

Housing: During acclimation in groups of five per sex in Makrolon type-4 cages with standard softwood bedding. Individually in Makrolon type-3 cages with standard softwood bedding (‘Lignocel’ J. Rettenmaier & Söhne GmbH & CoKG, Germany, imported by Provimi Kliba AG, Switzerland) during the treatment and observation periods.

4. Environmental conditions

Temperature: 22 ± 3°C

Humidity: 30-70%

Air changes: 10-15 per hour

Photoperiod: Cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS

1. **In life dates:** 27 May to 10 June 2009

2. **Animal assignment and treatment:**

All animals were selected by hand at time of delivery (5/sex). One day before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10% of the total body surface. Shortly prior to the application of the test material, the test item was weighed into a tared glass beaker on a precision balance and the vehicle was added (weight:volume). Homogeneity of the test item in the vehicle was maintained during administration using a magnetic stirrer. On test day 1, the test substance was applied at the limit dose of 2000 mg/kg bw (application volume 4 mL/kg bw) evenly on the intact skin with a syringe and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and fixed with an elastic adhesive bandage. Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dried with disposable paper towels. Thereafter, the sites were assessed for local skin reactions.

3. **Observations**

Animals were observed daily for mortality/viability and clinical signs of toxicity during the acclimation period, and during the first 30 minutes, 1, 2, 3 and 5 hours post dosing on test day 1. On days 2-15, animals were observed twice daily for mortality/viability and once daily for clinical signs of toxicity. Local dermal signs were observed once daily from day 2 (following dressing removal) through to day 15. Body weights were taken on test days 1 (prior to dosing), 8 and 15. Macroscopic examinations were completed on all animals at the end of the observation period.

4. **Statistics**

No statistical analysis was used.

I. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities occurred.

B. CLINICAL OBSERVATIONS

No clinical symptoms of systemic toxicity were observed during the study period.

Very slight to well-defined erythema was observed at the application site of all animals from removal of the dressing (test day 2) to test day 3, 4 or 5. Additionally, scaling was noted in all animals from test day 3 to 5 or to 11.

C. BODY WEIGHT

Overall body weight gain was within the range commonly recorded for rats of the strain and age used in this study. Slight body weight loss (1.4 to 7.3 g, 0.67% to 3.57% bw) was noted in three of five females from Day 1 to Day 8. All females gained weight from Day 8 to 15.

D. NECROPSY

No abnormality was detected at necropsy.

III. APPLICANT'S CONCLUSIONS

The dermal LD_{50} of MCW-2 480 EC, following a single single dose, for both sexes, was greater than 2000 mg/kg bw.

MCW-2 480 EC is assigned category 5 and does not require any labeling under GHS CLP.

According to US EPA criteria, MCW-2 480 EC is assigned to category III, signal word 'Caution'.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments:** This acute dermal toxicity study in the rat is classified as acceptable (fully reliable) and satisfies the guideline requirement for an acute dermal toxicity study (OPPTS 870.1200; OECD 402) in rats.

C. **Conclusions:** The acute dermal LD_{50} of MCW-2 480 EC was greater than 2000 mg/kg bw. According to the acute toxicity classification criteria for the PMRA, MCW-2 480 EC is of low acute toxicity via the dermal route, based on the LD_{50} value for males and females.

Under OCS criteria for acute toxicity endpoints, the test material is considered to be of low acute dermal toxicity.

D. **Deficiencies:** None.

Tier II Summary (PMRA #2181198, MRID 48574748) revised by the regulatory authorities July, 2013

Study Type Acute inhalation toxicity

IIA 5.2.3 – MRID 48574748, PMRA 2181198

Report:	Pothmann D. (2009) MCW-2 Tech. 4-Hour Acute Inhalation Toxicity Study in Rats Harlan Laboratories Ltd., Switzerland; unpublished report No. C14955, dated 16 January 2009. Sponsor reference No. R-23419 Dates of experimental work: 22 October to 5 November 2008
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 403, EC method B.2 and OPPTS 870.1300 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Data Confidentiality and Quality Assurance statements were provided.

Executive Summary:

In an acute inhalation toxicity study, a group of 5 male and 5 female HanRcc:WIST(SPF) rats was exposed by inhalation by nose-only, flow-past exposure, for four hours to fluensulfone (MCW-2 Tech., 96.2% a.i.) at the chemically determined mean concentration of 5.1 mg/L air (MMAD obtained at three times during exposure were 2.34, 2.18 and 2.16 µm, with GSD values of 1.74, 1.67 and 1.67, respectively). Exposure was followed by an observation period of 14 days.

All animals survived the end of the observation period. There were no clinical signs during exposure or the observation period. There were no effects on the body weight development that were considered to be related to exposure, and no macroscopic findings were noted at necropsy.

The 4-hour LC_{50} of fluensulfone was estimated to be greater than 5.1 mg/L air (chemically determined mean aerosol concentration).

According to the acute toxicity classification criteria for the PMRA, MCW-2 tech. (fluensulfone) is of low acute toxicity via the inhalation route (based on the LC_{50} value in male and female rats). Based on the EPA classification system, MCW-2 tech. falls under Category IV for acute inhalation toxicity. Under OCS criteria for acute toxicity endpoints, the test material is considered to be of low acute inhalation toxicity.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 tech. (Fluensulfone)

Description: Yellow solid

Lot/Batch: 36372130-291-PF1

Purity: 96.2%

CAS#: 318290-98-1

Stability: Stable until 31 December 2009

2. Vehicle: None. MCW-2 Tech. was used as supplied

3. Test animals:

Species: Rat

Strain: HanRcc: WIST(SPF)

Age: At exposure: males: 9 weeks; females: 10 weeks

Weight at dosing: Males: 244.9 to 262.9 g; females: 204.3 to 217.9 g

Source: Harlan Laboratories Ltd., Laboratory Animal Services, Switzerland

Acclimation period: Performed under a different study (Harlan Laboratory Study B68308) for 5 days

Diet: Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum* (except during the period when they were restrained in exposure tubes)

Water: Community tap water from Füllinsdorf *ad libitum* (except during the period when they were restrained in exposure tubes)

Housing: In groups of 5 of the same sex in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' Schill AG, Switzerland)

4. Environmental conditions:

Temperature: 22 ± 3 °C

Humidity: 30-70%

Air changes: 10-15 air changes per hour

Photoperiod: Light cycle of 12 hours light and 12 hours dark, radio program was played during most of the daytime light period

B. STUDY DESIGN AND METHODS:

1. In life dates: 22 October to 5 November 2008

2. Animals assignment and treatment

Only animals without any visible signs of illness were used for the study. A clinical health examination was performed on the day of exposure, before exposure start.

Inhalation exposure was performed using a system similar to that originally described by Sachsse et al. (K. Sachsse, L. Ullmann, G. Voss and R. Hess: Measurements of Inhalation Toxicity of Aerosols in Small Laboratory Animals. In: Proceedings of the Europ. Soc. for the Study of Drug Toxicity, 15, 239-251, Zürich, 1973 and K. Sachsse, L. Ullmann and K. Zbinden: Toxikologische Prüfungen von Aerosolen im Tierexperiment, "Chemische Rundschau" 29, 38, 1, 1976). The animals were confined separately in restraint tubes which were positioned radially around the flow-past, nose-only exposure chamber as described by Cannon et al. (W.C. Cannon, E.F Blanton and K.E. McDonald: the Flow-Past Chamber: An Improved Nose-Only Exposure System for Rodents, Am. Ind. Hyg. Assoc. J., 44, 923-928, 1983). The design of this chamber is based upon the fluid dynamic modeling of the test aerosol flow. The exposure system ensured a uniform distribution and provided a constant flow of test material to each exposure tube. The airflow at each port was 1.0 L/min, which is sufficient to minimize re-breathing of the test atmosphere as it is more than twice the respiratory minute volume of a rat.

3. Observations

Before commencement of the exposure of the group, technical trials were conducted (without animals) using the inhalation system foreseen for the study. The day of exposure was named test day 1. Observations for viability were recorded once before exposure, once/hour during exposure, once after exposure on day 1, and twice daily thereafter. Observations for clinical signs took place once/hour during exposure, once after exposure on day 1, and once daily thereafter. The body weight of each animal was recorded on test days 1 (prior to test substance exposure), 4, 8 and 15. All animals were necropsied and examined for abnormalities at the end of the observation period.

4. Generation of the test atmosphere/chamber description

The test aerosol was generated using a Glass Duran Nebulizer with a stainless steel injector. Fluensulfone was liquefied by warming it in the nebulizer kept in a PEG 400 bath at 140 °C. Both fluensulfone inside the glass nebulizer and the PEG 400 bath were continuously stirred with a magnetic stirrer during aerosol generation. The generation air was preheated in an oil bath at 130 °C.

The concentration of the test material (determined chemically and gravimetrically), the particle size distribution (determined gravimetrically), temperature, relative humidity, and oxygen concentration were measured on test atmosphere samples collected from the delivery tube at an empty port of the exposure chamber. Airflow rates were determined for the recording of temperature, relative humidity, and oxygen concentration and during the collection of samples for the determination of test aerosol concentration and particle size

using a dry-test meter and a pressure gauge.

Nominal Determination of Concentration: Test material usage was measured by weighing the nebulizer reservoir before and after exposure to determine the quantity of test material used. The weight used was then divided by the airflow volume to give the nominal concentration.

Gravimetric Determination of Concentration: Gravimetric determinations of aerosol concentration were performed five times during exposure. Samples were collected on a Millipore durapore filter, Type HVLP loaded in a 47 mm in-line stainless steel filter sampling device. Filters were weighed before and immediately after sampling. The test aerosol concentration was calculated from the amount of test item present on the filter and the sample volume.

Chemical Determination of Concentration: Chemical determinations of aerosol concentration were performed five times during exposure using the filters for the gravimetric determination. Filters were stored at 2 - 8˚C until analysis. Samples were analyzed using the HPLC method developed by Harlan Laboratories Ltd.

Particle Size Distribution and Mass Median Aerodynamic Diameter (MMAD): Particle size distribution was measured gravimetrically three times during exposure using a 7 stage cascade Mercer Impactor (Model 02-130, In-Tox. Products Inc,). MMAD and Geometric Standard Deviations were calculated on the basis of the results from the impactor using Microsoft Excel Software.

Oxygen Concentrations: Oxygen concentration of the test atmosphere was monitored continuously during exposure. Results were reported at 30 minute intervals. Oxygen concentration was maintained above 19% during the exposure period.

Airflow rate: Airflow rate was monitored indirectly during exposure through the generation and dilution system. The exposure airflow rate was adjusted before the start of exposure using calibrated flow-meters and pressure gauges. The actual airflow rate was recorded at 30 minute intervals.

5. Statistics

No statistical analyses were conducted.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred during the study period.

Exposure Concentration (mg/L)	Males		Females	
	Mortality	Time of death	Mortality	Time of death
5.1	0 / 5	NA	0 / 5	NA

No. of dead / No. of treated; NA: not applicable

B. CLINICAL OBSERVATIONS

There were no clinical signs during exposure and the observation period.

C. BODY WEIGHT

From test day 1 to test day 4, a slight body weight loss (2.9 g) was noted in one female. In addition, only minor weight gains (1.0 g or less) were observed in two females during the first four days of the observation period. These findings were considered to be due to the restraint during exposure period and not a test item related effect. From test day 4 onwards normal body weight developments were observed in these animals.

No effects on body weights were recorded in the remaining animals.

D. NECROPSY

No abnormality was detected at necropsy.

E. CHAMBER ATMOSPHERE CONDITIONS

Temperature, relative humidity and oxygen concentration during exposure were considered to be satisfactory for this type of study. Mean values ± standard deviation of 9 measurements carried out during exposure were as follows:

O_2 concentration (Vol %): 20.0 ± 0.1

Temperature (°C): 20.9 ± 0.3

Relative humidity (%): 1.3 ± 1.8

The nominal aerosol concentration was 5.5 mg/L air. The gravimetric aerosol concentration determined was 5.2 ± 0.2 mg/L air (5 measurements). The chemical aerosol concentration determined was 5.1 ± 0.2 mg/L air (5 measurements). Both gravimetric and chemically determined data show that the aerosol concentration was stable during the exposure period.

The Mass Median Aerodynamic Diameters (MMAD) obtained from 3 gravimetric measurements of particle size distribution during the exposure were similar (MMAD = 2.34 µm, 2.18 µm and 2.16 µm; GSD = 1.74, 1.67 and 1.67). This led to the conclusion that the particle size of the generated aerosol was fairly stable during the whole exposure period.

The MMADs were within the target range of 1 to 4 µm, with 83-89% of particles being smaller than 4 µm; hence, deposition of the particles can be assumed to have occurred in the upper and lower respiratory tract.

III. APPLICANT'S CONCLUSIONS

The LC_{50} of fluensulfone was estimated to be greater than 5.1 mg/L air (chemically determined mean aerosol concentration).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments:** This acute inhalation toxicity study in the rat is classified as acceptable (fully reliable) and satisfies the guideline requirement for an acute inhalation toxicity study (OPPTS 870.1300; OECD 403) in rats.

C. **Conclusions:** The 4-hour LC_{50} of fluensulfone was estimated to be greater than 5.1 mg/L air (analytically determined mean aerosol concentration; and MMAD obtained at three times during exposure were 2.34, 2.18 and 2.16 µm, with GSD values of 1.74, 1.67 and 1.67, respectively). According to the acute toxicity classification criteria for PMRA, MCW-2 tech. (fluensulfone) is of low acute toxicity via the inhalation route (based on the LC_{50} value in male and female rats), and based on the EPA classification criteria, MCW-2 tech. falls under Category IV for acute inhalation toxicity. Under OCS criteria for acute toxicity endpoints, the test material is considered to be of low acute inhalation toxicity.

D. **Deficiencies:** The method of animal group selection (i.e. randomization) and time to chamber equilibrium (t_{95}) were not specified in the study report; however, these items are not expected to have influenced study results.

Tier II Summary (PMRA #2181355, MRID 48574920) revised by the regulatory authorities July, 2013

Study Type: Acute inhalation toxicity to rats

IIIA 7.1.3 – MRID 48574920, PMRA 2181355

Report: Pothmann D. (2009)
MCW-2 480 EC 4-Hour Acute Inhalation Toxicity Study in the Wistar Rat
Harlan Laboratories Ltd., Switzerland; unpublished report No. C45454, Sponsor No. R-23430, dated 1 October 2009
Dates of experimental work: 15 to 29 June 2009

Sponsor: Makhteshim Chemical Works Ltd., Beer-Sheva, Israel

Guidelines: OECD 403, EEC method B.2 and OPPTS 870.1300

Deviations: None

GLP/Compliance: Yes. Signed and dated GLP, Data Confidentiality and Quality Assurance statements were provided.

Executive summary:

In an acute inhalation toxicity study, groups of 5 male and 5 female HanRcc:WIST(SPF) rats were exposed by nose-only, flow-past exposure, for four hours to MCW-2 480 EC (497 g a.i./L) at the chemically determined mean concentration of 6.0 mg/L air (MMAD = 1.8 µm, GSD = 2.12). The mean gravimetric concentration was 5.3 mg/L. Exposure was followed by an observation period of 14 days.

No mortality occurred during the study. Salivation and ruffled fur were observed in all animals during or after exposure. Decreased activity was recorded in all males and two females at the end of the exposure period and/or one hour after exposure. Rales were recorded in two males one hour after the end of exposure. There were no clinical signs in any animals from test day 2.

Slight body weight loss was noted in four males and all females between days 1 and 4. No body weight gain was recorded in this period in the remaining male. Thereafter normal body weight gain was recorded in all animals, although one female failed to regain the initial body weight by study termination.

No abnormality was detected at necropsy.

The acute inhalation LC_{50} of MCW-2 480 EC was greater than 6.0 mg/L air (chemically determined mean aerosol concentration).

MCW-2 480 EC is assigned category 5 and does not require any labeling under GHS CLP.

According to US EPA criteria, MCW-2 480 EC is assigned to category IV.

According to the acute toxicity classification criteria for the PMRA, MCW-2 480 EC is of low acute toxicity via the inhalation route based on the LC_{50} value in male and female rats.

Under OCS criteria for acute toxicity endpoints, MCW-2 480 EC is considered to be of low acute inhalation toxicity.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: MCW-2 480 EC

Description: Orange clear liquid

Batch No.: 286-010209-01

Purity: 497 g a.i./L

Expiry date: 1 February 2011

Stability of Test Item: Stable under storage conditions

2. Vehicle: None

3. Test animals

Species: Rat

Strain: Wistar [HanRcc: WIST(SPF)]

Age: Males: 9-10 weeks; females: 10-11 weeks

Weight at dosing: Males: 237.3 to 250.9 g; females: 211.6 to 218.9 g

Source: Harlan Laboratories Ltd., Netherlands

Acclimation period: Five days under laboratory conditions, after clinical health examination under study B68308

Diet: Pelleted standard Kliba-Nafag 3433 rat maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum* (except during exposure period when rats were restrained in exposure tubes).

Water: Community tap water from Füllinsdorf *ad libitum* (except during exposure period when rats were restrained in exposure tubes).

Housing: In groups of five per sex in Makrolon type-4 cages with wire mesh tops and standard softwood bedding (‘Lignocel’ J. Rettenmaier & Söhne GmbH & CoKG,

Germany, imported by Provimi Kliba AG, Switzerland)

4. Environmental conditions

Temperature:	22 ± 3°C
Humidity:	30-70%
Air changes:	10-15 per hour
Photoperiod:	Cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS

1. In life dates: 15 to 29 June 2009

2. Animal assignment and treatment:

A single group consisting of 5 male and 5 female rats was used. Only animals without any visible signs of illness were used for the study.

The animals were confined separately in restraint tubes which were positioned radially around the flow-past, nose-only exposure chamber. The design of this chamber is based upon the fluid dynamic modelling of the test aerosol flow. The exposure system ensured a uniform distribution and provided a constant flow of test material to each exposure tube. The flow of air at each tube was 1.0 L/min, which is sufficient to minimize re-breathing of the test atmosphere as it is more than twice the respiratory minute volume of a rat.

3. **Observations**

Animals were continuously exposed to the test material for 4 hours. Each animal was examined for viability/mortality and clinical signs of toxicity once per hour during exposure, and once after exposure on test day 1. During the observation period post dose, animals were observed twice daily for mortality/viability and once daily for clinical signs of toxicity. The body weight of each animal was recorded on day 1 (prior to exposure), 4, 8 and 15. All animals were necropsied at the end of the study.

4. **Generation of the test atmosphere/chamber description**

Before commencement of exposure, technical trials were conducted (without animals) using the inhalation system foreseen for the study.

The aerosol was generated with the test substance as supplied using a nebulizer connected to a syringe pump. The polyethylene injector inside the nebulizer was replaced by a stainless steel injector.

The aerosol concentration, the particle size distribution, relative humidity, temperature and oxygen concentration were measured on test aerosol samples taken at a representative exposure port.

The nominal concentration was determined by weighing the syringe and nebulizer

reservoir containing the test substance before and after exposure to determine the quantity used. The weight was then divided by the total air-flow volume to give the nominal concentration.

Gravimetric determinations of aerosol concentration were performed five times during exposure. The samples were collected on a Millipore® durapore filter, Type HVLP loaded in a 47 mm inline stainless steel filter sampling device. The filters were weighed before and immediately after sampling using a calibrated balance. The test aerosol gravimetric concentration was calculated from the amount of test substance present on the filter and the sample volume.

Chemical determinations of aerosol concentration were performed five times during exposure. The filters for gravimetric determination were transferred into appropriate labeled vials, forwarded at ambient conditions to those responsible for analytical chemistry and stored in the refrigerator (5 ± 3°C) until analysis. The samples were analyzed using an HPLC method developed by Harlan Laboratories Ltd.

The particle size distribution was measured gravimetrically twice during exposure using a 7 stage cascade Mercer Impactor. Mass Median Aerodynamic Diameters and Geometric Standard Deviations were calculated on the basis of the results from the impactor, using Microsoft Excel Software. The target range for the Mass Median Aerodynamic Diameter was 1 to 4 μm.

Oxygen concentration, temperature and relative humidity of the test atmosphere was measured continuously during exposure using a calibrated device. The oxygen concentration was maintained above 19% during each exposure period.

The airflow rate was monitored indirectly during exposure through the generation and dilution system. The actual airflow rate was recorded at 30 minute intervals.

5. Statistics

No statistical analysis was performed as only one group was used.

II. RESULTS AND DISCUSSION

A. INHALATION TECHNICAL DATA

Temperature, relative humidity and oxygen concentration during exposure were considered to be satisfactory for this type of study.

Temperature ranged between 21 to 21.3 °C (mean 21.2 °C); relative humidity between 0 and 3.3% (mean 0.6%); and oxygen concentration (vol. %) between 20.6 and 20.7 (mean 20.7).

The nominal aerosol concentration was 5.8 mg/L air.

The gravimetric aerosol concentration was determined to be 5.3 mg/L air. The aerosol concentration was stable during the exposure period.

The chemical aerosol concentration ranged between 5.8 and 6.3 mg/L, and the mean was determined to be 6.0 mg/L air. The aerosol concentration was stable during the exposure period.

The Mass Median Aerodynamic Diameters (MMAD) obtained from two gravimetric measurements of particle size distribution during the exposure were similar (MMAD = 1.79 µm and 1.81 µm, GSD = 2.12 and 2.11, respectively). This led to the conclusion that the particle size of the generated aerosol was fairly stable during the whole exposure period, and that ca. 86% of the particles were < 4 µm, assuring that deposition of the particles had occurred in both the upper and the lower respiratory tract.

B. MORTALITY

No mortalities occurred during the study.

C. CLINICAL OBSERVATIONS

Salivation and ruffled fur were observed in all animals during and/or after exposure. Decreased activity was recorded in all males and two females at exposure end and/or one hour after exposure. Rales were recorded in two males one hour after the end of exposure. There were no clinical signs from test day 2 onward in any of the animals.

D. BODY WEIGHT

Slight body weight loss was noted in four males and all females between days 1 and 4. No body weight gain was recorded in this period in the remaining male. Thereafter normal body weight gain was recorded in all animals, although one female failed to regain the initial body weight.

E. NECROPSY

No abnormality was detected at necropsy.

III. APPLICANT'S CONCLUSIONS

Treatment of HanRcc:WIST(SPF) rats with MCW-2 480 EC at a concentration of 6.0 mg/L for 4 hours resulted in transient effects on body weight, salivation, ruffled fur and decreased activity. The effect on body weight was considered to be test item related. However the restraint in tubes during exposure might have a cumulative effect.

The acute inhalation LC_{50} of MCW-2 480 EC obtained in this study was greater than 6.0 mg/L air (chemically determined mean aerosol concentration).

MCW-2 480 EC is assigned category 5 and does not require any labeling under GHS CLP.

According to US EPA criteria, MCW-2 480 EC is assigned to category IV.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

   Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments**: This acute inhalation toxicity study in the rat is classified as acceptable (fully reliable) and satisfies the guideline requirement for an acute inhalation toxicity study (OPPTS 870.1300; OECD 403) in rats.

C. **Conclusions**: The LC_{50} of the test material was estimated to be greater than 6.0 mg/L air (chemically determined mean aerosol concentration). According to the acute toxicity classification criteria for the PMRA, MCW-2 480 EC is of low acute toxicity via the inhalation route, based on the LC_{50} value in male and female rats.

   Under OCS criteria for acute toxicity endpoints, MCW-2 480 EC is considered to be of low acute inhalation toxicity.

D. **Deficiencies**: The method of animal group selection (i.e. randomization) and time to chamber equilibrium (t_{95}) were not specified in the study report; however, these omissions do not impact the review of this study.

Tier II Summary **(PMRA #2181200, MRID 48574750)** revised by the regulatory authorities July, 2013

Study Type Eye irritation

IIA 5.2.5 – MRID 48574750, PMRA 2181200

Report:	Simon C. (2009) MCW-2 tech. Primary Eye Irritation Study in Rabbits Harlan Laboratories Ltd., Switzerland; unpublished report No. C14977, dated 25 June 2009. Sponsor reference No. R-23421 Dates of experimental work: 15 to 23 October 2008
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 405, EC method B.5 and OPPTS 870.2400 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Executive Summary:

In a primary eye irritation study, 0.1 g of fluensulfone (MCW-2 tech., 96.78% a.i.) was instilled into the left eye of each of three young adult New Zealand White rabbits. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours after instillation.

No mortality occurred during the study and there were no clinical signs of toxicity recorded, nor were there any notable changes to the animals' body weights.

All irritation scores were zero. No abnormal findings were observed in the cornea, the iris, the conjunctivae or the sclera of any animal at any of the examinations. No corrosion was observed at any of the scoring intervals.

No staining of the treated eyes by the test item was observed and no clinical signs were observed.

The test item did not induce significant or irreversible damage to the rabbit eye.

Based on the results of this study, MCW-2 tech. (fluensulfone) is considered non-irritating to the rabbit eye according to the PMRA's classification scheme, and falls under Category IV for primary eye irritation according to the EPA's classification criteria. Under OCS criteria for acute toxicity endpoints, the test material is not considered to be an irritant of the eye of rabbits.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 tech. (Fluensulfone)

Description: Crystalline solid

Lot/Batch: 36372130-291-PF1

Purity: 96.78%

CAS#: 318290-98-1

Stability: Stable until 31 May 2009

2. Vehicle: No vehicle was used. MCW-2 tech. was first ground with a pestle and mortar. Thereafter 0.1 g of the test item was weighed and applied to each animal.

3. Test animals:

Species: Rabbit

Strain: New Zealand White, SPF

Age: Male: 13-14 weeks; females: 14 weeks

Weight at dosing: Male: 2272 g; females: 2162 – 2543 g

Source: Harlan Laboratories BV, The Netherlands

Acclimation period: 5 days

Diet: Pelleted standard Provimi Kliba 3418 rabbit maintenance diet *ad libitum* (Provimi Kliba AG, Switzerland)

Water: Community tap water from Füllinsdorf *ad libitum*

Housing: Individually in stainless steel cages equipped with feed hoppers and drinking water bowls. Wood blocks (Harlan Laboratories Ltd, Switzerland) and haysticks 4642 (Provimi Kliba AG, Switzerland) were provided for gnawing.

4. Environmental conditions:

Temperature: 17-23 °C

Humidity: 30-70%

Air changes: 10-15 air changes per hour

Photoperiod: Light cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS:

1. In life dates: 20 to 23 October 2008

2. Animal assignment and treatment

Animals were selected by hand at time of delivery. The eyes of the animals were examined one day prior to test item administration. Animals with overt signs of ocular injury or irritation which may have interfered with the interpretation of the results were not used in the test.

On the day of treatment, 0.1 g of fluensulfone was placed in the conjunctival sac of the left eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of test item. The right eye remained untreated and served as the reference control.

3. Observations

Observations of viability/mortality and clinical signs were taken daily from the beginning of the acclimation period through to the termination of the study. Body weights were measured on the day of test material application and at study termination. No necropsy was performed on animals.

Eye reactions were assessed according to the method of Draize, at approximately 1, 24, 48 and 72 hours after administration of the test material. Scleral reddening and ocular discharge were also assessed. Scores of each animal at 24, 48 and 72 hours post-dose were used in calculating the respective mean values (with exception of sclerae) for each type of lesion.

4. Statistics

No statistical method was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

No mortality or clinical signs were noted during the study. The body weights of all rabbits were considered to be within the normal range of variability. No necropsy was performed at study termination.

No abnormal findings were observed in the cornea or iris of any animal at any of the measurement intervals (all scores were zero).

III. APPLICANT'S CONCLUSIONS

Fluensulfone is considered non-irritating to the rabbit eye.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments:** This acute eye irritation study in the rabbit is classified as acceptable (fully reliable) and satisfies the guideline requirement for an acute eye irritation study (OPPTS 870.2400; OECD 405) in rabbits.

C. **Conclusions:** The Maximum Average Score (MAS) and the Mean Irritation Score (MIS) were both calculated to be zero. According to the acute toxicity classification criteria for PMRA, MCW-2 tech. (fluensulfone) is considered non-irritating to the rabbit eye, and based on the EPA classification criteria, MCW-2 tech. falls under Category IV for primary eye irritation. Under OCS criteria for acute toxicity endpoints, the test material is not considered to be an irritant to the eye of rabbits.

D. **Deficiencies:** None.

Note: The Tier II summary did not include a full review of this study. This review was prepared by the Pest Management Regulatory Agency, December 19, 2012.

Study Type: Primary Eye Irritation - Rabbit; OPPTS 870.2400; OECD 405.

Test Material (purity): MCW-2 480 EC (497 g a.i. / L)

Citation: Arcelin, G. (2009) MCW-2 480 EC Primary Eye Irritation Study in Rabbits. Harlan Laboratories, Ltd., Switzerland. Laboratory report number C45397. Study report date: 13-October-2009. Applicant Report Number R-23427. DACO 4.6.4. PMRA 2181352.

Sponsor: Makhteshim Chemical Works Ltd., Israel.

MRID: 48574922

Execurive Summary: In a primary eye irritation study, 0.1 ml of MCW-2 480 EC (497 g a.i. / L) was instilled into the conjunctival sac of the left eye of 13 week old New Zealand White rabbits (1 male, 2 females). Eyes were not washed post treatment. Animals were observed for 10 days. Eye reactions were assessed according to the Draize scale.

Slight to marked reddening and chemosis of the conjunctivae, and slight reddening of the sclera was observed in all animals up to 72 hours after treatment. One animal showed corneal opacity affecting over half the eye at 24 to 48 hours post dose. This animal also had delayed/reduced light reflex at the 24 hour observation point. One animal showed transient corneal opacity affecting less than one quarter of the area at 48 and 72 hours post treatment. All scores were zero by 7 days post treatment, except for slight reddening of the sclera persisting in one animal. The scleral reddening was absent by day 10 post dose.

The Maximum Average Score (MAS) for 24, 48 and 72 hours, used by the PMRA for acute toxicity classification, was calculated to be 17, and the Maximum Irritation Score (MIS) was calculated to be 22 (recorded at 24 hours). In this study, MCW-2 480 EC is **moderately irritating** to the eye based on the MAS score of 17 and the persistence of reddening of the sclera in one animal on Day 7. This level of hazard corresponds to the following label signal words and hazard statement: “Warning – Eye Irritant” for the Canadian label.

According to the US EPA criteria, the test material would be assigned to category III, with signal word “Caution.”

Under OCS criteria for acute toxicity endpoints, MCW-2 480 EC is considered to be a moderate irritant to the eye of rabbits.

This study is classified as acceptable (fully reliable). This study satisfies the guideline

requirement for a primary eye irritation study (OPPTS 870.2400; OECD 405) in the rabbit.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. Materials and Methods

A. Materials

Test material:	MCW-2 EC 480	
Description:	Clear orange liquid	
Lot/Batch #:	286-010209-01	
Purity:	497 g a.i./L	
Vehicle:	None	
Test species:	Rabbit	
Strain:	New Zealand White	
Age at dosing:	13 weeks	
Weight at dosing:	2528 g males, 2610 – 2615 g females	
Source:	Harlan France	
Housing:	Housed individually in stainless steel cages with feed hoppers and water bowls; wood blocks and hay sticks were provided for gnawing.	
Diet:	pelleted standard Provimi Kliba 3418 rabbit maintenance diet *ad libitum*	
Water:	community tap water from Füllinsdorf *ad libitum*	
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	17-23degrees C 30-70% 10-15/hr 12 hours dark/12 hours light, music during the daytime light period.
Acclimation:	5 days	

B. Study design and methods

1. Study experimentation dates	Start:	22-Jun-2009	End:	02-Jul-2009

2. Animal assignment and treatment – Animals were hand selected at the time of delivery and examined the day prior to administration of the test material. Any animals with overt signs of ocular injury or irritation were not used for the test. The test material (0.1ml/animal) was applied with a syringe in the conjunctival sac of the left eye of each animal (undiluted as delivered by the Sponsor). The lids were gently held together for about one second to present loss of the test material. The right eye remained untreated and served as the reference control. Eye reactions were assessed according to the Draize scale.

3. Observations – Animals were observed daily from acclimatization to study termination for viability/mortality and clinical signs of toxicity (systemic). Body weights were measured at the start of acclimatization, on the day of application of the test material, and at study termination. Eye reactions were assessed at 1, 24, 48 and 72 hours post treatment, as well as at 7 and 10 days after administration. Scleral reddening and ocular discharge were also assessed. Necropsies were not performed.

II. Results and Discussion

A. <u>Irritation response</u> – Slight to marked redness of the conjunctivae and chemosis were noted in all animals up to 72 hours post dose (Table 1). A moderate discharge was noted in all three animals 1 hour post treatment, persisting as slight (male) to marked (females) at 24 hours post dose. Slight corneal opacity, affecting half to the entire area, was observed in the male rabbit at 24 and 48 hours post treatment. This same animal also had delayed or reduced light reflex 24 hours post treatment. Slight corneal opacity, affecting one quarter of the area, was observed in one female at 48 and 72 hours post dose. Slight to marked reddening of the sclera was noted in all animals up to 72 hours post dose, with slight reddening persisting in one female up to 7 days post dose. No staining was observed in any treated eyes. Test item remnants were observed in one female at the 1 hour reading. All irritation scores were zero by day 10 post dose.

Table 1: Individual and mean eye irritation scores

Animal Number	Sex	Evaluation Interval	Corneal Opacity	Area of Corneal Opacity	Iris	Conjunctivae		Sclera
						Redness	Chemosis	
31	M		0	0	0	2	2	2
32	F	1 hour	0	0	0	2	2	2
33	F		0	0	0	2	2	2
31	M		1	4	1	3	3	3
32	F	24 hours	0	0	0	2	1	2
33	F		0	0	0	3	2	2
31	M		1	3	0	3	2	3
32	F	48 hours	0	0	0	2	1	2
33	F		1	1	0	3	2	2
31	M		0	0	0	1	1	2
32	F	72 hours	0	0	0	1	1	1
33	F		1	1	0	1	1	2
31	M		0	0	0	0	0	0
32	F	7 days	0	0	0	0	0	1
33	F		0	0	0	0	0	0
31	M		0	0	0	0	0	0
32	F	10 days	0	0	0	0	0	0
33	F		0	0	0	0	0	0

Data obtained from page 20 of the study report.

According to the PMRA acute toxicity classification criteria, the Maximum Average Score (MAS) for 24, 48 and 72 hours was calculated to be 17 and the Maximum Irritation Score (MIS) was calculated to be 22 (observed at 24 hours) (Table 2).

Table 2. Mean eye irritation scores according to the Draize scale (according to the acute toxicity classification criteria of the PMRA)

Time point	Corneal opacity[a]	Iris[b]	Conjunctivitis[c]	Total
1 hr	0	0	12	12/110
24 hrs	6.67	1.67	13.3	21.7/110
48 hrs	6.67	0	13.3	20/110
72 hrs	1.67	0	7.33	9/110
7 days	0	0	0.67	0.67/110
MAS: Mean at 24, 48, and 72 hour time points				17/110
Time when score equals 0	7 day	48 hrs	10 days	

a	Cornea:	degree of opacity x area involved x 5	Maximum 80
b	Iris:	degree of irritation x 5	Maximum 10
c	Conjunctivitis:	(redness + chemosis + discharge) x 2	Maximum 20
	Total Score:		Maximum 110

B. <u>Investigator's conclusions</u> – "The test item did not induce significant or irreversible damage to the rabbit eye."

C. <u>Reviewer's conclusions</u> –

Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

The Maximum Average Score (MAS) was calculated to be 17 and the Mean Irritation Score (MIS) was calculated to be 22. In this study, MCW-2 480 EC is considered moderately irritating to the rabbit eye according to the acute toxicity classification criteria for the PMRA. All scores were zero 7 days post dose, except one female had slight reddening of the sclera at this time point. According to the US EPA criteria, the test material would be assigned to category III. Under OCS criteria for acute toxicity endpoints, MCW-2 480 EC is considered to be a moderate irritant to the eye of rabbits.

D. <u>Deficiencies</u> – None.

Tier II Summary (PMRA #2181382, MRID 48574926) revised by the regulatory authorities July, 2013

Study Type: Eye irritation

IIIA 7.1.5 – MRID 48574926, PMRA 2181382

Report:	Mallaun M. (2011) MCW-2 480 EC: Primary Eye Irritation Study in Rabbits Harlan Laboratories Ltd., Switzerland; unpublished report No. D23956, Sponsor No. R-27475, dated 8 June 2011 Dates of experimental work: 19 April to 12 May 2011
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 405, EEC method B.5 and OPPTS 870.2400
Deviations:	None
GLP/Compliance:	Yes. Signed and dated GLP, Data Confidentiality and Quality Assurance statements were provided.

Executive summary:

In a primary eye irritation study, 0.1 mL of MCW-2 480 EC was instilled into the left eye of each of three young adult New Zealand White rabbits. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours, as well as 7, 10 and 14 days after instillation.

The instillation of MCW-2 480 EC into the eye resulted in slight corneal opacity, slight to moderate reddening and slight to marked chemosis of the conjunctivae, slight to moderate reddening of the sclera and slight to moderate ocular discharge. These effects were reversible and were no longer evident 10 days (2 animals) or 14 days (the remaining one animal) after treatment. The cornea of all animals showed positive fluorescein staining from 24 to 72 hours after treatment and reverted 7 days after treatment. No abnormal findings were observed for the iris light reflex and no corrosion was observed at any of the examinations. No staining of the treated eyes by the test item was observed. No clinical signs were observed.

The Maximum Average Score (MAS) for 24, 48 and 72 hours was determined to be 24, and the Maximum Irritation Score (MIS) was calculated to be 31 (recorded at 1 hour and 24 hours).

MCW-2 480 EC is assigned category 2 (H319: causes serious eye irritation) under GHS CLP.

As corneal effects had resolved, and no positive grades for the conjunctivae were recorded in

any animal by day 7, according to US EPA criteria MCW-2 480 EC is assigned to category III, signal word 'Caution'.

Based on the MAS and the persistent conjunctival redness in all animals and scleral reddening in one animal 7 days post treatment, MCW-2 480 EC is considered moderately irritating to the rabbit eye according to the acute toxicity classification criteria for the PMRA. This level of hazard corresponds to the following label signal words and hazard statement: "Warning – Eye Irritant" for the Canadian label.

Under OCS criteria for acute toxicity endpoints, MCW-2 480 EC is considered to be a moderate irritant to the eye of rabbits.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	MCW-2 480 EC
Description:	Clear orange liquid
Batch No.:	371-210110-02
Purity:	473.6 g a.i./L
Expiry date:	24 January 2012
Stability of Test Item:	Stable under storage conditions
2. Vehicle:	The test substance was applied undiluted
3. Test animals:	
Species:	Rabbit
Strain:	New Zealand White, SPF
Age:	Males: 14-15 weeks
Weight at dosing:	Males: 2666 – 3083 g
Source:	Harlan Laboratories U.K. Ltd., UK
Acclimation period:	At least 5 days
Diet:	Pelleted standard Teklad Global High Fiber Rabbit Diet 2031C *ad libitum* (Provimi Kliba AG, Switzerland)
Water:	Community tap water from Füllinsdorf *ad libitum*
Housing:	Individually in stainless steel cages equipped with feed hoppers and drinking water bowls. A piece of wood (imported by Indulab AG, Switzerland from ABEDD ® - LAB & VET GmbH, Austria) and a haystick 4642 (Provimi Kliba AG, Switzerland) were provided for

environmental enrichment.

4. Environmental conditions

Temperature:	17-23 °C
Humidity:	30-70%
Air changes:	10-15 per hour
Photoperiod:	Cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS

1. In life dates: 19 April to 10 May 2011 (one animal); 27 April 2011 to 12 May 2011 (two animals).

2. Animal assignment and treatment:

Animals were selected by hand at time of delivery. The eyes of the animals were examined one day prior to test substance administration. On the day of treatment, 0.1 mL of MCW-2 480 EC was placed with a syringe in the conjunctival sac of the left eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of test substance. The right eye remained untreated and served as the reference control.

A single animal was treated first, which resulted in corneal opacity, positive corneal fluorescein staining and further eye reactions. Upon reversion of the corneal opacity in the first treated animal, the remaining two animals were also treated.

3. Observations

Animals were observed daily for viability/mortality and clinical signs of toxicity (systemic) from acclimation to the end of the observation period. Body weights were measured at the start of the acclimation period, on the day of application of test material, and at the end of the observation period. Eye reactions were assessed at 1, 24, 48 and 72 hours post treatment, and on days 7, 10 and 14. Scleral reddening and ocular discharge were also assessed. To assess presence of corneal damage, fluorescein solution was instilled onto the cornea of both eyes 24 hours prior to dosing, at 24, 48, and 72 hours post dose, as well as 7 days post dose. Eyes were assessed using a hand slit lamp. No necropsies were performed.

4. Statistics

None.

II. RESULTS AND DISCUSSION

A. FINDINGS

No deaths occurred on study and no clinical signs of toxicity (systemic) were observed. The instillation of MCW-2 480 EC into the eye resulted in slight corneal opacity, slight to

moderate reddening and slight to marked chemosis of the conjunctivae, slight to moderate reddening of the sclera and slight to moderate ocular discharge (Table 1). Redness of the conjunctivae persisted in all animals on day 7 and in one animal on day 10, and scleral redness was observed in one animal on day 7. These effects were reversible and were no longer evident 10 days (2 animals) or 14 days (the remaining one animal) after treatment. The cornea of all animals showed positive fluorescein staining from 24 to 72 hours after treatment and reverted 7 days after treatment. No abnormal findings were observed for the iris light reflex and no corrosion was observed at any of the examinations. No staining of the treated eyes by the test item was observed. According to the PMRA acute toxicity classification criteria, the Maximum Average Score (MAS) for 24, 48 and 72 hours was calculated to be 24 and the Maximum Irritation Score (MIS) was calculated to be 31, which was observed at 1 hour and 24 hours (Table 2).

Table 1 Individual and mean eye irritation scores

Animal No.	Sex	Time	Cornea		Iris	Conjunctivae		Sclera	Ocular discharge
			Opacity	Area involved		Redness	Chemosis		
82	M	1 hour	1	4	0	2	2	2	0
83	M		1	4	0	2	2	2	2
84	M		1	4	0	2	3	0	1
82	M	24 hours	1	4	0	2	2	2	2
83	M		1	4	0	2	2	2	1
84	M		1	4	0	2	3	0	1
82	M	48 hours	1	4	0	2	1	2	0
83	M		0	0	0	2	1	2	0
84	M		1	4	0	2	2	2	1
82	M	72 hours	1	4	0	2	1	2	0
83	M		0	0	0	2	0	2	0
84	M		0	0	0	2	1	2	1
82	M	7 days	0	0	0	1	0	0	0
83	M		0	0	0	1	0	0	0
84	M		0	0	0	1	0	1	0
82	M	10 days	0	0	0	0	0	0	0
83	M		0	0	0	1	0	0	0
84	M		0	0	0	0	0	0	0
82	M	14 days	0	0	0	0	0	0	0
83	M		0	0	0	0	0	0	0
84	M		0	0	0	0	0	0	0
82	M	**Mean**	**1.00**		**0.00**	**2.00**	**1.33**		
83	M	**24+48+**	**0.33**		**0.00**	**2.00**	**1.00**		
84	M	**72 h**	**0.67**		**0.00**	**2.00**	**2.00**		

Data obtained from page 60 of the study report

Table 2: Mean eye irritation scores (according to the acute toxicity classification criteria of the PMRA)

Time point	Corneal opacity	Iris	Conjunctivitis	Total
1 hr	20.0	0	11.3	31.3 / 110
24 hrs	20.0	0	11.3	31.3 / 110
48 hrs	13.3	0	10.7	24.0 / 110
72 hrs	6.67	0	9.33	16.0 / 110
7 days	0	0	2.67	2.67 / 110
10 days	0	0	0.67	0.67 / 110
14 days	0	0	0	0 / 110
MAS: Mean at 24, 28 and 72 hour time points				24/ 110
Time when score equals 0	7 days	1 hr	14 days	

a Cornea: degree of opacity x area involved x 5 [Maximum 80]
b Iris: degree of irritation x 5 [Maximum 10]
c Conjunctivitis: (redness + chemosis + discharge) x 2 [Maximum 20]
Total Score: Maximum 110

III. APPLICANT'S CONCLUSIONS

MCW-2 480 EC is assigned category 2 (H319: causes serious eye irritation) under GHS CLP.

As corneal effects had resolved, and no positive grades for the conjunctivae were recorded in any animal by day 7, according to US EPA criteria MCW-2 480 EC is assigned to category III, signal word 'Caution'.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Health Evaluation Directorate, Pest Management Regulatory Agency, Health Canada.

B. **Reviewer's Comments**:

Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

This primary eye irritation study is classified as acceptable (fully reliable) and satisfies the guideline requirement for a primary eye irritation study (OPPTS 870.2400; OECD 405) in rabbits. The Maximum Average Score (MAS) for 24, 48 and 72 hours, used by the PMRA for acute toxicity classification, was calculated to be 24 and the Mean Irritation Score (MIS) was calculated to be 31 (recorded at 1 hour and 24 hours).

C. **Conclusions**: Based on the MAS score and the persistent conjunctival redness in all animals and scleral reddening in one animal 7 days post treatment, MCW-2 480 EC is considered moderately irritating to the rabbit eye according to the acute toxicity classification criteria for the PMRA. This level of hazard would warrant the following label signal words and hazard statement: "Warning – Eye Irritant."

Under OCS criteria for acute toxicity endpoints, MCW-2 480 EC is considered to be a moderate irritant to the eye of rabbits.

D. **Deficiencies**: None.

Tier II Summary **(PMRA # 2181199, MRID 48574749)** revised by the regulatory authorities July, 2013

Study Type: Skin irritation

IIA 5.2.4 – MRID 48574749, PMRA 2181199

Report:	Simon C. (2009) MCW-2 tech. Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) Harlan Laboratories Ltd., Switzerland; unpublished report No. C14966, dated 26 June 2009. Sponsor reference No. R-23420 Dates of experimental work: 6 to 13 October 2008
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 404, EC method B.4 and OPPTS 870.2500 Deviations: none
GLP/Compliance:	Signed and dated GLP, Quality Assurance, and Confidentiality statements were provided.

Executive Summary: In a primary skin irritation study, 0.5 g of fluensulfone (MCW-2 tech., 96.78% a.i.) moistened with ~0.5 mL purified water was applied to the intact left flank of each of three young adult New Zealand White rabbits (1 male and 2 females) for 4 hours. No mortality or clinical signs were noted during the study. One female had a slight body weight loss (-14 g or -0.6%) between treatment and the day of termination.

The scoring of skin reactions was performed 1, 24, 48 and 72 hours, as well as 7 days after removal of the semi-occlusive dressing and remaining test substance. The mean score was calculated across 3 scoring times (24, 48 and 72 hours after patch removal) for each animal for erythema/eschar grades and for oedema grades, separately. The mean erythema/eschar score of the three animals was 1.00, 0.33 and 0.33, respectively and the mean oedema score was 0.00 for all animals. The mean irritation score at 72 hours was 0.33.The application of fluensulfone to the skin resulted in very slight signs of irritation, such as erythema. This effect was reversible and was no longer evident 7 days after treatment, the end of the observation period for all animals. The test item caused no staining of the treated skin. No corrosive effects were noted on the treated skin of any animal at any of the measuring intervals and no clinical signs were observed.

The Maximum Average Score (MAS) of 24, 48 and 72 hours was calculated to be 0.56 and the Maximum Irritation Score (MIS) was calculated to be 1.0, at 1 and 24 hours. According to the acute toxicity classification criteria for PMRA, MCW-2 tech. (fluensulfone) is minimally irritating to the skin, and based on the EPA classification criteria, MCW-2 tech. falls under Category IV for primary skin irritation. Under OCS criteria for acute toxicity endpoints, the test material is considered to be a slight irritant to the skin of rabbits.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 tech. (Fluensulfone)

Description: Crystalline solid

Lot/Batch: 36372130-291-PF1

Purity: 96.78%

CAS#: 318290-98-1

Stability: Stable until 31 May 2009

2. Vehicle: MCW-2 tech. was first ground with a pestle and mortar. Thereafter 0.5 g (per animal) of the test item was weighed and moistened with approximately 0.5 mL of purified water before application.

3. Test animals:

Species: Rabbit

Strain: New Zealand White, SPF

Age: Male: 20 weeks; females: 20-21 weeks

Weight at dosing: Male: 2623 g; females: 2526 g – 2625 g

Source: Harlan Laboratories BV, The Netherlands

Acclimation period: 5 days

Diet: Pelleted standard Provimi Kliba 3418 rabbit maintenance diet *ad libitum* (Provimi Kliba AG, Switzerland)

Water: Community tap water from Füllinsdorf *ad libitum*

Housing: Individually in stainless steel cages equipped with feed hoppers and drinking water bowls. Wood blocks (Harlan Laboratories Ltd, Switzerland) and haysticks 4642 (Provimi Kliba AG, Switzerland) were provided for gnawing.

4. Environmental conditions:

Temperature: 17-23 °C

Humidity: 30-70%

Air changes: 10-15 air changes per hour

Photoperiod: Light cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS:

1. In life dates: 6 to 13 October 2008

2. Animal assignment and treatment

Animals were selected by hand at time of delivery. Four days before treatment, the left flank was clipped with an electric clipper, exposing an area of approximately 100 cm^2 (10 cm x 10 cm). The skin of the animals was examined one day before treatment, and regrown fur of all animals was clipped again. Animals with overt signs of skin injury or marked irritation which may have interfered with the interpretation of the results were not used in the test.

On the day of treatment, 0.5 g of fluensulfone was placed on a surgical gauze patch (ca. 2.5 cm x 2.5 cm) held in contact with the skin by means of an adhesive hypoallergenic aerated semiocclusive dressing and a restrainer bandage wrapped around the abdomen.

The duration of treatment was 4 hours. Then the dressing was removed and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible at that time.

3. Observations

Observations of mortality and viability, and clinical signs took place daily, from the acclimation period to the termination of the observation period. Body weights were measured at the start of the acclimation period, on the day of the application and at termination of the observation period. No necropsy was performed on the animals sacrificed at study termination.

Skin reaction was assessed according to the method of Draize at approximately 1, 24, 48 and 72 hours, as well as 7 days after exposure.

4. Statistics

No statistical method was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

No mortality or clinical signs were noted during the study. One female had a slight body weight loss (-0.6%) between treatment and the day of termination. The body weights of the other rabbits were considered to be within the normal range of variability. No necropsy was performed at termination.

All animals showed very slight erythema at the 1-hour reading which persisted in two animals up to 24 hours after treatment and in one animal up to the 72-hour reading. No abnormal findings were observed on the treated skin of any animal 7 days after treatment, the end of the observation time. Observed dermal reactions are reported in the table below.

Table 1. Individual and mean skin irritation scores

Animal No.	Sex	Scoring time	Erythema	Oedema
96	M		1	0
97	F	1 hour	1	0
98	F		1	0
96	M		1	0
97	F	24 hours	1	0
98	F		1	0
96	M		1	0
97	F	48 hours	0	0
98	F		0	0
96	M		1	0
97	F	72 hours	0	0
98	F		0	0
96	M		0	0
97	F	7 days	0	0
98	F		0	0
96	M	**Mean**	**1.00**	**0.00**
97	F	**24 + 48 + 72 hours**	**0.33**	**0.00**
98	F		**0.33**	**0.00**

Data obtained from page 22 of the study report

The Maximum Average Score (MAS) for 24, 48, and 72 hours, used by the PMRA for acute toxicity classification, was calculated to be 0.56 and the Maximum Irritation Score (MIS) was calculated to be 1.0, recorded at 1 hour and 24 hours (Table 2).

Table 2: Mean skin irritation scores according to Draize scale (according to the PMRA)

Time Point	Erythema[a]	Oedema[b]	Total
1 hr	1.0	0.0	1.0/8
24 hrs	1.0	0.0	1.0/8
48 hrs	0.33	0.0	0.33/8
72 hrs	0.33	0.0	0.33/8
7 days	0	0.0	0/8
MAS: Mean at 24, 48, and 72 hr time points			0.56/8
Time when score equals 0	7 days	1 hr	

a Erythema: Maximum 4
b Oedema: Maximum 4
Total Score: Maximum 8

III. APPLICANT'S CONCLUSIONS

Fluensulfone is not irritating to skin. Slight initial effects are reversible within 7 days.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments:** This acute primary skin irritation study in the rabbit is classified as acceptable (fully reliable) and satisfies the guideline requirement for an acute primary skin irritation study (OPPTS 870.2500; OECD 404) in rabbits.

C. **Conclusions:** Fluensulfone is minimally irritating to skin. Slight initial effects are reversible within 7 days. The Maximum Average Score (MAS) for 24, 48 and 72 hours was calculated to be 0.56 and the Maximum Irritation Score (MIS) was calculated to be 1.0, recorded at 1 and 24 hours. According to the acute toxicity classification criteria for PMRA, MCW-2 tech. (fluensulfone) is minimally irritating to the skin (based on the MAS and MIS scores in rabbits), and based on the EPA classification criteria, falls under

Category IV for primary skin irritation. Under OCS criteria for acute toxicity endpoints, the test material is considered to be a slight irritant to the skin of rabbits.

D. **Deficiencies:** None.

Note: The Tier II Summary did not include a full review of this study. This review was prepared by the Pest Management Regulatory Agency, December 19, 2012.

Study Type: Primary Dermal Irritation - Rabbit; OPPTS 870.2500: OECD 404.

Test Material (Guarantee): MCW-2 480 EC (497 g a.i./L)

Citation: Arcelin, G. (2009). MCW-2 480 EC: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application). Harlan Laboratories Ltd., Switzerland. Laboratory report number C45375. Study report date: 12-October-2009. Applicant Report Number R-23428. DACO 4.6.5. PMRA 2181353.

Sponsor: Makhteshim Chemical Works Ltd., Israel.

MRID: 48574921

Executive Summary: In a primary dermal irritation study, young adult New Zealand White rabbits were dermally exposed to 0.5 ml of MCW-2 480 EC (497 g a.i./L) as supplied by the Sponsor, for 4 hours, to approximately 6 cm^2 of the body surface area. Animals were then observed for 14 days. Irritation was assessed according to the Draize scale.

Slight to well-defined erythema and very slight to slight oedema were observed in all animals from 1 to 72 hours post exposure. Very slight erythema persisted in one female 7 days post exposure. Scaling was observed in one female 72 hours post dosing and in all animals from 7 to 14 days post exposure. No corrosive effects were noted on the treated skin and no clinical signs were observed. The Maximum Average Score (MAS) for 24, 48 and 72 hours, used by the PMRA for classification purposes, was determined to be 3.4. The Maximum Irritation Score (MIS) was calculated to be 3.7, which was observed at 24 and 48 hours post dose.

In this study, MCW-2 480 EC is **moderately irritating** to the skin based on the MAS of 3.4. This level of hazard corresponds to the following label signal words and hazard statement: "Warning – Skin Irritant" on the Canadian label. According to the US EPA criteria, the test material would be assigned to category IV. Under OCS criteria for acute toxicity endpoints, MCW-2 480 EC is considered to be a moderate irritant to the skin of rabbits.

This study is classified as acceptable (fully reliable). This study satisfies the guideline requirement for a primary dermal irritation study (OPPTS 870.2500; OECD 404) in the rabbit.

Compliance: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. Materials and Methods

A. Materials

Test material:	MCW-2 480 EC	
Description:	Clear orange liquid	
Lot/Batch #:	286-010209-01	
Purity:	497 g a.i./L	
Vehicle:	None	
Test species:	Rabbit	
Strain:	New Zealand White	
Age at dosing:	17 weeks ♂; 14 weeks ♀	
Weight at dosing:	2397g %, 2520 - 2617 g &	
Source:	Harlan France	
Housing:	Housed individually in stainless steel cages; wood blocks and haysticks were provided for gnawing.	
Diet:	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet *ad libitum*	
Water:	Community tap water from Füllinsdorf *ad libitum*	
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	20±3EC 30-70% 10-15/hr 12 hours dark/12 hours light
Acclimation:	5 days	

B. Study design and methods

1. Study experimentation dates	Start:	08-Jun-2009	End:	23-Jun-2009

2. Animal assignment and treatment - Animals (1 male, 2 females) were given a single dose of MCW-2 480 EC dermally as delivered by Sponsor. Four days prior to treatment, the left flank of each animal was clipped with an electric clipper exposing an area of approximately 100 cm^2 (10 x 10 cm). The skin was examined one day prior to treatment and regrown fur was clipped again. On the day of treatment, 0.5 ml of the test material was placed on a surgical gauze patch (approximately 2.5 x 2.5 cm) which was held in contact with the skin with an adhesive hypoallergenic aerated semi-occlusive dressing and a restrainer bandage wrapped around the abdomen. Dressings were left in place for 4 hours, after which the dressing was removed and flushed with lukewarm water. Animals were observed daily from acclimatization to study termination for viability/mortality and clinical signs of toxicity (systemic). Body weights were taken at the start of acclimatization, on the day of application, and at termination of the test. Skin irritation was assessed according to the Draize scale, at approximately 1, 24, 48 and 72 hours, and at 7, 10 and 14 days post exposure.

II. Results and Discussion

A. Irritation response – Slight to well-defined erythema and very slight to slight oedema were observed in all animals from 1 to 72 hours post exposure (Table 1). Very slight erythema persisted in one female 7 days post exposure. Scaling was observed in one female 72 hours post

dosing and in all animals from 7 to 14 days post exposure. No corrosive effects were noted on the treated skin and no clinical signs were observed.

Table 1. Individual and mean skin irritation scores

Animal Number	Sex	Evaluation Interval	Erythema	Oedema	Scaling
28	M	**1 hour**	1	1	
29	F		2	1	
30	F		2	1	
28	M	**24 hours**	2	1	
29	F		2	2	
30	F		2	2	
28	M	**48 hours**	2	1	
29	F		2	2	
30	F		2	2	
28	M	**72 hours**	2	1	
29	F		2	1	x
30	F		2	1	
28	M	**7 days**	0	0	x
29	F		0	0	x
30	F		1	0	x
28	M	**10 days**	0	0	x
29	F		0	0	x
30	F		0	0	x
28	M	**14 days**	0	0	x
29	F		0	0	X
30	F		0	0	x

Data obtained from page 21 of the study report.
x = scaling present

The Maximum Average Score (MAS) for 24, 48 and 72 hours, used by the PMRA for classification purposes, was determined to be 3.4 (Table 2). The Maximum Irritation Score (MIS) was calculated to be 3.7, which was observed at 24 and 48 hours post dose.

Table 2. Mean skin irritation scores according to the Draize scale (according to the PMRA)

Time point	Erythema[a]	Edema[b]	Total
1 hr	1.67	1.00	2.67/8
24 hrs	2.00	1.67	3.67/8
48 hrs	2.00	1.67	3.67/8
72 hrs	2.00	1.00	3.00/8
7 days	0.33	0	0.33/8
MAS: Mean at 24, 48, and 72 hour time points			3.44/8
Time when score equals 0	10 d	7 d	

a Erythema: Maximum 4
b Edema: Maximum 4
Total Score: Maximum 8

B. <u>Investigator's conclusions</u> – "The test item did not induce significant or irreversible damage to the skin."

C. <u>Reviewer's conclusions</u>

Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

The Maximum Average Score (MAS) was calculated to be 3.4 and the Mean Irritation Score (MIS) was calculated to be 3.7 at 24 and 48 hours. In this study MCW-2 480 EC was moderately irritating to the skin according to the acute toxicity classification criteria for the PMRA. According to the US EPA criteria, the test material would be assigned to category IV. Under OCS criteria for acute toxicity endpoints, MCW-2 480 EC is considered to be a moderate irritant to the skin of rabbits.

D. <u>Deficiencies</u> – None.

Tier II Summary (PMRA #2181381, MRID 48574925) revised by the regulatory authorities July, 2013

Study Type: Skin irritation

IIIA 7.1.4 – MRID 48574925, PMRA 2181381

Report: Mallaun M. (2011)
MCW-2 480 EC: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)
Harlan Laboratories Ltd., Switzerland; unpublished report No. D23945, Sponsor No. R-27474, dated 18 May 2011
Dates of experimental work: 05 April to 20 April 2011

Sponsor: Makhteshim Chemical Works Ltd., Beer-Sheva, Israel

Guidelines: OECD 404, EEC method B.4 and OPPTS 870.2500

Deviations: None

GLP/Compliance: Yes. Signed and dated GLP, Data Confidentiality and Quality Assurance statements were provided.

Executive summary: In a primary skin irritation study, 0.5 mL of undiluted MCW-2 480 EC was applied to the intact left flank of each of three young female New Zealand White rabbits for 4 hours. The scoring of skin reactions was performed at 1, 24, 48 and 72 hours, as well as 7, 10 and 14 days after removal of the semi-occlusive dressing.

Well-defined erythema and very slight to slight oedema was observed in all animals 1 hour after treatment. Very slight to well-defined erythema persisted in all animals until 10 days after treatment. Very slight oedema persisted until 72 hours in two animals. Two animals showed slight desquamation 10 days after treatment. All skin reactions had reverted 14 days after treatment, the end of the observation period for all animals.

The test item did not cause any staining of the treated skin. No corrosive effects were noted on the treated skin of any animal at any of the measuring intervals and no clinical signs were observed.

The Maximum Average Score (MAS) for 24, 48, and 72 hours, used by the PMRA for acute toxicity classification, was calculated to be 2.33, and the Maximum Irritation Score (MIS) at 1hour post dose was calculated to be 3.33.

MCW-2 480 EC is not classified under GHS CLP for skin irritation in rabbits.

According to US EPA criteria, MCW-2 480 EC is assigned to category IV.

According to the acute toxicity classification criteria of the PMRA, MCW-2 480 EC is mildly irritating to the skin based on the MAS of 2.33. This level of hazard corresponds to the following label signal words and hazard statement: “Caution – Skin Irritant” on the

Canadian label. Under OCS criteria for acute toxicity endpoints, MCW-2 480 EC is considered to be a moderate irritant to the skin of rabbits.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** MCW-2 480 EC

   Description: Clear orange liquid

   Batch No.: 371-210110-02

   Purity: 473.6 g a.i./L

   Expiry date: 24 January 2012

   Stability of Test Item: Stable under storage conditions

2. **Vehicle:** The test substance was applied undiluted

3. **Test animals:**

   Species: Rabbit

   Strain: New Zealand White, SPF

   Age: Females: 17 weeks

   Weight at dosing: Females: 2712 – 2990 g

   Source: Harlan Laboratories U.K. Ltd., UK

   Acclimation period: At least 8 days

   Diet: Pelleted standard Teklad Global High Fiber Rabbit Diet 2031C *ad libitum* (Provimi Kliba AG, Switzerland)

   Water: Community tap water from Füllinsdorf *ad libitum*

   Housing: Individually in stainless steel cages equipped with feed hoppers and drinking water bowls. A piece of wood (imported by Indulab AG, Switzerland from ABEDD® - LAB & VET GmbH, Austria) and a haystick 4642 (Provimi Kliba AG, Switzerland) were provided for environmental enrichment

4. **Environmental conditions**

   Temperature: 17-23 °C

   Humidity: 30-70%

   Air changes: 10-15 per hour

Photoperiod: Cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS

1. **In life dates:** 05 April to 20 April 2011

2. **Animal assignment and treatment:**

Animals were selected by hand at time of delivery.

Four days before treatment, the left flank (or both flanks) was clipped with an electric clipper, exposing an area of approximately 100 cm^2 (10 cm x 10 cm). The skin of the animals was examined one day before treatment, and regrown fur of all animals was clipped again.

On the day of treatment, 0.5 mL of MCW-2 480 EC was placed on a surgical gauze patch (ca. 2.5 cm x 2.5 cm) held in contact with the skin by means of an adhesive hypoallergenic aerated semi-occlusive dressing and a restrainer bandage wrapped around the abdomen. Exposure lasted 4 hours. Then the dressing was removed and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible at that time.

A single animal was treated first. As neither a corrosive effect nor a severe irritant effect was observed after the 4-hour exposure, the test was completed using the two remaining animals for an exposure period of 4 hours.

Observations:

3. Animals were observed daily from acclimatization to the end of the observation period for viability/mortality and clinical signs of toxicity (systemic). Animals were weighed at the start of acclimatization, on the day of application, and at the end of the observation period. Skin reactions were assessed according to the scoring system of Draize at 1, 24, 48 and 72 hours, as well as at 7, 10 and 14 days after treatment. In order to allow examination of the test sites, animal no. 79 was re-clipped 7 and 14 days after treatment and animal nos. 80 and 81 were re-clipped 10 days after treatment.

4. **Statistics**

None.

II. RESULTS AND DISCUSSION

A. FINDINGS

Results are summarized in Table 1. Slight to well defined erythema was present in all animals 1 hour to 10 days post exposure. Oedema was observed in all animals 1 hour post dose and in 2 animals 24 to 72 hours post exposure. No oedema was observed from 7 days to study termination. Desquamation was noted in two animals 10 days post exposure.

Table 1 Individual and mean skin irritation scores

Animal No.	Sex	Scoring time	Erythema	Oedema	Desquamation
79	F	1 hour	2	1	
80	F		2	2	
81	F		2	1	
79	F	24 hours	2	1	
80	F		2	1	
81	F		1	0	
79	F	48 hours	2	1	
80	F		2	1	
81	F		1	0	
79	F	72 hours	2	1	
80	F		2	1	
81	F		1	0	
79	F	7 days	1	0	
80	F		1	0	
81	F		1	0	
79	F	10 days	1	0	
80	F		1	0	1
81	F		1	0	1
79	M	14 days	0	0	
80	F		0	0	
81	F		0	0	
79	F	**Mean**	**2.00**	**1.00**	
80	F	**24 + 48 + 72 hours**	**2.00**	**1.00**	
81	F		**1.00**	**0.00**	

Data obtained from page 19 of the study report.

The Maximum Average Score (MAS) for 24, 48, and 72 hours, used by the PMRA for acute toxicity classification, was calculated to be 2.33 and the Maximum Irritation Score (MIS) was calculated to be 3.33, recorded at 1 hour (Table 2).

Table 2: Mean skin irritation scores according to Draize scale (according to the PMRA)

Time Point	Erythema[a]	Oedema[b]	Total
1 hr	2	1.33	3.33/8
24 hr	1.7	0.7	2.33/8
48 hr	1.7	0.7	2.33/8
72 hr	1.7	0.7	2.33/8
7 days	1	0	1/8
MAS: Mean at 24, 48, and 72 hr time points			2.33/8
Time when score equals 0	14 days	7 days	

a Erythema: Maximum 4
b Oedema: Maximum 4
Total Score: Maximum 8

III. APPLICANT'S CONCLUSIONS

MCW-2 480 EC is not classified under GHS CLP for skin irritation in rabbits.

According to US EPA criteria, MCW-2 480 EC is assigned to category IV.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments**: This primary skin irritation study is classified as acceptable (fully reliable) and satisfies the guideline requirement for an acute primary skin irritation study (OPPTS 870.2500; OECD 404) in rabbits.

C. **Conclusions**: According to the acute toxicity classification criteria of the PMRA, MCW-2 480 EC is mildly irritating to the skin based on the Maximum Average Score (MAS) of 2.33. According to the US EPA criteria, the test material would be assigned to category IV. Under OCS criteria for acute toxicity endpoints, MCW-2 480 EC is considered to be a moderate irritant to the skin of rabbits.

The applicant concluded that MCW-2 480 EC is not classified under GHS CLP for skin irritation in rabbits. However, based on the mean scores of 2.0 for erythema at the 24, 48 and 72 hour time points in two animals, the test material falls should be classified in category 3 under GHS CLP for skin irritation.

D. **Deficiencies**: None.

Tier II Summary (PMRA #2181201, MRID 48574751) revised by the regulatory authorities July, 2013

Study Type: Skin sensitization

IIA 5.2.6 – MRID 48574751, PMRA 2181201

Report:	Simon C. (2009) MCW-2 tech. Contact Hypersensitivity in Albino Guinea Pigs, Maximization-Test Harlan Laboratories Ltd., Switzerland; Main Study: unpublished report No. C14988, dated 26 June 2009. Sponsor reference No. R-23422. Positive Control Study: Study No. B86016. Dates of experimental work (Main Study): 15 October to 13 November 2008. Dates of experimental work (Positive control study): 19 March to 25 April 2008.
Sponsor:	Makhteshim Chemical Works, Ltd., Beer-Sheva, Israel
Guidelines:	OECD 406, EC method B.6 and OPPTS 870.2600 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Executive Summary:

In a skin sensitization study conducted according to the Maximization method, 10 male guinea pigs were induced by intradermal administration of fluensulfone (MCW-2 tech., 96.78% a.i.) at 3% in PEG 300 and in parallel in an emulsion of Freund's Complete Adjuvant (FCA)/physiological saline, followed 8 days later by an epidermal exposure to the test substance for 48 hours under occlusion at 75% in PEG 300, following pretreatment of the test areas with 10% Sodium-Lauryl-Sulfate (SLS) approximately 24 hours prior to application. The 5 male animals of the control group were treated intradermally with PEG 300 and FCA/physiological saline and epidermally with PEG 300 under occlusion. Two weeks after epidermal induction, the test animals were challenged by epidermal application of fluensulfone at 75% in PEG 300 and PEG 300 alone under occlusive dressing. Control animals received the same treatment. Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing.

Ten and 6 out of 10 test animals (100% and 60%, respectively) showed discrete/patchy erythema at 24 and 48 hours, respectively after the challenge treatment with fluensulfone at 75% in PEG 300. Two of 5 control animals (40%) showed the same skin reactions 24 hours after the challenge treatment. At 48 hours, 3 test animals (2 with a concurrent positive response, 1 with a negative response) were additionally observed with skin desquamation. No positive reactions were observed in either the test group or control animals at the site treated with only PEG 300.

Based on interpretation of challenge reactions, fluensulfone technicalwas considered to be a skin sensitizer in the guinea pig.

Under OCS criteria for acute toxicity endpoint, the test material is considered to be a sensitizer to the skin of guinea pigs (in a guinea pig maximization test).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 tech. (Fluensulfone)

Description: Crystalline solid

Lot/Batch: 36372130-291-PF1

Purity: 96.78%

CAS#: 318290-98-1

Stability: Stable until 31 May 2009

2. Vehicle and/or positive control: The vehicle was selected based on preliminary solubility testing which was performed before the study initiation date (excluded from the statement of compliance). Polyethylene glycol 300 (PEG 300) was a suitable vehicle to be used for the study. The pulverized test item prepared at 75% (w/w) in PEG 300 was well-soluble, described as a light yellow liquid and was considered to be the highest diluted and technically applicable concentration for dermal application during the pretest. For the intradermal application, the test item was tested at 15% in PEG 300 and went through a needle of 0.9 x 40 mm (highest needle size barely tolerated for guinea pigs). Due to the possible additional obstacle caused by the tissues during the intradermal injection, 15% (w/w) in PEG 300 was selected as the highest concentration for the intradermal injections in the pretest.

Test material was administered with Freund's Adjuvant – Complete (Sigma, USA, Lot No 068K8761), and physiological saline (Natrium chloratum 0.9%, B. Braun Medical AG, Switzerland, Charge No 7131A192).

For validation of sensitivity of the Maximization-Test as well as the sensitivity of the test system used, a known sensitizer ALPHA-HEXYLCINNAMALDEHYDE was selected as a positive control. Results obtained in the last performed test were included in the report.

3. Test animals:

Species:	Guinea pig
Strain:	Albino Dunkin Hartley, CRL:(HA)BR
Age:	Males, 7-8 weeks at start of pretest
Weight at dosing:	Pretest groups: 350 g – 389 g; Test and control group: 327 g – 431 g (at beginning of acclimatization)
Source:	Charles River Deutschland GmbH, Germany
Acclimation period:	14 days
Diet:	Pelleted standard Provimi Kliba 3418 guinea pig breeding/maintenance diet, containing Vitamin C (Provimi Kliba AG, Switzerland), *ad libitum*
Water:	Community tap water from Füllinsdorf, *ad libitum*
Housing:	Individually in Makrolon type-4 cages with standard softwood bedding ("Lignocel", Schill AG, Switzerland)

4. Environmental conditions:

Temperature:	22 ± 3 °C
Humidity:	30-70%
Air changes:	10-15 air changes per hour
Photoperiod:	Light cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS:

Positive control study: Intradermal induction of the test group was performed in the nuchal region with a 10% dilution of the test material (Alpha-hexylcinnamaldehyde) in PEG 300 in an emulsion of FCA/physiological saline. Control animals were injected with PEG 300 and FCA/physiological saline. For the epidermal induction phase, the test material and/or vehicle were dermally applied for 48 hours, one week following the intradermal induction. The test group was dosed at 10% of the test material in PEG 300, and animals in the control group were dosed with PEG 300 under occlusion. Two weeks following the epidermal induction phase, the control and test animals were challenged by epidermal application of the test material at 1% (left shoulder) and 0.1% (left flank) in PEG 300, and PEG 300 alone (right flank) under occlusive dressing.

Main Study: Based on results of a pretest, the concentrations of 3% fluensulfone in PEG 300 for the intradermal induction and 75% fluensulfone in PEG 300 for the dermal induction and challenge were selected for the main test. A reaction reading of 2 and presence of blanching were noted following intradermal injection at 3%, 5% and 10%, while oedema was also observed at 10%. A concentration of 75% in PEG 300 was considered the highest non-irritating concentration for dermal application.

The animal's fur was shaved with a fine clipper blade or equivalent just prior to the exposure.

Volumes administered were as follows: 0.1 mL/site for the intradermal injection or 0.2 to 0.3 mL on a filter paper for the epidermal administrations. The filter patch was covered by a strip of aluminum foil and firmly secured by an elastic plaster wrapped around the trunk of the animal and secured with impervious adhesive tape. The occlusive dressing was left in place for 24 (epidermal pretest and epidermal challenge) or 48 (epidermal induction) hours. Identical patching method was used for the epidermal pretest, epidermal induction and epidermal challenge.

Intradermal induction (test day 1):

An area of dorsal skin from the scapular region (approximately 6 x 8 cm) was clipped free of hair. Three pairs of intradermal injections (0.1 mL/site) were made just within the boundaries of a 4 x 6 cm area in the clipped region as follows:

Test Group: 1) 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.

2) fluensulfone at 3 % in PEG 300.

3) fluensulfone at 3 % in a 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.

Control Group: 1) 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.

2) PEG 300

3) 1:1 (w/w) mixture of PEG 300 in a 1:1 (v/v) mixture of Freund's Complete Adjuvant and physiological saline.

Epidermal induction (test day 8):

On test day 7, approximately 24 hours prior to the epidermal application the scapular area (approximately 6 x 8 cm) of the animals of the control and test group was clipped, shaved free of hair and the test area was pretreated with 0.5 mL of 10 % Sodium-Lauryl-Sulfate (SLS) in *paraffinum perliquidum* to enhance sensitization by provoking a mild inflammatory reaction. The SLS was massaged into the skin with a glass rod without bandaging.

On test day 8 the scapular area (approximately 6 x 8 cm) was again clipped and shaved free of hair prior to the application. A 2 x 4 cm patch of filter paper was saturated with the test item at 75% and placed over the injection sites of the test animals. The amount of test item preparation applied was approximately 0.3 g. The patch was covered with aluminum foil and firmly secured by an elastic plaster wrapped around the trunk of the animal and secured with impervious adhesive tape. The occlusive dressings were left in place for 48 hours. The epidermal application procedure ensured intensive contact of the test item. The guinea pigs of the control group were treated as described above with PEG 300 only, applied at a volume of approximately 0.3 mL.

The reaction sites were assessed 24 and 48 hours after removal of the bandage for erythema and oedema according to the method of Magnusson and Kligman.

Challenge (test day 22):

The test and control guinea pigs were challenged two weeks after the epidermal induction

application and were treated in the same way.

Two patches (3 x 3 cm) of filter paper were saturated with fluensulfone at the highest tested non-irritating concentration of 75% (applied to the left flank) and the vehicle only (PEG 300 applied to the right flank) using the same method as for the epidermal application. The amount of test item preparation applied was approximately 0.2 g and a volume of approximately 0.2 mL was used for the vehicle. The dressings were left in place for 24 hours. Reaction sites were assessed at 24 and 48 hours following removal of the bandage.

2. Animal assignment and observations

Animals were selected by hand at time of delivery. Three males were included in the pretest, 15 males (10 test and 5 control) in the main study and 15 males (10 test and 5 control) in the positive control study.

In the main study, observations of viability/mortality and clinical signs took place daily from the beginning of the acclimation period through to termination of the study. Skin responses were graded during the pretest, induction and challenge periods. Body weights were taken at the beginning of the acclimation period, at the end of the pretest, at test day 1 (day of treatment) and at the termination of the study. No necropsies were performed.

3. Statistics

Descriptive statistics (means and standard deviations) were calculated for body weights. No inferential statistics were used.

II. RESULTS AND DISCUSSION

A. FINDINGS

Positive Control Study: Discrete/patchy to moderate/confluent erythema with or without scaling was observed in 9/10 test animals at the 24- and 48-hour observation periods after challenge with alpha-hexylcinnamaldehyde at 1% in PEG 300 (left shoulder). Five test animals showed discrete/patchy erythema at the 24-hour reading after treatment with the test material at 0.1% in PEG 300 (left flank). No skin effects were observed in the control group.

Main Study: There were no mortalities and no signs of systemic toxicity during the study. Body weights were within normal range.

The expected and common findings were observed in the control and test group after the different applications using FCA intradermally. These findings consisted of erythema, oedema, necrotizing dermatitis, encrustation and exfoliation of encrustation. A detailed description of the effects observed with FCA was not included in the study report as the study author states that these effects are well known. Discrete/patchy erythema (score of 1) with or without scaling was observed in all animals of both the control and the treatment groups at the 24- and 48-hour reading following the epidermal induction (after pretreatment with 10% SLS in *paraffinum perliquidum*).

At challenge, no positive/skin reactions were observed in the flanks of the animals from either the control or the test group when treated with PEG 300 only (data not shown).

Discrete/patchy erythema was observed in two of five animals (40%) of the control group at the 24-hour reading and in none of the animals at the 48-hour reading when treated with the test item at 75% in PEG 300 (Table 1). Discrete/patchy erythema was observed in all animals (10/10 or 100%) of the test group at the 24-hour reading and in six of ten animals (60%) at the 48-hour reading when treated with the test item at 75% in PEG 300 (Table 1).

Table 1: Skin Reactions after Challenge Application

Animal No.	Sex	Reaction Readings After Removal of Bandage	
		24 hours	48 hours
C: 866	M	1	0
C: 867	M	0	0
C: 868	M	0	0
C: 869	M	0	0
C: 870	M	1	0
T: 871	M	1	1
T: 872	M	1	1
T: 873	M	1	1
T: 874	M	1	0
T: 875	M	1	0
T: 876	M	1	1
T: 877	M	1	1*
T: 878	M	1	1*
T: 879	M	1	0*
T: 880	M	1	0

C : control group treated with MCW-2 Tech., 75% in PEG 300 (left flank)
T: treatment group treated with MCW-2 Tech., 75% in PEG 300 (left flank)
*: scaling present; 1: discrete or patchy erythema (as per scoring system)
Data obtained from Tables 4 and 6 (page 34) of the study report.

In spite of the lack of evidence of irritation in the pretest animals, 40% of the control animals which were not exposed to the test item before the challenge procedure, showed skin irritation at the concentration of 75%. This phenomenon was regarded as being due to the biological

variability. Since the local reactions in the control group must be due to irritation, they cast doubt on the nature of the reactions apparent in the test animals, particularly since they are of the same grade. In such a case one approach is proposed by P. Evans [P. Evans: Regulatory Aspects of Contact Hypersensitivity (in: I. Kimber and Th. Maurer: Toxicology of Contact Hypersensitivity, 1996 Taylor and Francis ISBN 0 7484 0349 3)], is that the net percentage difference in test and control animals with positive readings should be calculated by subtracting the percentage of control animals giving skin reactions from the percentage of test animals showing these reactions at challenge. At the challenge concentration of 75% in PEG 300, MCW-2 tech. showed in the control group a discrete/patchy irritating potential (in 40% of the animals at the 24 hours and 0% at the 48 hours) as evidence from positive reactions. In the test group, discrete/patchy erythema was observed in 10 out of 10 test animals (100%) at the 24 hours. At the 48 hours in the test group, 60% of the test animals also had grade 1. Thus the net difference was 60% (i.e. 100% - 40%) at the 24 hours and was 60% (i.e. 60% - 0%) at the 48 hours, which indicates that the test substance was a sensitizer in this study.

III. APPLICANT'S CONCLUSIONS

Fluensulfone technical was found to be a skin sensitizer in the guinea pig Maximization test.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments:** This contact hypersensitivity study in albino Guinea pigs is classified as acceptable (fully reliable) and satisfies the guideline requirement for a skin sensitization study (OPPTS 870.2600; OECD 406) in Guinea pigs.

C. **Conclusions:** Fluensulfone technical was found to be a skin sensitizer in the Guinea pig Maximization test.

Under OCS criteria for acute toxicity endpoint, the test material is considered to be a sensitizer to the skin of guinea pigs (in a guinea pig maximization test).

D. **Deficiencies**: None.

Tier II Summary (PMRA #2181354 MRID 48574923) revised by the regulatory authorities July, 2013

Study Type: Dermal Sensitization

IIIA 7.1.6 – MRID 48574923. PMRA 2181354

Report: Arcelin G. (2009)
MCW-2 480 EC Contact Hypersensitivity in Albino Guinea Pigs, Maximization-Test
Harlan Laboratories Ltd., Switzerland; unpublished report No. C45421, Sponsor No. R23429, dated 19 October 2009
Dates of experimental work: 09 June to 6 July 2009

Sponsor: Makhteshim Chemical Works Ltd., Beer-Sheva, Israel

Guidelines: OECD 406, EEC method B.6 and OPPTS 870.2600

Deviations: None

GLP/Compliance: Yes. Signed and dated GLP, Data Confidentiality and Quality Assurance statements were provided.

Executive summary:

In a skin sensitization study conducted according to the Maximization method, 10 Albino Dunkin Hartley [CRL:(HA)BR, SPF] guinea pigs were induced by intradermal administration of MCW-2 480 EC at 15% in purified water and in parallel in an emulsion of Freund's Complete Adjuvant (FCA)/physiological saline, followed eight days later by an epidermal exposure for 48 hours under occlusion with the undiluted test substance. The 5 animals of the control group were treated intradermally with purified water and FCA/physiological saline and epidermally with purified water under occlusion. Two weeks after epidermal induction, the test animals were challenged by epidermal application of MCW-2 480 EC at 1% in purified water and purified water alone under occlusive dressing. Control animals received the same treatment. Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing.

All animals in the test group reacted positively after epidermal challenge with MCW-2 480 EC, while no skin reactions were recorded in any of the control animals. MCW-2 480 EC was considered to be a potential dermal sensitizer.

MCW-2 480 EC is assigned category 1 (H317: may cause an allergic skin reaction) under GHS CLP.

Under OCS criteria for acute toxicity endpoints, MCW-2 480 EC is considered to be a sensitizer to the skin of guinea pigs (in a guinea pig maximization test).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: MCW-2 480 EC

Description: Orange clear liquid

Batch No.: 286-010209-01

Purity: 497 g/L

Expiry date: 1 February 2011

Stability of Test Item: Stable under storage at room temperature, light protected

2. Vehicle and/or positive control: Purified water prepared at Harlan Laboratories Ltd. (deionised water which was processed and treated by the PURELAB Option-R unit. The latter links four purification technologies: reverse osmosis, adsorption, ion-exchange and photo oxidation) / The sensitivity and reliability of the experimental technique employed was assessed by use of ALPHA-HEXYLCINNAMALDEHYDE (a moderate skin sensitizer). The results from the most recent test run (Harlan Laboratories Study C50652, performed from 27-May-2009 to 20-Jul-2009) were included in the report.

3. Test animals

Species: Guinea pig

Strain: Albino Dunkin Hartley [CRL:(HA)BR, SPF]

Age: 4-6 weeks at start of acclimation period

Weight: Male: 289 g – 364 g at start of acclimation period

Source: Charles River Deutschland GmbH, Germany

Acclimation period: 12 days (main study)

Diet: Pelleted standard Provimi Kliba 3418 guinea pig breeding / maintenance diet, containing Vitamin C (Provimi Kliba AG, Switzerland), *ad libitum*

Water: Community tap water from Füllinsdorf, *ad libitum*

Housing: Individually in Makrolon type-4 cages with standard softwood bedding ("Lignocel", Switzerland)

4. Environmental conditions

Temperature:	22 ± 3°C
Humidity:	30-70%
Air changes:	10-15 per hour
Photoperiod:	Cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS

1. In life dates: Pretest Start: 27 May 2009; Main Study Start: 9 June 2009 (induction), 16 June 2009 (epidermal induction), and 30 June 2009 (challenge); Study Termination: 6 July 2009.

2. Animal assignment and treatment:

Animals were selected by hand at time of delivery.

A pre-test for choosing appropriate concentrations to be used in the main study was performed during acclimatisation of the control and test groups, using a single animal for the intradermal induction, and a total of 4 animals for the topical applications. For the intradermal injections, doses of 50%, 25% and 15% of the test item were tested. A skin irritation score of 2 was noted (moderate and confluent erythema) with swelling 24 hours post dose in the two high dose groups, whereas a score of 1 (discrete or patchy erythema) was noted in the low dose group. For the epidermal application, doses of 100%, 75%, 50% and 25% of the test material were examined. According to Magnusson-Kligman, and to the findings observed, the test item at 100% was considered to be the maximally tolerated concentration to stimulate a state of immune hypersensitivity in the epidermal induction procedure. In order to determine the highest non-irritating concentration to be used for the challenge procedure, a second pretest was performed with concentrations of 15%, 10%, 5% and 1% test material. Only animals exposed to 1% of the test material had irritation scores of zero. Therefore, based on the results of the pretest, the concentration of 15% in purified water was selected for the intradermal induction, and 100% and 1%, respectively, were selected for induction and challenge in the main study.

In the main study, the following procedures were performed.

For intradermal induction (test day 1), an area of dorsal skin from the scapular region (approximately 6 x 8 cm) was clipped free of hair. Three pairs of intradermal injections (0.1 mL/site) were made just within the boundaries of a 4 x 6 cm area in the clipped region. The first pair of injections were with a 1:1 (v/v) mixture of Freund's Complete Adjuvant (FCA) and physiological saline in both the treated and control animals; the second pair of injections were with the test substance at 15% in purified water or purified water only in treated and control animals, respectively; and the third pair of injections were with the test substance at 15% in a 1:1 (v/v) mixture of FCA

and physiological saline or FCA and physiological saline and purified water in the treated and control animals, respectively.

For the epidermal induction (test day 8), the scapular area (approximately 6 x 8 cm) was again clipped and shaved free of hair prior to the application. A 2 x 4 cm patch of filter paper was saturated with the test substance at 100% and placed over the injection sites of the test animals. The volume applied was approximately 0.3 mL. The patch was covered with aluminum foil and firmly secured by an elastic plaster wrapped around the trunk of the animal and secured with impervious adhesive tape. The occlusive dressings were left in place for 48 hours. The guinea pigs of the control group were treated in the same manner with purified water only applied at a volume of approximately 0.3 mL. The skin reactions at the application sites were assessed 24 and 48 hours after removal of the bandage for erythema and oedema according to the method of Magnusson and Kligman.

At challenge (test day 22), hair was clipped and shaved from a 5 x 5 cm area on the left and right flank of each guinea pig just prior to the application. Two patches (3 x 3 cm) of filter paper were saturated with the test substance at the highest tested non-irritating concentration of 1% (applied to the left flank) and the vehicle only (purified water applied to the right flank) using the same method as for the epidermal induction. The volume applied was approximately 0.2 mL and a volume of approximately 0.2 mL was used for the vehicle. The dressings were left in place for 24 hours.

Twenty-one hours after removal of the dressing the test sites treated with the test substance were depilated.

3. Data evaluation

During the epidermal induction, approximately 24 and 48 hours after removal of the bandage, application sites were assessed for erythema and oedema according to the method of Magnusson and Kligman. Following the challenge phase, approximately 24 hours after patch removal (48 hours from the start of the challenge application) the skin reactions were observed and recorded. Approximately 48 hours after patch removal, skin reactions were observed again (72 hours from the start of the challenge application) and recorded.

4. Observations

Animals were observed daily for viability/mortality and clinical signs of systemic toxicity from the time of delivery to study termination. Body weights were noted at the start of acclimatization, at the end of the pretest, on test day 1 (day of treatment), and at the termination of the study.

II. RESULTS AND DISCUSSION

A. FINDINGS

The expected and common findings after intradermal injection of FCA (erythema, oedema, necrotizing dermatitis, encrustation and exfoliation of encrustation) were observed in the control and test group.

No erythematous or oedematous reaction was observed in the animals treated with purified water only at dermal induction while discrete/patchy to moderate/confluent erythema was observed in all treated animals at the 24-hour and 48-hour readings after treatment with the test substance at 100%. At the same time, fissures were noted in five out of ten treated animals.

At challenge, no skin reactions were observed in the control animals treated with either purified water only or when treated with the test substance at 1% in purified water (Table 1). In treated animals, no skin reactions were observed in the flank treated with purified water only. Discrete/patchy to moderate/confluent erythema was observed in all animals at the 24-hour and 48-hour readings in the flank treated with the test substance at 1% in purified water.

Table 1: skin reactions after challenge procedure

Procedure at challenge	After 24 hours		After 48 hours	
	Positive / Total	% positive	Positive / Total	% positive
Control group				
MCW-2 480 EC 15 In purified water (left flank)	0 / 5	0	0 / 5	0
Purified water only (right flank)	0 / 5	0	0 / 5	0
Treated group				
MCW-2 480 EC 15 In purified water (left flank)	10 / 10	100	10 / 10	100
Purified water only (right flank)	0 / 10	0	0 / 10	0

No signs of systemic toxicity were observed in any of the animals. The body weight of the animals was within the range commonly recorded for animals of this strain and age.

III. APPLICANT'S CONCLUSIONS

Even though all test animals were affected in the challenge procedure, as the epidermal induction was performed at the highest epidermal concentration applicable of 100% (neat commercial formulation), MCW-2 480 EC was considered to be a weak sensitizer.

MCW-2 480 EC is assigned category 1 (H317: may cause an allergic skin reaction) under GHS CLP.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments**: This contact hypersensitivity study in albino Guinea pigs is classified as acceptable (fully reliable) and satisfies the guideline requirement for a skin sensitization study (OPPTS 870.2600; OECD 406) in Guinea pigs.

C. **Conclusions**: Based on the results of this study (i.e. the observations of erythema observed in all animals at 24 and 48 after the challenge phase), MCW-2 480 EC is considered to be a potential dermal sensitizer.

Under OCS criteria for acute toxicity endpoints, MCW-2 480 EC is considered to be a sensitizer to the skin of guinea pigs (in a guinea pig maximization test).

D. **Deficiencies**: None

Tier II Summary (PMRA #2181204, MRID 48575024) revised by the regulatory authorities, April 2013

Study Type 28-Day Oral Toxicity (mice)

IIA 5.3.1 – MRID 48575024, PMRA 2181204

Report:	Krötlinger F. and Rühl-Fehlert C. (2003) BYI 01921 Subacute oral toxicity in mice (4 weeks administration by diet). Bayer HealthCare, Experimental Toxicology, Germany; unpublished Report No. AT00816, dated 27 November 2003. Study No. T2071608. Sponsor reference No. R-23149 Dates of experimental work: 16 April 2002 to November 26, 2003
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines	OECD 407 (1995) and 67/548/EEC B.7 (although not stated in study report, EPA Test Guideline OPPTS 870.3050 also applies) Deviations: none
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In a 4-week dietary study (MRID 48575024, PMRA 2181204), groups of 5 male and 5 female Crl:CD1 mice received fluensulfone (BYI 01921, MCW-2 technical, 97%) in their diet at 0, 100, 500 and 2000 ppm (equivalent to 0/0, 30/41, 101/155, and 375/353 mg/kg bw/day in males/females, respectively).

No mortality and no clinical signs occurred. Food intake was decreased at 500 ppm (28-33%) and 2000 ppm (34-63%) in both sexes. Water consumption was slightly increased at 2000 ppm in females. Body weight loss was observed at 2000 ppm in the first week of treatment in both sexes. At the end of treatment the mean body weight difference from the controls was -13% in males at 2000 ppm.

Effects on red blood cell parameters were detected at 2000 ppm in both sexes, and were generally more pronounced (and statistically significant) in females (increases of erythrocyte and reticulocyte counts, concentration of hemoglobin, hematocrit values). Platelets were reduced in males at 500 ppm (13%; not statistically significant) and in females at 2000 ppm (20%; not statistically significant). Increased activities of plasma-ASAT and plasma-ALAT were recorded in both sexes at 2000 ppm and considered as correlates of the observed microscopic and organ weight changes in the liver. Very slight increases in the activity of APh were also noted in both sexes at 2000 ppm.

Liver weights (relative) were increased at 500 ppm and above in females. Histopathology revealed the following induced findings at 2000 ppm in both sexes:

- A cytoplasmic alteration in the periportal region, characterized by a dense cytoplasmic structure, eosinophilia (females) or slight basophilia (males)
- Degenerative changes of the periportal zone which encompassed also single cell necrosis and in some of the females mitotic figures
- Slight increase in number and size of bile ducts in the portal regions (bile duct hyperplasia) in one male and three females

The absolute kidney weights were decreased at 2000 ppm in males (20%), and absolute heart weights were decreased at 500 and 2000 ppm in males and at 2000 ppm in females (16-18%); relative weight in males did not show a dose response. However, no further correlates for an influence on kidneys or the heart were detected.

The NOAEL of the study was considered to be 100 ppm for both sexes (30 and 41 mg/kg bw/day in male and female mice, respectively). The LOAEL for both sexes is 500 ppm (101 and 155 mg/kg bw/day in male and female mice, respectively), based on decreased food consumption in both sexes and decreased platelets in males; based on the marginal changes at this dose, it is considered a threshold effect level.

This study is classified **acceptable/guideline (fully reliable)** and satisfies the requirement for a 28-day dietary study in the mouse (OECD 407; OPPTS 870.3050),

I. MATERIAL AND METHODS

1. Test Material	BYI 01921 (MCW-2)
Description:	Brown liquid
Lot/Batch:	NLL6692-7-5
Purity:	97.0%
CAS#:	318290-98-1
Stability:	Stable for the duration of the study
2. Vehicle	Standard diet (Provimi Kliba 3883.9.25 supplied by Provimi Kliba SA, Switzerland)
3. Test Animals	
Species	Mouse
Strain	Crl:CD1 (ICR)BR
Age	Males: approx. 5 weeks; Females: approx. 6 weeks
Weight	Males: 27-30 g; Females: 23-28 g
Source	Charles River, Sulzfeld, Germany
Acclimation period	At least 7 days
Diet	Standard diet (Provimi Kliba 3883.9.25 supplied by Provimi Kliba SA, Switzerland) *ad libitum*. The feed was provided in stainless steel automatic feeders inside the cages.
Water	Tap water *ad libitum*. Water was supplied in polycarbonate bottles (300 mL).
Housing	Individually in type II polycarbonate cages on low-dust wood granulate (Ssniff Spezialdiaten GmbH, Germany).
4. Environmental conditions	
Temperature:	22 ± 2°C
Humidity:	approximately 55 ± 5%
Air changes:	≤ 10 changes per hour
Photoperiod:	12 hours artificial lighting
B. STUDY DESIGN	
1. In-life dates	16 April to 16 May 2002

2. Animal Assignment and Treatment

Male and female mice were grouped by weight and assigned to dose groups (5/sex/dose) using random lists based on evenly distributed chance numbers by a software application. The test substance was administered in the feed from the start of the study until scheduled sacrifice at nominal doses of 0, 100, 500 and 2000 ppm.

3. Dose Selection Rationale

The doses were selected on the basis of the results of a 2-week dose range-finding study (Study No. T6071594; study was not submitted to regulatory authorities), in which 2 male mice/group were dosed with 0, 50, 150, 625, 2500, and 7000 ppm fluensulfone in the diet. At 7000 ppm, both animals showed apathy during days 3-8, and piloerection, palor and hunched back during days 4-8. No animal died. Food consumption was decreased (doses not specified). Body weights were decreased at 625 ppm and above (decreases of 10, 14 and 30% relative to controls at 625, 2500 and 7000 ppm, respectively).

4. Diet Preparation and Analysis

Fluensulfone was mixed into the diet intended for the following week in accordance with the dose scheme. A mixing granulator manufactured by Loedige (Paderborn) was used. Homogeneity and stability of the test substance and its correct concentration in the feed mixture were determined analytically. For the analytical determinations before the start of the study, an aliquot was taken from a 7000 ppm test diet and mixed with basal diet to obtain a 20 ppm sample. During the study, samples were taken from the individually prepared dose mixtures prepared at the beginning of the study.

The test substance was homogeneously distributed in the 20 and 7000 ppm test diets (relative standard deviations of 0.6-4.8%) and in samples of the 100 and 2000 ppm test diets (relative standard deviations of 1.5-2.5%). The 20 and 7000 ppm test diets were chemically stable for at least 15 days. The concentration of test material in the 100, 500 and 2000 ppm feed mixtures agreed to the target values within defined limits (range 94-105% of nominal values).

5. Statistics

The quantitative results for individual animals were used to calculate arithmetic group means and standard deviations. The results for the groups that received the test substance were compared with those for the control group and significant differences were indicated for $p < 0.05$ and $p < 0.01$ using the following methods:

Analysis of variance followed by Dunnett test: body weight, erythrocytes, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, platelets, albumin, protein, triglycerides, urea, absolute organ weights.

Kruskal-Wallis test followed by adjusted U test: leukocytes, reticulocytes, differential blood counts, albumin/globulin ratio, total bilirubin.

Adjusted Welsh test: ALAT, APh, ASAT, cholesterol.

Logarithmic analysis of variance and Dunnett test: relative organ weights.

C. METHODS

1. Observations

The experimental animals were inspected at least once a day.

2. Bodyweight

The body weights of the individual experimental animals were determined before the beginning of the study and weekly thereafter up to scheduled necropsy.

3. Food and water consumption and active ingredient intake

Food and water intakes of each individual mouse were determined once per week, based on the difference between the weight of the food/water made available and the weight of the remaining food/water that was not consumed. The consumption per animal per day and per kg body weight per day were determined from these primary data.

4. Ophthalmoscopic examination

Not performed.

5. Hematology and Clinical Chemistry

Blood samples for the laboratory determinations were collected on day 25 for hematology and day 28 for clinical chemistry from the retro-orbital venous plexus of animals anesthetized with CO_2/O_2 (80:20).

The following hematology parameters were determined:

differential blood count, morphology of erythrocytes, erythrocytes, hemoglobin, hematocrit, leucocyte count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, thrombocytes, reticulocytes.

The following clinical chemistry parameters were determined: alkaline phosphatase (APh), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), albumin, protein (total), cholesterol, urea, triglycerides, bilirubin (total), albumin/globulin ratio.

6. Urinalysis

Not performed.

7. Sacrifice and pathology

Animals were necropsied after exsanguination under deep ether anesthesia.

Adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes and thymus were weighed. A complete list of organs from animals of the control and high dose groups was examined microscopically (including adrenal glands, brain, epididymides, esophagus, femur, gall bladder, heart, intestine, kidneys, liver, lungs, lymph nodes, ovaries, oviducts, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicles, coagulation glands, skeletal muscle, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid glands, trachea, urinary bladder, uterus, vagina, gross lesions) and gross lesions, liver, gall bladder, lungs and spleen of animals of the mid and low-dose groups were also examined.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs

No clinical signs were noted during the study.

2. Mortality

No treatment-related mortalities occurred during the study. One male mouse each from the 500 and 2000 ppm groups died on day 25 because of blood sampling.

B. BODYWEIGHT AND BODYWEIGHT GAINS

Body weight loss was observed at 2000 ppm in week one in both sexes (Tables 1 and 2). At the end of treatment the difference in body weight from the controls was -13% in males (Table 1). At study termination, males from the 2000 ppm group showed no cumulative weight gain, while other groups gained 3-5 g. Females at 2000 ppm showed a cumulative weight loss of -2 g compared to gains of 1-2 g in the other groups.

Table 1: Mean ± SD body weights (g)

Day	0 ppm	100 ppm	500 ppm	2000 ppm
Male mice				
1	28 ± 0.5	28 ± 0.8	28 ± 1.1	28 ± 1.1
8	29 ± 0.8	30 ± 0.8	28 ± 1.5	26 ± 2.0** (↓10%)
15	31 ± 1.9	31 ± 0.8	30 ± 1.5	28 ± 2.1* (↓10%)
22	32 ± 1.1	32 ± 0.9	32 ± 1.1	30 ± 1.5** (↓6%)
29	32 ± 1.8	32 ± 1.3	31 ± 1.5	28 ± 1.9** (↓13%)
Female mice				
1	25 ± 0.8	26 ± 0.8	25 ± 1.8	26 ± 0.5
8	26 ± 0.9	26 ± 1.3	25 ± 1.1	25 ± 1.9
15	27 ± 1.7	27 ± 1.6	26 ± 1.1	25 ± 1.3
22	28 ± 2.1	28 ± 1.3	27 ± 0.8	26 ± 1.5
29	27 ± 1.9	28 ± 1.9	27 ± 1.1	26 ± 2.1

Data obtained from pages 79-80 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * $p<0.05$; ** $p<0.01$

Table 2: Mean ± SD body weight gains (g)

Day	0 ppm	100 ppm	500 ppm	2000 ppm
Male mice				
1-8	2 ± 0.5	2 ± 0	1 ± 1.5	-2 ± 1.1**
8-15	2 ± 1.3	1 ± 0	2 ± 0.8	2 ± 0.7
15-22	1 ± 1.1	1 ± 0.4	1 ± 0.5	2 ± 1.1
22-29	0 ± 1.2	0 ± 0.7	-1 ± 1	-2 ± 1.8
1-29[a]	5	4	3	0
Female mice				
1-8	0 ± 0.5	0 ± 0.5	0 ± 1	-2 ± 1.5*
8-15	1 ± 1	1 ± 0.4	1 ± 0	0 ± 0.7
15-22	1 ± 1.4	1 ± 0.4	1 ± 0.4	1 ± 1.1
22-29	-1 ± 0.9	0 ± 0.8	0 ± 0.5	-1 ± 1.8
1-29[a]	1	2	2	-2

Data obtained from pages 83-84 of the study report. * $p<0.05$; ** $p<0.01$. [a] Cumulative gain calculated by reviewer and not analyzed statistically.

C. FOOD AND WATER CONSUMPTION AND TEST SUBSTANCE INTAKE

At 500 ppm and above food intake (data not shown) was decreased in a dose-dependent fashion (per animal/day and per kg bw/day) in both sexes (at 500 ppm, 28-33% below controls; at 2000 ppm, males 34-40% and females 62-63% less than controls). Decreases were statistically significant for some weeks for males at both dose levels and for all weeks for the 2000 ppm females. The water consumption (data not shown) was slightly increased at 2000 ppm in females (11-17% relative to controls).

The intake of test substance in the individual treatment groups are indicated in Table 3.

Table 3: Mean achieved test substance intakes (mg/kg bw/day)

100 ppm		500 ppm		2000 ppm	
Males	Females	Males	Females	Males	Females
30.33	41.32	101.31	155.14	374.75	353.10

Data obtained from page 52 of the study report.

D. BLOOD ANALYSIS

1. Hematological findings

The erythrocyte and reticulocyte counts and the concentration of hemoglobin and hematocrit values were increased at 2000 ppm in both sexes (Table 4). Except for the reticulocyte counts, this effect was more pronounced in females. Platelet counts were slightly decreased in males from the 500 and 2000 ppm dose groups and in females from the 2000 ppm dose group. This decrease was also seen in the mouse 90-day study at 300 and 1500 ppm (MRID 48574753/ PMRA 2181205).

Table 4: Selected hematological parameters (mean ± SD)

Parameter	0 ppm	100 ppm	500 ppm	2000 ppm
Male mice				
Erythrocyte count (10^{12}/L)	9.33 ± 0.7	9.29 ± 0.3	9.67 ± 0.9	9.92 ± 0.5 (↑6%)
Hemoglobin (g/L)	154 ± 11	154 ± 6	161 ± 17	163 ± 11 (↑6%)
Hematocrit (L/L)	0.530 ± 0.03	0.525 ± 0.03	0.563 ± 0.07	0.567 ± 0.04 (↑7%)
Reticulocyte counts (0/00)	35 ± 2.9	36 ± 2.8	41 ± 3.6	44 ± 16 (↑26%)
Platelets	1248 ± 326	1162 ± 155	1089 ± 273 (↓13%)	1084 ± 154 (↓13%)
Female mice				
Erythrocyte count (10^{12}/L)	9.07 ± 0.4	9.47 ± 0.3	9.59 ± 0.5	9.98 ± 0.7* (↑10%)
Hemoglobin (g/L)	153 ± 6	157 ± 6	157 ± 10	168 ± 11* (↑10%)
Hematocrit (L/L)	0.512 ± 0.02	0.521 ± 0.00	0.541 ± 0.03	0.569 ± 0.04** (↑11%)
Reticulocyte counts (0/00)	38 ± 7.2	33 ± 3.0	36 ± 2.7	45 ± 9.8 (↑18%)
Platelets	1031 ± 137	1056 ± 107	1083 ± 162	823 ± 267 (↓20%)

Data obtained from pages 90-91 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * $p<0.05$; ** $p<0.01$

2. Blood chemistry findings

The activities of ASAT and ALAT were increased at 2000 ppm in both sexes (Table 5). Markedly increased values were noted in one male (no. 16: ASAT 232.5 U/L, ALAT 191.4 U/L) and two females (no.36: ASAT 118.4 U/L, ALAT 93.6 U/L, and no.40: ASAT 143.9 U/L, ALAT 93.5 U/L). Nevertheless, when these values were excluded from analysis, a slight increase of the activity of both enzymes could be seen. Very slight increases in the activity of APh were also noted in both sexes at 2000 ppm.

Table 5: Serum enzyme activities (mean ± SD)

Parameter	0 ppm	100 ppm	500 ppm	2000 ppm
Male mice				
ASAT (U/L)	47.0 ± 10	54.2 ± 9	49.8 ± 8~	113.6 ± 80~ (↑142%)
ALAT (U/L)	41.7 ± 4	44.9 ± 7	59.4 ± 21~	86.8 ± 71~ (↑108%)
APh (U/L)	251 ± 52	220 ± 51	257 ± 71~	286 ± 60~ (↑7%)
Female mice				
ASAT (U/L)	50.3 ± 4	52.4 ± 7	59.9 ± 11	93.1 ± 39 (↑85%)
ALAT (U/L)	30.5 ± 4	37.4 ± 8	36.4 ± 9	63.9 ± 28 (↑109%)
APh (U/L)	199 ± 41	197 ± 24	204 ± 19	222 ± 58 (↑12%)

Data obtained from pages 104-105 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). ~ number of available samples too low to analyze statistically.

E. SACRIFICE AND PATHOLOGY

1. Necropsy findings

There was no evidence of any treatment-related gross pathological findings at necropsy.

2. Organ weight

The absolute kidney weights were decreased at 2000 ppm in males (20% below controls), and the relative liver weights were increased at 500 ppm and above in females but did not follow a dose-dependent trend (Table 6).

Absolute heart weight was decreased in males from the 500 and 2000 ppm dose groups, but relative weight did not show a dose-related trend. Decreased absolute and relative heart weights were noted in females from the 2000 pm dose group.

A non-statistically significant increase in relative brain weight (14%) was noted for males from the 2000 ppm group (data not shown), which was likely secondary to the reduced body weight in this group.

Table 6: Selected absolute (mg) and *relative (mg/100 g)* organ weights (mean ± SD)

Organ		0 ppm	100 ppm	500 ppm	2000 ppm
Male mice					
Terminal BW (g)		33 ± 1.1	33 ± 1.5	32 ± 1.7	29 ± 1.7**
Liver	absolute	1924 ± 52	2083 ± 196	2026 ± 270	1741 ± 145
	relative	*5764 ± 126*	*6245 ± 601*	*6270 ± 635~*	*6055 ± 301~*
Kidneys	absolute	552 ± 60	553 ± 78	541 ± 55	440 ± 15* (↓20%)
	relative	*1652 ± 149*	*1661 ± 258*	*1673 ± 83~*	*1534 ± 88 ~*
Heart	absolute	194 ± 20	186 ± 30	163 ± 23 (↓16%)	159 ± 14 (↓18%)
	relative	*580 ± 58*	*555 ± 72*	*503 ± 49~ (↓13%)*	*555 ± 59~ (↓4%)*
Female mice					
Terminal BW (g)		27 ± 1.9	29 ± 1.8	28 ± 1.3	26 ± 1.9
Liver	absolute	1390 ± 213	1545 ± 56	1620 ± 67	1540 ± 217
	relative	*5051 ± 460*	*5415 ± 329*	*5834 ± 275* (↑15%)*	*5826 ± 622* (↑15%)*
Kidneys	absolute	343 ± 29	396 ± 21**	371 ± 22	364 ± 18
	relative	*1252 ± 91*	*1390 ± 123*	*1335 ± 58*	*1383 ± 110*
Heart	absolute	159 ± 31	168 ± 28	153 ± 18	134 ± 17 (↓16%)
	relative	*578 ± 93*	*584 ± 70*	*550 ± 45*	*511 ± 69 (↓12%)*

Data obtained from page 115-127 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * $p<0.05$; ** $p<0.01$; ~ number of available samples too low to analyze statistically.

5. Histopathology

Treatment-related findings were observed in the liver of the animals at 2000 ppm (Table 7). In the periportal region, a cytoplasmic alteration was observed which was characterized by a dense cytoplasmic structure, eosinophilia (in females) or slight basophilia (in males). Most of the animals also showed degenerative changes of this zone which encompassed single cell necrosis (3 males, 5 females), as well as mitotic figures in some females. In one male and three females, the bile ducts in the portal regions were slightly increased in number and size (bile duct hyperplasia). All other findings were distributed throughout all groups unrelated to treatment and known as spontaneous findings frequently occurring in animals of the species and strain in the performing laboratory.

Table 7: Histopathological findings in the liver

Lesion		0 ppm	100 ppm	500 ppm	2000 ppm
Male mice					
Cytoplasmic alteration	Total	-	-	-	3
	Grade 1	-	-	-	2
	Grade 2	-	-	-	1
Necrosis/degeneration	Total	-	-	-	1
	Grade 1	-	-	-	1
Bile duct hyperplasia	Total	-	-	-	1
	Grade 1	-	-	-	1
Kupffer cell foci	Total	-	-	1	1
	Grade 1	-	-	-	1
	Grade 2	-	-	1	-
Fatty change (ORO stain)	Total	-	-	-	2
	Grade 1	-	-	-	1
	Grade 2	-	-	-	1
Female mice					
Cytoplasmic alteration	Total	-	-	-	5
	Grade 1	-	-	-	2
	Grade 2	-	-	-	2
	Grade 3	-	-	-	1
Necrosis/degeneration	Total	-	-	-	5
	Grade 1	-	-	-	3
	Grade 2	-	-	-	2
Increased mitotic figures	Total	-	-	-	2
	Grade 1	-	-	-	2
Bile duct hyperplasia	Total	-	-	-	3
	Grade 1	-	-	-	2
	Grade 2	-	-	-	1
Kupffer cell foci	Total	-	2	-	1
	Grade 1	-	2	-	1
Fatty change (ORO stain)	Total	5	4	3	1
	Grade 1	5	3	3	1

Data obtained from pages 150-154 of the study report. Grade 1: minimal/very few/very small; Grade 2: slight/few/small; Grade 3: moderate/moderate number/moderate size.

III. CONCLUSIONS

Based on effects on food consumption and organ weights observed at 500 ppm, the NOAEL of the study was considered 100 ppm for both sexes (30 mg/kg bw/day and 41 mg/kg bw/day in male and female mice, respectively).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: Overall, the reviewer agrees with the study investigators' conclusions, with the following exceptions.

The study investigators did not identify any treatment-related effects on heart weight. However, there were apparent decreases in absolute heart weight in males from the 500 and 2000 ppm dose groups and in absolute and relative heart weight in females from the 2000 ppm dose group. In the 90-day dietary study in the mouse (Study R-23151), decreased heart weights were noted in males from the 1500 ppm dose group. The decrease in heart weight in that study was attributed to the lower body weights of males at that dose level. In addition, increased relative heart weights in males from the 1200 ppm dose group in the 18-month dietary study in the mouse (Study R-23354) were attributed to reduced body weight. In the current study, reduced heart weight was noted in the absence of a treatment-related effect on body weight at 500 ppm. However, relative heart weight did not show a dose-related change at the two highest doses. In the absence of pathological correlates, the toxicological significance of this decreased heart weight is unclear. Liver weight changes at 500 ppm, in the absence of corroborative clinical or histopathological changes, were considered adaptive.

The study investigators concluded that there were no toxicologically significant effects on platelet counts. An apparent slight treatment-related decrease in platelet counts was noted for males from the 500 and 2000 ppm dose groups and in females from the 2000 ppm dose group. These results are consistent with those of the 90-day dietary study in the mouse seen in males at 300 ppm and above, and as such are considered treatment-related and potentially adverse.

This subchronic toxicity study in the mouse is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement (OECD 407; OPPTS 870.3050) for a repeat-dose oral study in mice.

C. Conclusions: The NOAEL in this study is considered to be 100 ppm (30/41 mg/kg bw/day in males/females). The LOAEL is 500 ppm (101/155 mg/kg bw/day in males/females), based on decreased food consumption in both sexes and decreased platelet count in males. The LOAEL is considered a threshold effect level, based on the marginal extent of the changes observed.

D. Deficiencies: No deficiencies were identified.

Tier II Summary (PMRA #2181203, MRID 48575023) revised by the regulatory authorities, April 2013

Study Type: Oral 28-day toxicity - Rat

IIA 5.3.1 – MRID 48575023, PMRA2181203

Report:	Temerowski M. and Rühl-Fehlert C. (2002) BYI 01921 (MCW-2) Study for subacute oral toxicity in rats (Feeding Study over 4 Weeks). Bayer AG, Toxicology, Germany; unpublished Report No. PH-32327, dated 28 August 2002. Study No. T6069704. Sponsor reference No. R-23146. Dates of experimental work: 17 July 2000 to 17 August 2000
Sponsor/Submitter:	Makhteshim Chemical Works Ltd., Israel.
Guidelines:	US EPA OPPTS 870.3050, OECD 407 (1995) and 67/548/EEC B.7 Deviations: none; no functional observations and motor activity assessments performed
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In a 28-day oral toxicity study (MRID 48575023, PMRA 2181203), groups of 5 male and 5 female Wistar rats received fluensulfone (BYI 01921, MCW-2 technical, 98.4-98.9%, Lot No. NLL6692-3) in their diet at 0, 125, 500 and 2000 ppm (0/0, 10.4/12.2, 42.5/37.2, 152.0/165.9 mg/kg bw/day in males/females) for approximately four weeks. In addition to standard investigations, the clinical chemistry assessment included a determination of thyroid hormones (triiodothyronine, thyroxine, and thyroid stimulating hormone), liver samples were processed for enzyme determination, and immunotoxicity investigations were conducted. Clinical examinations included evaluation of sensory reactivity to stimuli, open field assessments and grip strength at the end of Week 4.

No mortality occurred. No treatment-related clinical signs were noted during the study and no

differences between the treated groups and controls were noted either during the weekly open field inspections or the sensory reactivity to stimuli of different types and assessment of grip strength conducted during week 4. Mean food consumption was slightly decreased in male animals receiving 2000 ppm during the first few days of treatment and throughout the study for females receiving 500 and 2000 ppm of the test material. Water consumption was slightly increased in high dose males and females.

Body weight development was impeded in male and female rats receiving 2000 ppm, and to a lesser extent in males receiving 500 ppm (8%). Overall body weight gain was decreased in mid (11-12%) and high dose (19-27%) male and female animals.

Hematological examinations revealed an increase in leukocyte and lymphocyte counts in females in all dose groups. All changes were considered to be treatment-related although only changes in mid (17-18%) and high dose (27-29%) groups were considered to be adverse. Slight reductions in hemoglobin levels were also noted in both sexes from the 500 and 200 ppm dose groups, while a slight reduction in mean corpuscular hemoglobin concentration (MCHC) was also observed in high dose females. Clinical chemistry revealed dose-related decreases in the activity of alanine aminotransferase (ALAT) in all treatment groups of both sexes. Decreases were also observed triglycerides in mid (25%) and high (53%) dose males. Glutamate dehydrogenase (GLDH) levels were decreased in mid and high dose males and in high dose females, but these enzyme changes were not considered adverse. Thyroxine (T4) was slightly decreased in males (12%) and females (17%) receiving 2000 ppm of the test material, possibly secondary to the effects on liver enzyme activities. Cholesterol was elevated in mid (17%) and high (39%) dose males and high dose females (14%). Total protein was increased only slightly (6%) in high dose males. Urinalysis did not indicate test compound-related effects.

Analysis of organ weight data showed statistically significant increases in mean relative liver and kidney weights in male rats treated at 2000 ppm, and relative spleen weight in females of the same group. Absolute and relative prostate weights were also decreased at the 2000 ppm dose level. Gross pathology revealed surface changes in the kidneys of males dosed at 500 ppm (1/5 animals) and 2000 ppm (5/5 animals). Histopathology revealed increased severity of basophilic tubules as well as degeneration and hyaline inclusions in the proximal tubules in mid and high dose males. At 2000 ppm focal tubular necrosis was observed in one male rat. While consistent with male rat alpha-2-microglobulin accumulation pathology, this cause was not conclusively demonstrated. Histopathology of the liver as well as of spleen revealed no toxicological correlates.

Monooxygenases (ECOD, EROD and ALD) were markedly decreased in all female dose groups. EROD and ALD were decreased to a lesser extent in all male dose groups. Decreases in ALD and EROD in the low dose groups were so minimal that they were not considered to be adverse. The phase II enzyme GS-T was clearly induced in all male dose groups and in females at 500 and 2000 ppm. The phase II enzyme epoxide hydrolase (EH) was induced in all male dose

groups and mid and high dose females. Glucuronyl transferase (GLU-T) levels were increased in mid and high dose males and in high dose females. The changes in liver enzyme levels were considered to be adaptive. Liver cell proliferation examinations gave no indication of test-compound related alterations regarding this endpoint.

Immunotoxicity examinations showed no substance induced effects on the splenic and bone marrow cell counts or in the antibody titers. A decrease in CD8 positive cells was observed in mid and high dose males and in high dose females but a dose response was not observed.

The NOAEL of the study is set at 125 ppm (equivalent to 10.4 and 12.2 mg/kg bw/day in male and female rats, respectively). The LOAEL in this study is considered to be 500 ppm (equivalent to 42.5 and 37.2 mg/kg bw/day in male and female rats, respectively), based on reduced body weights in males, reduced body weight gain in both sexes, decreased food consumption in females, increased white blood cells in females, increased cholesterol in males, and renal pathology in males.

This study is classified as **acceptable/guideline (fully reliable)** and satisfies the guideline requirement for a 28-day dietary study in the rat (OECD 407; OPPTS 870.3050).

I. MATERIAL AND METHODS

A. MATERIALS:

1. Test Material:	BYI 01921 (MCW-2)
Description:	Clear yellowish oily solution
Lot/Batch:	NLL6692-3
Purity:	98.4-98.9%
CAS#:	318290-98-1
Stability:	Stable for the duration of the study

2. Test Animals	
Species	Rat
Strain	Wistar (HsdCpb:WU)
Age	5-6 weeks
Weight	Males: 122-147 g; Females: 117-141 g
Source	Harlan Winkelmann GmbH, Germany
Acclimation period	5-6 days minimum
Diet	Standard diet (Altromin 1321 supplied by Altromin GmbH,

	Germany) *ad libitum*. The feed was provided in stainless steel automatic feeders inside the cages.
Water	Tap water *ad libitum*. Water was supplied in polycarbonate bottles (700 mL).
Housing	In groups of 5/sex in polycarbonate type Ill-cages during acclimation. Individually in type II-A cages on low-dust wood granulate (Ssniff Spezialdiaten GmbH, Germany), during the treatment period.

3. Environmental conditions

Temperature:	22 ± 2°C
Humidity:	approx. 55 ± 5%
Air changes:	approx. 15-20 changes per hour
Photoperiod:	12 hours artificial lighting

B. STUDY DESIGN

1. In-life dates 10 July to 17 August 2000 (treatment initiated 17 July)

2. Animal Assignment and treatment

Male and female rats were grouped by weight and assigned to dose groups (5/sex/group) using random lists. The test substance was administered in the feed from the start of the study until scheduled sacrifice (30 days of treatment for males and 31 days for females) at nominal concentrations of 0, 125, 500 and 2000 ppm.

3. Diet Preparation and Analysis

Fluensulfone was mixed into the diet intended for 4 days in accordance with the dose scheme. A mixing granulator manufactured by Loedige (Paderborn) was used. Homogeneity and stability of the test substance and its correct concentration in the feed mixture were determined analytically. The test substance was homogeneously distributed and its concentration in the feed mixtures agreed to the target values within defined limits (range of relative standard deviations = 1.11 – 6.36%). Fluensulfone was chemically stable for 3 to 4 days in the concentration range used, except for the concentration of 500 ppm, which failed to reach the defined limits after 4 days (78% instead of at least 80% content of the nominal value). Stability tests performed before the study start using a concentration range of 50 to 6000 ppm indicated that diets were stable for at least 3 days (87% of the nominal value).

4. Statistics

The quantitative results for individual animals were used to calculate arithmetic group means and standard deviations. The results for the groups that received the test substance were compared with those for the control group and significant differences were indicated for $p \leq 0.05$ and $p \leq 0.01$. For the statistical evaluation of samples drawn from continuously distributed random variates, three types of statistical test were used: Dunnett test, adjusted Welch test, or the Kruskal-Wallis test followed by a Mann-Whitney test, where appropriate. The statistical evaluation of data related to clinical chemistry, hematology, body and organ weights as well as feed intake was performed using SAS routines.

C. METHODS

1. Observations

On working days the experimental animals were inspected once a day for morbidity and mortality. General clinical examinations (in the home cage) were made daily and detailed clinical examinations in a standard arena (open field observations and observations during handing) were made once per week. In the fourth exposure week sensory reactivity to stimuli of different types (auditory, visual, touch, nociceptive) and assessment of grip strength were conducted additionally.

2. Bodyweight

The body weights of the individual experimental animals were determined before the beginning of the study and weekly thereafter up to scheduled necropsy.

3. Food consumption

The feed intake of each individual rat was determined once per week. These primary data were then used to calculate the following means for each feeding period of 7 days.

a. Feed consumption per animal and day (calculated from weekly determinations)
b. Feed consumption per kilogram body weight per day
c. Mean feed consumption per animal per day
d. Mean feed consumption per kg body weight per day
e. Cumulative Active Ingredient (AI) intake per animal
f. Mean AI intake per animal per day
g. Cumulative AI intake per kg body weight per day
h. Mean AI intake per kg body weight per day

4. Water consumption

Water consumption of each individual rat was determined weekly. These primary data were used to calculate mean water consumption.

5. Ophthalmoscopic examination

Not performed.

6. Hematology and Clinical Chemistry

Glucose was determined for fasted animals 1-2 days before scheduled necropsy. Blood samples for glucose analysis were taken in the morning from caudal veins of non-anaesthetized animals. The samples were deproteinized with perchloric acid (1+10). The blood for other tests was taken from animals under diethyl ether anaesthesia by cardiac puncture at the time of necropsy.
The following parameters were determined at hematology:

Blood erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, reticulocytes, prothrombin time, leucocytes, differential blood count.

The following parameters were determined at clinical chemistry:

Enzyme activity: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, glutamate dehydrogenase.

Substrates/ Electrolytes: cholesterol, triglycerides, glucose, creatinine, urea, bilirubin (total), protein (total), albumin, sodium, potassium, calcium, chloride and inorganic phosphate.

Hormones: Triiodothyronine (T3), Thyroxine (T4), Thyroid stimulating hormone (TSH).

7. Urinalysis

Urine was collected over a 16 hour period 2-3 days prior to the end of the study. Animals received a bolus of 5 mL of water orally and no feed was available during the urine collection period. The following parameters were determined:

Quantitative determinations: urine volume, specific gravity of urine, sediment of urine (microscopy).

Semiquantitative determinations: pH-value, protein, glucose, blood, bilirubin, ketone bodies, urobilinogen.

8. Sacrifice and pathology

Animals were necropsied after exsanguination under deep ether anesthesia. Brain, adrenal glands, heart, lungs, liver, spleen, thymus, kidneys, testes, epididymides, prostate, seminal vesicle, ovaries and uterus were weighed. The following organs were fixed for all animals and evaluated histopathologically for animals in the control and high dose groups (unless otherwise indicated): adrenal glands, aorta, brain, epididymides, esophagus, eyes, eye lids, exorbital lacrimal glands, head (nasal cavities), heart, duodenum, jejunum, ileum, cecum, colon, rectum, Peyer's patches, kidneys (all groups), larynx, liver (all groups), lungs (all groups), mandibular and mesenteric lymph nodes, optic nerves, ovaries, oviducts, pancreas, pharynx, pituitary glands, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic and lumbar), spleen, sternum, stomach, testes, thymus, thyroid glands, tongue, trachea, ureters, urethra, urinary bladder, uterus (with cervix), vagina, Zymbal's gland, and any organs or tissues with macroscopic findings (all groups).

During necropsy liver specimens were taken from each rat in order to investigate the following parameters:

Liver enzymes: 7-Ethoxycoumarin Deethylase (ECOD), 7-Ethoxyresorufin Deethylase (EROD), Aldrin Epoxidase (ALD), Epoxide Hydrolase (EH), UDP-Glucuronyl-Transferase(GLU-T), Glutathione-S Transferase (GS-T).

Substrates: Hepatic triglyceride and cytochrome P-450 concentration.

Other liver specimens were excised and frozen in order to analyze tissues for cell proliferation potential of the test compound using immunohistochemical staining of proliferating cell nuclear antigen as a marker for cell division.

At necropsy, blood (cardiac puncture), left femur and half of the spleen were taken and immediately placed in chilled test tubes containing sterile physiological saline solution. The following parameters were evaluated for immunotoxicity investigations:

Blood: antibody titer in sera (IgG, IgM and IgA) (Sandwich ELISA analyses)

Femur: cell counts in bone marrow

Spleen: splenic cell count, subpopulations of the spleen cells (FACScan-analyses)

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs

No treatment-related clinical signs were noted during the study and no differences between the treated groups and controls were noted either during the weekly open field inspections or the sensory reactivity to stimuli of different types and assessment of grip strength conducted during week 4.

2. Mortality

There were no mortalities during the study.

B. BODYWEIGHT AND BODYWEIGHT GAINS

Body weight development was impeded in male and female animals receiving 2000 ppm and, to a lesser extent, in males receiving 500 ppm (Table 1). At the end of the treatment period differences in body weights compared to control animals were -7.8% for males at 500 ppm, -8.3% for males treated at 2000 ppm and -10.6% for females receiving 2000 ppm. Overall body weight gains were decreased in mid and high dose groups compared to control animals. Treatment-related differences in body weight gain were -11.3% for males at 500 ppm, -19.1% for males treated at 2000 ppm, -11.9% for females at 500 ppm, and -26.9% for females receiving 2000 ppm.

Table 1: Mean (± standard deviation) body weights (g)

Day	0 ppm	125 ppm	500 ppm	2000 ppm
Male rats				
0	129 ± 4.21	134 ± 7.33	125 ± 1.64	136 ± 9.49
4	153 ± 3.39	157 ± 8.88	144 ± 5.02 (↓6%)	139***** ± 7.52 (↓9%)
7	169 ± 5.48	173 ± 10.8	158 ± 7.57 (↓7%)	153***** ± 8.04 (↓9%)
11	198 ± 6.40	201 ± 13.0	183 ± 11.2 (↓7%)	180 ± 12.5 (↓9%)
14	217 ± 6.80	218 ± 14.6	201 ± 14.4 (↓8%)	198 ± 15.9 (↓9%)
18	244 ± 8.51	245 ± 15.0	227 ± 14.8 (↓7%)	225 ± 20.7 (↓8%)
21	255 ± 10.2	256 ± 14.7	238 ± 17.9 (↓7%)	233 ± 22.6 (↓8%)
25	277 ± 11.1	276 ± 19.6	255 ± 19.5 (↓8%)	254 ± 26.9 (↓8%)
28	290 ± 11.9	287 ± 20.0	268 ± 22.6 (↓8%)	266 ± 28.9 (↓8%)
Overall BWG Day 0-28	**161 ± 10.9**	**153 ± 18.1**	**143 ± 21.8 (↓11%)**	**130 ± 23.0 (↓19%)**
Female rats				
0	132 ± 4.39	125 ± 7.84	135 ± 5.59	129 ± 5.13
4	145 ± 4.85	136 ± 7.67	148 ± 6.06	132****** ± 4.02(↓9%)
7	153 ± 5.50	144 ± 7.23	153 ± 6.88	139****** ± 6.19 (↓9%)
11	165 ± 6.84	157 ± 7.91	165 ± 6.89	149****** ± 7.44 (↓10%)
14	172 ± 6.88	164 ± 10.8	171 ± 9.39	158 ± 9.61 (↓8%)
18	186 ± 5.96	177 ± 11.4	183 ± 10.5	167***** ± 9.71 (↓10%)
21	186 ± 8.02	178 ± 9.73	184 ± 7.36	168****** ± 8.76 (↓10%)
25	194 ± 7.73	187 ± 9.53	195 ± 8.73	177***** ± 8.91 (↓8%)
28	204 ± 7.53	194 ± 6.94	198 ± 9.50	182****** ± 7.60 (↓11%)
Overall BWG Day 0-28	**72 ± 6.87**	**69 ± 3.49**	**63 ± 10.9 (↓12%)**	**53 ± 3.03 (↓27%)**

*: $p<0.05$; **: $p<0.01$; (% difference from control; calculated by the reviewer); BWG = body weight gain; n=5; Data obtained from pages 86-87 and the table on page 24 of the study report.

C. FOOD AND WATER CONSUMPTION AND TEST SUBSTANCE INTAKE

Mean daily feed consumption per kg body weight was slightly lower during the first days of treatment in males receiving 2000 ppm, and throughout the study in females receiving 500 ppm and 2000 ppm (Table 2). Food consumption was also decreased in male and female low dose groups on day 4 only. No changes were detected in males at 500 ppm or in either sex at 125 ppm from day 7 to study termination. None of the observed decreases were statistically significant when compared to controls.

Table 2: **Mean (± standard deviation) food consumption (g/kg bw/day)**

Study Day	0 ppm	125 ppm	500 ppm	2000 ppm
Male rats				
Day 4	110 ± 18.2	98.4 ± 5.15 (↓11%)	113 ± 24.3	72.7 ± 14.0 (↓34%)
Day 7	107 ± 8.39	108 ± 7.87	125 ± 33.9	97.3 ± 10.1 (↓9%)
Day 11	108 ± 24.0	98.1 ± 9.58	101 ± 12.9	97.2 ± 11.5 (↓10%)
Day 14	96.3 ± 6.59	91.9 ± 5.37	94.5 ± 10.6	91.5 ± 7.01
Day 18	90.7 ± 6.58	89.0 ± 7.62	93.3 ± 15.1	87.6 ± 7.73
Day 21	85.6 ± 9.26	93.0 ± 6.13	93.1 ± 5.06	85.7 ± 8.13
Day 25	78.3 ± 9.39	80.9 ± 7.31	86.8 ± 11.1	79.8 ± 4.74
Day 28	73.5 ± 2.21	80.8 ± 5.32	82.7 ± 10.8	75.4 ± 3.74
Female rats				
Day 4	104 ± 18.4	87.9 ± 9.96 (↓15%)	92.2 ± 10.1 (↓11%)	69.2 ± 5.64 (↓33%)
Day 7	105 ± 32.6	125 ± 70.4	88.5 ± 6.71 (↓16%)	93.3 ± 14.4 (↓11%)
Day 11	109 ± 30.1	128 ± 48.7	94.5 ± 24.5 (↓13%)	124 ± 44.3
Day 14	95.2 ± 21.0	102 ± 24.9	73.7 ± 12.4 (↓23%)	102 ± 31.2
Day 18	122 ± 59.8	112 ± 42.7	90.5 ± 19.2 (↓26%)	105 ± 42.0 (↓14%)
Day 21	107 ± 41.2	102 ± 39.4	90.3 ± 10.7 (↓16%)	87.5 ± 13.5 (↓18%)
Day 25	91.5 ± 23.5	99.7 ± 48.2	80.8 ± 4.38 (↓12%)	82.8 ± 12.4 (↓9%)
Day 28	95.1 ± 39.3	105 ± 49.5	80.0 ± 8.13 (↓16%)	85.9 ± 10.4 (↓10%)

Data obtained from pages 68-71 of the study report; n=5; ( ) = % different from controls, calculated by the reviewer.

The mean nominal daily compound consumption for each treated group for the duration of the study is reported in Table 3, together with corrected mean nominal daily compound consumption (based on the determination of concentration of fluensulfone in the test diet) which takes into account that the average test substance concentration within 4 days feeding intervals was 90%, 86% and 89% of the nominal values in ascending order of dose levels.

Table 3: Mean nominal test substance intake and corrected mean nominal daily compound consumption (mg/kg bw/day)

Concentration in Diet ppm	Mean nominal test compound intake	Corrected mean nominal daily compound consumption
Males		
0	0	0
125	11.6	10.4
500	49.4	42.5
2000	171.8	152.0
Females		
0	0	0
125	13.5	12.2
500	43.2	37.2
2000	187.2	165.9

Data obtained from pages 23, 72 and 73 of the study report.

The mean daily water intake per kg body weight was slightly increased in 2000 ppm male and female rats (Table 4). Statistically significant differences were not identified.

Table 4: Mean Water Intake (g/kg bw/day)

Study Day	0 ppm n = 5	125 ppm n = 5	500 ppm n = 5	2000 ppm n = 5
Males				
Day 7	145 ± 4.26	151 ± 17.0	149 ± 4.83	172 ± 23.7 (↑19%)
Day 14	119 ± 9.08	119 ± 7.04	127 ± 13.1	147 ± 28.7 (↑23%)
Day 21	112 ± 3.17	115 ± 12.8	119 ± 8.03	134 ± 19.3 (↑20%)
Day 28	97 ± 4.63	102 ± 13.1	104 ± 6.64	117 ± 16.8 (↑21%)
Females				
Day 7	138 ± 17.9	140 ± 11.0	136 ± 13.7	152 ± 17.1 (↑10%)
Day 14	123 ± 18.3	123 ± 11.3	119 ± 5.34	133 ± 13.6 (↑8%)
Day 21	111 ± 12.9	117 ± 16.5	116 ± 2.88	130 ± 20.0 (↑17%)
Day 28	105 ± 12.0	110 ± 14.7	108 ± 8.55	120 ± 15.7 (↑14%)

Data obtained from pages 23, 81 and 82 of the study report. ( ) = % difference from controls, calculated by the reviewer.

D. BLOOD ANALYSIS

1. Hematological findings

There were no effects of treatment on red blood cells, thrombocyte count and clotting times. A statistically significant difference in mean corpuscular hemoglobin concentration (MCHC) levels was noted in high dose males compared to controls (Table 5). Although not statistically significant, dose dependent decreases in hemoglobin levels were noted in males and females from the 500 and 2000 ppm dose groups. Despite the fact that these decreases were slight, these findings are considered to be treatment-related as they have been noted in other rat studies, as well as in studies conducted with other species dosed with fluensulfone.

Decreases in neutrophil counts were observed in all male dose groups; however, these observations were not statistically significant and not considered to be treatment-related or adverse due to the lack of dose response. Findings that, although not attaining statistical significance, were considered to be of toxicological concern include observations that leukocyte

and lymphocyte counts were increased in a dose dependent manner in females compared to concurrent and historical controls (Table 5). Increases in the 500 and 2000 ppm dose groups were considered to be adverse.

Table 5: Selected mean hematological parameters

Parameter	Historical Controls	0 ppm n = 5	125 ppm n = 5	500 ppm n = 5	2000 ppm n = 5
Male rats					
MCHC (g/l ERY)	307 ± 7.4 (n = 144)	309 ± 3.9	307 ± 5.6	310 ± 2.6	295** ± 4.3 (↓ 4%)
Hemoblobin (g/L)	150 ± 10.1 (n=143)	152 ± 5.9	152 ± 4.5	149 ± 3.6 (↓2%)	146 ± 5.9 (↓4%)
Neutrophils (10^9/L)	0.46 ± 0.213 (n=147)	0.57 ± 0.282	0.34 ± 0.098 (↓40%)	0.35 ± 0.154 (↓39%)	0.30 ± 0.107 (↓47%)
Leukocytes (10^9/L)	5.55 ± 1.356 (n=148)	6.48 ± 0.908	6.18 ± 1.226	6.00 ± 0.925	6.16 ± 1.290
Lymphocytes 10^9/L	4.81 ± 1.231 (n=148)	5.66 ± 0.691	5.65 ± 1.183	5.47 ± 0.784	5.62 ± 1.182
Female rats					
MCHC (g/l ERY)	312 ± 8.2 (n = 145)	318 ± 3.7	323 ± 5.4	321 ± 3.0	312 ± 5.3
Hemoblobin (g/L)	143 ± 11.4 (n=145)	149 ± 7.4	147 ± 4.0	146 ± 3.6 (↓2%)	142 ± 4.4 (↓5%)
Neutrophils (10^9/L)	0.32 ± 0.135 (n=148)	0.24 ± 0.087	0.20 ± 0.064	0.27 ± 0.127	0.32 ± 0.134
Leukocytes (10^9/L)	4.49 ± 1.368 (n=150)	4.51 ± 0.345	4.83 ± 0.455 (↑7%)	5.27 ± 0.896 (↑17%)	5.74 ± 0.584 (↑27%)
Lymphocytes (10^9/L)	3.94 ± 1.247 (n=150)	4.04 ± 0.354	4.43 ± 0.472 (↑10%)	4.77 ± 0.769 (↑18%)	5.21 ± 0.519 (↑29%)

Data obtained from pages 54-56 and 91-99 of the study report. **: $p<0.01$; ( ) = % different from concurrent controls, calculated by the reviewer.

2. Blood chemistry findings

Determinations of plasma enzyme activities (transaminases, alkaline phosphatase and glutamate dehydrogenase) revealed statistically significant and dose-related decreases in the activity of alanine aminotransferase (ALAT) in all treatment groups of both sexes (Table 6). Although not statistically significant, there was also a decrease in glutamate dehydrogenase (GLDH) in mid and high dose males, and in high dose females. A dose-dependent increase was noted in cholesterol in mid and high dose males attaining statistical significance at 2000 ppm, while an increase in cholesterol was noted for females at the highest dose level only (not statistically significant or dose dependent). Total protein was statistically significantly increased in high

dose males, but was not considered toxicologically significant due to the small increase (6%). Triglyceride concentration was decreased in mid and high dose males, with the decrease reaching statistical significance in the high dose group. The determination of all other substrates (albumin, glucose, bilirubin, creatinine and urea) did not reveal remarkable differences from controls. Blood electrolyte concentrations revealed no treatment-related differences from control. Inorganic phosphate was statistically significantly increased in 2000 ppm males, however this difference was considered incidental since it was only slight, appeared only in one sex and all individual values were within historical control values. The determination of thyroid hormones revealed slight decreases in T4 (thyroxine) levels in high dose males and females.

Table 6: Selected clinical chemistry parameters

Parameter	Historical Controls	0 ppm n = 5	125 ppm n = 5	500 ppm n = 5	2000 ppm n = 5
Male rats					
ALAT (U/L)	87.0 (n=179)	91.6 ± 11.21	63.0**± 4.93 (↓31%)	35.2**± 8.13 (↓62%)	11.8**± 5.11 (↓87%)
GLDH (U/L)	5.7 (n=154)	6.5 ± 2.38	6.1 ± 1.93	5.8 ± 3.34 (↓11%)	5.3 ± 1.23 (↓19%)
Cholesterol (nmol/L)	1.98 ± 0.373 (n=165)	1.55 ± 0.214	1.64 ± 0.150	1.82 ± 0.176 (↑17%)	2.19*± 0.303 (↑39%)
Triglycerides (nmol/L)	1.06 ± 0.41 (n=130)	1.73 ± 0.617	1.84 ± 0.314	1.30 ± 0.245 (↓25%)	0.82**± 0.395 (↓53%)
Total protein (g/L)	64.7 ± 2.93 (n=165)	60.9 ± 2.26	62.9 ± 1.77	60.8 ± 1.50	64.6*± 1.99 (↑6.4%)
T4 (nmol/L)	63 ± 13.4 (n=26)	61 ± 12.0	65 ± 6.1	72 ± 4.5	54 ± 6.8 (↓12%)
Phosphate (mmol/L)	3.23 ± 0.473 (n =90)	3.00 ± 0.213	3.10 ± 0.355	3.24 ± 0.211 (↑8%)	3.37 ± 0.156 (↑12%)
Female rats					
ALAT (U/L)	73.0 (n=189)	74.2 ± 8.63	54.6**± 8.93 (↓26%)	31.9**± 4.62 (↓57%)	11.0**± 3.42 (↓85%)
GLDH (U/L)	5.1 (n=123)	4.9 ± 5.01	4.3 ± 2.91	8.6 ± 7.81	2.4 ± 1.02 (↓52%)
Cholesterol (nmol/L)	1.87 ± 0.318 (n=174)	2.06 ± 0.353	1.93 ± 0.159	1.89 ± 0.283	2.34 ± 0.466 (↑14%)
Triglycerides (nmol/L)	0.83 ± 0.358 (n=145)	1.10 ± 0.161	1.02 ± 0.233	1.02 ± 0.172	1.19 ± 0.468
Total protein (g/L)	65.3 ± 3.38 (n=144)	64.7 ± 2.63	64.7 ± 0.84	64.8 ± 0.78	62.6 ± 2.06
T4 (nmol/L)	56 ± 17.5 (n=30)	58 ± 5.5	70 ± 8.5	53 ± 7.2	48 ± 7.3 (↓17%)
Phosphate (mmol/L)	2.64± 0.465 (n=74)	2.59 ± 0.273	2.42 ± 0.279	2.58 ± 0.406	2.39 ± 0.267

*: $p<0.05$; **: $p<0.01$; Data obtained from pages 26, 54-56 and 100-120 of the study report. ( ) = % different from concurrent controls, calculated by the reviewer.

E. URINALYSIS

Results of urinalysis were unremarkable in all groups.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

Mean absolute prostate and seminal vesicle weights were decreased in 500 and 2000 ppm dose groups; however, only relative prostate weight in high dose males was decreased; therefore, the changes in prostate weight at the 2000 ppm dose level are considered to be related to treatment. In females, absolute and relative uterus weights were elevated in 125 and 2000 ppm dose groups but not in the mid dose group. A slight increase in relative ovary weights was noted in high dose females. None of these organ weight changes were considered to be treatment-related due to the high variability in the data, the lack of a dose response, and/or the absence of any correlating clinical or pathology findings.

Mean relative kidney and liver weights in males treated at 2000 ppm were statistically significantly higher when compared with the concurrent controls. In female rats of this group, a statistically significant increase in relative spleen weight was also noted (Table 7).

Table 7: Selected absolute (mg) and relative (mg/100 g) organ weights

Organ	0 ppm n = 5	125 ppm n = 5	500 ppm n = 5	2000 ppm n = 5
Male rats				
Terminal body weight (g)	294 ±10.3	291 ± 20.6	267 ± 22.4 (↓11%)	263 ± 29.8 (↓11%)
Abs. Liver	13401 ± 752.2	14183 ± 741.6	13022 ± 1907.4	13719 ± 2184.8
Rel. Liver	4552 ± 185.2	4887 ± 143.7	4856 ± 313.8	5205** ± 243.4 (↑14%)
Abs. Kidney	2020 ± 232.4	2098 ± 165.6	2045 ± 241.5	2169 ± 280.4
Rel. Kidney	685 ± 54.6	722 ± 35.8	765 ± 46.7	827** ± 71.1 (↑21%)
Abs. Spleen	590 ± 34.0	554 ± 60.3	522 ± 106.1	553 ± 113.2
Rel. Spleen	201 ± 13.2	191 ± 20.9	194 ± 25.5	210 ± 29.0
Abs. Prostate	729 ± 209.8	787 ± 123.4	634 ± 54.9 (↓13%)	592 ± 76.6 (↓19%)
Rel. Prostate	248 ± 74.2	274 ± 60.1	238 ± 25.0	227 ± 34.1 (↓8%)
Abs. Seminal Vesicles	911 ± 75.3	909 ± 207.8	812 ± 118.7 (↓11%)	806 ± 217.7 (↓12%)
Rel. Seminal Vesicles	310 ± 29.6	312 ± 61.3	307 ± 60.3	305 ± 62.9
Female rats				
Terminal body weight (g)	203 ± 8.8	194 ± 5.9	200 ± 9.1	181** ± 6.9 (↓11%)
Abs. Liver	8559 ± 783.7	8136 ± 464.4	8914 ± 836.2	8097 ± 226.1
Rel. Liver	4211 ± 330.6	4186 ± 209.3	4470 ± 430.2	4467 ± 160.2
Abs. Kidney	1431 ± 119.2	1345 ± 73.2	1400 ± 118.0	1372 ± 103.2
Rel. Kidney	704 ± 43.5	692 ± 30.3	701 ± 33.6	756 ± 39.5
Abs. Spleen	452 ± 18.0	412 ± 46.8	450 ± 55.5	466 ± 41.8
Rel. Spleen	223 ± 11.1	212 ± 25.2	225 ± 18.9	257* ± 19.2 (↑15%)
Abs. Ovaries	124 ± 15.7	122 ± 7.1	128 ± 17.8	126 ± 37.7
Rel. Ovaries	61 ± 7.3	63 ± 2.7	64 ± 7.3	69 ± 19.7 (↑13%)
Abs. Uterus	651 ± 170.2	752 ± 191.8 (↑16%)	668 ± 136.8	748 ± 106.4 (↑15%)
Rel. Uterus	319 ± 71.2	387 ± 102.0 (↑31%)	333 ± 53.2	412 ± 50.7 (↑ 29%)

*: $p<0.05$; **: $p<0.01$; ( ) = % difference from controls, calculated by the reviewer; Data obtained from pages 28-29 and 147-162 of the study report.

2. Determination in Liver tissue

Changes were observed in liver enzyme levels that were related to treatment but considered adaptive and not adverse. Monooxygenases 7-Ethoxycoumarin Deethylase (ECOD), 7-Ethoxyresorufin Deethylase (EROD), Aldrin Epoxidase (ALD) were decreased dose-dependently in all female dose groups, attaining statistical significance at the mid and high doses (as well as the low dose for ECOD only). Decreases were also observed in EROD and ALD levels in all male dose groups, attaining statistical significance at 2000 ppm. Phase II enzymes Epoxide Hydrolase (EH) and Glutathione-S Transferase (GS-T) were clearly increased in all males in a dose-dependent manner, attaining statistical significance at 500 and 2000 ppm for both, and at all dose levels for GS-T. Glucuronyl Transferase (GLU-T) levels were statistically significantly increased in mid and high dose males. In females, EH and GS-T were increased in the 500 and 2000 ppm dose groups, and GLU-T was increased in females dosed with 2000 ppm. Hepatic triglyceride concentrations and the cytochrome P-450 levels showed no relevant differences between the treated and control groups.

In summary, treatment caused a decrease in monooxygenase, more pronounced in female rats, and an induction of Phase II enzymes in both sexes. The effects at the low dose level were marginal. Examination of liver cell proliferation with immunohistochemical staining of PCNA indicated no proliferative potential for fluensulfone in the liver.

Table 8: Activities of phase I and phase II enzymes in the liver

Parameter	0 ppm n = 5	125 ppm n = 5	500 ppm n = 5	2000 ppm n = 5
Male rats				
ECOD (nmol/g/min)	9.6 ± 1.9	8.1 ± 1.4	9.4 ± 2.6	9.7 ± 2.3
EROD (nmol/g/min)	0.56 ± 0.11	0.44 ± 0.11 (↓21%)	0.47 ± 0.17 (↓16%)	0.29* ± 0.15 (↓48%)
ALD (nmol/g/min)	171.9 ± 41.3	153.5 ± 26.8 (↓11%)	151.9 ± 14.8 (↓12%)	98.7** ± 20.4 (↓43%)
EH (nmol/g/min)	435 ± 81	494 ± 100 (↑14%)	690** ± 61 (↑59%)	1207** ± 273 (↑178%)
GS-T (µmol/g/min)	81 ± 8	100** ± 9 (↑24%)	116** ± 10 (↑43%)	152** ± 19 (↑88%)
GLU-T (nmol/g/min)	967 ± 133	939 ± 154	1172* ± 119 (↑21%)	1448** ± 283 (↑50%)
P450 (nmol/g)	38.3 ± 2.93	37.0 ± 2.40	38.3 ± 2.33	41.9 ± 3.76
Triglycerides (mcmol/g)	5.35 ± 0.970	5.40 ± 0.449	5.25 ± 0.472	5.42 ± 0.638
Female rats				
ECOD (nmol/g/min)	4.8 ± 0.9	3.4* ± 0.4 (↓29%)	2.4** ± 0.2 (↓50%)	2.2** ± 0.3 (↓54%)
EROD (nmol/g/min)	0.54 ± 0.25	0.37 ± 0.08 (↓32%)	0.18* ± 0.11 (↓67%)	0.19* ± 0.05 (↓65%)
ALD (nmol/g/min)	23.2 ± 5.2	19.0 ± 2.3 (↓18%)	11.1** ± 0.7 (↓52%)	9.4** ± 1.4 (↓60%)
EH (nmol/g/min)	360 ± 56	328 ± 44	493 ± 193 (↑37%)	629** ± 84 (↑75%)
GS-T (nmol/g/min)	85 ± 9	81 ± 3	119* ± 25 (↑40%)	135** ± 17 (↑59%)
GLU-T (µmol/g/min)	625 ± 86	620 ± 133	667 ± 225	863** ± 124 (↑38%)
P450 (nmol/g)	34.8 ± 2.98	37.1 ± 2.14	36.1 ± 1.73	36.6 ± 6.37
Triglycerides (mcmol/g)	5.96 ± 0.762	6.30 ± 0.393	5.72 ± 0.497	6.07± 0.429

*: $p<0.05$; **: $p<0.01$; Data obtained from pages 27, 121-122, and 138-143 of the study report.
( ) = % difference from concurrent controls, calculated by the reviewer.

3. Immunotoxicity investigations

Dose-dependent decreases in splenic cell counts were observed in all male dose groups and low and mid dose females (Table 9). The high dose female group could not be tested due to problems with the sample. A decrease in cell counts in the bone marrow of males treated with 2000 ppm of the test material was noted; however, an increase in bone marrow cell counts was observed in mid and high dose females. None of these changes were considered to be treatment-related based on the lack of a dose response.

Table 9: Mean spleen and bone marrow cell counts x 10^6 (per spleen or per 1.5 cm of bone)

Group	Spleen	Bone Marrow
Male rats		
Control	438.9 ± 61.5	12.0 ± 0.95
125 ppm	340.8 ± 104.9 (↓22%)	12.7 ± 2.56
500 ppm	355.8 ± 151.7 (↓19%)	12.2 ± 1.26
2000 ppm	329.0 ± 82.8 (↓25%)	9.9 ± 1.85 (↓18%)
Female rats		
Control	342.1 ± 93.9	5.8 ± 0.97
125 ppm	280.8 ± 70.3 (↓18%)	5.1 ± 0.55
500 ppm	251.0 ± 35.8 (↓27%)	6.6 ± 0.09 (↑14%)
2000 ppm	--	6.4 ± 1.77 (↑10%)

Data obtained from page 241 of the study report. ( ) = % difference from controls

Treatment with the test substance revealed no toxicologically relevant effects in the composition of the splenic subpopulations in both sexes except for a statistically significant decrease in the CD8 positive cells of the male rats at 500 ppm and at 2000 ppm and a decrease (not statistically significant) in CD 8 positive cells in female rats at 2000 ppm (Table 10).

Table 10: Determination of splenic cell subpopulations (mean ± standard deviation)

Group	CD8 ^{Total}
Male rats	
Control	22.41 ± 1.18
125 ppm	19.64 ± 2.32
500 ppm	17.86* ± 0.62 (↓20%)
2000 ppm	18.96* ± 2.80 (↓15%)
Female rats	
Control	21.44 ± 1.46
125 ppm	21.76 ± 1.72
500 ppm	20.34 ± 2.00
2000 ppm	18.63 ± 1.51 (↓13%)

* Statistically significant decrease compared to controls ($p<0.05$); Data obtained from pages 242 – 244 of the study report. ( ) % change from concurrent controls, calculated by reviewer.

With respect to changes in the antibody titer of IgA, IgM and IgG after treatment with the test substance, a statistically significant decrease was noted in the antibody titer of IgG of the male rats at the lowest and highest dose levels (125 ppm and 2000 ppm) (Table 11). According to the study report, the mean and standard deviation values calculated for the mid dose group were substantial due to one single animal with an unusually high titre. Individual animal data were not supplied in the study report. A decrease in the antibody titre of IgG of low and high dose females was also noted. These changes were not considered to be treatment-related.

Table 11: Antibody titer in the sera (ug/ml)

Group	IgG
Male rats	
Control	683.3 ± 119.4
125 ppm	581.9* ± 25.4 (↓15%)
500 ppm	860.7 ± 768.6
2000 ppm	553.4* ± 22.6 (↓19%)
Female rats	
Control	1206.4 ± 478.8
125 ppm	1073.7 ± 304.6 (↓11%)
500 ppm	1205.0 ± 217.5
2000 ppm	1063.5 ± 373.1 (↓12%)

* Statistically significant decrease; ( ) = % difference from controls, calculated by the reviewer; Data obtained from page 245 of the study report

4. Necropsy/Histopathology

Gross pathology revealed surface changes in the kidneys of male rats dosed at 500 ppm (1/5 animals) and 2000 ppm (5/5 animals). The kidneys of male rats receiving dietary concentrations of 500 ppm and 2000 ppm revealed an increased severity of basophilic tubules as well as degeneration and hyaline inclusions in the proximal tubules (Table 12). Degeneration of proximal tubules was most clear in the outer part of the renal cortex and affected tubules showed vacuolation. Hyaline droplets were most prominent in the middle cortex. At 2000 ppm focal tubular necrosis was observed in one male rat. In males dosed at 125 ppm and in females dosed up to and including 2000 ppm, there was no evidence of any induced kidney changes. No treatment-related changes were detected either in the liver or in the spleen to account for organ weight changes.

Table 12: Histopathological findings in kidneys of male rats

Lesion	0 ppm n = 5	125 ppm n = 5	500 ppm n = 5	2000 ppm n = 5
Basophilic tubules				
Total # of observations	5	5	5	4
Grade 1	4	5	3	1
Grade 2	1	-	2	3
Hyaline droplets				
Total # of observations	-	-	1	5
Grade 2	-	-	1	4
Grade 3	-	-	-	1
Degeneration proximal tubules				
Total # of observations	--	-	2	5
Grade 1	-	-	2	-
Grade 2	-	-	-	3
Grade 3		-	-	2
Mononuclear infiltration				
Total # of observations	1	1	-	2
Grade 1	1	1	-	2
Intratubular protein				
Total # of observations	-	--	2	1
Grade 1	-	-	2	-
Grade 2	-		-	1
Tubular necrosis				
Total # of observations	-	-	-	1
Grade 1	-	-	-	1

Grade 1: minimal/very few/very small; Grade 2: slight/few/small; Grade 3: moderate/moderate number/moderate size; Data obtained from pages 177 – 224 of the study report.

III. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments:** This 28-day dietary study in Wistar rats is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for a repeat dose 28-day oral toxicity study (OPPTS 870.3050; OECD 407) in rats.

C. **Conclusions:** The reviewer is in general agreement with the applicant's conclusions. Administration of fluensulfone to Wistar rats for approximately 4 weeks resulted in a NOAEL of 125 ppm (10.4 and 12.2 mg/kg bw/day for males and females, respectively), based on decreased body weight in males, decreased body weight gain in both sexes, effects on white blood cells in females, and some clinical chemistry changes and renal effects in males at the LOAEL of 500 ppm (42.5 and 37.2 mg/kg bw/day for males and females, respectively). Marginal functional effects on the liver (mainly phase II enzyme activities) were observed in both sexes at the NOAEL but without signs of cytotoxicity; these changes were not considered to be adverse. There were metabolic signs indicative of functional but non-adverse effects on the liver of male and female animals at 500 and 2000 ppm. The dose-correlated decrease in ALAT activity in peripheral blood is considered not to be an adverse effect at the low dose level. Alpha-2-microglobulin accumulation in phagolysosomes of the proximal convoluted tubuli of the kidneys (hyaline droplets) together with tubular degeneration is a phenomenon which is exclusively found in male rats. However, the localization of tubular degeneration and accumulation of hyaline droplets did not always match well; the hyaline droplets were most prominent in the middle cortex whereas tubular degeneration was most clear in the outer part of the cortex. Additionally, specific staining was not performed to identify the droplets, nor a temporal relationship of degeneration following accumulation demonstrated. Alpha-2-microglobulin accumulation does not develop in female Wistar rats and in either sex of higher species, including humans. Therefore, while the tubular degeneration and hyaline droplets in male rats in the 500 ppm and 2000 ppm groups are consistent with a male rat-specific alpha-2-microglobulin accumulation, the data are not considered conclusive for demonstrating this association.

D. **Deficiencies**: Sections 6.9 (Organ Weights) and 6.10 (Histopathology) of the "Materials and Methods" section were omitted from the study report. Individual immunotoxicity data was not provided, and the historical data that was referenced in the immunotoxicity investigation section were not provided in the study report. No other deficiencies were noted. These omissions did not affect the conclusions drawn from the study. It is noteworthy that an acceptable immunotoxicity study in the mouse (MRID 48574779 / PMRA 2181189) has been submitted.

Tier II Summary (PMRA #2181205, MRID 48574753) revised by the regulatory authorities, April 2013

Study Type: Oral 90-day toxicity - Mouse

IIA 5.3.2 – MRID 48574753, PMRA 2181205

Report:	Krötlinger F. (2003) BYI 01921 Study for Subchronic Oral Toxicity in Mice (Feeding study for 13 Weeks). Bayer HealthCare AG, Experimental Toxicology, Germany; unpublished Report No. AT00829, Sponsor reference No. R-23151, dated 2 December 2003. Study No. T7071801. Dates of experimental work: 27 June 2002 to 25 April 2003
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD No. 408, Council Recommendation (83/571/EEC), Annex I - Repeated Dose Toxicity, OPPTS 870.3100, Japanese MAFF (59 NohSan No.4200).
Deviations:	None
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary:

In a 90-day dietary toxicity study (MRID 48574753; PMRA 2181205), groups of 12 male and 12 female Crl:CD1 (ICR)BR mice received diets containing 0, 60, 300 and 1500 ppm (equivalent to 0/0, 11/18, 51/68 and 228/252 mg/kg bw/day in males/females, respectively) of fluensulfone (BYI 01921 technical, 97.0-97.8%, Batch No. NLL6692-7-5) for a period of 13 weeks. Additional satellite groups of 5 mice per sex were similarly treated at 0 or 1500 ppm for 4 weeks and then terminated for determination of enzyme levels in liver tissue.

There were several mortalities during the study: 2, 2, 4 and 4 males and 3, 0, 0 and 5 females for the control and treated groups in order of ascending doses. Apart from the death of a single female of the highest dose group, which was also the only animal in which clinical signs (piloerection, squatting position, poor general condition, and emaciation) were recorded, all mortalities were related to blood sampling.

At 1500 ppm food (males 22% and females 43% below controls; g/animal/day) and water intake (males 17% below controls; g/animal/day) were decreased. At 300 ppm food intake was decreased in females (15%). Body weight gain was significantly reduced at 1500 ppm in both sexes during the first week of treatment, with a body weight loss recorded for females.

At the end of treatment mean body weights of animals in the high dose group differed from the control values by approximately -10% for both sexes. Cumulative gain was 33% and 60% less than controls for males and females, respectively.

Hematological investigations showed increased erythrocyte counts, and hemoglobin and hematocrit values at 300 ppm and above in males, increased mean corpuscular volume (ranging from 5-15% above controls) and decreased mean corpuscular hemoglobin concentration (5%) in males at 1500 ppm, and increased reticulocyte counts in both sexes (32-36%) treated at 1500 ppm. Leukocyte counts were increased in males treated at and above 300 ppm (34-49%). Platelet levels were reduced in males at 300 ppm (17%) and above (23%) and in females at 1500 ppm (38%). Clinical chemistry showed increased concentrations of total bilirubin (45-77%) at 300 ppm and above in both sexes, and decreased urea concentrations in animals of both sexes treated at 1500 ppm (20-24%). The activities of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were increased at 1500 ppm in both sexes (55-107%), often attaining statistical significance.

No treatment-related macroscopic findings were noted at necropsy. At 1500 ppm liver weights were increased in males (relative) and females (absolute and relative). Additionally, due to the lower body weights, the weights of brain, heart, and epididymides were decreased in males at 1500 ppm. Histopathology revealed hepatocellular hypertrophy, accompanied by degenerative changes mainly of the periportal hepatocytes, minimal to slight pigment deposits, bile duct hyperplasia (observed in most animals) and proliferation of undifferentiated oval cells, and slightly reduced lipid content in both sexes treated at 1500 ppm. Hepatocellular hypertrophy, without any sign of liver cell damage, was also observed in females receiving 300 ppm. In the spleen, an increased incidence of animals with pigment deposition in the red pulp was recorded in both sexes at 1500 ppm.

Determinations in liver tissues in satellite animals sacrificed after 28 days of treatment showed no evidence for toxicologically significant effects on the concentration of cytochrome P450 at 1500 ppm. In male mice only the monooxygenase 7-ethoxycoumarin deethylase was marginally increased. The phase II enzymes epoxide hydrolase and glutathione-S-transferase were marginally increased, whereas UDP-glucuronyltransferase was not affected.

In female mice the monooxygenases were not significantly affected, whereas all phase II enzymes (epoxide hydrolase, glutathione-S-transferase, UDP-glucuronyltransferase) were significantly induced at 1500 ppm.

The NOAEL of the study was 60 ppm (equivalent to 11 mg/kg bw/day for males and 18 mg/kg bw/day for females) based on increased bilirubin in both sexes, hematology effects in the males and reduced food consumption in females, at the LOAEL of 300 ppm (51 mg/kg bw/day for males and 68 mg/kg bw/day for females).

This study is classified **acceptable/guideline (fully reliable)** and satisfies the guideline requirement for a subchronic oral toxicity study in the mouse (OECD 408; OPPTS 870.3100).

I. MATERIAL AND METHODS

A. MATERIALS

1.Test Material:	BYI 01921 (MCW-2 TECH)
Description:	Yellow oil
Batch No.:	NLL6692-7-5
Purity:	97% / 97.8%
CAS#:	318290-98-1
Expiry date:	14 August 2002 / 31 January 2003
Stability of Test Item:	Stable in the diet for at least 15 days
2. Vehicle:	Provimi Kliba SA (3883.9.25)
3.Test animals	
Species:	Mouse
Strain:	Crl:CD1 (ICR)BR
Age:	Males: about 5 weeks old; females about 6 weeks old (based on body weight)
Weight at dosing:	Males: 25-31 g; females: 24-29 g
Source:	Charles River, Sulzfeld, Germany
Acclimation period:	At least 9 days
Diet:	Provimi Kliba SA (3883.9.25) *ad libitum,* supplied from stainless steel automatic feeders inside the cages
Water:	Tap water *ad libitum*, supplied in bottles of approximately 300 mL
Housing:	Individually in Makrolon® cages Type II on low-dust wood granules (supplied by Ssniff Spezialdiäten Inc., Germany)

4.Environmental conditions

Temperature:	22 ± 2°C
Humidity:	55 ± 5%
Air changes:	≤10 per hour
Photoperiod:	12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

1. In life dates: 27 June to 27 September 2002

2. Animal assignment and treatment:

At the start of the study, male and female mice were grouped by weight and assigned to dose groups using random lists. Animals of the main groups (12/sex/dose) were treated for a total of 91 days at nominal dose levels of 0, 60, 300 or 1500 ppm. Animals of the satellite groups (5/sex/dose) were treated for a total of 28 days at nominal dose levels of 0 or 1500 ppm.

3. Dose selection rationale

The doses were selected based on the results of a 4-week dietary study in mice (Study Number T2071608; Sponsor Report Number R-23149; MRID 48575024; PMRA 2181204). In that study, 5 mice/sex/dose were administered fluensulfone in the diet at nominal doses of 0, 100, 500 and 2000 ppm. The study investigators concluded that food intake was decreased at 500 ppm and above in both sexes, that water consumption was slightly increased at 2000 ppm in females, and that body weight was reduced at 2000 ppm in both sexes. Effects on red blood cell parameters were detected at 2000 ppm in both sexes and on platelets in males at 500 ppm. Liver weights were increased at 500 ppm and above in females, and several microscopic findings were detected in the liver at 2000 ppm in one or both sexes (cytoplasmic alteration, eosinophilia, basophilia, cell necrosis, mitotic figures, and bile duct hyperplasia). Other findings included increased activities of ASAT and ALAT in both sexes at 2000 ppm and decreased absolute kidney weight in females at 2000 ppm.

4. Diet Preparation and Analysis

Fluensulfone was mixed into the diet intended for the following week in accordance with the dose scheme. A mixing granulator manufactured by Loedige (Paderborn) was used. Homogeneity and stability of the test substance and its correct concentration in the feed mixture were determined analytically. For the analytical determinations before the start of the study, an aliquot was taken from a 7000 ppm test diet and mixed with basal diet to obtain a 20 ppm sample. During the study, samples were taken from the individually prepared dose mixtures at three time points.

The test substance was homogeneously distributed in the 20 and 7000 ppm test diets (relative standard deviations of 0.6-4.8%) and in samples of the 60 and 1500 ppm test diets (relative

standard deviations of 0.8-2.6%). The 20 and 7000 ppm test diets were chemically stable for at least 15 days, with concentrations within 8% of target. The concentration of test material in the 60, 300 and 1500 ppm feed mixtures agreed to the target values within defined limits (range 94-107% of nominal values).

5. Statistics

The quantitative results for individual animals were used to calculate arithmetic group means and standard deviations. The results for the groups that received the test substance were compared with those for the control group and significant differences indicated for $p < 0.05$ and $p < 0.01$ using the following methods:

Analysis of variance followed by Dunnett test: body weight, erythrocytes, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, platelets, albumin, creatinine, protein, triglycerides, urea, absolute organ weights.

Kruskal-Wallis test followed by adjusted U test: leukocytes, reticulocytes, differential blood counts, albumin/globulin ratio, total bilirubin.

Adjusted Welsh test: alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, cholesterol, cytochrome P450.

Logarithmic analysis of variance and Dunnett test: relative organ weights.

Student’s t-test: 7-ethoxycoumarin deethylase, 7-ethoxyresorufin deethylase, aldrin epoxidase, epoxide hydrolase, glutathione-S-transferase, and UDP-glucuronyltransferase.

C. METHODS

1. Observations

The experimental animals were inspected at least once a day. Detailed clinical examinations were performed weekly.

2. Bodyweight

The body weights of the individual experimental animals were determined before the beginning of the study and weekly thereafter up to scheduled necropsy.

3. Food and water consumption and active ingredient intake

Food and water intakes of each individual mouse were determined once per week, based on the difference between the weight of the food/water made available and the weight of the remaining food/water that was not consumed. The consumption per animal per day and per kg body weight per day were determined from these primary data.

4. Ophthalmoscopic examination

Not performed.

5. Hematology and Clinical Chemistry

Blood samples for the laboratory determinations were collected on day 86 for hematology and day 91 for clinical chemistry from the retro-orbital venous plexus of animals anesthetized with CO_2/O_2 (80:20).

The following hematology parameters were determined: differential blood count, morphology of erythrocytes, erythrocytes, hemoglobin, hematocrit, leucocyte count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, thrombocytes, reticulocytes.

The following clinical chemistry parameters were determined: alkaline phosphatase (APh), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), albumin, protein (total), cholesterol, creatinine, urea, triglycerides, bilirubin (total), albumin/globulin ratio.

6. Urinalysis

Not performed.

7. Sacrifice and pathology

The animals in the satellite groups were necropsied on study day 29 and the animals from the main dose groups were necropsied on study days 92 (males) and 93 (females). Animals were necropsied after exsanguination under deep ether anesthesia.

In the main dose groups, adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, uterus and thymus were weighed. A complete list of organs from animals of

the control and high dose groups was examined microscopically (including adrenal glands, aorta, brain, epididymides, esophagus, eyes, exorbital lacrimal gland, femur, harderian glands, nasal cavity, heart, intestine, kidneys, larynx, liver/gall bladder, lungs, lymph nodes, optic nerves, ovaries, oviducts, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicles with coagulation glands, skeletal muscle, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid glands, tongue, trachea, urinary bladder, uterus, vagina, gross lesions) and gross lesions, kidney, liver/gall bladder, lungs and spleen of animals of the mid and low-dose groups were also examined.

In the satellite groups, livers were weighed and liver tissue was analyzed for cytochrome P-450, 7-ethoxycoumarin deethylase, 7-ethoxyresorufin deethylase, aldrin epoxidase, epoxide hydrolase, glutathione-S-transferase, and UDP-glucuronyltransferase.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Actual achieved intakes of test substance

The actual achieved intakes of the test substance were as follows:

Dietary levels (ppm)	Actual dosage (mg/kg bw/day)	
	Males	Females
60	11.06	18.33
300	50.85	68.47
1500	228.53	252.49

The analytical data revealed that the homogeneity of the formulations was achieved, and the content checks confirmed that during the study appropriate and equal mixture procedures were followed (see Section I.B.4 of this review).

The mean test material intake for mice in the satellite group was 269 mg/kg bw/day for males and 269 mg/kg bw/day for females (calculated by the reviewer as an average of the mean weekly values from pages 153-154 of the study report).

2. Mortality and clinical signs

One female from the 1500 ppm dose group (animal no. 89) exhibited piloerection, squatting position, poor general condition, and emaciation on day 71. No other treatment-related clinical signs were noted. There were several mortalities during the study: 2, 2, 4 and 4 males and 3, 0, 0 and 5 females from the 0, 60, 300 and 1500 ppm dose groups (main study), respectively. Apart from animal no. 89 who died on day 75, all mortalities were reported to be related to blood sampling at study termination. Necropsy findings in animal no. 89

included dark-red lungs.

3. Body weight and food and water consumption

Mean body weight values throughout the study period are summarized in Table 1. Body weight gain was reduced in males at 1500 ppm during the first week of treatment (males in the 1500 ppm group did not gain weight during the first week whereas a body weight gain of 2 g was observed in all other groups). In females, body weight loss was observed in the same week. At the end of treatment mean body weights in the 1500 ppm group differed from the control values by approximately -10% for both sexes. Cumulative gain was decreased at 1500 ppm in males (33% below control) and females (60% below control). Concomitant to effects on mean body weight/body weight gain, a reduction in food intake was observed in both sexes at 1500 ppm, and in females at 300 ppm (Table 2). The water consumption was also decreased in males at 1500 ppm (Table 2).

Table 1: Mean ± SD body weights and cumulative weight gain (g)

Day	Control	60 ppm	300 ppm	1500 ppm
Males				
1	29 ± 1.3	28 ± 1.4	28 ± 2.0	28 ± 1.7
8	30 ± 1.7	31 ± 1.6	30 ± 2.0	28 ± 1.7** (↓7%)
29	34 ± 2.1	33 ± 3.0	33 ± 2.6	31 ± 2.2** (↓9%)
64	38 ± 2.1	37 ± 3.7	37 ± 2.3	35 ± 2.5* (↓8%)
92	38 ± 2.4	38 ± 3.7	37 ± 2.2	34 ± 2.1* (↓10%)
Gain 1-92[a]	9	10	9	6 (↓33%)
Females				
1	26 ± 1.1	26 ± 1.1	27 ± 1.0	26 ± 1.1
8	26 ± 1.7	26 ± 1.7	28 ± 1.0	25 ± 1.7
29	28 ± 1.2	28 ± 1.8	30 ± 1.4**	27 ± 1.7
64	30 ± 1.8	31 ± 2.2	32 ± 1.8	29 ± 1.4
92	31 ± 2.1	31 ± 2.5	33 ± 1.8	28 ± 1.2* (↓10%)
Gain 1-92[a]	5	5	6	2 (↓60%)

Data obtained from pages 93-94 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). [a] Cumulative body weight gain was calculated by the reviewer from mean body weight values and not analyzed statistically. * $p < 0.05$; ** $p < 0.01$

Table 2: Mean daily food and water intakes (g/animal/day – *g/kg bw/day*)

Up to day	Control	60 ppm	300 ppm	1500 ppm
Food intake				
Males				
91	6.31 *180.92*	6.41 *184.40*	5.83 *168.82*	4.92 (↓22%) *152.36 (↓16%)*
Females				
91	8.25 *282.80*	8.90 *305.55*	7.03 (↓15%) *228.23(↓19%)*	4.69 (↓43%) *168.33 (↓40%)*
Water intake				
Males				
91	5.71 *162.49*	6.10 *175.02*	5.74 *164.53*	4.73 (↓17%) *146.37 (↓12%)*
Females				
91	5.31 *182.10*	5.52 *188.62*	5.26 *169.66*	5.09 *182.34*

Data obtained from pages 26-27 of the study report. () Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

4. Hematology

Hematological investigations showed increased erythrocyte counts (RBC), and hemoglobin (Hb) and hematocrit (Ht) values at 300 ppm and above in males; increased mean corpuscular volume (MCV) and decreased mean corpuscular hemoglobin concentration (MCHC) in males at 1500 ppm; and increased reticulocyte counts in both sexes treated at 1500 ppm. Decreases in the counts of erythrocytes and in the concentration of hemoglobin and hematocrit values were recorded in females at 60 ppm. However, they were not dose-related and were considered incidental. Leukocyte (WBC) counts were increased in males treated at and above 300 ppm. Platelet counts were reduced in males at 300 ppm (17%) and 1500 ppm (23%), and in females at 1500 ppm (38%).

Table 3: Selected haematological parameters (mean ± SD)

Parameter	Control	60 ppm	300 ppm	1500 ppm
Males				
RBC (10^{12}/L)	10.58 ± 1.1	10.22 ± 1.2	11.56 ± 1.2 (↑9%)	11.51 ± 1.1 (↑9%)
Hb (g/L)	159 ± 19	152 ± 18	174 ± 20 (↑9%)	172 ± 15 (↑8%)
Ht (L/L)	0.561 ± 0.06	0.550 ± 0.07	0.633 ± 0.08* (↑13%)	0.643 ± 0.05* (↑15%)
MCV (fl)	53.1 ± 1.3	53.8 ± 1.5	54.7 ± 2.0	56.0 ± 2.0** (↑5%)
MCHC (g/L RBC)	282 ± 7	276 ± 9	275 ± 9	267 ± 8** (↓5%)
Reticulocyte (0/00)	28 ± 3.6	30 ± 4.2	31 ± 4.1	37 ± 6.7** (↑32%)
WBC (10^9/L)	6.96 ± 1.7	6.68 ± 3.6	9.33 ± 2.0* (↑34%)	10.35 ± 4.7 (↑49%)
Platelets (10^9/L)	1082 ± 284	1218 ± 294	900 ± 336 (↓17%)	836 ± 282 (↓23%)
Females				
RBC (10^{12}/L)	11.06 ± 1.0	10.05 ± 0.9*	10.79 ± 1.1	11.48 ± 0.7
Hb (g/L)	170 ± 16	155 ± 12*	165 ± 17	177 ± 11
Ht (L/L)	0.613 ± 0.06	0.550 ± 0.04*	0.590 ± 0.06	0.653 ± 0.05
MCV (fl)	55.4 ± 1.9	54.8 ± 1.5	54.7 ± 1.9	56.9 ± 1.8
MCHC (g/L RBC)	278 ± 10	282 ± 8	279 ± 8	271 ± 5
Reticulocyte (0/00)	28 ± 6	31 ± 8	29 ± 7	38 ± 9* (↑36%)
WBC (10^9/L)	6.73 ± 3.3	6.08 ± 1.5	8.33 ± 1.9	7.07 ± 2.8
Platelets (10^9/L)	823 ± 440	1064 ± 217	856 ± 294	511 ± 303 (↓38%)

Data obtained from pages 107-108 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * $p < 0.05$; ** $p < 0.01$

5. Clinical chemistry

Clinical chemistry analysis showed increased concentrations of total bilirubin at 300 ppm and above in both sexes, and decreased urea concentrations in animals of both sexes treated at 1500 ppm. Bilirubin was decreased in females at 60 ppm without any dose-relationship and was considered to be incidental. The activities of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and alkaline phosphatase (APh) were increased at 1500 ppm in both sexes, often attaining statistical significance. No effect on cholesterol, triglycerides, protein, albumin, or globulin was noted.

Table 4: Selected clinical chemistry parameters

Parameter	Control	60 ppm	300 ppm	1500 ppm
Males				
ASAT (U/L)	52.1 ± 13	52.0 ± 10	54.7 ± 21	84.6 ± 15** (↑62%)
ALAT (U/L)	41.0 ± 9	35.3 ± 4	39.4 ± 21	84.7 ± 39* (↑107%)
APh (U/L)	148 ± 26	161 ± 46	160 ± 60	229 ± 41** (↑55%)
Bilirubin (µmol/L)	1.1 ± 0.3	1.2 ± 0.4	1.6 ± 1.2 (↑45%)	1.6 ± 0.5 (↑45%)
Urea (mmol/L)	8.63 ± 1.9	9.35 ± 1.2	8.12 ± 0.1	6.87 ± 0.8* (↓20%)
Females				
ASAT (U/L)	55.9 ± 12	55.1 ± 40	56.1 ± 8	87.3 ± 20* (↑56%)
ALAT (U/L)	38.5 ± 14	34.5 ± 9	52.4 ± 19	71.4 ± 32 (↑85%)
APh (U/L)	149 ± 24	159 ± 26	177 ± 55	231 ± 58* (↑55%)
Bilirubin (µmol/L)	1.3 ± 0.4	0.8 ± 0.5*	2.0 ± 0.5** (↑54%)	2.3 ± 0.6** (↑77%)
Urea (mmol/L)	8.33 ± 1.6	8.32 ± 0.8	7.13 ± 1.2	6.29 ± 1.5** (↓24%)

Data obtained from pages 126-129 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * $p < 0.05$; ** $p < 0.01$

6. Terminal examinations

6.1 Macroscopic examination

At necropsy, the liver or spleen was swollen in some males (liver – 0, 2, 1, 2; spleen – 0, 1, 1, 1 in the 0, 60, 300 and 1500 ppm dose groups, respectively; n=12). The distribution of these findings was not clearly dose-related and thus considered unrelated to treatment.

6.2 Organ weight

At 1500 ppm, liver weights were increased in males (relative) and females (absolute and relative). Additionally, due to the lower body weights, the weights of brain, heart, and epididymides were decreased in males at 1500 ppm. Statistically significant decreases were recorded in the weights of kidneys (60 ppm, absolute and relative in males) and brain (60 ppm, absolute in males); however, these changes were not dose-related. Although not dose-dependent, uterine weights (absolute and relative) were statistically significantly increased at 300 ppm and increased to a lesser extent at 1500 ppm with respect to controls. There were no treatment-related pathological lesions observed in the uterus, although the uteri collected from the low- and mid-dose groups were not examined histologically.

At interim sacrifice, the relative liver weight was statistically significantly increased in males from the 1500 ppm dose group when compared to controls (↑17%; data not shown).

Table 5: Selected absolute (mg) *and relative (mg/100 g)* organ weights (mean ± SD)

Organ		Control	60 ppm	300 ppm	1500 ppm
Males					
Terminal body weight (g)		38 ± 2	38 ± 4	37 ± 2	34 ± 2* (↓10%)
Brain	absolute	522 ± 11	486 ± 26**	501 ± 18	492 ± 22* (↓6%)
	relative	*1377 ± 78*	*1282 ± 94*	*1360 ± 115*	*1452 ± 91*
Heart	absolute	203 ± 26	195 ± 22	195 ± 17	168 ± 14** (↓17%)
	relative	*536 ± 65*	*515 ± 81*	*527 ± 47*	*495 ± 21*
Liver	absolute	1912 ± 140	1790 ± 274	1981 ±152	1881 ± 204
	relative	*5036 ± 293*	*4698 ± 562*	*5372 ± 547*	*5531 ± 466 (↑10%)*
Kidneys	absolute	635 ± 78	558 ± 74*	590 ± 52	603 ± 64
	relative	*1673 ± 211*	*1466 ± 150**	*1598 ± 152*	*1781 ± 231*
Epididymides	absolute	126 ± 10	115 ± 11	119 ± 12	108 ± 10** (↓14%)
	relative	*332 ± 23*	*305 ± 41*	*321 ± 36*	*319 ± 31*
Females					
Terminal body weight (g)		31 ± 2	31 ± 2	33 ± 2*	29 ± 1
Brain	absolute	511 ± 26	503 ± 35	505 ± 33	481 ± 21
	relative	*1650 ± 101*	*1628 ± 152*	*1537 ± 96*	*1670 ± 111*
Heart	absolute	172 ± 17	155 ± 14	162 ± 16	156 ± 24
	relative	*554 ± 57*	*501 ± 55*	*492 ± 46*	*542 ± 105*
Liver	absolute	1641 ± 133	1547 ± 131	1703 ± 153	1813 ± 250 (↑10%)
	relative	*5291 ± 302*	*4992 ± 318*	*5175 ± 370*	*6275 ± 764** (↑18%)*
Kidneys	absolute	405 ± 36	388 ± 24	420 ± 44	416 ± 44
	relative	*1307 ± 93*	*1257 ± 111*	*1274 ± 109*	*1440 ± 123*
Uterus	absolute	182 ± 56	184 ± 67	287 ± 94** (↑58%)	212 ± 65 (↑16%)
	relative	*586 ± 177*	*596 ± 228*	*878 ± 292* (↑50%)*	*738 ± 243 (↑26%)*

Data obtained from pages 192-204 of the study report. ( ) Values in parentheses represent the percent change relative to mean control values (calculated by the reviewer). * $p < 0.05$; ** $p < 0.01$

6.3 Histopathology

In the liver, hepatocellular hypertrophy was observed in both sexes at 1500 ppm. At that dose, hepatocellular degeneration, mainly of periportal hepatocytes, was also observed which was most prominent below the liver capsule or in one liver lobe, whereas other localizations appeared to be less affected. Small amounts of pigment were often seen next to the portal triads and may represent residues of degenerated hepatocytes. In the degenerated areas, the Kupffer cells lining the sinus appeared to be more prominent than in the normal liver tissue. Diffuse bile duct proliferation (both sexes) and oval cell proliferation (in males more frequently than in females) were also observed in the affected areas. In the cryocuts stained with Oil Red O (ORO), the lipid content was slightly reduced at 1500 ppm. This finding was clearer in males than in females. At 300 ppm, an increased incidence of liver cell hypertrophy, which was mostly rated as minimal, was observed in females. However at 300 ppm, there was no evidence of any liver cell damage and this finding is therefore considered adaptive.

In the spleen, increased hematopoiesis was recorded in all groups including control. The grade of this finding was mostly moderate (males) or moderate to marked (females). Hematopoiesis is increased to compensate for the loss from blood sampling and is unrelated to the test compound. It is probably the cause of a grossly swollen spleen observed in some of the animals. Pigment mostly rated as minimal was observed in the red pulp in all groups including control. At 1500 ppm, the incidence (in females also the severity) was higher than in the other groups. This increase is regarded as a treatment-related finding. With special stains for lipopigment (Schmorl stain) or ferrous pigment (Prussian Blue), in selected controls and dosed animals, the pigment was iron-containing and (partly) positive with the Schmorl stain.

Table 6: Incidence of microscopic findings in selected organs (males / females)

Organ		Control	60 ppm	300 ppm	1500 ppm
Liver (# examined)		12 / 12	12 / 12	12 / 12	12 / 12
Hepatocyte hypertrophy	Total	- / 1	- / 1	- /6	12 / 10
	Grade 1	- / 1	- / 1	- / 6	- / -
	Grade 2	- / -	- / -	- / -	3 / -
	Grade 3	- / -	- / -	- / -	9 / 10
Bile duct hyperplasia	Total	- / -	- / -	- / -	11 / 10
	Grade 1	- / -	- / -	- / -	2 / -
	Grade 2	- / -	- / -	- / -	4 / 5
	Grade 3	- / -	- / -	- / -	5 / 5
Oval cell proliferation	Total	- / -	- / -	- / -	7 / 2
	Grade 1	- / -	- / -	- / -	5 / 1
	Grade 2	- / -	- / -	- / -	2 / 1
Degeneration	Total	- / -	- / -	- / -	10 / 10
	Grade 1	- / -	- / -	- / -	1 / -
	Grade 2	- / -	- / -	- / -	3 / 2
	Grade 3	- / -	- / -	- / -	6 / 7
	Grade 4	- / -	- / -	- / -	- / 1
Kupffer cell foci	Total	4 / 2	3 / 1	2 / 2	- / -
	Grade 1	4 / 1	2 / -	2 / 2	- / -
	Grade 2	- / 1	1 / -	- / -	- / -
	Grade 3	- / -	- / 1	- / -	- / -
Fatty change	Total	4 / 4	5 / 6	5 / 3	1 / 2
(ORO staining)	Grade 1	4 / 4	4 / 5	4 / 3	1 / 2
	Grade 2	- / -	- / 1	- / -	- / -
	Grade 3	- / -	1 / -	1 / -	- / -
Pigment deposits	Total	- / -	- / -	- / -	7 / 9
	Grade 1	- / -	- / -	- / -	5 / 6
	Grade 2	- / -	- / -	- / -	2 / 3
Spleen (# examined)		12 / 12	12 / 12	12 / 12	12 / 12
Increased hematopoiesis	Total	11 / 12	11 / 12	10 / 12	11 / 10
	Grade 1	1 / -	- / -	- / -	1 / 1
	Grade 2	- / 2	2 / -	2 / -	1 / -
	Grade 3	9 / 6	8 / 6	8 / 4	9 / 7
	Grade 4	1 / 4	1 / 6	- / 8	- / 2
Pigment deposits	Total	5 / 5	5 / 6	6 / 8	12 / 11
	Grade 1	4 / 5	5 / 6	6 / 8	11 / 8
	Grade 2	1 / -	- / -	- / -	1 / 2
	Grade 3	- / -	- / -	- / -	- / 1

Data obtained from pages 226-249 of the study report. Grade 1 = minimal / very few / very small; Grade 2 = slight / few / small; Grade 3 = moderate / moderate number / moderate size; Grade 4 = marked / many / large

6.4 Enzyme activity in homogenised liver tissue

Analyses of liver tissue collected from satellite animals sacrificed after 28 days of treatment showed no evidence for toxicologically significant effects on the concentration of cytochrome P450 at 1500 ppm (Table 7). At 1500 ppm in male mice only, the monooxygenase 7-ethoxycoumarin deethylase (ECOD) was increased. The phase II enzymes epoxide hydrolase (EH) and glutathione-S-transferase (GS-T) were increased, whereas UDP-glucuronyltransferase (GLU-T) was not affected.

In female mice the monooxygenases were not significantly affected, whereas all phase II enzymes (epoxide hydrolase, glutathione-S-transferase, UDP-glucuronyltransferase) were significantly induced at 1500 ppm.

Neither 7-ethoxyresorufin deethylase (EROD) nor aldrin epoxidase (ALD) was affected by treatment in males or females.

Table 7: Liver homogenate determinations

Parameter	Control		1500 ppm	
	Male	Female	Male	Female
P450 (nmol/g)	62.1 ± 6.3	47.4 ± 3.9	58.9 ± 5.6	46.0 ± 4.9
ECOD (nmol/g min)	17.0 ± 5.3	34.3 ± 3.6	29.1 ± 3.7** (↑71%)	36.2 ± 7.0
EROD (nmol/g min)	1.47 ± 0.25	1.31 ± 0.15	1.73 ± 0.66	1.14 ± 0.57
ALD nmol/g min)	64.9 ± 9.5	72.0 ± 6.4	83.4 ± 21.3	77.8 ± 23.8
EH (nmol/g min)	698 ± 137	439 ± 98	868 ± 76* (↑24%)	1172 ± 203** (↑167%)
GS-T (µmol/g min)	543 ± 100	234 ± 42	755 ± 104* (↑39%)	598 ± 107** (↑156%)
GLU-T (µmol/g min)	1333 ± 179	1151 ± 232	1371 ± 144	1635 ± 194** (↑42%)

Data obtained from pages 137 and 146-147 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * $p < 0.05$; ** $p < 0.01$

III. CONCLUSIONS

The NOAEL of the study was 60 ppm (equivalent to 11 mg/kg/day for males and 18 mg/kg bw/day for females) based on hematology effects in the males, increased bilirubin levels in both sexes, and reduced food consumption in females at the LOAEL of 300 ppm (51 mg/kg bw/day form males and 68 mg/kg bw/day for females).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: The study investigators noted that there were no toxicologically significant effects on platelet counts. An apparent treatment-related decrease in platelet counts was noted for males from the 300 and 1500 ppm dose groups and in females from the 1500 ppm dose group. The mean value in females from the 1500 ppm dose group fell below the typical range of platelet levels in CD-1 mice (700-1400 $10^3/mm^3$, as reported in Handbook of Toxicology, Second Edition, M.J. Derelanko and M.A. Hollinger, Eds., 2001).

The study investigators also indicated that the dose level of 60 ppm was a No-Effect-Level (NOEL). While many of the effects noted at 300 ppm would be considered adaptive (i.e. hepatocellular hypertrophy in the absence of other hepatocellular damage) or minimal (e.g. increased in RBC parameters of less than 10% from controls), substantial elevations in WBC (34%) were noted in males and bilirubin levels (45-54%) were noted in both sexes at that dose level, and males demonstrated decreased platelets (17%), the toxicological significance of which is not certain. Therefore, the dose level of 60 ppm (11/18 mg/kg bw/day in males/females) will be considered the NOAEL and the dose level of 300 ppm (51/68 mg/kg bw/day in males/females) will be considered the LOAEL in this study.

C. Conclusions: The NOAEL in this study is considered to be 60 ppm (11/18 mg/kg bw/day in males/females). The LOAEL is 300 ppm (51/68 mg/kg bw/day in males/females), based on decreased platelet count in males, increased bilirubin in both sexes and decreased food consumption in females.

This subchronic toxicity study in the mouse is acceptable/guideline (fully reliable) and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3100; OECD 408) in mice.

D. Deficiencies: No deficiencies were identified.

Tier II Summary (PMRA 2181206, MRID 48574754) revised by the regulatory authorities, April 2013

Study Type: Oral 90-day toxicity - Rat

IIA 5.3.2 – MRID 48574754, PMRA 2181206

Report:	Schladt L. and Rühl-Fehlert C. (2003) BYI 01921 Study on Subchronic Toxicity in Wistar Rats (Dietary Administration for 3 Months with a Subsequent Recovery Period over 1 Month).Study No. T0071363, Report No. AT00814, 29 October 2003, Sponsor Reference No. R-23150. Sponsor: Bayer HealthCare, Experimental Toxicology, Germany Dates of experimental work: Main Groups: 22 April 2002 to 29 July 2002; Recovery Groups, recovery period: 22 July 2002 to 22 August 2002.
Sponsor / Submitter:	Submitter: Makhteshim Chemical Works, Ltd., Israel. Sponsor: Bayer CropScience AG.
Guidelines:	OECD No. 408, OPPTS 870.3100, 67/548/EEC B.7, Japanese MAFF (12-Nousan no. 8147, Nov. 24, 2000, amended in Jun. 26, 2001)
Deviations:	None
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary:

In a 90-day dietary toxicity study (MRID 48574754, PMRA 2181206), groups of 10 male and 10 female Wistar rats (Hsd Cpb:WU) received diets containing 0, 60, 120, 500 or 2000 ppm of fluensulfone (BYI 01921, MCW-2 technical, 97%, Batch No. NLL6692-7-5) for a period of 3 months. The mean daily intakes in order of ascending concentration were 0, 4.31, 8.26, 34.86 or 139.01 mg/kg bw/day in males and 0, 4.85, 11.68, 53.10 or 148.73 mg/kg bw/day in females of the main groups. Dosages were chosen on the basis of a 28-day range-finding study (MRID 48575023, PMRA 2181203). Additional recovery groups were similarly treated at 0 or 2000 ppm (10/sex/dose) and then maintained without treatment for a recovery period of 28 days. Along with the standard guideline evaluations, all groups of animals were assessed for changes in functional observational battery (FOB) and motor activity (MA) at the end of the dosing period. Fluoride levels in ash of bone (left femur) and teeth lower left incisor) were examined in all animals of the main and recovery groups. Additional groups of 5 males were treated for one week at 0 or 2000 ppm for investigation of alpha-2µ-globulin accumulation in kidneys.

No animals died during the study and no observations related to neurotoxicity were noted during the study.

Body weights were statistically significantly reduced by treatment in both males and females at 2000 ppm throughout the study (at study termination, 9% below controls for both sexes). Although no statistical difference occurred, mean body weights of males treated at 500 ppm during the study were also lower compared with the control group. Females of this dose group were not affected. Body weights of recovery animals were also statistically significantly decreased at the end of the recovery period (8-15% compared to 11-21% at the end of the treatment period). In the main group overall body weight gain was decreased in mid (9%) and high (15%) dose males and in high dose females (15%); recovery groups also showed decreased body weight gain (17-20%). Food consumption was decreased in 2000 ppm females of the main group as well as in recovery females. Water intake was increased in males at 500 ppm and in males and females at 2000 ppm and in recovery animals.

At 2000 ppm there were some changes detected at hematological investigations, most of which were reversible during the recovery period. In females the HQuick (thromboplastin time) was significantly decreased at 2000 ppm (6%). In the 2000 ppm dose groups, statistically significant decreases were observed in hemoglobin, MCH, and MCHC in males, and in hemoglobin and MCHC levels in females (2-7%). Reticulocyte counts were increased in high dose females (44%). Despite the low magnitude of the decreases in MCHC, the changes were considered to be treatment-related due to the statistical significance and because such decreases have been observed in another long term rat study, as well as studies in other species.

Clinical laboratory tests gave evidence for effects on liver function in males at 120 ppm and above, and in females at 500 ppm and above, including some effects indicating an adaptive response of the liver like a significant increase of relative liver weight at the end of the treatment period for both males and females treated at 2000 ppm, accompanied by hepatocellular hypertrophy at microscopic examination. Effects on enzyme activities and hepatocellular hypertrophy were no longer recorded at the end of the recovery period. Significantly decreased activities of alanine aminotransferase (ALAT) were observed in peripheral blood of males and females at 500 ppm and 2000 ppm. At the end of the recovery period, ALAT levels were slightly increased compared to controls; however, these were not statistically significant differences. These changes were not considered adverse.

At the end of the treatment period concentrations of urea (22%) were significantly increased at 2000 ppm in males. In females, triglycerides were increased in all dose groups (statistically significant at 500 and 2000 ppm; 73% and 65%, respectively) and cholesterol was increased at 500 ppm (25%) and 2000 ppm (49%), while creatinine was decreased at 2000 ppm. Statistically significant decreases in cholesterol and triglycerides were observed in recovery males.

At 2000 ppm the concentration of calcium was slightly but statistically significantly increased in males of the main group and the concentration of phosphate was statistically significantly increased in females of the main group.

At 2000 ppm relative weights of kidneys were significantly increased in both sexes (males: 21%, females: 14%), but not at the end of the recovery period. In this dose group changes of the surface of the kidneys were observed in 5 males at necropsy at the end of the treatment period. Histopathological investigations revealed hyaline droplet nephropathy possibly due to alpha-2-µ-globulin accumulation in males at 500 ppm and 2000 ppm. This is a phenomenon unique to the male rat and is regarded not to be of toxicological relevance for humans. Hyaline droplet accumulation was reversible within the recovery period, while other tubular changes observed at 500 ppm and above after the treatment period were still discernible in 2000 ppm males after the recovery period (i.e. basophilic cortical tubules, tubulosclerosis, Schmorl-positive material in tubules, cystic tubules and mononuclear infiltrates). In females, a slightly increased grade of Schmorl-positive pigment was seen in renal tubules at 2000 ppm. This change was reversible within the recovery period and did not cause any damage. The histopathological investigations of the kidneys of males from the control and 2000 ppm dose groups using the Azan stain revealed an immunohistochemical reaction that was stronger and distributed more widely throughout the renal cortex of animals dosed at 2000 ppm compared to controls.

The histopathological investigations of the kidneys of males treated for one week with 2000 ppm revealed no differences between control and 2000 ppm males using the Azan stain. With immunohistochemical staining for alpha-2µ-globulin, the reaction was slightly more intense and more widely distributed in control than in treated males.

Urinalysis revealed decreased urine protein concentration and excretion in high dose males, and decreased urine protein excretion in recovery animals. Some of these findings may be attributable to increased water intake in mid and high dose males and in high dose females, and in recovery animals.

In the forestomach a diffuse basal cell hyperplasia was observed in both sexes at 500 ppm and 2000 ppm. At the end of recovery period, this change partly regressed, but was still detectable in a few animals.

In the pharynx an increased incidence of minimal mononuclear infiltrates was recorded at the end of the treatment period in 500 and 2000 ppm males and females treated at 2000 ppm. These findings were still observed in recovery males. In the adrenal glands of males an increased incidence of cortical vacuolation was observed at 2000 ppm. This finding was reversible within the recovery period.

At necropsy at the end of the treatment period discoloration of teeth was observed in 6/10 males at 2000 ppm, but no corresponding histopathological changes were observed.

Treatment with the test substance increased the content of fluoride in ashes from bones and teeth of treated male and female rats in a dose-related manner. Increases 85% to 140% (not statistically significant) were observed in the main study animals at 60 ppm (recovery groups not were tested at the low and mid doses). Statistically significantly increases in fluoride concentrations were observed at 120 ppm (157% to 233% higher than controls) and above (487% to 1852%). At the end of the recovery period, fluoride concentrations, although still significantly increased in ashes from bones and teeth of male and female rats compared to the corresponding controls, had started to decline somewhat in males and females previously treated with 2000 ppm indicating a possible reversibility of this effect. Histopathological examinations revealed no abnormalities in teeth and investigated bones.

The measured accumulation of fluoride in ashes from bones and teeth is not necessarily, in itself, an adverse effect. The visible discoloration of the teeth was possibly associated with accumulation of fluoride but was only observed at 2000 ppm (the highest dose tested), and free of histopathological signs of damage in both sexes. Therefore, it is concluded that there is no dental fluorosis in this study at levels below 2000 ppm and certainly no severe dental fluorosis at any dose level.

As only adaptive effects on the liver were observed at 120 ppm, this dose level, equivalent to 8.23 mg/kg bw/day for males and 11.68 mg/kg bw/day for females, is considered to be the NOAEL of the study. Based on the numerous effects in the kidneys, observations in the pharynx and forestomach, increased cholesterol in females, slightly decreased body weight and body weight gain in males, the dose of 500 ppm in the diet (34.86 and 53.10 mg/kg bw/day in males and females, respectively) is considered to be the LOAEL.

This study in is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for a repeated dose 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rats.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BYI 01921 (MCW-2 TECH)
Description:	Yellow oil
Batch No.:	NLL6692-7-5
Purity:	97%
CAS#:	318290-98-1
Expiry date:	14 August 2002
Stability of Test Item:	Stable in the diet for at least 15 days
2. Vehicle:	Provimi Kliba SA 25 G4 (3883.9.25)
3. Test animals	
Species:	Rat
Strain:	Wistar (Hsd Cpb:WU)
Age:	5 weeks old at delivery
Weight at dosing:	Males: mean 131 g (range: 113-150 g); females: mean 117 g (range: 104-128 g)
Source:	Harlan Winkelmann GmbH, Germany
Acclimation period:	7 days
Diet:	Fixed-formula standard diet (supplied by Provimi Kliba AG, Switzerland). Diet mixtures (Provimi Kliba SA 25 G4 (3883.9.25 meal) containing the test compound were provided in stainless steel containers for *ad libitum* consumption.
Water:	Tap water supplied in bottles of approximately 300 mL for *ad libitum* consumption.
Housing:	Individually in Makrolon® cages Type IIa on low-dust wood granules (supplied by Ssniff Spezialdiaten Inc., Germany; manufacturer: J. Rettenmeier, Ellwangen-Holzmühle).
4. Environmental conditions	
Temperature:	22 ± 2°C
Humidity:	55 ± 5%
Air changes:	≥10 per hour

Photoperiod: 12 hours rhythm from 5:00 to 17:00 hours (artificial illumination: approx. 140 lux, for work in the room approx. 380 lux).

From 17:00 to 5:00 hours orientation light (approx. 3-5 lux)

B. STUDY DESIGN AND METHODS

1. In life dates: 22 April to 22 August 2002

2. Animal assignment and treatment:

The animals were allocated to their final cage number on the basis of a random list generated with evenly distributed chance numbers especially generated for the study (whereby the first random number on the list gave the actual position of the animal to be moved into cage No, 1, and so on). The cages were then placed one after the other in shelves in the order of increasing numbers.

Animals of the main groups (10/sex/group) were treated for a total of 99 days at nominal concentrations of 0, 60, 120, 500 and 2000 ppm. Animals of the recovery groups (10/sex in control and 2000 ppm groups) were treated for a total of 91 days and then received untreated diet for 31 days. A satellite group (5 males in a control group and 5 males in a 2000 ppm group) used for investigations on kidneys were treated for one week.

3. Methods:

a. Observations:

Animals were inspected twice daily for morbidity and mortality (once on weekends and holidays). General clinical examinations were made daily and detailed clinical examinations were performed weekly. Once before the start of the study, and weekly thereafter, animals were placed into an open field for behavioral observations.

b. Body Weight and Food Consumption:

Body weights of individual animals were determined just prior to the start of the study and weekly thereafter up to scheduled necropsy. Body weights were also recorded on the day of necropsy in order to calculate relative organ weights. Individual food and water intakes were determined weekly. This data was then used to calculate group means for each period of 7 days. Food intake was calculated from the weight of food offered at the start of the measurement period minus the weight of the feed at the end of the period.

c. Clinical Laboratory Investigations:

Hematological and clinical chemistry investigations were performed on all animals. Blood samples were taken during week 14 for the main group and week 18 for the recovery group. Clinical chemistry evaluations were conducted during weeks 13/14 for the main group and weeks 17/18 for the recovery group. Urinalysis was conducted during week 13 for the main group and week 17 for the recovery group. Urine samples were collected at room temperature during a period of 16 hours. During this time, water was offered *ad libitum* but feed was not supplied. Blood samples for glucose concentrations were taken directly after the urine sampling period from the caudal vein of fasted (16 hours), non-anaesthetized animals. Blood samples for determining other parameters were collected in the morning from the retro-orbital venous plexus of fasted (approximately 16 hours) animals anaesthetized with diethyl ether. Liver samples were taken during necropsy.

The following hematological parameters were determined in the peripheral blood: differential blood count, erythrocyte morphology, erythrocyte count, erythrocyte indices, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, hemoglobin concentration, hematocrit, leukocyte count, amount of reticulocytes, thrombocyte count, thromboplastin time. The following parameters were determined for clinical chemistry evaluations: alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, glutamate dehydrogenase, gamma glutamyl transferase, albumin, bilirubin, cholesterol, creatinine, total protein, albumin/globulin ratio, triglycerides, urea, glucose, chloride, calcium, inorganic phosphate, potassium and sodium. Urinalysis parameters examined included: blood, bilirubin, glucose, ketone bodies, pH, sediment, urobilinogen, density, volume and protein.

d. Functional Observational Battery and Motor Activity:

In week 12, the Functional Observational Battery (FOB) was performed with all animals of main and recovery groups, and in week 16 with all animals of the recovery groups. Motor activity assessments were conducted in week 12 (main group) and week 16 (recovery group). Motor activity (MA) and locomotor activity (LMA) were examined as activity for the entire 70-minute session and activity during each 10-minute interval. MA was measured as the number of beam interruptions that occurred during the test session. LMA was measured by eliminating consecutive counts given for a beam meaning that only one interruption of a given beam was counted until the animal relocated in the maze and interrupted one of the other beams. Habituation was evaluated as a reduction in activity during the test session.

e. Ophthalmology:

An ophthalmological examination was performed on all animals prior to the start of dosing. Control and high dose animals were examined at the end of the treatment and recovery periods.

f. Sacrifice and Pathology:

Animals killed in moribund state and all animals scheduled for necropsy were euthanized by exsanguination under deep diethyl anesthesia. Animals underwent necropsy, and their organs and tissues were subjected to thorough gross pathological examination. The following organs were fixed and evaluated for histopathology: adrenal glands, aorta, brain (cerebrum, cerebellum, brain stem), epididymides, esophagus, eyes, exorbital lacrimal glands, femur, harderian glands, head (nasal cavity), heart, intestine (duodenum, jejunum, ileum, cecum, colon, rectum), kidneys, larynx, liver, lungs, lymph nodes (mandibular, mesenteric), optic nerves, ovaries, oviducts, pancreas, pharynx, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, skull cap, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, teeth, testes, thymus, tongue, trachea, urinary bladder, uterus, and vagina. Some tissues/organs were only examined from control and high dose animals from the main group, some were examined from control and high dose main and satellite groups, and others were examined from all groups. The following organs were weighed before fixation: brain, heart, liver, spleen, kidneys, thymus, adrenal glands, uterus, ovaries, epididymides, and testes.

Kidneys from control and high dose males from the main group and males from the satellite group (dosed for one week) were examined following Azan staining and immunohistochemical staining with antibody to alpha 2-µ-globulin.

Examinations of homogenized liver samples included determination of activities of aminopyrine-N-demethylase, p-nitroanisole-O-demethylase, alanine aminotransferase, and cytochrome P-450. For fluoride determinations in bone and teeth, one femur (left) and one tooth (left incisor of lower jaw) of each animal (main and recovery groups) was taken at necropsy and frozen until determination of fluoride concentration could be conducted.

4. Data evaluation/Statistics:

The results for the groups that received the test compound were compared with those for the control group. The statistical evaluation of data related to clinical chemistry, hematology, body and organ weights as well as feed and water intake was performed using SAS® routines. Evaluations of body and organ weight data were done using the Dunnett-test in connection with ANOVA. A Kruskal-Wallis test with a Steel-test was performed for food and water data.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Actual achieved intakes of test substance

The actual achieved intakes of the test substance were as follows:

Dietary level (ppm)	Actual Dose (mg/kg bw/day)	
	Males	Females
Main Group		
60	4.31	4.85
120	8.26	11.68
500	34.86	53.10
2000	139.01	148.73
Recovery Group		
2000	141.63	155.92

Data obtained from pages 48 and 49 of the study report.

The test diet was prepared weekly was mixed and stored at room temperature. The analytical data revealed that the homogeneity of the formulations (relative standard deviation range: 0.57 – 4.76%) was achieved, and the content checks confirmed that during the study appropriate and equal mixture procedures were followed. All the samples were within ± 10% of nominal values (93-104% of nominal). Analytical data revealed that mixtures were stable at room temperature for at least 15 days (stability range: 92-104%).

2. Clinical signs/Mortality

Results of daily in-cage observations, weekly clinical examinations and observations in the open field revealed no treatment-related findings. No animals died during the study.

3. FOBs and Motor activity assessments

The investigations in the home cage, during handling, in the open field and the determination of grip strength showed no relevant signs or symptoms indicating evidence for neurotoxicity (data not presented). The activity determination over the entire 70 minute observation period failed to reveal any significant effect on motor and locomotor activity. The results of the 10-minute intervals indicate that treated and control groups reacted in a similar way and that habituation (defined as a decrement in activity during the test session) followed the same curve and had the same slope. In particular, there were no significant differences in the first 10-minute intervals which are regarded as best indicator of increases or decreases in activity, before the animals habituate to a minimal level of activity.

4. Body weight and food and water consumption

Body weights were reduced by treatment at 2000 ppm throughout the study with statistically significant decreases observed beginning on Day 8. At the end of the treatment period the difference from control was 9% for both males and females of the main groups (Table 1).

Although no statistical difference occurred, mean body weights of males treated at 500 ppm during the study were lower compared with the control group resulting in a 7% difference at the end of the study. Females of this dose group were not affected.

At the end of the treatment period body weights of males of the recovery groups were 21% and those of females 11% lower compared to the respective control group. At the end of the recovery period the differences were 15% for males and 8% for females, indicating a slight recovery (Table 2).

In the main treatment groups, overall body weight gain was decreased in mid and high dose males by 9 and 15%, respectively, and by 15% in high dose females. In the recovery groups, overall body weight gain was also decreased in both males and females by 20 and 17%, respectively.

Table 1: Mean ± S.D. body weights (g) – Main groups

Day	Control n = 10	60 ppm n = 10	120 ppm n = 10	500 ppm n = 10	2000 ppm n = 10
Males					
1	128 ± 7.16	130 ± 7.45	130 ± 9.26	126 ± 8.02	133 ± 7.62
30	297 ± 16.8	304 ± 18.0	312 ± 27.2	283 ± 26.8 (↓5%)	262**± 16.7 (↓12%)
64	401 ± 22.7	405 ± 23.6	418 ± 35.6	379 ± 36.6 (↓6%)	356**± 15.8 (↓11%)
99	438 ± 28.3	445 ± 28.9	459 ± 39.6	409 ± 44.9 (↓7%)	397* ± 14.4 (↓9%)
Overall BWG	310	315	329	283 (↓9%)	264 (↓15%)
Females					
1	119 ±5.33	119 ± 5.26	117 ± 5.85	114 ± 5.55 (↓4%)	116 ± 7.09 (↓3%)
30	193 ± 13.5	199 ± 17.0	184 ± 9.34	181 ± 16.2 (↓6%)	175* ± 18.0 (↓9%)
64	228 ± 14.6	238 ± 24.2	219 ± 12.3	220 ± 15.8 (↓4%)	208* ± 20.2 (↓9%)
99	243 ± 16.2	253 ± 27.8	234 ± 14.5	235 ± 16.9 (↓3%)	221 ± 25.2 (↓9%)
Overall BWG	124	134	117 (↓6%)	121 (↓3%)	105 (↓15%)

* $p < 0.05$; ** $p < 0.01$; ( ) = % difference from controls; BWG = body weight gain, calculated by the reviewer. Data obtained from pages 52 and 232-235 of the study report.

Table 2: Mean ± S.D. body weights (g) – Recovery groups

Day	Males		Females	
	Control n = 10	2000 ppm n = 10	Control n = 10	2000 ppm n = 10
Treatment period				
1	137 ± 7.20	133 ± 5.34	115 ± 6.26	118 ± 4.56
30	315 ± 23.4	245** ± 15.8 (↓22%)	187 ± 12.6	168** ± 10.6 (↓10%)
64	420 ± 34.6	328** ± 22.7 (↓22%)	223 ± 15.1	199** ± 12.0 (↓11%)
92	458 ± 39.7	363** ± 24.1 (↓21%)	238 ± 17.0	212** ± 14.6 (↓11%)
Recovery Period				
99	465 ± 40.7	382** ± 24.5 (↓18%)	240 ± 17.9	217** ± 15.1 (↓10%)
120	482 ± 41.4	408** ± 28.1 (↓15%)	245 ± 17.3	226* ± 15.2 (↓8%)
Overall BWG Days 1 - 120	345	275 (↓20%)	130	108 (↓17%)

* $p < 0.05$; ** $p < 0.01$; ( ) = % difference from controls; BWG = body weight gain, calculated by the reviewer. Data obtained from pages 52 and 236-239 of the study report.

Food intake of females was decreased at 2000 ppm (not statistically significant), both in the main and in the recovery groups. There were no relevant differences in mean daily food intake of any group of male animals compared with the respective control group (Table 3).

Table 3: Mean Daily Food Intake (mg/kg bw/day) (n = 10)

Main Groups		**Recovery Groups**	
Males		**Males - Treatment Period**	
0 ppm	70.30 ± 22.79	0 ppm	70.37 ± 20.30
60 ppm	71.80 ± 21.51	2000 ppm	70.82 ± 14.88
120 ppm	68.87 ± 23.38	**Males - Recovery Period**	
500 ppm	69.71 ± 22.03	0 ppm	48.25 ± 3.87
2000 ppm	69.51 ± 15.72	2000 ppm	52.62 ± 3.91
Females		**Females - Treatment Period**	
0 ppm	0	0 ppm	109.61 ± 20.47
60 ppm	2000	2000 ppm	77.96 ± 15.11 (↓29%)
120 ppm	97.35 ± 24.41	**Females - Recovery Period**	
500 ppm	106.21 ± 30.19	0 ppm	75.46 ± 6.80
2000 ppm	74.37± 15.51 (↓16%)	2000 ppm	68.06 ± 5.00 (↓10%)

Data obtained from page 47 of the study report. ( ) = % difference from controls, calculated by the reviewer. Standard deviation calculated by PMRA reviewer.

Water intake was increased in males at 500 ppm and in males and females at 2000 ppm. Differences from the control values were 11% in males at 500 ppm, and 23% in males and 15% in females at 2000 ppm. For males and females of the 2000 ppm recovery group following treatment, the differences were 23% and 13%, respectively (Table 4).

During the recovery period, mean daily water intake of animals previously treated at 2000 ppm was increased, compared to that of the respective control group, by 29% for males and of 9% for females.

Table 4: Mean daily water intake (g/animal/day – g/kg bw/day) (n = 10)

Up to day	Control	60 ppm	120 ppm	500 ppm	2000 ppm
Males – Main groups					
g/animal/day	25.92 ± 1.66	27.28 ± 1.47	27.58 ± 1.74	27.45 ± 1.52 (↑6%)	28.62 ± 2.27 (↑10%)
g/kg bw/day	79.55 ±23.82	82.12 ±24.44	81.32 ±25.20	88.60 ±25.09 (↑11%)	98.22 ±26.80 (↑23%)
Females – Main groups					
g/animal/day	19.70 ±0.769	19.36 ±1.197	18.74 ±0.843	19.83 ±0.836	20.68 ±1.273 (↑5%)
g/kg bw/day	96.29 ±16.82	91.62 ±17.35	94.97 ±16.67	99.13 ±17.50	110.82±20.1 (↑15%)
Males – Recovery groups – Treatment period					
g/animal/day	27.22 ±1.926				26.67 ±1.370
g/kg bw/day	80.88 ±26.66				99.52 ±25.47 (↑23%)
Males – Recovery groups – Recovery period					
g/animal/day	25.10 ±1.042				26.67 ±1.928
g/kg bw/day	53.10±2.955				68.39± 6.727 (↑29%)
Females – Recovery groups – Treatment period					
g/animal/day	19.37 ±0.935				19.74 ±0.547
g/kg bw/day	97.67 ±15.72				110.81±19.7 (↑13%)
Females – Recovery groups – Recovery period					
g/animal/day	19.08 ±0.700				19.03 ±0.435
g/kg bw/day	78.74 ±3.066				85.88 ±2.984 (↑9%)

Data obtained from pages 50 and 210-221 of the study report. ( ) = % difference from controls, calculated by the reviewer. Standard deviations were calculated by the reviewer.

5. Hematology

In females the HQuick (thromboplastin time) value at 2000 ppm was statistically significantly decreased (6%) (Table 5). In the 2000 ppm dose groups, statistically significant decreases were observed in hemoglobin and MCHC in males and females, and in MCH in males only. Reticulocyte counts were increased in high dose females only.

With the exception of the decreased MCHC in males at 2000 ppm, these changes were reversible during the recovery period. Despite the low magnitude of changes observed in MCHC levels, they are considered to be treatment-related due to the statistical significance and because such decreases have also been observed in another long term rat study, as well as studies in other species dosed with fluensulfone.

A statistically significant decrease in platelet count was observed in 500 ppm females, but there was no dose response relationship for this finding and, as such, was not considered to be related to treatment.

Table 5: Mean Hematological Findings (n = 10)

Dose group (ppm)	Hemoglobin (g/l)	MCH (pg)	MCHC (g/l ERY)	Reticulocytes (o/oo)	Thrombocyte count (10^9/l)	H Quick (thromboplastin time) (sec)
Males – Main Group						
Historical controls (n = # of animals)	155 ± 5.0 (n = 59)	17.3 ± 0.89 (n = 59)	322 ± 10.9 (n = 59)	24 ± 5.3 (n = 59)	1304 ± 133.7 (n = 58)	31.8 ± 2.23 (n = 77)
0	151 ± 3.2	16.7 ± 0.32	318 ± 4.7	19 ± 2.7	1321 ± 84.5	30.2 ± 1.27
60	147 ± 6.8	16.3 ± 0.49	319 ± 2.8	21 ± 4.9	1346 ± 141.9	28.9 ± 1.24
120	147 ± 5.2	16.6 ± 0.51	317 ± 4.9	21 ± 4.1	1298 ± 115.5	29.0 ± 1.88
500	152 ± 6.6	16.6 ± 0.50	316 ± 5.6	22 ± 3.8	1374 ±126.2	29.9 ± 2.01
2000	144* ± 4.4 (↓5%)	16.0** ±0.56 (↓4%)	310** ± 3.6 (↓2%)	22 ± 2.7	1266 ± 136.7 (↓4%)	29.8 ± 1.71
Females – Main Group						
Historical controls	153 ± 6.0 (n =60)	17.8 ± 0.62 (n = 60)	321 ± 11.6 (n = 60)	23 ± 5.7 (n = 60)	1307 ± 144.3 (n = 60)	29.2 ± 2.06 (n = 77)
0	150 ± 3.6	17.4 ± 0.46	323 ± 6.5	16 ± 3.4	1441 ± 116.8	27.8 ± 1.23
60	149 ± 3.9	17.7 ± 0.58	323 ± 5.5	19 ± 4.6	1331 ± 143.6	27.4 ± 1.09
120	149 ± 3.8	17.4 ± 0.43	327 ± 4.4	18 ± 3.5	1327 ± 71.1	28.1 ± 1.56
500	149 ± 2.8	17.4 ± 0.48	324 ± 4.6	17 ± 3.1	1296* ± 72.3 (↓10%)	27.3 ± 1.14
2000	144** ± 4.6 (↓4%)	17.3 ± 0.55	317* ± 4.5 (↓2%)	23* ± 4.3 (↑44%)	1334 ± 109.6 (↓7%)	26.1* ± 0.97 (↓6%)
Males – Recovery Group						
0	150 ± 5.8	16.3 ± 0.75	319 ± 3.5	15 ± 3.6	1327 ± 85.3	30.9 ± 3.38
2000	150 ± 3.0	16.2 ± 0.64	312** ± 4.0 (↓2%)	15 ± 2.0	1326 ± 146.7	32.7 ± 1.94
Females – Recovery Group						
0	150 ± 4.6	17.4 ± 0.37	318 ± 3.7	16 ± 2.6	1385 ± 144.2	29.9 ± 2.37
2000	148 ± 3.9	17.2 ± 0.50	317 ± 4.4	16 ± 3.4	1335 ± 140.7	29.4 ± 1.67

Data obtained from pages 57-58 and 254-258; historical control values obtained from pages 97-98. ( ) = % difference from concurrent controls, calculated by the reviewer. * Significantly different from controls at $p \leq 0.05$; ** Significantly different from controls at $p \leq 0.01$.

6. Clinical chemistry

Clinical-chemistry investigations revealed statistically significantly decreased activities of alanine aminotransferase (ALAT) in peripheral blood of males and females at 500 ppm and 2000 ppm which were outside of the historical ranges (Table 6). Statistical significance was attained also in males treated at 60 ppm however this change was regarded not to be of toxicological relevance as all individual values were in the range of 2 standard deviations of historical control values and no dose-response was noted. At the end of the recovery period, ALAT levels were slightly increased compared to controls; however, these differences were not statistically significant (Table 7).

The concentration of urea was statistically significantly increased at 2000 ppm in males in the main group; although within the historical range, the increase is considered to be related to treatment. No significant difference was noted at the end of the recovery period.

In females of the main group, cholesterol was increased at 500 and 2000 ppm, attaining statistical significance at 2000 ppm, triglycerides were increased in all dose groups (statistically significant at 500 and 2000 ppm), and creatinine was statistically significantly decreased at 2000 ppm. No significant differences were noted in triglycerides or cholesterol in recovery group females. At the end of the recovery period a statistically significant decrease in cholesterol and triglycerides in males, and in creatinine, total bilirubin, and protein in females was observed; albumin/globulin ratio (A/G) was significantly increased in males.

At 2000 ppm the concentration of calcium was slightly but statistically significantly increased in males of the main group and the concentration of phosphate was statistically significantly increased in females of the main group. No significant differences were observed in these parameters at the end of the recovery period.

Table 6: Clinical chemistry endpoints – Main groups

Parameter	Historical Controls	Control (n = 10)	60 ppm (n = 10)	120 ppm (n = 10)	500 ppm (n = 10)	2000 ppm (n = 10)
Males						
ALAT (U/L)	32.5 ± 6.24 (n = 79)	36.0 ± 4.59	30.4* ± 3.58 (↓16%)	32.4 ± 5.57 (↓10%)	25.5** ± 5.08 (↓29%)	15.2** ± 3.19 (↓58%)
Cholesterol (mmol/L)	2.16 ±0.472 (n = 86)	2.13 ± 0.496	2.16 ± 0.497	2.44± 0.637	2.68 ± 0.885	2.41 ± 0.463
Trigylcerides (mmol/L)	0.75±0.270 (n = 80)	1.07± 0.492	1.16 ± 0.339	1.21± 0.421	1.35 ± 0.804	1.21 ± 0.404
Creatinine (µmol/L)	50 ± 3.6 (n = 89)	47 ± 3.5	47 ± 3.0	47 ± 2.6	48 ± 3.6	47 ± 2.5
Urea (mmol/L)	6.06±0.931 (n =89)	4.98± 0.415	5.55 ± 0.792	5.49± 0.781	5.69 ± 0.670	6.06**±0.767 (↑22%)
Bilirubin (µmol/L)	1.5 ± 0.33 (n = 88)	1.5 ± 0.30	1.3 ± 0.29	1.4 ± 0.18	1.3 ± 0.24	1.5 ± 0.20
Protein (g/L)	65.8 ± 2.66 (n = 88)	64.5 ± 2.39	64.2 ± 2.57	63.2 ± 2.66	64.8 ± 2.92	67.3 ± 1.97
A/G	0.74±0.039 (n = 10)	1.12± 0.095	1.07 ± 0.089	1.10± 0.048	1.07 ± 0.108	1.09 ± 0.062
Calcium (mmol/l)	2.47±0.063 (n = 88)	2.50 ± 0.042	2.51 ± 0.037	2.51± 0.050	2.48 ± 0.100	2.58**±0.049 (↑3%)
Phosphate (mmol/l)	2.04±0.191 (n = 85)	2.15± 0.179	2.14 ± 0.136	2.11± 0.082	2.08 ± 0.130	2.30 ± 0.130
Females						
ALAT (U/L)	30.4 ± 8.93 (n = 78)	30.0 ± 3.42	30.2 ± 5.22	31.1 ± 5.78	23.6** ± 3.04 (↓21%)	15.0 **± 3.45 (↓50%)
Cholesterol (mmol/L)	2.00±0.359 (n = 89)	1.89± 0.474	2.11 ± 0.359	1.84 ± 0.272	2.37 ± 0.586 (↑25%)	2.81**±0.446 (↑49%)
Trigylcerides (mmol/L)	0.48±0.111 (n = 83)	0.48± 0.109	0.62 ± 0.154 (↑29%)	0.70 ± 0.235 (↑46%)	0.83**±0.304 (↑73%)	0.79**±0.144 (↑65%)
Creatinine (µmol/L)	52 ± 3.4 (n = 68)	52 ± 3.9	51 ± 3.5	51 ± 3.9	53 ± 3.2	48** ± 2.0 (↓8%)
Urea (mmol/L)	6.63±1.297 (n = 89)	5.77± 0.604	6.15 ± 0.796	5.90± 0.804	6.46 ± 0.963	6.06 ± 0.748

Parameter	Historical Controls	Control (n = 10)	60 ppm (n = 10)	120 ppm (n = 10)	500 ppm (n = 10)	2000 ppm (n = 10)
Total Bilirubin (µmol/L)	1.7 ± 0.34 (n = 89)	1.9 ± 0.23	1.8 ± 0.43	1.9 ± 0.19	1.7 ± 0.32	2.2 ± 0.53
Protein (g/L)	67.3 ± 3.13 (n = 77)	66.5 ± 5.22	64.4 ± 2.59	66.6 ± 3.08	66.4 ± 2.72	65.2 ± 2.65
A/G	0.91±0.063 (n = 10)	1.20± 0.083	1.16 ± 0.064	1.23± 0.069	1.19 ± 0.070	1.18 ± 0.050
Calcium (mmol/l)	2.43±0.057 (n = 77)	2.48± 0.074	2.45 ± 0.090	2.46± 0.099	2.44 ± 0.083	2.54 ± 0.077
Phosphate (mmol/l)	1.56±0.308 (n = 84)	1.62± 0.149	1.63 ± 0.170	1.62± 0.191	1.56 ± 0.345	2.01**±0.248 (↑24%)

Data obtained from pages 61-63 and pages 278-290 of the study report. ( ) = % difference from concurrent controls, calculated by the reviewer. * $p \leq 0.05$, ** $p \leq 0.01$

Table 7: Clinical chemistry endpoints – recovery animals

Parameter	Control (n = 10)	2000 ppm (n = 10)
Males		
ALAT (U/l)	36.5 ± 7.09	38.7 ± 9.60
Cholesterol (mmol/L)	2.98 ± 1.409	1.81* ± 0.265 (↓39%)
Trigylcerides (mmol/L)	1.65 ± 0.813	0.93* ± 0.169 (↓44%)
Creatinine (µmol/L)	48 ± 3.8	48 ± 2.8
Urea (mmol/L)	6.44 ± 1.446	5.68 ± 0.849
Bilirubin (µmol/L)	1.8 ± 0.29	1.9 ± 0.39
Protein (g/L)	67.9 ± 2.93	66.2 ± 2.63
Albumin/globulin (g/l)	0.93 ± 0.091	1.06** ± 0.056 (↑14%)
Calcium (mmol/l)	2.46 ± 0.070	2.43 ± 0.052
Phosphorus (mmol/l)	1.80 ± 0.188	1.95 ± 0.177
Females		
Parameter	**Control (n = 10)**	**2000 ppm (n = 10)**
ALAT (U/l)	37.2 ± 7.47	41.2 ± 18.32
Cholesterol (mmol/L)	2.00 ± 0.362	1.87 ± 0.266
Trigylcerides (mmol/L)	0.54 ± 0.064	0.59 ± 0.113
Creatinine (µmol/L)	54 ± 4.3	50* ± 3.6 (↓7%)
Urea (mmol/L)	5.97 ± 0.807	6.30 ± 0.571
Bilirubin (µmol/L)	2.5 ± 0.51	2.1* ± 0.23 (↓16%)
Protein (g/L)	68.8 ± 1.94	66.8* ± 2.21 (↓3%)
Albumin/globulin (g/l)	1.15 ± 0.059	1.18 ± 0.074
Calcium (mmol/l)	2.38 ± 0.066	2.35 ± 0.039
Phosphorus (mmol/l)	1.36 ± 0.170	1.41 ± 0.202

Data obtained from pages 61-63 and pages 278-290 of the study report. ( ) = % different from controls, calculated by the reviewer. * $p \leq 0.05$; ** $p \leq 0.01$

7. Urinalysis

No statistically significant effects were noted at the end of the treatment period. Decreases in urinary protein concentration and protein excretion (volume) were noted in high dose males of the main group (Table 8a). In high dose females, increased urine volume was noted in both the main and recovery groups, and decreased protein concentration was noted in the recovery group (Table 8a and 8b).

Large decreases in protein concentration and excretion were noted in males of the recovery group although, according to the study report, two animals in the control recovery groups had higher values as compared to other control animals (and historical controls) thus resulting in relatively high mean control values for protein concentration and excretion. The differences in protein concentration and excretion in recovery males compared to controls were 260% and 72%, respectively. However, if the outliers are left out of the calculations for the means for the controls, levels for the treatment groups would still be 56% and 33% different from controls, respectively, for urinary protein and protein excretion (Table 8b).

Table 8a: Urinalysis – Main group

Parameter	Historical Control	0 (n = 10)	60 (n = 10)	120 (n = 10)	500 (n = 10)	2000 (n = 10)
Males						
Vol (ml)	10.3 ± 4.86 (n = 178)	11.4 ± 4.62	12.4 ± 3.96	14.3 ± 3.32	11.6 ± 3.59	11.5 ± 2.89
Density (g/l)	1021 ± 8.2 (n =148)	1023 ± 7.3	1020 ± 5.1	1018 ± 3.7	1022 ± 8.1	1022 ± 7.9
Prot (g/l)	1.43± 1.561 (n =97)	1.91± 1.082	1.95±2.051	1.94±2.302	3.75±3.177	1.55± 0.519 (↓19%)
Prot*Vol (excretion) (mg)	12.0 ± 9.98 (n =97)	22.1± 14.49	20.8±18.76	26.7±31.77	44.2±38.56	17.1±5.14 (↓23%)
Females						
Vol (ml)	6.1 ± 2.73 (n = 160)	8.7 ± 2.59	10.2 ± 4.00	8.7 ± 2.62	8.1 ± 4.74	14.7 ± 9.19 (↑69%)
Density (g/l)	1022 ± 8.6 (n =139)	1018 ± 7.3	1018 ± 9.9	1020 ± 6.0	1024 ± 11.1	1013 ± 10.3
Prot (g/l)	0.25± 0.148 (n =89)	0.16± 0.043	0.20 ±0.120	0.35 ±0.451	0.19 ±0.067	0.15 ±0.073
Prot*Vol (excretion) (mg)	1.4 ± 0.68 (n =89)	1.3 ± 0 .13	1.8 ± 0.94	2.6 ± 2.72	1.4 ± 0.77	1.9 ± 1.25

Data obtained from pages 64-65 and 314-317of the study report. ( ) = % difference from controls, calculated by the reviewer. * $p \leq 0.05$; ** $p \leq 0.01$

Table 8b: Urinalysis – Recovery group

Parameter	0 (n = 10)	2000 ppm (n = 10)
Males		
Vol (ml)	10.0 ± 6.93	10.1 ± 4.52
Density (g/l)	1033 ± 16.2	1025 ± 11.7
Prot (g/l)	6.15 ± 8.204 (value without outliers: 3.9 ± 6.312)	1.71*± 1.119 (↓260%) (compared to controls without outliers: ↓56%)
Prot*Vol (excretion) (mg)	52.4 ± 73.10 (value without outliers: 22.6 ± 19.341)	15.1 ± 9.26 (↓72%) (compared to controls without outliers: ↓33%)
Females		
Vol (ml)	7.4 ± 3.67	11.2 ± 7.28 (↑51%)
Density (g/l)	1024 ± 9.9	1017* ± 8.1 (↓0.7%)
Prot (g/l)	0.21 ± 0.155	0.15 ± 0.138 (↓29%)
Prot*Vol (excretion) (mg)	1.3 ± 0.42	1.2 ± 0.51

Data obtained from pages 64-65 and 314-317of the study report. ( ) = % difference from controls, calculated by the reviewer. * $p \leq 0.05$; ** $p \leq 0.01$

8. Ophthalmology

There were no treatment-related abnormalities observed in the eyes of high dose animals at study termination.

9. Terminal examinations

a. Macroscopic examination

At the end of the treatment period changes on the surface of kidneys (5, compared to none in the control animals and other dose groups) and discoloration of teeth (6, compared to none in the control and other dose groups) were noted in males at 2000 ppm. At the end of the recovery period, fracture of an incisor was observed in two males previously treated at 2000 ppm.

b. Organ weights

Mean terminal body weight was statistically significantly reduced, compared to controls, in males at 2000 ppm at the end of the treatment period, as well as at the end of the recovery period while slight (non statistical) reductions were also observed in females after treatment and after recovery at 2000 ppm (Table 9). In the main groups, relative weights of livers were significantly increased in both sexes at 2000 ppm. The difference, compared to the control, was 17% for males and 9% for females. Absolute liver weight was increased by 7% over controls in males only. Relative weights of kidneys were statistically significantly increased in both sexes at 2000 ppm. The difference compared to the control was 21% for males and 14% for females. Absolute kidney weight was increased 9% over controls in males only. At the end of the recovery period, absolute liver and kidney weights were statistically significantly decreased in males (14% and 16%, respectively), and differences in relative organ weights were no longer evident.

Statistically significant decreases in absolute thymus (21%), heart (18%) and spleen (19%) weights in males, as well as the increases in relative brain (4%, males), testes (21%) and uterus (24%) weights recorded at the end of the recovery period were considered related to the lower body weight of the previously treated animals, as supported by the lack of any treatment-related findings in these organs at histopathology.

Table 9: Mean absolute (mg) and relative (mg/100g) organ weights (n=10)

Organ	0 ppm	60 ppm	120 ppm	500 ppm	2000 ppm
Males – Main group					
Body Weight	439 ± 28	445 ± 29	460 ± 39	409 ± 44	397 * ± 14 (↓10%)
Abs. Liver	16383 ± 1847	16549 ±2156	16874 ±2064	16573 ± 2954	17364 ± 1000 (↑7%)
Rel. Liver	3732 ± 317	3711 ± 357	3667 ± 285	4040 ± 432	4371*± 193 (↑17%)
Abs. Kidney	2601 ± 273	2613 ±174	2718 ± 348	2573 ± 451	2857 ± 312 (↑9%)
Rel. Kidney	593 ± 52	589 ± 51	590 ± 43	633 ± 108	718**± 68 (↑21%)
Females – Main group					
Body Weight	245 ± 14	255 ± 29	235 ± 15	235 ± 13	223 ± 26 (↓9%)
Abs. Liver	8763 ± 482	8944±1148	8808 ± 742	8497 ± 632	8688 ± 1147
Rel. Liver	3581 ± 216	3504 ± 201	3754 ± 234	3611 ± 193	3890*± 294 (↑9%)
Abs. Kidney	1506 ± 154	1602 ± 229	1536 ± 126	1554 ± 179	1561 ± 192
Rel. Kidney	614 ± 47	626 ± 28	656 ± 56	660 ± 65	700**± 51 (↑14%)
Males – Recovery group					
Body Weight	474 ± 41				402** ± 28 (↓15%)
Abs. Liver	18384± 3260				15773*± 2109 (↓14%)
Rel. Liver	3867 ± 504				3914 ± 324
Abs. Kidney	2860 ± 484				2391* ± 230 (↓16%)
Rel. Kidney	603 ± 85				595 ±37
Females – Recovery group					
Body Weight	237 ± 18				220* ± 15 (↓7%)
Abs. Liver	8381 ± 490				7886 ± 630
Rel. Liver	3543 ± 192				3595 ± 217
Abs. Kidney	1526 ± 125				1468 ± 123
Rel. Kidney	645 ± 46				671 ± 67

Data obtained from pages 73-73 and 341-360 of the study report. ( ) = % different from controls, calculated by the reviewer; Rel. = relative; Abs. = absolute

c. Histopathology

Histopathology revealed treatment-related changes in the kidneys, liver, forestomach, pharynx and adrenal glands (Table 10).

In the kidneys of males findings typical for the histopathological picture of alpha-2-microglobulin nephropathy (increased severity of basophilic cortical tubules, sclerotic cortical tubules partially with thickening of Bowman's capsule of the glomeruli of the affected nephrons and Schmorl-positive material, increased severity of hyaline droplet accumulation in proximal tubules, cystic tubules containing cellular debris at the corticomedullary junction, mononuclear cell infiltrates) were observed in males at 500 ppm and/or 2000 ppm. Immunohistochemical staining for alpha-2-microglobulin with Azan stain revealed an immune reaction that was stronger and more widely distributed throughout the renal cortex in animals dosed at 2000 ppm compared to controls in the animals dosed for 90 days. In the satellite group of rats dosed for one week, no differences between control and 2000 ppm males were noted using the Azan stain, and the reaction was slightly more intense in control than in treated males.

In females, a slightly increased degree of Schmorl-positive material (Lipofuscin pigment) and increased incidences of pigment deposits were seen in renal tubules at 2000 ppm.

In the liver, minimal to slight (males) or minimal (females) hepatocellular hypertrophy was recorded at 2000 ppm.

In the forestomach, basal cell hyperplasia was recorded in both sexes at 500 ppm and above.

In the pharynx an increased incidence of mononuclear infiltrates was recorded at 500 and 2000 ppm in males and in 2000 ppm females. The degree was minimal in all affected animals.

In the adrenal glands, an increased incidence of cortical vacuolation of the zona fasciculata was seen in males at 2000 ppm.

In the teeth and investigated bones no abnormalities were recorded.

At the end of the recovery period, hyaline droplet accumulation in the kidneys of males was minimal or slight and similar to negative controls and was thus reversible. The tubular changes (increased basophilia, sclerosis, pigment, cystic tubules containing debris at the corticomedullary junction) and increased number of mononuclear cell infiltrates in the kidneys were still discernible in 2000 ppm males. Changes observed in the forestomach had regressed in terms of grade and incidence by the recovery period; however, hyperplasia was still observed in some treated males and females. Incidences of mononuclear infiltrates in the pharynx were also still observed in treated males (incidence in treated females was the same as controls). The minimal cortical vacuolation of the adrenal gland noted in two males in the recovery group were considered to be in the range of normal variability and thus this finding was considered to be somewhat reversible. Pigment in the kidneys of females, and liver cell hypertrophy in males and females were also reversible.

Table 10a: Notable findings observed during histopathological examination - Males

Finding	Main Groups					Recovery	
	0	60	120	500	2000	0	2000
KIDNEYS, # examined	10	10	10	10	10	10	10
Basophilic Cortical Tubules	8	9	9	10	10	10	9
Grade 1	5	7	5	4	1	6	-
Grade 2	3	2	4	5	-	2	5
Grade 3	-	-	-	1	9	2	4
Tubulosclerosis	-	-	-	2	10	2	9
Grade 1	-	-	-	1	1	2	5
Grade 2	-	-	-	1	9	-	4
Schmorl-Positive Material in Tubules	4	2	1	1	7	2	7
Grade 1	4	2	1	1	-	2	7
Grade 2	-	-	-	-	7	-	-
Hyaline Droplets	10	10	10	10	10	7	10
Grade 1	6	6	4	-	-	6	6
Grade 2	4	4	5	5	-	1	4
Grade 3	-	-	1	5	2	-	-
Grade 4	-	-	-	-	8	-	-
Cystic Tubules (corticomedullary junction)	-	-	-	-	7	-	5
Mononuclear Infiltrates	3	4	1	5	10	5	9
Grade 1	3	4	1	4	9	5	8
Grade 2	-	-	-	1	-	-	1
LIVER, # examined	10	10	10	10	10	10	10
Hypertrophy of Hepatocytes	-	-	-	-	10	-	-
Grade 1	-	-	-	-	6	-	-
Grade 2	-	-	-	-	4	-	-
STOMACH, # examined	10	10	10	10	10	10	10
Hyperplasia, forestomach	-	1	-	4	8	-	3
PHARYNX, # examined	10	10	10	10	10	10	10
Mononuclear infiltrates, Oropharynx Grade 1	2	2	1	4	6	-	4
	2	2	1	4	6	-	4
ADRENAL GLANDS, # examined	10	10	10	10	10	10	10
↑ cortical vacuolation	1	1	2	2	7	-	2

Data obtained from pages 76-77 and 615-634 of the study report.

Table 10b: Notable findings observed during histopathological examination - Females

Finding	Main Groups					Recovery	
	0	60	120	500	2000	0	2000
KIDNEYS, # examined	10	10	10	10	10	10	10
Schmorl-Positive Material in Tubules	10	10	10	10	10	8	8
Grade 1	8	8	8	6	-	5	8
Grade 2	2	2	2	4	9	3	-
Grade 3	-	-	-	-	1	-	-
Pigment Deposits	-	-	-	-	6	-	-
Grade 1	-	-	-	-	6	-	-
LIVER, # examined	10	10	10	10	10	10	10
Hypertrophy of Hepatocytes	-	-	-	-	6	-	-
Grade 1	-	-	-	-	6	-	-
STOMACH, # examined	10	10	10	10	10	10	10
Hyperplasia, forestomach	-	-	-	4	10	-	2
PHARYNX, # examined	10	10	10	10	10	10	10
Mononuclear infiltrates, Oropharynx	3	4	2	2	5	2	2
Grade 1	3	4	2	2	5	2	2

Data obtained from pages 76-77 and 615-634 of the study report.

d. Enzyme activity in homogenized liver tissue

The concentration of cytochrome P-450 was slightly, but statistically significantly increased at 2000 ppm in females (Table 11). The activity of O-demethylase (O-Dem) was statistically significantly decreased in males at 2000 ppm and in females at 500 ppm and above. The activity of N-demethylase (N-Dem) was statistically significantly decreased in males at 120 ppm and above, and in females at 500 ppm and above. At the end of the recovery period no statistically significant differences from the control values occurred in these parameters. The activity of N-Dem was slightly increased in recovery animals (males and females.)

Activity of alanine aminotransferase (ALAT) in liver tissue was significantly decreased at 2000 ppm in both in males and in females. At the end of the recovery period this activity was statistically significantly increased in males and females: this was considered to be possibly due to an over-expression after the long inhibition of this enzyme activity during the treatment period.

Table 11: Determinations in liver tissue – Main and Recovery groups (n = 10)

Dose (ppm)	O-Dem mU/g	N-Dem mU/g	P450 nmol/g	ALAT U/g
Males – Main Group				
0	10.7 ±1.07	114.2 ± 15.45	44.4 ± 2.96	101.4 ± 10.94
60	11.0 ± 1.17	111.1 ± 16.56	39.9 ± 5.08	91.4 ± 12.58
120	10.2 ± 1.57	90.8* ± 19.30 (↓20%)	39.3* ± 1.95	106.6 ± 15.76
500	10.1 ± 1.17	93.9* ± 16.52 (↓18%)	40.7 ± 5.41	102.2 ± 10.87
2000	8.4** ± 1.49 (↓22%)	78.5** ± 17.04 (↓31%)	44.3 ± 4.74	77.1** ± 10.08 (↓24%)
Females – Main Group				
0	10.4 ± 1.01	55.7 ± 6.98	40.0 ± 4.04	82.1 ± 10.10
60	9.4 ± 1.72	49.2 ± 7.14	41.6 ± 3.40	76.3 ± 21.25
120	9.8 ± 1.17	50.9 ± 12.47	41.0 ± 2.91	82.1 ± 9.87
500	7.9** ± 1.20 (↓24%)	46.6* ± 6.70 (↓16%)	42.5 ± 4.39	75.8 ± 9.79
2000	6.2** ± 1.04 (↓40%)	41.8** ± 5.48 (↓25%)	45.2*± 2.77 (↑13%)	54.0 **± 7.76 (↓34%)
Males – Recovery Group				
0	11.6 ± 2.19	98.8 ± 21.61	34.4 ± 3.67	100.0 ± 13.58
2000	10.8 ± 1.76	109.8 ± 23.02 (↑11%)	36.5 ± 3.60	114.6* ± 16.10 (↑15%)
Females – Recovery Group				
0	11.4 ± 0.82	48.9 ± 9.50	29.0 ± 2.98	81.1 ± 9.81
2000	11.8 ± 0.70	54.3 ± 6.55 (↑11%)	31.5 ± 3.07	100.9** ± 8.35 (↑24%)

Data obtained from pages 67 and 135-139 or the study report. ( ) = % different from controls, calculated by the reviewer. * $p \leq 0.05$; ** $p \leq 0.01$

e. Determination of fluoride in ashes from bones and teeth

The content of fluoride in ashes from bones and teeth of treated male and female rats was dose-dependently increased (Tables 12 and 13). Despite statistical significance only being attained at 120 ppm and above in both sexes, the magnitude of the increases of fluoride levels in all treated groups compared to controls was large enough to consider a treatment-related effect down to the lowest dose tested. At the end of the recovery period, fluoride concentrations were still significantly increased in ashes from bones and teeth of previously treated male and female rats compared to the corresponding controls, although levels had started to decline somewhat in males and females treated with 2000 ppm, indicating a possible reversibility of this effect may be occurring.

Table 12: Fluoride content in ashes from femurs (mg fluoride/g ash)

Control	60 ppm	120 ppm	500 ppm	2000 ppm
Males – Main Group				
0.29 ± 0.015	0.60 ± 0.026 (↑107%)	0.89* ± 0.053 (↑207%)	2.32* ± 0.127 (↑700%)	5.66* ± 0.363 (↑1852%)
Males – Recovery Group				
0.31 ± 0.021				4.54* ± 0.342 (↑1365%)
Females – Main Group				
0.37 ± 0.029	0.68 ± 0.041 (↑84%)	0.95* ± 0.058 (↑157%)	2.17* ± 0.148 (↑487%)	4.22* ± 0.273 (↑1041%)
Females – Recovery Group				
0.40 ± 0.026				3.63* ± 0.306 (↑808%)

Data obtained from page 68 and 149-154 of the study report. Standard deviations were calculated by the PMRA reviewer. ( ) = % different from controls, calculated by the reviewer. * $p < 0.05$.

Table 13: Fluoride content in ashes from teeth (mg fluoride/g ash)

Control	60 ppm	120 ppm	500 ppm	2000 ppm
Males – Main Group				
0.09 ± 0.018	0.20 ± 0.013 (↑122%)	0.30* ± 0.013 (↑233%)	0.82* ± 0.055 (↑811%)	2.71* ± 0.460 (↑2911%)
Males – Recovery Group				
0.09 ± 0.020				1.15 * ± 0.207 (↑1178%)
Females – Main Group				
0.10 ± 0.023	0.24 ± 0.040 (↑140%)	0.31* ± 0.032 (↑210%)	0.80* ± 0.055 (↑700%)	1.87* ± 0.162 (↑1770%)
Females – Recovery Group				
0.11 ± 0.011				0.95* ± 0.209 (↑764%)

Data obtained from pages 68 and 149-154 of the study report. ( ) = % different from control, calculated by the reviewer. * $p < 0.05$

III. CONCLUSIONS

Treatment of rats with fluensulfone for 3 consecutive months was well tolerated up to 2000 ppm (approximately 139 mg/kg bw/day in males and 149 mg/kg bw/day in females), with no mortality. Body weight/body weight gain was affected by treatment in both male and female animals receiving 2000 ppm, and marginally in males treated at 500 ppm. Food consumption was decreased only in females given 2000 ppm while water intake was increased in males at 500 ppm and in animals of both sexes at 2000 ppm. Recovery animals previously treated at 2000 ppm also showed increased water intakes compared to the control values.

Hematology showed some changes in animals treated at 2000 ppm, most of which recovered in animals remaining untreated for 4 weeks. In females the HQuick (thromboplastin time) was significantly decreased at 2000 ppm. In the 2000 ppm dose groups, statistically significant decreases were observed in hemoglobin, MCH, and MCHC in males, and in hemoglobin and MCHC levels in females. Reticulocyte counts were increased in high dose females. Despite the low magnitude of the decreases in MCHC, the changes were considered to be treatment-related due to the statistical significance and because such decreases have been observed in another long term rat study, as well as studies in other species dosed with fluensulfone.

Clinical chemistry investigations gave evidence for effects on liver function in males at 120 ppm and above, and in females at 500 ppm and above. Concentrations of urea were significantly increased at 2000 ppm in males. In females, triglycerides were increased at all doses, cholesterol was increased at 500 and 2000 ppm, and creatinine was decreased at 2000 ppm.

Adaptive changes of the liver were also noted in animals treated at 500 and 2000 ppm at the end of the treatment period and these recovered after the recovery period. Alterations in clinical chemistry parameters, organ weight and histopathological findings were noted in the kidneys of animals treated at 2000 ppm and in males at 500 ppm. All findings were reversible in females. Some of the findings in males were related to accumulation of alpha-2-µ-globulin and were partially reversible after the recovery period. Kidney effects still present in recovery males included basophilic cortical tubules, tubulosclerosis, Schmorl-positive material in the kidney, cystic tubules, and mononuclear infiltrates of the kidney.

Urinalysis revealed decreased urine protein concentration and decreased urine protein excretion in high dose males. Decreased urine protein concentration was noted in both male and female recovery animals, and decreased urine protein excretion was still observed in recovery males. Increased urine volume was observed in high dose females of the main group, as well as in recovery animals. Some of these findings may have been due to increased water intake in mid and high dose males and in high dose females, and in recovery animals.

In the forestomach a diffuse basal cell hyperplasia was observed in both sexes at 500 ppm and 2000 ppm. This finding could have been related to a possible local irritating effect of the substance. At the end of recovery period, this change had regressed in terms of severity and incidence but was still detectable in a few animals. The toxicological relevance of forestomach hyperplasia is unclear since humans have no forestomach.

Observations of increased incidence of mononuclear infiltrates in the pharynx in mid and high dose males and in high dose females, normalized for 500 ppm males and for 2000 ppm females during the recovery period. Findings in the adrenal glands of males treated at 2000 ppm also normalized during the recovery period.

At necropsy at the end of the treatment period discoloration of teeth was observed in 6/10 males at 2000 ppm, but no corresponding histopathological changes were observed. Treatment with the test substance increased the content of fluoride in ashes from bones and teeth of treated male and female rats in a dose-related manner. Statistically significant increases in fluoride concentrations were observed at 120 ppm and above. At the end of the recovery period, fluoride concentrations, although still significantly increased in ashes from bones and teeth of male and female rats compared to the corresponding controls, had started to decline in males and females previously treated with 2000 ppm indicating possible reversibility of this effect. Histopathological examinations revealed no abnormalities in teeth and investigated bones.

The authors of the study established the NOAEL at 60 ppm (equivalent to 4.3 mg/kg bw/day for males and 4.8 mg/kg bw/day for females). It is not clear from the study report if this conclusion was based on the statistically significant increased content of fluoride in ashes from bones and teeth of rats treated at 120 ppm and above. The measured accumulation of fluoride in ashes from bones and teeth is not necessarily, in itself, an adverse effect. The visible discoloration of the teeth (possibly associated with accumulation of fluoride) was only observed at 2000 ppm (the highest dose tested). Therefore, it is concluded that there is no apparent dental fluorosis in this study at levels below 2000 and certainly no severe dental fluorosis at any dose level.

As only adaptive effects on the liver were observed at 120 ppm, this dose level, equivalent to 8.26 mg/kg bw/day for males and 11.68 mg/kg bw/day for females, is considered to be the NOAEL of the study. The dose of 500 ppm in the diet (34.86 and 53.10 mg/kg bw/day in males and females, respectively) is considered to be the LOAEL based on the numerous effects in the kidneys, observations in the pharynx and forestomach, increased cholesterol in females, and decreased body weight gain in males at this dose

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study in is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for a repeated dose 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rats.

C. Conclusions: The reviewer is in agreement with the overall conclusions drawn by the applicant in the Tier II summary except for identification of 120 ppm as a LOAEL. The LOAEL is considered to be 500 ppm (34.86 mg/kg bw/day for males and 53.10 mg/kg bw/day for females), based on effects in the kidneys, observations in the pharynx and forestomach, increased cholesterol in females, and a marginally decreased body weight and body weight gain in males. The NOAEL for this study is set at 120 ppm (8.26 mg/kg bw/day for males and 11.68 mg/kg bw/day for females).

D. Deficiencies: The standard deviations for some parameters were not provided in the study report. This omission does not affect the conclusions of the study.

Tier II Summary (PMRA #2181207, MRID 48574755) revised by the regulatory authorities, April 2013

Study Type: Oral 90-day toxicity (dog)

IIA 5.3.3 - MRID 48574755, PMRA 2181207

Report:	Braun L. (2009) MCW-2 TECH 90-Day Oral (Feeding) Toxicity Study in the Beagle Dog. Harlan Laboratories Ltd., Switzerland; unpublished report No. C15552, dated 26 October 2009. Sponsor reference No. R-23350 Dates of experimental work: 28 August 2008 to 21 October 2009
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD No. 409, OPPTS 870.3150
Deviations:	On a few occasions the temperature and relative humidity of the animal rooms were out of the target range which was likely due to the cleaning procedures. These minor deviations had no impact on the outcome of the study.
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary:

In a dietary study (MRID 48574755, PMRA 2181207), groups of 4 male and 4 female Beagle dogs received fluensulfone (MCW-2 tech., 96.67-96.78%, Batch No. 36372130-291-PF1) in their diets at 0, 5, 50 or 500 ppm (0/0, 0.2/02, 1.6/1.8 and 17.1/18.0 mg/kg bw/day in males/females) for 13 consecutive weeks. Similar sized recovery groups for the control and the high dose were treated for 13 consecutive weeks and sacrificed after an additional 28-day period without treatment.

There were no deaths or clinical signs related to treatment. At 500 ppm, body weight gains were reduced by 20% to 55% compared to controls in both sexes, while reductions in body weight of up to 17% were noted in females. Food consumption was reduced in females at 50 and 500 ppm. Decreased plasma and liver ALAT activity were observed at 500 ppm as well as increased liver weight, Kupffer cell pigmentation in two males and one female, and an isolated incidence of hepatocellular hypertrophy in one female suggesting that the liver is a target organ. Both sexes dosed with the 500 ppm test diet exhibited increases in reticulocytes and decreases in bilirubin, albumin, and albumin/globulin ratios. Thyroid stimulating hormone levels were also elevated in both sexes from the 500 ppm dose group. Other effects at 500 ppm included reduced hemoglobin and protein levels in males, and decreased glucose levels and thymus weights in females.

Indications of phase I enzyme induction were seen at 50 and 500 ppm in females for 6-beta testosterone hydroxylase and of phase II enzymes at 500 ppm only for microsomal epoxide hydrolase. The results exclude a potent effect on liver enzymes by fluensulfone as the changes were considered biologically not relevant and likely to be a response of the constant load on the liver with a xenobiotic.

Other findings at 50 ppm included a slight decrease in plasma and hepatic ALAT activity in both sexes, a slight and transient decrease in bilirubin in males, and slightly reduced food consumption

in females. At 5 ppm, there was a slight reduction in hepatic ALAT activity in females only.

All the effects observed at 5 and 50 ppm were slight and reversible, and considered unlikely to represent an adverse effect; therefore, the NOAEL was considered to be 50 ppm (equivalent to an achieved intake of 1.6 mg/kg/day in males and 1.8 mg/kg bw/day in females). The LOAEL is 500 ppm (equivalent to an achieved intake of 17.1 mg/kg bw/day in males and 18.0 mg/kg bw/day in females) based on decreased plasma and liver ALAT, decreased body weight gains and decreased body weight in females, liver effects, hematological findings, altered clinical chemistry parameters, and increased TSH levels.

This 90-day oral toxicity study in the dog is classified as **acceptable/guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OECD 409; OPPTS 870.3150) in dogs.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	MCW-2 TECH (Fluensulfone)
Description:	Yellow solid
Batch No.:	36372130-291-PF1
Purity:	96.67% - 96.78% (determined by gas chromatography assays at the two different dates)
CAS#:	318290-98-1
Assay dates:	30 November 2008 & 30 May 2009
Stability of Test Item:	Stable throughout the duration of the study
2. Vehicle:	Pelleted standard Kliba 3353 dog maintenance diet (Provimi Kliba AG, Switzerland), batch nos. 36/08, 41/08, 59/08, and 71/08.
3. Test animals	
Species:	Dog
Strain:	Pure-bred Beagle
Age:	5.5 to 6.5 months
Weight at dosing:	Range: 6.6 to 10.7 kg
Source:	Harlan Laboratories Ltd., Switzerland
Acclimation period:	Approx. 2 weeks
Diet:	350 ± 1 g per day and per dog of pelleted standard diet (Provimi Kliba 3353 dig maintenance diet, Provimi Kliba AG, Switzerland) was presented to all animals at the same time daily. Any remaining diet was withdrawn approximately four hours after feeding.

Water: Domestic tap water was supplied *ad libitum* by an automatic watering system.

Housing: Individual or group housing in kennels with minimum of 2.0 square meters floor space per dog. Dogs were separated during feeding periods or at the discretion of the study director to facilitate recording of clinical signs or performance of special investigations. The animals in each treatment group were housed in adjacent kennels. Kennels were supplied with autoclaved standard softwood bedding, changed at least once a week.

4. Environmental conditions

Temperature: 22 ± 3°C. On a few occasions, the temperature was recorded at 17-18°C. These deviations had no impact on the outcome of the study.

Humidity: 30-70%. On a few occasions, the relative humidity exceeded 70%, probably due to the cleaning procedures. These deviations had no impact on the outcome of the study.

Air changes: Approx. 10-15 per hour

Photoperiod: 12-hour fluorescent light / 12-hour dark cycle. Music was played during the major part of the light period.

B. STUDY DESIGN

1. In life dates: 28 August 2008 (delivery of the animals) to 15 January 2009

2. Animal assignment and treatment:

Shortly after arrival the dogs were temporarily allocated to groups by a computer generated random algorithm; the spare animals were allocated to group 5 and assigned numbers 49 and 50. Thereafter, pre-treatment investigations were carried out on all dogs. Shortly before the start of treatment, the data obtained were reviewed. The allocation was adjusted using the spare female dog due to low body weight in one female assigned to the control group. The rejected animals did not take further part in the study after commencement of treatment.

The pre-test period lasted one week. Thereafter dogs were treated for 13 consecutive weeks at dietary dose levels of 0, 5, 50 or 500 ppm. All males and females of the main groups were sacrificed on day 92 and day 93, respectively. The recovery animals (0 and 500 ppm dose groups) were sacrificed after an additional 28-day period without treatment.

3. Dose Selection Rationale

Doses were selected based on the results of a 28-day dietary study in dogs with fluensulfone (Study B89087, MRID 48574752, PMRA 2181202), in which the NOAEL was determined to be 200 ppm based on effects on body weight and organ weight at 900 ppm.

4. Diet Preparation and Analysis

Treated diets were prepared every two weeks and stored at room temperature in disposable paper bags by dose group.

Fluensulfone was warmed in the original container in a water bath up to maximum of approximately 40 °C until the test item was liquid. The liquid test item was then transferred into a glass beaker (wrapped with aluminum foil) and mixed using a magnetic stirrer on a heating plate (maximum approximately 40°C). The test item was divided into aliquots in brown glass containers for the feed preparations. The required amounts of fluensulfone were weighed into tared glass beakers (wrapped with aluminum foil) and kept at maximum approximately 40 °C on a heating plate until use for feed preparation. The test substance was mixed with microgranulated feed, and pellets were prepared. Water (approximately 1:10 v/w) was added to aid pelleting. The pellets were dried with warm air for approximately 48 hours before storage. The pellets for the control group were prepared separately as described above but without test item.

Samples for determination of concentration, homogeneity and stability (14 days) of the dose formulations were taken from the feed preparation for the first week of the administration period. Additionally, samples for determination of concentration (by HPLC coupled to a UV/VIS detector and quantified with the area under the peak) were taken from the feed preparation for the last week of the administration period.

The diet samples (prepared during the first and last week of the treatment period) investigated during the study were found to comprise fluensulfone in the range of 94% to 133% of the target level. The homogeneous distribution was considered acceptable because single results found did not deviate by more than 2.3% from the corresponding mean. In addition, fluensulfone was found to be stable in diet when kept 14 days under storage conditions as recoveries were within 5.6% of the time-zero (homogeneity) mean.

5. Data evaluation

The following statistical methods were used to analyze body weight, clinical laboratory parameters and organ weights.

- If the variables were assumed to follow a normal distribution, the Dunnett-test (many to one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups and the control groups for each sex.

- The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data was not assumed to follow a normal distribution.

Additional methods of statistical analysis were used at the discretion of the statistician.

C. METHODS

1. Observations

Dogs were inspected twice a day for morbidity and mortality from commencement of the pre-test period. General clinical examinations (in the pen) were made twice daily from commencement of the pre-test period.

Veterinary examinations, including pupil reflex, corneal reflex, and patellar reflex, cardiac and pulmonary auscultation, were performed one during the pre-test, once during week 13, and at the end of the recovery period.

2. Bodyweight

Body weight was recorded at least once weekly from commencement of the pre-test period, weekly during treatment and before necropsy. All weights were recorded before feeding.

3. Food consumption

The feed intake of each individual dog was determined daily from commencement of the pre-test period. The daily ration was weighed before and after feeding.

4. Water consumption

Not determined.

5. Ophthalmoscopic examination

Observations of the cornea, lens, conjunctiva, sclera, iris and fundus were performed using an opththalmoscope following the instillation of 0.5% tropicamide on all dogs once during the pre-test period and once during week 13 of the treatment period and at the end of the recovery period.

6. Hematology and Clinical Chemistry

Blood was collected from the jugular vein of all animals following an overnight fast (dogs were allowed access to water *ad libitum*) once during the pre-test period, at weeks 6 and 13 of the treatment period, and at the end of the recovery period for the analysis of hematology and clinical chemistry. The following parameters were determined at hematology:

> Erythrocyte count, Hemoglobin concentration, Hematocrit, Mean corpuscular volume, Red cell volume distribution width, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration, Hemoglobin concentration distribution width, Platelet count, Reticulocyte count, Reticulocyte maturity index, Total leukocyte count, Differential leukocyte count, Thromboplastin time and Activated partial thromboplastin time

The following parameters were determined at clinical chemistry:

> Glucose, Urea, Creatinine, Bilirubin (total), Cholesterol (total), Triglycerides, Phospholipids, Aspartate aminotransferase, Alanine aminotransferase, Glutamate dehydrogenase, Creatine kinase, Alkaline phosphatase, Gamma-glutamyl-transferase, Calcium, Phosphorus, Sodium, Potassium, Chloride, Protein (total), Albumin, Globulin, and Albumin/Globulin Ratio

In addition, blood samples were collected from all animals at the same time as the clinical laboratory investigations for T3, T4, and TSH analyses.

7. Urinalysis

Urine was collected from all animals into a specimen vial using a catheter once during the pre-test period, at weeks 6 and 13 of the treatment period, and at the end of the recovery period. The following parameters were determined:

> Color, Appearance, Relative density, pH, Protein, Glucose, Ketones, Urobilinogen, Bilirubin, Erythrocytes, Leukocytes

8. Sacrifice and pathology

All animals were anesthetized by intravenous injection of sodium pentobarbital and sacrificed by exsanguination. All animals were necropsied and descriptions of all macroscopic abnormalities

were recorded.

Samples of tissues and organs were collected from all animals at necropsy and fixed, unless otherwise indicated, in neutral phosphate-buffered 4% formaldehyde solution (10% formalin). Selected organs (Table 1) were weighed prior to fixation (paired organs were weighed separately; organ to terminal body weight ratios and organ to brain weight ratios were determined). Bone marrow smears from the sternum from all animals were taken, stained and retained for possible further investigation.

Table 1: Summary of tissues collected for pathology examination

Organ/tissue	Weighed	Collected	Examined
Adrenal gland	X	X	X
Aorta		X	X
Bone - sternum, femur including articular surface		X	X
Bone marrow - femur		X	X
Brain - including sections of medulla/pons, cerebral and cerebellar cortex	X	X	X
Epididymides (fixed in Bouin's solution)	X	X	X
Esophagus		X	X
Eyes with optic nerve (fixed in Davidson's solution)		X	X
Female mammary gland area, (male mammary gland area)		X	X
Gallbladder		X	X
Heart	X	X	X
Kidneys	X	X	X
Large intestine - cecum, colon, rectum		X	X
Larynx		X	
Liver	X	X	X
Lungs, instilled via trachea with formalin at approximately 30 cm H_2O pressure		X	X
Lymph nodes - retropharyngeal, mesenteric		X	X
Ovaries	X	X	X
Oviducts		X	X
Pancreas		X	X
Pituitary gland		X	X
Peyer's Patches		X	X
Prostate gland		X	X
Salivary glands - mandibular, parotid, (sublingual)		X	X
Sciatic nerve		X	X
Skeletal muscle		X	X
Skin - and subcutaneous tissue		X	X
Small intestine - duodenum, jejunum, ileum		X	X
Spinal cord - cervical, midthoracic, lumbar segments		X	X
Spleen	X	X	X
Stomach		X	X

Testes (fixed in Bouin's solution)	X	X	X
Thymus	X	X	X
Thyroid gland with parathyroid gland	X	X	X
Tongue		X	X
Trachea		X	X
Ureter		X	X
Urinary bladder		X	X
Uterus with (vagina), cervix	X	X	X
All gross lesions		X	X

A liver sample was taken at the scheduled necropsy after 13 weeks from all animals, minced into small pieces and frozen in liquid nitrogen. Liver subcellular fractions were prepared and the following parameters were determined: Protein content of subcellular fractions, Microsomal cytochrome P450 content (differential spectroscopy), CYP1A1 (microsomal 7-ethoxyresorufin O-dealkylation), CYP1A2 (microsomal 7-methoxyresorufin O-dealkylation), CYP2B1 (microsomal pentoxy-resorufin O-dealkylation), CYP2B11 (microsomal 7-benzyloxyresorufin O-dealkylation), CYP3A (microsomal testosterone 6β-hydroxylation), Cytosolic glutathione S-transferase (GST, glutathione conjugation of 1-chloro-2,4-dinitrobenzol), Microsomal uridine diphosphoglucuronosyl-transferase (UDPGT, glucuronidation of 3-methyl-2-nitrophenol), Microsomal epoxide hydrolase (hydrolysis of styrene oxide), and Cytosolic alanine aminotransferase (ALAT, transamination of ketoglutarate).

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Actual achieved intakes of test substance

The actual achieved intakes of the test substance were as follows:

Dietary level (ppm)	**Actual dosage (mg/kg bw/day)**	
	Males	**Females**
5	0.2	0.2
50	1.6	1.8
500	17.1	18.0
Dietary level (ppm)	**Combined sexes**	
5	0.2	
50	1.7	
500	17.6	

2. Clinical signs

The clinical signs observed, such as loose and watery feces, occurred in animals from all groups, including the controls, and, in the absence of a treatment relationship, were considered not to be

related to treatment.

3. Body weight and food consumption

In males from the 500 ppm dose group, decreases in mean body weight of 3-9% relative to controls were observed throughout the treatment period (Table 2), but a decrease of 7% relative to controls was observed during the pre-test period and as such the decrease during the treatment period is not considered to be related to treatment. In females from the 500 ppm dose group, decreases in mean body weight of 10-17% relative to controls were observed from day 43 through to the end of the treatment period. Mean body weight values for females in the 500 ppm dose group remained within 10% of the control mean throughout the recovery period.

Male and female animals receiving 500 ppm gained slightly less weight than the controls (mean of 1.2 kg vs. 1.5 kg in the control group for males, and 1.2 kg vs. 2.7 kg in the control group for females).

Table 2. Mean (± S.D.) body weights (kg)

Day	0 ppm	5 ppm	50 ppm	500 ppm
Male dogs				
Pre-test day 1	9.0 ± 1.4	9.2 ± 1.1	8.8 ± 0.8	8.4 ± 1.0
1	9.1 ± 1.3	9.4 ± 1.1	9.0 ± 0.9	8.8 ± 1.0
15	9.6 ± 1.2	9.7 ± 1.2	9.5 ± 0.8	9.1 ± 1.0
36	10.4 ± 1.2	10.4 ± 1.2	10.3 ± 0.7	9.6 ± 1.0
64	11.0 ± 1.3	10.9 ± 1.2	11.2 ± 0.7	10.1 ± 1.1
85	11.3 ± 1.4	11.3 ± 1.4	11.4 ± 0.7	10.3 ± 1.1
92	10.6 ± 1.7	11.5 ± 1.3	10.8 ± 0.9	10.0 ± 1.3
Recovery day 1	11.9 ± 1.1	--	--	10.6 ± 1.0
Recovery day 29	12.0 ± 0.8	--	--	10.8 ± 0.8
Female dogs				
Pre-test day 1	7.7 ± 0.9	7.4 ± 0.8	7.6 ± 0.8	7.2 ± 0.5
1	7.8 ± 0.8	7.5 ± 1.0	7.6 ± 0.9	7.5 ± 0.5
15	8.4 ± 0.9	8.1 ± 1.3	7.8 ± 0.9	7.8 ± 0.6
36	9.1 ± 0.9	8.8 ± 1.5	8.4 ± 1.0	8.3 ± 0.8
64	9.7 ± 1.0	9.4 ± 1.9	8.9 ± 1.2	8.6 ± 0.9 (↓11%)
85	10.2 ± 1.2	9.7 ± 2.0	9.5 ± 1.2	8.9 ± 0.8 (↓13%)
92	10.3 ± 1.2	9.7 ± 2.1	9.1 ± 1.2	8.6 ± 0.9 (↓17%)
Recovery day 1	10.0 ± 1.3	--	--	9.0 ± 1.0
Recovery day 29	10.0 ± 1.4	--	--	9.0 ± 1.0

N=8 for the 0 and 500 ppm dose groups and N=4 for the 5 and 50 ppm dose groups except for Day 92 and Recovery days 1 and 29 where N=4 for all dose groups. Data obtained from pages 82-85 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

No effects on food consumption were noted in male dogs during the treatment period. In females, dose-related decreases in food consumption were observed sporadically throughout the treatment period, with overall food consumption (overall mean of means during the treatment period) reduced by 9% and 11% compared to the control mean for females from the 50 and 500 ppm dose groups, respectively.

4. Ophthalmoscopic and veterinary examinations

Neither the ophthalmoscopic nor the veterinary examination revealed any change related to the treatment.

5. Hematology

Selected hematology parameters are summarized in Table 3. A slight, dose-related and statistically significant decrease in hemoglobin (HGB) was noted in males from the 500 ppm dose group at week 13. All hemoglobin values for individual animals fell within the 95% limits of the historical control data (Table 4).

Dose related and statistically significant decreases in mean corpuscular hemoglobin concentration (MCHC) were observed in males and females from the 500 ppm dose group at weeks 6 and 13, and in females at the end of the recovery period. These decreases were slight, and values for males at weeks 6 and 13 and for females at week 6 fell within 95% limits of historical control values, while at week 13, 4/8 females had values slightly below the 95% limits of the historical control values.

A trend toward increased reticulocyte counts was also observed at 500 ppm in males, but more remarkably in females, as males demonstrated an increase over concurrent controls at the pre-test sampling time, and values for individual males fell within the 95% limits of the historical control values. In females, there was no difference during the pre-test period, the changes from concurrent control at weeks 6 and 13 were more substantial than in males, and values in 2 females at week 6 approached the upper range of the 95% limit of the historical control values, while at week 13 one female exceeded the 95% limits of the historical control values.

Similar patterns (reduced HGB and MCHC and increased reticulocytes) were also noted in the 1-year dietary study in dogs with fluensulfone starting after 6 weeks of dosing with a 500 ppm test diet. Therefore, an effect of treatment cannot be ruled out, although the noted changes in these hematology parameters are not considered to be adverse due to the small magnitude of the changes.

Table 3. Mean selected hematology findings

Parameter	Time Point	0 ppm	5 ppm	50 ppm	500 ppm
Male dogs					
HGB	Pre-test	8.3	8.5	7.9	8.3
	Week 6	9.1	9.4	8.6	9.0
	Week 13	9.3	8.9	9.2	8.7* (↓6%)
	Recovery	9.4	--	--	9.6
MCHC (mmol/L)	Pre-test	21.39	21.68	21.49	21.27
	Week 6	22.31	22.67*	22.65	21.73** (↓3%)
	Week 13	21.48	21.48	21.51	20.34** (↓5%)
	Recovery	21.79	--	--	21.20
Retic. (G/L)	Pre-test	68	65	66	84 (↑23%)
	Week 6	54	67	47	87* (↑61%)
	Week 13	52	43	35	73* (↑40%)
	Recovery	44	--	--	76 (↑73%)
Retic. (relative)	Pre-test	0.011	0.011	0.011	0.015 (↑36%)
	Week 6	0.008	0.011	0.008	0.014* (↑75%)
	Week 13	0.008	0.008	0.006	0.011 (↑37%)
	Recovery	0.007	--	--	0.010 (↑43%)
Female dogs					
HGB	Pre-test	9.1	9.3	9.4	9.3
	Week 6	9.7	9.6	9.9	9.2
	Week 13	9.6	9.8	10.0	9.1
	Recovery	9.7	--	--	9.3
MCHC (mmol/L)	Pre-test	21.68	21.75	21.82	21.51
	Week 6	22.50	22.47	22.75	21.47** (↓5%)
	Week 13	21.66	21.76	21.52	20.15** (↓7%)
	Recovery	22.08	--	--	21.36** (↓3%)
Retic. (G/L)	Pre-test	63	82	75	73
	Week 6	44	59	48	89** (↑102%)
	Week 13	37	37	58	98** (↑165%)
	Recovery	44	--	--	53
Retic. (relative)	Pre-test	0.009	0.013	0.012	0.011
	Week 6	0.007	0.010	0.008	0.014* (↑100%)
	Week 13	0.005	0.006	0.009	0.015** (↑200%)
	Recovery	0.007	--	--	0.007

N=8 for the 0 and 500 ppm dose groups at pre-test and weeks 6 and 13; N=4 for the 5 and 50 ppm dose groups at all time points and the 0 and 500 ppm dose groups at end of recovery. Data obtained from pages 94-97 of the study report. Standard deviations were not provided in the report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * statistically significantly different from the controls ($p<0.05$). ** statistically significantly different from the controls ($p<0.01$). HGB = hemoglobin. MCHC = mean corpuscular hemoglobin concentration. Retic. = reticulocytes.

Table 4. Historical control values for selected hematological parameters

Parameter	Age	Males		Females	
		Mean ± S.D.	95% limits	Mean ± S.D.	95% limits
HGB (mmol/L)	≤ 28 weeks 29-48 weeks	9.0 ± 0.8 9.0 ± 0.8	7.5-10.4 7.6-10.6	9.4 ± 1.0 9.5 ± 0.8	7.6-11.3 8.1-11.0
MCHC (mmol/L)	≤ 28 weeks 29-48 weeks	21.10 ± 0.62 21.36 ± 0.72	20.13-22.61 20.08-22.80	21.02 ± 0.66 21.39 ± 0.68	20.16-23.07 20.16-22.75
Retic. (G/L)	≤ 28 weeks 29-48 weeks	58 ± 26 65 ± 27	20-126 26-124	58 ± 27 65 ± 28	19-126 21-128
Retic. (relative)	≤ 28 weeks 29-48 weeks	0.009 ± 0.004 0.010 ± 0.005	0.003-0.020 0.004-0.020	0.009 ± 0.004 0.010 ± 0.004	0.003-0.021 0.003-0.020

Data obtained from pages 480-483 of the study report. Studies were conducted between June 2002 and May 2007. Sample sizes ranged from 286 to 294 animals. HGB = hemoglobin. MCHC = mean corpuscular hemoglobin concentration. Retic. = reticulocytes.

No changes were recorded in other hematological parameters that were considered to be related to treatment with fluensulfone.

Some inter-group variations achieved statistical significance but they either did not show a dose-response relationship, were sporadic, or reflected differences present during pretest, and were therefore not considered to be related to treatment with fluensulfone.

6. Clinical chemistry

Statistically significantly decreased plasma alanine aminotransferase (ALAT) was observed after 6 weeks in males at 50 and 500 ppm (Table 5a). Females in these dose groups also exhibited slight decreases from controls that did not attain statistical significance (Table 5b). Two of four males from the 50 ppm dose group and 1/8 male from the 500 ppm dose group had values that fell slightly below the 95% limit of the historical control data (Table 6). None of the females in the 50 ppm dose group and 1/8 females in the 500 ppm dose group had ALAT values below the 95% limits of the historical control values. At 13 weeks no statistically significant differences in ALAT were recorded; however, the values were still lower than the controls in male animals at 50 ppm. None of the individual values at week 13, in either males or females, were outside the range of historical control data. At the end of recovery, slightly increased, but not statistically significant ALAT values were recorded in males and females at 500 ppm when compared to controls. The individual values in males at the end of recovery fell within the historical control range, while the values for 2/4 females at 500 ppm at the end of recovery exceeded historical values.

Statistically significant reductions in total bilirubin were noted in males and females from the 500 ppm dose group at weeks 6 and 13 and in males from the 50 ppm dose group at week 6 when compared to concurrent controls. A slight decrease (not statistically significant) persisted in females to the end of the recovery period. The individual values for these animals all fell within the historical control range.

Decreased total protein (males only), albumin, and albumin/globulin ratios were recorded after 6 and 13 weeks for male and female animals at 500 ppm. A slight reduction in albumin persisted in females to the end of the recovery period. With the exception of albumin values for 2/8 females at week 6 and 2/8 males at week 13, which fell below the historical control range, the protein,

albumin and albumin/globulin ratio values for the 500 ppm dose group were within the historical control range for the performing laboratory.

The glucose level was slightly decreased in female animals at 500 ppm in comparison with the controls in weeks 6 and 13 attaining statistical significance in week 13. Individual values fell below the historical control range for 2/4 females at week 6 and 5/8 females at week 8.

Table 5a: Mean selected clinical chemistry parameters – male animals

Parameter	0 ppm	5 ppm	50 ppm	500 ppm
Pre-test				
ALAT (U/L)	32.8	41.7	34.7	38.7
Bilirubin (µmol/L)	1.50	1.40	1.25	1.40
Protein (g/L)	56.26	54.37	54.84	54.70
Albumin (g/L)	31.39	31.81	31.43	30.40
A/G ratio	1.27	1.44	1.37	1.27
Week 6				
ALAT (U/L)	42.2	46.7	23.9** (↓43%)	28.3** (↓33%)
Bilirubin (µmol/L)	1.88	1.64	1.30* (↓31%)	1.34** (↓29%)
Protein (g/L)	58.48	57.89	57.21	53.95** (↓8%)
Albumin (g/L)	33.43	33.86	31.43	28.22** (↓16%)
A/G ratio	1.35	1.38	1.18	1.06** (↓21%)
Week 13				
ALAT (U/L)	43.8	49.5	32.2 (↓26%)	42.6
Bilirubin (µmol/L)	2.07	1.44	1.73	1.36* (↓34%)
Protein (g/L)	59.15	56.21	60.82	53.76** (↓9%)
Albumin (g/L)	33.55	33.07	33.68	29.03** (↓13%)
A/G ratio	1.30	1.37	1.35	1.17 (↓10%)
End of recovery				
ALAT (U/L)	44.3	--	--	63.4 (↑43%)
Bilirubin (µmol/L)	1.90	--	--	2.03
Protein (g/L)	58.45	--	--	58.30
Albumin (g/L)	32.00	--	--	32.61
A/G ratio	1.21	--	--	1.32

N=8 for the 0 and 500 ppm dose groups at pre-test and weeks 6 and 13; N=4 for the 5 and 50 ppm dose groups at all time points and the 0 and 500 ppm dose groups at end of recovery. Data obtained from pages 101-103 of the study report. Standard deviations were not provided in the report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).* statistically significantly different from the control mean ($p < 0.05$); ** statistically significantly different from the control mean ($p < 0.01$). ALAT = alanine aminotransferase. A/G = albumin/globulin ratio.

Table 5b: Mean selected clinical chemistry parameters – female animals

Parameter	Control	5 ppm	50 ppm	500 ppm
Pre-test				
Glucose	5.72	6.35	5.59	5.69
Bilirubin (µmol/L)	1.65	1.85	1.75	1.60
ALAT (U/L)	35.5	35.1	63.3**	40.8
Protein	54.88	53.91	54.60	54.27
Albumin	32.64	33.02	33.52	32.06
A/G ratio	1.49	1.58	1.60	1.47
Week 6				
Glucose	5.25	5.89**	5.53	4.99 (↓5%)
Bilirubin (µmol/L)	2.26	2.33	2.20	1.45* (↓36%)
ALAT (U/L)	44.2	39.4	34.2 (↓23%)	33.0 (↓25%)
Protein	56.74	57.81	56.08	54.47
Albumin	33.73	34.83	33.05	30.42** (↓10%)
A/G ratio	1.42	1.47	1.45	1.29* (↓9%)
Week 13				
Glucose	5.15	5.66	5.59	4.57** (↓11%)
Bilirubin (µmol/L)	2.34	2.17	2.42	1.60* (↓32%)
ALAT (U/L)	44.7	35.9	47.7	51.2
Protein	57.95	58.31	58.90	55.91
Albumin	34.78	35.51	35.64	31.48** (↓9%)
A/G ratio	1.52	1.59	1.51	1.31* (↓14%)
End of recovery				
Glucose	5.45	--	--	5.52
Bilirubin (µmol/L)	2.15	--	--	1.82 (↓15%)
ALAT (U/L)	38.4	--	--	80.3 (↑109%)
Protein	59.11	--	--	56.14
Albumin	34.42	--	--	31.76** (↓7%)
A/G ratio	1.48	--	--	1.31

N=8 for the 0 and 500 ppm dose groups at pre-test and weeks 6 and 13; N=4 for the 5 and 50 ppm dose groups at all time points and the 0 and 500 ppm dose groups at end of recovery. Data obtained from pages 104-106 of the study report. Standard deviations were not provided in the report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).* statistically significantly different from the control mean ($p < 0.05$); ** statistically significantly different from the control mean ($p < 0.01$). ALAT = alanine aminotransferase. A/G = albumin/globulin ratio.

Table 6. Historical control values for selected clinical chemistry parameters

Parameter	Age	Males		Females	
		Mean ± S.D.	95% limits	Mean ± S.D.	95% limits
Glucose (mmol/L)	≤ 28 weeks	5.79 ± 0.44	5.01-6.63	5.78 ± 0.54	4.83-6.70
	29-48 weeks	5.73 ± 0.47	4.74-6.60	5.64 ± 0.49	4.64-6.57
ALAT (U/L)	≤ 28 weeks	39.6 ± 13.4	20.6-77.9	38.3 ± 11.8	21.9-69.0
	29-48 weeks	44.2 ± 42.6	23.6-95.6	41.1 ± 41.3	23.3-77.3
Bilirubin (µmol/L)	≤ 28 weeks	1.58 ± 0.40	0.91-2.46	1.77 ± 0.51	0.80-2.87
	29-48 weeks	1.70 ± 0.42	0.99-2.60	1.91 ± 0.49	1.03-2.96
Protein (g/L)	≤ 28 weeks	56.86 ± 3.45	49.68-63.28	56.16 ± 3.33	50.56-63.06
	29-48 weeks	57.07 ± 3.12	50.90-63.62	56.81 ± 3.01	51.22-62.97
Albumin (g/L)	≤ 28 weeks	31.34 ± 2.29	26.12-35.84	33.38 ± 2.81	28.44-37.74
	29-48 weeks	32.01 ± 2.30	27.12-36.05	33.33 ± 2.20	28.48-37.26
A/G ratio	≤ 28 weeks	1.28 ± 0.20	0.90-1.74	1.53 ± 0.23	1.17-2.07
	29-48 weeks	1.29 ± 0.18	0.93-1.63	1.44 ± 0.20	1.02-1.84

Data obtained from pages 484-487 of the study report. Studies were conducted between June 2002 and May 2007. Sample sizes ranged from 87 to 2005 animals. ALAT = alanine aminotransferase.

7. Urinalysis

No treatment-related effects were noted at any evaluation time point.

8. Thyroid hormone analyses

Generally, no differences in T3 and T4 levels were observed in the treated groups when compared to the control values (data not shown).

There seemed to be a tendency toward higher TSH values in male (Table 7a) and female (Table 7b) dogs treated at 500 ppm when compared to the control values. However, only one mean value was significantly different (TSH in females at week 6). The mean TSH levels also appeared elevated in the 5 ppm dose group, but an increase over controls at this dose level was noted during the pre-test period. There were no apparent differences after the recovery period.

Table 7a: TSH levels (ng/mL) in serum of male dogs

Group	Pre-test		Week 6		Week 13		End of recovery	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Control	0.16 ± 0.19	0.04 – 0.61	0.18 ± 0.14	0.05 – 0.47	0.19 ± 0.14	0.05 – 0.47	0.21 ± 0.19	0.07 – 0.48
5 ppm	0.10 ± 0.06	0.05 – 0.18	0.10 ± 0.05	<0.03 – 0.16	0.10 ± 0.07	< 0.03 - 0.20	--	--
50 ppm	0.08 ± 0.02	0.06 – 0.10	0.14 ± 0.04	0.09 – 0.17	0.12 ± 0.04	0.09 – 0.18	--	--
500 ppm	0.16 ± 0.07	< 0.03 – 0.26	0.38 ± 0.28 (↑111%)	0.05 – 0.96	0.37 ± 0.36 (↑95%)	0.05 – 1.20	0.28 ± 0.14	0.15 – 0.49

N=8 for the 0 and 500 ppm dose groups at pre-test and weeks 6 and 13; N=4 for the 5 and 50 ppm dose groups at all time points and the 0 and 500 ppm dose groups at end of recovery. Data obtained from page 504 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).For all individual values <0.03 ng/mL, a value of 0.03 ng/mL was used for the calculation of the mean

Table 7b: TSH levels (ng/mL) in serum of female dogs

Group	Pre-test		Week 6		Week 13		End of recovery	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Control	0.08 ± 0.06	0.03 – 0.23	0.12 ± 0.09	0.06 – 0.26	0.09 ± 0.06	0.03 – 0.18	0.12 ± 0.07	0.07 – 0.22
5 ppm	0.35 ± 0.22	0.16 – 0.59	0.24 ± 0.16	0.11 – 0.48	0.19 ± 0.09	0.09 – 0.31	--	--
50 ppm	0.10 ± 0.02	0.07 – 0.12	0.12 ±0.04	0.08 – 0.16	0.10 ± 0.05	0.05 – 0.17	--	--
500 ppm	0.15 ± 0.12	0.08 – 0.38	0.38 ± 0.21* (↑217%)	0.13 – 0.69	0.27 ± 0.23 (↑200%)	0.12 – 0.81	0.15 ± 0.08	0.08 – 0.24

N=8 for the 0 and 500 ppm dose groups at pre-test and weeks 6 and 13; N=4 for the 5 and 50 ppm dose groups at all time points (except N=3 for the 50 ppm dose group at pre-test) and the 0 and 500 ppm dose groups at end of recovery. Data obtained from page 504 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).* statistically significantly different from the controls ($p<0.05$).

9. Terminal examinations

9.1 Macroscopic examination

There were no gross lesions that could be attributed to treatment with fluensulfone. All gross lesions recorded were considered to be within the range of normal background alterations.

9.2 Organ weight

Increased liver weights were observed for males and females receiving 500 ppm at the end of the treatment period, with only relative-to-body weight liver weight attaining statistical significance in females (Table 8). This was not apparent at the end of recovery.

In females from the 500 ppm dose group, decreases in absolute and relative thymus weight were observed. It was noted that one female in the control group (animal #26) demonstrated a fairly high thymus weight when compared to other control animals [absolute thymus weight of 13.32 g versus 6.73-7.11 g in other animals; relative (to body weight) thymus weight of 0.13% versus 0.06-0.08% in other animals; relative (to brain weight) thymus weight of 18.20% versus 8.68-10.07% in other animals). However, even when the mean organ weights were recalculated excluding the values for animal #26, the mean thymus weights of females from the 500 ppm dose group were still reduced by 29-39% when compared to the re-calculated control mean.

Apparent increases in mean uterus weight at 500 ppm and in mean ovary weight at all dose groups was observed in females, but the data were highly variable, and reductions in these organ weight when compared to controls were noted following the recovery period. These changes are not considered to be related to treatment.

Table 8: Organ weight at terminal sacrifice *(recovery animals in italics)*

Parameter	Control	5 ppm	50 ppm	500 ppm
Males				
Terminal bw (g)	10,641 ± 1691 *11,918 ± 820*	11,456 ± 1315	10,843 ± 858	9975 ± 1330 (↓6%) *10,879 ± 1000*
Liver – Abs. (g)	286.0 ± 63.8 *(337.3 ± 80.8)*	295.0 ± 68.3	280.6 ± 22.5	315.0 ± 50.8 (↑10%) *(309.8 ± 24.7)*
Liver – Rel. to bw (%)	2.7 ± 0.3 *(2.8 ± 0.6)*	2.6 ± 0.7	2.6 ± 0.2	3.2 ± 0.2 (↑18%) *(2.9 ± 0.2)*
Liver – Rel. to brain wt (%)	376.0 ± 59.8 *(415.6 ± 63.9)*	390.1 ± 102.1 [341.9 ± 41.4][#]	374.2 ± 27.5	390.6 ± 38.5 (↑4%) *(373.9 ± 44.1)*
Females				
Terminal bw (g)	10,516 ± 1180	9750 ± 2018	9301 ± 1259 (↓11%)	8681 ± 836 (↓17%)
Liver – Abs. (g)	318.2 ± 47.3 *(267.8 ± 9.3)*	304.7 ± 30.5	299.1 ± 63.8	343.6 ± 19.1 (↑8%) *(254.3 ± 21.5)*
Liver – Rel. to bw (%)	3.1 ± 0.4 *(2.7 ± 0.4)*	3.2 ± 0.4	3.2 ± 0.3	4.0 ± 0.3** (↑29%) *(2.9 ± 0.1)*
Liver – Rel. to brain wt (%)	425.4 ± 55.3 *(353.7 ± 14.7)*	432.6 ± 20.2	397.0 ± 84.7	483.6 ± 56.5 (↑14%) *(327.6 ± 38.9)*
Thymus – Abs. (g)	8.54 ± 3.19 [6.95 ± 0.20][#] *(8.42 ± 2.31)*	6.82 ± 3.28	6.72 ± 1.31	4.24 ± 1.35 (↓39%)[##] *(6.73 ± 1.03)*
Thymus – Rel. to bw (%)	0.09 ± 0.03 [0.07 ± 0.01][#] *(0.09 ± 0.02)*	0.07 ± 0.02	0.08 ± 0.02	0.05 ± 0.01 (↓29%)[##] *(0.08 ± 0.01)*
Thymus – Rel. to brain wt (%)	11.50 ± 4.50 [9.27 ± 0.72][#] *(11.05 ± 2.68)*	9.35 ± 3.59	8.89 ± 1.50	5.83 ± 1.16 (↓37%)[##] *(8.67 ± 1.56)*

Heart – Abs. (g)	73.2 ± 5.8 *(71.7 ± 5.8)*	72.8 ± 7.5	67.0 ± 10.0 (↓8%)	66.0 ± 7.6 (↓10%) *(71.0 ± 5.7)*
Heart – Rel. to bw (%)	0.7 ± 0.1 *(0.8 ± 0.1)*	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1 *(0.8 ± 0.1)*
Heart – Rel. to brain wt (%)	98.2 ± 8.8 *(94.6 ± 7.5)*	103.2 ± 2.8	88.3 ± 6.7	92.7 ± 12.8 *(91.3 ± 8.4)*
Uterus – Abs. (g)	33.42 ± 27.26 *(23.20 ± 4.18)*	28.46 ± 15.12	25.18 ± 13.06	46.41 ± 15.81 *(14.14 ± 7.62)*
Uterus – Rel. to bw (%)	0.31 ± 0.24 *(0.24 ± 0.08)*	0.31 ± 0.17	0.26 ± 0.11	0.53 ± 0.14 *(0.16 ± 0.08)*
Uterus – Rel. to brain wt (%)	44.93 ± 36.29 *(30.79 ± 6.50)*	40.95 ± 21.49	32.86 ± 16.00	65.18 ± 24.16 *(18.26 ± 10.29)*
Ovary (right) – Abs. (g)	0.568 ± 0.245 *(0.870 ± 0.432)*	0.845 ± 0.573	0.865 ± 0.525	0.947 ± 0.379 *(0.395 ± 0.153)*
Ovary (right) – Rel. to bw (%)	0.006 ± 0.002 *(0.009 ± 0.006)*	0.008 ± 0.004	0.009 ± 0.005	0.011 ± 0.005 *(0.004 ± 0.001)*
Ovary (right) – Rel. to brain wt (%)	0.759 ± 0.313 *(1.162 ± 0.608)*	1.165 ± 0.688	1.136 ± 0.662	1.347 ± 0.589 *(0.511 ± 0.212)*

N=4/sex/dose/time point. Data obtained from pages 114-137 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). # mean ± S.D. excluding outlier (calculated by the reviewer). ## Percent change from re-calculated control mean excluding outlier. ** statistically significantly different from the controls ($p<0.01$). Abs. = absolute. Rel. = relative. bw = body weight. wt = weight

9.3 Histopathology

A slight diffuse hepatocellular hypertrophy was noted in a single female treated at 500 ppm at the end of the treatment period (versus no incidences in lower dose groups). None of the recovery females showed a similar finding. Pigmented Kupffer cells were also noted in two males and one female from the 500 ppm dose group (versus no incidences in lower dose groups).

9.4 Liver enzyme determinations

Dosing with fluensulfone had no effect on the protein content of the 100g supernatants, cytosolic and microsomal fractions, or on the microsomal cytochrome P450 content.

Treatment of males (Table 9a) and females (Table 9b) was without an effect on the microsomal phase I enzymes activities 7-ethoxy- (EROD - CYP1A1), 7-methoxy (MROD - CYP1A2), and 7-pentoxy-resorufin O-dealkylase (PROD - CYP2B1).

Mean testosterone 6β-hydroxylase (6β-OHT, CYP3A) was slightly but not statistically significantly increased in females after treatment with 50 and 500 ppm fluensulfone. Microsomal epoxide hydrolase (EH) was increased compared to the controls in males and females at 500 ppm, attaining statistical significance in females only. The effect on EH was considered by the study investigators not to be biologically relevant, since this enzyme is responsible for phase II metabolism (detoxification and excretion) and the slight increase of its activity might be a response to the constant load of the liver with a xenobiotic.

Treatment was without an effect on the phase II enzyme activities of microsomal uridine diphosphoglucuronosyl-transferase (UDPGT) and cytosolic glutathione S-transferase (GST) in both sexes.

Following treatment with 5, 50 and 500 ppm, a tendency to decreased ALAT activities was observed in males and females at all concentrations (but the decrease was minimal in males at 5 ppm); however, they were considered by the study investigators not to be biologically relevant and likely a response of the constant load on the liver with a xenobiotic.

The results indicate that fluensulfone is not a potent inducer of liver enzymes.

Table 9a: Activities of phase I and phase II enzymes in the liver of male dogs (% of control)

Parameter	0 ppm	5 ppm	50 ppm	500 ppm
Protein content of 100g supernatant (mg/g liver)	100	99.6	108.4	97.7
Protein content of cytosolic fractions (mg/g liver)	100	91.9	106.8	91.3
Protein content of microsomal fractions (mg/g liver)	100	79.1	91.5	100.6
ALAT activity in 100g supernatants (U/g liver)	100	97.0	72.9	42.6**
Microsomal cytochrome P450 content (nmol/g liver)	100	78.0	90.5	122.5
MROD activity (nmol/min/g liver)	100	71.2	88.8	95.4
EROD activity (nmol/min/g liver)	100	101.0	272.0	196.9
PROD activity (nmol/min/g liver)	100	67.1	98.7	138.7
6 ß -OHT activity (nmol/min/g liver)	100	131.5	89.9	126.7
GST activity (µmol/min/g liver)	100	100.7	109.8	132.0
UDPGT activity (µmol/min/g liver)	100	73.1	76.0	83.2
EH activity (µmol/min/g liver)	100	83.1	92.1	147.5

Data obtained from pages 696-719 of the study report. ** statistically significantly different from the controls ($p<0.01$).

Table 9b: Activities of phase I and phase II enzymes in the liver of female dogs (% of control)

Parameter	0 ppm	5 ppm	50 ppm	500 ppm
Protein content of 100g supernatant (mg/g liver)	100	104.2	96.8	95.4
Protein content of cytosolic fractions (mg/g liver)	100	87.3*	94.6	83.3
Protein content of microsomal fractions (mg/g liver)	100	86.0	94.2	104.1
ALAT activity in 100g supernatants (U/g liver)	100	72.2	71.1	70.1
Microsomal cytochrome P450 content (nmol/g liver)	100	86.0	90.3	141.3
MROD activity (nmol/min/g liver)	100	114.1	162.1	102.5
EROD activity (nmol/min/g liver)	100	82.3	225.7*	91.4
PROD activity (nmol/min/g liver)	100	106.2	98.1	136.0
6 ß -OHT activity (nmol/min/g liver)	100	94.4	149.8	162.2
GST activity (µmol/min/g liver)	100	121.5	134.5	138.1
UDPGT activity (µmol/min/g liver)	100	86.7	88.8	108.0
EH activity (µmol/min/g liver)	100	91.6	107.6	156.9*

N=4/sex/dose. Data obtained from pages 696-719 of the study report. * statistically significantly different from the controls ($p<0.05$).

III. CONCLUSIONS

Decreased plasma and liver ALAT activities were observed at 500 ppm as well as increased liver weight, Kupffer cell pigmentation in two males and one female, and an isolated incidence of hepatocellular hypertrophy in one female suggesting that the liver is a target organ. At 500 ppm, body weight gains were reduced by 20% to 55% compared to controls in both sexes, while reductions in body weight of up to 17% were noted in females. Both sexes dosed with the 500 ppm test diet exhibited increases in reticulocytes and decreases in bilirubin, albumin, and albumin/globulin ratios. Thyroid stimulating hormone levels were also elevated in both sexes from the 500 ppm dose group. Other effects at 500 ppm included reduced hemoglobin and protein levels in males, and decreased glucose levels and thymus weights in females.

Indications of phase I enzyme induction were seen at 50 and 500 ppm in females for 6-beta testosterone hydroxylase and of phase II enzymes at 500 ppm only for microsomal epoxide hydrolase. The results exclude a potent effect on liver enzymes by fluensulfone as the changes were considered biologically not relevant and likely to be a response of the constant load on the liver with a xenobiotic.

Other findings at 50 ppm included a slight decrease in plasma and hepatic ALAT activity in both sexes, a slight and transient decrease in bilirubin in males, and slightly reduced food consumption in females. At 5 ppm, there was a slight reduction in hepatic ALAT activity in females only.

All the effects observed at 5 and 50 ppm were slight and reversible, and considered unlikely to represent an adverse effect; therefore, the NOAEL was considered to be 50 ppm (equivalent to an achieved intake of 1.6 mg/kg/day in males and 1.8 mg/kg bw/day in females).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: The study investigators considered the reductions in body weight gain, hematological findings, and liver effects (increased weight, changes in liver enzyme activities, and slight diffuse hepatocellular hypertrophy in one female) noted at 500 ppm to be slight and reversible and unlikely to represent an adverse effect. The reviewer noted additional treatment-related effects that the study investigators did not consider to be treatment-related, including the reduced body weight in females, decreased hemoglobin in males, and the increased reticulocytes, decreased MCHC, decreased bilirubin and Kupffer cell pigmentation in both sexes. The study investigators also discounted the higher TSH levels at 500 ppm as being unrelated to treatment due to the lack of a dose relationship and the fact that the value for only one time point in one sex attained statistical significance. In males, the lack of a dose response can be attributed to the control mean which was slightly elevated than the means for the 5 and 50 ppm dose groups, while in females, the lack of a dose response can be attributed to the higher mean in the 5 ppm dose group. However, these trends were noted during the pre-test, and when the values obtained at weeks 6 and 13 are compared to pre-test values, a clear increase in TSH levels is apparent in both sexes at 500 ppm. The study investigators did not note the reduced thymus weight in females at 500 ppm; this effect is likely a secondary effect to the decreased body weight noted in these animals.

This study in dogs is acceptable/guideline (fully reliable) and satisfies the guideline requirement for a 90-day oral toxicity study in dogs (OPPTS 870.3150; OECD 409).

C. Conclusions: Overall, the reviewer considers the effects at 500 ppm to be indicative of an adverse effect of treatment. Therefore, the NOAEL and LOAEL in this study are considered to be 50 and 500 ppm, respectively.

D. Deficiencies: Sorbital dehydrogenase was not measured during the clinic chemistry analysis. Volume was not assessed during urinalysis. The nose, pharynx, and peripheral nerve were not collected upon sacrifice and the gall bladder was not weighed. These omissions do not alter the conclusions or acceptability of the study. No other deficiencies were noted.

Tier II Summary (PMRA #2181209, MRID 48574780) revised by the regulatory authorities, April 2013

Study Type: Percutaneous 28-day toxicity (rodents)

IIA 5.3.7 - MRID 48574780, PMRA 2181209

Report	Sommer E.W. (2011) MCW-2 TECH: 4-Week Dermal Toxicity Study in Rats Harlan Laboratories Ltd, Switzerland; unpublished Report D09837, dated 22 December 2011. Sponsor reference No. R-23348 Dates of experimental work: 31 May to 2 December 2011
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines	OECD 410, EPA OPPTS 870.3200 and EEC method B.9 Deviations: none
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In a repeated dose dermal toxicity study (MRID 48574780, PMRA 2181209), groups of 10 male and 10 female Wistar rats were dermally exposed for 6 hours each day for 5 consecutive days per week over a period of 4 weeks (28 days) to fluensulfone (MCW-2 tech., 94.44%, Lot No. 36372130-291-PFI) at doses of 0 (control), 80, 400 or 2000 mg/kg bw/day. Carboxymethylcellulose (0.5% CMC) was used as the vehicle and the exposed area of approximately 25 cm^2 (approximately 10% of the total body surface) was covered by a semiocclusive dressing.

No mortality occurred, and no clinical signs of toxicity were observed. During clinical observations, no test item-related effects were recorded at the treated skin sites. Increased concentrations of fluoride in bones and teeth were noted in treated animals of all dose groups but were not considered adverse, because no signs of dental fluorosis were observed during the study, and no abnormality in bone structure was observed at microscopic examination.

Transient reductions in food consumption were noted in males at 400 mg/kg bw/day and in both sexes at 2000 mg/kg bw/day. A slight reduction in body weight gain, driven by a decrease in body weight gain during week 1 of the study, was noted in males at the 2000 mg/kg bw/day dose level. In females dosed with 2000 mg/kg bw/day fluensulfone, slight alterations in hematological (increased red cell volume distribution width, decreased mean corpuscular hemoglobin concentration, and increased reticulocytes) and clinical chemistry (increased cholesterol, reduced serum ALAT) parameters were observed. No effect on hepatic ALAT activity was observed.

The combination of the vehicle (CMC) with fluensulfone led to minimally increased incidence and/or severity of acanthosis/hyperkeratosis noted upon histological examination of treated skin of animals dosed at 400 and 2000 mg/kg bw/day when compared with the vehicle alone. These dermal effects were considered indicative of a slight irritant potential of the test item. Because of

the moderate degrees of acanthosis/hyperkeratosis observed in this study, these changes were considered adaptive and likely to be reversible after cessation of treatment, and therefore not adverse.

The NOAEL was determined to be 400 mg/kg bw/day, based on reduced body weight gain, increased prothrombin time and increased relative spleen weight in males and increased red cell distribution width, increased reticulocytes and increased cholesterol in females at 2000 mg/kg bw/day.

This 28-day dermal toxicity study in the rat is classified as **acceptable/guideline** and satisfies the guideline requirement for a 28-day dermal toxicity study (OPPTS 870.3200/OECD 410) in rats.

I. MATERIAL AND METHODS

1. Test Material	MCW-2 TECH (fluensulfone)
Description:	Solid, yellow
Lot/Batch:	36372130-291-PFI
Purity:	94.44%
CAS#:	318290-98-1
Stability:	Stable for the duration of the study
2. Vehicle	Aqueous solution of 0.5% Carboxymethylcellulose (CMC - Carboxymethylcellulose sodium salt, medium viscosity 400 - 800 cps) in purified water
3. Test Animals	
Species	Rat
Strain	Wistar [RccHan^{TM}: WIST(SPF)]
Age	8 weeks at start of dosing
Weight	Males: 173.95 to 193.70 g (mean: 181.55 g); Females: 124.80 to 149.40 g (mean: 135.34 g) at acclimatization
Source	Harlan Laboratories, B.V., The Netherlands
Acclimation period	6 days
Diet	Pelleted standard Kliba Nafag 3433 rodent maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum*
Water	Community tap-water from Itingen *ad libitum* in water bottles
Housing	Individually in Makrolon type-3 cages with wire mesh tops and standard softwood bedding (J. Rettenmaier & Söhne GmbH & Co., Germany, imported by Provimi Kliba SA, Switzerland) including paper enrichment (Enviro-dri from Lilico, Biotechnology, UK)

4. Environmental conditions

Temperature	22 ± 3°C
Humidity	30 - 70% (71% measured on a single occasion; no effect on the study)
Air change	10 - 15 per hour
Photoperiod	12-hour fluorescent light/12-hour dark cycle with music during the light period

B. STUDY DESIGN

1. In-life dates 31 May to 5 July 2011

2. Animal assignment and treatment

Animals were assigned to groups using a computer-generated random algorithm.

Treatment was epidermal on the clipped intact skin of the back, on an exposed area of approximately 10% of the total body surface. The fur on the back of each rat was closely clipped as necessary (in individual animals the fur was clipped 1 to 4 times in 4 weeks) exposing an area of approximately 25 cm². The necessary amount of dosing formulation for each animal (as automatically calculated based on body weight by the Tox Control System-software; dosing volume of 5 mL/kg bw) was filled into a syringe and transferred onto the gauze pad. Afterwards, the gauze pad was placed onto the skin and fixed with the bandage. The gauze pad was placed on the bandage (30 cm x 5 cm) with the test item facing upwards. The clipped back of the rat was then brought in contact with the test item on the gauze pad and the bandage was firmly wrapped around the trunk of the animal. A second bandage (30 cm x 2.5 cm) was then wrapped around the first bandage to fix its borders and to assure a tight semi-occlusive dressing.

Control animals were bandaged only, without application of test item, but aqueous 0.5% CMC was applied to the gauze pad beforehand. Treated animals were dosed with 80, 400 or 2000 mg/kg bw/day of fluensulfone.

The application was semi-occlusive for 6 hours per day for 4 cycles of 5 consecutive days per week over a period of 28 days (accounting for a total of 20 applications) with an exposure-free period of 2 days after each cycle. After each 6 hour daily exposure period, the dressing and gauze were removed carefully and the treated area was gently rinsed with lukewarm tap water (and with the use of a separate sponge for each dose group), and the skin was dried with a disposable paper towel.

3. Dose Preparation and Analysis

The dose formulations were prepared daily. Fluensulfone was warmed in the original container in a water bath up to maximum 40°C until the test item became fluid. Thereafter, the whole amount of liquid test item was transferred into a glass flask (most of the time wrapped with aluminum foil) and mixed using a magnetic stirrer on a heating plate (maximum temperature of approximately 40°C). The required amount of fluensulfone was weighed into a tared glass flask (most of the time wrapped with aluminum foil) on a tared Mettler balance and then kept at a maximum temperature of approximately 40°C. The vehicle (0.5% CMC, medium viscosity, prepared with purified water)

was added (w/v), and the dose formulation mixed using a suitable laboratory mixer. The mixtures were vigorously stirred using a magnetic stirrer at a maximum temperature of approximately 40°C until administration onto the gauze pad. Homogeneity of fluensulfone in the vehicle was maintained during the daily administration period using a magnetic stirrer.

On the first treatment day samples from the control group as well as 3 samples (top, middle and bottom) of about 2 g of the dose formulation at 400 mg/mL were taken prior to dosing for analysis of concentration and homogeneity. The aliquots for analysis of dose formulations were frozen (-20 ± 5 °C), delivered on dry ice to the person responsible for the analysis and stored at -20 ± 5°C until analysis.

The samples (total amount transferred or homogenized before taking subsamples) were analyzed by HPLC coupled to a UV detector following an analytical procedure provided by the Sponsor, adapted at Harlan Laboratories Ltd. and tested in a previous study (MRID 48574770, PMRA 2181224). Fluensulfone was used as the analytical standard.

4. Statistics

The following statistical methods were used to analyze the grip strength, locomotor activity, food consumption, body weight, ophthalmoscopic examinations, fluoride determination in bones and teeth, macroscopic findings, organ weights and ratios, as well as clinical laboratory data:

- The Dunnett-test (many to one t-test) based on a pooled variance estimate was applied if the variables could be assumed to follow a normal distribution for the comparison of the treated groups and the control groups for each sex (i.e. for food consumption, body weight, grip strength, locomotor activity, clinical laboratory data, organ weights and ratios).
- The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data could not be assumed to follow a normal distribution (i.e. for analysis of the clinical laboratory data and data obtained in the fluoride determination for bones and teeth).
- Fisher's Exact-Test was used for analysis of ophthalmoscopic and macroscopic findings.

C. METHODS

1. Observations

Checks for viability/mortality were performed twice daily. General clinical observations were carried out once during the acclimatization period and daily thereafter. Skin reactions for erythema and edema were checked before application and after removal of dressing and gauze each treatment day.

Detailed behavioral observations were performed weekly. In addition, during the last week of treatment and before application for that day (day 24 or 25), relevant parameters from a modified Irwin screen test (functional observations), including grip strength measurements and locomotor activity assessments, were evaluated on all rats. These observations were based upon the procedures used for the detailed behavioral observations. Any abnormal findings were recorded and graded in severity. The hind- and forelimb grip strength measurement was performed using a push-pull strain gauge. Locomotor activity was measured quantitatively with an automated system [AMS Foehr Medical Instruments GmbH (FMI) and DeMeTec GmbH). With this system, the animals remain in their homecage, which is placed within the sensor frames, each equipped with

three infrared beams that measure locomotor activity in the x- and y-direction. Activity of the animals was recorded for 10-minute intervals over a period of 60 minutes. Interval data and the total activity over 60 minutes were reported.

Ophthalmoscopic examination was performed after instillation of a mydriaticum in all animals during acclimatization. During week 4, animals of the control and high dose groups were examined after instillation of a mydriaticum before the treatment was applied for that day. As there were no test item-related findings in the high dose group, animals of the low and mid dose groups were not examined.

2. Bodyweight

Body weights were recorded weekly.

3. Food consumption

Food consumption was determined weekly.

4. Haematology and Clinical Chemistry

After 4 weeks, blood samples were drawn from the retro-orbital plexus from all animals under light isoflurane anesthesia. The animals were fasted for approximately 18 hours before blood sampling but allowed access to water *ad libitum*. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms. Urine was collected from all animals during the 18 hours fasting period into a specimen vial, using a metabolism cage.

The following parameters were determined at hematology:

> Erythrocyte count, Hemoglobin concentration, Hematocrit, Mean corpuscular volume, Red cell volume distribution width, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration, Hemoglobin concentration distribution width, Platelet count, Reticulocyte count, Reticulocyte maturity index, Total leukocyte count, Differential leukocyte count, Thromboplastin time, Activated partial thromboplastin time, and Methemoglobin (slides were also prepared for the evaluation of Heinz bodies but this analysis was not conducted as no changes were seen in the methemoglobin level)

The following parameters were determined at clinical chemistry:

> Glucose, Urea, Creatinine, Bilirubin (total), Cholesterol (total), Triglycerides, Aspartate aminotransferase, Alanine aminotransferase, Lactate dehydrogenase, Creatine kinase, Alkaline phosphatase, Gamma-glutamyl-transferase, Calcium, Phosphorus, Sodium, Potassium, Chloride, Protein (total), Albumin, Globulin, and Albumin/Globulin Ratio

The following urinalysis parameters were determined:

> Color, Appearance, Volume, Relative density, pH, Protein, Glucose, Ketones, Nitrite, Urobilinogen, Bilirubin, Erythrocytes, Leukocytes

5. Sacrifice and pathology

All animals were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination. Organ weights were recorded, and their organ to terminal body weight ratios determined.

An aliquot of the livers from treated animals was collected at necropsy, and after weighing, defined parts were snap frozen in liquid nitrogen. They were stored at -80 ± 10 ºC until analysis for alanine aminotransferase (ALAT). Additional liver samples of untreated control animals of the same age were collected after the end of the in life part of the study, frozen in liquid nitrogen and stored at -80° C until subcellular fractionation and ALAT determination. Since the experimental variation was not unacceptably high, and an effect on ALAT activity in the high dose group could be excluded when compared with control data, the remaining samples from the mid dose group were not analyzed.

Samples of a complete list of tissues and organs (Table 1) were collected from all animals at necropsy and fixed in neutral phosphate buffered 4% formaldehyde solution (unless otherwise stated). In addition, the right femur and the incisors of the maxilla of all surviving animals were collected for fluoride determination using ion chromatography. The samples were stored at room temperature in plastic bags (Teflon®-free) until shipment to the responsible study scientist. The samples from the first 5 surviving animals per sex in the control and high dose group were initially analyzed, and the remaining samples from the intermediate groups were then also analyzed after significant effects were observed in the samples from the high dose group. The fluoride samples were stored frozen at -80 ± 10°C upon receipt until work up.

Slides of the test site, and from all organs and tissues collected at terminal sacrifice from the animals of the control and high-dose groups were examined by the study pathologist. The same applied to all occurring gross lesions. As test item-related morphologic changes were detected on some treated/untreated skin sites in high-dose animals, the corresponding treated/untreated skin sites from the mid- and low-dose groups were also examined.

Table 1: Summary of tissues collected for pathology examination

Organ/tissue	Weighed	Collected	Examined
Adrenal gland	X	X	X
Aorta		X	X
Bone - sternum, femur including articular surface		X	X
Bone marrow - femur		X	X
Brain - including medulla/pons, cerebral and cerebellar cortex	X	X	X
Cecum		X	X
Colon		X	X
Duodenum		X	X
Epididymides (fixed in Bouin's solution)	X	X	X
Esophagus		X	X
Eyes with optic nerve (fixed in Davidson's solution)		X	X
Harderian gland (fixed in Davidson's solution)		X	X
Heart (including auricles)	X	X	X
Ileum, with Peyer's patches		X	X
Jejunum, with Peyer's patches		X	X
Kidneys	X	X	X
Larynx		X	X
Lacrimal gland, exorbital		X	X
Liver	X	X	X
Lungs, filled with formalin at necropsy		X	X
Lymph nodes – mesenteric, mandibular and popliteal		X	X
Mammary gland area		X	X
Nasal cavity		X	X
Ovaries	X	X	X
Pancreas		X	X
Pharynx		X	X
Pituitary gland		X	X
Prostate gland including coagulating glands		X	X
Rectum		X	X
Salivary glands - mandibular, sublingual		X	X
Sciatic nerve		X	X
Seminal vesicles		X	X
Skeletal muscle		X	X
Skin (treated and untreated)		X	X
Spinal cord - cervical, midthoracic, lumbar segments		X	X
Spleen	X	X	X
Stomach		X	X
Testes (fixed in Bouin's solution)	X	X	X
Thymus	X	X	X
Thyroid gland with parathyroid gland	X	X	X
Tongue		X	X
Trachea		X	X
Ureter		X	X
Urinary bladder (filled with formalin at necropsy)		X	X
Uterus with cervix and vagina		X	X
All gross lesions		X	X

II. RESULTS AND DISCUSSION

A. ANALYTICS

A mean concentration of 85.6% fluensulfone was recovered in the dose formulation prepared at 400 mg/mL in 0.5% CMC. Fluensulfone was homogeneously distributed in the dosing formulation as the deviation from the mean was up to 4.4% of the nominal concentration.

B. OBSERVATIONS

1. Clinical signs

No fluensulfone-related effects were recorded at the treated skin sites during clinical observations. In addition, no fluensulfone-related clinical signs or behavioral changes were observed.

A slight erythema (grade 1) was present at the application site on one day only in the first half of week 1 in two males (no. 21 and 22) dosed at 400 mg/kg bw/day. Given the low incidence and the small magnitude in the occurrence of this transient effect, the lack of any skin effect in the other treated male groups, this clinical sign was considered to be incidental to treatment.

2. Mortality

There was no mortality in the study.

3. Ophthalmic examination

No treatment-related changes to the eyes were observed upon ophthalmic examination.

4. Neurological assessments

FOB, grip strength evaluation and locomotor activity assessments did not reveal any treatment-related findings.

Mean hindlimb grip strength in males dosed at 400 mg/kg bw/day was statistically significantly increased when compared to controls (Table 2); however, this alteration did not show any dose-response relationship and was therefore not considered to be treatment-related.

Table 2. Mean ± S.D. grip strength

Parameter	Dose (mg/kg bw/day)			
	0	80	400	2000
Males				
Forelimb grip strength (kg)	1.32 ± 0.09	1.30 ± 0.20	1.46 ± 0.18	1.36 ± 0.11
Hindlimb grip strength (kg)	0.90 ± 0.10	0.90 ± 0.11	1.05 ± 0.14* (↑17%)	0.99 ± 0.17
Females				
Forelimb grip strength (kg)	1.15 ± 0.16	1.13 ± 0.16	1.16 ± 0.18	1.20 ± 0.10
Hindlimb grip strength (kg)	0.79 ± 0.07	0.85 ± 0.16	0.77 ± 0.08	0.82 ± 0.12

* Statistically significantly different from controls ($p<0.05$). Data obtained from pages 81-82 of the study report. N=10/sex/dose.

There was a statistically significant decrease in locomotor activity in females dosed at 400 mg/kg bw/day when compared with controls (Table 3), and a slight decrease in locomotor activity in males dosed at 400 and 2000 mg/kg bw/day which did not attain any statistical significance or follow a dose-dependent trend. These observations are not considered to be treatment-related.

Table 3. Mean ± S.D. locomotor activity (counts)

Interval	Dose (mg/kg bw/day)			
	0	**80**	**400**	**2000**
Males				
0-10 min	540 ± 66	664 ± 166	607 ± 101	543 ± 115
10-20 min	345 ± 79	390 ± 143	407 ± 123	349 ± 115
20-30 min	245 ± 104	200 ± 135	184 ± 103	226 ± 142
30-40 min	189 ± 94	127 ± 124	80 ± 75	125 ± 110
40-50 min	106 ± 83	50 ± 67	51 ± 69	103 ± 108
50-60 min	62 ± 58	23 ± 23	57 ± 77	42 ± 63
Total	1486 ± 228	1454 ± 476	1385 ± 302	1389 ± 419
Females				
0-10 min	548 ± 113	688 ± 144*	452 ± 131	543 ± 114
10-20 min	315 ± 84	383 ± 151	181 ± 127*	319 ± 87
20-30 min	139 ± 127	135 ± 154	49 ± 79	226 ± 113
30-40 min	109 ± 107	83 ± 110	33 ± 48	148 ± 112
40-50 min	104 ± 116	54 ± 104	27 ± 31	57 ± 96
50-60 min	108 ± 123	101 ± 110	83 ± 146	35 ± 55
Total	1322 ± 259	1443 ± 519	824 ± 326*	1328 ± 391

* Statistically significantly different from controls ($p<0.05$). Data obtained from pages 84-87 of the study report. N=10/sex/dose.

C. BODYWEIGHT AND BODYWEIGHT GAINS

The slight reduction in food consumption in females dosed at 2000 mg/kg bw/day during week 1 (see section D below) had no effect on body weight when compared to controls (Table 4). However, body weight gain (Table 5) in males dosed at 2000 mg/kg bw/day was statistically significantly reduced on day 8, and was still slightly reduced at treatment end, without attaining statistical significance.

Table 4. Mean ± S.D. body weights (g)

Test Day	Dose (mg/kg bw/day)			
	0	80	400	2000
Males				
Day 1	235 ± 5	236 ± 6	234 ± 8	238 ± 4
Day 8	268 ± 8	264 ± 11	263 ± 11	264 ± 8
Day 15	293 ± 11	289 ± 14	291 ± 12	289 ± 10
Day 22	316 ± 12	315 ± 16	314 ± 16	310 ± 14
Day 28	327 ± 12	325 ± 18	323 ± 19	318 ± 14 (↓3%)
BWG	92	89	89	80 (↓13%)
Females				
Day 1	163 ± 8	162 ± 8	164 ± 7	165 ± 7
Day 8	178 ± 9	178 ± 9	181 ± 11	178 ± 11
Day 15	191 ± 8	191 ± 10	195 ± 13	191 ± 13
Day 22	201 ± 16	207 ± 12	205 ± 18	205 ± 13
Day 28	206 ± 12	214 ± 12	209 ± 18	209 ± 14
BWG	43	52	45	44

N=10/sex/dose. Data obtained from pages 95-96 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). BWG = body weight gain from days 1 to 28, calculated by the reviewer based on the mean body weight values on days 28 and 1.

Table 5. Mean ± S.D. body weight gains (% of initial body weight)

Test Day	Dose (mg/kg bw/day)			
	0	80	400	2000
Males				
Day 8	14 ± 3	12 ± 2	12 ± 2	11 ± 2* (↓21%)
Day 15	24 ± 3	22 ± 3	24 ± 4	22 ± 3
Day 22	34 ± 4	33 ± 4	34 ± 5	30 ± 5 (↓12%)
Day 28	39 ± 4	37 ± 5	38 ± 6	34 ± 5 (↓13%)
Females				
Day 8	9 ± 3	10 ± 2	10 ± 3	8 ± 3
Day 15	17 ± 4	18 ± 4	19 ± 4	15 ± 4
Day 22	23 ± 7	27 ± 6	25 ± 7	24 ± 5
Day 28	27 ± 6	31 ± 4	28 ± 7	26 ± 5

N=10/sex/dose. Data obtained from pages 98-99 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * Statistically significantly different from the control mean ($p<0.05$).

D. FOOD CONSUMPTION

During week 4, food consumption was statistically significantly reduced in males dosed at 400 and 2000 mg/kg bw/day (Table 6). However, the overall food consumption in males dosed at 400 and 2000 mg/kg bw/day was reduced by only 3 and 5%, respectively, when compared to controls, without attaining any statistical significance. Food consumption in females was not affected.

Table 6. Mean ± S.D. absolute food consumption (g/animal/day)

Study Week	Dose (mg/kg bw/day)			
	0	80	400	2000
Males				
Pre-test	21.36 ± 0.95	21.39 ± 1.17	22.17 ± 1.80	21.65 ± 0.95
Week 1	23.11 ± 1.57	22.28 ± 1.62	22.33 ± 1.71	21.98 ± 1.44
Week 2	24.59 ± 1.64	24.16 ± 3.63	24.70 ± 1.61	23.94 ± 1.81
Week 3	23.86 ± 1.70	23.73 ± 1.34	23.40 ± 1.63	22.94 ± 1.58
Week 4	25.40 ± 1.52	24.72 ± 1.93	23.14 ± 2.20* (↓9%)	22.93 ± 1.70* (↓10%)
Overall (mean of means)	24.24	23.72	23.39	22.95 (↓5%)
Females				
Pre-test	15.53 ± 0.66	15.65 ± 1.89	15.37 ± 1.40	16.74 ± 1.40
Week 1	17.10 ± 1.27	16.69 ± 1.21	16.77 ± 1.36	16.04 ± 1.77 (↓6%)
Week 2	18.20 ± 1.88	17.95 ± 1.13	17.52 ± 1.49	17.83 ± 1.51
Week 3	17.05 ± 2.50	18.04 ± 1.42	17.08 ± 1.53	17.64 ± 1.31
Week 4	17.71 ± 1.54	19.63 ± 2.17	17.46 ± 1.98	17.78 ± 1.44
Overall (mean of means)	17.52	18.08	17.21	17.32

N=10/sex/dose. Data obtained from pages 89-90 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * Statistically significantly different from the control mean ($p<0.05$).

Relative food consumption was reduced during week 4 in males dosed at 400 and 2000 mg/kg bw/day (Table 7). Relative food consumption was statistically significantly reduced during week 1 in females dosed at 2000 mg/kg bw/day but returned to normal during week 2. However, the overall relative food consumption in males and females was not affected by treatment with the test item.

Table 7. Mean ± S.D. relative food consumption (g/kg bw/day)

Study Week	Dose (mg/kg bw/day)			
	0	80	400	2000
Males				
Pre-test	117.96 ± 5.07	116.51 ± 6.56	122.53 ± 8.78	120.11 ± 7.81
Week 1	86.21 ± 3.58	84.35 ± 4.17	84.78 ± 4.95	83.24 ± 4.36
Week 2	83.90 ± 2.80	83.55 ± 11.75	84.90 ± 3.50	82.78 ± 4.49
Week 3	75.43 ± 3.36	75.48 ± 2.67	74.40 ± 2.92	73.94 ± 3.90
Week 4	80.35 ± 3.32	78.58 ± 4.75	73.48 ± 3.79** (↓8%)	73.93 ± 4.54** (↓8%)
Overall (mean of means)	81.47	80.49	79.39	78.47
Females				
Pre-test	116.53 ± 6.86	114.14 ± 11.05	112.04 ± 9.93	125.41 ± 10.55
Week 1	96.19 ± 6.36	93.76 ± 4.77	92.79 ± 5.24	89.73 ± 5.76* (↓7%)
Week 2	95.16 ± 7.92	93.94 ± 5.27	89.87 ± 3.79	93.31 ± 3.54
Week 3	84.59 ± 9.00	87.34 ± 5.52	83.19 ± 3.97	86.12 ± 4.63
Week 4	88.63 ± 10.24	95.02 ± 8.91	84.88 ± 4.57	86.84 ± 6.25
Overall (mean of means)	91.14	92.52	87.68	89.00

N=10/sex/dose. Data obtained from pages 92-93 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * Statistically significantly different from the control mean ($p<0.05$). ** Statistically significantly different from the control mean ($p<0.01$).

E. BLOOD AND URINALYSIS

1. Haematological findings

The values for absolute basophil counts in males dosed at 400 mg/kg bw/day and in females dosed at 400 and 2000 mg/kg bw/day as well as the relative basophil count in females dosed at 2000 mg/kg bw/day were slightly lower than the corresponding control values (Table 8), but no dose response was observed.

The prothrombin time (presented as a ratio of normal activity) was slightly longer in males dosed at 2000 mg/kg bw/day (the mean presented in the study report was 0.89 but the mean calculated by the reviewer was 0.93; the latter mean is presented in Table 8 below).

In females from the 2000 mg/kg bw/day dose group, the mean values for the red cell volume distribution width (RDW) and reticulocyte counts were increased while the mean corpuscular hemoglobin concentration (MCHC) was slightly but statistically significantly reduced.

Table 8. Selected hematology values

Parameter	Dose (mg/kg bw/day)			
	0	80	400	2000
Males				
Basophils (G/L)	0.05 ± 0.02	0.04 ± 0.02	0.03 ± 0.01*	0.04 ± 0.01
Basophils (rel)	0.006 ± 0.001	0.006 ± 0.001	0.005 ± 0.001	0.005 ± 0.002
RDW (rel)	0.125 ± 0.018	0.126 ± 0.027	0.116 ± 0.002	0.117 ± 0.009
MCHC (mmol/L)	23.04 ± 0.65	23.04 ± 0.23	22.77 ± 0.16	22.69 ± 0.47
Reticulocytes (G/L)	110 ± 23	121 ± 17	126 ± 9	114 ± 24
Prothrombin time (rel)	0.84 ± 0.05	0.88 ± 0.08	0.87 ± 0.04	0.93 ± 0.09* (↑11%)
Females				
Basophils (G/L)	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01* (↓33%)	0.02 ± 0.01** (↓33%)
Basophils (rel)	0.006 ± 0.002	0.006 ± 0.002	0.005 ± 0.001	0.004 ± 0.001* (↓33%)
RDW (rel)	0.119 ± 0.007	0.119 ± 0.006	0.119 ± 0.004	0.131 ± 0.033 (↑10%)
MCHC (mmol/L)	23.33 ± 1.08	22.70 ± 0.47	22.92 ± 0.55	22.32 ± 0.36** (↓4%)
Reticulocytes (G/L)	147 ± 59	189 ± 57	152 ± 25	206 ± 58* (↑40%)
Prothrombin time (rel)	0.85 ± 0.04	0.86 ± 0.06	0.87 ± 0.06	0.87 ± 0.07

N= 9 for females in the control group and for males in the 2000 mg/kg bw/day dose group (one sample was coagulated) and 10/sex/dose for all other dose groups. * Statistically significantly different from the control mean ($p<0.05$). ** Statistically significantly different from the control mean ($p<0.01$). Data obtained from pages 107-110 of the study report. Standard deviations were not provided in the study report and were calculated by the reviewer. Values in parentheses represent the percent change from controls (calculated by the reviewer).

2. Blood chemistry findings

The value for glucose was statistically significantly increased in females dosed at 400 mg/kg bw/day, the value for phosphorus was statistically significantly increased in males dosed at 400 mg/kg bw/day, and the values for glucose and potassium were statistically significantly increased in males dosed at 80, 400 and 2000 mg/kg bw/day (Table 9). However, these alterations did not follow a dose dependent trend and were not considered to be related to treatment. Although not statistically significant, a slight elevation in cholesterol and a slight reduction in alanine aminotransferase (ALAT) activity were noted in females at 2000 mg/kg bw/day.

Table 9. Selected clinical chemistry values

Parameter	Dose (mg/kg bw/day)			
	0	80	400	2000
Males				
Glucose (mmol/L)	4.88 ± 0.43	5.64 ± 0.82*	5.77 ± 0.64** (↑18%)	5.55 ± 0.41* (↑14%)
Cholesterol (mmol/L)	1.67 ± 0.27	1.84 ± 0.41	1.65 ± 0.27	1.85 ± 0.33
Potassium (mmol/L)	3.55 ± 0.12	3.84 ± 0.15** (↑8%)	3.85 ± 0.21** (↑8%)	3.93 ± 0.17** (↑11%)
Phosphorus (mmol/L)	2.13 ± 0.09	2.15 ± 0.08	2.28 ± 0.13*	2.24 ± 0.15
ALAT (U/L)	28.9 ± 7.2	28.8 ± 6.4	28.1 ± 4.3	28.2 ± 5.6
Females				
Glucose (mmol/L)	4.59 ± 0.73	5.31 ± 0.97	5.61 ± 0.74* (↑22%)	5.33 ± 0.69
Cholesterol (mmol/L)	1.46 ± 0.41	1.58 ± 0.50	1.56 ± 0.39	1.75 ± 0.42 (↑20%)
Potassium (mmol/L)	3.26 ± 0.23	3.12 ± 0.23	3.19 ± 0.20	3.45 ± 0.31
Phosphorus (mmol/L)	1.84 ± 0.21	1.79 ± 0.21	1.79 ± 0.21	1.91 ± 0.24
ALAT (U/L)	24.5 ± 5.6	23.8 ± 3.8	22.0 ± 2.8	21.3 ± 3.9 (↓13%)

N= 9 for females in the control group and for males in the 2000 mg/kg bw/day dose group (one sample was coagulated) and 10/dose at 80, 400 and 2000 mg/kg bw/day. * Statistically significantly different from the control mean ($p<0.05$). ** Statistically significantly different from the control mean ($p<0.01$). Data obtained from pages 112-113 of the study report. Standard deviations were not provided in the study report and were calculated by the reviewer. Values in parentheses represent the percent change from controls (calculated by the reviewer).

3. Urinalysis

No changes were noted in any of the urinalysis parameters examined that were attributable to treatment.

F. SACRIFICE AND PATHOLOGY

1. Organ Weights

For males in the 2000 mg/kg bw/day dose group, the relative spleen to body weight ratios were statistically significantly higher than controls. No dose-response relationship was observed in the absolute weights of the spleens. Additionally, the absolute body weight for males in the high dose group (2000 mg/kg/day) was slightly (3%) but not significantly lower than in controls. The lower body weight of the 2000 mg/kg bw/day males may be accountable for the apparent increase in the relative spleen weights of the high dose males. No dose-related findings were observed in organ weights of female rats.

Table 10. Terminal body weight and spleen weights

Parameter	Dose (mg/kg bw/day)			
	0	80	400	2000
Males				
Terminal bw (g)	294.8 ± 8.4	297.0 ± 17.3	299.5 ± 15.4	286.6 ± 22.2 (↓3%)
Absolute spleen wt (g)	0.62 ± 0.07	0.67 ± 0.08	0.64 ± 0.06	0.68 ± 0.06
Relative spleen wt (%)	0.21 ± 0.02	0.22 ± 0.02	0.21 ± 0.01	0.24 ± 0.01* (↑14%)
Females				
Terminal bw (g)	190.9 ± 9.0	198.6 ± 15.3	198.9 ± 17.6	196.0 ± 14.6
Absolute spleen wt (g)	0.50 ± 0.06	0.50 ± 0.09	0.50 ± 0.14	0.49 ± 0.08
Relative spleen wt (%)	0.26 ± 0.03	0.25 ± 0.04	0.25 ± 0.06	0.25 ± 0.03

Data obtained from pages 118-126 of the study report. * Statistically significantly different from the control mean ($p<0.05$).

2. Gross Pathology

No treatment-related macroscopic findings were noted in this study. At the skin application sites there were no differences recorded between control and treated animals. A few macroscopic findings were recorded during necropsy, sparsely distributed among control and/or treated groups; they were considered to be within the range of background alterations that are commonly observed in rats of the used strain and age in 4-week studies, and judged incidental, thus reflecting the normal individual variability.

3. Histopathology

Treatment with either the vehicle 0.5% CMC alone, or fluensulfone in the vehicle led to minimally increased incidence and/or severity of acanthosis/hyperkeratosis in treated skin of males dosed at 400 and 2000 mg/kg bw/day and females dosed at 2000 mg/kg bw/day (Table 11) when compared to untreated sites of control animals (Table 12). The presence of fluensulfone seems to minimally enhance this finding.

Table 11: Incidence and mean severity of acanthosis/hyperkeratosis in the treated skin

	Dose (mg/kg bw/day)							
	0		80		400		2000	
	M	F	M	F	M	F	M	F
Affected animals	10	2	10	3	10	2	10	6
Grade 1	2	2	0	3	0	2	0	6
Grade 2	8	0	10	0	4	0	5	0
Grade 3	0	0	0	0	6	0	5	0
Mean severity	1.8	1.0	2.0	1.0	2.6	1.0	2.5	1.0

1 = minimal; 2 = slight; 3 = moderate; Data obtained from page 376 of the study report; N=10/sex/dose.

Table 12: Incidence and mean severity of acanthosis/hyperkeratosis in the untreated skin

	Dose (mg/kg bw/day)							
	0		80		400		2000	
	M	F	M	F	M	F	M	F
Affected animals	1	2	1	2	2	3	2	2
Grade 1	1	1	1	2	2	2	2	2
Grade 2	0	1	0	0	0	1	0	0
Mean severity	1.0	1.5	1.0	1.0	1.0	1.3	1.0	1.0

1 = minimal; 2 = slight; 3 = moderate; Data obtained from page 376 of the study report; N=10/sex/dose.

4. ALAT determination in liver

Mean hepatic ALAT activities following daily dermal treatment with 80 and 2000 mg/kg bw/day fluensulfone for 28 days are presented in Table 13. As livers of the control animals were accidentally not collected in the study, an additional 5 untreated animals per sex of similar age were sacrificed and hepatic ALAT activity determined. No treatment-related effect on hepatic ALAT activity was apparent. There was no dose response between the low- and high dose groups in the study (despite of a 25-fold increase in dose).

Table 13: Hepatic ALAT activities (mean ± S.D. of 5 animals)

Group	ALAT (U/g liver)		ALAT (U/mg protein)	
	M	F	M	F
Untreated	60.3 ± 10.4	47.5 ± 5.7	0.396 ± 0.066	0.353 ± 0.050
80 mg/kg bw/day	103.5 ± 15.5	69.1 ± 7.2	0.649 ± 0.061	0.435 ± 0.062
2000 mg/kg bw/day	104.1 ± 9.5	67.3 ± 11.2	0.670 ± 0.102	0.404 ± 0.054

Data obtained from page 38 of the study report.

5. Fluoride determination in bones and teeth

The mean fluoride content in bones and incisors was statistically significantly increased in males and females at all dose levels when compared to controls (Table 14), except for the teeth in females dosed at 80 mg/kg bw/day in which the increase in fluoride concentration did not attain statistical significance.

Table 14: Mean fluoride concentration (ppm) in bones and teeth

Matrix	Sex	Dose (mg/kg bw/day)			
		0	80	400	2000
Teeth	M	133.4 ± 9.6	230.2 ± 38.3* (↑73%)	398.7 ± 104.6** (↑199%)	420.6 ± 101.8** (↑215%)
	F	111.1 ± 57.0	267.1 ± 108.4 (↑140%)	316.5 ± 23.2** (↑185%)	490.6 ± 122.8** (↑341%)
Bone	M	314.0 ± 17.7	502.6 ± 39.5 (↑60%)*	759.1 ± 53.9* (↑142%)	1092.0 ± 63.6* (↑248%)
	F	351.4 ± 27.3	462.3 ± 20.7 (↑31%)*	735.1 ± 93.7* (↑109%)	1304.0 ± 84.3* (↑271%)

* Statistically significantly different from the control mean ($p<0.05$). ** Statistically significantly different from the control mean ($p<0.01$). Data obtained from page 499-502 of the study report. N=5/sex/dose. Standard deviations were calculated by the reviewer. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

III. CONCLUSIONS

Dermal administration of fluensulfone to Wistar rats at doses of 80, 400 and 2000 mg/kg bw/day for 6 hours each day for 5 days weekly over a period of 28 days resulted in no toxicologically significant findings.

The increased concentrations of fluoride in bones and teeth of treated animals of all dose groups were not considered adverse, because no signs of dental fluorosis were observed during the study, and no abnormality in bone structure was observed at microscopic examination.

The combination of the vehicle (CMC) with fluensulfone led to minimally increased incidence and/or severity of acanthosis/hyperkeratosis in treated skin of males dosed at 400 mg/kg bw/day and in males and females dosed at 2000 mg/kg bw/day when compared with the vehicle alone. The dermal effects could be indicative of a slightly irritant potential of the test item. Since the findings represent an adaptive change to the vehicle and vehicle/test item, and because the moderate degrees of acanthosis/hyperkeratosis observed in this study are considered likely to be reversible after cessation of treatment, the finding was judged to represent an adaptive and not adverse change. In conclusion, fluensulfone did not induce any adverse skin effects.

Transient reductions in food consumption were noted in males at 400 mg/kg bw/day and in both sexes at 2000 mg/kg bw/day. A slight reduction in body weight gain, driven by a decrease in body weight gain during week 1 of the study, was noted in males at the 2000 mg/kg bw/day dose level. In females dosed with 2000 mg/kg bw/day fluensulfone, slight alterations in hematological (increased red cell volume distribution width, decreased mean corpuscular hemoglobin concentration, and increased reticulocytes) and clinical chemistry (increased cholesterol, reduced serum ALAT) parameters were observed. No effect on hepatic ALAT activity was observed.

The NOAEL was determined to be 400 mg/kg bw/day, based on reduced body weight gain, increased prothrombin time and increased relative spleen weight in males and increased red cell distribution width, increased reticulocytes and increased cholesterol in females at 2000 mg/kg bw/day.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments/Conclusions: The study investigators concluded that there were no treatment-related effects on body weight, body weight gain, food consumption, hematology, or clinical chemistry. The reviewer identified several effects for which the relationship to treatment cannot be discounted. Although the changes in several of the noted effects could be considered minimal when examined in isolation, the treatment-related alterations at 2000

mg/kg bw are consistent with findings in other toxicity studies conducted with fluensulfone and, when considered collectively, are indicative of slight systemic toxicity.

Therefore, the reviewer considers the LOAEL in this study to be 2000 mg/kg bw/day, based on statistically significant changes in prothrombin time and relative spleen weight in males, and reticulocytes in females, as well as the non-statistically significant decrease in body weight gain in males, and increases in cholesterol and red cell distribution width in females. The NOAEL for both males and females is 400 mg/kg bw/day.

OCS considers the increase in red cell distribution width in females at 2000 mg/kg bw/day to likely be incidental and not adverse based on the magnitude, the lack of statistical significance, and the large variability at the high dose.

This dermal toxicity study in the rat is classified as acceptable/guideline and satisfies the guideline requirement for a repeat-dose dermal toxicity study (OPPTS 870.3200; OECD 410) in rats.

C. Deficiencies: Livers of the control animals were accidentally not collected in the study; an additional 5 untreated animals per sex of similar age were sacrificed and hepatic ALAT activity determined. The results were slightly lower than those in the treated animals from the study. However, there was no treatment-related effect on hepatic ALAT activity. Clotting time and sorbitol dehydrogenase were missing from the hematology and clinical chemistry analyses. The bile duct and peripheral nerve were not collected upon sacrifice and the uterus was only collected and not weighed. These omissions do not alter the conclusions or acceptability of the study. No other deficiencies were noted.

Addendum to Tier II Summary (PMRA #2257061, MRID 49067003) revised by the regulatory authorities, April 2013

Study Type 90–day inhalation toxicity (rodents)

IIA 5.3.6 – MRID 49067003, PMRA 2257061

Report:	Staal, Y.C.M. and Jonker, D. (2012) Sub-chronic (90-day) inhalation toxicity study in rats with MCW-2. TNO Triskelion, The Netherlands; unpublished Report No. V20052, dated 15 August 2012 (Amendment No. 1 dated 3 September 2012). Sponsor reference No. R-28490 Dates of experimental work: 30 June 2011 to 14 August 2012 (including the dose range-finding study)
Sponsor:	Maktheshim Chemical Works Ltd., Beer Sheva, Israel
Guidelines:	OECD No. 413 (2009), OPPTS 870.3465 (1998), 440/2008 EC B.29 (2008)
Deviations:	Occasionally, the temperature or relative humidity in the animal room was out of the target range. On 2 November 2011 (exposure day 47), the exposure period was about 3 hours instead of 6 hours due to a power failure. The nominal concentration could not be calculated for the low-concentration test atmosphere of the main study because this test atmosphere was generated by dilution of the mid- concentration test atmosphere. These deviations were considered not to have affected the validity of the study.
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary:

In a 90-day inhalation toxicity study (MRID 49067003; PMRA 2257061), groups of 10 male and 10 female Wistar Crl:WI(Han) rats were exposed nose-only to fluensulfone (MCW-2 Technical, 96.8%, Batch No. 411-033-05) for 6 hours/day, 5 days/week for 13 consecutive weeks (resulting in 65 and 66 exposure days for males and females, respectively) to target concentrations of 0, 0.04, 0.2 or 1.0 mg/L (MMAD ± GSD of 1.02 µm ± 1.59, 1.16 µm ± 1.71, and 1.19 µm ± 1.60 for increasing test concentration). The achieved concentrations were 0.040, 0.204 and 0.983 mg/L for the 0.04, 0.2 and 1.0 mg/L target concentrations, respectively. A 14-day range-finding study was conducted on 5 rats/sex/dose at 0, 0.035, 0.35 or 3.5 mg/L (6 hours/day, 5 days/week) to identify appropriate dose levels.

No mortality occurred during the study. Piloerection in combination with hunched posture and/or blepharospasm was noted in a few animals of the mid- and high-concentration groups shortly after exposure on the first exposure day, but not thereafter. Reduced body temperatures were recorded in females from the mid- and high-concentration groups (decreases of 0.57 and 1.47°C, respectively, from the control mean) at the end of the 90-day exposure period; a reduction of 0.54°C was observed in males from the high concentration group but was not statistically significant. In male rats, body weight development was affected from day 3 onwards at all concentrations. The terminal body weights were decreased by 7%, 12% and 14% at the low-, mid- and high-concentration, respectively. The exposed males showed weight loss during exposure days, followed by catch-up growth during the weekend (when they were not exposed). Cumulative weight gain in males was reduced by 17%, 29% and 36% (low, mid, high dose, respectively). Female body weights were decreased only at the high-concentration during the first half of the study and were comparable to the control afterwards; however cumulative gain was reduced by 16%. Feed consumption of the exposed males from all groups and of females from the high concentration group was slightly lower compared to controls throughout the study. Water consumption was increased, concentration-dependently, in all concentration groups in both sexes.

Hematology results showed a concentration-dependent increase in prothrombin time in females at all exposure concentrations (7%, 10% and 19%) and in males from the high-concentration group only (16%), an increase in reticulocytes in males from the high-concentration group (26%), a decrease in platelet counts in females from the high-concentration group (9%), and slight decreases (2-3%) in mean corpuscular hemoglobin concentration in females from the mid- and high-concentration groups. Clinical chemistry values showed changes in alanine aminotransferase (ALAT; decreased 53%) and bilirubin (increased 53-56%) in both sexes at the high-concentration. ALAT was also decreased in males of the mid-concentration group (29%). In addition, males showed changes in glucose (decreased 31%) and inorganic phosphate (increased 18%) at all concentrations, in potassium (increased 13%) at the mid- and high-concentration, and in phospholipids (increased 17%) and alkaline phosphatase (ALP; increased 15%) at the high-concentration. Females additionally showed increases in ALP at the mid- and high-concentrations (10-31%), and increases in triglycerides (82%)

and potassium (10%) at the high-concentration.

The relative weights of the kidneys (12-20%) and liver (7-19%) were increased at the mid- and high concentrations in both sexes. In females, the absolute weights of these organs were increased as well. In addition, males had decreased thymus weights (absolute and relative) at all concentrations (15-45%), increased adrenal weights at the mid- and high concentrations, and increased relative heart weight at the high-concentration. The changes in kidney, liver, thymus and adrenal weight showed a concentration-related response. Relative lung weights were slightly elevated in both sexes at the high concentration (9-15%). The other organ weight changes observed occurred in males only and were likely to be secondary to the lower body weight in this sex (higher relative weights of the brain at the mid dose and above and testes at the high dose, lower absolute weight of the spleen at the mid dose and above).

Microscopic examination revealed treatment-related changes in the epiglottis and the nose (mainly at the second level of the nasal cavity). No histopathological correlates to the weight changes of liver, adrenals and thymus were observed. Females exhibited a slightly increased incidence of corticomedullary mineralization of the kidney at the high concentration when compared to controls (the kidney was not examined at lower concentrations), but no histopathological correlates to the change in kidney weight were noted in males.

The changes in the epiglottis were characterized by squamous metaplasia in all exposed animals, a concentration-dependent increase in the incidence of epithelial hyperplasia (starting at the low-concentration in males and the mid-concentration in females), and an increase in the presence of focal mononuclear cell infiltrates in males (starting at the low-concentration). Alterations in the nasal cavity consisted of squamous epithelial hyperplasia in males starting at the low-concentration and in females starting at the mid-concentration, squamous metaplasia in both sexes starting at the mid-concentration, squamous epithelial hyperkeratosis starting at the mid-concentration in females and at the high-concentration in males, and an increased inflammatory load characterized by focal mononuclear cell infiltrates (in both sexes starting at the mid-concentration) and focal polymorphonuclear leukocytic infiltration (in females at the high-concentration).

Pale lower incisors at necropsy were observed in females at all exposure concentrations (2, 4 and 9 affected at the low, mid and high concentrations, respectively) and in males starting at the mid-concentration (7 and 10 affected at the mid and high concentrations, respectively), while pale upper incisors were seen in 10 males and 3 females at the high concentration. Microscopically, remnants of enamel in the upper incisors (following decalcification of samples) were recorded in both sexes at the highest concentration only (8 animals of each sex). Fluoride content of bone and teeth was not analyzed.

In conclusion, rats exposed to fluensulfone by inhalation (6 hours/day, 5 days/week, 13 consecutive weeks) showed various treatment-related changes. Potentially adverse changes which were present at the lowest concentration consisted of:

- weight loss on exposure days in males
- prolonged prothrombin time in females
- decreased thymus weight in males (without histopathological effects)
- histopathological changes in the epiglottis (squamous metaplasia, epithelial hyperplasia, focal mononuclear cell infiltrate) in both sexes and in the nasal cavity (squamous epithelial hyperplasia) in males.

For most of these changes, their magnitude/incidence increased with increasing exposure concentration. Squamous metaplasia in the epiglottis was present in all exposed animals.

The lowest concentration tested (actual concentration 0.040 mg/L) was a Lowest-Observed-Adverse-Effect-Concentration (LOAEC or LOAEL) based on decreased weight gain in males, increased prothrombin time in females, decreased thymus weight in males and histopathology in the epiglottis in both sexes. A NOAEC (NOAEL) was not identified (<0.040 mg/L).

This study is classified as **acceptable/guideline (fully reliable)** and satisfies the guideline requirements for a subchronic inhalation study in the rat (OECD 413, OPPTS 870.3465).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	MCW-2 (Fluensulfone technical grade)
Description:	Yellow solid
Batch No.:	411-033-05
Purity:	96.8%
CAS#:	318290-98-1
Expiry date:	17 May 2013
Stability of Test Item:	Stable when stored under refrigeration. Not compromised by the test atmosphere generation procedure (i.e. melted at about 45-50 ºC and filtered to remove large particles and then heated in a water bath at 50 ºC to generate the test atmosphere)
2. Vehicle:	Humidified air. The test atmosphere for the low-concentration group in the main study was obtained by dilution (5-fold) of the test atmosphere for the mid-concentration group
3. Test animals	
Species:	Rat
Strain:	Wistar [Crl:WI(Han)]
Age:	8 weeks old at randomization (shortly before the first exposure day)
Weight at dosing:	Males: mean 225 g; females: mean 165 g (main study) Males: mean 198 g; females: mean 153 g (range-finding study)
Source:	Charles River Deutschland, Germany
Acclimation period:	11 days (main study) 7 days (range-finding study)
Diet:	Rat & Mouse No. 3 Breeding Diet, RM3 (supplied by SDS Special Diets Services, UK), *ad libitum* except during exposure and the fasting period before the scheduled sacrifice. The feed was provided as a powder in stainless steel cans, covered by a perforated stainless steel plate that served to prevent spillage. The feed in the feeders was replaced with fresh portions once weekly and filled up as needed.
Water:	Tap water supplied in polypropylene bottles, cleaned weekly and filled as needed; provided *ad libitum* except during exposure and the fasting period before the scheduled sacrifice.

Housing: In groups of 5, separated by sex, in macrolon cages with a bedding of wood shavings (Lignocel, Type ¾, Rettenmaier, Germany) and strips of paper (Enviro-dri) as environmental enrichment (Lillico, UK).

Sentinel animals were housed similarly in groups of 2 (range-finding study) or 4 (main study).

During exposure, the animals were secured individually in plastic animal holders (Battelle) in the exposure nose-only unit. Immediately after each exposure, the animals were returned to their home cages.

4. Environmental conditions

Temperature: 22 ± 2°C - During the main study, the temperature was just below 20°C (minimum of 19.8°C for periods of 10 to 30 minutes) on 7 occasions. These deviations had no impact on the study.

Humidity: 45 - 65% - During the main study, the relative humidity exceeded the upper limit on 9 occasions with a maximum relative humidity of 73% and a maximum duration of 5 hours. On one occasion the relative humidity was below 45% for about 10 minutes (lowest value recorded: 39.8%). These deviations had no impact on the study.

Air changes: Approximately10 per hour

Photoperiod: Artificial light (fluorescent tubes) with a sequence of 12 hours light and 12 hours dark

B. STUDY DESIGN AND METHODS

1. In life dates: 17 August to 30 November 2011 (main study)

13 July to 5 August 2011 (range-finding study)

2. Animal assignment and treatment:

A 14-day range-finding study, consisting of four groups of 5 rats/sex each, was performed prior to the full 90-day study (data were presented in the main report for the 90-day study). In the range-finding study, rats were exposed to target concentrations of 0, 0.035, 0.35 or 3.5 mg/L (MMAD ± GSD of 1.45 µm ± 2.41, 1.32 µm ± 1.71 and 1.44 µm ± 1.66 at 0.035, 0.35 and 3.5 mg/L, respectively). At the high concentration level (3.5 mg/L), animals showed clinical signs (piloerection, soiled fur, blepharospasm, hunched posture, hypoactivity), weight loss, decreased food consumption, increased water consumption, organ weight changes (increased relative lung, kidney and liver weights; decreased absolute and relative spleen weight) and white discolouration at the base of the lower incisors. Animals exposed to the mid-concentration level (0.35 mg/L) showed growth retardation, decreased food consumption, and organ weight changes (increased relative kidney weight and decreased absolute and relative spleen weight in males only). The low-concentration level (0.035 mg/L) was a no-observed-effect-concentration.

Based on these results, the target concentrations selected for the 90-day study were 0.04, 0.2, and 1.0 mg/L.

One day before the initiation of exposure, the animals were allocated to the various groups by computer randomization proportionally to body weight (males and females separately).

The animals were exposed to the test atmosphere in nose-only exposure units consisting of a cylindrical column, surrounded by a transparent cylinder. The column had a volume of approximately 60 liters and consisted of a top assembly with the entrance of the unit, one rodent tube section and at the bottom the base assembly with the exhaust port. Each rodent tube section had 20 ports for animal exposure. Several empty ports were used for test atmosphere sampling (for determination of the actual concentration) and measurement of temperature and relative humidity. The animals were secured in plastic animal holders (Battelle), positioned radially through the outer cylinder around the central column. The rats of each group were placed in an alternating order (male, female etc.). Each week, the animals were rotated within their chamber. The remaining ports were closed. Only the nose of the rats protruded into the interior of the column.

The unit was illuminated externally by normal laboratory fluorescent tube lighting. The air entering the unit was controlled at 22 ± 2°C and the relative humidity was maintained between 30 and 70%.

The animals were exposed 5 days per week, 6 hours per day, over a 13-week study period (65 and 66 exposure days in total for female and male rats, respectively), for the last time on the day before scheduled sacrifice.

On 2 November 2011 (exposure day 47), the exposure period was about 3 hours instead of 6 hours due to a power failure.

The time to attain 95% of the steady state concentration (T_{95}) was calculated to be about 6 minutes. The animals were placed in the exposure unit after stabilization of the test atmosphere.

3. Generation of test atmosphere

The inhalation equipment was designed to expose the rats to a continuous supply of fresh test atmosphere. Prior to use, fluensulfone was melted at about 45-50°C and filtered to remove large particles which could obstruct the generation and exposure system. To generate the test atmosphere, fluensulfone was heated in a water bath at 50°C. A controlled amount of fluensulfone, controlled by a peristaltic pump with electrically heated tubing was nebulized using an air-driven atomizer. The atomizer of the control, mid- and high-concentration groups was supplied with a stream of humidified air, whereas the test atmosphere for the low-concentration group was obtained by dilution (5-fold) of the test atmosphere for the mid-concentration group. Part of the mid-concentration test atmosphere was extracted, using a venturi and subsequently diluted with humidified air. The output of the venturi was indirectly monitored by the pressure of the driving humidified air stream. The humidified dilution air was measured with a mass stream meter. Before the aerosol entered the exposure units, the aerosol was cooled in a water bath. The exposure unit for the control animals was supplied with a stream of humidified air only.

4. Monitoring of exposure conditions

Actual concentration: The actual concentration of the test material in the test atmosphere was measured by gravimetric filter analysis for each exposure level at least 3 times on each exposure day (except on 2 November 2011 when the exposure was stopped after about 3 hours because of a power failure in Zeist). The test atmosphere was sampled in the breathing zone of the animals. Samples were obtained by passing test atmosphere (generally about 50, 10 or 5 L at the low-, mid- and high-concentration, respectively) at about 5 L/min through fiber glass filters. The actual concentration was calculated by dividing the total amount of test material present on each filter by the volume of the sample taken.

Chemical analysis of the test atmosphere was done using a High Performance Liquid Chromatography (HPLC) method with UV detection (273 nm) and a mobile phase gradient of acetonitrile and water for confirmation of the gravimetric analysis and for demonstration of the integrity of the test material. For confirmation, the concentration of the test material in the test atmosphere of each group was chemically analyzed (in duplicate) in the range-finding and main study, when a new subsample of test material was used (first day of use). For integrity, duplicate samples from the low-, mid- and high-concentration test atmospheres were taken when the subsample of test material contained in the jar in the exposure equipment had been used for one week during the range-finding study.

Nominal concentration and generation efficiency: The nominal concentration was determined by dividing the daily amount of test material used (by weight) by the total daily volume of air passed through the inhalation chamber. The nominal concentration for the low-concentration group of the main study could not be calculated because the test atmosphere of this group was derived by dilution of the test atmosphere of the mid-concentration group. The generation efficiency was calculated from the actual and the nominal concentration (efficiency = actual concentration as percentage of the nominal concentration).

Particle size: Particle size distribution measurements were carried out using a cascade impactor once weekly for each concentration level during the study and at least once during preliminary generation of the test atmosphere for each exposure condition. The Mass Median Aerodynamic Diameter (MMAD) and the Geometric Standard Deviation (GSD) were calculated.

Total air flow, temperature, relative humidity, oxygen and carbon dioxide concentration: The temperature and relative humidity (measured by a RH/T device) and the total air flow of each test atmosphere were recorded at least hourly during exposure. The oxygen concentration was measured on the first day of the range-finding study. As the target concentrations in the main study were in the concentration range used in the range-finding study, oxygen was not measured in the main study. The carbon dioxide concentration in the test atmosphere was calculated from the air flow, the assumed carbon dioxide concentration in inhaled air (0.04% v/v) and in exhaled air (4% v/v), the respiratory minute volume of the animals (RMV) and the number of animals in the exposure unit. The RMV (L/min) was calculated as 0.608 x body weight $(kg)^{0.852}$.

5. Statistical analysis of the results

Body weight data collected after initiation of treatment were analyzed using 'AnCova & Dunnett's Test' with 'Automatic' data transformation method. Day 0 body weight data were used as covariate in the analysis of the post-treatment data unless removed during data preprocessing. The statistical method is an automatic decision tree consisting of:

(1) Data preprocessing tests. These tests start with transformation 'None' (Identity). First, suitability of the covariate was checked (criteria: sufficient cases, at least 2; variability of covariate non-zero; covariate effects sufficiently parallel over the groups, significance level parallelism test 0.01). Next, normality of data distribution (Shapiro-Wilks test; significance level 0.05) and homogeneity of variances (Levene test; significance level 0.05) were checked. If any of these 3 checks failed they were repeated using Log transformation. If checks on log-transformed, covariate-adjusted data failed, the covariate was removed and the normality and homogeneity checks were repeated. If these checks passed on transformations 'None' or 'Log', data were analyzed without covariate. If they failed, data were rank-transformed and the covariate was reinstated.

(2) A group test assessing whether or not group means were all equal (one-way analysis of covariance [Ancova], or one-way analysis of variance [Anova] if the covariate is removed). If the group test showed no significant non-homogeneity of group means ($p \geq 0.05$), group summary tables do not show whether or not a covariate was used in the analysis.

(3) Post-hoc analysis. If the group test showed significant ($p < 0.05$) non-homogeneity of group means, pairwise comparisons with the control group were conducted by Dunnett's multiple comparison test (significance levels 0.01 and 0.05).

Pretreatment body weight, rectal temperature, hematology, clinical chemistry and organ weight data were analyzed by the 'Generalized Anova/Ancova Test' with 'Automatic' data transformation method. This test is an automatic decision tree consisting of:

(1) Data preprocessing tests. First, normality of data distribution (Shapiro-Wilks test) and homogeneity of variances (Levene test) were checked (initial transformation 'None' [Identity]). If any of these checks failed ($p < 0.05$) they were repeated using Log transformation. If checks on log-transformed data failed, data were rank-transformed.

(2) A group test assessing whether or not group means were all equal (parametric for untransformed or log-transformed data: one-way analysis of variance [Anova]; non-parametric for rank transformed data: Kruskal-Wallis test).

(3) Post-hoc analysis. If the group test showed significant ($p < 0.05$) non-homogeneity of group means, pairwise comparisons with the control group were conducted by Dunnett's multiple comparison test (parametric after Anova, non-parametric after Kruskal-Wallis; significance levels 0.01 and 0.05).

Feed and water consumption data were analyzed by Dunnett's multiple comparison test.

Incidences of histopathological changes were analyzed by Fisher's exact probability test.

Tests were performed as two-sided tests with results taken as significant where the probability of the results is <0.05 or <0.01.

Because numerous variables were subjected to statistical analysis, the overall false positive rate (Type I errors) was greater than suggested by a probability level of 0.05. Therefore, the final interpretation of results was based not only on statistical analysis but also on other considerations such as dose-response relationships and whether the results were significant in light of other biological and pathological findings.

C. OBSERVATIONS

1. Clinical signs:

Each animal was observed daily in the morning hours (prior to exposure) by cage-side observations and, if necessary, handled to detect signs of toxicity. During exposure, a group-wise observation was made about halfway through the 6-hour exposure period. All animals were thoroughly checked again after exposure. On weekends, only one check per day was carried out. All abnormalities, signs of ill health, reaction to treatment and mortality were recorded.

2. Ophthalmoscopic examination:

Ophthalmoscopic observations were made prior to the start of the exposure period in all rats and in the last week of the exposure period in all rats of the control and high concentration groups. The eye examination was carried out using an ophthalmoscope after induction of mydriasis by a solution of atropine sulphate. As no treatment-related ocular changes were observed, eye examinations were not extended to the animals of the intermediate concentration groups at the end of the treatment period.

3. Body temperature:

The body temperature (rectal) of all animals was measured in the last week of exposure shortly (2-5 minutes) after exposure (females on study day 84, males on study day 85). Additionally, rectal temperature was measured just before necropsy (females on day 91, males on day 92).

4. Body weight:

The body weight of each animal was recorded once before the start of exposure, once prior to exposure on the first exposure day (day 0) and twice weekly thereafter (Mondays and Fridays). At the end of the in-life phase, the animals were weighed on the day before overnight fasting prior to their scheduled sacrifice, and on the day of sacrifice.

5. Food consumption:

Food consumption (g/animal/day) was measured per cage by weighing the feeders. The food consumption was measured from the start of treatment (day 0) over successive 7-day periods (or a 6-day period at the start of the main study).

6. Water consumption:

Water consumption (g/animal/day) was measured per cage by weighing the drinking bottles daily on five exposure days in weeks 1, 6, and 12.

7. Hematology and clinical chemistry:

Blood samples were taken from the abdominal aorta of overnight fasted rats (water was freely available) whilst under pentobarbital anesthesia at sacrifice. Potassium-EDTA was used as anticoagulant. The following hematology parameters were measured:

> Red blood cells, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocytes, total white blood cells, differential white blood cells (lymphocytes, neutrophils, eosinophils, basophils, monocytes), prothrombin time, platelets,

For the assessment of clinical chemistry, plasma was prepared by centrifugation. The following clinical chemistry parameters were determined:

> Alkaline phosphatase activity, aspartate aminotransferase activity, alanine aminotransferase activity, gamma glutamyl transferase activity, total bilirubin, total cholesterol, phospholipids, triglycerides, creatinine, urea, inorganic phosphate, calcium, chloride, potassium, sodium

8. Pathology:

Early in week 14, after overnight fasting (water was freely available), the surviving animals were killed on two consecutive working days (females on study day 91, males on day 92) by exsanguination from the abdominal aorta under pentobarbital anesthesia (intraperitoneal injection of sodium pentobarbital) and then examined grossly for pathological changes. The teeth were checked for obvious discolouration.

Samples of the tissues and organs listed in Table 1 were preserved in a neutral aqueous phosphate-buffered 4% solution of formaldehyde (10% solution of formalin). The organs indicated were weighed (paired organs were weighed together) as soon as possible after dissection to avoid drying and the relative organ weights (g/kg bw) were calculated from the absolute organ weights and the terminal body weight.

The tissues for microscopic examination were embedded in paraffin wax, sectioned at 5 µm and stained with hematoxylin and eosin. Histopathological examination (by light microscopy) was performed on the organs and tissues listed in Table 1 of all animals in the control and high-concentration groups. In addition, all gross lesions were examined in the low- and mid-concentrations groups. As treatment-related microscopic changes were observed in the larynx, nose and upper incisors, examination of these tissues was extended to the low- and mid-concentration groups. Compared to the groups examined initially (control and high-concentration), the teeth of the animals of the intermediate-concentration groups were decalcified slightly longer.

Table 1: Summary of tissues collected for pathology examination

Organ/tissue	Weighed	Collected	Examined
Adrenal gland	X	X	X
Aorta		X	X
Bone - sternum, femur with joint		X	X
Bone marrow - sternum		X	X
Brain (brain stem, cerebrum, cerebellum)	X	X	X
Cecum		X	X
Colon		X	X
Duodenum		X	X
Epididymides	X	X	X
Esophagus		X	X
Eyes		X	X
Heart	X	X	X
Ileum		X	X
Jejunum		X	X
Kidneys	X	X	X
Larynx (three levels, one including the epiglottis)		X	X
Lacrimal gland, exorbital		X	X
Liver	X	X	X
Lungs with trachea and larynx (after weighing, the lung was infused with fixative; each lung lobe was examined at one level, including main bronchi)	X	X	X
Lymph nodes – axillary, cervical, mediastinal,		X	X
Mammary gland (female)		X	X
Mandible (lower jaw)*		X	
Nasopharyngeal tissues with teeth [six levels including the nasopharyngeal duct and the draining lymphatic tissue (nose-associated lymphoid tissue or NALT)]		X	X
Ovaries	X	X	X
Pancreas		X	X
Parathyroid gland			
Pharynx		X	X
Pituitary gland		X	X
Prostate gland		X	X
Rectum		X	X
Salivary glands – parotid, sublingual, submaxillary		X	X
Sciatic nerve		X	X
Seminal vesicles/coagulating glands		X	X
Skeletal muscle (thigh)		X	X
Skin (flank)		X	X
Spinal cord (cervical, mid-thoracic, lumbar)		X	X
Spleen	X	X	X
Stomach [non-glandular (forestomach) and glandular (fundus, pylorus)]		X	X

Teeth (in upper jaw)		X	X
Testes	X	X	X
Thymus	X	X	X
Thyroid gland	X	X	X
Tongue		X	X
Trachea/bronchi (three levels of trachea examined, including one through the carina of the bifurcation of the extrapulmonary bronchi and one transverse section)		X	X
Ureter		X	X
Urethra			
Urinary bladder		X	X
Uterus (with cervix)	X	X	X
All gross lesions		X	X

* The mandible (containing the lower incisors) was preserved in formalin to enable possible future examinations. Though the lower incisors of animals exposed to the test material showed pale discolouration, microscopic examination of these teeth was considered not to be necessary because the upper incisors, which also showed treatment-related pale discoloration, were assumed to show similar microscopic changes as the lower incisors.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Monitoring of exposure conditions

Results pertaining to the generated test atmosphere were as follows:

Parameter		Low concentration	Mid concentration	High concentration
Concentration (mg/L air)	Target	0.04	0.2	1.0
	Achieved	0.040 ± 0.005	0.204 ± 0.027	0.983 ± 0.067
MMAD (µm)		1.02 ± 0.07	1.16 ± 0.10	1.19 ± 0.05
GSD		1.59 ± 0.07	1.71 ± 0.27	1.60 ± 0.10
Nominal concentration (mg/L)		NA	0.308	1.209
Generation efficiency		NA	67%	81%

The chemical analysis showed that the amount of test material in the vapor phase was below the detection limit at all concentration levels and that there was no indication of decomposition of the test material during the study.

The average (± SD) air flows were 29.5 (± 0.2), 36.2 (± 2.7), 37.8 (± 0.3) and 30.6 (± 0.7) L/min for the control, low-, mid- and high-concentration groups, respectively. Measured temperatures

during exposure were within the range of 20-24°C, except on two occasions. Mean relative humidity during exposure was generally within the range of 30-70%, except on three occasions. The carbon dioxide concentrations in the test atmospheres, calculated for study days 0, 45 and 90, were 0.45-0.60 vol% for the control group, 0.38-0.51 vol% for the low-concentration, 0.35-0.46 vol% for the mid-concentration and 0.42-0.55 vol% for the high-concentration. For each group, the calculated carbon dioxide concentration increased over the course of the study due to the increase in the body weight of the animals and, consequently, their respiratory minute volume.

2. Mortality and clinical signs

No deaths occurred during the study period. For males, treatment-related clinical abnormalities were seen in the mid- and high-concentration groups. For females, treatment-related clinical signs were seen in all groups exposed to the test material. These findings are summarized in Table 2.

On the first exposure day, shortly after termination of the exposure period, a number of animals of the mid-concentration group (males only) and the high-concentration group showed piloerection in combination with hunched posture and/or blepharospasm (females only). These transient symptoms of discomfort were ascribed to treatment and were no longer observed during the remainder of the study.

Light colored discoloration of the incisors was observed in all male animals of the mid- and high-concentration groups and in some females of the low-, mid- and high-concentration groups. The discoloration was first observed in the lower incisors (around days 24-34) and, about five weeks later, in the upper incisors in the high-concentration group only. These mild changes were considered by the study investigators not to be adverse but do indicate uptake of fluoride by the teeth.

Soiled fur (yellow to orange-brown discoloration on the ventral side) occurred in nearly all female animals exposed to the test material and in a few male animals of the mid- and high-concentration groups; this finding occurred earlier in the study with increasing exposure concentration. No toxicological significance was attributed to this finding.

In the second half of the exposure period, most female animals, controls included, showed skin encrustations and/or sparsely haired area(s) on their back. In addition, one female of the mid-concentration group had a skin wound. In males, skin encrustations were seen in only 2 animals of the high-concentration group. These findings were related to the repeated restraint in Batelle tubes (which have longitudinal slits at the side of the back of the animals) and, therefore, considered not to be an effect of the test material.

No abnormalities were seen at the group-wise observations made about halfway through each 6-hour exposure period.

Table 2. Selected clinical signs – number of animals (number of observations)

Observation	Days	Concentration (mg/L)			
		0	**0.04**	**0.2**	**1.0**
Males					
Hunched posture	0 (after exposure)	0	0	0	1 (1)
Piloerection	0 (after exposure)	0	0	2 (2)	2 (2)
Blepharospasm	0 (after exposure)	0	0	2 (2)	0
Lower incisors light coloured	24-92	0	0	10 (499)	10 (651)
Upper incisors light coloured	58-92	0	0	0	10 (335)
Soiled fur	76-92	0	0	2 (2) *day 92 only*	4 (27)
Encrustation of skin	83-92	0	0	0	2 (13)
Females					
Hunched posture	0 (after exposure)	0	0	0	5 (5)
Piloerection	0 (after exposure)	0	0	0	5 (5)
Blepharospasm	0 (after exposure)	0	0	0	3 (3)
Lower incisors light coloured	30-91	0	2 (66)	10 (297)	10 (555)
Upper incisors light coloured	80-91	0	0	0	3 (36)
Soiled fur	20-91	0	8 (232)	10 (400)	10 (474)
Encrustation of skin	27-91	8 (91)	10 (153)	8 (108)	3 (23)
Sparsely haired area	41-91	7 (123)	10 (165)	9 (152)	7 (63)

Data obtained from pages 67-68 of the study report. N= 10/sex/concentration.

3. Body weight and food and water consumption

In males, mean body weights were statistically significantly decreased at all concentration levels from day 3 onwards (selected values in Table 3). The decreases showed a concentration-related response and were reduced by approximately 3-9%, 5-13%, and 5-16% relative to controls at the 0.04, 0.02, and 1.0 mg/L exposure levels. Compared to controls, the mean terminal body weights

of low-, mid- and high-concentration males were decreased by 7%, 12% and 14%, respectively. The weight change results (Table 4) showed that the treated males lost weight, concentration-dependently, on the days when they were exposed (periods of 4 successive exposure days). In the 3-day periods which included the weekends (exposure on Friday, no exposure on Saturday and Sunday), the treated males gained more weight than controls (the differences were statistically significant in most weeks).

In females, the effect on body weight was less marked than in males. Statistically significant differences in mean body weight occurred sporadically only in the high-concentration group during the first half of the study. Throughout the study period, mean body weights of females from the high-concentration group were decreased by approximately 3-5% relative to controls. As in males, this was due to weight loss/retarded growth on exposure days (Table 5). The mean terminal body weight of high-concentration females was only 3% lower than that of controls (this difference was not statistically significant). No treatment-related differences in body weight were noted in females at the two lower exposure concentrations.

Table 3. Selected mean ± S.D. body weight values in males and females (g)

Study Day	Exposure Concentration (mg/L)			
	0	**0.04**	**0.2**	**1.0**
Males				
0	225.73 ± 11.10	225.74 ± 9.60	224.40 ± 9.27	224.39 ± 9.63
10	257.28 ± 14.63	242.40 ± 11.85** (↓6%)	234.99 ± 8.52** (↓9%)	232.71 ± 10.25** (↓10%)
45	319.29 ± 16.78	297.18 ± 19.27** (↓7%)	281.36 ± 13.15** (↓12%)	275.69 ± 11.91** (↓14%)
90	348.82 ± 20.51	327.25 ± 19.33** (↓6%)	311.16 ± 14.70** (↓11%)	302.63 ± 13.29** (↓13%)
Females				
0	166.19 ± 5.80	164.93 ± 6.12	164.26 ± 8.93	164.31 ± 6.63
10	179.59 ± 8.49	176.83 ± 7.27	175.05 ± 7.93	170.66 ± 5.50** (↓5%)
45	206.92 ± 10.26	202.08 ± 10.60	203.04 ± 11.43	195.70 ± 9.18
90	219.22 ± 10.64	214.88 ± 11.41	218.56 ± 12.02	209.05 ± 10.95 (↓5%)

Data obtained from pages 72-77 of the study report. * Statistically significantly different from the control mean ($p<0.05$). ** Statistically significantly different from the control mean ($p<0.01$). Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 4: Mean ± S.D. body weight changes in males (g)

Time period	Control	0.04 mg/L	0.2 mg/L	1.0 mg/L
Days 0 to 3	7.88 ± 3.57	-0.77 ± 3.44**	-3.58 ± 3.00**	-5.58 ± 4.01**
Days 3 to 6	14.14 ± 2.76	14.70 ± 1.83	15.61 ± 2.22	16.60 ± 2.22
Days 6 to 10	9.53 ± 3.10	2.73 ± 3.07**	-1.44 ± 3.70**	-2.70 ± 2.83**
Days 10 to 13	12.56 ± 3.16	11.93 ± 1.54	14.10 ± 2.37	15.18 ± 1.89*
Days 13 to 17	5.76 ± 3.90	1.07 ± 3.29**	-0.37 ± 3.51**	-3.52 ± 1.55**
Days 17 to 20	9.36 ± 2.65	13.36 ± 1.43**	12.28 ± 2.77*	14.64 ± 2.24**
Days 20 to 24	3.54 ± 4.17	-0.35 ± 3.06*	-1.36 ± 3.04**	-6.45 ± 2.30**
Days 24 to 27	6.59 ± 2.63	11.18 ± 2.71**	10.86 ± 1.82**	11.23 ± 3.10**
Days 27 to 31	5.98 ± 2.05	0.54 ± 3.26**	-2.02 ± 3.36**	-4.23 ± 1.71**
Days 31 to 34	7.23 ± 2.68	10.93 ± 2.93**	10.91 ± 1.35**	13.07 ± 1.78**
Days 34 to 38	5.02 ± 1.98	-1.83 ± 2.37**	-3.77 ± 2.14**	-4.01 ± 0.97**
Days 38 to 41	4.96 ± 2.30	9.05 ± 2.21**	9.34 ± 2.38**	10.17 ± 2.50**
Days 41 to 45	1.01 ± 5.09	-1.10 ± 3.06	-3.60 ± 1.85**	-3.10 ± 2.18**
Days 45 to 48	7.26 ± 2.11	7.87 ± 2.01	9.59 ± 2.64*	10.81 ± 1.65**
Days 48 to 52	2.61 ± 4.43	-4.10 ± 3.40**	-5.39 ± 2.97**	-5.66 ± 1.10**
Days 52 to 55	5.68 ± 3.88	10.14 ± 3.27*	9.32 ± 1.19	10.19 ± 2.43*
Days 55 to 59	-0.23 ± 2.83	-4.24 ± 2.95**	-3.58 ± 2.54*	-6.68 ± 2.06**
Days 59 to 62	4.80 ± 2.63	6.38 ± 2.10	6.89 ± 2.70	7.79 ± 3.38
Days 62 to 66	1.82 ± 2.64	-1.94 ± 3.32*	-3.34 ± 2.54**	-4.88 ± 3.58**
Days 66 to 69	4.40 ± 3.56	7.36 ± 2.42	9.40 ± 1.82**	10.59 ± 1.98**
Days 69 to 73	-1.07 ± 4.39	-3.24 ± 2.22	-5.61 ± 2.36*	-6.72 ± 2.31**
Days 73 to 76	2.03 ± 2.44	6.31 2.96**	8.80 ± 1.57**	7.51 ± 1.45**
Days 76 to 80	-3.47 ± 3.83	-4.10 ± 3.44	-7.13 ± 2.46*	-6.41 ± 2.00
Days 80 to 83	2.88 ± 3.88	6.33 ± 3.35	7.68 ± 1.85*	8.99 ± 2.33**
Days 83 to 87	1.13 ± 3.10	-1.03 ± 4.13	-3.10 ± 1.64*	-5.05 ± 2.04**
Days 87 to 90	1.69 ± 3.42	4.33 ± 3.16	6.27 ± 2.63**	6.46 ± 2.42**
Days 0 to 90	**123.09 ± 16.93**	**101.51 ± 15.80** (↓17%)**	**86.76 ± 13.83** (↓29%)**	**78.24 ± 7.45** (↓36%)**

* Statistically significantly different from the control mean ($p<0.05$). ** Statistically significantly different from the control mean ($p<0.01$). Data obtained from pages 78-81 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

Table 5: Mean ± S.D. body weight changes in females (g)

Time period	Control	0.04 mg/L	0.2 mg/L	1.0 mg/L
Days 0 to 3	2.39 ± 4.36	2.20 ± 3.30	3.34 ± 4.95	-2.68 ± 2.63*
Days 3 to 6	8.59 ± 4.77	8.66 ± 3.69	7.78 ± 4.50	8.28 ± 3.79
Days 6 to 10	2.42 ± 2.64	1.04 ± 4.89	-0.33 ± 3.09	0.75 ± 3.13
Days 10 to 13	3.93 ± 4.84	6.13 ± 5.34	8.52 ± 2.98	10.09 ± 4.94*
Days 13 to 17	4.51 ± 3.72	2.11 ± 5.54	-0.98 ± 4.55*	-0.73 ± 4.04*
Days 17 to 20	3.93 ± 6.07	7.29 ± 3.94	7.28 ± 4.33	3.49 ± 2.55
Days 20 to 24	2.51 ± 3.48	-0.55 ± 2.86	2.22 ± 2.06	0.60 ± 3.94
Days 24 to 27	4.57 ± 5.04	3.34 ± 4.43	3.90 ± 2.02	4.84 ± 4.26
Days 27 to 31	-0.26 ± 2.66	0.13 ± 3.97	0.23 ± 2.24	-1.53 ± 2.99
Days 31 to 34	2.70 ± 6.46	7.57 ± 5.14	5.13 ± 4.06	7.59 ± 5.55
Days 34 to 38	1.47 ± 2.14	-2.19 ± 4.27*	-0.80 ± 3.27	-2.27 ± 4.81**
Days 38 to 41	5.63 ± 4.99	1.99 ± 3.45	5.03 ± 3.68	3.75 ± 3.17
Days 41 to 45	-1.66 ± 1.89	-0.57 ± 2.66	-2.54 ± 2.47	-0.79 ± 3.12
Days 45 to 48	4.10 ± 3.23	5.10 ± 5.99	6.45 ± 3.62	6.76 ± 2.57
Days 48 to 52	0.64 ± 3.82	-2.92 ± 3.53	-3.94 ± 4.13*	-1.61 ± 3.11
Days 52 to 55	2.18 ± 3.12	5.94 ± 3.21*	6.54 ± 3.20*	2.74 ± 5.69
Days 55 to 59	-2.39 ± 5.41	-1.54 ± 2.45	-4.81 ± 2.99	-2.34 ± 3.33
Days 59 to 62	3.09 ± 4.62	1.78 ± 3.19	2.73 ± 2.25	2.83 ± 3.09
Days 62 to 66	-2.37 ± 3.35	-3.41 ± 4.10	-1.26 ± 3.16	-2.39 ± 2.78
Days 66 to 69	1.18 ± 2.79	4.99 ± 5.20	4.24 ± 4.00	3.70 ± 3.79
Days 69 to 73	-0.70 ± 2.51	-3.01 ± 2.79	-2.47 ± 3.35	-1.64 ± 3.82
Days 73 to 76	2.49 ± 4.97	1.22 ± 4.77	3.05 ± 5.09	5.87 ± 3.84
Days 76 to 80	-5.55 ± 4.18	-2.76 ± 2.33	-3.54 ± 5.41	-4.60 ± 2.53
Days 80 to 83	6.64 ± 3.00	5.57 ± 4.56	6.50 ± 3.30	1.03 ± 4.09**
Days 83 to 87	0.56 ± 4.73	-0.54 ± 3.45	-0.15 ± 2.76	1.12 ± 3.49
Days 87 to 90	2.43 ± 5.41	2.38 ± 6.24	2.18 ± 4.30	1.88 ± 3.58
Days 0 to 90	**53.03 ± 6.44**	**49.95 ± 9.55**	**54.30 ± 9.47**	**44.74 ± 10.36 (↓16%)**

* Statistically significantly different from the control mean ($p<0.05$). ** Statistically significantly different from the control mean ($p<0.01$). Data obtained from pages 82-85 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

Throughout the study, males of the low-, mid- and high-concentration groups consumed somewhat (between 6% and 17%) less feed than controls (data not presented). Except in the first two exposure weeks, the differences were not statistically significant. In females, feed consumption was decreased only at the high-concentration level in the first week of the exposure period (14% relative to controls).

At the end of the first exposure week (day 6-7), water consumption was statistically significantly increased at the mid-concentration (females only; 19%) and the high-concentration (both sexes; 16-24%). A non-statistically significant increase was also noted in females at the low-concentration level (15%). Thereafter (measurements in weeks 6 and 12), water consumption was increased, concentration-dependently, at the all concentrations in both sexes. At the high-concentration, water intake was increased by approximately 30-50% and the differences from controls were statistically significant. At the mid- and low-concentrations, the increases (8-20% at the low-concentration, 7-32% at the mid-concentration) were not always statistically significant.

4. Ophthalmoscopic examination and body temperature

Ophthalmoscopic examination did not reveal any treatment-related abnormalities.

Body temperature (rectal) was statistically significantly decreased, concentration-dependently, in female animals of the mid- and high-concentration groups shortly after termination of exposure on day 84 (mean ± S.D. of 38.04 ± 0.32, 37.70 ± 0.54, 37.47 ± 0.34** and 36.57 ± 0.57** °C, in order of ascending dosage, with ** indicating statistical significance at $p<0.01$). A slight, non-statistically significant decrease in body temperature was noted in males from the high concentration group only (mean ± S.D. of 37.37 ± 0.61, 37.23 ± 0.24, 37.13 ± 0.49 and 36.83 ± 0.50°C, in order of ascending dosage).

Body temperature measured on the morning of scheduled sacrifice showed no intergroup differences in males or females (mean ± S.D. in males of 37.21 ± 0.46, 37.47 ± 0.57, 37.71 ± 0.44 and 37.63 ± 0.31°C, mean ± S.D. in females of 38.27 ± 0.48, 38.14 ± 0.40, 38.01 ± 0.52 and 37.89 ± 0.41 °C, in order of ascending dosage).

5. Hematology

Red blood cell and coagulation parameters showed the following statistically significant differences between exposed rats and controls (Table 6):

- A statistically significantly higher percentage of reticulocytes in males of the high-concentration group.
- A statistically significantly lower MCHC in females of the mid- and high-concentration group.
- Statistically significantly longer prothrombin time in females at all concentration levels. The differences from controls showed a concentration-related response.

A slight (not statistically significant) increase in prothrombin time was also observed in males from the high-concentration group. In addition, a slight (not statistically significant) reduction in platelet count was observed in females from the high-concentration group. Total and differential white blood cell values were comparable between the groups.

Table 6. Selected mean ± S.D. hematology parameters

Parameter	Exposure Concentration (mg/L)			
	0	0.04	0.2	1.0
Males				
Reticulocytes (%)	0.869 ± 0.172	0.795 ± 0.169	0.785 ± 0.142	1.095 ± 0.213* (↑26%)
Prothrombin time (sec)	51.86 ± 32.39	53.85 ± 21.35	84.27 ± 50.44[#]	60.44 ± 21.32 (↑16%)
Platelets (10^9/L)	686.1 ± 74.2	668.5 ± 62.1	625.2 ± 56.7	645.7 ± 52.2
MCHC (mmol/L)	20.96 ± 0.32	20.80 ± 0.45	20.56 ± 0.21	20.52 ± 0.56
Females				
Reticulocytes (%)	1.283 ± 0.532	1.094 ± 0.344	1.094 ± 0.214	1.199 ± 0.251
Prothrombin time (sec)	32.76 ± 1.33	34.97 ± 1.91* (↑7%)	36.13 ± 2.61** (↑10%)	39.06 ± 4.07** (↑19%)
Platelets (10^9/L)	778.2 ± 89.2	742.8 ± 73.4	722.0 ± 57.3	710.4 ± 97.6 (↓9%)
MCHC (mmol/L)	21.38 ± 0.21	21.14 ± 0.36	20.84 ± 0.43** (↓2%)	20.78± 0.18** (↓3%)

Data obtained from pages 88-89 of the study report. * Statistically significantly different from the control mean ($p<0.05$). ** Statistically significantly different from the control mean ($p<0.01$). Values in parentheses represent the percent change from controls (calculated by the reviewer). [#] Three of 10 males had prothrombin times of 131, 132 and 189 seconds, compared to a range of 39-84 in the remaining 7 animals; when the high values for these three males are excluded, the re-calculated mean ± S.D. becomes 55.7 ±16 seconds.

6. Clinical chemistry

Compared to controls, animals exposed to the test material showed the following changes (Table 7):

- A dose-dependent increase in alkaline phosphatase (ALP) at the high-concentration in males and females and at the mid-concentration in females.

- Lower alanine aminotransferase (ALAT) at the mid-concentration in males and at the high-concentration in both sexes. The differences showed a concentration-related response.

- A dose-related increase in bilirubin at the high-concentration in both sexes.

- Lower glucose in all groups of males exposed to the test material, most markedly in the high-concentration group, but without a clear concentration-related response.

- Lower total protein and albumin at all concentrations in females (not statistically significant for total protein at the mid-concentration and for albumin at the high-concentration). There was no concentration-related response. The differences in total protein and albumin in females were considered to be unrelated to treatment because they were only slight and not concentration-related.

- Higher phospholipids at the high-concentration in males.

- Higher triglycerides at the high-concentration in females.

- Higher phosphate at all concentrations in males. The differences tended to increase with increasing exposure concentration. The phosphate value for the concurrent male control group was below the historical control range (range of means of 2.076 to 3.366 mmol/L from inhalation and gavage studies) while the values in the exposed groups were at the lower end of this range.

- A dose-related increase in potassium at the mid- and high-concentration in males and at the high-concentration in females.

Table 7. Selected mean ± S.D. clinical chemistry parameters

Parameter	Exposure Concentration (mg/L)			
	0	0.04	0.2	1.0
Males				
ALP (U/L)	97.8 ± 18.0	99.0 ± 26.0	102.8 ± 19.9	112.4 ± 15.7 (↑15%)
ALAT (U/L)	46.1 ± 11.1	42.5 ± 8.3	32.9 ± 8.2** (↓29%)	21.4 ± 6.3** (↓53%)
Bilirubin (µmol/L)	1.87 ± 0.46	1.94 ± 0.26	2.14 ± 0.26 (↑14%)	2.92 ± 0.39** (↑56%)
Protein (g/L)	60.6 ± 2.1	59.7 ± 0.9	59.0 ± 1.1	60.7 ± 3.2
Albumin (g/L)	31.9 ± 0.9	31.7 ± 0.9	31.7 ± 0.7	32.3 ± 1.5
Glucose (mmol/L)	8.00 ± 1.105	6.705 ± 1.371* (↓16%)	6.795 ± 0.908* (↓15%)	5.520 ± 0.780** (↓31%)
Phospholipids (mmol/L)	1.328 ± 0.140	1.317 ± 0.119	1.382 ± 0.117	1.555 ± 0.183** (↑17%)
Triglycerides (mmol/L)	0.445 ± 0.097	0.447 ± 0.122	0.450 ± 0.102	0.466 ± 0.091
Phosphate (mmol/L)	1.832 ± 0.260	2.072 ± 0.229* (↑13%)	2.126 ± 0.175** (↑16%)	2.158 ± 0.155** (↑18%)
Potassium (mmol/L)	4.06 ± 0.33	4.20 ± 0.32	4.54 ± 0.42* (↑12%)	4.61 ± 0.32** (↑13%)
Females				
ALP (U/L)	61.0 ± 16.3	61.2 ± 14.3	72.9 ± 19.1 (↑19%)	79.9 ± 9.1* (↑31%)
ALAT (U/L)	35.3 ± 8.2	37.3 ± 11.5	29.8 ± 10.4	18.8 ± 4.6** (↓47%)
Bilirubin (µmol/L)	2.18 ± 0.40	2.33 ± 0.37	2.45 ± 0.58	3.34 ± 0.70** (↑53%)
Protein (g/L)	63.2 ± 2.3	59.8 ± 1.9**	60.9 ± 2.0	60.6 ± 2.7*
Albumin (g/L)	34.1 ± 1.3	31.7 ± 0.9**	32.2 ± 1.3**	32.9 ± 1.0
Glucose (mmol/L)	5.006 ± 0.578	4.680 ± 0.486	5.009 ± 1.271	4.787 ± 0.599
Phospholipids (mmol/L)	1.459 ± 0.264	1.371 ± 0.181	1.404 ± 0.213	1.445 ± 0.198
Triglycerides (mmol/L)	0.348 ± 0.084	0.446 ± 0.094	0.407 ± 0.102	0.632 ± 0.264** (↑82%)
Phosphate (mmol/L)	1.881 ± 0.417	1.864 ± 0.283	1.862 ± 0.396	1.978 ± 0.297
Potassium (mmol/L)	4.03 ± 0.44	4.27 ± 0.51	4.21 ± 0.43	4.43 ± 0.49 (↑10%)

Data obtained from pages 92-95 of the study report. * Statistically significantly different from the control mean ($p<0.05$). ** Statistically significantly different from the control mean ($p<0.01$). Values in parentheses represent the percent change from controls (calculated by the reviewer).

7. Terminal examinations

7a. Macroscopic examination

The only treatment-related alteration observed at gross necropsy consisted of a pale appearance of the incisors. The affected teeth were paler in comparison to the normal yellow-brown appearance of incisors of control rats. The lower incisors were affected at all concentrations in females (2, 4 and 9 females at the low-, mid- and high-concentration, respectively) and at the mid- and high-concentration in males (7 and 10 males, respectively). The upper incisors were affected at the high-concentration only (in 10 males and 3 females). The color change was not uniform but showed striation. The light colored stripes reflected the exposure regime: bands of five light stripes separated by narrow yellow-brown stripes reflected the 6-hour exposure periods on 5 successive days. On both sides of these striped bands there was a broader yellow-brown band which reflected the exposure-free weekend.

Most females, controls included, showed skin encrustations and/or sparsely haired area(s) on their back. These lesions were due to mechanical damage of the skin during restraint in the Batelle tubes.

The remaining macroscopic findings were unremarkable. They represent normal background pathology in rats of this strain and age and occurred only incidentally.

7b. Organ weights

The organ weight results (summarized in Tables 8 and 9 for males and females, respectively) showed the following differences between animals exposed to the test material and controls:

- Statistically significantly higher relative brain weight at the mid- and high-concentration and slightly but statistically significantly lower absolute brain weight at the high-concentration in males only.

- Higher relative lung weight at the high-concentration in males (statistically significant) and females (not statistically significant).

- Statistically significantly higher relative heart weight at the high-concentration in males.

- Statistically significantly higher relative adrenal weight at the mid- and high-concentration in males, accompanied by slightly higher (not statistically significant) absolute adrenal weights in these animals. Statistically significantly higher adrenal weights were also observed in females at the low- and mid-concentration. In the absence of a significant change in adrenal weight in females of the high-concentration group, the differences in the intermediate concentration groups were not ascribed to treatment.

- Higher kidney weight at the mid- and high-concentration in males (relative weight only) and females (absolute and relative weight). There was a concentration-related response for these findings.

- Higher relative liver weight at the mid- and high-concentration in both sexes. These differences showed a concentration-related response. Absolute liver weight was statistically significantly increased in females of the high-concentration group and slightly (not statistically significant) increased in females of the mid-concentration group.

- Lower absolute and relative thymus weight at all concentrations in males. This effect was concentration-related.

- Lower absolute spleen weight at the mid- and high-concentration in males. The differences showed no concentration-related response.

- Higher relative testes weight at the high-concentration.

- Lower (not statistically significant) absolute thyroid weights in males from the mid- and high-concentration groups and lower absolute and relative thyroid weights in females from all concentration groups. These changes were slight, not statistically significant, did not follow strong concentration-related trend, were not accompanied by any corresponding pathological findings, and were therefore not considered to be related to treatment.

- Higher absolute and relative weight of the ovaries at the mid-concentration. As this was not confirmed at the high-concentration, the differences at the mid-concentration were not considered to be related to treatment.

- Lower (not statistically significant) absolute and relative uterus weights in females from the mid- and high-concentration groups. These changes were slight, not statistically significant, did not follow strong concentration-related trend, and were not accompanied by any corresponding pathological findings, and were therefore not considered to be related to treatment.

The changes in kidney (males), liver, thymus and adrenal weight showed a concentration-related response; however, there were no corroborative histopathological changes in these organs. There was an increased incidence of corticomedullary mineralization of the kidney in high-concentration females when compared to controls (the kidney was not assessed in animals from lower concentration groups).

The other organ weight changes observed occurred in males only and were likely to be secondary to the lower body weight in this sex.

Table 8. Mean ± S.D. absolute (g) and relative (g/kg bw) organ weights and terminal body weights (g) in male rats

Organ/Tissue	Exposure Concentration (mg/L)			
	0	0.04	0.2	1.0
Terminal bw	326 ± 21	304 ± 19* (↓7%)	287 ± 15** (↓21%)	279 ± 13** (↓14%)
Brain – abs. – rel.	1.982 ± 0.081 6.090 ± 0.374	1.910 ± 0.099 6.299 ± 0.421	1.915 ± 0.054 6.678 ± 0.339** (↑10%)	1.873 ± 0.053** (↓5%) 6.715 ± 0.272** (↑10%)
Lung – abs. – rel.	1.342 ± 0.112 4.111 ± 0.198	1.307 ± 0.079 4.308 ± 0.294	1.253 ± 0.044 4.371 ± 0.279	1.323 ± 0.063 4.746 ± 0.322** (↑15%)
Heart – abs. – rel.	1.018 ± 0.118 3.124 ± 0.350	0.957 ± 0.051 3.153 ± 0.165	0.929 ± 0.067 3.239 ± 0.273	0.968 ± 0.079 3.466 ± 0.252* (↑11%)
Kidney – abs. – rel.	1.964 ± 0.208 6.011 ± 0.375	1.907 ± 0.149 6.274 ± 0.306	1.953 ± 0.141 6.823 ± 0.721** (↑13%)	2.010 ± 0.152 7.195 ± 0.417** (↑20%)
Adrenal – abs. – rel.	0.0566 ± 0.0078 0.1738 ± 0.0233	0.0583 ± 0.0097 0.1925 ± 0.0354	0.0647 ± 0.0094 (↑14%) 0.2256 ± 0.0347** (↑30%)	0.0656 ± 0.0083 (↑16%) 0.2354 ± 0.0323** (↑35%)
Liver – abs. – rel.	7.669 ± 0.738 23.48 ± 1.30	7.226 ± 0.636 23.76 ± 1.18	7.362 ± 0.380 25.65 ± 1.39** (↑9%)	7.615 ± 0.509 27.29 ± 1.80** (↑16%)
Thymus – abs. – rel.	0.2427 ± 0.0537 0.739 ± 0.133	0.1903 ± 0.0314* (↓21%) 0.625 ± 0.085* (↓15%)	0.1546 ± 0.0273** (↓36%) 0.537 ± 0.086** (↓27%)	0.1333 ± 0.0216** (↓45%) 0.477 ± 0.073** (↓35%)
Spleen – abs. – rel.	0.5648 ± 0.0486 1.736 ± 0.170	0.5188 ± 0.0357 1.713 ± 0.165	0.4730 ± 0.0327** (↓16%) 1.652 ± 0.167	0.5041 ± 0.0910** (↓11%) 1.808 ± 0.334
Thyroid – abs. – rel.	0.0164 ± 0.0038 0.0505 ± 0.0121	0.0156 ± 0.0028 0.0513 ± 0.0085	0.0144 ± 0.0031 (↓12%) 0.0502 ± 0.0116	0.0143 ± 0.0024 (↓13%) 0.0510 ± 0.0072
Testes – abs. – rel.	3.469 ± 0.290 10.670 ± 1.120	3.350 ± 0.315 11.035 ± 1.005	3.314 ± 0.239 11.572 ± 1.158	3.334 ± 0.277 11.931 ± 0.751* (↑12%)

Data obtained from pages 96-97 and 100-101 of the study report; bw = body weight; Abs. = absolute; Rel. = relative to body weight; * Statistically significantly different from controls ($p<0.05$); ** Statistically significantly different from controls ($p<0.01$). Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 9. Mean ± S.D. absolute (g) and relative (g/kg bw) organ weights and terminal body weights (g) in female rats

Organ/Tissue	Exposure Concentration (mg/L)			
	0	0.04	0.2	1.0
Terminal bw	201 ± 9	198 ± 10	203 ± 12	195 ± 9
Brain – abs. – rel.	1.809 ± 0.075 8.998 0.460	1.817 ± 0.090 9.177 ± 0483	1.829 ± 0.076 9.046 ± 0.616	1.792 ± 0.078 9.213 0.431
Lung – abs. – rel.	1.037 ± 0.075 5.156 ± 0.371	1.040 ± 0.050 5.252 ± 0.253	1.092 ± 0.081 5.389 ± 0.312	1.100 ± 0.147 5.637 ± 0.561 (↑9%)
Heart – abs. – rel.	0.710 ± 0.047 3.528 ± 0.193	0.675 ± 0.053 3.408 ± 0.264	0.699 ± 0.038 3.452 ± 0.169	0.709 ± 0.067 3.638 ± 0.248
Kidney – abs. – rel.	1.291 ± 0.075 6.417 ± 0.330	1.366 ± 0.103 6.894 ± 0.454	1.447 ± 0.136* (↑12%) 7.159 ± 0.828* (↑12%)	1.483 ± 0.141** (↑15%) 7.618 ± 0.641** (↑19%)
Adrenal – abs. – rel.	0.0720 ± 0.0059 0.3578 ± 0.0266	0.0819 ± 0.0066 0.4138 ± 0.0360*	0.0875 ± 0.0155** 0.4318 ± 0.0736**	0.0769 ± 0.0125 0.3937 ± 0.0505
Liver – abs. – rel.	5.054 ± 0.426 25.09 ± 1.61	5.248 ± 0.406 26.45 ± 1.14	5.474 ± 0.530 (↑8%) 26.97 ± 1.65* (↑7%)	5.811 ± 0.621** (↑15%) 29.82 ± 2.46** (↑19%)
Thymus – abs. – rel.	0.1775 ± 0.0284 0.882 ± 0.141	0.1852 ± 0.0256 0.937 ± 0.144	0.1826 ± 0.0338 0.902 ± 0.165	0.1472 ± 0.0378 0.754 ± 0.183
Spleen – abs. – rel.	0.4259 ± 0.0630 2.115 ± 0.301	0.4167 ± 0.0342 2.105 ± 0.180	0.4528 ± 0.0780 2.229 ± 0.330	0.4235 ± 0.0537 2.180 ± 0.298
Thyroid – abs. – rel.	0.0147 ± 0.0041 0.0729 ± 0.0192	0.0134 ± 0.0042 (↓8%) 0.0673 ± 0.0202 (↓8%)	0.0129 ± 0.0051 (↓12%) 0.0632 ± 0.0229 (↓13%)	0.0122 ± 0.0019 (↓17%) 0.0627 ± 0.0091 (↓14%)
Ovaries – abs. – rel.	0.0854 ± 0.0112 0.4251 ± 0.0605	0.0891 ± 0.0119 0.4493 ± 0.0549	0.1047 ± 0.0166* 0.5197 ± 0.1005*	0.0927 ± 0.0161 0.4750 ± 0.0713
Uterus – abs. – rel.	0.6766 ± 0.2657 3.385 ± 1.401	0.6050 ± 0.2584 3.050 ± 1.271	0.5290 ± 0.2087 (↓22%) 2.583 ± 0.959 (↓24%)	0.5020 ± 0.2122 (↓26%) 2.552 ± 0.996 (↓25%)

Data obtained from pages 98-99 and 102-103 of the study report; bw = body weight; Abs. = absolute; Rel. = relative to body weight; * Statistically significantly different from controls ($p<0.05$); ** Statistically significantly different from controls ($p<0.01$). Values in parentheses represent the percent change from controls (calculated by the reviewer).

7c. Histopathology

In agreement with the macroscopically observed encrustations on the back of many female animals, dermatitis and scab formation from contact with the inhalation apparatus were observed in the affected skin. No abnormalities were seen in the standardized flank skin/subcutis tissue sections.

Microscopic examination revealed treatment-related alterations in the epiglottis, nose and incisors as described below and summarized in Table 10.

Four of ten females from the high-concentration group exhibited corticomedullary mineralization of the kidney versus none in the control group. The kidney was examined in only one female

from the mid-concentration group (no evidence of corticomedullary mineralization was observed in that animal) while the kidney was not examined in any females from the low-concentration group; therefore, a dose-response could not be demonstrated.

Epiglottis: The epiglottis was affected at all concentrations in both sexes. The changes were characterized by squamous metaplasia (in all exposed animals), a concentration-dependent increase in the incidence of epithelial hyperplasia (from the low-concentration in males and from the mid-concentration in females), and an increase in the presence of focal mononuclear cell infiltrate (in males at all concentrations; statistical significance was reached only at the mid- and high-concentration in males). The epiglottis could not be assessed in 2 animals (one male each from the low- and mid-concentration groups) because no epiglottis tissue was present in the larynx sections (because epiglottis tissue is not visible when the sections of the larynx were prepared and therefore was lost).

Nasal cavity: The second level of the nasal cavity was affected at all concentrations in males and at the mid- and high-concentration in females. The changes consisted of squamous epithelial hyperplasia (at all concentrations in males, at the mid- and high-concentration in females), squamous metaplasia (at the mid- and high-concentration in both sexes), and squamous epithelial hyperkeratosis (at the high-concentration in males and at the mid- and high-concentration in females). The incidences of these changes increased with increasing exposure concentration. In addition, increased inflammation (characterized by an increased presence of focal mononuclear cell infiltrate and focal polymorphonuclear leukocytic infiltration) was observed at the mid- and high-concentration (although the focal mononuclear cell infiltrate was limited to one female at the mid-concentration). At the third level of the nasal cavity, squamous epithelial hyperplasia, squamous metaplasia and/or squamous epithelial hyperkeratosis were seen in one male of the high-concentration group and a few females of the mid-concentration group.

These microscopic changes indicate that the test material irritated the epithelium of the epiglottis and nasal cavity. In response to chronic irritation, transitional epithelial cells (epiglottis) and respiratory epithelial cells (nasal cavity) adapt and transform into less vulnerable squamous epithelial cells. Depending on the concentration, the squamous epithelial cells can be hyperplastic (epiglottis and nasal cavity) and/or show hyperkeratosis (nasal cavity). In agreement with chronic irritation, the incidence of inflammatory cells was increased, though not always statistically significantly.

Upper incisors: After decalcification in preparation for histopathological examination, remnants of enamel were observed in the upper incisors of most males (8/10) and females (8/10) of the high-concentration group, and in one male of the mid-concentration group. The teeth of the intermediate-concentration animals were decalcified slightly longer than those of the control and high-concentration animals. Enamel is removed by acid decalcification during processing of the teeth for microscopy. Hence, no remnants of enamel are present in microscopic slides of control teeth.

The presence of remnants of enamel might have resulted from an increased resistance to decalcification (during processing of the teeth for microscopic examination). Fluoride ions from the fluorine containing test material may have increased resistance to acid through remineralization of the enamel to form fluoroapatite. This is not considered by the study investigators to be an adverse effect; however, it does indicate uptake of fluoride and deposition in the teeth. Fluoride levels in the teeth and bone were not analyzed in this study. The lower incisors were preserved in formalin but not examined microscopically.

Table 10: Incidence of microscopic findings in epiglottis and nasal cavity

Organ	Control	0.04 mg/L	0.2 mg/L	1 mg/L
Males				
Epiglottis – No. examined	**10**	**9**	**9**	**10**
Squamous metaplasia	0	9***	9***	10***
Epithelial hyperplasia	0	6*	7**	10***
Focal mononuclear cell infiltrate	1	6	7*	7*
Nasal cavity – 2nd level – No. examined	**10**	**10**	**10**	**10**
	0	7**	10***	10***
Squamous metaplasia	0	0	2	10***
Squamous epithelial hyperkeratosis	0	0	0	4
Focal mononuclear cell infiltrate	0	0	3	3
Focal polymorphonuclear leukocytic infiltrate	0	0	0	0
Females				
Epiglottis – No. examined	**10**	**10**	**10**	**10**
Squamous metaplasia	0	10***	10***	10***
Epithelial hyperplasia	0	0	9***	10***
Focal mononuclear cell infiltrate	3	6	7	4
Nasal cavity – 2nd level – No. examined	**10**	**10**	**10**	**10**
Squamous epithelial hyperplasia	0	0	7**	10***
Squamous metaplasia	0	0	5*	9***
Squamous epithelial hyperkeratosis	0	0	1	7**
Focal mononuclear cell infiltrate	0	0	1	6*
Focal polymorphonuclear leukocytic infiltrate	0	0	0	5*

* Statistically significantly different from controls ($p<0.05$). ** Statistically significantly different from controls ($p<0.01$). *** Statistically significantly different from controls ($p<0.001$). Data obtained from pages 33-34 of the study report.

III. CONCLUSIONS

In conclusion, rats exposed to fluensulfone by inhalation (6 hours/day, 5 days/week, 13 consecutive weeks) showed various treatment-related changes.

Potentially adverse changes which were present at the lowest concentration consisted of:

- weight loss on exposure days in males
- prolonged prothrombin time in females
- decreased thymus weight in males (without histopathological effects)
- histopathological changes in the epiglottis (squamous metaplasia, epithelial hyperplasia, focal mononuclear cell infiltrate) in both sexes and in the nasal cavity (squamous epithelial hyperplasia) in males.

For most of these changes, their magnitude/incidence increased with increasing exposure concentration. Squamous metaplasia in the epiglottis was present in all exposed animals.

As treatment-related changes occurred at all exposure concentrations, a No-Observed-Adverse-Effect-Concentration (NOAEC) could not be determined based on the results of this study. The lowest concentration tested (actual concentration 0.040 mg/L) was a Lowest-Observed-Adverse-Effect-Concentration (LOAEC).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments/Conclusions: While certain findings that the reviewer noted as being potentially treatment-related were considered by the study investigators to be unrelated to treatment, the reviewer agrees with the study investigators' conclusions regarding most findings and the LOAEC in this study. Fluoride concentrations in the teeth and bone were not determined in this study; however, it is noted that changes to tooth enamel were observed during daily clinical examinations and at necropsy, primarily in mid- and high-dose animals of both sexes, but also in two low-dose females.

This subchronic inhalation toxicity study in the rat is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for a subchronic inhalation study (OPPTS 870.3465; OECD 413) in the rat.

C. Deficiencies: No deficiencies were identified.

Tier II Summary **(PMRA #2181224, MRID 48574770)** revised by the regulatory authorities, April 2013

Note that the regulatory authorities differ in the conclusions reached regarding the interpretation of the skeletal variation findings (see section IV – Evaluation, Summary and Conclusions)

Study Type: Teratogenicity by the oral route in the rabbit

IIA 5.6.11 – MRID 48574770, PMRA 2181224

Report:	Ceccatelli R. (2009) MCW-2 TECH Prenatal Developmental Toxicity Study in the Himalayan Rabbit Harlan Laboratories Ltd., Switzerland; unpublished report No. B92687, dated 10 November 2009. Sponsor reference No. R-23357 Dates of experimental work: (in-life phase) 26 August 2008 to 10 October 2008
Sponsor:	Makhteshim Chemical Works, Ltd., Beer-Sheva, Israel
Guidelines:	OECD 414, EC 2004/73 method B.31, OPPTS No. 870.3700, JMAFF, 12 Nohsan No. 8147
Deviations:	The room temperature of animal room was 20 ± 3°C instead of 18 ± 3°C. This deviation was not considered to have any impact on the outcome and integrity of the study.
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality Statements were provided.

Executive summary:

In a teratogenicity study (MRID 48574770, PMRA 2181224), 4 groups of 20 female Himalayan rabbits were mated and then administered daily doses of fluensulfone (MCW-2 tech., 96.67%, Batch No. 36372130-291-PF1) in 0.5% aqueous carboxymethylcellulose at 0, 2.5, 10 or 40 mg/kg bw at a dosing volume of 5 mL/kg bw on gestation days (GD) 6 through 27. Maternal evaluations during gestation included clinical observations and changes in body weight gain and food consumption. All does were sacrificed on GD 28, at which time gross external and internal necropsies were performed. The gravid uterus was weighed and examined, and the foetuses were removed by caesarean section. Fetuses were removed from the uterus, sexed, weighed individually, examined for gross external abnormalities, and sacrificed by a subcutaneous injection of sodium pentobarbital. All fetuses were examined for visceral abnormalities. The heads of half of the fetuses were serially sectioned and examined. The other half of fetuses was used for skeletal examination.

The total number of females with live fetuses was 20, 19, 19 and 17, respectively, in order of ascending dosages. One female each at 2.5 and 40 mg/kg bw/day was not pregnant, and one female and two females respectively in groups treated at 10 and 40 mg/kg bw/day had

resorption sites only.

All does survived until the scheduled necropsy, and no clinical signs related to treatment were observed.

Mean body weight gain was slightly reduced in does treated at 40 mg/kg bw/day between GD 6 and 9 (control to high dose gains during this interval were 13, 24, 10 and -29 g, respectively), attaining statistical significance on GD 7 and 8. This effect was accompanied by a reduction in food intake noted between GD 18 and 24 (-22.2% compared to the control group). When body weight gain was corrected for gravid uterine weight, does in the 10 and 40 mg/kg bw/day dose groups lost 29% and 39% more weight from GD 6 to 28 than control does, respectively. In the range-finding study, marked weight loss and decreased food consumption along with clinical signs were observed at 50 mg/kg bw/day from GD 6-12 and mortality was observed at 60 mg/kg bw/day in a dose toleration study; therefore, the transient weight change at 40 mg/kg bw/day was considered treatment-related.

The relevant reproduction data (post-implantation loss and the mean number of fetuses per doe) were not affected by treatment with the test item at any dose level. No macroscopic findings related to treatment were noted at necropsy of the does.

Fetal data showed no treatment-related effects on sex ratio. Fetal body weight was slightly reduced (7% below controls) at 40 mg/kg bw/day. Head sectioning, visceral examination and skeletal and cartilage examinations of fetuses revealed only one finding related to treatment: incomplete ossification of digit 5 of the medial phalanx in both forelimbs. The maternal and developmental LOAEL is 40 mg/kg bw/day based on the reduced body weight gain and effects on food consumption noted in maternal animals and the reduced fetal body weight and delayed ossification; the NOAEL for both maternal and developmental toxicity was considered to be 10 mg/kg bw/day. Although maternal body weight effects were marginal at the LOAEL, the LOAEL is considered a threshold effect level, based on the significant toxicity observed in the range-finding studies at 50 and 60 mg/kg bw/day.

The developmental toxicity study in the rabbit is classified as **acceptable/guideline** and satisfies the guideline requirement for a developmental toxicity study in the rabbit (OECD 414; OPPTS 870.3700).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: MCW-2 TECH

Description: Solid

Batch No.: 36372130-291-PF1

Purity: 96.67%

CAS#: 318290-98-1

Expiry date: 31 December 2009

Stability of Test Item: Stable in the dosing formulations for at least 7 days when stored at room temperature

2. Vehicle: 0.5% aqueous carboxymethylcellulose (CMC)

3. Test animals

Species: Rabbit

Strain: Himalayan (SPF)

Age: Females: 17 to 29 weeks at day 0 *post coitum*

Weight at dosing: 2087 to 3193 g at day 0 *post coitum*

Source: Charles River Germany

Acclimation period: Minimum 7 days

Diet: Pelleted standard Kliba Nafag 3418 rabbit maintenance diet (Provimi Kliba SA, Switzerland) *ad libitum* (batch nos. 19/08 and 46/08)

Water: Community tap-water from Füllinsdorf *ad libitum* in water bottles

Housing: Individually in stainless steel cages equipped with an automatic cleaning system. A piece of wood and a haystick were also provided for environmental enrichment.

4. Environmental conditions

Temperature: 20 ± 3°C

Humidity: 30-70%

Air changes: 10-15 per hour

Photoperiod: 12-hour fluorescent light / 12-hour dark cycle with music during the light period

B. STUDY DESIGN AND METHODS

1. In life dates 26 August (starting of pairing) to 10 October 2008

2. Animal assignment and treatment

After acclimatization, females were placed in cages with sexually mature males (1:1) from the same source and strain until copulation had been observed. After mating, the females were removed and placed in individual cages. The day of mating was designated day 0 post coitum or gestation day (GD) 0. Male rabbits from the same source and strain were used for the mating only. These male rabbits were in the possession of Harlan Laboratories Ltd. and were not considered part of the test system. The fertility of these males had been proven and was continuously monitored.

The randomization procedure was based on body weight, adjusted if necessary, so that a similar number of rabbits were allocated to each group on each day of mating and ensuring an acceptable distribution of males to which the females were mated.

Twenty females were allocated to each group and doses of 0, 2.5, 10 and 40 mg/kg bw/day were administered by oral gavage on GD 6 through 27. Dosing volume (5 mL/kg bw) was adjusted daily based on doe body weight during the dosing period.

3. Dose selection rationale

The dose levels were selected based on a previous pilot developmental toxicity study in Himalayan rabbits (Bayer study T4062934) using dose levels of 8, 20, and 50 mg/kg bw/day (this study was not provided to regulatory authorities). According to the study investigators, the dose of 50 mg/kg bw/day resulted in severe maternal toxicity associated with moderate to severe body weight loss between GD 6 and 11 and a variety of signs such as cold ears, transiently decreased food intake, and decreased and discolored urine. It was reported that deaths were observed at 60 mg/kg bw/day in dose toleration studies (Bayer study T2071004 and T3071573), which were not provided to regulatory authorities.

4. Dose formulation preparation and analysis

The dose formulations were prepared weekly using the test item as supplied by the Sponsor. The test item was heated overnight in a water bath at 40 ± 5°C. The next morning, the test item was weighed into a glass beaker on a tared precision balance and approximately 80% of the vehicle was added. The test item was stirred using a magnetic stirrer for approximately 5 minutes. The remaining vehicle was added and the formulation was placed in the water bath again for approximately 30 minutes. Directly before administration, the formulations were removed from the water bath. Homogeneity of the test item in the vehicle was maintained during the administration period using a magnetic stirrer. The vehicle alone was stirred but not heated.

Homogeneity of the test item in the vehicle was maintained during the daily administration period using a magnetic stirrer.

Dose formulations were stored at room temperature (20 ± 5°C) in glass beakers.

On the first day of dosing, one sample from the control group as well as three samples (top, middle and bottom) of about 2 g of each concentration were taken prior to dosing for analysis of concentration and homogeneity after the formulations had been removed from the water bath.

Samples of about 2 g of each concentration were taken from the middle only to confirm stability (over 4 hours and 7 days). Since the test item content in the 10 and 40 mg/kg bw/day dose groups was found to be out of the accepted range of ±20% of the nominal content, new dose formulations were prepared four days after the initiation of dosing using the same procedure. Samples to confirm the stability (over 4 hours and 7 days) were also re-taken.

During the last week of dosing, samples were taken from the middle to confirm concentration.

The samples were analysed by HPLC coupled to a UV detector.

5. Observations

Viability / mortality was recorded twice daily and clinical signs were recorded once a day.

Food consumption was recorded for the following periods: GD 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-21, 21-24 and 24-28. Body weight was recorded daily from GD 0 until GD 28.

6. Termination of the study

At the scheduled necropsy on GD 28, females were sacrificed by an intravenous injection of sodium pentobarbital (Eutha 77®) and the fetuses removed by Caesarean section.

All females were subjected to a macroscopic examination with emphasis on the uterus and its contents. The uteri (and contents) of all females with live fetuses were weighed during necropsy on GD 28 to enable the calculation of the corrected body weight gain.

7. Fetal pathology

Fetuses were removed from the uterus, sexed, weighed individually, examined for gross external abnormalities, sacrificed by a subcutaneous injection of sodium pentobarbital and subjected to the following procedures:

1. The fetuses were dissected; the organs examined and any abnormal findings were recorded.
2. After the skin had been removed, the cranium was examined for the degree of ossification.
3. For one half of the foetuses, the heads were separated from the trunks and fixed in Bouin's fixative. They were serially sectioned and examined (for evaluation of the internal structures of the heads, including the eyes, brain, nasal passages and tongue). Descriptions of any abnormal findings were recorded. After examination, the sections were preserved in a solution of ethyl alcohol and glycerin (one head per container). For the other half of the foetuses, the heads were not separated but processed and stained as described in the next paragraph.
4. From all fetuses the skin with the exception of over the paws and the dorsal-cervical fat pads was removed and discarded. The trunks of the fetuses without heads and the fetuses with heads were processed through solutions of ethanol, glacial acetic with Alcian blue (for cartilage staining), potassium hydroxide with Alizarin red S (for clearing and staining ossified bone) and aqueous glycerin for preservation and storage. The skeletons were examined and all abnormal findings and variations were recorded. The specimens were preserved individually in plastic vials.
5. Fetuses with abnormalities were photographed.

7. Data evaluation and statistical analysis

From the recorded reproduction data, the following parameters were calculated: pre- and post-implantation losses, embryonic and fetal deaths, live and dead fetuses, abnormal fetuses, fetal sex ratios and fetal body weights.

For reproduction data, group mean values were calculated both on a litter basis and on a percentage per group basis. Mean fetal weights were calculated from the individual weights both on a per group and on a per litter basis.

Historical control data were provided to allow comparison with concurrent controls. Historical control data for reproduction and fetal malformations and variations were included from studies conducted from 2004 - 2006. Two sets of data were provided; the first set came from five studies with a total of 575 fetuses from 91 litters and the second set from six studies with a total of 763 fetuses from 1113 litters. Data were presented as both fetal and litter incidences.

Food consumption, body weights and reproduction data were analyzed using the following statistical methods:

- The Dunnett-test (many to one t-test) based on a pooled variance estimate was applied if the variables could be assumed to follow a normal distribution for the comparison of the treated groups and the control groups for each sex.
- The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data could not be assumed to follow a normal distribution.
- Fisher's exact-test was applied if the variables could be dichotomized without loss of information.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Analytical results

At the initial sampling from the first preparation, fluensulfone content in the samples from the 10 and 40 mg/kg bw/day dose groups was found to be out of the accepted range of ±20% of the nominal content (40% and 26% of nominal concentration, respectively, for the 10 and 40 mg/kg bw/day dose formulations). As a consequence, 6 animals in each of the 10 and 40 mg/kg bw/day groups possibly received doses lower than desired for 1-4 days. The subsequent control of all recorded raw data and procedure, including order of preparation, weighing of test item, volumes of vehicle added, and sample collection confirmed the correct procedure of formulation preparation and did not give any evidence of the reason for the dissatisfying results. The first dose formulation had been performed under the study based inspection of the quality assurance. Control of the analytical raw data or procedures also did not give any evidence for analytical issues of concern. As the analytical results in the 10 and 40 mg/kg bw/day dose formulations prepared thereafter were consistent, homogeneous distribution was confirmed, and the fluensulfone formulations were demonstrated to be stable at room temperature, an undetected bias during sample collection or processing was considered probable to have occurred. This was supported by the fact that the in life data for these animals were comparable to the other animals in the same dosage groups, and therefore it was supposed that all dose formulations used had the correct contents.

The new dose formulations were prepared 4 days after the start of administration and verified analytically. Mean recoveries from all samples ranged between 85% and 102% of nominal, thus confirming the correct preparation and that correct dosing of all animals was assured from the fourth day of administration.

The test item was found to be stable in dose formulations when stored for 7 days at room temperature (concentrations of 3-11% of initial concentrations). Concentrations in samples taken from the top, middle and bottom of the dose formulations varied from the mean concentration by 0.6% to 8.1%, demonstrated homogeneity of the dose formulations.

2. Maternal toxicity

All does survived until the scheduled necropsy, and no clinical signs related to treatment were observed.

Mean body weights were generally comparable among the treated and control groups throughout the study period (Table 1). In rabbits treated at 40 mg/kg bw/day, a slight mean body weight loss (Table 1) was observed at the onset of the treatment between GD 6 and 9 (from control to high dose, mean body weight change of 13, 24, 10 and -29 g, respectively). Mean food consumption was also reduced between GD 18 and 21 (decreased 8.5% compared to the control group) and attaining statistical significance between GD 21 and 24 (decreased 22% compared to the control group).

When body weight gain was corrected for gravid uterine weight, does in the 10 and 40 mg/kg bw/day dose groups lost 29% and 39% more weight from GD 6 to 28 than control does, respectively.

During macroscopic examination of maternal animals, no treatment-related abnormal findings were noted.

Table 1: Body weight and body weight gain (mean ± SD)

Gestation Day	Control	2.5 mg/kg bw/day	10 mg/kg bw/day	40 mg/kg bw/day
Body weight (g)				
0	2505 ± 274	2491 ± 289	2524 ± 254	2490 ± 250
6	2551 ± 256	2546 ± 269	2576 ± 250	2561 ± 236
9	2564 ± 248	2570 ± 261	2586 ± 253	2532 ± 232
12	2583 ± 239	2580 ± 259	2609 ± 244	2566 ± 206
18	2673 ± 242	2666 ± 235	2692 ± 235	2662 ± 213
22	2687 ± 240	2679 ± 224	2715 ± 229	2669 ± 206
24	2709 ± 237	2705 ± 223	2735 ± 225	2695 ± 195
28	2773 ± 239	2753 ± 224	2782 ± 213	2761 ± 198
Gravid uterine weight	301 ± 94	288 ± 59	308 ± 64	310 ± 83
Body weight gain (g)[a]				
6 - 9	13	24	10	-29
6 - 28	222	207	206	200 (↓10%)[b]
Corrected 6 - 28 (%)[c]	-79 ± 92 (-3 ± 3%)	-81 ± 76 (-3 ±3%)	-102 ± 73 (-4 ± 3%)	-110 ± 136 (-4 ±5%)

[a] Calculated from the mean body weight values.
[b] Value in parentheses represents the percent change from the control mean (calculated by the reviewer).
[c] Corrected for gravid uterine weight. Values in parentheses represent the corrected body weight gain as a percent of the body weight on GD 6.
Data obtained from pages 43- 52 of the study report.

Table 2: Food consumption (g/animal/day - mean ± SD)

Gestation Day	Control	2.5 mg/kg bw/day	10 mg/kg bw/day	40 mg/kg bw/day
0 - 3	100 ± 30	110 ± 25	105 ± 30	109 ± 15
3 - 6	107 ± 33	113 ± 20	113 ± 17	114 ± 21
6 - 9	95 ± 26	113 ± 21	103 ± 22	92 ± 32
9 - 12	100 ± 19	108 ± 20	106 ± 20	99 ± 30
12 - 15	94 ± 23	103 ± 27	100 ± 26	99 ± 29
15 - 18	94 ± 26	93 ± 27	99 ± 39	94 ± 44
18 - 21	106 ± 26	105 ± 26	110 ± 22	97 ± 40 (↓8.5%)
21 - 24	108 ± 24	102 ± 20	105 ± 17	84 ± 26* (↓22%)
24 - 28	88 ± 27	82 ± 16	88 ± 14	87 ± 22

* $p < 0.05$. Data obtained from page 40 of the study report. Values in parentheses represent the percent change from the control mean, calculated by the reviewer.

3. Caesarean data

Relevant reproduction parameters (pre- and post-implantation loss and number of fetuses) were not affected by treatment (Table 3).

There were no treatment-related effects on the sex ratio of the fetuses. The statistically significantly higher percentage of males noted at 40 mg/kg bw/day was due to the lower percentage of male fetuses which occurred in the control group (51.8% versus 38.3% in control). The control value was below the range of historical control values (44.1-53.8%; from 5 studies conducted between 2004 and 2006).

At the highest dose level of 40 mg/kg bw/day, weight of male fetuses was statistically significantly reduced compared to controls (30.4 g versus 34.1 g on an individual basis and 31.6 g vs. 34.8 g on a litter basis). Total mean fetal weight was also reduced attaining statistical significance when calculated on a fetal basis (31.3 g versus 33.7 g), possibly due to the noted decrease in the weight of the male fetuses. This was considered likely due to the lower food intake observed in does during GD 18 and 24. The recorded fetal body weight values were within historical control range (minimum average of 29.1 g and maximum average of 33.6 g on an individual basis for both sexes combined; from 5 studies conducted between 2004 and 2006), but an effect of treatment cannot be ruled out.

At 10 mg/kg bw/day, the mean weight of total fetuses was statistically significantly lower when calculated on an individual basis (32.5 g versus 33.7 g in the control). This was likely due to the slightly higher number of fetuses since when calculated on a litter basis it did not attain statistical significance.

Table 3: Caesarean data

Parameter	Control	2.5 mg/kg bw/day	10 mg/kg bw/day	40 mg/kg bw/day
Number of mated females	20	20	20	20
# not pregnant	0	1	0	1
# with resorptions only	0	0	1	2
Number of does with live fetuses	20	19	19	17
Corpora lutea - Total	141	126	142	125
Mean ± SD	7.1 ± 1.5	6.6 ± 1.1	7.5 ± 1.6	7.4 ± 2.1
Pre-implantation loss - Total	15	10	12	8
% of corpora lutea	10.6	7.9	8.5	6.4
Mean ± SD	0.8 ± 1.1	0.5 ± 1.0	0.6 ± 1.6	0.5 ± 0.9
# does affected	9	6	6	5
Implantation sites - Total	126	116	130	117
% of corpora lutea	89.4	92.1	91.5	93.6
Mean ± SD	6.3 ± 1.8	6.1 ± 1.5	6.8 ± 1.7	6.9 ± 2.3
Post-implantation loss - Total	11	10	11	7
% of corpora lutea	8.7	8.6	8.5	6.0
Mean ± SD	0.6 ± 0.9	0.5 ± 0.7	0.6 ± 0.7	0.4 ± 0.6
# does affected	8	8	9	6
Implantation site scars	0	0	0	0
Total resorptions	11	10	11	7
Early resorptions - Total	8	7	11	6
% of implantation sites	6.3	6.0	8.5	5.1
Mean ± SD	0.4 ± 0.9	0.4 ± 0.6	0.6 ± 0.7	0.4 ± 0.5
# does affected	5	6	9	6
Late resorptions - Total	3	3	0	1
% of implantation sites	2.4	2.6	--	0.9
Mean ± SD	0.2 ± 0.4	0.2 ± 0.5	--	0.1 ± 0.2
# does affected	3	2	--	1
Total fetuses	115	106	119	110
% of implantation sites	91.3	91.4	91.5	94.0
Mean ± SD	5.8 ± 1.9	5.6 ± 1.4	6.3 ± 1.7	6.5 ± 2.2
Live fetuses	115	106	119	110
Dead fetuses	0	0	0	0
Abnormal fetuses	0	0	0	0
Sex ratio (% males)	38.3	50.0	47.9	51.8^
Fetal weight (g) - litter basis				
Total - Mean ± SD	34.1 ± 2.9	33.4 ± 2.6	32.8 ± 2.7	31.9 ± 3.2 (↓6%)
Males - Mean ± SD	34.8 ± 3.6	34.1 ± 3.0	33.2 ± 3.1	31.6 ± 3.3* (↓9%)
Females - Mean ± SD	33.7 ± 3.0	32.1 ± 3.4	32.6 ± 2.6	32.7 ± 3.4
Fetal weight (g) - individual basis				
Total - Mean ± SD	33.7 ± 3.7	33.1 ± 3.4	32.5 ± 3.7* (↓4%)	31.3 ± 3.7** (↓7%)
Males - Mean ± SD	34.1 ± 4.1	33.7 ± 3.4	32.7 ± 3.6 (↓4%)	30.4 ± 3.7** (↓11%)
Females - Mean ± SD	33.5 ± 3.4	32.4 ± 3.4	32.3 ± 3.8 (↓4%)	32.2 ± 3.5 (↓4%)

^: Fisher's Exact Test significant at 5%; * and **: Dunnett-Test based on pooled variance significant at 5% or 1%, respectively. Data obtained from pages 25 and 53-54 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

Examination of fixed sectioned fetal heads did not reveal any treatment-related effects: the only abnormality noted was a tissue mass in the nasopharyngeal tract of one fetus at 10 mg/kg bw/day. The incidence and type of variations also did not indicate any treatment-related effect.

During visceral examination of the fetuses, findings were noted in all litters from all groups and in 60%, 65%, 61% and 57% of examined fetuses, respectively, in the 0, 2.5, 10 and 40 mg/kg bw/day dose groups.

Selected findings from the external and visceral examinations are summarized in Table 4. As the type and frequency of the observed variations and abnormalities did not show any dose dependency or they were within the range of the historical control data, these findings were not considered to be related to the test substance.

External abnormalities were noted in one fetus in the 2.5 mg/kg bw/day group and included meningoencephalocele (head cranium frontal region dorsal opening, 6 mm, with brain tissue and fluid enclosed in membraneous sac) and occipital region with small dorsal projection. External abnormalities were also noted in two fetuses at 10 mg/kg bw/day (one fetus with head cranium flat and with small dorsal projection and lumbar spina bifida and another fetus with skull fontanelle misshapen and enlarged, and cranial bones with small holes and fluid leakage).

Visceral abnormalities were noted in two fetuses at 2.5 mg/kg bw/day (aortic arch dilated in one fetus, heart interventricular septal defect in another fetus) and in two fetuses at 10 mg/kg bw/day (abdominal wall thin in one fetus, heart interventricular septal defect in another one). One fetus at 40 mg/kg bw/day was missing a gall bladder and bile duct.

Table 4: Selected external and visceral malformations and variations

Parameter	Control	2.5 mg/kg bw/day	10 mg/kg bw/day	40 mg/kg bw/day
Viable fetuses				
Litters evaluated	20	19	19	17
Fetuses evaluated	115	106	119	110
Any finding				
Fetal incidence	69 (60%)	69 (65%)	72 (61%)	63 (57%)
Litter incidence	20 (100%)	19 (100%)	19 (100%)	17 (100%)
Malformations				
Fetal incidence	0	3 (3%)	4 (3%)	1 (1%)
Litter incidence	-	3 (16%)	4 (21%)	1 (6%)
Eye internal opacity (external variation)				
Fetal incidence	7 (6%)	6 (6%)	10 (8%)	8 (7%)
Litter incidence	4 (20%)	5 (26%)	5 (26%)	5 (29%)
Forelimb flexed (external variation)				
Fetal incidence	1 (1%)	0	2 (2%)	1 (1%)
Litter incidence	1 (5%)	-	2 (11%)	1 (6%)
Trachea cartilage disorganized (visceral variation)				
Fetal incidence	3 (3%)	1 (1%)	2 (2%)	2 (2%)
Litter incidence	2 (10%)	1 (5%)	2 (11%)	2 (12%)
Thymus long cranial (visceral variation)				
Fetal incidence	11 (10%)	12 (11%)	7 (6%)	3 (3%)
Litter incidence	6 (30%)	7 (37%)	7 (37%)	3 (18%)
Common carotid artery supernumerary branch (visceral variation)				
Fetal incidence	8 (7%)	14 (13%)	12 (10%)	17 (15%)
Litter incidence	6 (30%)	10 (53%)	6 (32%)	10 (59%)
Lung abnormal lobation (visceral variation)				
Fetal incidence	20 (17%)	18 (17%)	30 (25%)	26 (24%)
Litter incidence	13 (65%)	8 (42%)	14 (74%)	15 (88%)
Liver cyst(s) or white lesion(s) (visceral variation)				
Fetal incidence	15 (13%)	15 (14%)	11 (9%)	14 (13%)
Litter incidence	12 (60%)	8 (42%)	7 (37%)	8 (47%)
Renal vein supernumerary or branched (visceral variation)				
Fetal incidence	3 (3%)	3 (3%)	3 (3%)	1 (1%)
Litter incidence	3 (15%)	3 (16%)	3 (16%)	1 (6%)

Data obtained from pages 61-62 of the study report.

During skeletal examination of the fetuses, abnormalities and variations were noted in 20% of examined fetuses (in 60% of litters) in the control group, in 26% of examined fetuses (in 74% of litters) in the group treated at 2.5 mg/kg bw/day, in 20% of examined fetuses (in 79% of litters) in the group treated at 10 mg/kg bw/day, and in 15% of examined fetuses (in 65% of litters) in the group treated at 40 mg/kg bw/day.

Bone abnormalities were noted in two fetuses at 2.5 mg/kg bw/day (misshapen skull bones or severe incomplete ossification in one fetus, lumbar scoliosis in another one) and in two fetuses at 10 mg/kg bw/day (misshapen skull bones or severe incomplete ossification).

The type and frequency of these findings did not correlate with dose levels and were considered to be a result of the biological variability and not related to treatment with the test substance. The frequency of common variations did not indicate any treatment-related effect (Table 5).

Table 5: Selected skeletal malformations and variations

Parameter	Control	2.5 mg/kg bw/day	10 mg/kg bw/day	40 mg/kg bw/day
Viable fetuses				
Litters evaluated	20	19	19	17
Fetuses evaluated	115	106	119	110
Skulls evaluated	57	53	61	55
Any finding				
Fetal incidence	23 (20%)	29 (27%)	26 (22%)	20 (18%)
Litter incidence	12 (60%)	15 (79%)	15 (79%)	12 (71%)
Malformations				
Fetal incidence	0	2 (1%)	2 (2%)	0
Litter incidence	-	2 (11%)	2 (11%)	-
Skull suture bone (bone variation)				
Fetal incidence	4 (7%)	8 (15%)	6 (10%)	4 (7%)
Litter incidence	3 (15%)	5 (26%)	5 (26%)	4 (24%)
Skull parietal unossified area / line (bone variation)				
Fetal incidence	3 (5%)	2 (4%)	10 (16%)	2 (4%)
Litter incidence	3 (15%)	2 (11%)	8 (42%)	2 (12%)
Cervical rib (bone variation)				
Fetal incidence	2 (2%)	5 (5%)	0	3 (3%)
Litter incidence	2 (10%)	3 (16%)	-	2 (12%)
Fused sternebrae (bone variation)				
Fetal incidence	10 (9%)	4 (4%)	3 (3%)	2 (2%)
Litter incidence	7 (35%)	4 (21%)	2 (11%)	2 (12%)
Incompletely ossified 5th digit of the medial phalanx, left (bone variation)				
Fetal incidence	0	1 (1%)	1 (1%)	11 (10%)**
Litter incidence	-	1 (5%)	1 (5%)	5 (29%)*
Incompletely ossified 5th digit of the medial phalanx, right (bone variation)				
Fetal incidence	0	1 (1%)	1 (1%)	10 (9%)**
Litter incidence	-	1 (5%)	1 (5%)	5 (29%)*
Incompletely ossified sternebra 5 (bone variation)				
Fetal incidence	27 (23%)	37 (35%)*	48 (40%)**	26 (24%)
Litter incidence	14 (70%)	18 (95%)	17 (89%)	11 (65%)
Non-ossified sternebra 5 (bone variation)				
Fetal incidence	12 (10%)	20 (19%)	22 (18%)	7 (6%)
Litter incidence	5 (25%)	12 (63%)*	11 (58%)*	5 (29%)
Shortened costal cartilage 10, left (cartilage variation)				
Fetal incidence	52 (45%)	44 (42%)	46 (39%)	36 (33%)*
Litter incidence	17 (85%)	17 (89%)	14 (74%)	12 (71%)
Shortened costal cartilage 10, right (cartilage variation)				
Fetal incidence	60 (52%)	48 (45%)	53 (45%)	43 (39%)*

Litter incidence	17 (85%)	16 (84%)	16 (84%)	14 (82%)

Data obtained from pages 64-68 of the study report; * Significantly different from the control group, $p<0.05$; ** Significantly different from the control group, $p<0.01$.

The incidence of incomplete ossification of digit 5 of medial phalanx in both forelimbs was statistically significantly increased in the 40 mg/kg bw/day dose group when calculated on litter and fetal basis. Recorded values were within the range of the historical control data (fetal incidence of 1-25% from 5 studies conducted between 2004 and 2006; left: 25, 13, 9 22, 1%; right: 25, 13, 9, 23, 2%).The study investigators did not consider this finding to be test item-related. However, the reviewer cannot rule out an effect of treatment when concurrent controls, which also fell within the range of historical values, are used as the basis for comparison. Other statistical significances noted in the group treated at 2.5 and/or 10 mg/kg bw/day did not show a dose-dependent pattern and thus they were considered to be incidental.

During cartilage examination of the foetuses, abnormalities and variations were noted in 3% of examined fetuses (in 15% of litters) in the control group, in 6% of examined fetuses (in 32% of litters) at 2.5 mg/kg bw/day, in 5% of examined fetuses (in 21% of litters) at 10 mg/kg bw/day and in 6% of examined fetuses (in 35% of litters) at 40 mg/kg bw/day.

Cartilage abnormalities were noted in two fetuses at 10 mg/kg bw/day. One fetus was noted to have dorsal cervical vertebral arch incompletely fused and a second fetus was noted to have a lumbar vertebral arch displayed or not dorsally fused. No other cartilage abnormalities were noted.

The type and frequency of common variations did not indicate any test item-related effects.

At 40 mg/kg bw/day, the incidence of shortened left and right costal cartilage 10 was reduced compared to the control group when calculated on a fetus basis. Since no statistically significant differences were observed when calculated on a litter basis, this finding was not considered to be test item-related. The statistically significant findings noted at 2.5 and/or 10 mg/kg bw/day did not follow a dose dependent pattern and therefore were considered incidental.

III. CONCLUSIONS

In rabbits, treatment at 40 mg/kg bw/day resulted in slight maternal toxicity consisting in reduction of food consumption between GD 18 and 24, and body weight loss at the beginning of the treatment only, and decreased overall body weight gain. The statistically significantly lower fetal weights and incomplete ossification of digit 5 of the medial phalanx in both forelimbs recorded at 40 m/kg bw/day were within the range of the historical control data, but an effect of treatment on these parameters could not be ruled out. Based on the slight maternal toxicity on food consumption, the NOAEL for maternal and fetal organisms was considered to be 40 mg/ kg bw/day.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: The study investigators used the terms "embryonic resorptions" and "fetal resorptions" while the reviewer used the terms early and late resorptions, respectively. The study investigators also used the term abnormality while the reviewer used the term malformation.

The study investigators noted that food consumption was reduced in high-dose females at the initiation of treatment from GD 6 to 9; however, the reviewer noted that the food consumption for high dose females differed from control by only 3 g/animal, well within the range of variability noted in the dose groups, and therefore did not consider there to be a decrease in food consumption during this time period.

The study investigators concluded that the NOAEL for both maternal and developmental toxicity was 40 mg/kg bw/day, the highest dose tested. The reviewer has considered the body weight loss and reduced body weight gain noted in maternal animals, and the reduced fetal weight and incomplete ossification of digit 5 of the medial phalanx in both forelimbs to be related to treatment and adverse. The reviewer disagrees with the study investigators and considers that the maternal and developmental LOAELs are 40 mg/kg bw/day based on the reduced body weight gain and effects on food consumption noted in maternal animals and the reduced body weight and delayed ossification; the NOAELs for both maternal and developmental toxicity was considered to be 10 mg/kg bw/day. Although maternal body weight effects were marginal at the LOAEL, it is considered a threshold effect level for maternal toxicity, based on the significant maternal weight loss between GD 6-12 and clinical signs observed in the range-finding study at 50 mg/kg bw/day, and significant effects including mortality at 60 mg/kg bw/day.

OCS considers that the incidences of incomplete ossification of digit 5 of the medial phalanx in the forelimbs, which are within the historical control range, are likely incidental and not treatment-related. Consequently, OCS considers that the NOAEL for developmental toxicity of 10 mg/kg bw/day is based on fetal weight findings only.

The developmental toxicity study in the rabbit is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rabbit.

C. Conclusions: In this developmental toxicity study in rabbits, offspring effects were slight and were observed in the presence of mild maternal toxicity. No evidence of teratogenicity was observed in this study.

D. Deficiencies: Details pertaining to the conduct of the studies from which historical control data were obtained, such as route of exposure were not provided. No other deficiencies were identified.

Tier II Summary (PMRA #2181223, MRID 48574771) revised by the regulatory authorities, April 2013

Study Type: Teratogenicity test by the oral route in the rat

IIA 5.6.10 – MRID 48574771, PMRA 2181223

Report:	Young A.D. (2003) Technical Grade BYI 01921: A Prenatal Developmental Toxicity Study in the Wistar Rat. Bayer CropScience LP, Stillwell, Kansas, USA; unpublished report No. 02-T12-LD, dated 4 September 2003. Sponsor reference No. R-23155 Dates of experimental work: starting 22 July 2002
Sponsor:	Bayer CropScience LP, North Carolina, USA
Guidelines:	OECD 414, OPPTS No. 870.3700, JMAFF Guideline 12-Nousan No. 8147 and EU No. 87/302/EEC
Deviations:	None
GLP/Compliance:	Signed and dated GLP, Quality Assurance and Data Confidentiality Statements were provided.

Executive summary: In an oral teratogenicity study (MRID 48575771, PMRA 2181223), groups of 30 Wistar Crl:WI(HAN) female rats were mated and then administered daily dosages of fluensulfone (BYI 01921, MCW-2 tech., 96.1-97.0%, Batch No. NLL-6692-7-5) in 2% Cremophor, 0.5% CMC, and deionized water at 8, 50, or 300 mg/kg bw/day at a dosing volume of 10 mL/kg bw/day on days 6-19 of gestation, inclusive. All dams were sacrificed on gestation day (GD) 20. All fetuses were evaluated for external anomalies. Approximately half of all fetuses from each litter were examined for visceral effects at necropsy and also evaluated for cranial effects using the Wilson's technique. The remaining fetuses underwent an evaluation for general skeletal (including cartilage) development.

Maternal compound-related findings observed in dams during the treatment period at 300 mg/kg bw/day included body weight declines (up to 11% from control values; 6% when corrected for uterine weight) and body weight gain reduction (35% during treatment when corrected for uterine weight), coupled with decreased food consumption and increased absolute and relative liver and kidney weights. There were no other compound-related findings observed.

There were no reproductive effects attributed to the test compound nor were there any significant differences in the litter size, the median percent male fetuses, or placental weights in any group tested. One litter from the 300 mg/kg bw/day dose group contained four dead fetuses, which may have been secondary to maternal body weight effects, since the dam showed weight loss and stopped eating during the last few days of treatment. A decline in female fetal weights of 8% was observed at 300 mg/kg bw/day and was considered to be secondary to maternal toxicity. No compound-related effects were observed on fetal external, visceral, or skeletal malformations or variations, or on the incidence of total malformations or variations.

Incidences of several skeletal variations, including incompletely ossified parietal bone, interparietal bone and squamosal bone, as well as incidences of enlarged sagittal suture, anterior fontanelle, posterior fontanelle and squamosal suture were reduced compared to concurrent controls on both a fetal and litter basis in the 300 mg/kg bw/day dose group. These findings may represent a "more progressed" ossification of the fetal skeleton, perhaps related to the deposition of fluoride into bone during pre-natal development.

Based on decreased body weight gain and food consumption in dams and on lower body weight of female pups observed at the maternal and developmental LOAELs of 300 mg/kg bw/day, the maternal and developmental NOAELs were 50 mg/kg bw/day.

This study is classified as **acceptable/guideline (fully reliable)** and satisfies the guideline requirement for a developmental toxicity study in the rat (OPPTS 870.3700; OECD 414).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BYI 01921 (Fluensulfone) (BYI 01921 was an earlier development code for the active substance, MCW-2)
Description:	Due to the low melting point, this test substance appeared in a crystallized state or as yellow oil. When doses were prepared the test material was yellow oil when the 2% Cremophor was added.
Batch No.:	NLL6692-7-5
Purity:	97.0% and 96.1% were measured for samples tested on 6 May 2002 and 14 August 2002, respectively
CAS#:	318290-98-1
Expiry date:	Not stated
Stability of Test Item:	Stable in the dosing formulations
2. Vehicle:	A mixture of 2% Cremophor, 0.5% CMC, and deionised water
3. Test animals	
Species:	Rat
Strain:	Wistar [Crl:WI(HAN)]
Age:	Males: 16 weeks; females: 13 weeks
Weight at dosing:	Mean group values at beginning of treatment (day 6) for pregnant females ranged between 224.8 and 228.8 g
Source:	Charles River Laboratories, NC, USA
Acclimation period:	At least 6 days

Diet:	Purina Mills Rodent Lab Chow 5002 meal, available *ad libitum*
Water:	Tap water (municipal water supply of Kansas City) supplied to each cage by pressure-activated water nipples, available *ad libitum*
Housing:	During the co-housing phase a maximum of two females were housed with one male and those females found sperm positive were housed individually in polycarbonate cages with corn cob bedding

4. Environmental conditions

Temperature:	19 to 25°C
Humidity:	30-70%
Air changes:	Minimum of 10-15 per hour
Photoperiod:	12 hours of light [6:00 a.m. to 6:00 p.m.] alternating with 12 hours of darkness

B. STUDY DESIGN AND METHODS

1. In life dates: Starting date: 22 July 2002; end date not stated

2. Animal assignment and treatment:

The females were assigned to either a control or one of three treated groups using a weight stratification-based computer program (INSTEM Computer Systems, UK) and those females falling within +/- 20% of the mean for all females were placed on study.

Doses were administered by oral gavage at a dosage volume of 10 mL/kg bw on gestation day (GD) 6 through GD 19. Dosing volume was adjusted daily based on dam body weight during the dosing period.

3. Dose selection rationale:

Doses were selected based on the results of a pilot prenatal developmental toxicity study conducted in the rat. Fluensulfone was administered in that study at doses of 0, 10, 50, 200 and 400 mg/kg bw/day. Maternal weight gain and feed consumption were decreased and liver and kidney weights were increased at 200 mg/kg bw/day and above. This pilot study was not provided to the regulatory authorities.

4. Dose preparation and analysis:

Fluensulfone was suspended in a mixture of 2% Cremophor, 0.5% CMC, and deionized water as vehicle. The nominal dose levels were 0, 8, 50 and 300 mg/kg bw/day. The actual dose levels based on analytically confirmed concentrations were 0, 7.7, 48.6, and 292.0 mg/kg bw/day. Dose vehicle was prepared one day in advance of the dose suspension preparation and stored at room temperature. Following preparation, the concentration of the test compound at each dose level was determined and the stock suspensions refrigerated.

On the days of dosing, an aliquot of each stock suspension was taken for use in the animal room, from which the appropriate animals were dosed. Dosing suspensions and aliquots were continuously stirred on a stir plate until dispersion to the animals.

Homogeneity and 52-day stability of the compound in the vehicle under the storage conditions employed in this study were confirmed in the pilot prenatal developmental toxicity study at concentrations that bracketed the dosages used in the main study. After 52 days of storage in the refrigerator (2-8°C), the concentration of fluensulfone in the dosing solutions was 104-109% of the initial concentration. Results for the homogeneity analysis were not provided in the report.

5. Maternal Observations and Evaluations:

Inseminated females underwent a detailed examination for clinical signs twice daily from GD 0 to GD 20, once in the morning prior to dosing and again in the afternoon, with the exception of weekends and holidays when these observations were performed only once. The detailed evaluation for clinical signs included both observing the animal in the cage and removing the animal to perform a physical examination. Checks for mortality, which consisted of a cageside observation that characterized mortality, moribundity, and overt toxicity, were performed at least twice a day.

Body weights were recorded daily from GD 0 to GD 20. Food consumption was measured on GD 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20.

On GD 20, dams were terminated by CO_2 asphyxiation and a gross external examination and internal necropsy was performed. The ovaries were excised, corpora lutea counted, and pregnancy status determined. The liver, kidneys, and intact uterus were removed and weighed, and resorptions, if any, were characterized. Fetuses were removed from the uterine wall and each implant noted. The placentas were trimmed of extraneous tissue, blotted, and weighed.

6. Fetal Evaluations: Fetuses were sacrificed by intreperitoneal injection of 0.01-0.05cc Fatal Plus (Vortech Pharmaceuticals). All fetuses were individually identified, sexed, weighed and externally examined. Approximately half of all fetuses from each litter were fixed *in toto* in 70% alcohol and processed and evaluated for general skeletal and cartilage development. The remaining fetuses underwent a gross wet visceral examination and were then placed in Bouin's solution. Prior to fetal cranial examination, the fetuses were transferred to 70% alcohol. Sectioning of the head was performed according to the method of Wilson.

7. Data evaluation:

The data were analysed with the litter as the primary experimental unit.

Parametric data (including dam body weights and food consumption) were analysed using an Analysis of Variance (ANOVA), and if significant differences were observed, a Dunnett's Test. Fetal and placental weights were specifically analysed via the Healy's Test if significance was observed in the ANOVA.

Nonparametric data (e.g. litter size and number of corpora lutea) were first analysed by the Kruskal-Wallis test and then subjected to Dunn's Test if significant differences were identified.

Nonparametric dichotomous data (e.g. number normal/abnormal) were initially analysed by the Chi-Square Test and if significance was observed between groups then by the Fisher's Exact Test with the Bonferroni adjustment. Differences between the control and test compound-treated groups were considered statistically significant when $p \leq 0.05$ or $p \leq 0.01$.

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Maternal toxicity

The number of pregnant females was 24 in the control group and 24, 26 and 20 in the treated groups, in order of ascending dosage.

Incidental deaths occurred in both the 8 mg/kg bw/day (3 dams) and 300 mg/kg bw/day (4 dams) dose groups. There were no compound-related clinical findings observed in any dose level up to and including 300 mg/kg bw/day.

Body weight declines (Table 1) were observed at 300 mg/kg bw/day starting on day 7 (decreases of 3-5% days 7 to 9) with statistical significance from day 10-20 (range of 8-11% decreased compared to controls; mean decrease of 9.2%). Total gain throughout gestation (minus uterine weight) was declined by 35% when compared to controls. Feed consumption (Table 2) was also decreased at this dose level from Days 6-12 (mean 36%).

Table 1: Body weight and body weight gain (mean ± SE)

Gestation Day	Control	8 mg/kg bw/day	50 mg/kg bw/day	300 mg/kg bw/day
Body weight (g)				
0	208.2 ± 2.33	207.7 ± 2.88	211.0 ± 2.35	206.1 ± 2.43
6	228.8 ± 2.59	224.8 ± 3.42	228.7 ± 2.38	228.3 ± 2.90
7	230.4 ± 2.80	227.1 ± 3.68	228.7 ± 2.49	223.5 ± 3.28 (↓3%)
10	241.0 ± 2.85	235.9 ± 4.30	240.4 ± 2.66	220.2 ± 3.38** (↓9%)
15	258.5 ± 4.09	255.8 ± 3.53	262.8 ± 2.71	237.1 ± 4.39** (↓8%)
20	310.0 ± 5.06	299.9 ± 5.20	314.1 ± 4.24	284.2 ± 5.17** (↓8%)
Gravid uterine weight	60.1 ± 3.36	49.6 ± 3.97	62.9 ± 2.30	51.0 ± 2.37
Final weight (corrected)[a]	249.9 ± 4.44	250.3 ± 3.69	251.2 ± 2.97	233.3 ± 4.44* (↓6%)
Body weight gain (g)				
0 - 20	101.8 ± 4.05	92.3 ± 4.26	103.0 ± 2.81	78.1 ± 4.81** (↓23%)
6 - 20	81.2 ± 3.81	75.1 ± 3.92	85.4 ± 3.23	55.9 ± 4.41** (↓31%)
Total net gain (corrected)[b]	41.7 ± 2.90	42.7 ± 2.75	40.2 ± 1.82	27.1 ± 3.37** (↓35%)

[a]: final dam weight less the weight of the intact uterus
[b]: from day 0, corrected for gravid uterine weight
* $p < 0.05$; ** $p < 0.01$
Data obtained from pages 21-23 and 38 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer)

Table 2: Food consumption (g/kg bw/day - mean ± SE)

Gestation Day	Control	8 mg/kg bw/day	50 mg/kg bw/day	300 mg/kg bw/day
0 - 2	79.0 ± 3.80	79.9 ± 2.78	73.7 ± 2.69	77.6 ± 3.33
2 – 4	81.5 ± 2.59	82.0 ± 2.57	84.8 ± 1.86	83.9 ± 2.61
4 – 6	84.9 ± 1.70	79.2 ± 1.65	83.2 ± 2.68	86.1 ± 2.23
6 – 8	83.8 ± 3.03	82.4 ± 2.36	78.4 ± 2.63	52.6 ± 3.83** (↓37%)
8 – 10	85.8 ± 3.01	78.0 ± 2.97	82.0 ± 2.64	52.5 ± 3.10** (↓39%)
10 - 12	86.6 ± 2.82	78.7 ± 2.73	82.3 ± 2.14	60.8 ± 3.87** (↓30%)
12 - 14	87.3 ± 2.54	83.6 ± 2.91	82.9 ± 2.21	97.4 ± 9.80
14 – 16	89.0 ± 3.61	76.4 ± 3.03*	82.9 ± 1.32	81.4 ± 4.06
16 – 18	87.5 ± 2.95	83.1 ± 2.66	82.6 ± 2.29	75.9 ± 5.13
18 - 20	71.9 ± 2.32	76.0 ± 2.39	74.7 ± 2.46	75.1 ± 8.71

** $p < 0.01$
Data obtained from pages 25-26 of the study report. Values in parentheses represent the percent change from the control mean, calculated by the reviewer.

The mean terminal body weight of dams from the 300 mg/kg bw/day dose group was 8% lower than the mean control value. Relative liver and kidneys weights were increased 14-15% compared to the controls (Table 3). There were no changes in absolute organ weights or gross pathology observations attributed to the test compound in any dose group. Consequently, the observed increases in relative liver and kidney weights are considered to be attributed to the reduced body weights.

Table 3: Organ weights (mean ± SE)

Parameter	Control	8 mg/kg bw/day	50 mg/kg bw/day	300 mg/kg bw/day
Final body weight	310 ± 5.1	300 ± 5.2	314 ± 4.2	284 ± 5.2** (↓8%)
Liver - absolute	12.430 ± 0.2808	11.958 ± 0.3043	13.010 ± 0.2359	13.113 ± 0.4024
Liver – relative	4.007 ± 0.0607	3.979 ± 0.0549	4.143 ± 0.0498	4.598 ± 0.0844** (↑15%)
Kidneys - absolute	1.769 ± 0.0364	1.726 ± 0.0296	1.776 ± 0.0259	1.848 ± 0.0340
Kidneys - relative	0.573 ± 0.0132	0.578 ± 0.0110	0.567 ± 0.0077	0.654 ± 0.0171** (↑14%)

** $p < 0.01$. Data obtained from pages 34-35 of the study report. Values in parentheses represent the percent change from the control mean.

2. Caesarean data

No compound-related effects were observed for any reproductive parameter in any dose group tested (Table 4). The fertility index was 83.3%, 90.0%, 86.7%, and 80.0%, for the control, 8, 50, and 300 mg/kg bw/day dose groups, respectively. The mating index was 100% for all dose groups and the gestation index was 100% for all dose groups with the exception of the 8 mg/kg bw/day dose group which was 91.7% due to two animals with only resorption sites. No abortions were noted.

No differences were seen in the number of corpora lutea or the number of implantation sites. There were no compound-related effects observed on resorptions, early or late; or pre or post-implantation loss.

Table 4: Caesarean data

Parameter	Control	8 mg/kg bw/day	50 mg/kg bw/day	300 mg/kg bw/day
Fertility index (%)	83.3	90.0	86.7	80.0
Gestation index (%)	100	91.7	100	100
Mating index (%)	100	100	100	100
Dams with resorption sites only	0	2	0	0
Dams with viable fetuses	24	22	26	20
Dams with nonviable fetuses	0	0	0	1
# dams found dead	0	3	0	4
Total # corpora lutea	319	310	349	269
Mean ± SE	13.3 ± 0.37	12.9 ± 0.62	13.4 ± 0.35	13.4 ± 0.68
Range	11 - 20	6 - 20	11 - 18	9 - 23
Total # implantations	271	259	317	207
Mean ± SE	11.3 ± 0.59	10.8 ± 0.60	12.2 ± 0.46	10.4 ± 0.52
Range	1 - 15	1 - 15	5 - 17	5 - 15
Pre-implantation loss	48	57	36	63
Mean ± SE/dam	2.0 ± 0.59	2.4 ± 0.45	1.4 ± 0.41	3.2 ± 0.81
Mean ± SE %/dam	14.5 ± 4.35	18.7 ± 4.14	9.8 ± 2.74	20.6 ± 4.60
Range	0 - 92	0 - 83	0 - 67	0 - 69
Post-implantation loss	14	39	21	14
Mean ± SE/dam	0.6 ± 0.19	1.6 ± 0.54	0.8 ± 0.21	0.7 ± 0.27
Mean ± SE %/dam	5.6 ± 1.83	18.1 ± 5.74	6.2 ± 1.70	6.7 ± 2.68
Range	0 - 30	0 - 100	0 - 25	0 - 50
Nonviable fetuses	0	0	0	4
Mean ± SE/dam	-	-	-	0.2 ± 0.20
Mean ± SE %/dam	-	-	-	2.0 ± 2.00
Resorptions: early+late	14	39	21	10
Mean ± SE/dam	0.6 ± 0.19	1.6 ± 0.54	0.8 ± 0.21	0.5 ± 0.15
Range	0 - 3	0 - 12	0 - 3	0 - 2
Mean ± SE %/dam	5.6 ± 1.83	18.1 ± 5.74	6.2 ± 1.70	4.7 ± 1.43
Resorptions: early	14	39	20	10
Mean ± SE/dam	0.6 ± 0.19	1.6 ± 0.54	0.8 ± 0.20	0.5 ± 0.15
Mean ± SE/dam	5.6 ± 1.83	18.1 ± 5.74	5.9 ± 1.58	4.7 ± 1.43
Resorptions: late	0	0	1	0
Mean ± SE/dam	-	-	0.0 ± 0.04	-
Mean ± SE %/dam	-	-	0.3 ± 0.32	-

Data obtained from pages 40-41 of the study report

A statistically significant ($p<0.05$) decline in fetal weight for females only (8%) was observed at 300 mg/kg bw/day (Table 5), which was considered to be secondary to the maternal body weight and food consumption effects already noted in this group. Statistical significance was observed for the number of viable fetuses at 300 mg/kg bw/day due to one female (animal no. 657) which had four dead fetuses (as well as five live fetuses). This dam started to lose weight after GD 17, and did not consume any food from GD 18 to 20. The mean number of viable fetuses per litter for the 300 mg/kg bw/day dose group fell within the performing laboratory's historical control for this strain of rat (mean ± SE 10.2 ± 0.25; range of 9.5-11.4; 6 studies). It is not clear if this finding is secondary to the maternal body weight effect or incidental. There were no differences in the litter size, median percent of male fetuses, or placental weights observed between the control and the treated groups.

Table 5: Litter data

Parameter	Control	8 mg/kg bw/day	50 mg/kg bw/day	300 mg/kg bw/day
# litters	24	24	26	20
Litter size: Mean ± SE	10.7 ± 0.63	9.2 ± 0.76	11.4 ± 0.44	9.9 ± 0.50
Range	1 - 15	0 - 14	5 - 15	5 - 14
Total # fetuses	257	220	296	197
Viable fetuses	257	220	296	193
Mean ± SE/litter	10.7 ± 0.63	9.2 ± 0.76	11.4 ± 0.44	**9.6 ± 0.56****
Fetuses/implants (%)[a]	94.4 ± 1.83	81.9 ± 5.74	93.8 ± 1.70	93.3 ± 2.68
% viable	100	100	100	98
Nonviable fetuses	0	0	0	**4**
% nonviable	0	0	0	2
Median % male fetuses	57	50	59	50
Fetal weight (g)[a]	3.6 ± 0.05	3.6 ± 0.05	3.6 ± 0.04	3.4 ± 0.07
Males[a]	3.6 ± 0.04	3.6 ± 0.05	3.7 ± 0.04	3.5 ± 0.08
Females[a]	3.6 ± 0.06	3.5 ± 0.05	3.5 ± 0.04	**3.3 ± 0.07* (↓8%)**
Weight of placentas (g)[a]	0.47 ± 0.019	0.46 ± 0.009	0.43 ± 0.008	0.44 ± 0.013

* $p < 0.05$; ** $p < 0.01$. [a] mean ± SE. Data obtained from page 43 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

There were no external malformations or variations observed in any dose group tested.

No effects on either the fetal or litter incidences of visceral malformations or variations were observed in any group (Table 6). Incidental visceral malformations included: enlarged ventricle, observed in 1 fetus from 1 litter at 50 mg/kg bw/day; discoloured kidney, observed in 3 fetuses from 3 litters in the control group; and a hole in the hard palate, observed in 1 fetus from 1 litter in the control group. Based on the sporadic observation and variability of these findings, they were not considered treatment-related.

Table 6: Summary of visceral malformations and variations

Parameter	Control	8 mg/kg bw/day	50 mg/kg bw/day	300 mg/kg bw/day
Viable fetuses				
Litters evaluated	23	22	26	20
Fetuses evaluated	121	105	141	93
Variations				
Fetal incidence	16 (13.2%)	12 (11.4%)	14 (9.9%)	21 (22.6%)
Litter incidence	10 (43.5%)	11 (50.0%)	9 (34.6%)	13 (65.0%)
Malformations				
Fetal incidence	3 (2.5%)	0	1 (0.7%)	0
Litter incidence	3 (13.0%)	-	1 (3.8%)	-
Left side umbilical artery (variation)				
Fetal incidence	15 (12.4%)	12 (11.4%)	11 (7.8%)	20 (21.5%)
Litter incidence	10 (43.5%)	11 (50.0%)	8 (30.8%)	13 (65.0%)
Enlarged ventricle (malformation)				
Fetal incidence	0	0	1 (0.7%)	0
Litter incidence	-	-	1 (3.8%)	-
Uterine horn (variation)				
Fetal incidence	0	0	1 (0.7%)	0
Litter incidence	-	-	1 (3.8%)	-
Dilated ureter (variation)				
Fetal incidence	0	0	2 (1.4%)	1 (1.1%)
Litter incidence	-	-	2 (7.7%)	1 (5.0%)
Discolored kidney (malformation)				
Fetal incidence	3 (2.5%)	0	0	0
Litter incidence	3 (13.0%)	-	-	-
Hole in hard palate (variation)				
Fetal incidence	1 (0.8%)	0	0	0
Litter incidence	1 (4.3%)	-	-	-

Data obtained from pages 49 and 51-53 of the study report. Values in parentheses indicate percent change from controls (calculated by the study author).

There were no treatment-related skeletal malformations or variations observed in any group up to and including 300 mg/kg bw/day. Malformations considered incidental to treatment were extra thoracic arches and centra in a single fetus of 1 litter at 300 mg/kg bw/day. Incidental, albeit statistically significant skeletal variations, of one form or another, were observed, in the 50 and/or 300 mg/kg bw/day dose groups and included: incomplete ossification of the frontal, parietal, interparietal, supraoccipital, squamosal, and xiphoid bones; wavy/curved ribs; rudimentary ribs; and enlargement of the sagittal suture, squamosal suture, and anterior and posterior fontanelles (Table 7). Most of these findings represented decreases compared to controls, and all fell within the incidence in the historical controls or did not elicit a dose response relationship.

However, incidences of incompletely ossified parietal bone, interparietal bone and squamosal bone, as well as incidences of enlarged sagittal suture, anterior fontanelle, posterior fontanelle and squamosal suture were reduced compared to concurrent controls on both a fetal and litter basis in the 300 mg/kg bw/day dose group. These findings may represent a “more progressed” ossification of the fetal skeleton, perhaps related to the deposition of fluoride into bone during pre-natal development.

Table 7: Summary of skeletal malformations and variations

Parameter	Dose (mg/kg bw/day)				Historical Control
	0	8	50	300	
Viable fetuses					
Fetuses evaluated	136	115	155	100	522
Litters evaluated	24	22	26	20	97
Variations					
Fetal incidence	134 (98.5%)	115 (100%)	153 (98.7%)	100 (100%)	n/a
Litter incidence	23 (95.8%)	22 (100%)	26 (100%)	20 (100%)	n/a
Malformations					
Fetal incidence	0	0	0	1 (1.0%)	n/a
Litter incidence	-	-	-	1 (5.0%)	n/a
Incompletely ossified frontal bone (variation)					
Fetal incidence	21 (15.4%)	13 (11.3%)	7 (4.5%)**	10 (10.0%)	14% (1-32%)
Litter incidence	12 (50.0%)	11 (50.0%)	7 (26.9%)	8 (40.0%)	36% (48-74%)
Incompletely ossified parietal bone (variation)					
Fetal incidence	58 (42.6%)	40 (34.8%)	27 (17.4%)**	13 (13.0%)**	37% (6-64%)
Litter incidence	18 (75.0%)	18 (81.8%)	16 (61.5%)	7 (35.0%)*	69% (27-100%)
Incompletely ossified interparietal bone (variation)					
Fetal incidence	76 (55.9%)	65 (56.5%)	67 (43.2%)	32 (32.0%)**	48% (24-63%)
Litter incidence	20 (83.3%)	21 (95.5%)	23 (88.5%)	16 (80.0%)	83% (65-100%)
Incompletely ossified supraoccipital bone (variation)					
Fetal incidence	56 (41.2%)	48 (41.7%)	37 (23.9%)**	34 (34.0%)	50% (26-77%)
Litter incidence	20 (83.3%)	18 (81.8%)	16 (61.5%)	14 (70.0%)	82% (61-100%)
Incompletely ossified squamosal bone					
Fetal incidence	23 (16.9%)	18 (15.7%)	10 (6.5%)*	5 (5.0%)*	16% (3-34%)
Litter incidence	13 (54.2%)	13 (59.1%)	6 (23.1%)	4 (20.0%)	37% (15-78%)
Enlarged sagittal suture (variation)					
Fetal incidence	39 (28.7%)	22 (19.1%)	18 (11.6%)**	6 (6.0%)**	26% (4-43%)
Litter incidence	14 (58.3%)	13 (59.1%)	13 (50.0%)	4 (20.0%)*	59% (19-87%)
Enlarged anterior fontanelle (variation)					
Fetal incidence	41 (30.1%)	23 (20.0%)	19 (12.3%)**	11 (11.0%)**	25% (4-42%)
Litter incidence	15 (62.5%)	14 (63.6%)	14 (53.8%)	7 (35.0%)	61% (23-91%)
Enlarged posterior fontanelle (variation)					
Fetal incidence	42 (30.9%)	32 (27.8%)	18 (11.6%)**	7 (7.0%)**	25% (5-38%)
Litter incidence	15 (62.5%)	15 (68.2%)	13 (50.0%)	5 (25.0%)	53% (23-83%)

Table 7 (continued): Summary of skeletal malformations and variations

Parameter	Dose (mg/kg bw/day)				Historical Controls
	0	8	50	300	
Rudimentary ribs (variation)					
Fetal incidence	14 (10.3%)	23 (20.0%)	24 (15.5%)	25 (25.0%)*	11% (4-18%)
Litter incidence	7 (29.2%)	10 (45.5%)	14 (53.8%)	12 (60.0%)	39% (9-57%)
Wavy/curved ribs (variation)					
Fetal incidence	20 (14.7%)	15 (13.0%)	8 (5.2%)*	7 (7.0%)	12% (3-23%)
Litter incidence	8 (33.3%)	10 (45.5%)	7 (26.9%)	3 (15.0%)	31% (11-45%)
Incompletely ossified xiphoid (variation)					
Fetal incidence	94 (69.1%)	85 (73.9%)	103 (66.5%)	91 (91%)**	61% (35-71%)
Litter incidence	23 (95.8%)	22 (100%)	24 (92.3%)	20 (100%)	93% (85-100%)
Enlarged squamosal suture (variation)					
Fetal incidence	13 (9.6%)	8 (7.0%)	4 (2.6%)*	1 (1.0%)*	9% (2-18%)
Litter incidence	8 (33.3%)	7 (31.8%)	2 (7.7%)	1 (5.0%)	27% (11-48%)

* $p<0.05$; ** $p<0.01$. Data obtained from pages 55 and 57-74 of the study report. Values in parentheses indicate percent incidence (calculated by study author). Historical control data provided for four studies, pages 405-419 of the study report, and represent an overall mean and range of means.

There were no treatment-related increases in the total malformations or variations (Table 8).

Table 8: Summary of external, visceral and skeletal malformations and variations

Parameter	Control	8 mg/kg bw/day	50 mg/kg bw/day	300 mg/kg bw/day
Viable fetuses				
Litters evaluated	24	22	26	20
Fetuses evaluated	257	220	296	193
MALFORMATIONS				
Fetal incidence	3 (1.2%)	0	1 (0.3%)	1 (0.5%)
Litter incidence	3 (12.5%)	0	1 (3.8%)	1 (5.0%)
Fetuses /Litter[a]	1.00 ± 0.559	-	0.32 ± 0.321	0.45 ± 0.455
VARIATIONS				
Fetal incidence	150 (58.4%)	127 (57.7%)	167 (56.4%)	121 (62.7%)
Litter incidence	23 (95.8%)	22 (100%)	26 (100%)	20 (100%)
Fetuses/Litter[a]	57.42 ± 3.602	58.96 ± 1.987	56.14 ± 1.519	62.21 ± 2.657
TOTAL AFFECTED FETUSES				
Fetal incidence	3 (1.2%)	0	1 (0.3%)	5 (2.5%)
Litter incidence	3 (12.5%)	-	1 (3.8%)	2 (10.0%)
Fetuses/Litter[a]	1.00 ± 0.559	-	0.32 ± 0.321	2.68 ± 2.245

[a] mean % ± SE

III. CONCLUSIONS

Fluensulfone was not teratogenic in rats. Based on decreased body weight gain and food consumption in dams and on lower body weight of female pups observed at the maternal and developmental LOAELs of 300 mg/kg bw/day, the maternal and developmental NOAELs were 50 mg/kg bw/day.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: The developmental toxicity study in the rat is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rat.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions. The study investigators did not consider the increase in non-viable fetuses noted at 300 mg/kg bw/day to be related to treatment. The reviewer notes that this finding may have been secondary to maternal body weight effects. Nevertheless, the effect was limited to one litter, and is not a clear indication of a developmental effect.

In addition, incidences of incompletely ossified parietal bone, interparietal bone and squamosal bone, as well as incidences of enlarged sagittal suture, anterior fontanelle, posterior fontanelle and squamosal suture were reduced compared to concurrent controls on both a fetal and litter basis in the 300 mg/kg bw/day dose group. These findings may represent a "more progressed" ossification of the fetal skeleton, perhaps related to the deposition of fluoride into bone during pre-natal development.

D. Deficiencies: Results for the homogeneity determinations of the dosing solutions were not provided in the study report. Information pertaining to the historical control studies (dates of conduct, route of administration, vehicle) was not provided. No other deficiencies were identified.

Tier II Summary (PMRA # 2181222, MRID 48574769 revised by the regulatory authorities, April 2013

Study Type **Two generation reproductive study in the rat**

IIA 5.6.1 – MRID 48574769, PMRA 2181222

Report:	Ceccatelli R. (2011) MCW-2 TECH. Two-Generation Reproduction Toxicity Study in the Han Wistar Rat. Harlan Laboratories Ltd., Switzerland; unpublished report No. B92654, dated 20 May 2011. Sponsor reference No. R-23355 Dates of experimental work: 5 August 2008 to 19 May 2011
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 416, EC method B.35, OPPTS 870.3800 and MAFF 12 Noh San No. 8147 Deviations: none
GLP/Compliance:	Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In a two generation reproduction study, groups of 24 Wistar HanRcc:WIST(SPF) rats/sex/dose received fluensulfone [MCW-2 TECH (96.67-96.78% a.i., Batch No. 36372130-291-PF1)] in their diets at concentrations of 0, 30, 250 or 1800 ppm. In addition to the standard evaluations performed in a guideline reproductive toxicity study, fluoride content of teeth (incisor) and bone (femur) was assessed in F1 parental animals and F2 offspring. The overall average daily compound intake (weighted for each period of the study) was as follows: P adults: 0, 1.9, 15.4, or 114.2 mg/kg bw/day in males, 0, 2.7, 22.8 or 164.8 mg/kg bw/day in females; F1 adults: 0, 2.0, 17.0 or 129.7 mg/kg bw/day in males, 0, 2.9, 23.3 or 169.1 mg/kg bw/day in females; both generations combined: 0, 2.0, 16.2, or 122.0 mg/kg bw/day in males, 0, 2.8, 23.0, or 169.1 mg/kg bw/day in females). The premating average daily compound intake for both generations combined was 0, 2.2, 18.3 or 137 mg/kg bw/day in males and 0, 2.5, 20.3 or 149 mg/kg bw/day in females.

Parental toxicity: High dose male and female body weights were reduced in both generations throughout the treatment period (at end of premating, 10-12% less for males and 4-7% less for females), although high dose animals of the F1 generation started the post-weaning period with lower body weights than controls that did not increase in magnitude over the dosing period. Cumulative premating body weight gain was reduced by 21% and 12% for P males and females, respectively, but was not decreased in F1 animals. Body weight gain was also lower for P (8%) and F1 (10%) females during gestation, but not during lactation. In all other dose groups, body weight gain was either similar to controls or was increased to compensate for the initial losses.

Treatment with the test item at 1800 ppm resulted in a reduction of relative food consumption (g/kg bw/day) during the first week of dosing of the P generation. Due to lower body weights observed in the 1800 ppm dose group, absolute food consumption (g/animal/day) was reduced throughout most of the study at this dose.

Increased kidney and liver weights were seen in high dose animals of both generations and adrenal weights were reduced in high dose P females. Relative (to body weight) testes and epididymides weights were increased in the 1800 ppm F1 males. Other organ weight changes were noted at the high dose that were considered to be secondary effects of a lowered body weight as organ to body weight ratios were not affected.

Centrilobular hypertrophy of the liver at minimal to slight severity (P males, F1 males and females) and reduced glycogen deposits (P males and females, F1 males) were noted in parental animals dosed at 1800 ppm. The hypertrophy finding correlated with the increase in liver weight and was considered to be of metabolic nature and of adaptive character as there were no further indicators of liver injury. Glycogen deposits were reduced, likely due to the lower body weight. The incidence and severity of renal tubular hyaline droplets were increased in males of P and F1-parental animals. The incidence and/or severity of renal tubular basophilia, mononuclear cell infiltration and tubular casts were also increased in males of both generations. These lesions were considered to be the secondary lesion resulting from hyaline droplets described above. Follicular cell hypertrophy of the thyroid gland was recorded at minimal to slight severity in males of the P and F1 generations.

Reproductive toxicity: There were no treatment-related effects on reproductive performance.

Offspring toxicity: In both 1800 ppm F1 and F2 pups, pup body weights were reduced during lactation (10% below controls at PND 31). Post-natal pup losses before post-natal day 4 were observed in both the high dose P and F1 generations. From control to high dose, the percent loss was 0.4, 0.6, 1.4 and 2.6% (offspring from P mating) and 3.9, 3.1, 3.3 and 9.0% (offspring from F1 mating).

Spleen weights were reduced in both F1 and F2 high dose pups. Absolute and relative (to brain weight) thymus weights were reduced in the F1 pups and F2 pups, which were considered to be related to the lower body weights of these animals. During the histopathological examination, the severity of glycogen deposits was observed to be reduced in the F2 male and female pup livers, and a decrease in incidence of glycogen deposits was seen in the high dose female pups. This finding was likely associated with the lower body weights. The livers of F1 pups were not examined histologically.

Fluoride evaluation: In the F1 parental animals, mean fluoride content was dose dependently higher in bone (femur) and in teeth (incisors) at 30, 250 and 1800 ppm. With increasing dose, fluoride content of bones in males was increased by 100%, 494% and 1789% relative to controls; in females the increase was 72%, 332% and 1094%. In teeth, increases in males were 32%, 305% and 1600% and in females were 58%, 329% and 1077%. In F2 pups, the mean content of fluoride was higher in teeth of males at 30 ppm and above (35%, 64% and 206% with increasing dose), in bones and teeth of females at 250 ppm (33%) and 1800 ppm (206% in bone, 145% in teeth), and in bones of males at 1800 ppm (389%). Since there were no effects of discoloration of the teeth or fragility of bones and teeth in either F1 adults or F2 pups, it is concluded that there was no evidence of fluorosis in this study.

The parental LOAEL in males and females is 1800 ppm (overall study intake of 122.0 mg/kg bw/day in males and 169.1 mg/kg bw/day in females; premating intake of 137 mg/kg bw/day in males and 149 mg/kg bw/day in females) based on decreased body weight, body weight gain, food consumption and increased liver and kidney weights and histopathology changes in the liver and kidney. The parental NOAEL is 250 ppm (overall study intake of 16.2 mg/kg bw/day in males and 23.0 mg/kg bw/day in females; premating intake of 18.3 mg/kg bw/day in males and 20.3 mg/kg bw/day in females).

The reproductive NOAEL in males and females is 1800 ppm (overall study intake of 122.0 mg/kg bw/day in males and 169.1 mg/kg bw/day in females; premating intake of 137 mg/kg bw/day in males and 149 mg/kg bw/day in females). The reproductive LOAEL was not established as no treatment-related effects on reproductive performance were observed.

The offspring LOAEL in males and females is 1800 ppm (overall study intake of 122.0 mg/kg bw/day in males and 169.1 mg/kg bw/day in females; premating intake of 37 mg/kg bw/day in males and 149 mg/kg bw/day in females) based on decreased pup weights and spleen weights and an increase in post-natal pup loss between days 0 and 4 in the F1 and F2 offspring. The offspring NOAEL is 250 ppm (overall study intake of 16.2 mg/kg bw/day in males and 23.0 mg/kg bw/day in females; premating intake of 18.3 mg/kg bw/day in males and 20.3 mg/kg bw/day in females).

This study is classified as **acceptable/guideline (fully reliable)** and satisfies the guideline requirement (OPPTS 870.3800; OECD 416) for a two-generation reproductive toxicity study in the rat.

A. MATERIAL AND METHODS

1. Test Material	MCW-2 TECH (Fluensulfone)
Description:	Solid, yellow
Lot/Batch:	36372130-291-PF1
Purity:	96.67%-96.78%
CAS#:	318290-98-1
Stability:	Stable for the duration of the study. Test diets were shown to be stable for up to 24 days at room temperature.
2. Vehicle	Pelleted standard Kliba Nafag 3433 rat/mouse maintenance diet (Provimi Kliba SA, Switzerland)
3. Test Animals	
Species	Rat
Strain	HanRcc: WIST(SPF)
Age	7 weeks (at start of treatment)
Weight at start	190 to 237g males, 141-179g females (P generation)
Source	Harlan Laboratories Ltd. Laboratory Animal Services, Switzerland
Acclimation period	7 days
Diet	Pelleted standard Kliba Nafag 3433 rat/mouse maintenance diet (Provimi Kliba SA, Switzerland); *ad libitum*
Water	Community tap-water from Füllinsdorf; *ad libitum*
Housing	Individually in Makrolon type-3 cages with wire mesh top and sterilized standard softwood bedding (‘Lignocell’ Schill AG, Switzerland). During the pre-pairing period, cages with males were interspersed among those holding females to promote the development of regular estrus cycles.
4. Environmental conditions	
Temperature	22 ± 3°C
Humidity	21.2-70%
Air change	10-15 per hour
Photoperiod	12 hours light : 12 hours dark, with music during the light period

B. STUDY DESIGN

1. In-life dates 5 August 2008 to 17 May 2009 (initiation of dosing 12 August 2008)

2. Animal Assignment and treatment

Animals were randomly assigned based on a computer-generated algorithm to dose groups of 24 animals per sex and received diets containing 0, 30, 250 or 1800 ppm of fluensulfone. Body weights, recorded on the day of allocation, were taken into consideration to ensure similar mean body weights in all groups. Selection of F1 and F2 pups for different allocations (culling, necropsy, behavior tests and breeding) was based on randomly assigned pup numbers on day 1 post-partum.

Treatment was continuous throughout the study period, approximately 273 days. P animals were treated during the pre-pairing period (70-days), during the pairing and post pairing periods in males, and during the pairing, gestation and lactating periods in females for breeding of the F1 litters. A period of 14 days was allowed for mating. Males failing to copulate within this period were replaced by another male from the same group. Any female for which no evidence of mating was detected (sperm cells in daily vaginal smears or a copulating plug) after two mating periods of 14 days was housed individually.

Following weaning of the F1 litters on day 21 post-partum, F1 animals were selected for the next generation. Treatment was considered by the study investigators to have commenced when the selected F1 animals were about 4 weeks old, although animals were maintained on their respective diets from weaning. Treated diets were administered to the F1 generation through to adulthood (at least 91-day pre-pairing period) and also during the pairing, gestation and lactation periods related to the generation of the F2 litters.

3. Diet Preparation and Analysis

Dietary admixtures were prepared every two weeks using the test item as supplied. Fluensulfone was warmed in the original container in a water bath up to approximately 40°C until the test item was fluid. Thereafter, the whole amount of fluid test item was transferred into a glass beaker (wrapped with aluminum foil) and mixed using a magnetic stirrer on a heating plate (maximum approx. 40 °C). The test item was divided into three aliquots. The required amount of fluensulfone was weighed into tared glass beakers (wrapped with aluminum foil) and kept on a heating plate until use for feed preparation. The test item was mixed with microgranulated feed for each dose group. An appropriate amount of water was added to aid pelleting. The pellets were air dried for approximately 48 hours before storage. Control feed for the animals of group 1 were prepared similarly, but without test item.

Based on previous stability analyses (RCC study no. B76814), the fluensulfone feed is stable at room temperature for at least 24 days. At the start of the P generation pre-pairing period, samples of the test diet were drawn for the determination of content, homogeneity and stability (24 days). Additional samples of test diet were drawn at the beginning of the P lactation period and F1 pre-pairing and lactation periods for content and homogeneity determination. For assessment of content and homogeneity, a 100 g sample was collected from each top, middle and bottom dietary admixture of the respective diet preparation. For stability assessment, a 100 g sample was drawn from the middle of the dietary admixture on the day of the preparation.

Samples of the control feed from the first preparation were taken from the feed hoppers and from the store bag for concentration analysis.

4. Statistics

Food consumption, body weight and reproduction data were statistically analyzed using the following methods:

- Means and standard deviations of various data were calculated.
- The Dunnett-test (many to one t-test) based on a pooled variance estimate was applied if the variables could be assumed to follow a normal distribution for the comparison of the treated groups and the control groups for each sex.
- The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data could not be assumed to follow a normal distribution.
- The Fisher’s exact test was applied to macroscopic findings.

C. METHODS

1. Observations

Animals were observed twice daily for mortality and daily for cage-side clinical observations. Additionally, a detailed clinical examination was conducted on a weekly basis outside the home cage.

2. Bodyweight

All animals were weighed on the first day of dosing and thereafter at weekly intervals, with the exception of the pairing period. After mating, females were weighed on days 0, 7, 14 and 21 post coitum. Dams that produced litters were weighed on days 1, 4, 7, 14 and 21 post-partum and on the day the animals were sacrificed.

3. Food consumption

Food consumption was recorded weekly together with the recording of body weights. During the pairing period, no food consumption was recorded. During the lactation period, food consumption of the females was recorded until day 14 post-partum (i.e. until pups started consumption of the test diet). Relative food consumption ratios and intake of the test item expressed as mg/kg bw/day were calculated.

4. Vaginal smears

Vaginal smears for P and F1 females were taken for 21 days prior to pairing and throughout the pairing period until evidence of mating was detected.

5. Sexual development markers

For the F1 animals selected for breeding of the F2 generation, the age and body weight at which vaginal opening or preputial separation occurred were recorded.

6. Reproductive Indices

The following parameters were recorded/calculated: the duration of gestation, implantation rate, post-implantation losses, mean litter size, pup sex ratios, viability indices, and indices of mating, gestation, birth and lactation. In addition, mean and median pre-coital time was determined.

Birth index = (No. of pups born live/No. of implantations) x 100

Viability index = (No of pups alive on Day 4 post-partum/No. of pups born alive) x 100

Weaning index = (No. of pups alive on Day 21 post-partum/No. of pups alive on Day 4 post-partum) x 100

7. Litter examinations

The offspring were examined as soon as possible after completion of delivery for litter size, number of live and still births, and any gross abnormalities. Observations for number of missing (cannibalized) or dead pups and abnormal findings were performed daily. Sex ratio was recorded on Days 0 (the day on which a female had delivered all her pups) or 1, 4 and 21 post-partum. Body weight was recorded on Days 0 or 1, 4, 7, 14 and 21 post-partum. In addition, the onset of ear and eye opening, lower incisor eruption and hair growth were recorded.

8. Sacrifice and pathology

All P and F1 adult animals selected for breeding were sacrificed when they were no longer necessary for the assessment of reproductive effects.

F1 and F2 pups were culled by random selection to yield as nearly as possible 4 males and 4 females per litter on day 4 post-partum. F1 pups not selected for the F1 generation pairing and the remaining F2 pups were sacrificed after weaning. Females for which no pregnancy was detected were sacrificed three weeks after the last possible mating date. Females that lost their litters were sacrificed either directly after litter loss or with the other dams after weaning.

All animals were sacrificed by an injection of sodium pentobarbital (Eskonarcon). All animals sacrificed or found dead were subjected to a detailed macroscopic examination for any structural abnormalities or pathological changes and to establish the cause of death. Specimens of abnormal tissue were fixed in neutral phosphate buffered 4% formaldehyde solution. Dead pups, except those excessively cannibalized, were examined macroscopically.

For the parental animals, special attention was directed at the organs of the reproductive system. The uteri of all dams were placed in a solution of ammonium sulfide to visualize possible hemorrhagic areas of implantation sites and the number of implantation sites was noted.

The following organ weights were recorded for all P and F1 parental animals on day 21 post-partum, or shortly thereafter: Brain (incl. entire brainstem), Kidney, Pituitary, Adrenal glands, Liver, Spleen, Thyroid, Target Organs, Seminal vesicles with coagulating glands and their fluids (as one unit), Epididymides (total weight as caudal separately), Testes, Prostate, Ovaries and Uterus (including cervix, excluding oviducts). Paired organs were weighed separately.

The following organ weights were recorded from one randomly selected male and female pup from each F1 litter (on day 21 post-partum precisely): Brain, Spleen and Thymus.

The following organ weights were recorded from one randomly selected male and female pup from each F2 litter (on day 21 post-partum precisely): Brain, Spleen, Thymus, Kidney, Liver, Adrenal glands and Thyroid.

At necropsy the following tissues were collected from all parental animals and from one male and one female pup (selected for organ weight recording) in each F2 litter and fixed in neutral phosphate buffered 4% formaldehyde solution unless otherwise indicated: Gross lesions, Pituitary, Adrenal glands, Prostate, Seminal vesicles with coagulating gland, Right testis and epididymidis (in Bouin’s fixative), Ovaries, Uterus and cervix and Vagina. The Liver, Kidney, and Thyroid gland were also collected from P and F1 parental animals and F2 pups (these tissues were not collected from F1 pups).

Incisors and femur bones were also collected from all F1 animals and F2 pups. Right femurs and one half of the incisors were stored at -80°C for fluoride determination and left femurs and the other half of incisors in 4% formaldehyde solution in case of further evaluation. Fluoride determination was performed in right femur bones and in right incisors of ten F1 parental animals per sex and group and ten F2 pups (at PND 21) per sex and group.

Slides of all organs and tissues collected at terminal sacrifice from the animals of the control and high-dose groups were examined by the study pathologist. The same applied to all occurring gross lesions, and to all animals which died spontaneously or had to be terminated *in extremis*. Because of possible test item-related findings noted in the high dose group, the liver, kidneys and thyroid gland were also examined in mid- and low-dose groups of males in the P generation and of both sexes of the F1 generation adults, and the liver was examined in all dose groups in the F2 pups.

Ovarian histopathology, in addition to qualitative examination, included quantitative evaluation of primordial follicles, growing follicles and antral follicles from 10 sections per ovary in the first ten females of the P and F1 generations in the controls and high dose group. Additionally, corpora lutea were counted on one section per ovary. Histological examination of ovaries was carried out on any females that did not give birth.

Special emphasis was made on the stages of spermatogenesis and histopathology of interstitial cell structure. In addition, microscopic examination of the reproductive organs of all infertile males was made. At necropsy of adult males an epididymal sperm sample was obtained from the left caudal epididymis of each male. One hundred sperm were counted microscopically for determination of percentage of not motile, stationary motile and progressively motile sperm. A sperm sample from the left vas deferens was used for morphological assessment after fixation and Eosin staining. Five hundred sperm per sample were evaluated microscopically. The left caudal epididymis and left testis were taken for determination of homogenization-resistant spermatids and caudal epididymal sperm reserve. These evaluations were performed in the first instance only for the control and the high dose males. In the absence of a treatment-related effect the slides for the low and mid dose males were not evaluated.

II. RESULTS AND DISCUSSION

A. ANALYTICAL RESULTS

The diet samples investigated during the study were found to contain fluensulfone in the range of 81% to 118% of the target concentration. The homogeneous distribution of fluensulfone in the diet preparations was demonstrated because single results did not deviate more than 15% from the corresponding mean. In addition, the stability of the test item in the diet was demonstrated in samples taken 24 days after preparation. The variation limits ranged from 5% to 11% from the time-zero value. The absence of fluensulfone in the control diet was confirmed.

B. OBSERVATIONS

1. Clinical signs

P GENERATION

No fluensulfone-related clinical signs or observations were noted during the study. Findings like localized hair loss, crusts or irregular hair growth occurred in individual animals without following a dose-related pattern and were considered to be incidental.

F1 GENERATION

Findings like localized hair loss or crusts, tail kinked or tail apex kinked, mydriasis, ocular opacity and red circumorbital ring occurred in individual animals and were considered to be incidental. One male treated at 250 ppm had a longer incisor which had to be cut regularly; as none of the animals treated at 1800 ppm ever showed a similar abnormal growth of the incisors, this finding was considered incidental.

2. Mortality

P GENERATION

All animals of P generation survived until the scheduled necropsy.

F1 GENERATION

At 1800 ppm, one female was sacrificed on day 84 of the pre-pairing period because of several crusts on the shoulder and cervical area; sores over the body were confirmed at necropsy. One male of this group was found dead on day 13 of the pairing period; there were no previous clinical signs, necropsy revealed marked centrilobular necrosis with thrombus in the liver. At 30 ppm, one female was found dead on day 7 of the gestation period, the possible cause of death was established as a histiocytic sarcoma (affecting the liver, spleen, lung, ovary, adrenal glands and lymph node). Since these deaths occurred in individual animals and did not give any indication of dose-dependency, none of these deaths were attributed to the treatment.

C. BODYWEIGHT AND BODYWEIGHT GAIN

P GENERATION

Body weights and body weight gains in both males and females were affected at 1800 ppm (Tables 1a-d). In males, mean body weights/body weight gains were statistically significantly reduced during the pre-pairing period (10%/21%). During the post pairing period body weight gain at the high dose was similar to that of the control, although the mean body weight remained statistically significantly lower.

In females, mean body weights/body weight gains were slightly but statistically significantly reduced at the high dose during most of the pre-pairing period (4-5%/12%). Mean body weights remained statistically significantly lower during the gestation period through day 7 of the lactation period. Mean body weight gain was similar to the control during the gestation period and statistically significantly increased during the lactation period. In addition, mean body weight gain was slightly and non-statistically increased in the mid dose females during the lactation period.

Table 1a. Body weight (g) - pre-mating P generation (Mean ± S.D.)[a]

Study Day	0 ppm	30 ppm	250 ppm	1800 ppm
P Generation Males - Pre-mating				
Initial BW	209 ± 11.8	212 ± 10.0	209 ± 8.8	210 ± 8.8
BW Day 8	246 ± 15.0	249 ± 10.7	244 ± 9.7	229 ± 11.1** (↓7%)
BW Day 15	279 ± 18.5	284 ± 12.1	278 ± 14.0	261 ± 12.6** (↓6%)
BW Day 29	321 ± 24.7	330 ± 16.4	317 ± 20.3	295 ± 15.0** (↓8%)
BW Day 43	353 ± 26.4	365 ± 19.2	348 ± 25.2	320 ± 19.1** (↓9%)
BW Day 57	375 ± 30.5	386 ± 21.7	369 ± 27.0	339 ±20.3** (↓10%)
Final Pre-mating BW	392 ± 32.7	402 ± 23.8	387 ± 30.8	354 ± 21.0** (↓10%)
Overall BWG Days 1-70[b]	183	190	178	144 (↓21%)
P Generation Females - Pre-mating				
Initial BW	162 ± 7.2	160 ± 8.9	160 ± 8.2	160 ± 8.3
Final Pre-mating BW	243 ± 12.8	245 ± 14.5	243 ± 13.0	231 ± 10.6** (↓5%)
BW Day 8	177 ± 7.4	177 ± 10.6	178 ± 7.9	170 ± 8.5* (↓4%)
BW Day 15	192 ± 8.2	192 ± 10.0	192 ± 8.3	184 ± 9.1** (↓4%)
BW Day 29	207 ± 8.5	206 ± 11.2	211 ± 8.7	201 ± 10.5
BW Day 43	221 ± 9.6	225 ± 12.1	225 ± 9.4	214 ± 10.9
BW Day 57	235 ± 13.0	238 ± 16.1	236 ± 12.6	226 ± 11.0* (↓4%)
Overall BWG Days 1-70[b]	81	85	83	71 (↓12%)

[a] Data obtained from pages 160, 162, 171 and 174 in the study report. N=24.
[b] Calculated by reviewer using mean BW values
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BW body weight, BWG body weight gain
Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 1b. Body weight gain (%) - pre-mating P generation (Mean ± S.D.)[a]

Study Day	0 ppm	30 ppm	250 ppm	1800 ppm
P Generation Males - Pre-mating				
BWG Day 8	18 ± 2.9	17 ± 2.3	17 ± 2.8	9 ± 3.4**
BWG Day 15	34 ± 4.8	34 ± 3.9	33 ± 4.5	25 ± 4.6**
BWG Day 29	54 ± 7.9	55 ± 6.1	51 ± 6.8	41 ± 6.7**
BWG Day 43	69 ± 8.4	72 ± 7.1	66 ± 8.7	53 ± 8.6**
BWG Day 57	80 ± 9.6	82 ± 8.3	76 ± 9.4	62 ± 9.5**
BWG Day 70	88 ± 10.5	89 ± 9.2	85 ± 10.7	69 ± 9.3**
P Generation Females - Pre-mating				
BWG Day 8	9 ± 2.8	11 ± 3.6	11 ± 2.6*	6 ± 2.8**
BWG Day 15	19 ± 3.4	20 ± 4.1	20 ± 3.9	15 ± 3.2**
BWG Day 29	28 ± 3.7	29 ± 4.8	32 ± 4.3**	26 ± 4.6
BWG Day 43	36 ± 4.8	41 ± 6.1*	40 ± 5.9	34 ± 5.1
BWG Day 57	45 ± 7.6	49 ± 10.1	47 ± 6.5	41 ± 5.8
BWG Day 70	50 ± 6.6	54 ± 8.1	52 ± 7.8	45 ± 6.1*

[a] Data obtained from pages 165 and 167 in the study report. N=24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BWG body weight gain

Table 1c. Body weight (g) – post-mating P generation (Mean ± S.D.)[a]

Study Day	0 ppm	30 ppm	250 ppm	1800 ppm
P Generation Males – Post-mating				
Initial BW	407 ± 35.6	415 ± 23.1	404 ± 30.6	371 ± 22.1** (↓9%)
BW Day 8	414 ± 35.5	423 ± 23.2	411 ± 31.3	378 ± 21.1** (↓9%)
BW Day 22	424 ± 36.2	435 ± 25.8	422 ± 33.0	388 ± 22.7** (↓9%)
Final BW	439 ± 37.3	452 ± 26.2	436 ± 35.2	402 ± 23.4** (↓8%)
Overall BWG Days 1-36[b]	32	37	32	31
P Generation Females - Gestation				
Initial BW	242 ± 13.6	247 ± 16.4	243 ± 12.7	231 ± 10.8* (↓5%)
BW Day 7	265 ± 15.2	268 ± 16.3	263 ± 13.7	252 ± 12.8* (↓9%)
BW Day 14	288 ± 16.7	295 ± 16.5	288 ± 15	273 ± 14.7** (↓5%)
Final BW	353 ± 20.6	361 ± 24.3	350 ± 21.3	330 ± 20.2** (↓7%)
Overall BWG Days 0-21[b]	108	114	107	99 (↓8%)
P Generation Females - Lactation				
Initial BW	253 ± 16.2	260 ± 20.8	250 ± 16.6	232 ± 14.6** (↓8%)
BW Day 4	266 ± 13.6	271 ± 17.9	264 ± 14.8	252 ± 18.6* (↓5%)
BW Day 7	271 ± 14.6	283 ± 19.9	272 ± 16.3	255 ± 15.1* (↓6%)
BW Day 14	295 ± 15.7	307 ± 19.6	299 ± 17.7	285 ± 19.9
Final BW	296 ± 16.4	306 ± 18.3	301 ± 11.1	289 ± 18.7
Overall BWG Days 1-21[b]	43	46	51 (↑19%)	57 (↑32%)

[a] Data obtained from pages 161, 163, 164, 172, 175 and 176 in the study report. N=24.
[b] Calculated by reviewer using mean BW values
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BW body weight; BWG body weight gain
Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 1d. Body weight gain (%) – post-mating P generation females (Mean ± S.D.)[a]

Study Day	0 ppm	30 ppm	250 ppm	1800 ppm
P Generation Females - Gestation				
BWG Day 7	9 ± 2.3	9 ± 2.9	9 ± 2.1	9 ± 1.5
BWG Day 14	19 ± 2.8	20 ± 3.5	19 ± 2.9	18 ± 2.2
BWG Day 21	46 ± 5.7	46 ± 6.4	44 ± 6.3	43 ± 5.2
P Generation Females - Lactation				
BWG Day 4	5 ± 2.9	4 ± 3.9	6 ± 3.6	9 ± 3.5**
BWG Day 7	8 ± 4.2	9 ± 5.5	10 ± 4.6	12 ± 5.3*
BWG Day 14	17 ± 5.6	18 ± 7.6	20 ± 5.9	23 ± 5.7**
BWG Day 21	17 ± 4.7	18 ± 7.0	21 ± 6.5	25 ± 6.4**

[a] Data obtained from pages 168-169 in the study report. N=24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BWG body weight gain

F1 GENERATION

Male and female body weights were affected at 1800 ppm (Tables 2a-d). In males, mean body weights were statistically significantly reduced during the pre-pairing and post pairing periods from day 1 (21%) of the pre-mating period and remained somewhat lower (12%) at the end of the premating period, a result of the decreased F1 pup weights at the high dose prior to weaning.

In females, mean body weight was also statistically significantly reduced during the pre-pairing (22% on Day 1 and 7% at the end of premating) and gestation periods, and days 7 - 21 of the lactation period at the high dose.

Both males and females in the 1800 ppm dose groups gained more weight (as a percentage of their initial body weight) than their respective control groups during the pre-mating period (statistical significance was attained in females only). Females from the high dose group showed a slight, non-statistically significant reduction in overall body weight gain (10%) during the gestation period.

Table 2a. Body weight (g) - pre-mating F1 generation (Mean ± S.D.)[a]

Study Day	0 ppm	30 ppm	250 ppm	1800 ppm
F1 Generation Males - Pre-mating				
Initial BW	111 ± 17.9	107 ± 19.0	103 ± 17.7	88 ± 12.2** (↓21%)
BW Day 8	162 ± 19.7	159 ± 23.0	151 ± 27.2	131 ± 16.4** (↓19%)
BW Day 15	207 ± 21.7	205 ± 25.2	195 ± 31.1	170 ± 17.9** (↓18%)
BW Day 29	287 ± 24.6	286 ± 29.6	270 ± 42.9	245 ± 21.1** (↓17%)
BW Day 43	335 ± 29.1	336 ± 32.0	316 ± 43.6	287 ± 25.2** (↓14%)
BW Day 57	369 ± 31.2	371 ± 34.8	350 ± 48.7	319 ± 28.1** (↓14%)
BW Day 71	393 ± 33.7	391 ± 36.9	374 ± 52.0	338 ± 30.9** (↓14%)
BW Day 85	409 ± 36.8	411 ± 39.0	395 ± 47.7	356 ± 34.5** (↓13%)
Final Pre-mating BW	413 ± 37.9	418 ± 39.5	404 ± 47.1	364 ± 33.5** (↓12%)
Overall BWG Days 1-91[b]	302	311	301	278
F1 Generation Females - Pre-mating				
Initial BW	101 ± 11.3	99 ± 14.0	96 ± 13.7	79 ± 11.7** (↓22%)
BW Day 8	134 ± 11.4	134 ± 14.6	130 ± 13.9	112 ± 15.4** (↓16%)
BW Day 15	154 ± 12.1	155 ± 13.3	150 ± 13.3	132 ± 14.7** (↓14%)
BW Day 29	186 ± 12.7	190 ± 16.4	184 ± 14.1	165 ± 15.9** (↓11%)
BW Day 43	206 ± 15.5	211 ± 16.3	206 ± 15.4	186 ± 17.3** (↓10%)
BW Day 57	220 ± 16.0	227 ± 17.6	220 ± 16.3	202 ± 17.1** (↓8%)
BW Day 71	233 ± 16.4	242 ± 18.5	234 ± 16.7	214 ± 17.3** (↓8%)
BW Day 85	241 ± 18.8	251 ± 19.4	245 ± 18.3	229 ± 18.3 (↓5%)
Final Pre-mating BW	243 ± 18.4	250 ± 18.5	243 ± 17.1	227 ± 17.2** (↓7%)
Overall BWG Days 1-91[b]	142	151	147	148

[a] Data obtained from pages 261, 263, 272 and 275 in the study report. N=24.
[b] Calculated by reviewer using mean BW values
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BW body weight; BWG body weight gain
Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 2b. Body weight gain (%) - pre-mating F1 generation (Mean ± S.D.)[a]

Study Day	0 ppm	30 ppm	250 ppm	1800 ppm
F1 Generation Males - Pre-mating				
BWG Day 8	47 ± 8.8	50 ± 8.2	47 ± 15.6	50 ± 5.7
BWG Day 15	88 ± 17.8	94 ± 17.2	91 ± 25.9	97 ± 19.1
BWG Day 29	162 ± 36.3	173 ± 33.8	165 ± 48.5	185 ± 45.8
BWG Day 43	207 ± 48.3	221 ± 46.5	212 ± 59.6	235 ± 69.2
BWG Day 57	239 ± 57.1	255 ± 56.0	246 ± 72.4	274 ± 79.1
BWG Day 71	260 ± 64.3	275 ± 61.9	271 ± 78.6	296 ± 85.8
BWG Day 85	275 ± 67.3	294 ± 66.1	291 ± 77.9	318 ± 92.6
F1 Generation Females - Pre-mating				
BWG Day 8	33 ± 7.8	36 ± 8.4	37 ± 8.3	42 ± 7.7**
BWG Day 15	53 ± 10.7	58 ± 17.3	59 ± 15.9	70 ± 19.8**
BWG Day 29	85 ± 15.6	94 ± 23.9	95 ± 24.2	113 ± 30.1**
BWG Day 43	105 ± 18.4	116 ± 28.8	118 ± 28.3	141 ± 35.8**
BWG Day 57	119 ± 20.1	132 ± 31.9	134 ± 34.0	161 ± 40.8**
BWG Day 71	132 ± 20.1	147 ± 33.9	148 ± 37.5	176 ± 45.9**
BWG Day 85	140 ± 22.4	157 ± 37.9	161 ± 42.8	196 ± 47.7**

[a] Data obtained from pages 266 and 268 in the study report. N=24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BWG body weight gain

Table 2c. Body weight (g) – post-mating F1 generation (Mean ± S.D.)[a]

Study Day	0 ppm	30 ppm	25 ppm	1800 ppm
F1 Generation Males – Post-mating				
Initial BW	440 ± 39.3	439 ± 40.3	425 ± 45.8	389 ± 35.3** (↓12%)
BW Day 8	447 ± 39.9	448 ± 40.0	434 ± 48.8	396 ± 34.6** (↓11%)
BW Day 25	456 ± 41.8	463 ± 43.6	447 ± 53.9	411 ± 38.2** (↓10%)
Final BW	461 ± 41.6	469 ± 45.9	454 ± 52.7	415 ± 38.0** (↓10%)
Overall BWG Days 1-29[b]	21	30	29	26
F1 Generation Females - Gestation				
Initial BW	246 ± 17.8	257 ± 23.0	248 ± 17.4	232 ± 18.3* (↓6%)
BW Day 7	265 ± 21.4	272 ± 21.6	264 ± 18.2	248 ± 19.4* (↓7%)
BW Day 14	291 ± 24.5	297 ± 24.6	286 ± 20.2	270 ± 21.9** (↓7%)
Final BW	359 ± 29.0	362 ± 37.9	353 ± 35.6	333 ± 31.0* (↓7%)
Overall BWG Days 0-21[b]	113	105	105	101 (↓10%)
F1 Generation Females - Lactation				
Initial BW	264 ± 28.5	276 ± 24.4	266 ± 21.8	248 ± 22.2 (↓6%)
BW Day 4	276 ± 25.9	289 ± 22.0	281 ± 20.7	260 ± 23.0 (↓6%)
BW Day 7	282 ± 23.4	297 ± 21.0	288 ± 19.5	265 ± 21.2** (↓6%)
BW Day 14	302 ± 21.7	313 ± 19.8	306 ± 21.3	276 ± 21.2** (↓9%)
Final BW	297 ± 22.4	306 ± 17.1	299 ± 18.5	278 ± 21.7** (↓6%)
Overall BWG Days 1-21[b]	33	30	33	30

[a] Data obtained from pages 262, 264, and 265 in the study report. N=24.
[b] Calculated by reviewer using mean BW values
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BW body weight; BWG body weight gain
Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 2d. Body weight gain (%) – post-mating F1 generation females (Mean ± S.D.)[a]

Study Day	0 ppm	30 ppm	250 ppm	1800 ppm
F1 Generation Females - Gestation				
BWG Day 7	8 ± 2.8	7 ± 1.8	6 ± 19	7 ± 1.9
BWG Day 14	18 ± 3.9	17 ± 3.2	15 ± 3.1*	16 ± 2.1
BWG Day 21	46 ± 6.7	42 ± 9.1	42 ± 11.9	44 ± 4.9
F1 Generation Females - Lactation				
BWG Day 4	5 ± 4.1	5 ± 3.9	6 ± 4.4	5 ± 3.0
BWG Day 7	7 ± 5.8	8 ± 4.3	8 ± 4.9	7 ± 4.5
BWG Day 14	15 ± 6.3	14 ± 6.2	15 ± 6.7	12 ± 4.8
BWG Day 21	13 ± 6.6	11 ± 5.9	12 ± 7.0	12 ± 5.5

[a] Data obtained from pages 269-270 in the study report. N=24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BWG body weight gain

D. FOOD CONSUMPTION

P GENERATION

Treatment at 1800 ppm affected food consumption during the pre-mating period in males and females (Tables 3a and 3b).

In males, mean absolute food consumption (g/animal/day) was statistically significantly reduced (7-11%) at the high dose on days 1 - 8 and between days 15 and 70 during the pre-pairing period. During the post pairing period, mean absolute food consumption was not affected by dosing (Table 3c). Mean relative food consumption (g/kg bw/day) was also statistically significantly reduced during the first week of treatment. Afterwards mean relative food consumption during the pre-pairing period was not affected by treatment. All other statistically significant changes that occurred were considered to be of incidental nature since there was no dose-dependency. During the post pairing period mean relative food consumption generally increased, attaining statistical significance between days 1 and 22 and between days 29 and 36 (Table 3d).

In females, mean absolute food consumption (g/animal/day) was slightly but statistically significantly decreased (5-7%) at the high dose from days 1 - 29 during the pre-pairing period. During the post-mating period, absolute food consumption was reduced on days 7 - 21 of the gestation period. There were no changes in absolute food consumption during the lactation period. Relative food consumption was not changed during gestation or lactation (Tables 3c and 3d).
Treatment at lower dietary concentrations was considered to not have an effect on mean food consumption and/or relative food consumption in either males or females. The statistical significances noted were single occurrences or were not dose related.

Table 3a. Absolute food consumption (g/animal/day) – pre-mating P generation (Mean ± S.D.)[a]

Study Days	0 ppm	30 ppm	250 ppm	1800 ppm
P Generation Males - Pre-mating				
Days 1-8	21.0 ± 1.6	21.3 ± 1.7	20.1 ± 1.7	17.5 ± 2.7** (↓17%)
Days 8-15	25.1 ± 2.1	26.7 ± 1.8* (↑6%)	25.3 ± 2.1	24.2 ± 1.9
Days 15-22	21.4 ± 1.9	22.5 ± 1.8	21.1 ± 1.8	19.5 ± 1.3** (↓9%)
Days 22-29	24.5 ± 2.0	26.4 ± 1.7** (↑8%)	23.9 ± 1.9	22.6 ± 1.7** (↓8%)
Days 36-43	24.0 ± 1.9	25.2 ± 1.3* (↑5%)	23.0 ± 1.9	22.0 ± 1.5** (↓8%)
Days 50-57	22.8 ± 1.9	23.5 ± 1.4	22.0 ± 1.9	21.1 ± 1.5** (↓7%)
Days 64-70	23.1 ± 1.7	23.7 ± 1.4	22.3 ± 1.9	21.2 ± 1.2** (↓8%)
Mean Consumption	22.9	23.8	22.3	21.0 (↓8%)
P Generation Females - Pre-mating				
Days 1-8	15.3 ± 1.5	15.5 ± 1.7	15.4 ± 1.2	13.0 ± 2.3** (↓15%)
Days 8-15	18.9 ± 1.3	18.7 ± 1.1	18.4 ± 1.0	17.8 ± 1.2** (↓6%)
Days 15-22	15.3 ± 1.1	15.1 ± 1.1	15.0 ± 0.9	14.3 ± 1.1** (↓7%)
Days 22-29	18.2 ± 1.2	18.5 ± 1.2	18.0 ± 1.1	17.2 ± 1.6* (↓5%)
Days 36-43	17.4 ± 1.5	17.9 ± 1.1	17.1 ± 1.3	16.7 ± 1.4
Days 50-57	16.7 ± 1.9	17.1 ± 1.6	16.4 ± 1.2	16.1 ± 1.8
Days 64-70	16.4 ± 2.3	16.1 ± 1.3	16.1 ± 1.4	15.4 ± 1.8
Mean Consumption	16.6	16.8	16.4	15.7 (↓5%)

[a] Data obtained from pages 143 and 145 in the study report. N=24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 3b. Relative food consumption (g/kg bw/day) – pre-mating P generation (Mean ± S.D.)[a]

Study Days	0 ppm	30 ppm	250 ppm	1800 ppm
P Generation Males - Pre-mating				
Days 1-8	85.5 ± 5.2	85.7 ± 5.6	82.3 ± 5.2	76.3 ± 10.3** (↓11%)
Days 8-15	90.0 ± 4.7	94.0 ± 4.7* (↑4%)	91.1 ± 5.2	92.8 ± 6.1
Days 29-36	66.5 ± 4.7	69.1 ± 3.0* (↑4%)	68.3 ± 3.4	69.1 ± 3.6* (↑4%)
Days 43-50	61.8 ± 3.6	60.6 ± 3.0	59.0 ± 3.3** (↓4%)	61.4 ± 3.1
Days 64-70	59.2 ± 3.3	59.0 ± 3.4	57.7 ± 3.4	60.1 ± 2.9
Mean of Means	69.6	70.7	68.5	69.5
P Generation Females - Pre-mating				
Days 1-8	86.5 ± 7.2	87.5 ± 6.8	86.7 ± 7.1	76.8 ± 13.4* (↓11%)
Days 15-22	75.8 ± 4.3	75.6 ± 4.2	74.1 ± 3.8	74.8 ± 5.6
Days 29-36	75.2 ± 4.3	78.6 ± 4.1	77.1 ± 6.0	77.5 ± 6.3
Days 64-70	67.6 ± 7.3	65.7 ± 4.8	66.3 ± 4.6	66.8 ± 7.2
Mean of Means	77.6	78.0	76.3	76.5

[a] Data obtained from pages 155 and 157 in the study report. N=24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 3c. Absolute food consumption (g/animal/day) – post-mating P generation (Mean ± S.D.)[a]

Study Days	0 ppm	30 ppm	250 ppm	1800 ppm
P Generation Males – Post-mating				
Days 1-8	22.0 ± 1.5	22.8 ± 1.2	22.3 ± 1.9	22.3 ± 1.5
Days 8-15	22.8 ± 1.5	24.4 ± 1.4** (↑7%)	23.8 ± 2.1	22.4 ± 1.3
Days 15-22	22.0 ± 1.8	23.1 ± 1.3	22.2 ± 2.1	21.1 ± 1.2
Days 22-29	22.9 ± 2.0	24.0 ± 1.2	22.9 ± 2.0	21.8 ± 1.3
Days 29-36	21.4 ± 1.7	22.6 ± 1.4* (↑5%)	21.5 ± 1.9	20.7 ± 1.2
Mean Consumption	22.2	23.4	22.5	21.7
P Generation Females - Gestation				
Days 0-7	19.2 ± 2.4	19.4 ± 1.8	18.5 ± 1.5	18.9 ± 2.1
Days 7-14	20.9 ± 2.6	21.6 ± 1.5	20.5 ± 1.6	19.2 ± 3.2* (↓6%)
Days 14-21	22.2 ± 2.1	23.2 ± 2.3	21.5 ± 1.9	20.9 ± 2.5 (↓6%)
Mean Consumption	20.8	21.4	20.2	19.6 (↓6%)
P Generation Females - Lactation				
Days 1-7	36.4 ± 10.4	37.1 ± 7.5	37.8 ± 10.3	35.2 ± 11.4
Days 7-14	52.0 ± 4.6	55.7 ± 4.4	54.2 ± 4.2	50.8 ± 9.3
Mean Consumption	44.2	46.4	46.0	43.0

[a] Data obtained from pages 144, 146-147 in the study report. N=24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 3d. Relative food consumption (g/kg bw/day) – post-mating P generation (Mean ± S.D.)[a]

Study Days	0 ppm	30 ppm	250 ppm	1800 ppm
P Generation Males – Post-mating				
Days 1-8	53.2 ± 3.3	54.0 ± 3.5	54.2 ± 3.6	59.1 ± 3.0** (↑11%)
Days 8-15	54.4 ± 3.4	56.7 ± 3.4* (↑4%)	57.0 ± 3.6* (↑5%)	58.7 ± 2.7** (↑8%)
Days 15-22	51.9 ± 3.6	53.2 ± 3.1	52.8 ± 3.5	54.5 ± 2.8* (↑5%)
Days 29-36	49.0 ± 3.7	50.1 ± 3.3	49.4 ± 3.2	51.6 ± 2.6* (↑5%)
Mean Consumption	52.3	53.7	53.4	55.8
P Generation Females - Gestation				
Days 0-7	72.6 ± 6.5	72.2 ± 5.3	70.3 ± 5.4	74.9 ± 6.8
Days 7-14	72.3 ± 5.5	73.2 ± 4.3	71.1 ± 4.0	70.1 ± 10.7
Days 14-21	62.8 ± 4.1	64.2 ± 3.6	61.3 ± 3.7	63.0 ± 5.1
Mean Consumption	69.2	69.9	67.6	69.3
P Generation Females - Lactation				
Days 1-7	137.4 ± 39.8	137.4 ± 29.6	144.7 ± 40.4	142.6 ± 44.8
Days 7-14	176.8 ± 13.8	181.0 ± 9.5	181.9 ± 9.6	183.1 ± 30.4
Mean Consumption	157.1	159.2	163.3	162.9

[a] Data obtained from pages 156, 158-159 in the study report. N=24.

* Significantly different from control, $p<0.05$

** Significantly different from control, $p<0.01$

Values in parentheses represent the percent change from controls (calculated by the reviewer).

F1 GENERATION

Treatment at 1800 ppm affected food consumption during the pre-mating period in males and females (Tables 4a and 4b).

In males, mean absolute food consumption (g/animal/day) was statistically significantly reduced at the high dose throughout most of the pre-pairing period. During the post-pairing period (Table 4c), mean absolute food consumption was reduced during days 1 - 8 and statistically significantly reduced during days 8 - 15. Food consumption was not considered to be affected by treatment after the second week of the post-mating period. Relative food consumption (g/kg bw/day) was statistically significantly increased during almost the entire pre-pairing and post pairing periods at the high dose due to lower body weights (Table 4d). Absolute food consumption of males treated at 250 ppm was lower through day 57 of the pre-pairing period but without attaining statistical significance. Afterwards, the absolute food consumption was comparable to the control value for these animals. Relative food consumption for the 250 ppm males was similar to controls throughout the pre-mating period, except for days 85-91 when a statistically significant increase was observed.

In females, mean absolute food consumption (g/animal/day) was generally reduced in the 1800 ppm group during the pre-pairing and post-pairing periods. Absolute food consumption was statistically significantly decreased on days 1 - 50 and on days 57 - 64 of the pre-pairing period and on days 14 - 21 of the gestation period. During the lactation period, absolute food consumption was statistically significantly reduced on days 7 - 14. Relative food consumption (g/kg bw/day) was statistically significantly increased between days 1 - 15 and 22 - 29 of the pre-pairing period. Afterwards, there were only slight fluctuations which were considered to be incidental.

Table 4a. Absolute food consumption (g/animal/day) – pre-mating F1 generation (Mean ± S.D.)[a]

Study Days	0 ppm	30 ppm	250 ppm	1800 ppm
F1 Generation Males - Pre-mating				
Days 1-8	19.7 ± 2.4	19.5 ± 2.9	18.2 ± 3.9 (↓8%)	16.7 ± 2.5** (↓15%)
Days 8-15	23.2 ± 2.3	23.3 ± 2.7	22.0 ± 3.5 (↓5%)	20.8 ± 2.1* (↓10%)
Days 15-22	24.3 ± 2.1	24.2 ± 2.3	23.0 ± 3.3 (↓5%)	21.8 ± 2.2** (↓10%)
Days 22-29	25.6 ± 2.5	25.8 ± 2.2	24.0 ± 4.2 (↓6%)	23.7 ± 2.2
Days 36-43	24.8 ± 2.4	25.3 ± 1.9	23.4 ± 3.4 (↓6%)	22.6 ± 2.3* (↓9%)
Days 50-57	24.2 ± 2.2	24.6 ± 1.9	22.6 ± 4.1 (↓7%)	22.1 ± 2.2* (↓9%)
Days 64-71	23.2 ± 2.6	23.6 ± 1.8	22.6 ± 2.7	21.9 ± 2.2 (↓6%)
Mean Consumption	23.6	23.9	22.7	21.7 (↓8%)
F1 Generation Females - Pre-mating				
Days 1-8	16.5 ± 1.6	16.6 ± 2.1	15.7 ± 1.8	14.4 ± 2.1** (↓13%)
Days 8-15	17.7 ± 1.8	18.3 ± 1.9	17.3 ± 1.5	16.0 ± 1.8** (↓10%)
Days 15-22	17.5 ± 1.7	17.9 ± 2.0	16.8 ± 1.5	15.5 ± 1.5** (↓11%)
Days 22-29	17.7 ± 1.5	18.4 ± 1.9	17.5 ± 1.5	16.4 ± 1.4* (↓7%)
Days 36-43	18..0 ± 2.2	18.9 ± 1.9	17.7 ± 1.7	16.5 ± 1.5* (↓8%)
Days 50-57	17.6 ± 2.7	18.5 ± 1.8	17.0 ± 1.6	16.3 ± 1.5 (↓7%)
Days 64-71	17.1 ± 2.7	18.0 ± 1.8	16.8 ± 1.9	16.4 ± 1.7
Mean Consumption	17.3	18.0	16.9	16.0 (↓7%)

[a] Data obtained from pages 244 and 246 in the study report. N=24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 4b. Relative food consumption (g/kb bw/day) – pre-mating F1 generation (Mean ± S.D.)[a]

Study Days	0 ppm	30 ppm	250 ppm	1800 ppm
F1 Generation Males - Pre-mating				
Days 1-8	121.4 ± 5.5	122.6 ± 5.1	119.2 ± 9.4	127.4 ± 5.7** (↑5%)
Days 8-15	112.2 ± 7.1	114.2 ± 6.7	112.7 ± 10.2	122.6 ± 5.2** (↑9%)
Days 15-22	96.9 ± 5.7	97.9 ± 5.6	97.9 ± 7.2	103.8 ± 8.6** (↑7%)
Days 22-29	89.2 ± 4.9	90.6 ± 4.7	88.5 ± 9.0	96.8 ± 6.4** (↑10%)
Days 36-43	73.9 ± 3.6	75.5 ± 3.4	74.1 ± 4.4	78.8 ± 3.4** (↑7%)
Days 50-57	65.5 ± 3.4	66.4 ± 3.2	64.1 ± 7.3	69.3 ± 2.9* (↑6%)
Days 64-71	59.1 ± 3.4	60.6 ± 3.9	60.8 ± 3.9	64.9 ± 4.0** (↑10%)
Days 71-78	58.1 ± 3.0	59.8 ± 3.4	59.3 ± 4.3	60.8 ± 6.3 (↑5%)
Days 85-91	53.3 ± 2.5	55.3 ± 3.1	57.7 ± 6.1** (↑8%)	61.5 ± 4.8** (↑15%)
Mean Consumption	76.7	78.1	77.6	82.6
F1 Generation Females - Pre-mating				
Days 1-8	122.5 ± 5.4	123.7 ± 6.4	120.9 ± 5.1	129.1 ± 7.0** (↑5%)
Days 8-15	115.5 ± 5.5	117.9 ± 7.1	115.2 ± 6.4	121.1 ± 6.6** (↑5%)
Days 22-29	95.4 ± 5.1	96.9 ± 6.0	95.1 ± 6.7	99.7 ± 6.0* (↑5%)
Days 36-43	87.1 ± 6.7	89.7 ± 8.2	85.8 ± 6.0	88.8 ± 6.6
Days 85-91	67.8 ± 7.8	67.9 ± 5.8	65.0 ± 5.4	69.2 ± 8.5
Mean Consumption	87.3	88.5	85.7	89.8

[a] Data obtained from pages 256 and 258 in the study report. N=24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 4c. Absolute food consumption (g/animal/day) – post-mating F1 generation (Mean ± S.D.)[a]

Study Days	0 ppm	30 ppm	250 ppm	1800 ppm
F1 Generation Males – Post-mating				
Days 1-8	24.0 ± 2.7	25.0 ± 1.8	23.5 ± 2.2	22.7 ± 2.3 (↓5%)
Days 8-15	23.9 ± 2.6	25.1 ± 2.1	23.2 ± 2.5	22.1 ± 2.22* (↓8%)
Days 15-25	23.4 ± 2.4	24.7 ± 2.0	23.5 ± 2.3	22.7 ± 2.3
Days 25-29	22.6 ± 2.7	24.0 ± 2.1	23.1 ± 2.1	21.9 ± 2.0
Mean Consumption	23.5	24.7	23.3	22.3 (↓5%)
F1 Generation Females – Gestation				
Days 0-7	19.0 ± 1.9	19.6 ± 2.2	18.6 ± 2.2	18.1 ± 1.6 (↓5%)
Days 7-14	21.7 ± 3.2	22.0 ± 2.6	21.0 ± 2.0	20.3 ± 2.2 (↓6%)
Days 14-21	23.5 ± 3.3	23.6 ± 2.7	22.1 ± 2.5	20.7 ± 2.2** (↓12%)
Mean Consumption	21.4	21.7	20.6	19.7 (↓8%)
F1 Generation Females – Lactation				
Days 1-7	33.5 ± 4.5	35.8 ± 4.8	36.1 ± 4.5	33.6 ± 6.0
Days 7-14	54.9 ± 4.9	54.4 ± 5.8	53.0 ± 4.2	48.3 ± 4.8** (↓12%)
Mean Consumption	44.2	45.1	44.6	41.0 (↓7%)

[a] Data obtained from pages 245, 247-248 in the study report. N=24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 4d. Relative food consumption (g/kg bw/day) – post-mating F1 generation (Mean ± S.D.)[a]

Study Days	0 ppm	30 ppm	250 ppm	1800 ppm
F1 Generation Males – Post-mating				
Days 1-8	53.6 ± 2.9	56.0 ± 2.8	54.5 ± 5.6	57.4 ± 3.4** (↑7%)
Days 8-15	53.1 ± 3.1	55.5 ± 3.0	52.8 ± 7.6	54.6 ± 3.3
Days 15-25	51.3 ± 2.7	53.4 ± 2.8	52.9 ± 4.8	55.2 ± 3.6** (↑8%)
Days 25-29	49.0 ± 3.1	51.1 ± 2.0	51.2 ± 5.6	53.0 ± 3.3** (↑8%)
Mean Consumption	51.7	54.0	52.9	55.0
F1 Generation Females – Gestation				
Days 0-7	71.3 ± 6.3	71.4 ± 6.0	69.8 ± 6.5	73.2 ± 5.5
Days 7-14	74.4 ± 5.8	73.4 ± 5.1	72.4 ± 4.4	75.1 ± 6.0
Days 14-21	65.2 ± 6.0	64.9 ± 4.5	62.5 ± 4.0	62.2 ± 4.0
Mean Consumption	70.3	69.9	68.2	70.2
F1 Generation Females – Lactation				
Days 1-7	121.9 ± 15.1	124.5 ± 17.3	128.6 ± 15.0	129.2 ± 18.0
Days 7-14	181.9 ± 13.3	174.1 ± 18.2	174.3 ± 7.8	175.2 ± 14.0
Mean Consumption	151.9	149.3	151.4	152.2

[a] Data obtained from pages 257, 259-260 in the study report. N=24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
Values in parentheses represent the percent change from controls (calculated by the reviewer).

E. ACHIEVED TEST SUBSTANCE INTAKES

Mean achieved test substance intakes for each period during the study and overall study average weighted values are summarized in Table 5.

Table 5: Average weighted* achieved test substance intakes (mg/kg bw/day)

Period	Generation	30 ppm	250 ppm	1800 ppm
Male rats				
Pre-pairing	P (70 days – 10 weeks)	2.1	17.1	125.1
	F1 (91 days – 13 weeks)	2.3	19.4	148.6
After-pairing	P (56 days – 8 weeks)	1.6	13.3	100.5
	F1 (56 days – 8 weeks)	1.6	13.2	99.1
Average weighted* P generation		1.9	15.4	114.2
Average weighted* F1 generation		2.0	17.0	129.7
Mean both generations**		2.0	16.2	122.0
Female rats				
Pre-pairing	P (70 days – 10 weeks)	2.3	19.1	137.7
	F1 (91 days – 13 weeks)	2.7	21.4	161.7
Gestation	P	2.1	16.9	124.8
	F1	2.1	17.1	126.3
Lactation	P	4.8	40.8	293.2
	F1	4.5	37.9	273.9
Average weighted* P generation		2.7	22.8	164.4
Average weighted* F1 generation		2.9	23.3	173.8
Mean both generations**		2.8	23.0	169.1

Values extracted from study page 45 and pages 177-181 and 278-282 of the study report.
*Average weighted Males, P: (mean pre-pairing x 10) + (mean post-pairing x 8) / 18; F1: (mean pre-pairing x 13) + (mean post-pairing x 8) / 21
*Average weighted Females, P: (mean pre-pairing x 10) + (mean gestation x 3) + (mean lactation x 3) / 16; F1: (mean pre-pairing x 13) + (mean gestation x 3) + (mean lactation x 3) / 19
**Mean both generations calculated using unrounded (3 decimals) average values for each generation

F. VAGINAL SMEARS

P GENERATION

The estrous cycles measured during the pre-pairing period (between days 50 and 70) did not give any indication of treatment-related effects (Table 6a). Although the mean number of days between cycles was similar, the number of animals that experienced irregular cycles (both shortened and prolonged) was increased in the high dose group (7 animals compared to 2 in the control). This was not considered to be treatment-related due to the lack of a dose response for each type of irregularity.

Table 6a. Estrous cycles for the P generation

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
P Generation Females				
Mean # Days	5.1 ± 1.9	4.8 ± 1.7	5.4 ± 3.3	4.8 ± 1.6
Regular Cycles – 4 days	16	15	16	11
– 4 to 5 days	1	1	3	3
– 5 days	0	0	0	0
Irregular Cycles – Shortened	1	4	0	4
– Prolonged	1	1	0	3
Extended estrous	0	0	0	1
Acyclic Cycle	6	5	5	4

[a] Data obtained from pages 184-185 in the study report. N=24.

F1 GENERATION

The mean duration of estrus cycle of females receiving 1800 ppm was longer than that of the control group (Table 6b). This was due to persistent diestrus in six females resulting in estrous cycles lasting longer than 8 days. Consequently, there was a reduction in the number of animals with regular cycles, and an increased number of animals with acyclic cycles at the 1800 ppm dose level. However, all of the six females in persistent diestrus mated successfully and achieved pregnancy. The study author suggested that the observation of estrous could have been missed if the smear procedure triggered the animals to go into estrous and pass it within 24 hours before the next smear. In a meeting between the sponsor and the reviewing authority on March 4, 2011, the sponsor referred to an expert opinion that prolongation of the estrous cycle in this study was related to the nutritional status of the animals and not endocrine-mediated.

Table 6b. Estrous cycles for the F1 generation

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
F1 Generation Females				
Mean # Days	4.8 ± 2.8+	4.3 ± 0.9	5.4 ± 3.1	6.3 ± 3.9
Regular Cycles – 4 days	18**	18	15	8##
– 4 to 5 days	3	2	3	5
– 5 days	0	0	1	1
Irregular Cycles – Shortened	0	1	0	0
– Prolonged	0	2	1	1
Acyclic Cycle	3*	1	4	9

[a] Data obtained from pages 287-288 in the study report. N=22-24.
+ Statistically significant ($p<0.05$) based on the Kruskal-Wallis Test; it does not appear as though additional pair-wise tests were conducted to determine which doses demonstrated inter-group differences.
* Statistically significant ($p<0.05$) based on the Chi-Square Test.
** Statistically significant ($p<0.01$) based on the Chi-Square Test.
Significantly different from control ($p<0.01$) based on Fisher's Exact Test.

G. REPRODUCTIVE PERFORMANCE

P GENERATION

No treatment-related effects on mating performance, fertility, duration of gestation implantation rate and post-implantation loss, litter size at first litter check and during the lactation period, birth index and weaning index were observed. Results are summarized in Table 7a.

A single female treated at 1800 ppm mated on day 1 of the second pairing period, while all others mated during the first pairing period. Taking this into account, mean pre-coital time was not considered influenced by treatment. During the first pairing, mean pre-coital time was 2.5 days, 3.5 days, 3.2 days and 3.6 days in the control, low, mid and high dose groups respectively.

One female in the control group (animal #99) had a high post implantation loss (9), and therefore the control mean number of post-implantation losses was higher (2.0 ± 2.40). By excluding this female from the evaluation, the mean number of post implantation losses in the control group is 1.5 ± 1.01 and within the range of the historical control data (means of 0.2 to 1.7 from 7 studies).

The litter size at the first litter check was similar in all dose groups. There were 3 dead pups at first litter check in litter no. 99 in the control group, and one dead pup each in the low, mid and high dose groups.

According to the study author, the postnatal loss up to day 4 post-partum was not considered to be affected by treatment. The statistically significantly higher number of pups lost in the high dose group (8 pups compared to 1 in control) was within the range of the historical control data (range between 2 and 14 pups) and therefore not considered by the study author to be a treatment-related effect. Consequently, the slight statistically significantly lower viability index at the high dose was judged by the study author not to be biologically relevant. Most of the post-natal loss occurred on day 2 post-partum.

However, of the seven historical studies from which the historical control range was taken, one study had a relatively high number of losses (14 versus 2 to 5 in the other six studies). The post-natal loss at the high dose in this study exceeds the historical range of 2 to 5 as well as the concurrent control. For these six historical studies, the range for post-natal loss days 0-4 post-partum as a proportion of living pups is 0.7-1.6 and the range for the mean post-natal loss is 0.1-0.2. The values for these parameters in the high dose in this study also exceed these historical ranges. Additionally, this finding was observed in the offspring from the F1 mating.

Table 7a. Breeding data for the P generation

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
No. males/females paired	24/24	24/24	24/24	24/24
No. females mated	24	24	24	24
No. pregnant females[#]	23	24	22	24
No. females giving birth[##]	22	24	22	24
No. females weaning young	22	24	22	24
Duration of gestation (days)				
Mean ± SD	21.6 ± 0.59	21.4 ± 0.58	21.6 ± 0.49	21.5 ± 0.51
Implantations				
Total	313	338	298	340
Mean ± SD	13.6 ± 2.13	14.1 ± 1.72	13.5 ± 2.30	14.2 ± 1.58
Post implantation loss				
% of implantations	14.4	8.6	6.7	11.2
Litters affected (No.)	20	16	13*	16
Total No.	45	29*	20**	38
Mean ± SD	2.0 ± 2.40	1.2 ± 1.53	0.9 ± 0.97	1.6 ± 1.41
Dead pups at first litter check				
Litters affected (No.)	1	1	1	1
Total No.	3	1	1	1
Mean ± SD	0.1 ± 0.63	0.0 ± 0.20	0.0 ± 0.21	0.0 ± 0.20
Living pups at first litter check				
% of males / females	44 / 56	48 / 52	53 / 47*	46 / 54
Total No.	268	309	278	302
Mean ± SD	11.7 ± 3.41	12.9 ± 2.07	12.6 ± 2.48	12.6 ± 1.79
Postnatal loss days 0-4 post-partum				
% of living pups	0.4	0.6	1.4	**2.6**
HC range (%; 7 studies)		0.7 – 4.8		
Litters affected (No.)	1	2	3	**6**
Total No.	1	2	4	**8***
HC range (7 studies)		2 – 14		
Mean ± SD	0.0 ± 0.21	0.1 ± 0.28	0.2 ± 0.50	**0.3 ± 0.64**
Living pups day 4 post-partum				
Total No.	174	192	176	192
Mean ± SD	7.6 ± 1.70	8.0 ± 0.00	8.0 ± 0.00	8.0 ± 0.00
Breeding loss days 5-21 post-partum				
% of living pups at day 4	0.0	0.5	0.0	0.5
Litters affected (No.)	0	1	0	1
Total No.	0	1	0	1
Mean ± SD	0.0 ± 0.0	0.0 ± 0.20	0.0 ± 0.0	0.0 ± 0.20
Living pups day 21 post-partum				
% of males / females	50 / 50	53 / 47	51 / 49	48 / 52
Total No.	174	191	176	191
Mean ± SD	7.6 ± 1.70	8.0 ± 0.20	8.0 ± 0.0	8.0 ± 0.20
Birth index (%)	85.6	91.4*	93.3**	88.8
Viability index (%)	99.6	99.4	98.6	97.4*
Weaning index (%)	100.0	99.5	100.0	99.5

Female no. 112 in the control group and female nos. 161 and 166 in the 250 ppm group were not pregnant.
Female no. 99 in the control group had 3 dead pups at first litter check.
Fisher's exact test at 5% (*) or 1% (**) level.
Data obtained from pages 41, 188-189 and 2036-2037 in the study report.

F1 GENERATION

No effects on mating performance, fertility, duration of gestation, implantation rate and post-implantation loss, litter size at first litter check and during the lactation period, or weaning index were observed. Results are summarized in Table 7b.

During the first mating period, the mean precoital time was 4.0 days in controls, 2.9 days in the 30 ppm group, 4.1 days in the 250 ppm group and 5.0 days in the 1800 ppm group. One 30 ppm female and one 1800 ppm female mated during the second pairing period after the male was replaced. Mean precoital times for these two females were 1.0 and 4.0 days, respectively.

The number of post implantation losses (total number and litters affected) was statistically significantly lower in the mid and high doses due to a higher post- implantation loss in the control group.

Total postnatal loss was statistically significantly higher during post-natal days 0-4 in the 1800 ppm dose group. This was mainly due to one dam with a complete litter loss (12 pups) and another dam that lost 10 of its 15 pups and there was no effect on the number of litters affected. Most of the post-natal loss occurred on day 2 post-partum. Pup loss between day 5 and 21 post-partum was not affected by the treatment.

Data from ten historical studies were provided for breeding data for the F1 generation. The range of post-natal losses from LD 0 to 4 in these historical studies was 0 to 23 total pups lost. A few of these studies had relatively high numbers of losses (23, 18 and 9 versus 0 to 4 in the other seven studies). The post-natal loss at the high dose in this study exceeds the historical range as well as the concurrent control. Additionally, this finding was observed in the offspring from the P mating.

Table.7b.-Breeding data for the F1 generation

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
No. males/females paired	24/24	24/24	24/24	23/23
No. females mated	24	24	24	23
No. females pregnant[#]	24	22	22	23
No. females giving birth[##]	24	22	22	23
No. females weaning young[+]	23	22	22	22
Duration of gestation (days)				
Mean ± SD	21.7 ± 0.55	21.2 ± 3.40	21.8 ± 0.39	21.7 ± 0.47
Implantations				
Total	316	276	288	292
Mean ± SD	13.2 ± 2.30	12.5 ± 2.91	13.1 ± 1.82	12.7 ± 2.16
Post implantation loss				
% of implantations	10.4	7.6	5.6	4.5
Litters affected (No.)	18	13	9*	10*
Total No.	33	21	16*	13**
Mean ± SD	1.4 ± 1.31	1.0 ± 1.17	0.7 ± 1.12	0.6 ± 0.79
Dead pups at first litter check				
Total No.	0	0	0	0
Living pups at first litter check				
% of males / females	47 / 53	46 / 54	49 / 51	43 / 57
Total No.	283	255	272	279
Mean ± SD	11.8 ± 2.92	11.6 ± 2.81	12.4 ± 1.89	12.1 ± 2.22
Postnatal loss days 0-4 post-partum				
% of living pups	3.9	3.1	3.3	**9.0**
HC range (%; 10 studies)			0-8.5	
Litters affected (No.)	9	4	5	5
Total No.	11	8	9	**25***
HC range (10 studies)			0-23	
Mean ± SD	0.5 ± 0.66	0.4 ± 1.09	0.4 ± 0.85	**1.1 ± 3.16**
Living pups day 4 post-partum				
Total No.	184	170	176	170
Mean ± SD	7.7 ± 1.63	7.7 ± 0.88	8.0 ± 0.00	7.4 ± 1.83
Breeding loss days 5-21 post-partum				
% of living pups at day 4	1.6	0.6	0.6	0.0
Litters affected (No.)	1	1	1	0
Total No.	3	1	1	0
Mean ± SD	0.1 ± 0.61	0.0 ± 0.21	0.0 ± 0.21	0.0 ± 0.00
Living pups day 21 post-partum				
% of males / females	49 / 51	50 / 50	50 / 50	48 / 52
Total No.	181	169	175	170
Mean ± SD	7.5 ± 1.72	7.7 ± 0.89	8.0 ± 0.21	7.4 ± 1.83
Birth index (%)	89.6	92.4	94.4*	95.5**
Viability index (%)	96.1	96.9	96.7	91.0*
Weaning index (%)	98.4	99.4	99.4	100.0

Female no. 291 in the 1800 ppm dose group was sacrificed for ethical reasons.
Female no. 338 in the 30 ppm dose group and female no. 346 in the 250 ppm dose group were not pregnant. Female no. 342 in the 30 ppm dose group was found dead on day 7 of gestation. Female no. 347 in the 250 ppm dose group had only two implantation sites and did not deliver any pups.
+ Female no. 299 in the control group had only one pup which was lost on day 1. Female no. 377 in the 1800 ppm dose group lost its entire litter on day 2 post-partum.
Fisher's exact test at 5% (*) or 1% (**) level. Data obtained from pages 51, 291-292 and 2099-2100 in the study report.

H. SACRIFICE AND PATHOLOGY

1. Organ Weights

P GENERATION

In the 1800 ppm males (Table 8a), brain weight relative to body weight was increased by 9% due to a lower final body weight. Absolute and relative weights of liver and both kidneys were statistically significantly increased. There were no changes in prostate and seminal vesicle weights relative to body weight. Therefore, the statistically decreased absolute prostate weight and prostate weight relative to brain weight and the non-statistical decrease in absolute seminal vesicle weight were considered to be a secondary effect of the decreased body weight at this dose.

Absolute and relative kidney weights were statistically significantly increased in the 1800 ppm females (Table 8b). At this dose, relative liver weight to body weight was statistically increased by 8% and adrenal weights were statistically decreased by approximately 15%.

Higher absolute and relative (to brain weight) weights of kidneys were recorded in females treated at 30 ppm; this was considered incidental since there was no dose-dependency.

Table 8a. Absolute (g) and relative (%) organ weights - P generation males (Mean ± S.D.)[a]

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
P Generation Males				
Final BW	425.7 ± 37.5	440.1 ± 26.5	420.9 ± 36.0	392.3 ± 24.1** (↓8%)
Abs. Brain	2.00 ± 0.09	2.02 ± 0.08	2.00 ± 0.09	2.00 ± 0.05
Rel. Brain (to BW)	0.47 ± 0.04	0.46 ± 0.02	0.48 ± 0.05	0.51 ± 0.03** (↑9%)
Abs. Liver	12.05 ± 0.93	12.28 ± 1.03	12.22 ± 1.24	13.51 ± 0.98** (↑11%)
Rel. Liver (to BW)	2.84 ± 0.15	2.79 ± 0.17	2.91 ± 0.21	3.45 ± 0.20** (↑21%)
Rel. Liver (to brain wt)	603.07 ± 42.84	608.16 ± 43.55	610.03 ± 57.95	676.63 ± 42.66** (↑12%)
Abs. Kidney (R)	1.09 ± 0.11	1.14 ± 0.07	1.09 ± 0.10	1.30 ± 0.15** (↑19%)
Rel. Kidney (R) (to BW)	0.26 ± 0.02	0.26 ± 0.02	0.26 ± 0.02	0.33 ± 0.03** (↑27%)
Rel. Kidney (R) (to brain wt)	54.28 ± 4.32	56.64 ± 2.98	54.57 ± 4.40	64.92 ± 7.28** (↑20%)
Abs. Kidney (L)	1.08 ± 0.11	1.14 ± 0.09	1.09 ± 0.09	1.25 ± 0.13** (↑16%)
Rel. Kidney (L) (BW)	0.25 ± 0.02	0.26 ± 0.02	0.26 ± 0.02	0.32 ± 0.03** (↑28%)
Rel. Kidney (L) (to brain wt)	54.12 ± 4.79	56.47 ± 3.37	54.29 ± 4.39	62.47 ± 6.21** (↑15%)
Abs. Prostate	0.94 ± 0.20	0.89 ± 0.18	0.85 ± 0.17	0.80 ± 0.15* (↓15%)
Rel. Prostate (to BW)	0.22 ± 0.05	0.20 ± 0.03	0.20 ± 0.04	0.20 ± 0.03
Rel. Prostate (to brain wt)	47.24 ± 10.70	43.74 ± 7.91	42.69 ± 8.33	40.12 ± 7.54* (↓15%)
Abs. Seminal Vesicle	1.50 ± 0.25	1.64 ± 0.34	1.39 ± 0.26	1.33 ± 0.28 (↓11%)
Rel. Seminal Vesicle (to BW)	0.35 ± 0.05	0.37 ± 0.09	0.33 ± 0.08	0.34 ± 0.07
Rel. Seminal Vesicle (to brain wt)	75.09 ± 11.46	81.23 ± 17.86	69.41 ± 13.64	66.64 ± 14.13 (↓11%)

[a] Data obtained from pages 197-202 in the study report. N=22-24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BW = body weight
wt = weight
R = right
L = left
Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 8b. Absolute (g) and relative (%) organ weights - P generation females (Mean ± S.D.)[a]

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
Final BW	289.0 ± 14.9	298.7 ± 17.5	288.6 ± 14.2	278.3 ± 22.3
Abs. Kidney (R)	0.93 ± 0.06	0.98 ± 0.07* (↑5%)	0.95 ± 0.05	1.02 ± 0.06** (↑10%)
Rel. Kidney (R) (to BW)	0.32 ± 0.02	0.33 ± 0.03	0.33 ± 0.02	0.37 ± 0.02** (↑16%)
Rel. Kidney (R) (to brain wt)	49.35 ± 2.60	52.18 ± 3.13** (↑6%)	51.43 ± 2.10	54.16 ± 3.93** (↑10%)
Abs. Kidney (L)	0.90 ± 0.06	0.95 ± 0.08* (↑6%)	0.92 ± 0.06	1.00 ± 0.06** (↑10%)
Rel. Kidney (L) (to BW)	0.31 ± 0.02	0.32 ± 0.03	0.32 ± 0.02	0.36 ± 0.02** (↑16%)
Rel. Kidney (L) (to brain wt)	47.53 ± 2.85	50.53 ± 3.84** (↑6%)	49.73 ± 2.31	53.15 ± 3.77** (↑12%)
Abs. Liver	13.71 ± 1.18	14.12 ± 1.28	13.82 ± 1.11	14.23 ± 1.47
Rel. Liver (to BW)	4.74 ± 0.30	4.73 ± 0.35	4.79 ± 0.37	5.11 ± 0.31** (↑8%)
Rel. Liver (to brain wt)	728.07 ± 58.41	755.82 ± 2.84	749.07 ± 66.85	757.06 ± 84.44
Abs. Adrenal (R)	0.043 ± 0.006	0.044 ± 0.006	0.042 ± 0.005	0.036 ± 0.004** (↓16%)
Rel. Adrenal (R) (to BW)	0.015 ± 0.002	0.015 ± 0.002	0.015 ± 0.002	0.013 ± 0.002** (↓13%)
Rel. Adrenal (R) (to brain wt)	2.267 ± 0.346	2.350 ± 0.286	2.265 ± 0.287	1.902 ± 0.228** (↓16%)
Abs. Adrenal (L)	0.046 ± 0.009	0.049 ± 0.006	0.046 ± 0.005	0.040 ± 0.004** (↓13%)
Rel. Adrenal (L) (to BW)	0.016 ± 0.003	0.016 ± 0.002	0.016 ± 0.002	0.014 ± 0.002* (↓13%)
Rel. Adrenal (L) (to brain wt)	2.468 ± 0.484	2.619 ± 0.305	2.497 ± 0.307	2.120 ± 0.256** (↓14%)

[a] Data obtained from pages 203-206 in the study report. N=22-24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BW = body weight
wt = weight
R = right
L = left
Values in parentheses represent the percent change from controls (calculated by the reviewer).

F1 GENERATION

Relative brain weights were statistically significantly increased in both males (Table 8c) and females (Table 8d) at the high dose, but absolute weights were comparable.

In males treated at 1800 ppm, absolute and relative weight of kidneys and relative weights of liver were statistically significantly increased. Absolute and relative (to brain weight) spleen, pituitary and seminal vesicle weights were noted to be statistically decreased in the high dose males. The decreases were consistent with the decrease in body weight and there were no effects in the weights of these organs relative to body weight; therefore, these findings were considered to be secondary effects of this lower body weight. The weights of the testes and epididymides relative to body weight were statistically significantly increased.

In females treated at 1800 ppm, relative (to body weight) weights of kidneys and of liver were statistically significantly increased. Statistically significantly decreased values in absolute spleen, adrenals, ovary, and uterus weights were considered to be due to lower body weight as the organ to body weight ratios were not affected for any of these organs.

There were no other organ weight effects in F1 animals.

Table 8c. Absolute (g) and relative (%) organ weights - F1 generation males (Mean ± S.D.)[a]

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
Final BW	448.9 ± 39.5	458.0 ± 44.0	442.2 ± 51.01	403.4 ± 37.5 (↓10%)
Abs. Brain	2.06 ± 0.10	2.07 ± 0.10	2.04 ± 0.09	2.01 ± 0.09
Rel. Brain (to BW)	0.46 ± 0.04	0.46 ± 0.03	0.47 ± 0.05	0.50 ± 0.04** (↑9%)
Abs. Pituitary	0.009 ± 0.002	0.009 ± 0.001	0.009 ± 0.001	0.008 ± 0.002** (↓11%)
Rel. Pituitary (to BW)	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000
Rel. Pituitary (to brain wt)	0.443 ± 0.069	0.444 ± 0.068	0.446 ± 0.068	0.386 ± 0.071* (↓13%)
Abs. Kidney (R)	1.13 ± 0.14	1.16 ± 0.12	1.14 ± 0.14	1.27 ± 0.16** (↑12%)
Rel. Kidney (R) (to BW)	0.25 ± 0.02	0.25 ± 0.02	0.26 ± 0.02	0.32 ± 0.03** (↑28%)
Rel. Kidney (R) (to brain wt)	55.08 ± 5.49	56.03 ± 4.42	55.72 ± 6.04	63.12 ± 6.68** (↑15%)
Abs. Kidney (L)	1.11 ± 0.13	1.14 ± 0.10	1.12 ± 0.14	1.25 ± 0.15** (↑13%)
Rel. Kidney (L) (to BW)	0.25 ± 0.02	0.25 ± 0.02	0.25 ± 0.03	0.31 ± 0.02** (↑24%)
Rel. Kidney (L) (to brain wt)	53.66 ± 5.23	55.21 ± 4.10	54.95 ± 6.11	62.15 ± 6.07** (↑16%)
Abs. Liver	13.01 ± 1.31	13.78 ± 1.39	13.24 ± 1.52	13.98 ± 1.44
Rel. Liver (to BW)	2.90 ± 0.18	3.02 ± 0.26	3.00 ± 0.21	3.47 ± 0.23** (↑20%)
Rel. Liver (to brain wt)	632.02 ± 57.75	665.23 ± 60.18	648.91 ± 71.82	694.53 ± 65.10** (↑10%)
Abs. Spleen	0.86 ± 0.13	0.83 ± 0.11	0.81 ± 0.13	0.73 ± 0.12** (↓15%)
Rel. Spleen (to BW)	0.19 ± 0.03	0.18 ± 0.03	0.18 ± 0.03	0.18 ± 0.02
Rel. Spleen (to brain wt)	41.49 ± 5.53	40.10 ± 4.33	39.50 ± 6.05	36.47 ± 5.44** (↓12%)
Abs. Testis (R)	1.96 ± 0.16	2.00 ± 0.36	2.01 ± 0.13	1.96 ± 0.18
Rel. Testis (R) (to BW)	0.44 ± 0.04	0.44 ± 0.08	0.46 ± 0.05	0.49 ± 0.03** (↑11%)
Abs. Testis (L)	1.99 ± 0.14	2.02 ± 0.36	2.04 ± 0.19	2.01 ± 0.20
Rel. Testis (L) (to BW)	0.45 ± 0.04	0.44 ± 0.08	0.46 ± 0.06	0.50 ± 0.04** (↑11%)
Abs. Epididymis (R)	0.727 ± 0.076	0.740 ± 0.099	0.735 ± 0.079	0.715 ± 0.059
Rel. Epididymis (R) (to BW)	0.162 ± 0.015	0.162 ± 0.021	0.167 ± 0.015	0.178 ± 0.012** (↑10%)
Abs. Epididymis (L)	0.575 ± 0.047	0.584 ± 0.087	0.578 ± 0.058	0.579 ± 0.049
Rel. Epididymis (L) (to BW)	0.129 ± 0.013	0.128 ± 0.019	0.132 ± 0.017	0.144 ± 0.013** (↑12%)
Abs. Seminal Vesicle	1.28 ± 0.31	1.24 ± 0.22	1.24 ± 0.24	1.11 ± 0.18* (↓13%)
Rel. Seminal Vesicle (to BW)	0.29 ± 0.07	0.27 ± 0.04	0.28 ± 0.05	0.27 ± 0.04
Rel. Seminal Vesicle (to brain wt)	62.18 ± 14.94	59.80 ± 11.26	60.51 ± 11.13	55.00 ± 8.99 (↓11%)

[a] Data obtained from pages 300-308 in the study report. N=22-24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BW = body weight; wt = weight; R = right; L = left
Values in parentheses represent the percent change from controls (calculated by the reviewer).

Table 8d. Absolute (g) and relative (%) organ weights - F1 generation females (Mean ± S.D.)[a]

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
Final BW	288.3 ± 22.9	293.5 ± 18.8	285.9 ± 17.1	264.1 ± 22.3** (↓8%)
Abs. Brain	1.90 ± 0.08	1.90 ± 0.09	1.91 ± 0.09	1.86 ± 0.11
Rel. Brain (to BW)	0.66 ± 0.06	0.65 ± 0.03	0.67 ± 0.05	0.71 ± 0.06* (↑7%)
Abs. Kidney (R)	0.94 ± 0.09	0.95 ± 0.11	0.96 ± 0.10	0.94 ± 0.12
Rel. Kidney (R) (to BW)	0.33 ± 0.03	0.32 ± 0.03	0.34 ± 0.03	0.36 ± 0.04** (↑10%)
Abs. Kidney (L)	0.90 ± 0.08	0.92 ± 0.07	0.92 ± 0.09	0.92 ± 0.10
Rel. Kidney (L) (to BW)	0.31 ± 0.02	0.32 ± 0.02	0.32 ± 0.03	0.35 ± 0.02** (↑13%)
Abs. Liver	13.59 ± 1.30	13.50 ± 1.28	14.06 ± 1.47	13.70 ± 1.48
Rel. Liver (to BW)	4.72 ± 0.33	4.61 ± 0.40	4.92 ± 0.47	5.18 ± 0.29** (↑10%)
Abs. Adrenal (R)	0.041 ± 0.005	0.041 ± 0.005	0.042 ± 0.006	0.035 ± 0.004** (↓15%)
Rel. Adrenal (R) (to BW)	0.014 ± 0.002	0.014 ± 0.002	0.015 ± 0.002	0.013 ± 0.002
Rel. Adrenal (R) (to brain wt)	2.175 ± 0.226	2.160 ± 0.252	2.186 ± 0.288	1.881 ± 0.244** (↓14%)
Abs. Adrenal (L)	0.045 ± 0.007	0.044 ± 0.006	0.045 ± 0.007	0.039 ± 0.007** (↓13%)
Rel. Adrenal (L) (to BW)	0.016 ± 0.002	0.015 ± 0.002	0.016 ± 0.002	0.015 ± 0.002
Rel. Adrenal (L) (to brain wt)	2.377 ± 0.361	2.292 ± 0.290	2.365 ± 0.344	2.082 ± 0.332* (↓12%)
Abs. Spleen	0.80 ± 0.12	0.75 ± 0.12	0.74 ± 0.11	0.66 ± 0.11** (↓18%)
Rel. Spleen (to BW)	0.28 ± 0.04	0.26 ± 0.04	0.26 ± 0.04	0.25 ± 0.04
Rel. Spleen (to brain wt)	41.87 ± 6.49	39.72 ± 6.83	39.05 ± 5.91	35.26 ± 5.41** (↓16%)
Abs. Ovaries (R)	0.055 ± 0.010	0.055 ± 0.011	0.049 ± 0.010	0.046 ± 0.009** (↓16%)
Rel. Ovaries (R) (to BW)	0.019 ± 0.003	0.019 ± 0.004	0.017 ± 0.003	0.017 ± 0.003
Rel. Ovaries (R) (to brain wt)	2.909 ± 0.523	2.878 ± 0.581	2.568 ± 0.502	2.451 ± 0.438* (↓16%)
Abs. Ovaries (L)	0.053 ± 0.013	0.055 ± 0.008	0.051 ± 0.012	0.046 ± 0.011 (↓13%)
Rel. Ovaries (L) (to BW)	0.018 ± 0.004	0.019 ± 0.003	0.018 ± 0.004	0.018 ± 0.004
Rel. Ovaries (L) (to brain wt)	2.801 ± 0.657	2.904 ± 0.400	2.695 ± 0.601	2.485 ± 0.528 (↓11%)
Abs. Uterus	0.82 ± 0.17	0.81 ± 0.17	0.83 ± 0.16	0.69 ± 0.18* (↓16%)
Rel. Uterus (to BW)	0.29 ± 0.06	0.28 ± 0.06	0.29 ± 0.06	0.26 ± 0.06
Rel. Uterus (to brain wt)	43.32 ± 8.67	42.64 ± 8.67	43.53 ± 7.57	36.82 ± 9.73* (↓15%)

[a] Data obtained from pages 300-308 in the study report. N=22-24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BW = body weight; wt = weight; R = right; L = left
Values in parentheses represent the percent change from controls (calculated by the reviewer).

2. Gross and Histopathology

P GENERATION

There were no treatment-related macroscopic findings in males and females.

Treatment at 1800 ppm induced microscopic changes in the liver, kidneys and thyroid gland (Table 9a). Minimal centrilobular hypertrophy of the liver was noted in 9 males; this correlated with the recorded increased organ weight. As there were no further indicators of liver injury, the study authors considered this lesion to be of metabolic nature and of adaptive character. Glycogen deposits were reduced in animals of both sexes; this was considered by the study authors to be related to the lower body weight.

In the kidneys, the incidence and mean severity of hyaline droplets was increased in males. The incidence and/or severity of tubular basophilia, mononuclear cell infiltration and tubular cast were also increased in males. According to the study author, these lesions were considered to be the secondary lesions to the hyaline droplets described above.

Minimal follicular hypertrophy of the thyroid gland was noted in 4 males. A possible relationship to liver enzyme inductions was suggested by the study author.

Table 9a. Incidence/Mean Severity of Microscopic Findings in the P generation[a]

ppm	0		30		250		1800	
Liver (# examined)	**24 M**	**24 F**	**24 M**	**24 F**	**24 M**	**24 F**	**24 M**	**24 F**
-Centrilobular hypertrophy	0	0	0	0	0	0	9/1.0	0
-Glycogen deposits	20/1.0	18/1.3	19/1.1	19/1.2	20/1.0	18/1.1	15/1.0	9/1.0
Kidneys (# examined)	**24 M**	**24 F**	**24 M**	**1 F**	**24 M**	**1 F**	**24 M**	**24 F**
- Hyaline droplets	5/1.0	0	2/1.0	0	4/1.0	0	24/1.8	0
-Tubular basophilia	7/1.1	2/1.0	5/1.0	0	9/1.0	1/2.0	24/1.7	2/1.0
-Mononuclear cell infiltration	6/1.2	3/1.0	5/1.0	0	4/1.0	1/2.0	11/1.2	2/1.0
-Tubular cast	0	0	0	0	0	0	19/1.7	0
Thyroid Gland (# examined)	**24 M**	**24 F**	**24 M**	**N/E**	**24M**	**N/E**	**24 M**	**24 F**
-Follicular hypertrophy	0	0	0	-	0	-	4/1.0	0

[a] Data obtained from pages 1369-1370 in the study report
N/E not examined

F1 GENERATION

There were no treatment-related macroscopic findings in males or females.

At the high dose, minimal to slight centrilobular hypertrophy of the liver was noted in 12 males and 3 females. The incidence of glycogen deposit was slightly reduced in males only.

In the kidneys, the incidence and mean severity of hyaline droplets was increased in 1800 ppm males along with the incidence and/or severity of tubular basophilia, mononuclear cell infiltration and tubular casts. Follicular hypertrophy of the thyroid gland was noted in 6 males, with a mean severity of 1.2.

Table 9b. Incidence/Mean Severity of Microscopic Findings in the F1 generation[a]

Finding	0 ppm		30 ppm		250 ppm		1800 ppm	
Liver (# examined)	**24 M**	**24 F**	**24 M**	**24 F**	**24 M**	**24 F**	**24 M**	**24 F**
-Centrilobular hypertrophy	0	0	0	0	0	0	12/1.1	3/1.0
-Glycogen deposits	24/1.2	21/1.7	21/1.2	18/1.5	20/1.1	19/1.6	16/1.1	22/1.3
Kidneys (# examined)	**24 M**	**24 F**	**24 M**	**1 F**	**24 M**	**1 F**	**24 M**	**24 F**
-Hyaline droplets	6/1.0	0	4/1.0	0	3/1.0	0	23/1.8	0
-Tubular basophilia	5/1.0	3/1.0	3/1.0	1/1.0	4/1.0	0	20/1.2	2/1.2
-Mononuclear cell infiltration	7/1.0	4/1.0	4/1.0	2/1.0	6/1.0	1/1.0	14/1.0	5/1.0
-Tubular cast	1/1.0	0	0	0	0	0	9/1.0	0
Thyroid Gland (# examined)	**24 M**	**24 F**	**24 M**	**N/E**	**24M**	**N/E**	**24 M**	**24 F**
-Follicular hypertrophy	0	0	0	-	0	-	6/1.2	0

[a] Data obtained from pages 1371-1372 in the study report.
N/E = not examined

Fluoride Determinations

Mean fluoride content was statistically significantly increased in the femur of all groups of treated male and female F1 parent animals in a dose-related manner (Table 10). Mean fluoride content of the incisors was also increased in all male and female dose groups; statistically significant increases were noted in all female dose groups and in males receiving 250 and 1800 ppm. These levels of fluoride were not associated with any discoloration or other abnormality of teeth or bones.

Table 10. Fluoride Analysis (ppm) in the F1 generation[a]

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
F1 Generation Males –Bones				
Mean Fluoride Content	348.4	696.4* (↑100%)	2070* (↑494%)	6583* (↑1789%)
Rel. SD (%)	7.4	7.2	6.3	5.9
F1 Generation Males-Teeth				
Mean Fluoride Content	232.9	308.6 (↑32%)	944.0* (↑305%)	3960* (↑1600%)
Rel. SD (%)	10.7	11.4	14.0	9.1
F1 Generation Females – Bones				
Mean Fluoride Content	518.8	893* (↑72%)	2242* (↑332%)	6192* (↑1094%)
Rel. SD (%)	12.2	7.9	8.1	7.6
F1 Generation Females –Teeth				
Mean Fluoride Content	295.8	466.5* (↑58%)	1269* (↑329%)	3483* (↑1077%)
Rel. SD (%)	15.7	16.0	9.2	11.9

[a] Data obtained from pages 2010-2025 in the study report. N=22-24.
*Significantly different from control, $p<0.05$.

3. Sperm Analysis Data

P GENERATION

Sperm motility, morphology and head count were not affected by treatment (Table 11a). There was a high degree of variability in the control males for the mean progressively motile sperm and mean stationary motile sperm. This was due to one male (animal #15) that exhibited 100% stationary sperm. When this animal is excluded from the calculation of the mean, the mean progressively motile sperm becomes 68% ± 12% and the mean stationary motile becomes 19% ± 7%.

Table 11a. Seminology in the P generation[a]

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
Motility				
Progressive motile (%)	65 ± 18	70 ± 9	71 ± 9	67 ± 8
Stationary motile (%)	23 ± 18	19 ± 7	19 ± 7	20 ± 5
Not motile (%)	12 ± 7	11 ± 4	11 ± 4	13 ± 5
Morphology				
Normal, complete sperm (%)	89.3 ± 5.5	Not examined	Not examined	89.0 ± 6.0
Normal head only (tail detached) (%)	5.2 ± 4.6	Not examined	Not examined	5.1 ± 4.6
Complete sperm, misshapen hook (%)	1.8 ± 1.5	Not examined	Not examined	1.9 ± 1.1
Complete sperm, abnormally curved hook (%)	2.9 ± 1.4	Not examined	Not examined	3.3 ± 1.7
Complete sperm, reversed head (%)	0.1 ± 0.4	Not examined	Not examined	0.2 ± 0.6
Abnormal head only (tail detached) (%)	0.8 ± 0.7	Not examined	Not examined	0.6 ± 0.5
Sperm Head Count				
Testis (millions/g)	127.31 ± 14.68	Not examined	Not examined	126.54 ± 15.28
Cauda epididymis (millions/g)	749.38 ± 153.58	Not examined	Not examined	731.05 ± 139.10

[a] Data obtained from pages 194-196 in the study report. N=22-24.

F1 GENERATION

Sperm motility, morphology and head count were not affected by treatment (Table 11b). A statistically significant decrease in progressively motile sperm and an accompanying increase in stationary motile sperm were noted in the 30 ppm dose group but were not extended to the higher dose groups.

Table 11b. Seminology in the F1 generation[a]

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
Motility				
Progressive motile (%)	68 ± 10	62 ± 6*	66 ± 9	64 ± 7
Stationary motile (%)	18 ± 5	24 ± 7**	22 ± 8	23 ± 6
Not motile (%)	14 ± 10	14 ± 4	12 ± 4	13 ± 4
Morphology				
Normal, complete sperm (%)	89.2 ± 6.8	Not examined	Not examined	90.8 ± 3.6
Normal head only (tail detached) (%)	4.8 ± 6.4	Not examined	Not examined	3.0 ± 1.6
Complete sperm, misshapen hook (%)	1.4 ± 1.4	Not examined	Not examined	1.3 ± 1.0
Complete sperm, abnormally curved hook (%)	4.1 ± 1.9	Not examined	Not examined	4.2 ± 1.4
Complete sperm, reversed head (%)	0.1 ± 0.1	Not examined	Not examined	0.0 ± 0.1
Abnormal head only (tail detached) (%)	0.6 ± 0.5	Not examined	Not examined	0.7 ± 0.8
Sperm Head Count				
Testis (millions/g)	137.21 ± 12.26	Not examined	Not examined	138.95 ± 13.05
Cauda epididymis (millions/g)	751.38 ± 157.14	Not examined	Not examined	740.46 ± 98.77

[a] Data obtained from pages 297-299 in the study report. N=23-24.

I. PUP DATA

1. Abnormal Findings at Birth (First Litter Check) and during Lactation to Weaning

F1 PUPS

No test item-related abnormal findings were noted at first litter check and during lactation.

F2 PUPS

No test item-related abnormal findings were noted at first litter check and during lactation.

2. Sex Ratios

F1 PUPS

The sex ratios were not affected by treatment. At first litter check the sex ratios (% males/% females) were 44/56, 48/52, 53/47 and 46/54 at 0, 30, 250 and 1800 ppm, respectively. The percentage of females at 250 ppm was statistically significantly reduced compared to controls, but this finding was considered incidental since there was no dose-dependency.

F2 PUPS

The sex ratios (% males/% females) were not affected by treatment (47/53, 46/54, 49/51 and 42/58 at 0, 30, 250 and 1800 ppm, respectively).

3. Pup Weights to Weaning (Day 21 Post-Partum)

F1 PUPS

In the group receiving the 1800 ppm test diet, the mean body weight of pups was reduced during the lactation period (Table 12a). Post-partum mean body weights of pups were slightly reduced on days 1, 4 and 7 at the high dose compared to controls and were statistically reduced on days 14 and 21. Mean body weights of male pups were also statistically significantly decreased on day 4. The combined male and female overall pup body weight gains were lower at the 1800 ppm dose compared to controls by 11%.

Table 12a. Body weight (g) – F1 pups (Mean ± S.D.)[a]

Study Day	0 ppm	30 ppm	250 ppm	1800 ppm
Males				
BW Day 0	6.2 ± 0.32	-	5.7	5.9
BW Day 1	5.9 ± 0.57	5.8 ± 0.58	5.9 ± 0.62	5.6 ± 0.65 (↓5%)
BW Day 4	8.5 ± 1.20	8.2 ± 0.91	8.5 ±1.12	7.8 ± 1.01* (↓8%)
BW Day 7	13.9 ± 1.58	13.8 ± 1.39	14.0 ± 1.44	12.9 ± 1.53 (↓7%)
BW Day 14	29.6 ± 2.53	30.5 ± 2.10	30.4 ± 2.11	27.3 ± 2.35** (↓8%)
BW Day 21	47.9 ± 4.48	49.2 ± 3.55	48.4 ± 3.68	43.3 ± 3.78** (↓10%)
Females				
BW Day 0	5.8 ± 0.30	-	5.1	6.0
BW Day 1	5.6 ± 0.52	5.5 ± 0.6	5.7 ± 0.62	5.4 ± 0.57 (↓4%)
BW Day 4	8.2 ± 1.14	8.0 ± 0.96	8.2 ± 1.16	7.6 ± 0.97 (↓7%)
BW Day 7	13.6 ± 1.52	13.4 ± 1.33	13.5 ± 1.59	12.6 ± 1.16 (↓7%)
BW Day 14	29.3 ± 2.34	29.9 ± 1.92	29.6 ± 2.54	26.8 ± 2.59** (↓9%)
BW Day 21	46.9 ± 3.80	48.1 ± 3.10	47.1 ± 4.24	42.5 ± 4.17** (↓10%)
Overall BWG Days 1-21 (both sexes combined)	41.8	43.0	42.0	37.4 (↓11%)

[a] Data obtained from pages 212-214 in the study report. N=22-24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BW body weight; BWG body weight gain
Values in parentheses represent the percent change from controls (calculated by the reviewer).

F2 PUPS

Mean pup weights were similar in all groups up to Day 7 (Table 12b). On Day 14 and Day 21 both male and female high dose pups experienced statistical decreases in body weight by 7% and 10%, respectively. This is also reflected in the overall combined body weight gain by a reduction of 12% in pups dosed at 1800 ppm.

Table 12b. Body weight (g) – F2 pups (Mean ± S.D.)[a]

Study Day	0 ppm	30 ppm	250 ppm	1800 ppm
Males				
BW Day 0	-	6.0	6.0 ± 0.24	5.9
BW Day 1	6.1 ± 0.53	6.5 ± 0.66	6.4 ± 0.69	6.2 ± 0.56
BW Day 4	8.9 ± 1.29	9.8 ± 1.45	9.3 ± 1.09	9.1 ± 0.95
BW Day 7	14.4 ± 2.03	15.5 ± 1.65	14.9 ± 1.72	14.7 ± 1.68
BW Day 14	30.3 ± 2.41	31.4 ± 2.59	30.3 ± 2.61	28.2 ± 2.08* (↓7%)
BW Day 21	49.1 ± 3.85	51.4 ± 3.91	48.8 ± 4.28	44.1 ± 3.45** (↓10%)
Females				
BW Day 0	-	5.4	5.7 ± 0.47	5.6
BW Day 1	5.8 ± 0.55	6.1 ± 0.56	6.0 ± 0.55	5.9 ± 0.56
BW Day 4	8.6 ± 1.31	9.3 ± 1.18	8.9 ± 1.15	8.8 ± 0.99
BW Day 7	14.1 ± 1.59	14.9 ± 1.35	14.4 ± 1.88	13.9 ± 1.53
BW Day 14	29.6 ± 2.27	30.4 ± 2.23	29.3 ± 2.94	27.6 ± 2.16* (↓7%)
BW Day 21	47.4 ± 3.79	49.2 ± 3.72	46.5 ± 4.47	42.7 ± 3.44** (↓10%)
Overall BWG Days 1-21 (both sexes combined)	42.3	44.2	41.5	37.4 (↓12%)

[a] Data obtained from pages 315-317 in the study report. N=22-24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BW body weight; BWG body weight gain
Values in parentheses represent the percent change from controls (calculated by the reviewer).

4. Developmental Indices

F1 PUPS

The mean time of onset of coat development was statistically significantly shorter (8.8 days versus 9.6 days in the control) in the group receiving 1800 ppm (Table 13a). All pups from all dose groups developed their first coat at 8-10 days of age. Mean time of incisor eruption was also statistically significantly but very slightly shorter in this group. However the mean time was similar to that of the 250 ppm dose which did not attain any statistical significance.

There were no changes in the mean time of pinna unfolding and opening of eyes in any treated group.

Table 13a. Developmental indices (days) – F1 pups (Mean ± S.D.)[a]

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
F1 Pups				
Incisor eruption	8.0 ± 0.41	7.8 ± 0.36	7.6 ± 0.51	7.6 ± 0.56*
Onset of coat development	9.6 ± 0.50	9.1 ± 0.83*	9.2 ± 0.73	8.8 ± 0.66*
Pinna unfolding	3.3 ± 0.65	3.5 ± 0.60	3.1 ± 0.67	3.4 ± 0.65
Opening of eye	15.3 ± 0.62	15.3 ± 0.54	15.1 ± 0.68	15.4 ± 0.77

[a] Data obtained from pages 215 in the study report. N=22-24.
* Significantly different from control, p<0.05

F2 PUPS

No changes in the mean time of pinna unfolding, incisor eruption, onset of coat development or opening eyes were noted (Table 13b). The statistically significant reduction in time to eye opening in the 30 ppm dose group was not considered to be related to treatment given the lack of dose response.

Table 13a. Developmental indices (days) – F2 pups (Mean ± S.D.)[a]

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
F2 Pups				
Incisor eruption	7.0 ± 0.26	6.9 ± 0.35	7.1 ± 0.48	7.0 ± 0.39
Onset of coat development	10.0 ± 0.37	10.0 ± 0.65	10.1 ± 0.35	10.0 ± 0.21
Pinna unfolding	3.1 ± 0.90	2.7 ± 0.54	2.7 ± 0.61	2.9 ± 0.75
Opening of eye	15.1 ± 0.64	14.5 ± 0.47*	14.9 ± 0.67	15.2 ± 0.71

[a] Data obtained from pages 318 in the study report. N=22-23.
* Significantly different from control, p<0.05

5. Sexual Maturation

F1 PUPS

There were slight delays in the onset of preputial separation (1.3 days) and vaginal patency (1.4 days) at the high dose group (Table 14). These slight delays are considered to be a secondary effect of the lower animal body weights at this dose rather than a direct developmental effect of treatment.

Table 14. Sexual maturation – F1 pups (Mean ± S.D.)[a]

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
Time of preputial separation (days)	26.9 ± 1.3	26.6 ± 1.3	27.2 ± 1.8	28.2 ± 2.0
Body weight at time of preputial separation (g)	72.22 ± 9.32	72.30 ± 8.29	73.87 ± 10.42	68.43 ± 8.81 (↓5%)
Time of vaginal patency (days)	35.4 ± 1.9	34.8 ± 1.8	34.5 ± 1.7	36.8 ± 2.8
Body weight at time of vaginal patency (g)	109.87 ± 12.08	109.32 ± 12.76	104.18 ± 14.81	99.66 ± 11.56* (↓9%)

[a] Data obtained from pages 216-217 in the study report. N=22-24.
* Significantly different from control, $p<0.05$
Values in parentheses represent the percent change from controls (calculated by the reviewer).

F2 PUPS

Not applicable: the study was terminated at weaning of F2 pups (day 21 post-partum).

6. Organ Weight Data

F1 PUPS

In the 1800 ppm pups, mean absolute weight of spleen and ratio of spleen weight relative to brain weight were statistically significantly reduced in males and females (Table 15a). Spleen weight relative to body weight was also reduced although not statistically. Absolute thymus weight and relative thymus weight to brain weight ratio were statistically significantly reduced in the high dose males. In females, the absolute thymus weight and relative thymus weight to brain weight ratio were reduced non-statistically by 8%. These were considered likely to be secondary effects to the lower body weight as there were no effects on the organ weights relative to body weight.

Table 15a. Selected absolute (g) and relative (%) organ weights, F1 pups (Mean ± S.D.)[a]

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
F1 Pups Males				
Final BW	47.1 ± 4.9	48.3 ± 3.6	46.9 ± 3.5	42.6 ± 4.1** (↓10%)
Abs. Brain	1.43 ± 0.06	1.45 ± 0.07	1.44 ± 0.05	1.43 ± 0.05
Rel. Brain (to BW)	3.06 ± 0.26	3.01 ± 0.19	3.08 ± 0.22	3.38 ± 0.25 (↑10%)
Abs. Thymus	0.198 ± 0.030	0.194 ± 0.027	0.186 ± 0.030	0.170 ± 0.029** (↓14%)
Rel. Thymus (to BW)	0.421 ± 0.051	0.401 ± 0.046	0.397 ± 0.060	0.399 ± 0.059
Rel. Thymus (to brain wt)	13.838 ± 1.892	13.350 ± 1.627	12.928 ± 1.960	11.837 ± 1.832** (↓14%)
Abs. Spleen	0.23 ± 0.05	0.23 ± 0.04	0.23 ± 0.04	0.19 ± 0.04** (↓17%)
Rel. Spleen (to BW)	0.49 ± 0.07	0.48 ± 0.06	0.48 ± 0.06	0.44 ± 0.06 (↓10%)
Rel. Spleen (to brain wt)	16.11 ± 3.24	15.98 ± 2.36	15.74 ± 2.57	13.17 ± 2.22** (↓18%)
F1 Pups Females				
Final BW	45.3 ± 4.0	46.8 ± 3.3	45.6 ± 4.5	41.6 ± 4.2** (↓10%)
Abs. Brain	1.40 ± 0.05	1.40 ± 0.06	1.39 ± 0.05	1.39 ± 0.06
Rel. Brain (to BW)	3.11 ± 0.26	3.01 ± 0.17	3.07 ± 0.23	3.36 ± 0.26** (↑8%)
Abs. Thymus	0.205 ± 0.035	0.205 ± 0.031	0.195 ± 0.032	0.186 ± 0.028 (↓8%)
Rel. Thymus (to BW)	0.454 ± 0.070	0.438 ± 0.051	0.426 ± 0.050	0.449 ± 0.070
Rel. Thymus (to brain wt)	14.626 ± 2.236	14.600 ± 1.805	13.991 ± 2.061	13.395 ± 1.952 (↓8%)
Abs. Spleen	0.23 ± 0.04	0.24 ± 0.04	0.23 ± 0.05	0.20 ± 0.04** (↓13%)
Rel. Spleen (to BW)	0.50 ± 0.06	0.52 ± 0.07	0.50 ± 0.08	0.47 ± 0.07 (↓6%)
Rel. Spleen (to brain wt)	16.07 ± 2.52	17.17 ± 2.55	16.60 ± 3.27	14.03 ± 2.49* (↓13%)

[a] Data obtained from pages 219-223 in the study report. N=21-24.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BW = body weight
wt = weight
Values in parentheses represent the percent change from controls (calculated by the reviewer).

F2 PUPS

Relative brain weights to body weight were statistically significantly increased in both sexes.

Absolute spleen weight and relative spleen to body and to brain weight ratios were statistically significantly reduced in male and female pups at 1800 ppm (Table 15b). In male pups, absolute thymus and thymus relative to brain weight ratios were statistically reduced at 250 and 1800 ppm; although the thymus weight/body weight ratio at 1800 ppm was not reduced. A statistically significant decrease of 9% was observed in the thymus weight/body weight ratio at 250 ppm, which was not dose-dependent. In females, slight, non-statistically significant decreases in absolute thymus and thymus relative to brain weight ratios were also noted at 1800 ppm.

The relative organ to body weight ratio of the right kidney in female pups at 1800 ppm was slightly but statistically significantly higher than the control value. Since there were no changes in male kidney weights or changes in the left female kidney weight, or in the absolute or relative to brain weight values for the right kidney, the increase in the right kidney relative to body weight was considered to be unrelated to treatment. There were no treatment related effects on liver or thyroid gland weights.

Table 15b. Selected absolute (g) and relative (%) organ weights, F2 pups (Mean ± S.D.)[a]

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
F2 Pups - Males				
Final BW	48.1 ± 4.1	50.6 ± 4.7	47.0 ± 4.6	43.4 ± 3.4** (↓10%)
Abs. Brain	1.47 ± 0.06	1.47 ± 0.05	1.45 ± 0.05	1.46 ± 0.05
Rel. Brain (to BW)	3.07 ± 0.21	2.93 ± 0.27	3.11 ± 0.26	3.38 ± 0.24** (↑13%)
Abs. Thymus	0206 ± 0.025	0.207 ± 0.028	0.184 ± 0.027* (↓11%)	0.179 ± 0.026** (↓10%)
Rel. Thymus (to BW)	0.428 ± 0.045	0.411 ± 0.053	0.390 ± 0.039* (↓9%)	0.415 ± 0.054
Rel. Thymus (to brain wt)	14.001 ± 1.620	14.067 ± 2.031	12.597 ± 1.518* (↓10%)	12.303 ± 1.633** (↓13%)
Abs. Spleen	0.24 ± 0.04	0.24 ± 0.05	0.22 ± 0.05	0.19 ± 0.04** (↓21%)
Rel. Spleen (to BW)	0.51 ± 0.07	0.47 ± 0.07	0.45 ± 0.08	0.43 ± 0.08** (↓16%)
Rel. Spleen (to brain wt)	16.56 ± 2.51	16.16 ± 3.40	14.77 ± 3.24	12.80 ± 2.80** (↓23%)
F1 Pups - Females				
Final BW	46.3 ± 3.9	48.1 ± 4.3	45.5 ± 4.4	41.5 ± 3.7** (↓10%)
Abs. Brain	1.40 ± 0.04	1.43 ± 0.06	1.40 ± 0.06	1.40 ± 0.04
Rel. Brain (to BW)	3.05 ± 0.24	2.99 ± 0.29	3.10 ± 0.26	3.38 ± 0.27** (↑11%)
Abs. Kidney (R)	0.25 ± 0.03	0.27 ± 0.02	0.25 ± 0.03	0.24 ± 0.02
Rel. Kidney (R) (to BW)	0.55 ± 0.03	0.56 ± 0.03	0.56 ± 0.04	0.58 ± 0.04* (↑5%)
Rel. Kidney (R) (to brain wt)	18.15 ± 1.80	18.77 ± 1.44	17.99 ± 1.46	17.15 ± 1.47
Abs. Thymus	0.198 ± 0.030	0.214 ± 0.034	0.192 ± 0.046	0.184 ± 0.028 (↓7%)
Rel. Thymus (to BW)	0.428 ± 0.055	0.443± 0.052	0.420 ± 0.088	0.444 ± 0.060
Rel. Thymus (to brain wt)	14.128 ± 2.050	14.967 ± 2.290	13.672 ± 3.036	13.198 ± 1.925 (↓6%)
Abs. Spleen	0.22 ± 0.05	0.23 ± 0.24	0.22 ± 0.05	0.17 ± 0.04** (↓23%)
Rel. Spleen (to BW)	0.48 ± 0.08	0.47 ± 0.06	0.48 ± 0.008	0.42 ± 0.07* (↓13%)
Rel. Spleen (to brain wt)	15.77 ± 3.17	15.97 ± 2.83	15.78 ± 3.32	12.44 ± 2.60** (↓21%)

[a] Data obtained from pages 319-324 in the study report. N=21-23.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$
BW = body weight
wt = weight
Values in parentheses represent the percent change from controls (calculated by the reviewer).

7. Necropsy findings

F1 PUPS

There were no treatment related macroscopic findings in the F1 pups. Discoloration or dark red foci were noted in the thymus of 2 mid dose males and 2 high dose males. Dark red or reddish foci were present on the thymus in one control group female, one low dose female, and 2 females each from the mid and high dose groups.

F2 PUPS (including histopathology and fluoride determinations)

Uni/bilateral pelvic dilation of the kidney was observed in pups in all groups. The incidence of this finding did not indicate any test item-related effect. Other findings included single incidences of red brown foci on kidney, and reduction in size of the right adrenal. These findings were not attributed to treatment with the test item.

Histologically, the severity of glycogen deposits was slightly reduced in pups of both sexes at 1800 ppm and the incidence of glycogen deposits was slightly reduced in the high dose females (Table 16); this was considered by the study authors to be related to the lower body weight of these animals.

Table 16. Incidence/Mean Severity of Microscopic Findings in F2 pups[a]

Finding	0 ppm		30 ppm		250 ppm		1800 ppm	
Liver (# examined)	**23 M**	**23 F**	**22 M**	**21 F**	**22 M**	**22 F**	**22 M**	**22 F**
-Glycogen deposits	23/2.0	23/1.8	22/2.0	21/1.8	22/2.0	22/1.8	22/1.4	18/1.4

[a] Data obtained from pages 1320 in the study report

Fluoride Determinations

Mean content of fluoride was statistically significantly increased in the bone (femur) and teeth (incisors) of male and female pups dosed at 1800 ppm (Table 17). At 250 ppm, mean fluoride content was statistically significantly increased in teeth but not in bones of male and female pups. Slight but non-statistically significant increases in fluoride content of bone were noted in females at 250 ppm and of bone in males at 30 ppm. Overall, fluoride content in teeth increased with dose.

Table 17. Fluoride Analysis (ppm) in the F2 generation[a]

Parameter	0 ppm	30 ppm	250 ppm	1800 ppm
F2 Generation Males –Bones				
Mean Fluoride Content	91.04	59.52	90.54	445.4* (↑389%)
Rel. SD (%)	11.6	9.9	28.3	45.3
F2 Generation Males-Teeth				
Mean Fluoride Content	64.39	86.66 (↑35%)	105.5* (↑64%)	196.8* (↑206%)
Rel. SD (%)	12.7	16.5	44.7	16.5
F2 Generation Females – Bones				
Mean Fluoride Content	98.95	68.49	131.6 (↑33%)	421.6* (↑326%)
Rel. SD (%)	17.2	18.8	19.5	45.3
F2 Generation Females –Teeth				
Mean Fluoride Content	85.43	91.32	113.2* (↑33%)	208.9* (↑145%)
Rel. SD (%)	12.0	10.1	8.6	15.9

[a] Data obtained from pages 2026-2029 in the study report. N=22-24.
*Significantly different from control, $p<0.05$, significance was not tested at higher levels
Values in parentheses represent the percent change from controls (calculated by the reviewer).

III. APPLICANT'S CONCLUSIONS

"A NOAEL (No Observed Adverse Effect Level) for general toxicity in parental animals of both generations was established at 250 ppm, based on the effects on body weight development throughout the study.

A NOAEL for general toxicity in F1 and F2 pups was established at 250 ppm based on reduced pup weight noted at 1800 ppm.

A NOAEL for reproductive effects in P and F1 parental generations was established at 1800 ppm, as there were no effects on any other measures of reproductive function including gonadal function, mating behavior, fertility, viability of offspring, breeding, conception, gestation and parturition."

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer comments: The study author stated that the increase in post-natal loss up to Day 4 in the high dose P generation was not treatment-related since the number of losses remained within the historical control range (2-14). However, of the seven studies where the range was taken from, one study had an abnormally high number of losses. Omitting this study, the range of loss would be 2-5. Therefore, the post-natal loss at the high dose would have exceeded both the historical control range and the concurrent control. In the F1 generation, a high number of post-natal losses were seen again at the high dose. The study author explained that this was not considered to be treatment-related because the increase was mainly due to two animals that experienced a high number of loss and that there was no change on a litter basis. However, given that there was a higher post-natal loss in the P generation and no other groups experienced an increase in pup loss, the reviewer considers that this was a treatment-related effect in spite of a lack of change in the number of litters affected.

In the F1 parent animals, mean fluoride content was dose dependently higher in bone (femur) of males and females and in teeth (incisors) of females at 30, 250 and 1800 ppm. In F2 pups, at 1800 ppm mean content of fluoride was statistically significantly higher in bone and in the teeth of males and females. Fluoride content was also significantly increased in pups dosed at 250 ppm. The deposition of fluoride in F2 pups was much lower when compared to the F1 parental generation. The study author believes that this was mainly due to a shorter exposure period as F2 pups commence to eat by themselves only during the last week of the lactation period. Since there was no evidence of discoloration of the teeth or fragility of bones and teeth in either F1 adults or F2 pups, it is concluded that there is no fluorosis in this study. Thus, the deposition of fluoride in bone and teeth did not appear to cause any adverse health effects.

C. Conclusions: The parental LOAEL in males and females is 1800 ppm (overall intake of 122.0 mg/kg bw/day in males and 169.1 mg/kg bw/day in females; premating intake of 137 mg/kg bw/day in males and 149 mg/kg bw/day in females) based on decreased body weight, body weight gain, food consumption and increased liver and kidney weights and histopathology changes in the liver and kidney. The parental NOAEL is 250 ppm (overall study intake of 16.2 mg/kg bw/day in males and 23.0 mg/kg bw/day in females; premating intake of 18.3 mg/kg bw/day in males and 20.3 mg/kg bw/day in females).

The reproductive NOAEL in males and females is 1800 ppm (overall study intake of 122.0 mg/kg bw/day in males and 169.1 mg/kg bw/day in females; premating intake of 137 mg/kg bw/day in males and 149 mg/kg bw/day in females) based on an increase in post-natal pup loss between days 0-4 in both P and F1 generations. The reproductive LOAEL was not determined as no treatment-related effects on reproductive performance were observed.

The offspring LOAEL in males and females is 1800 ppm (overall study intake of 122.0 mg/kg bw/day in males and 169.1 mg/kg bw/day in females; premating intake of 137 mg/kg bw/day in males and 149 mg/kg bw/day in females) based on decreased pup weights and spleen weights and an increase in post-natal pup loss between days 0 and 4 in the F1 and F2 offspring. The offspring NOAEL is 250 ppm (overall study intake of 16.2 mg/kg bw/day in males and 23.0 mg/kg bw/day in females; premating intake of 18.3 mg/kg bw/day in males and 20.3 mg/kg bw/day in females).

This study is acceptable/guideline (fully reliable) and satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800; OECD 416) in rats.

D. Study deficiencies: No study deficiencies were noted.

Tier II Summary (PMRA #2181208~~2181141~~, MRID 48574756~~48575035~~) revised by the US EPA, March 13, 2014

Study Type: Oral One year toxicity (dog)

IIA 5.3.4– MRID 48574756, PMRA 2181208

Report	Braun L. (2011) MCW-2 TECH: 52-Week Oral (Feeding) Toxicity Study in the Beagle Dog with an 8-Week Recovery Period. Harlan Laboratories Ltd., Switzerland; unpublished report No. C57031, dated 14 July 2011. Sponsor reference No. R-23351 Dates of experimental work: 12 August 2009 to 13 July 2011
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines	OECD No. 452, and OPPTS 870.4100 Deviations: none
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In a 1 year oral toxicity study (MRID 48574756, PMRA 2181208), fluensulfone (MCW-2 tech., 95.70-98.65%, Batch No. 36372130-291-PF1) was administered in the diet to groups of 4 male and 4 female Beagle dogs at 0, 5, 50 100 and 500 ppm (0.0, 0.1/0.1, 1.5/1.5, 3.1/3.3. 16.0/16.2 mg/kg bw/day in male/females) for a period of 52 weeks. Similar sized recovery groups for the control and the high dose were treated for 52 consecutive weeks and sacrificed after an additional 8-week period without treatment.

Oral administration of fluensulfone to Beagle dogs in the diet at a dose of 500 ppm resulted in transient changes in consistency of feces, reductions in food intake in females and lower body weight gains, mean body weights and terminal body weights, effects on hematology and biochemistry parameters and on the liver.

A mild reduction in hemoglobin concentration and associated parameters with reticulocytosis, as well as the presence of brownish pigment (likely hemosiderin) in the liver and increased platelets were observed. Changes in albumin and protein profile (reductions) were also noted. Several organ weight changes (increased kidney weight, reduced spleen weight in females, and increased relative adrenal gland weight in males) were noted at 500 ppm without any pathological correlates. Females from the 500 ppm dose group exhibited slight reductions in serum glucose levels as well. Decreases in plasma alanine aminotransferase activity were observed at 50, 100 and 500 ppm. An inhibition of hepatic alanine aminotransferase activity was evident after 52 weeks of treatment at the same dose levels. These changes were not considered to be adverse since decreases of these enzymes had no impact on the health status of the animals. In addition, no effect was observed in animals previously treated at 500 ppm after the 8-week recovery period. The phase II enzyme glutathione S-transferase was induced at 500 ppm in both sexes and after the recovery period the effect was still increased in males but of no statistical significance, or not present in the females. A slight induction of epoxide hydrolase

was also evident in both sexes at 500 ppm. Slightly increased liver weight in females and minimal to slight degrees of diffuse hepatocellular hypertrophy in both sexes were observed at 500 ppm. The findings were still evident at the end of recovery in a single female. The changes in consistency of feces were also evident in a dose-related manner at the lower dose of 100 ppm. Also at 100 ppm, males demonstrated a slight reduction in partial thromboplastin time, and females exhibited reduced body weights and associated organ weight changes in addition to slight reductions in serum bilirubin levels; at this dose level the effects were not considered adverse.

A dose-related increase of fluoride content was evident at doses of 50, 100 and 500 ppm in the ashes from bone and teeth, attaining statistical significance only at the high dose level. No signs of dental fluorosis were observed during the study and no abnormality in bone structure was observed at microscopic examination and therefore the deposition of fluoride was not considered adverse.

The NOAEL is 100 ppm (3.1/3.3 mg/kg bw/day, M/F) based on reductions in body weights, changes in hematological and clinical chemistry parameters, and organ weights in both sexes at the LOAEL of 500 ppm (16.0/16.2 mg/kg bw/day, M/F).

This chronic study in the dog is classified as **acceptable/guideline** and satisfies the guideline requirement for a chronic oral study (OECD 452; OPPTS 870.4100) in dogs.

I. MATERIAL AND METHODS

1. Test Material	MCW-2 TECH (fluensulfone)
Description:	Solid, yellow
Lot/Batch:	36372130-291-PF1
Purity:	95.70-98.65%
CAS#:	318290-98-1
Stability:	Stable until 31 May 2011
2. Vehicle	Pelleted standard Kliba Nafag 3353 dog maintenance diet (Provimi Kliba AG, Switzerland)
3. Test Animals	
Species	Dog
Strain	Beagle
Age	5.5 to 6.5 months at treatment start
Weight	6.2 to 9.9 kg at treatment start
Source	Harlan Winkelmann GmbH, Germany
Acclimation period	Approximately 1 month
Diet	350 ± 1 g of pelleted standard Kliba Nafag 3353 dog maintenance diet (Provimi Kliba AG, Switzerland), batch nos.

	43/09, 70/09, 06/10 and 40/10. Any remaining diet was withdrawn approximately 4 hours after feeding.
Water	Domestic tap water (chlorinated to approximately 0.5 ppm), *ad libitum*, by an automatic watering system.
Housing	Individual or group housing (2 animals/group/sex) in solid floor pens with minimum of 2.0 square meters floor space per dog. Autoclaved softwood bedding was provided and changed at least once weekly. Dogs were individually housed during feeding periods or at the discretion of the study director to facilitate recording of clinical signs.

4. Environmental conditions

Temperature	22 ± 3°C
Humidity	30 - 70%. Values for humidity above these ranges (maximum approx. 89%) occasionally occurred, usually following room cleaning. These transient variations were not considered to have any influence on the study.
Air change	10 - 15 per hour.
Photoperiod	12-hour fluorescent light / 12-hour dark cycle with at least 8 hours music during the light period.

B. STUDY DESIGN

1. In-life dates	15 July 2009 to 13 October 2010

2. Animal Assignment and treatment

Shortly after arrival the dogs were temporarily allocated to groups by a computer generated random algorithm, with two additional spare animals. Thereafter, pre-treatment investigations were carried out on all dogs. Shortly before the start of treatment, the data obtained were reviewed. The allocation was adjusted by exchanging animals for the spare animals as well as between groups due to clinical biochemistry values or for better inter-group mean body weights. The rejected animals did not take further part in the study after commencement of treatment. Four animals per sex were assigned to each group and received the test diet for 52 weeks. An additional four animals per sex in the control and 500 ppm dose group levels received the test diet for 52 weeks and were sacrificed after an additional 8-week recovery period without treatment.

The dose groups and achieved mean daily intakes are given in Table 1 below:

Table 1: Achieved mean daily intake

Dietary concentration	0 ppm	5 ppm	50 ppm	100 ppm	500 ppm
	Daily intake (mg/kg bw/day)				
Males	0.0	0.1	1.5	3.1	16.0
	Recovery: 0.0				Recovery: 0.0
Females	0.0	0.1	1.5	3.3	16.2
	Recovery: 0.0				Recovery: 0.0

3. Dose Selection Rationale

Dose levels were selected based on the results of the 90-day dietary study in dogs (MRID 48574755, PMRA 2181207), in which dose levels of 0, 5, 50 and 500 ppm were used. The study investigators of that study concluded that the NOAEL was 500 ppm based on slight and reversible effects noted at that dose that were considered unlikely to represent adverse effects; however, the reviewer concluded that the effects at 500 ppm were indicative of an adverse effect of treatment and set the NOAEL for the 90-day study in dogs at 50 ppm. An additional dose group of 100 ppm was included in this study with the intention of improving the safety margin for the NOEL/NOAEL.

4. Diet Preparation and Analysis

Diets were prepared weekly to every 2 weeks and stored at room temperature in disposable paper bags.

Fluensulfone was warmed in the original container in a water bath up to a maximum of approximately 40°C until it became liquid. Thereafter, the whole amount of fluid test item was transferred into a glass beaker wrapped with aluminum foil and mixed using a magnetic stirrer on a heating plate (maximum of approximately 40 °C). The test item was divided into aliquots in labeled brown glass containers. The required amount of fluensulfone was weighed into tared glass beakers (wrapped with aluminum foil) and kept at a maximum of approximately 40°C on a heating plate until use for feed preparation. The test item was mixed with microgranulated feed for each dose group and pellets were prepared. Water (approximately 1:10 volume/weight ratio) was added to aid pelleting. The pellets were dried with warm air for approximately 48 hours before storage.

The pellets for the control group were prepared as described above but without test item.

Samples for determination of concentration and homogeneity of the dose formulations were taken from the admixture used in the first week of the administration period. Additionally, samples for determination of concentration were taken from the admixture used in weeks 13, 26, 39, and 52 of the administration period. Confirmation of stability (21 days at room temperature) of the dose formulations was determined from the admixture used in week 52 of the administration period.

Analyses were performed according to a HPLC-method developed at the performing laboratory in a separate study. The diet samples were stored deep-frozen (-15°C to -25°C) until analysis. Fluensulfone was used as analytical standard.

5. Statistics

The following statistical methods were used to analyze body weight, clinical laboratory data and organ weights:

- The Dunnett-test (many to one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups and the control groups for each sex.
- The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data could not be assumed to follow a normal distribution.

C. METHODS

1. Clinical observations

Observations for viability were recorded at least twice daily from commencement of the pre-test period.

Each animal was examined at least twice daily, starting from the pre-test period, for any change in behavior, reaction to treatment or ill-health. A description of any abnormality was recorded. A detailed observation was performed once weekly. The weekly observation included a visual assessment of tooth color. Throughout the study individual animals were examined by a veterinarian.

Veterinary examinations were performed on all animals pre-test, at weeks 13, 26 and 52, and at week 8 of the recovery period. The examinations included pupil reflex, corneal reflex, and patellar reflex, cardiac and pulmonary auscultation (including basic neurological assessment). In addition, a visual examination of the teeth of all animals for possible discoloration was performed during the scheduled veterinary examinations and was observed once in one animal at pre-test only (brown discolored teeth in one male of the highest dose level). During treatment the investigations were performed after dosing.

When necessary, therapeutic treatments were used for specific common health problems, not related to treatment with the test item (e.g. subcutaneous injections of antibiotics for a painful and swollen abscess on the left forepaw, antiseptic rinses for cleansing open wounds or cuts, antibacterial ophthalmic ointment for conjunctivitis and purulent discharge of one eye).

During the last day of the recovery period one control male was killed in extremis due to marked signs of toxicity (increased heart rate, weak pulse, recumbency, dehydration).

2. Bodyweight

The body weight of each animal was recorded before feeding at least once weekly during the pre-test, treatment and recovery periods and before necropsy.

3. Food consumption

Food consumption was recorded daily from commencement of the pre-test period. The daily ration was weighed before and after feeding. The mean and individual values for each week were reported.

4. Ophthalmoscopic examination

Each animal was examined for abnormalities of the eyes at least 20 minutes after the instillation of 0.5% tropicamide solution (Mydriaticum Dispersa, CIBA Vision AG, Switzerland) using a binocular indirect ophthalmoscope (all pupil model, Keeler Instruments, Inc., USA) and the results were recorded. The observation area included the cornea, conjunctiva, sclera, iris, lens and fundus.

Ophthalmoscopic examinations were performed in all animals at pre-test, at weeks 13, 26 and 52 of the treatment period, and at week 8 of the recovery period.

5. Hematology and Clinical Chemistry

Blood samples, drawn from the jugular vein, were collected from all animals pre-test, at weeks 6, 13, 26 and 52 of the treatment period, and at week 8 of the recovery period for hematology and clinical chemistry determinations. The dogs were fasted overnight but allowed access to water *ad libitum*. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms.

The following parameters were determined at hematology:

Erythrocyte count, Hemoglobin concentration, Hematocrit, Mean corpuscular volume, Red cell volume distribution width, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration, Hemoglobin concentration distribution width, Platelet count, Reticulocyte count, Reticulocyte maturity index, Total leukocyte count, Differential leukocyte count, Thromboplastin time and Activated partial thromboplastin time

The following parameters were determined at clinical chemistry:

Glucose, Urea, Creatinine, Bilirubin (total), Cholesterol (total), Triglycerides, Phospholipids, Aspartate aminotransferase, Alanine aminotransferase, Creatine kinase, Alkaline phosphatase, Sorbitol dehydrogenase, Lactate dehydrogenase, Glutamate dehydrogenase, Gamma-glutamyl-transferase, Calcium, Phosphorus, Sodium, Potassium, Chloride, Protein (total), Albumin, Globulin, and Albumin/Globulin Ratio

At pre-test, at week 52 of the treatment period, and at week 8 of the recovery period, 3.5 mL of blood were collected into blood collection tubes without anticoagulant for Total triiodothyronine (Total T3), Total thyroxine (Total T4) and Thyroid-stimulating hormone (TSH) analyses. Following centrifugation, each serum sample was divided into 2 aliquots and transferred to plastic (polypropylene) tubes and stored at -80 ± 10 °C in the dark until transfer on dry ice for hormone analysis. Samples were analyzed using commercially available kits (Siemens, Switzerland: Immulite Total T3, Immulite Canine Total T4 and Immulite Canine TSH).

Additional blood samples from all animals were collected into 4 mL lithium heparin tubes at weeks 1, 2, 4 (twice), 8, 10, 17, and 39 (days 7, 14, 22, 28 55, 69, 115 and 273) of the treatment period, and at week 4 of the recovery period for liver parameter determinations.

6. Urinalysis

Urine samples, collected into a specimen vial using a catheter, were obtained from all animals at pre-test, at weeks 6, 13, 26 and 52 of the treatment period, and at week 8 of the recovery period. The dogs were fasted overnight but allowed access to water *ad libitum*. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms.

The following parameters were determined:

Color, Appearance, Relative density, pH, Protein, Glucose, Ketones, Urobilinogen, Bilirubin, Erythrocytes, Leukocytes

7. Sacrifice and pathology

All animals were anesthetized by intravenous injection of sodium pentobarbital (Esconarkon®, Veterinaria AG) and killed by exsanguination at the end of the scheduled treatment or at the end of the scheduled recovery periods. One control male was killed in extremis before the end of the recovery period. A description of all macroscopic abnormalities was recorded.

Samples of a complete list of tissues and organs (Table 2) were collected from all animals at necropsy and fixed, unless otherwise indicated, in neutral phosphate-buffered 4% formaldehyde solution (10% formalin). Bone marrow smears from the sternum from all animals were taken, stained and retained for possible further investigation.

At the planned necropsies the right femur and one vertebra (L3) of each animal were collected, wrapped in saline soaked gauze and plastic wrap, placed in labeled plastic bags on dry ice, frozen within 4 hours following euthanasia and stored at -20 ± 5 °C for possible later investigations. As no further investigations were performed, these bone samples were discarded.

Organs were weighed before fixation. Paired organs were weighed separately. Relative organ weights were calculated on the basis of the body weight and brain weight.

All organ and tissue samples were processed, embedded and cut at a nominal thickness of 4 µm and stained with hematoxylin and eosin. Slides of all organs and tissues collected at necropsy were examined by the study pathologist. An attempt was made to correlate gross observations with microscopic findings. A peer review of the findings was also performed.

Table 2: Summary of tissues collected for pathology examination

Organ/tissue	Weighed	Collected	Examined
Adrenal gland	X	X	X
Aorta		X	X
Bone - sternum, femur including articular surface		X	X
Bone marrow - femur		X	X
Brain - including sections of medulla/pons, cerebral and cerebellar cortex	X	X	X
Epididymides (fixed in Bouin's solution)	X	X	X
Esophagus		X	X
Eyes with optic nerve (fixed in Davidson's solution)		X	X
Female mammary gland area, (male mammary gland area)		X	X
Gallbladder		X	X
Heart	X	X	X
Kidneys	X	X	X
Large intestine - cecum, colon, rectum		X	X
Larynx		X	
Liver	X	X	X
Lungs, instilled via trachea with formalin at approximately 30 cm H_2O pressure		X	X
Lymph nodes - retropharyngeal, mesenteric		X	X
Ovaries	X	X	X

Oviducts		X	X
Pancreas		X	X
Pharynx		X	X
Pineal gland		X	X
Pituitary gland		X	X
Peyer's Patches		X	X
Prostate gland		X	X
Salivary glands - mandibular, parotid, sublingual		X	X
Sciatic nerve		X	X
Skeletal muscle		X	X
Skin - with subcutaneous tissue		X	X
Small intestine - duodenum, jejunum, ileum		X	X
Spinal cord - cervical, midthoracic, lumbar segments		X	X
Spleen	X	X	X
Stomach		X	X
Testes (fixed in Bouin's solution)	X	X	X
Thymus	X	X	X
Thyroid gland with parathyroid gland	X	X	X
Tongue		X	X
Trachea		X	X
Ureter		X	X
Urinary bladder		X	X
Uterus with (vagina), cervix	X	X	X
All gross lesions		X	X

8. Additional investigations

Fluoride Determination

Two samples each of the left femur and two incisors were collected from all animals at the scheduled necropsies (after 52 weeks treatment and 8 weeks recovery) for fluoride determination. The samples were placed into labeled plastic bags and stored frozen at -80 ± 10 °C until analysis. The samples were transferred on dry ice to the analytics laboratory. The fluoride determination was performed according to an ion chromatographic method developed at the performing laboratory in a separate study.

Liver Enzyme Determinations

Samples of the liver were collected from all animals at the scheduled necropsies (after 52 weeks treatment and 8 weeks recovery) for liver enzyme determinations. The samples were minced into small pieces and aliquots of 4 - 5 g each were placed into labeled plastic bags and frozen in liquid nitrogen. The samples were transferred on dry ice to the analytical laboratory and stored at -80 ± 10°C until analysis. The assay was performed at Harlan Laboratories Ltd. (Füllinsdorf) under internal laboratory quality control conditions to assure reliable test results.

Liver subcellular fractions were prepared from the main study animals and the following parameters were determined:

- Protein content of subcellular fractions
- Microsomal cytochrome P450 content (differential spectroscopy)
- CYP1A1 (microsomal 7-ethoxyresorufin O-dealkylation; EROD
- Cytosolic glutathione S-transferase (GST, glutathione conjugation of 1-chloro-2,4-dinitrobenzol)
- Microsomal epoxide hydrolase (hydrolysis of styrene oxide)
- Cytosolic alanine aminotransferase (ALAT, transamination of ketoglutarate)

From the recovery animals only liver homogenate and cytosol were prepared and analyzed for the following enzyme activities:

- Protein content of subcellular fractions
- Cytosolic glutathione S-transferase (GST, glutathione conjugation of 1-chloro-2,4-dinitrobenzol)
- Cytosolic alanine aminotransferase (ALAT, transamination of ketoglutarate)

II. RESULTS AND DISCUSSION

A. ANALYSIS OF FEED PREPARATIONS

The content of fluensulfone in 71 out of a total of 72 diet samples ranged from 81 to 114% of the nominal concentration. The content of one sample taken in week 39 from the 100 ppm test diet was 78% of the nominal concentration.

The distribution of fluensulfone in the diet preparations was homogeneous as no single results deviated more than 9.8% from the corresponding mean. In addition, the test item was found to be stable in diet when kept for 21 days at room temperature based on the recoveries which were 92% to 102% of the time-zero (homogeneity) mean.

The absence of fluensulfone in the control diet was confirmed.

B. OBSERVATIONS

1. Mortality and Clinical signs

No mortality occurred during the treatment period and all animals appeared in good general health throughout the treatment period.

One control male was killed in extremis on the last day of the 8-week recovery period (day 56) due to marked clinical signs. A convulsion and stiff gait were noted during the veterinary examination on the previous day (day 55) and signs of general poor condition (increased heart rate, weak pulse, recumbency and dehydration) were present the next morning (day 56).

Loose or watery feces were recorded in all groups with a dose-related increased incidence primarily in the animals of the 100 and 500 ppm treated groups.

The remaining signs, vomiting of mucus or feed, red particles/red discolored feces or red urine, occurred in single animals in all groups including controls and were, therefore, not considered to be related to treatment. In addition, hairless areas, wounds, nodules and thickened/swollen areas noted during the study in individual animals were not treatment-related due to the isolated occurrences in different groups.

External signs of oestrus were evident in all females of all groups throughout the study which is consistent with the age of the animals, and the oestrus cycle was not affected by treatment as there were no differences between control and any treated groups.

2. Detailed clinical (veterinary) observations

There were no differences noted in the color of the teeth between control animals and any animal in the treated groups during the veterinary examinations in the treatment and recovery periods.

There were no findings which were considered to be related to treatment.

C. BODYWEIGHT AND BODYWEIGHT GAINS

Overall lower mean body weight and body weight gain were recorded in the animals treated at 500 ppm when compared with the control group (Table 3). Starting around week 26, mean body weights in males from the 500 ppm dose group were often statistically significantly decreased by 11-18% when compared to the control mean. Mean body weights in the 5, 50 and 100 ppm dose groups were generally lower than controls throughout the study, but were not dose related. In females from the 100 and 500 ppm dose groups, mean body weights were decreased by 6-15% and 8-17% relative to controls, respectively, throughout most of the study period, although statistical significance was not reached. By the end of the recovery period, mean body weights of animals from the 500 ppm dose group were still reduced compared to controls, but to a lesser degree.

Mean body weight gains were statistically significantly reduced in males from the 500 ppm group from day 8 and continuing throughout most of the treatment period. The overall mean body weight gain (as recorded on day 358 and as a percent of initial body weight) of males at 500 ppm was 43% lower than the body weight gain of control males. As with the body weight data, a dose-response relationship was not observed for body weight gains of males from the lower dose groups. Females from the 100 and 500 ppm dose groups exhibited overall body weight gains that were 9% and 38% lower than controls, respectively (not statistically significant).

Table 3. Select mean (± S.D.) body weights (kg)

Day	0 ppm	5 ppm	50 ppm	100 ppm	500 ppm
Male dogs					
Pre-test	8.2 ± 0.9	8.7 ± 0.6	7.9 ± 0.9	8.0 ± 0.8	8.3 ± 0.5
1	8.5 ± 0.8	9.1 ± 0.5	8.2 ± 1.0	8.4 ± 0.8	8.6 ± 0.5
64	10.7 ± 0.6	11.0 ± 0.6	10.4 ± 1.4	10.5 ± 0.8	10.1 ± 0.6
148	11.9 ± 0.8	11.7 ± 0.7	11.1 ± 1.4	11.3 ± 0.7	10.7 ± 0.9* (↓10%)
218	12.6 ± 0.8	12.1 ± 0.7	11.7 ± 1.5	11.8 ± 1.5	11.0 ± 0.9* (↓13%)
302	13.1 ± 0.9	12.2 ± 1.1	11.9 ± 1.4	12.1 ± 1.7	11.3 ± 1.2* (↓14%)
358	13.4 ± 1.0	12.3 ± 1.2	12.1 ± 1.6	12.1 ± 2.1	11.4 ± 1.2* (↓15%)
End of recovery	13.4 ± 1.4	--	--	--	11.9 ± 1.6 (↓11%)
Female dogs					
Pre-test	7.7 ± 0.9	7.4 ± 0.6	7.3 ± 0.7	7.5 ± 0.8	7.5 ± 1.0
1	7.9 ± 0.9	7.7 ± 0.6	7.6 ± 0.8	7.4 ± 1.0	7.7 ± 0.9
64	9.7 ± 1.0	9.6 ± 0.4	9.5 ± 0.8	8.2 ± 1.7 (↓15%)	8.9 ± 0.9 (↓8%)
148	10.7 ± 1.3	10.8 ± 0.7	10.9 ± 1.2	9.7 ± 1.8 (↓9%)	9.7 ± 1.1 (↓9%)
218	10.9 ± 1.4	11.3 ± 1.1	11.0 ± 0.9	9.9 ± 1.9 (↓9%)	9.5 ± 1.1 (↓13%)
302	11.9 ± 1.6	12.2 ± 1.3	11.2 ± 1.8	10.7 ± 2.4 (↓10%)	10.1 ± 1.3 (↓15%)
358	12.0 ± 2.0	12.6 ± 1.5	11.5 ± 1.4	10.9 ± 2.3 (↓9%)	10.1 ± 1.5 (↓16%)
End of recovery	10.9 ± 0.6	--	--	--	10.3 ± 1.6 (↓6%)

N=8 for the 0 and 500 ppm dose groups and N=4 for all other dose groups except for the control group in males at the end of the recovery period where N=3. Data obtained from pages 101-124 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * statistically significantly different from the controls ($p<0.05$). ** statistically significantly different from the controls ($p<0.01$).

D. FOOD CONSUMPTION

A transient slight decrease in mean food consumption was recorded in the first week of treatment (12% compared with pre-test and 6% compared to controls) in the females treated at 500 ppm. Thereafter, slightly lower mean food intake for the high dose group females was recorded sporadically throughout the treatment period, not attaining statistical significance compared to the control group, due to fluctuations in individual animals. Food consumption in the high dose animals was stable or improved during the recovery period. No effect of treatment on food consumption was noted in males.

E. OPHTHALMOSCOPY

There were no ophthalmic findings which were considered to be related to treatment.

F. BLOOD ANALYSIS

1. Hematological findings

Decreases in mean hemoglobin (HGB), mean corpuscular hemoglobin (MCH, males only) and mean corpuscular hemoglobin concentration (MCHC) were recorded in males (Table 4) and females (Table 5) treated at 500 ppm when compared with control and pre-test values. The values were often statistically significant and were observed generally from week 6. Decreases in HGB values attained statistical significance in females at weeks 6 and 13 and in males at weeks 26 and 52. In addition, statistically significant increases in mean hemoglobin concentration distribution width (HDW) and generally statistically significant mean absolute and relative reticulocyte counts (Reti, abs and rel) were evident in weeks 6, 13, 26 and/or 52 in males and females of the high dose group when compared with control values; decreases were also evident when compared to pre-test values. Mean platelet count (Plat) was slightly increased at week 52 in animals of the high dose group attaining statistical significance in the females. Mean platelet levels of males from the 100 ppm dose group appeared elevated over controls at week 52 but were similar to pre-test values. These differences were not apparent at the end of the 8-week recovery period.

Reductions in partial thromboplastin time (PTT) were noted in males from the 100 and 500 ppm dose groups at weeks 6 and 13 when compared to controls, with statistical significance attained for the values at 500 ppm. These changes did not persist to the week 26 or 52 time points. It should be noted that the mean PTT value reported for males from the 500 ppm dose group at week 6 appears to have been calculated using values from 7/8 dogs (the value of 30.3 seconds for animal no. 25 appears to have been excluded from the calculation of this mean). The mean PTT did not appear to be affected in females.

A statistically significant decrease in mean red blood cell count as well as a statistically significant increase in mean corpuscular volume (data not shown) were noted in females from the 500 ppm dose group at week 6 only, these findings are considered to be incidental based on the lack of a dose-response at this time point and the absence of changes compared to controls at other time points. At week 52, a statistically significant increase in mean white blood cell count (data not shown) was observed in females from the 500 ppm dose group, but was also considered incidental due to the lack of a dose response, highly variable data, and the fact that all of the individual values were within the range of historical values (95% limits).

Slight but statistically significant increases in red cell volume distribution width (RDW) were observed in 500 ppm males at weeks 26 and 52 and in 500 ppm females at week 52, as well as in 500 ppm males at the end of the 8-week recovery period.

Table 4: Selected hematology parameters – Male dogs (means)

Dose (ppm)	HGB (mmol/L)	MCH (fmol)	MCHC (mmol/L)	HDW (mmol/L)	Reti abs (G/L)	Reti rel	Plat (G/L)	PTT (sec)	RDW rel
Pre-test									
0	8.2	1.37	20.77	1.01	57	0.010	419	24.2	0.135
5	8.1	1.36	21.03	1.05	59	0.009	324	22.8	0.140
50	8.0	1.37	21.21	1.08	52	0.009	391	21.2	0.136
100	7.8	1.33	20.80	1.05	63	0.011	443	21.8	0.139
500	8.3	1.34	20.94	1.04	65	0.011	384	21.2	0.137
Week 6									
0	8.7	1.37	21.00	1.08	57	0.009	373	22.4	0.140
5	8.8	1.38	21.29	1.13	61	0.009	299	19.4	0.143
50	8.5	1.35	21.03	1.11	51	0.008	339	18.5	0.138
100	8.9	1.35	21.08	1.13	61	0.010	403	17.6 (↓21%)	0.141
500	8.4	1.33	20.18** (↓4%)	1.44** (↑33%)	108** (↑89%)	0.020* (↑122%)	396	17.9* (↓20%)	0.139
Week 13									
0	9.5	1.40	21.38	1.05	58	0.008	348	22.7	0.138
5	10.0	1.42	21.53	1.10	73	0.011	261	18.5	0.139
50	9.5	1.39	21.48	1.07	50	0.007	339	18.2	0.137
100	9.7	1.38	21.35	1.11	60	0.008	380	16.9 (↓26%)	0.138
500	9.1	1.32* (↓6%)	20.23** (↓5%)	1.40** (↑33%)	102** (↑76%)	0.016 (↑100%)	387	17.7* (↓22%)	0.140
Week 26									
0	10.2	1.39	20.76	1.03	67	0.010	346	23.1	0.131
5	10.1	1.41	21.22	1.05	49	0.007	279	22.6	0.129
50	9.7	1.39	21.09	1.05	52	0.007	332	19.6	0.131
100	9.9	1.36	20.71	1.07	74	0.011	423	20.8	0.130
500	9.1* (↓11%)	1.31* (↓6%)	19.78** (↓5%)	1.47** (↑43%)	108* (↑61%)	0.017 (↑70%)	398	23.1	0.138* (↑5%)
Week 52									
0	10.8	1.45	21.76	1.08	60	0.008	342	22.8	0.132
5	10.4	1.45	21.82	1.14	65	0.009	278	21.2	0.133
50	10.5	1.43	21.67	1.13	79	0.012	398	21.0	0.135
100	10.0	1.40	21.55	1.13	62	0.010	451	20.5	0.134
500	9.3** (↓14%)	1.34** (↓8%)	20.47** (↓6%)	1.53** (↑42%)	108* (↑80%)	0.015* (↑87%)	457 (↑34%)	22.0	0.140** (↑6%)
Week 8 of recovery									
0	12.4	1.41	21.00	1.01	58	0.007	257	21.0	0.131
500	10.7 (↓14%)	1.39	21.42	1.13	72	0.010	318	20.3	0.142* (↑8%)

*: significant at 5%; **: significant at 1%. N=8 for the 0 and 500 ppm dose groups at pre-test and during the treatment period; N=4 for the 5, 100 and 50 ppm dose groups at all time points and the 0 and 500 ppm dose groups at end of recovery. Data obtained from pages 151-155 of the study report. Standard deviations were not provided in the report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

Table 5: Selected hematology parameters – Female dogs (means)

Dose (ppm)	Hb (mmol/L)	MCH (fmol)	MCHC (mmol/L)	HDW (mmol/L)	Reti abs (G/L)	Reti rel	Plat (G/L)	PTT (sec)	RDW rel
Pre-test									
0	8.5	1.36	20.81	0.98	64	0.009	382	22.9	0.133
5	8.5	1.35	20.97	1.03	69	0.011	418	22.6	0.136
50	8.2	1.39	21.22	1.11*	76	0.011	377	22.5	0.135
100	9.1	1.39	21.20	1.14**	98	0.016	391	21.3	0.142
500	8.8	1.38	20.81	1.04	90	0.014	439	22.5	0.138
Week 6									
0	9.5	1.37	21.15	1.10	74	0.011	347	17.9	0.141
5	9.5	1.36	21.12	1.11	72	0.011	352	17.3	0.138
50	8.6	1.39	21.11	1.11	49	0.008	361	18.0	0.138
100	9.9	1.40	21.37	1.12	92	0.014	343	17.6	0.141
500	8.4** (↓12%)	1.36	20.10** (↓5%)	1.40** (↑27%)	108 (↑46%)	0.017 (↑54%)	454 (↑31%)	19.2	0.137
Week 13									
0	10.2	1.41	21.55	1.03	58	0.008	324	18.9	0.137
5	10.0	1.38	21.37	1.02	53	0.007	360	18.4	0.136
50	9.9	1.43	21.63	1.04	50	0.008	334	18.8	0.136
100	10.2	1.44	21.61	1.02	63	0.010	350	18.7	0.137
500	9.2* (↓10%)	1.38	20.36** (↓5%)	1.39** (↑35%)	109* (↑88%)	0.016* (↑100%)	422* (↑30%)	20.1	0.138
Week 26									
0	9.7	1.38	20.78	1.08	68	0.010	373	22.2	0.131
5	10.3	1.39	21.33	1.07	67	0.009	382	21.9	0.130
50	9.5	1.42	21.13	1.05	51	0.006	434	23.0	0.131
100	10.5	1.41	20.98	1.05	72	0.010	374	20.6	0.127
500	9.6	1.36	19.76** (↓5%)	1.39** (↑29%)	118** (↑73%)	0.017* (↑70%)	461 (↑24%)	23.2	0.135
Week 52									
0	10.6	1.44	21.72	1.07	57	0.007	387	22.8	0.130
5	10.5	1.43	21.85	1.14	72	0.009	417	22.2	0.135
50	9.9	1.46	21.80	1.13	63	0.009	406	23.4	0.134
100	10.5	1.45	21.73	1.13	78	0.011	398	20.8	0.135
500	9.8	1.40	20.66** (↓5%)	1.41** (↑32%)	110** (↑93%)	0.015* (↑114%)	557* (↑44%)	23.6	0.136** (↑5%)
Week 8 of recovery									
0	10.9	1.41	21.86	1.09	74	0.008	374	20.6	0.141
500	10.5	1.41	21.51	1.10	70	0.009	375	20.0	0.137

*: significant at 5%; **: significant at 1%. N=8 for the 0 and 500 ppm dose groups at pre-test and during the treatment period; N=4 for the 5, 100 and 50 ppm dose groups at all time points and the 0 and 500 ppm dose groups at end of recovery. Data obtained from pages 156-160 of the study report. Standard deviations were not provided in the report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

2. Blood chemistry findings

Statistically significant decreases in mean plasma alanine aminotransferase activity (ALAT) were recorded from week 1 (day 7) to week 8 (day 55) in males (Table 6) and females (Table 7) receiving 50, 100 and 500 ppm when compared with control values; decreases were also evident when compared to pre-test values. A transient recovery was recorded in week 10 (day 69) and week 17 (day 115) in males and females, with decreases recorded (often achieving statistical significance) in weeks 13, 26, 39 and 52. These differences were not evident in week 4 or week 8 of the recovery period. There were no changes recorded in mean plasma aspartate aminotransferase activity (ASAT) during the treatment period.

Statistical analysis revealed mean sorbitol dehydrogenase values (SDH) generally lower throughout the treatment period at 500 ppm, and during recovery week 8 for the females previously treated at 500 ppm when compared with control and pre-test values. The activity appears to be inconsistent within groups and large fluctuations between individual animals, including in the control group, were recorded. Due to the pre-existing (pre-test) slightly reduced SDH in animals at 500 ppm, large individual fluctuations within each group and in the absence of a clear dose-response, this was not considered to be related to treatment.

Slight, occasionally statistically significant, decreases in mean total protein, albumin and/or albumin/globulin (A/G) ratios were recorded in weeks 6, 13, 26 and 52 in male and female treated at 500 ppm when compared with control values; decreases were also evident when compared to pre-test values. These differences were not apparent at the end of the 8-week recovery period.

Mean glucose and bilirubin levels were also reduced in females from the 500 ppm dose group generally throughout the treatment period, and females from the 100 ppm dose level had reduced bilirubin values at the end of the treatment period (week 52) only. These differences were not apparent at the end of the 8-week recovery period.

Table 6: Selected clinical chemistry parameters – Male dogs (means)

Dose (ppm)	ALAT (U/L)	SDH (U/L)	Protein (g/L)	Albumin (g/L)	A / G ratio
Pre-test					
0	40.5	25.3	57.46	30.25	1.20
5	40.0	21.1	59.16	32.14	1.19
50	34.8	24.6	56.67	29.51	1.16
100	37.2	23.1	57.23	31.09	1.23
500	37.5	21.7	56.65	30.67	1.19
Week 1 (day 7)					
0	38.0	24.4			
5	35.5	25.0			
50	20.4** (↓46%)	22.3			
100	17.3**(↓54%)	21.8			
500	15.6** (↓59%)	18.4*			
Week 2 (day 14)					
0	38.3	25.1			
5	33.1	27.2			
50	15.0** (↓61%)	23.8			
100	9.5** (↓75%)	24.7			
500	7.8** (↓80%)	20.6*			
Week 3 (day 22)					
0	48.9	22.9			
5	32.2	24.6			
50	15.7* (↓68%)	17.8			
100	15.9* (↓67%)	23.0			
500	13.0** (↓73%)	12.0**			
Week 4 (day 28)					
0	40.8	24.7			
5	31.8	13.7*			
50	16.9** (↓58%)	18.5			
100	15.8** (↓61%)	21.4			
500	17.1** (↓58%)	16.6			
Week 6					
0	41.2	20.8	57.81	31.39	1.17
5	34.4	19.6	61.36	33.36	1.14
50	16.2** (↓61%)	20.2	57.76	30.81	1.11
100	14.3** (↓65%)	18.4	58.36	31.95	1.23
500	12.3** (↓70%)	18.5	55.19	28.25 (↓10%)	1.05 (↓10%)

Dose (ppm)	ALAT (U/L)	SDH (U/L)	Protein (g/L)	Albumin (g/L)	A / G ratio
Week 8 (day 55)					
0	41.4	21.8			
5	34.0	24.2			
50	11.9** (↓71%)	20.3			
100	10.2** (↓75%)	22.9			
500	8.8** (↓79%)	19.6			
Week 10 (day 69)					
0	42.6	20.7			
5	54.0	16.0			
50	35.7 (↓16%)	18.1			
100	32.8 (↓23%)	15.7			
500	35.0 (↓18%)	15.1			
Week 13					
0	42.6	21.7	59.98	32.45	1.24
5	62.5	17.6	63.06	35.10	1.26
50	16.9 (↓60%)	18.8	58.79	31.46	1.15
100	15.7 (↓63%)	17.6	60.00	32.86	1.22
500	15.6* (↓63%)	17.3	56.75 (↓5%)	28.38* (↓28%)	0.97 (↓22%)
Week 17 (day 115)					
0	49.3	23.5			
5	94.8	19.7			
50	120.5 [27.2] (↓45%)[#]	20.6			
100	27.1 (↓45%)	22.0			
500	23.0 (↓53%)	16.9			
Week 26					
0	48.8	23.5	56.01	34.10	1.57
5	41.3	22.8	56.61	33.72	1.46
50	18.3** (↓62%)	22.1	54.16	32.37	1.48
100	9.9** (↓80%)	20.3	52.31	30.05	1.33
500	10.0** (↓80%)	20.7	51.24 (↓8%)	27.65** (↓19%)	1.12* (↓29%)
Week 39 (day 273)					
0	51.0	30.0			
5	46.6	28.4			
50	19.5** (↓60%)	21.7			
100	15.2** (↓70%)	21.1			
500	15.1** (↓70%)	17.7*			

Dose (ppm)	ALAT (U/L)	SDH (U/L)	Protein (g/L)	Albumin (g/L)	A / G ratio
Week 52					
0	49.2	16.6	62.93	34.84	1.25
5	101.0	12.7	62.95	33.95	1.22
50	12.7 (↓74%)	9.4	62.29	31.82	1.10
100	9.5 (↓81%)	12.0	59.10	29.80	1.14
500	10.5 (↓79%)	10.3	55.29* (↓12%)_	26.52** (↓24%)	0.93* (↓26%)
Week 4 of recovery					
0	46.6	21.1			
500	45.7	22.9			
Week 8 of recovery					
0	78.2	6.6	62.71	35.55	1.30
500	51.3	6.9	59.05	32.39	1.26

*: significant at 5%; **: significant at 1%; # The value in square brackets represents the mean re-calculated after excluding the value for one animal (400 U/L) and the value in parentheses represent the percent change from the control mean for the re-calculated mean. N=8 for the 0 and 500 ppm dose groups at pre-test and during the treatment period; N=4 for the 5, 100 and 50 ppm dose groups at all time points and the 0 and 500 ppm dose groups during the recovery period. Data obtained from pages 167-173 of the study report. Standard deviations were not provided in the report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

Table 7: Selected clinical chemistry parameters – Female dogs (means)

Dose level (ppm)	ALAT (U/L)	SDH (U/L)	Protein (g/L)	Albumin (g/L)	A / G ratio	Glucose (mmol/L)	Bilirubin (µmol/L)
Pre-test							
0	35.4	18.2	57.79	33.26	1.40	4.92	2.00
5	38.7	12.8	54.46	31.43	1.34	5.31	1.45
50	36.2	14.4	57.41	32.36	1.28	5.33	1.70
100	40.3	11.3	57.03	32.31	1.30	5.52	1.80
500	32.3	15.0	57.39	32.93	1.37	4.89	1.70
Week 1 (day 7)							
0	33.9	21.7					
5	35.4	17.4					
50	21.0** (↓38%)	16.1					
100	19.1** (↓44%)	17.2					
500	15.9** (↓53%)	11.1					
Week 2 (day 14)							
0	36.3	23.4					
5	32.5	20.8					
50	15.9** (↓56%)	20.6					

Dose level (ppm)	ALAT (U/L)	SDH (U/L)	Protein (g/L)	Albumin (g/L)	A / G ratio	Glucose (mmol/L)	Bilirubin (µmol/L)
100	12.8** (↓65%)	21.5					
500	4.5** (↓88%)	17.5					
Week 3 (day 22)							
0	39.1	22.1					
5	30.9	21.7					
50	15.3** (↓61%)	18.6					
100	18.0** (↓54%)	17.4					
500	17.5** (↓55%)	17.1					
Week 4 (day 28)							
0	37.8	20.8					
5	33.5	17.8					
50	18.9** (↓50%)	15.0					
100	20.5** (↓46%)	15.1					
500	17.8** (↓53%)	12.4					
Week 6							
0	36.3	19.9	59.80	34.65	1.40	5.30	1.90
5	38.8	19.1	57.43	33.67	1.40	5.41	1.70
50	15.6** (↓57%)	13.9*	56.40	32.33	1.35	5.49	1.95
100	14.7** (↓59%)	15.1	57.07	32.72	1.33	5.86	2.00
500	11.7** (↓68%)	15.6*	55.53* (↓7%)	30.09** (↓13%)	1.21* (↓14%)	5.00 (↓6%)	1.90
Week 8 (day 55)							
0	37.3	20.7					
5	32.2	23.9					
50	16.3** (↓56%)	18.0					
100	13.6** (↓63%)	18.7					
500	7.3**(↓80%)	18.7					
Week 10 (day 69)							
0	38.2	18.3					
5	38.9	18.1					
50	33.1 (↓13%)	14.1					

Dose level (ppm)	ALAT (U/L)	SDH (U/L)	Protein (g/L)	Albumin (g/L)	A / G ratio	Glucose (mmol/L)	Bilirubin (µmol/L)
100	35.0 (↓8%)	16.4					
500	29.1 (↓24%)	14.9					
Week 13							
0	33.4	20.4	60.75	35.29	1.39	5.14	2.10
5	29.9	21.3	59.06	34.56	1.37	5.46	1.85
50	18.4** (↓45%)	15.4	58.86	33.63	1.33	5.47	1.85
100	15.1** (↓55%)	20.3	59.87	33.07	1.24	5.40	1.90
500	14.6** (↓56%)	21.1	59.62	31.63 (↓10%)	1.14* (↓18%)	4.85 (↓6%)	1.75 (↓17%)
Week 17 (day 115)							
0	36.8	22.5					
5	33.4	24.5					
50	38.0	19.7					
100	21.3 (↓42%)	25.4					
500	20.5* (↓44%)	17.5					
Week 26							
0	37.2	24.6	56.54	35.40	1.68	5.42	1.90
5	37.1	22.7	55.56	34.51	1.65	5.43	1.70
50	16.1** (↓57%)	18.4	53.55	32.75	1.64	5.44	1.75
100	16.9** (↓55%)	19.3	52.71	32.88	1.72	5.42	1.35
500	15.5** (↓58%)	19.5	52.56 (↓7%)	31.06* (↓12%)	1.42 (↓15%)	4.74* (↓12%)	1.70
Week 39 (day 273)							
0	36.2	25.5					
5	36.1	27.2					
50	19.2** (↓47%)	20.3					
100	14.7** (↓59%)	25.4					
500	10.8** (↓70%)	16.6					
Week 52							
0	33.0	9.0	63.48	36.72	1.41	5.39	1.85
5	36.9	3.8	61.31	35.85	1.43	5.72	1.89
50	17.8** (↓46%)	6.4	58.92	31.45	1.27	5.56	1.73
100	18.9** (↓43%)	7.5	57.89	32.51	1.30	5.64	1.48* (↓20%)

Dose level (ppm)	ALAT (U/L)	SDH (U/L)	Protein (g/L)	Albumin (g/L)	A / G ratio	Glucose (mmol/L)	Bilirubin (µmol/L)
500	12.4** (↓62%)	10.4	56.17** (↓11%)	29.69* (↓19%)	1.16 (↓18%)	4.80 (↓11%)	1.47 (↓20%)
Week 4 of recovery							
0	37.9	17.2					
500	40.2	15.7					
Week 8 of recovery							
0	40.5	9.3	64.91	36.95	1.36	5.51	1.93
500	40.7	2.9*	59.77	34.08	1.28	5.75	2.24

*: significant at 5%; **: significant at 1%. N=8 for the 0 and 500 ppm dose groups at pre-test and during the treatment period; N=4 for the 5, 100 and 50 ppm dose groups at all time points and the 0 and 500 ppm dose groups during the recovery period. Data obtained from pages 174-180 of the study report. Standard deviations were not provided in the report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

3. Thyroid hormone analysis

No differences concerning TSH, Total T3 and Total T4 levels in dog serum were observed in any treated groups at pre-test, after 52 weeks of treatment and after 8 weeks of recovery when compared with the control values.

G. URINALYSIS

There were no changes in urinalysis which were considered to be related to treatment.

H. SACRIFICE AND PATHOLOGY

1. Organ Weights

Slightly increased mean liver weight and statistically significant liver to body weight ratios were recorded at the end of treatment in females treated at 500 ppm. A slight increase in relative liver weight (not statistically significant) was also noted in males at this dose level, and in females at 100 ppm.

A dose-related increase in the weight of the left thyroid gland was noted in males at 50 ppm and above, but a similar trend was not observed for the weight of the right thyroid gland in males, nor was any effect noted in the thyroid gland of females. In the absence of any correlating pathological findings, these changes in thyroid gland weight are not considered to be related to treatment.

Absolute thymus weights were reduced in males from all dose groups and in females at the 100 and 500 ppm dose levels. In the 90-day dietary study in dogs, reduced thymus weights were noted in females (18 mg/kg bw/day), which were attributed to a secondary effect of reduced body weights. No effect on thymus weight or body weight was noted in males in that study. In the current study, the dose response in males is somewhat flat, and body weight was reduced in males at the high dose only, while females showed body weight reductions at the 100 and 500 ppm dose levels that were similar in magnitude to the reductions in thymus weight. No corresponding pathological findings were noted in the thymus for either sex. Therefore, the only change in thymus weight considered to

be treatment related are the reductions in absolute weight in females at 100 and 500 ppm, although these reductions are considered to be secondary to the reduced body weight in these animals.

Slightly increased absolute and/or relative kidney weights were recorded in the males and unilaterally in the females treated at 500 ppm at the end of treatment, with statistical significance attained for relative kidney weight in males only. Increases in absolute and relative adrenal gland weight were noted in males at all dose levels, but the dose response was fairly flat for absolute weights at all doses, and for relative weights until the high dose level, at which statistical significance was attained for the right adrenal gland. This increase in relative adrenal gland weight is considered to be reflective of differences in terminal body weight.

In females from the 500 ppm dose group, absolute and relative spleen weights were reduced at the end of treatment relative to controls and were considered treatment-related, despite the lack of statistical significance.

None of the organ weight changes were evident at the end of the recovery period.

Table 8: Selected organ weights - Male dogs (Mean ± SD) (*recovery data in italics*[+])

Organ	0 ppm	5 ppm	50 ppm	100 ppm	500 ppm
Terminal bw (g)	13,519 ± 845 *13,373 ± 1410*	12,074 ± 1051	11,810 ± 1535	12,046 ± 1758	10,976 ± 915* (↓19%) *11,869 ± 1592 (↓11%)*
Liver - Abs. (g)	379.9 ± 69.6 *326.7 ± 71.0*	322.0 ± 28.1	315.1 ± 25.9	371.9 ± 73.2	351.1 ± 92.6 *330.6 ± 77.7*
Rel. to bw (%)	2.8 ± 0.4 *2.5 ± 0.5*	2.7 ± 0.3	2.7 ± 0.3	3.1 ± 0.4	3.2 ± 0.7 (↑14%) *2.8 ± 0.3*
Thyroid L - Abs. (g)	0.396 ± 0.086 *0.580 ± 0.102*	0.366 ± 0.030	0.460 ± 0.161 (↑16%)	0.475 ± 0.100 (↑20%)	0.476 ± 0.122 (↑20%) *0.520 ± 0.156*
Rel. to bw (%)	0.0029 ± 0.0005 *0.0044 ± 0.0009*	0.0031 ± 0.0002	0.0039 ± 0.0010 (↑34%)	0.0040 ± 0.0007 (↑38%)	0.0044 ± 0.0012 (↑52%) *0.0044 ± 0.0007*
Thyroid R – Abs. (g)	0.432 ± 0.096 *0.441 ± 0.030*	0.398 ± 0.077	0.444 ± 0.119	0.476 ± 0.086 (↑10%)	0.466 ± 0.160 (↑8%) *0.533 0.254 (↑21%)*
Rel. to bw (%)	0.0032 ± 0.0006 *0.0033 ± 0.0004*	0.0033 ± 0.0008	0.0037 ± 0.0006	0.0040 ± 0.0008	0.0043 ± 0.0015 (↑34%) *0.0044 ± 0.0015 (↑33%)*
Thymus – Abs. (g)	6.66 ± 0.68 *8.77 1.16*	5.80 ± 1.91 (↓13%)	4.81 ± 2.65 (↓28%)	4.87 ± 1.00 (↓27%)	4.58 ± 1.24 (↓31%) *8.68 2.89*
Rel. to bw (%)	0.05 ± 0.01 *0.07 ± 0.01*	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.01 *0.08 ± 0.02*

Kidney R – Abs. (g)	29.97 ± 3.03 *28.40 ± 1.38*	26.31 ± 2.12	25.89 ± 2.42	32.05 ± 5.74	36.42 ± 7.59 (↑21%) *27.59 ± 3.37*
Rel. to bw (%)	0.22 ± 0.02 *0.21 ± 0.02*	0.22 ± 0.02	0.22 ± 0.02	0.27 ± 0.02	0.34 ± 0.09** (↑54%) *0.24 ± 0.02*
Kidney L – Abs. (g)	30.38 ± 3.22 *27.43 ± 1.10*	26.32 ± 1.85	27.17 ± 2.23	31.15 ± 5.69	37.19 ± 8.93 (↑22%) *27.98 ± 4.18*
Rel. to bw (%)	0.23 ± 0.02 *0.21 ± 0.02*	0.22 ± 0.01	0.23 ± 0.02	0.26 ± 0.02	0.34 ± 0.10* (↑48%) *0.23 ± 0.02*
Adrenal R – Abs. (g)	0.647 ± 0.068 *0.853 ± 0.142*	0.768 ± 0.074 (↑19%)	0.743 ± 0.129 (↑15%)	0.821 ± 0.079 (↑27%)	0.848 ± 0.189 (↑31%) *0.774 ± 0.218*
Rel. to bw (%)	0.0048 ± 0.0003 *0.0064 ± 0.0008*	0.0064 ± 0.0010 (↑33%)	0.0063 ± 0.0009 (↑31%)	0.0070 ± 0.0018 (↑46%)	0.0078 ± 0.0019*(↑62%) *0.0066 ± 0.0017*
Adrenal L – Abs. (g)	0.737 ± 0.028 *0.808 ± 0.197*	0.776 ± 0.036	0.754 ± 0.099	0.819 ± 0.103 (↑11%)	0.872 ± 0.207 (↑18%) *0.735 0.116*
Rel. to bw (%)	0.0055 ± 0.0003 *0.0060 ± 0.0009*	0.0065 ± 0.0007 (↑18%)	0.0065 ± 0.0010 (↑18%)	0.0070 ± 0.0018 (↑27%)	0.0080 ± 0.0021 (↑45%) *0.0063 ± 0.0013*

[+]: data from 3 animals for the control group; R: right; L: left; Abs. = absolute; Rel. = relative. * and **: Dunnett-test based on pooled variance significant at 5% and 1%, respectively. Data obtained from pages 188-238 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

Table 9: Selected organ weights - Female dogs (Mean ± SD) (*recovery data in italics)*

Organ	0 ppm	5 ppm	50 ppm	100 ppm	500 ppm
Terminal bw (g)	12,558 ± 2323 *10,889 ± 565*	12,243 ± 1701	11,294 ± 1255	10,713 ± 2342 (↓15%)	9931 ± 1683 (↓21%) *10,319 ± 1636*
Liver – Abs (g)	316.9 ± 43.7 *326.3 ± 30.6*	313.7 ± 88.5	277.8 ± 24.0	315.7 ± 25.2	365.8 ± 108.8 (↑15%) *295.9 ± 69.3*
Rel. to bw (%)	2.5 ± 0.2 *3.0 ± 0.4*	2.5 ± 0.4	2.5 ± 0.3	3.1 ± 0.6 (↑24%)	3.7 ± 0.7* (↑48%) *2.9 ± 0.3*
Thymus – Abs. (g)	5.41 ± 1.74 *5.64 ± 1.63*	5.99 ± 3.27	5.58 ± 1.30	4.61 ± 1.25 (↓15%)	4.41 ± 0.75 (↓18%) *8.99 ± 4.46*
Rel. to bw (%)	0.04 ± 0.01 *0.05 ± 0.02*	0.05 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	0.05 ± 0.01 *0.09 ± 0.05*
Kidney R – Abs. (g)	26.45 ± 9.41 *22.47 ± 1.26*	23.69 ± 1.58	22.46 ± 3.00	24.64 ± 2.99	25.03 ± 3.18 *23.09 ± 1.90*
Rel. to bw (%)	0.21 ± 0.06 *0.21 ± 0.02*	0.20 ± 0.02	0.20 ± 0.02	0.24 ± 0.05 (↑14%)	0.26 ± 0.06 (↑24%) *0.23 ± 0.03*
Kidney L – Abs. (g)	22.66 ± 5.06 *23.24 ± 1.00*	23.48 ± 2.02	23.74 ± 2.92	25.07 ± 2.62	25.01 ± 2.37 *22.84 ± 2.48*

Rel. to bw (%)	0.19 ± 0.02 *0.22 ± 0.02*	0.20 ± 0.01	0.21 ± 0.02	0.24 ± 0.04 (↑26%)	0.26 ± 0.06 (↑37%) *0.22 ± 0.03*
Spleen – Abs. (g)	76.74 ± 39.04 *76.69 ± 23.15*	82.11 ± 33.08	104.60 ± 35.10	71.04 ± 48.87	38.63 ± 10.37 (↓50%) *86.97 ± 47.38*
Rel. to bw (%)	0.61 ± 0.26 *0.71 ± 0.24*	0.70 ± 0.31	0.92 ± 0.24	0.63 ± 0.38	0.39 ± 0.11 (↓36%) *0.88 ± 0.50*

R: right; L: left; Abs. = absolute; Rel. = relative. * and **: Dunnett-test based on pooled variance significant at 5% and 1%, respectively. Data obtained from pages 188-238 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

2. Gross Necropsy and Histopathology

There were no macroscopic findings considered to be related to treatment. A number of organs were discolored dark red, including the intestinal tract mucosa in the control animal which was killed in extremis.

Minimal to slight degrees of diffuse hepatocellular hypertrophy, as well as brownish pigment in sinusoidal cells, were recorded in animals treated at 500 ppm (Table 10). At the end of the recovery period, there was still minimal hepatocellular hypertrophy in one female and minimal brownish pigment in almost all high dose group animals (3 males and 4 females). Congestion was present in the mediastinal lymph node of three males from the 500 ppm dose group. This tissue was only examined in these animals and not in males from lower dose groups or in recovery group animals.

Table 10. Summary of histopathological lesions

Finding	Severity	0 ppm	5 ppm	50 ppm	100 ppm	500 ppm
Males – Main Groups						
Liver - Hepatocellular hypertrophy	Minimal Slight Total	0/4 0/4 0/4	0/4 0/4 0/4	0/4 0/4 0/4	0/4 0/4 0/4	1/4 2/4 3/4
Liver – Brownish pigment	Minimal Slight Total	0/4 0/4 0/4	0/4 0/4 0/4	0/4 0/4 0/4	1/4 0/4 1/4	2/4 2/4 4/4
Mediastinal lymph node – congestion	Present					3/4
Males – Recovery Groups						
Liver - Hepatocellular hypertrophy	Total	0/4				0/4
Liver – Brownish pigment	Minimal Total	0/4 0/4	--	--	--	3/4 3/4
Females – Main Groups						
Liver - Hepatocellular hypertrophy	Minimal Total	0/4 0/4	0/4 0/4	0/4 0/4	0/4 0/4	2/4 2/4
Liver – Brownish pigment	Minimal Total	1/4 1/4	1/4 1/4	0/4 0/4	0/4 0/4	3/4 3/4
Mediastinal lymph node – congestion	Present				1/4	1/4
Females – Recovery Groups						
Liver - Hepatocellular hypertrophy	Minimal Total	0/4 0/4				1/4 1/4
Liver – Brownish pigment	Minimal Total	0/4 0/4				4/4 4/4

I. ADDITIONAL INVESTIGATIONS

1. Fluoride Determination

A dose-related increase of fluoride content was recorded in the bone and teeth of animals dosed at 50, 100 and 500 ppm (Table 11). The mean values were statistically significant at the high dose only when compared with control values, while no significance was attained in animals treated at 50 or 100 ppm. The statistically significant increase in fluoride content in the bone and teeth of the high dose group was still evident at the end of the 8-week recovery period. There were no differences between the control and low dose (5 ppm).

Table 11: (Mean (ppm) ± Relative S.D. (%) fluoride content

Parameter	0 ppm		5 ppm		50 ppm		100 ppm		500 ppm	
	M	F	M	F	M	F	M	F	M	F
52 weeks										
Bones	536.9 ± 15.7	660.4 ± 22.8	587.8 ± 16.1	589.1 ± 8.9	767.5 ± 12.3 (↑43%)	911.2 ± 13.5 (↑38%)	897.2 ± 6.8 (↑67%)	953.5 ± 17.4 (↑44%)	2079 ± 22.0* (↑287%)	2166 ± 20.0* (↑228%)
Teeth	417.4 ± 29.7	529.7 ± 31.2	455.1 ± 19.6	489.6 ± 14.7	555.5 ± 7.8 (↑33%)	791.2 ± 18.7 (↑49%)	705.0 ± 21.4 (↑69%)	682.4 ± 23.4 (↑29%)	984.3 ± 22.3* (↑136%)	1232 ± 52.1* (↑132%)
Recovery week 8										
Bones	605.7 ± 26.0	644.4 ± 14.0							2216 ± 14.5* (↑266%)	2661 ± 2.3* (↑313%)
Teeth	317.0 ± 29.0	437.5 ± 40.2							540.9 ± 31.1 (↑71%)	1409 ± 65.5 (↑222%)

*: significantly different from control (Dunnett's t-test) at 5%. Data obtained from pages 1159-1162 of the study report. Values in parentheses represent the percent change from controls (calculated by the reviewer).

2. Liver Enzyme Determinations

Fluensulfone caused a statistically significant inhibition of hepatic ALAT activity in males and females after 52 weeks of treatment at 50 ppm and above (Table 12). In recovery animals previously treated at 500 ppm however, no effect on hepatic ALAT activity was observed in either sex, demonstrating that this effect was reversible. The effects on hepatic ALAT are not considered to be adverse because there was no associated liver toxicity, no effect on the overall health of the animals after one year of treatment and the changes were fully reversible.

The phase II enzyme glutathione S-transferase was induced in both sexes treated at 500 ppm. In female recovery animals, however, no effect on glutathione S-transferase activity was observed while in male recovery animals glutathione S-transferase activity was still increased but without attaining statistical significance. Microsomal epoxide hydrolase, another phase II enzyme, was significantly induced at 500 ppm. The mean microsomal 7-ethoxyresorufin o-deethylase (EROD) activity was reduced in both males and females from the 500 ppm dose group; decreases in this parameter are not considered toxicologically relevant.

Table 12: Mean ± S.D. selected liver enzyme activities (% of control)

Parameter	0 ppm		5 ppm		50 ppm		100 ppm		500 ppm	
	M	F	M	F	M	F	M	F	M	F
52 weeks										
EROD (nmol/min/g liver)	0.71 ± 0.44	0.71 ± 0.13	0.95 ± 0.53 (133%)	0.49 ± 0.18 (71%)	0.86 ± 0.35 (120%)	0.95 ± 0.33 (134%)	0.85 ± 0.17 (118%)	0.88 ± 0.64 (124%)	0.46 ± 0.15 (64%)	0.45 ± 0.19 (64%)
ALAT (U/g liver)	189 ± 27.1	166 ± 41.8	180 ± 42.2 (95%)	158 ± 38.8 (96%)	79.7 ± 15.3 (42%)**	94.4 ± 32.1 (57%)*	63.3 ± 8.78 (34%)**	49.9 ± 11.1 (30%)**	30.7 ± 6.78 (16%)**	29.1 ± 4.87 (18%)**
GST (nmol/min/g liver)	40.5 ± 13.2	45.3 ± 37.6	23.3 ± 17.0 (58%)	60.3 ± 28.4 (133%)	40.7 ± 41.3 (100%)	55.2 ± 43.0 (122%)	52.5 ± 24.2 (130%)	31.5 ± 10.3 (69%)	76.9 ± 18.9 (190%)	81.3 ± 21.7 (179%)
mEH (nmol/min/g liver)	321 ± 25.9	243 ± 39.8	253 ± 41.8 (79%)	246 ± 82.7 (101%)	266 ± 29.8 (83%)	293 ± 58.4 (121%)	271 ± 45.3 (85%)	265 ± 53.8 (109%)	495 ± 122 (154%)**	377 ± 57.4 (155%)*
Recovery week 8										
ALAT (U/g liver)	150 ± 33.1	128 ± 28.2							119 ± 19.1 (79%)	125 ± 32.7 (98%)
GST (nmol/min/g liver)	15.7 ± 5.27	26.8 ± 18.4							32.5 ± 18.7 (208%)	31.5 ± 11.4 (118%)

ALAT: alanine aminotransferase in 100g supernatants; GST: cytosolic glutathione S-transferase activity; mEH: microsomal epoxide hydrolase activity; * and **: significantly different from control (Dunnett's t-test) at 5% and 1%, respectively. Data obtained from pages 1184-1203 of the study report.

III. CONCLUSIONS

Based on decreased body weight and body weight gains, hematological and organ weight effects at 500 ppm in the diet, the NOAEL was considered to be 100 ppm (3.1 mg/kg bw/day in males and 3.3 mg/kg bw/day in females).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: In this study, no treatment-related effects were noted at the lowest dose tested of 5 ppm. At 50 ppm and above, effects included reduced serum and hepatic ALAT activity, and increased fluoride content in bones and teeth. These findings are not considered to be adverse in the absence of other indications of hepatic toxicity or skeletal or dental effects. At 100 ppm, males exhibited a slightly reduced partial thromboplastin time, which in isolation is not considered to be adverse.

Females from the 100 ppm dose level exhibited reduced body weights in the range of 6 to 15% compared to controls, reduced body weight gain, slight reductions in bilirubin, and changes in organ weight resulting from the reduced growth of these animals. The decreased body weight noted in females at 100 ppm was only greater than 10% on day 64 and this finding was not consistent with the rest of the findings at this dose level. Therefore, decreased body weight in females at 100 ppm was not considered adverse. Similarly, the overall reduction in body weight gain was 9% relative to controls at 100 ppm, versus 38% at the next highest dose of 500 ppm. Overall, the effects noted in females at 100 ppm are not considered to reflect toxicologically significant findings. The effects noted at 500 ppm are more substantial and reflective of adversity.

This study in dogs is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for a 1-year oral toxicity study in dogs (OPPTS 870.4100; OECD 452).

C. Conclusions: The NOAEL is 100 ppm (3.1/3.3 mg/kg bw/day, M/F) based on reductions in body weights, changes in hematological and clinical chemistry parameters, and organ weights in both sexes at the LOAEL of 500 ppm (16.0/16.2 mg/kg bw/day, M/F).

D. Deficiencies: Volume was not assessed during urinalysis. The nose and peripheral nerve were not collected upon sacrifice. These omissions do not alter the conclusions or acceptability of the study. No other deficiencies were noted.

Tier II Summary (PMRA # 2181217, MRID 48574765) revised by the US EPA, March 14, 2014

Note that the regulatory authorities have set different NOAELs/LOAELs based on different interpretation of body weight findings in males and chronic interstitial inflammation in females (see section IV – Evaluation, Summary and Conclusions)

Study Type: Chronic Toxicity/Oncogenicity Study - Rat

IIA 5.5.1 and 5.5.2 – MRID 48574765, PMRA 2181217

Report:	Kaiser St. (2011) MCW-2 TECH: 104-Weeks Combined Chronic Toxicity and Oncogenicity (Feeding) Study in the Rat. Harlan Laboratories Ltd., Switzerland; unpublished report No. B80188, dated 19 July 2011. Sponsor reference No. R-23353 Dates of experimental work: 20 February 2008 to 25 February 2010 (allocation A); 20 February 2008 to 22 February 2009 (allocation B)
Sponsor/Submitter:	Makhteshim Chemical Works Ltd, Israel.
Guidelines	OECD No. 453, EEC Method B.32, and OPPTS 870.4300 Deviations: none
GLP/ Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In an oral combined chronic toxicity/oncogenicity study in rats (MRID 48574765; PMRA 2181217), 4 groups of HANRcc Wist(SPF) rats (allocation A, oncogenicity animals), 50 males and 50 females each, were treated for 104 weeks with 0, 30, 200 or 1200 ppm (0/0, 1.4/1.7, 9.6/11.6, and 57.7/69.3 mg/kg bw/day in males/females) of fluensulfone (MCW-2 tech., 96.7%, Batch No. NLL6692-3) in their diets. A satellite group (allocation B, chronic toxicity animals) of 20 males and 20 females per group was similarly treated for 52 weeks at the same dose levels (0/0, 1.6/1.9, 11.0/13.1, 66.3/75.2 mg/kg bw/day in males/females).

Usual investigations were conducted on all animals (viability/mortality, clinical signs, food/water consumption, body weights, ophthalmoscopic, hematology, clinical chemistry, urinalysis, etc.). In addition, a functional observational battery (FOB) and locomotor activity assessments were performed on allocation B animals at week 52. In 5 allocation B animals per group, a part of the liver was used for enzyme activity determinations. After 52 and 104 weeks,

the right femur and the incisors of the maxilla of ten animals per sex and group were also taken for fluoride determination.

Treatment of Wistar rats with fluensulfone at dietary concentrations of 30, 200, 1200 ppm for up to 104 weeks had no effects on survival, clinical signs or ophthalmoscopic findings. Grip strength and the results of the functional observational battery were also not affected by treatment for 52 consecutive weeks. A slight decrease in overall locomotor activity was noted in high dose males. No increase in palpable nodules or masses was observed at any dose level at either the interim and terminal sacrifices, and no increase in any type of neoplastic finding was recorded at terminal sacrifice.

Food consumption in males treated with 1200 ppm was slightly lower during the first 4 weeks of treatment, but no further effects on food consumption were recorded thereafter. Water consumption was also increased in high dose males and females. Absolute mean body weight and body weight gain were decreased by the treatment in a dose-dependent manner in males treated at 200 and 1200 ppm; however, the changes at 200 ppm were marginal (5-7%), although statistically significant for some weeks. At 1200 ppm, body weight and body weight gain were decreased by 11% and 15%, respectively, at study termination. In high dose females, body weight was decreased during the last 11 weeks of the study (decreased by 6% at study termination) and body weight gain was reduced from week 2 onwards (decreased by 13%).

After 52 weeks a number of changes in hematology and biochemistry parameters were noted, mainly in animals treated with 1200 ppm. At 200 ppm and above, slightly decreased prothrombin time (5%) was noted in males. Dose-dependent decreases in neutrophil and white blood cell counts were observed in all female dose groups. Clinical chemistry findings in the low and mid dose groups included changes in ALAT, sodium, chloride, calcium, phosphorus, total protein and globulin, in males and/or females. Clinical chemistry and organ weight data revealed some effects on the liver, limited to the 1200 ppm group. In the absence of correlating histopathological findings these changes were considered to be non-adverse metabolic adaptations. Other organ weight changes noted in high dose groups included increased kidney weights in males and females, and increased adrenal weights in males. No effects on adrenals were noted at histopathology. During the urinalysis, increased ketones were observed in 1200 ppm males and females, and increased leukocytes in high dose males.

After 104 weeks a few changes in hematology parameters, mainly in red blood cell parameters, were noted in animals treated with 1200 ppm. Decreased hemoglobin concentration distribution width (HDW) was noted in females at 200 and 1200 ppm. Decreased mean corpuscular hemoglobin (MCH) was observed in high dose males, and decreases in mean corpuscular hemoglobin concentration (MCHC) were noted in all males at 78 weeks, as well as in 1200 ppm males and 200 and 1200 ppm females at 104 weeks. Neutrophil counts were also increased in high dose females, and eosinophil (EOS) levels were increased in high dose males.

At necropsy after 52 weeks, no gross lesions that could be attributed to treatment with fluensulfone were observed. Microscopically, hyperkeratosis of the esophagus of minimal severity was observed in animals of both sexes treated with 1200 ppm, but no abnormality was noted in lungs.

At necropsy after 104 weeks, an increased number of foci in the lungs was recorded in the treated animals, compared to controls, attaining statistical significance only in males at 1200 ppm. Enlarged livers were recorded in 4 females (versus one in the control group), as well as cysts in the livers of 8 females (versus 2 in the control group) and foci in the liver of 4 females (versus 0 in the control group) treated at 1200 ppm. In males, a slight increase in foci in the prostate was noted in mid and high dose groups (5 in each treatment group versus one in the control group). No correlative microscopic observations were noted in the prostate. Microscopically, an increased incidence of hyperkeratosis in the esophagus was recorded in animals of both sexes treated at 200 and 1200 ppm. Since the severity grade was minimal in most cases, the hyperkeratosis was considered to be of no toxicological significance, and possibly from a slight irritating effect of fluensulfone. The incidence of chronic interstitial inflammation in the lungs was increased in males and females treated at 1200 ppm and in females treated at 200 ppm but was only statistically significant for males at 1200 ppm. This lesion was characterized by focal/multifocal changes consisting of interstitial or intra-alveolar inflammatory cells associated with hypertrophied reactive type II pneumocytes, associated with the presence of foamy intra-alveolar macrophages, which was also increased in mean severity grade. There were no treatment-related hyperplastic changes or tumors recorded. At 104 weeks, slight increases were observed in chronic nephropathy, tubular basophilia and mononuclear foci in the kidneys and mononuclear infiltrates in the pharynx of females treated at 1200 ppm as compared to controls.

Investigations of liver enzymes from liver tissues demonstrated that the phase II enzymes uridine diphosphoglucuronosyl-transferase (UDPGT) and glutathione S-transferase (GST) were induced in 200 and 1200 ppm females and in 1200 ppm males, and epoxide hydrolase (mEH) was induced in all male and female dose groups. These enzymes were more significantly induced in females than males.

After 52 weeks of treatment, fluoride measurements revealed a marked dose-dependent increase, several fold above controls, of the fluoride content in the ashes from bones and teeth at 200 and 1200 ppm. Only very slight increases in fluoride content in bones (up to 28% above controls) were noted in 30 ppm animals. No increase of the fluoride content in the ashes from teeth was recorded in animals treated with 30 ppm.

After 104 weeks of treatment, the fluoride content in the ashes from bones and teeth of all treated animals was markedly increased. At 30 ppm, significant increases of 47-81% above controls were seen in bones and non-statistically significant increases of 20-66% were seen in teeth. Changes in fluoride levels were not considered adverse as there were no associated structural signs of dental fluorosis (discoloration of the teeth) or skeletal fluorosis (mobility problems, changes in external appearance of bones, changes in bone histopathology) at any dose.

The NOAEL was established at 200 ppm (9.6/11.6 mg/kg/day, M/F) based on decreased body weight in males, hematology and clinical chemistry findings in both sexes, histopathological changes in the lungs of males, and histopathological changes in the esophagus of both sexes seen at the LOAEL of 1200 ppm (57.7/69.9 mg/kg/day, M/F).

Fluensulfone showed no carcinogenic potential in rats. Dosing was considered adequate in both sexes based on the findings of decreased body weight and weight gain, slight hematological effects and microscopic findings in the lung and esophagus.

This study is classified **acceptable/guideline (fully reliable)** and satisfies the guideline requirement (OPPTS 870.4300; OECD 453) for a combined oral chronic toxicity/carcinogenicity study in the rat.

I. MATERIAL AND METHODS

1. Test Material	MCW-2 TECH (fluensulfone)
Description:	Solid, yellow
Lot/Batch:	36372130-291-PF1
Purity:	96.7%
CAS#:	318290-98-1
Stability:	Stable until 31 May 2010
2. Vehicle	Provimi Kliba Nafag 3433 rodent maintenance diet (Provimi Kliba AG, Switzerland)
3. Test Animals	
Species	Rat
Strain	HanRcc: WIST(SPF) – males and females
Age	5 weeks at delivery; 6-7 weeks old at start of dosing
Weight	At acclimatization: Males: 83.4 g to 127.0 g (mean 103.0 g); Females: 71.9 g to 113.7 g (mean 94.4 g)
Source	Harlan Laboratories Ltd., Switzerland
Acclimation period	9 days
Diet	Pelleted standard Provimi Kliba Nafag 3433 rodent maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum*
Water	Community tap water from Itingen *ad libitum*
Housing	In groups of 5 in Makrolon type-4 cages with wire mesh tops and sterilized standard softwood bedding (‘Lignocel’ Schill AG, Switzerland).
4. Environmental conditions	
Temperature	22 ± 3°C. Values outside of this range occasionally occurred, usually following room cleaning, and were considered not to have any influence on the study.
Humidity	30-70%. Values outside of this range occasionally occurred, usually following room cleaning, and were considered not to have any influence on the study.
Air change	10-15 air changes per hour
Photoperiod	Light cycle of 12 hours light and 12 hours dark, music during the daytime light period.

B. STUDY DESIGN

1. In-life dates 20 February 2008 to 25 February 2010 (allocation A); 20 February 2008 to 22 February 2009 (allocation B).

2. Animal Assignment and treatment

The animals were assigned to groups using a computer-generated random algorithm. Animals in "Allocation A" (50 animals/sex/group) were treated for 104 weeks, up to a maximum of 105 weeks and 2 days (due to the high number of animals), and those in "Allocation B" (20 animals/sex/group) were treated for 52 weeks, up to a maximum of 53 weeks. Animals received the test material in the diet at nominal concentrations of 0, 30, 200, or 1200 ppm (Table 1).

Table 1: Study Design for a Combined Chronic Toxicity/Carcinogenicity study in Rats

Allocation **Dietary concentration**		**Number of animals**	**Group 1** **0 ppm**	**Group 2** **30 ppm**	**Group 3** **200 ppm**	**Group 4** **1200 ppm**
		Daily intake (mg/kg bw/day)				
Males	**A**	50	0	1.4	9.6	57.7
	B	20	0	1.6	11.0	66.3
Females	**A**	50	0	1.7	11.6	69.3
	B	20	0	1.9	13.1	75.2

A: Oncogenicity animals (at least 104 weeks of treatment)
B: Chronic toxicity animals (at least 52 weeks of treatment)

3. Diet Preparation and Analysis

Fresh batches of the feed pellets for the study were prepared every two weeks. In treatment week 43, the 2-week interval was shortened to a one week interval. Thereafter, the feed pellets were prepared every two weeks.

Fluensulfone was warmed in the original container in a water bath up to maximum of approximately 40°C until the test item was fluid. Thereafter, the whole amount of fluid test item was transferred into a glass beaker (wrapped with aluminum foil) and mixed using a magnetic stirrer on a heating plate (maximum temperature of approximately 40°C). The test item was divided into 3 aliquots.

The required amount of fluensulfone was weighed into tared glass beakers (wrapped with aluminum foil) and kept at a maximum temperature of approximately 40°C on a heating plate until use for feed preparation. The test item was mixed with microgranulated feed for each dose group. An appropriate amount of water was added to aid pelleting. The pellets were dried with air for approximately 48 to 96 hours before storage.

Control feed for the animals of group 1 were prepared similarly, but without test item.

Feed preparations were stored at room temperature (17 to 23°C) in disposable paper bags until use. They were determined to be stable for at least 24 days at room temperature in previous studies.

Stability, concentration and homogeneity of the actual test item batch in the feed were determined with the first feed preparations. The concentration and homogeneity were determined every three months thereafter with the exception of the interval between April 30, 2009 and November 12, 2009, and the interval between January 21, 2010 and February 4, 2010. In addition, the concentration was determined in the first 5 feed preparations. Double samples for the stability of the test item were assessed after 7, 14 and 21 days. One sample was stored under study conditions and one was stored protected from light.

Additional samples of the control feed from the first preparation were taken from the feed hoppers as well from the storage bag for concentration analysis.

Analyses were performed using an HPLC-method, previously developed at the performing laboratory. The diet samples were stored deep-frozen (-20°C ± 5°C) until analysis. The test item was used as analytical standard. (See “Results”, section A “Analysis of Feed Preparations” for results).

4. Statistics

The following methods were used to analyze the water consumption, food consumption, body weight, grip strength, locomotor activity, clinical laboratory data, organ weights and ratios as well as macroscopic findings:

• If the variables were assumed to follow a normal distribution, the Dunnett-test (many to one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups with control groups for each sex.

• The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data cannot be assumed to follow a normal distribution.

• The Fisher’s exact test

• Armitage / Cochran Trend Test for non-neoplastic lesions, if appropriate.

C. METHODS

1. Observations

Observations for viability and mortality were recorded twice daily. General cage-side observations were recorded once daily during acclimatization and once daily during the treatment period. Detailed clinical observations, including palpation for tissue masses on each animal outside the home cage, were performed weekly during acclimatization and treatment periods.

2. Bodyweight

Body weights were recorded weekly during acclimatization and treatment weeks 1 to 14, and once every 4 weeks thereafter.

3. Food and water consumption

Food consumption was recorded weekly during acclimatization and treatment weeks 1 to 14, and once every 4 weeks thereafter.

The water consumption was recorded once during week 78 over a period of 24 hours, using an on-line electronic recording system consisting of a Mettler balance connected to the testing facility computer.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were performed on all animals during acclimatization, on all surviving allocation B animals in the control and high dose groups at the end of the treatment and on 10 allocation A animals per sex in the control and high dose groups at termination of the study. A Miroflex 2 or a Heine Beta 200 ophthalmoscope (Heine Optotechnik GmbH & Co. KG, Germany) was used.

5. Functional Observation Battery and Motor Activity

At week 52, relevant parameters from a modified Irwin screen test, as part of a functional observational battery (FOB) were performed on all Allocation B rats. Any abnormal findings were recorded and graded in severity. The table below summarizes the clinical and behavioral observations and their frequency.

Category	Score	Parameter	D	P	W Weeks 1-51	F Week 52
APPEARANCE	1-3	Piloerection	X	X	X	X
	1-3	Salivation	X	X	X	X
	1	Hunched posture	X	X	X	X
MOTOR	1-3	Ataxia	X	X	X	X
	1-3	Tremor / twitching	X	X	X	X
	1	Prostration	X	X	X	X
	1	Circling		X	X	X
	1-3	Spasm		X	X	X
BEHAVIOR	1-3	Hyperactivity	X	X	X	X
	1-3	Somnolence	X	X	X	X
	1-3	Increased exploration		X	X	X
	1-3	Reduced grooming		X	X	X
	1-3	Vocalization		X	X	X
RESPIRATION	1	Dyspnea	X	X	X	X
	1	Tachypnea	X	X	X	X
	1	Bradypnea	X	X	X	X
REFLEXES	1	Blink		X	X	X
	1	Pinna		X	X	X
	1	Iridic light reflex		X	X	X
	1	Push-off (hind leg)		X	X	X
	1	Pain response		X	X	X
	1	Startle / hearing		X	X	X
MISCELLANEOUS	1-3	Lacrimation		X	X	X
	1	Limbs cyanotic		X	X	X
	1	Mydriasis		X	X	X
	1	Miosis		X	X	X
	1	Exophthalmos		X	X	X
	1-3	Reduced muscle tone		X	X	X

D: daily cage-side; P: pre-test; W: weekly; F: FOB; X: observed; 1-3: severity of finding noted. Data obtained from page 30 of the study report.

Hindlimb and forelimb grip strength measurements were performed using a push-pull strain gauge (Mecmesin, AFG 25N) at week 52.

Locomotor activity was measured quantitatively at week 52 with Activity Monitor AMS (DeMeTec GmbH, Germany). Decreased or increased activity was recorded. Activity of the animals (low-beam counts) was recorded for 10-minute intervals over a period of 60 minutes.

6. Clinical Laboratory Investigations

Allocation B animals: Blood and urine samples for clinical laboratory investigations (hematology, clinical chemistry, and urinalysis) were collected from all allocation B animals during weeks 13 and 26 and after 52 weeks. Blood samples were drawn from the retro-orbital plexus from all allocation B animals under light isoflurane anesthesia. The animals were fasted in metabolism cages for approximately 18 hours before blood and urine sampling but allowed access to water *ad libitum*. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms.

Allocation A animals: Blood samples for hematology (erythrocyte count, total leukocyte count, differential leukocyte count, and blood smears only) were collected under isoflurane anesthesia from all allocation A animals after weeks 78 and 104. The animals were not fasted before blood sampling. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms.

Hematology:

The following hematological parameters were examined: erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, red cell volume distribution width, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, hemoglobin concentration distribution width, reticulocyte count, reticulocyte maturity index, leukocyte count (total), differential leukocyte count (neutrophils, eosinophils, basophils, lymphocytes, monocytes, large unstained cells), thrombocyte count. The following parameters were examined for Allocation B animals only: prothrombin time (thromboplastin time), activated partial thromboplastin time. Blood spears were prepared from Allocation A animals but were not evaluated.

Clinical Chemistry:

The following parameters were examined for clinical chemistry investigations: glucose, urea, creatinine, total bilirubin, total cholesterol, triglycerides, phospholipids, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, glutamate dehydrogenase, alkaline phosphatase, gamma-glutamyl-transferase, creatine kinase, sodium, potassium, chloride, calcium, phosphorus, total protein, albumin, globulin, and albumin/globulin ratio.

Urinalysis:

The following parameters were determined for urinalysis: urine volume, specific gravity, colour, appearance, pH, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes, leukocytes.

7. Sacrifice and pathology

All animals were weighed and necropsied. Descriptions of all macroscopic abnormalities were recorded. All animals surviving to the end of the observation period and all animals found dead or sacrificed *in extremis* were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination.

From the first five animals of each group in allocation B, liver samples (approximately 5 g) were taken for liver enzyme determination (see point 8 below).

From the surviving first ten animals of each group in allocations A and B, the right femur and the incisors of the maxilla were used for fluoride determination (see point 8 below).

Samples of a complete list of tissues and organs were collected from all animals at necropsy and, unless otherwise indicated, fixed in neutral phosphate buffered 4% formaldehyde solution. Additional tissues (such as ear tattoo) were retained in accordance with standard operating procedures but were not processed or examined further.

From allocation B and A animals, adrenal glands, brain, epididymides, heart including auricles, kidneys, liver, ovaries, prostate including coagulating gland, spleen, testes, thymus, thyroid (including parathyroid gland, if possible) and uterus were weighed before fixation and recorded on the scheduled dates of necropsy. Relative organ weights were calculated on the basis of the body weight and brain weight. Samples were also examined from the following tissues/organs: aorta, bone (sternum, femur including joint), cecum, colon, duodenum, esophagus, eyes with optic nerve, ileum, incisors, jejunum with Peyer's patches, larynx, lungs, lymph nodes, mammary gland area, nasal cavity, pancreas, pharynx, pituitary gland, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, stomach, tongue, trachea, urinary bladder, and vagina.

Sections of all organs and tissues of the control and high-dose groups and all gross lesions from all animals were examined by the study pathologist. Organ and tissue samples taken from animals which died spontaneously or which were killed *in extremis* were evaluated similarly to those organs taken from animals of the high-dose group. Due to test item-related morphologic changes detected in the lungs and esophagus of high-dose animals, these organs were also processed and examined from the mid- and low-dose group.

8. Additional investigations

Liver Enzyme Determination

At scheduled necropsy after 52 weeks of treatment, additional liver samples (approximately 5 g) taken from the first 5 animals of each sex and group in allocation B (total of 40 animals) were minced in small pieces, weighed and frozen in liquid nitrogen. Liver pieces were placed in plastic bags which were labeled with the Study and Animal Number. The samples were kept at -80°C until shipment on dry ice for liver enzyme determination. Liver subcellular fractions were prepared and the following parameters determined:

• Protein content of subcellular fractions

• Microsomal cytochrome P450 content (differential spectroscopy)

• CYP1A1 (microsomal 7-ethoxyresorufin O-dealkylation)

• CYP1A2 (microsomal 7-methoxyresorufin O-dealkylation)

• CYP2B1 (microsomal 7-pentoxyresorufin O-dealkylation)

• CYP3A (microsomal testosterone 6β-hydroxylation)

• CYP4A1 (microsomal lauric acid 12-hydroxylation)

• Cytosolic glutathione S-transferase (GST, glutathione conjugation of 1-chloro-2,4-dinitrobenzol)

• Microsomal uridine diphosphoglucuronosyl-transferase (UDPGT, glucuronidation of 3-methyl-2-nitrophenol)

• Microsomal epoxide hydrolase (mEH, hydrolysis of styrene oxide)

• Cytosolic alanine aminotransferase (ALAT, transamination of ketoglutarate)

• Peroxisomal beta Oxidation

Fluoride determination

At scheduled necropsy after 52 and 104 weeks, the right femur and the incisors of the maxilla of the first surviving 10 animals per sex and group were taken for fluoride determination (allocation A: in total 80 animals; allocation B: in total 80 animals). The samples were stored at room temperature in plastic bags (Teflon® free) until shipment to the responsible study scientist. The fluoride determination was performed according to Zober [Fluorid, Bestimmung in Knochen. In: Analytische Methoden zur Prüfung gesundheitsschädlicher Arbeitsstoffe, Band 2, Analysen in biologischen Material (1. Lieferung), Senatskommision zur Prüfung gesundheitsschädlicher Arbeitsstoffe der DFG, Verlag Chemie, Weinheim (1987)] at room temperature. Analyses were performed using ion chromatography. The fluoride samples were stored frozen at -80 ± 10 °C until work up. A defined fluoride solution was used as analytical standard.

II. RESULTS

A. ANALYSIS OF FEED PREPARATIONS

The content of fluensulfone in 111 out of a total of 117 diet samples ranged from 82% to 107% of nominal concentration. The content of the three feed samples of dose group 2 (30 ppm) prepared on November 12, 2009 were 125% to 128% of nominal, while the three group 2 (30 ppm) diets prepared on February 4, 2010 were 139% to 141% of nominal.

The maximum variation from the mean of homogeneity samples which were taken from the top, middle and bottom of the samples ranged from 0.2% to 7.1%.

The absence of fluensulfone in the control diet was confirmed.

The stability of fluensulfone was tested in samples taken 7, 14 and 21 days after preparation and stored at room temperature. The variation values, except one, were found to be <10% from the time-zero value (range: 1.2% to 13.5%).

In conclusion, the results indicate the accurate preparation and use of fluensulfone and the control diet during this study. Diet samples were found to contain acceptable concentrations, to be homogeneously prepared, and sufficiently stable under the storage conditions.

B. OBSERVATIONS

1. Clinical signs

There were no fluensulfone-related clinical signs observed during the study.

All clinical signs noted generally affected single animals of all dose groups including controls and were those commonly seen in rats of this strain and age kept under the conditions of the study. Their incidence, nature and group distribution did not distinguish treated groups from controls. The most frequent clinical signs noted were hair loss, wounds, scars and eye lesions.

2. Mortality

Mortality was not affected by treatment with fluensulfone (Tables 2 and 3).

Table 2: Mortality data for animals allocated to 52-week sacrifice (Allocation B)

Dose (ppm)	Spontaneous death				Killed *in extremis*				Planned necropsy			
	Males		Females		Males		Females		Males		Females	
0	0	0%	2	10%	0	0%	0	0%	20	100%	18	90%
30	0	0%	0	0%	0	0%	0	0%	20	100%	20	100%
200	0	0%	0	0%	0	0%	0	0%	20	100%	20	100%
1200	0	0%	0	0%	0	0%	0	0%	20	100%	20	100%

Data obtained from page 40 of the study report.

Table 3: Mortality data for animals allocated to 104-week sacrifice (Allocation A)

Dose (ppm)	Spontaneous death				Killed *in extremis*				Planned necropsy			
	Males		Females		Males		Females		Males		Females	
0	3	6%	2	4%	8	16%	12	24%	39	78%	36	72%
30	4	8%	2	4%	4	8%	12	24%	42	84%	36	72%
200	8	16%	10	20%	7	14%	7	14%	35	70%	33	66%
1200	4	8%	4	8%	8	16%	8	16%	38	76%	38	76%

Data obtained from page 39 of the study report

3. FOB, Grip Strength and Locomotor Activity

Locomotor activity was assessed at week 52 in Allocation B animals. Overall mean locomotor activity was decreased by 14% in high dose males. Decreases in activity were noted at the 0-10 minute interval, as well as at the 30-40 and 50-60 minute intervals (Table 4). In females, total overall activity was increased by 20% at the high dose. This increase is largely due to a 70% increase at the 50-60 minute interval. Overall, values were variable throughout the assessment period. No treatment-related effects were recorded during the FOB or grip strength assessments.

Table 4: Mean locomotor activity per group (± S.D.) at 52 weeks (Allocation B):

Interval	**Control n = 20**	**30 ppm n = 20**	**200 ppm n = 20**	**1200 ppm n = 20**
MALES				
0-10 minutes	240 ± 107	221 ± 80 (↓8%)	199 ± 114 (↓17%)	186 ± 107 (↓23%)
10-20 minutes	66 ± 80	72 ± 36	71 ± 64	80 ± 60 (↑21%)
20-30 minutes	49 ± 48	49 ± 46	67 ± 65 (↑37%)	50 ± 33
30-40 minutes	39 ± 54	45 ± 52	70 ± 50 (↑79%)	28 ± 25 (↓28%)
40-50 minutes	40 ± 53	53 ± 42	43 ± 54	41 ± 42
50-60 minutes	39 ± 47	30 ± 46	17 ± 25 (↓56%)	25 ± 33 (↓36%)
Total	474 ± 238	470 ± 215	467 ± 196	410 ± 218 (↓14%)
Interval	**Control n = 18**	**30 ppm n = 20**	**200 ppm n = 20**	**1200 ppm n = 20**
FEMALES				
0-10 minutes	237 ± 76	224 ± 80	237 ± 107	254 ± 93
10-20 minutes	110 ± 69	86 ± 46	110 ± 92	113 ± 66
20-30 minutes	83 ± 54	68 ± 59	90 ± 106	84 ± 56
30-40 minutes	69 ± 79	60 ± 73	52 ± 66	77 ± 62
40-50 minutes	60 ± 56	59 ± 56	47 ± 63	41 ± 50
50-60 minutes	59 ± 58	65 ± 69	68 ± 65	100 ± 84 (↑70%)
Total	556 ± 311	562 ± 256	604 ± 389 (↑9%)	669 ± 255 (↑20%)

Data obtained from pages 215-218 of the study report. ( ) = % different from controls, calculated by the reviewer.

C. BODYWEIGHT AND BODYWEIGHT GAINS

Absolute mean body weight and body weight gain were decreased in a dose-dependent manner in males treated at 200 and 1200 ppm, although not always achieving statistical significance at 200 ppm (Tables 5 and 6). Mean body weight of males treated at 1200 ppm was 11% lower than controls on day 715, thus indicating that a Maximum Tolerated Dose (MTD) was reached in this study. In high dose females, body weight was decreased during the last 11 weeks of the study in the range of 3 to 6%. These decreases did not reach statistical significance. Body weight gain was statistically significantly reduced in females at 1200 ppm from week 2 onwards. Overall body weight gain was decreased by 6% in 200 ppm males, by 14% in 1200 ppm males, and by 10% in 1200 ppm females.

Table 5: Mean (± S.D.) Body Weight and Overall Body Weight Gain (g)

Study Day	Control	30 ppm	200 ppm	1200 ppm
MALES				
Day 1	166 ± 15	164 ± 13	163 ± 14	167 ± 14
Day 15	257 ± 22	253 ± 18	250 ± 19	249* ± 17 (↓3%)
Day 85	431 ± 43	424 ± 37	411** ± 39 (↓5%)	408** ± 35 (↓5%)
Day 239	542 ± 61	535 ± 54	517* ± 50 (↓5%)	510** ± 48 (↓6%)
Day 603	688 ± 88	677 ± 71	639* ± 84 (↓7%)	624** ± 82 (↓9%)
Day 715	703 ± 87	691 ± 78	668 ± 93 (↓5%)	628** ± 99 (↓11%)
BWG	537	527	505 (↓6%)	461 (↓14%)
FEMALES				
Day 1	131 ± 10	132 ± 10	132 ± 10	133 ± 11
Day 15	172 ± 12	175 ± 12	174 ± 13	171 ± 13
Day 85	246 ± 17	248 ± 19	244 ± 21	244 ± 19
Day 239	284 ± 25	290 ± 29	286 ± 37	277 ± 28 (↓3%)
Day 603	390 ± 53	396 ± 58	393 ± 62	370 ± 58 (↓5%)
Day 715	402 ± 53	408 ± 64	404 ± 63	378 ± 61 (↓6%)
BWG	271	276	272	245 (↓10%)

Data obtained from pages 320-327 of the study report. ( ) = % difference from control (calculated by the reviewer). * $p < 0.05$; ** $p < 0.01$. N=70/sex/dose at start of study. BWG = overall body weight gain, calculated by the reviewer from the mean body weight values on days 715 and 1 (not analyzed statistically).

Table 6: Mean (± S.D.) Body Weight Gain (% of initial bw)

Study Day	Control	30 ppm	200 ppm	1200 ppm
MALES				
Day 1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Day 15	55.2 ± 4.7	54.0 ± 4.1	53.6 ± 4.9	49.1** ± 5.2
Day 85	160.7 ± 19.1	158.7 ± 15.8	152.7* ± 18.7	144.7** ± 15.5
Day 239	228.3 ± 29.5	226.5 ± 25.2	217.9 ± 26.8	205.9** ± 23.9
Day 603	315.4 ± 43.9	316.0 ± 35.8	293.8* ± 46.8	273.7** ± 40.8
Day 715	325.1 ± 48.8	324.6 ± 44.7	313.9 ± 47.5	275.2** ± 51.2 (↓15%)
FEMALES				
Day 1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Day 15	31.2 ± 4.6	32.6 ± 5.1	31.6 ± 4.6	28.4** ± 4.9
Day 85	88.0 ± 9.8	88.4 ± 11.5	85.0 ± 11.3	83.6* ± 10.7
Day 239	117.7 ± 16.8	120.0 ± 20.3	116.7 ± 23.5	108.4* ± 17.4
Day 603	198.5 ± 36.4	200.2 ± 41.9	195.3 ± 41.1	178.6 ± 42.1
Day 715	208.6 ± 37.1	208.3 ± 40.6	202.2 ± 39.7	181.4* ± 42.9 (↓13%)

Data obtained from pages 330 – 337 of the study report. * $p < 0.05$; ** $p < 0.01$. N=70/sex/dose at start of study.

D. FOOD AND WATER CONSUMPTION

Slightly lower absolute (g/animal/day) and relative (g/kg bw/day) food consumption was recorded in both sexes at all dose levels during the first 1-2 weeks of dosing (Table 7). This effect is commonly seen in feeding studies, is caused by the change of feed (e.g. due to different taste of the feed) and is not considered to be of toxicological relevance.

Lower absolute food consumption was recorded in males treated at 1200 ppm during the first 4 weeks of treatment. Afterwards statistically significantly lower absolute food consumption was recorded at several intervals. However, the lower absolute food consumption was likely caused by lower body weights, as relative food consumption in the same animals was similar to, or higher than in the controls. The mean of means for food consumption for each dose group was comparable for both sexes, indicating overall similar food consumption.

The 200 ppm group males occasionally had statistically significant reduced absolute food consumption; however, the relative food consumption values were not affected. Therefore these changes were not considered adverse.

Relative water consumption was statistically significantly increased during week 78 in males treated at 1200 ppm (15%). Relative water consumption was also increased in females of this dose group (7%), although the increase was slight and not statistically significant (Table 8).

Table 7a: Absolute (g/animal/day) and relative (g/kg bw/day) mean (± S.D.) food consumption in males

Study Days		Control	30 ppm	200 ppm	1200 ppm
1 – 8	absolute	19.9 ± 0.9	19.0 ± 0.8** (↓4%)	18.9 ± 0.7** (↓5%)	18.4 ± 0.7** (↓7%)
	relative	93.3 ± 2.6	89.4 ± 2.0** (↓4%)	89.7** ± 3.0 (↓4%)	87.8 ± 2.4** (↓6%)
8 – 15	absolute	22.2 ± 0.8	21.4 ± 0.9* (↓4%)	21.3 ± 0.6* (↓4%)	21.2 ± 0.8** (↓4%)
	relative	86.4 ± 1.7	84.7 ± 2.0	85.2 ± 2.4	85.4 ± 2.5
15 – 22	absolute	21.8 ± 0.8	21.4 ± 1.2 (↓2%)	21.4 ± 0.7 (↓2%)	21.3 ± 0.8 (↓2%)
	relative	75.2 ± 2.2	75.0 ± 2.7	76.2 ± 2.8	76.5 ± 2.3
22 – 29	absolute	23.3 ± 0.9	22.4 ± 1.0 (↓3%)	22.6 ± 1.8 (↓3%)	21.4** ± 0.7 (↓8%)
	relative	73.2 ± 2.4	71.9 ± 1.7	73.8 ± 4.8	70.2 ± 1.9* (↓4%)
176 – 183	absolute	21.7 ± 0.9	21.9 ± 1.0	21.6 ± 1.0	21.4 ± 0.7
	relative	42.1 ± 1.5	42.9 ± 1.9	43.4 ± 1.6	43.6 ± 1.5
372 – 379	absolute	22.2 ± 1.1	22.2 ± 0.8	21.4 ± 1.2 (↓4%)	21.0 ± 1.2* (↓5%)
	relative	35.7 ±1.0	36.4 ± 1.4	36.5 ± 1.1	36.0 ± 1.2
568 – 575	absolute	22.8 ± 1.1	22.6 ± 1.0	20.7 ± 1.3* (↓9%)	20.8 ± 2.7* (↓9%)
	relative	34.1 ± 1.6	34.4 ± 1.3	33.4 ± 1.8	34.5 ± 6.6
708 – 715	absolute	20.6 ± 1.8	20.9 ± 1.4	20.0 ± 1.8 (↓3%)	19.8 ± 1.8 (↓4%)
	relative	29.4 ± 2.1	29.8 ± 1.5	30.1 ± 2.5	32.1 ± 3.0* (↑9%)

Data obtained from pages 281 – 298 of the data report. n = 14 from the beginning of the study until reading for days 344 – 351. n = 10 for reading on days 372-379 to study termination. * $p < 0.05$; ** $p < 0.01$; ( ) = % difference from control, calculated the by reviewer.

Table 7b: Absolute (g/animal/day) and relative (g/kg bw/day) mean (± S.D.) food consumption in females

Study Days		Control	30 ppm	200 ppm	1200 ppm
1 – 8	absolute	14.7 ± 0.8	14.0 ± 0.5* (↓5%)	13.9 ± 0.7** (↓5%)	13.4 ± 0.6** (↓9%)
	relative	95.4 ± 4.4	90.8 ± 1.9** (↓5%)	89.9 ± 3.7** (↓6%)	86.9 ± 2.3** (↓9%)
8 – 15	absolute	14.9 ± 0.5	15.0 ± 0.5	15.0 ± 0.7	14.7 ± 0.6
	relative	86.7 ± 1.4	86.1 ± 2.3	86.2 ± 3.1	86.2 ± 2.7
15 – 22	absolute	14.6 ± 0.5	15.0 ± 0.6 (↑3%)	15.3 ± 1.0* (↑5%)	15.2 ± 0.7 (↑4%)
	relative	77.8 ± 1.5	79.7 ± 2.0	82.3 ± 3.5** (↑6%)	82.2 ± 2.7** (↑6%)
22 – 29	absolute	15.4 ± 0.7	15.6 ± 0.8	15.7 ± 0.8	15.3 ± 0.7
	relative	77.2 ± 2.4	78.3 ± 2.5	79.0 ± 3.2	77.0 ± 3.4
176 – 183	absolute	13.7 ± 1.1	13.9 ± 1.3	14.3 ± 1.0 (↑4%)	14.2 ± 0.9 (↑4%)
	relative	51.5 ± 4.0	51.4 ± 3.7	53.0 ± 3.2	53.5 ± 3.9
372 – 379	absolute	15.8 ± 0.8	15.9 ± 0.8	15.7 ± 2.3	15.7 ± 0.9
	relative	47.9 ± 2.7	47.5 ± 2.2	46.7 ± 4.8	48.9 ± 2.2
568 – 575	absolute	16.6 ± 2.0	17.0 ± 1.2	16.7 ± 1.2	15.5 ± 1.5 (↓7%)
	relative	44.8 ± 4.2	44.1 ± 2.4	43.9 ± 4.2 (↓2%)	43.1 ± 5.6 (↓4%)
708 – 715	absolute	14.8 ± 1.5	15.2 ± 1.2	14.6 ± 1.6	13.9 ± 1.6 (↓6%)
	relative	37.1 ± 3.0	37.0 ± 4.1	36.3 ± 3.7	36.7 ± 4.2

Data obtained from pages 281 – 298 of the data report. n = 14 from the beginning of the study until reading for days 344 – 351. n = 10 for reading on days 372-379 to study termination. * $p < 0.05$; ** $p < 0.01$; ( ) = % difference from control, calculated by the reviewer.

Table 8: Mean water consumption (g/animal/day and mg/kg bw/day)

	Control n = 10	30 ppm n = 10	200 ppm n = 10	1200 ppm n = 10
MALES				
g/animal/day	27.2 ± 2.4	25.8 ± 2.6	26.8 ± 3.5	28.7 ± 4.0 (↑5%)
mg/kg bw/day	40.7 ± 3.8	39.3 ± 4.4	42.8 ± 7.3	46.9* ± 6.1 (↑15%)
FEMALES				
g/animal/day	26.3 ± 4.9	27.3 ± 3.5	27.0 ± 5.1	26.8 ± 3.6
mg/kg bw/day	70.7 ± 12.9	71.3 ± 7.3	71.7 ± 17.6	75.9 ± 10.4 (↑7%)

Data obtained from pages 300-304 of the study report. ( ) = % difference from control, calculated by the reviewer. * $p < 0.05$; ** $p < 0.01$.

E. OPHTHALMOSCOPY

No ophthalmoscopic findings of toxicological relevance were recorded.

F. BLOOD ANALYSIS

1. Hematological findings

The following changes noted at 52 weeks were considered to be related to treatment with fluensulfone (Tables 9 and 10):

- Increased absolute red blood cell count (RBC) in males at 1200 ppm (5%);

- Decreased hemoglobin concentration (Hb) in females at 1200 ppm (3%);

- Decreased mean corpuscular hemoglobin (MCH) in males at 1200 ppm (5%);

- Decreased mean corpuscular hemoglobin concentration (MCHC) in males (2%) and females (1%) at 1200 ppm;

- Dose dependent decreased neutrophil count (Neut) in all female dose groups (19, 23, 38%), attaining statistical significance at 1200 ppm;

- Decreased lymphocytes in 1200 ppm females (17%);

- Decreased prothrombin time (PT) in males at 200 and 1200 ppm (5% and 9%, respectively), and increased partial prothrombin time (PTT) in males at 1200 ppm (10%);

- LUC (large unstained cell) counts were increased in 1200 ppm males (33%);

- White blood cell counts (WBC) counts were decreased in a dose-dependent manner in all female treatment groups at week 52 (10%, 13%, and 24% at 30, 200 and 1200 ppm, respectively).

In most of the parameters similar changes were also recorded after 13 and 26 weeks.

The following changes noted at 78 and/or 104 weeks were considered to be related to treatment with fluensulfone:

- Decreased hemoglobin concentration (Hb) in females at 1200 ppm (2%);

- Increased absolute RBC in males at 1200 ppm (4%);

- Decreased MCH in males at 1200 ppm (3%);

- Decreased MCHC in males at all doses at 78 weeks (1-3%) and at 1200 ppm (1%) at 104 weeks, as well as in females at 200 and 1200 ppm (1% and 2%, respectively);

- Decreased hemoglobin concentration distribution width (HDW) in females at 200 and 1200 ppm (5% and 6%, respectively), as well as a slight decrease in females at 30 ppm at week 78 only which is not considered toxicologically relevant;

- Increased neutrophil counts (Neut) in females (53%) at 1200 ppm;

- Decreased lymphocyte counts (Lymph) of 19% in females in both 200 and 1200 ppm dose groups at week 78;

- Increased eosinophil (EOS) values in 1200 ppm males (50%).

At week 104, one control male (animal #12) demonstrated very high values for several white blood cell parameters and means were re-calculated after excluding these outlying values (values of 165 G/L for total WBC, 19.76 G/L for large unstained cells, and 140 G/L for lymphocytes).

At 78 weeks, males in the 200 ppm dose group also exhibited a statistically significant increase in mean red blood cell count when compared to controls, but this finding is considered to be spurious as changes were not observed at any other time point.

Changes in several other parameters achieving statistical significance were considered unrelated to treatment since they lacked a dose-relationship and/or were clearly within the historical control range.

Table 9a: Selected (± S.D.) mean red blood cell parameters – Male rats

Dose (ppm)	RBC (T/L)	Hb (mmol/L)	MCH (fmol)	MCHC (mmol/L)	HDW (mmol/L)
Week 13 (allocation B animals)					
0	8.80 ± 0.51	10.4 ± 0.4	1.18 ± 0.06	23.34 ± 0.49	1.83 ± 0.11
30	8.77 ± 0.38	10.3 ± 0.4	1.17 ± 0.04	23.65 ± 0.31*	1.91 ± 0.09
200	8.80 ± 0.37	10.2 ± 0.3	1.17 ± 0.04	23.29 ± 0.30	1.84 ± 0.13
1200	9.06 ± 0.32 (↑3%)	10.3 ± 0.3	1.13 ± 0.01** (↓4%)	22.83 ± 0.46** (↓2%)	1.78 ± 0.14
Week 26 (allocation B animals)					
0	8.81 ± 0.49	9.7 ± 0.3	1.10 ± 0.06	22.43 ± 0.47	1.69 ± 0.11
30	8.94 ± 0.35	9.7 ± 0.3	1.09 ± 0.04	22.37 ± 0.32	1.74 ± 0.11
200	8.81 ± 0.28	9.5 ± 0.3	1.08 ± 0.04	21.15 ± 0.32*	1.69 ± 0.15
1200	9.22 ± 0.41** (↑5%)	9.7 ± 0.3	1.06 ± 0.04** (↓4%)	21.88 ± 0.31** (↓2%)	1.60 ± 0.13
Week 52 (allocation B animals)					
0	8.89 ± 0.53	10.1 ± 0.3	1.14 ± 0.07	23.10 ± 0.43	1.81 ± 0.14
30	9.01 ± 0.43	10.1 ± 0.4	1.13 ± 0.01	23.46 ± 0.37**	1.86 ± 0.12
200	9.11 ± 0.39	10.2 ± 0.3	1.12 ± 0.05	23.04 ± 0.38	1.79 ± 0.15
1200	9.34 ± 0.42** (↑5%)	10.1 ± 0.3	1.08 ± 0.04** (↓5%)	22.59 ± 0.32** (↓2%)	1.74 ± 0.15
Week 78 (allocation A animals)					
0	8.95 ± 0.40	9.8 ± 0.3	1.09 ± 0.05	21.44 ±0.37	1.58 ± 0.08
30	9.05 ± 0.40	9.8 ± 0.4	1.08 ± 0.04	21.11 ± 0.39** (↓1%)	1.57 ± 0.08
200	9.16 ± 0.41*	9.8 ± 0.3	1.07 ± 0.05	20.95 ± 0.45** (↓2%)	1.56 ± 0.12
1200	9.13 ± 0.45 (↑2%)	9.6 ± 0.4	1.06 ± 0.04** (↓3%)	20.88 ± 0.30** (↓3%)	1.53 ± 0.12
Week 104 (allocation A animals)					
0	8.54 ± 0.60	9.4 ± 0.5	1.10 ± 0.06	21.42 ± 0.39	1.63 ± 0.09
30	8.46 ± 0.77	9.4 ± 0.8	1.11 ± 0.05	21.47 ± 0.34	1.62 ± 0.10
200	8.65 ± 0.54	9.6 ± 0.4	1.11 ± 0.05	21.43 ± 0.42	1.62 ± 0.13
1200	8.91 ± 0.46* (↑4%)	9.6 ± 0.4	1.07 ± 0.05 (↓3%)	21.20 ± 0.38* (↓1%)	1.62 ± 0.15

T: tera (10^{12}); m: milli (10^{-3}); mol: mole; f: femto (10^{-15}); *: significant at 5%; **: significant at 1%; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 354-368 of the study report. No standard deviation values were provided in the study report and thus were calculated by the reviewer. Weeks 13, 26 and 52, n = 20. Weeks 78 and 104, n = 50.

Table 9b: Selected mean (± S.D.) red blood cell parameters – Female rats

Dose (ppm)	RBC (T/L)	Hb (mmol/L)	MCH (fmol)	MCHC (mmol/L)	HDW (mmol/L)
Week 13 (allocation B animals)					
0	8.01 ± 0.23	9.9 ± 0.2	1.24 ± 0.04	23.27 ± 0.30	1.53 ± 0.10
30	7.80 ± 0.40	9.7 ± 0.3	1.25 ± 0.05	23.51 ± 0.35*	1.56 ± 0.09
200	7.91 ± 0.39	9.8 ± 0.3	1.24 ± 0.04	23.15 ± 0.26	1.49 ± 0.09
1200	7.90 ± 0.37	9.6 ± 0.4* (↓3%)	1.22 ± 0.03	22.90 ± 0.29** (↓2%)	1.51 ± 0.11
Week 26 (allocation B animals)					
0	8.04 ± 0.34	9.3 ± 0.3	1.16 ± 0.03	22.22 ± 0.42	1.40 ± 0.10
30	7.97 ± 0.40	9.3 ± 0.3	1.17 ± 0.05	22.33 ± 0.37	1.41 ± 0.09
200	7.97 ± 0.34	9.3 ± 0.3	1.17 ± 0.04	22.28 ± 0.27	1.35 ± 0.10
1200	7.93 ± 0.30	9.1 ± 0.3* (↓2%)	1.15 ± 0.03	21.93 ± 0.29* (↓1%)	1.36 ± 0.09
Week 52 (allocation B animals)					
0	8.02 ± 0.41	9.8 ± 0.4	1.22 ± 0.04	23.25 ± 0.42	1.45 ± 0.10
30	8.15 ± 0.37	10.0 ± 0.3	1.22 ± 0.06	23.49 ± 0.42	1.47 ± 0.09
200	8.14 ± 0.37	9.9 ± 0.3	1.21 ± 0.05	23.13 ± 0.43	1.40 ± 0.10
1200	7.79 ± 0.64	9.5 ± 0.6* (↓3%)	1.22 ± 0.06	22.95 ± 0.37 (↓1%)	1.41 ± 0.15
Week 78 (allocation A animals)					
0	7.90 ± 0.42	9.3 ± 0.3	1.18 ± 0.05	21.57 ± 0.4	1.46 ± 0.09
30	7.77 ± 0.39	9.2 ± 0.3	1.19 ± 0.03	21.63 ± 0.45	1.41 ± 0.08* (↓3%)
200	7.95 ± 0.33	9.2 ± 0.3	1.16 ± 0.04	21.33 ± 0.41* (↓1%)	1.36 ± 0.07** (↓7%)
1200	7.76 ± 0.44	9.0 ± 0.3** (↓3%)	1.16 ± 0.05	21.23 ± 0.48** (↓2%)	1.37 ± 0.10** (↓6%)
Week 104 (allocation A animals)					
0	7.61 ± 0.43	9.1 ± 0.3	1.20 ± 0.05	21.98 ± 0.36	1.46 ± 0.08
30	7.47 ± 0.44	9.0 ± 0.4	1.20 ± 0.04	21.95 ± 0.36	1.45 ± 0.11
200	7.70 ± 0.33	9.1 ± 0.3	1.19 ± 0.04	21.70 ± 0.41** (↓1%)	1.39 ± 0.11* (↓5%)
1200	7.51 ± 0.54	8.9 ± 0.5** (↓2%)	1.19 ± 0.07	21.57 ± 0.34** (↓2%)	1.37 ± 0.12** (↓6%)

T: tera (10^{12}); m: milli (10^{-3}); mol: mole; f: femto (10^{-15}); *: significant at 5%; **: significant at 1%; nd: not determined; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 354-368 of the study report. Data obtained from pages 354-368 of the study report. No standard deviation values were provided in the study report and thus were calculated by the reviewer. Weeks 13, 26 and 52, n = 20. Weeks 78 and 104, n = 50.

Table 10a: Selected mean (± S.D.) white blood cell and clotting parameters – Male rats

Dose (ppm)	Neu (G/L)	Lymph (G/L)	PT (a)	PTT (sec)	LUC (G/L)	WBC (G/L)
Week 13 (allocation B animals)						
0	1.38 ± 0.36	4.62 ± 0.85	0.92 ± 0.05	24.4 ± 9.8	0.06 ± 0.02	6.36 ± 1.14
30	1.47 ± 0.44	4.57 ± 1.37	0.91 ± 0.03	23.8 ± 1.6*	0.06 ± 0.02	6.36 ± 1.72
200	1.53 ± 0.41	5.50 ± 1.12*	0.92 ± 0.08	23.6 ± 1.9	0.07 ± 0.03	7.39 ± 1.40
1200	1.28 ± 0.38	5.34 ± 0.91	0.87 ± 0.07 (↓5%)	26.0 ± 1.8** (↑7%)	0.08 ± 0.03* (↑33%)	7.00 ± 1.18
Week 26 (allocation B animals)						
0	1.29 ± 0.40	3.84 ± 0.72	0.87 ± 0.03	25.3 ± 1.4	0.07 ± 0.03	5.42 ± 1.01
30	1.56 ± 0.35	4.31 ± 0.84	0.89 ± 0.03	26.1 ± 1.3	0.08 ± 0.03	6.22 ± 1.08
200	1.52 ± 0.34	3.94 ± 0.96	0.86 ± 0.04	26.8 ± 2.3*	0.07 ± 0.02	5.76 ± 1.18
1200	1.50 ± 0.60	4.32 ± 0.93	0.81 ± 0.07** (↓7%)	27.8 ± 2.1** (↑10%)	0.09 ± 0.03* (↑29%)	6.18 ± 1.33
Week 52 (allocation B animals)						
0	1.51 ± 0.72	3.19 ± 0.57	0.86 ± 0.04	24.5 ± 2.2	0.06 ± 0.04	5.07 ± 1.17
30	1.97 ± 0.61	3.76 ± 1.07	0.85 ± 0.03	25.0 ± 1.5	0.07 ± 0.03	6.16 ± 1.45*
200	2.01 ± 0.63*	3.79 ± 0.93	0.82 ± 0.04* (↓5%)	25.1 ± 1.7	0.07 ± 0.02	6.21 ± 1.35*
1200	1.47 ± 0.45	3.78 ± 0.57	0.78 ± 0.08** (↓9%)	27.0 ± 2.2** (↑10%)	0.08 ± 0.03* (↑33%)	5.65 ± 0.99
Dose (ppm)	**Neu (G/L)**	**Lymph (G/L)**	**Eos (G/L)**	**Mono (G/L)**	**LUC (G/L)**	**WBC (G/L)**
Week 78 (allocation A animals)						
0	1.79 ± 0.86	4.20 ± 0.97	0.13 ± 0.05	0.20 ± 0.09	0.07 ± 0.04	6.44 ± 1.66
30	1.69 ± 0.43	3.86 ± 0.72	0.12 ± 0.05	0.18 ± 0.05	0.06 ± 0.02*	5.94 ± 1.02
200	1.72 ± 0.47	4.16 ± 0.84	0.12 ± 0.05	0.19 ± 0.06	0.06 ± 0.02	6.29 ± 1.15
1200	2.02 ± 0.63	4.04 ± 0.76	0.14 ± 0.06	0.19 ± 0.06	0.07 ± 0.02	6.49 ± 1.22
Week 104 (allocation A animals)						
0	2.20 ± 2.39	7.35 ± 21.85 (without outlier: 3.89 ± 1.43)	0.10 ± 0.05	0.20 ± 0.09	0.58 ± 3.00 (without outlier: 0.07 ± 0.08)	10.45 ± 25.6 (without outlier: 6.39 ± 3.41)
30	1.90 ± 1.43	3.42 ± 0.70	0.12 ± 0.08	0.18 ± 0.06	0.04 ± 0.02	5.69 ± 1.58
200	1.77 ± 0.50	3.51 ± 0.61	0.11 ± 0.04	0.17 ± 0.06	0.05 ± 0.03	5.61 ± 0.93
1200	2.39 ± 1.72	4.11 ± 0.99	0.15 ± 0.06** (↑50%)	0.23 ± 0.11 (↑15%)	0.06 ± 0.03	6.95 ± 2.39

G: giga (10^9); a: Clotting assay, thromboplastin from rabbit brain tissue, results as ratio of normal activity; *: significant at 5%; **: significant at 1%; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 354-368 of the study report. No standard deviation values were provided in the study report and thus were calculated by the reviewer. Weeks 13, 26 and 52, n = 20. Weeks 78 and 104, n = 50.

Table 10b: Selected mean (± S.D.) white blood cell and clotting parameters – Female rats

Dose (ppm)	Neu (G/L)	Lymph (G/L)	PT (a)	PTT (sec)	LUC (G/L)	WBC (G/L)
Week 13 (allocation B animals)						
0	0.71 ± 0.23	3.03 ± 0.75	0.94 ± 0.04	29.2 ± 4.5	0.05 ± 0.02	3.96 ± 0.81
30	0.72 ± 0.21	2.23 ± 0.74*	0.92 ± 0.06	30.0 ± 3.6	0.03 ± 0.01**	3.11 ± 0.78*
200	0.76 ± 0.21	2.79 ± 0.94	0.95 ± 0.07	26.8 ± 4.2	0.04 ± 0.02	3.74 ± 1.04
1200	0.80 ± 0.17	2.70 ± 1.08	0.97 ± 0.04*	26.1 ± 3.3	0.04 ± 0.02*	3.71 ± 1.22
Week 26 (allocation B animals)						
0	0.79 ± 0.27	2.01 ± 0.54	0.90 ± 0.03	35.2 ± 5.4	0.04 ± 0.02	2.97 ± 0.59
30	0.74 ± 0.33	2.55 ± 0.83*	0.89 ± 0.04	36.5 ± 7.1	0.05 ± 0.03	3.49 ± 0.99
200	0.79 ± 0.24	2.20 ± 0.46	0.90 ± 0.04	34.2 ± 5.5	0.05 ± 0.03	3.18 ± 0.64
1200	0.65 ± 0.21	1.95 ± 0.60	0.89 ± 0.04	32.6 ± 4.6	0.05 ± 0.02	2.79 ± 0.71
Week 52 (allocation B animals)						
0	0.95 ± 0.40	2.27 ± 0.60	0.86 ± 0.05	29.3 ± 5.3	0.04 ± 0.01	3.46 ± 0.85
30	0.77 ± 0.24 (↓19%)	2.11 ± 0.51	0.86 ± 0.05	29.2 ± 3.7	0.04 ± 0.02	3.11 ± 0.62 (↓10%)
200	0.73 ± 0.29 (↓23%)	2.08 ± 0.51	0.86 ± 0.04	27.1 ± 2.8	0.04 ± 0.01	3.02 ± 0.72 (↓13%)
1200	0.59 ± 0.19** (↓38%)	1.88 ± 0.52 (↓17%)	0.86 ± 0.05	31.2 ± 5.7	0.03 ± 0.02	2.64 ± 0.62** (↓24%)
Dose (ppm)	**Neu (G/L)**	**Lymph (G/L)**	**Eos (G/L)**	**Mono (G/L)**	**LUC (G/L)**	**WBC (G/L)**
Week 78 (allocation A animals)						
0	1.16 ± 0.42	2.61 ± 0.68	0.08 ± 0.04	0.14 ± 0.04	0.05 ± 0.02	4.06 ± 0.83
30	1.08 ± 0.48	2.38 ± 0.45	0.08 ± 0.02	0.12 ± 0.03	0.04 ± 0.02	3.73 ± 0.68
200	1.15 ± 0.41	2.12 ± 0.50** (↓19%)	0.08 ± 0.03	0.12 ± 0.03	0.04 ± 0.02	3.53 ± 0.69**
1200	1.29 ± 0.55	2.12 ± 0.57** (↓19%)	0.08 ± 0.03	0.13 ± 0.05	0.04 ± 0.02	3.68 ± 0.92
Week 104 (allocation A animals)						
0	1.19 ± 0.52	2.31 ± 0.70	0.09 ± 0.05	0.13 ± 0.05	0.03 ± 0.01	3.76 ± 1.04
30	1.36 ± 1.28	2.22 ± 0.50	0.09 ± 0.06	0.15 ± 0.09	0.03 ± 0.01	3.86 ± 1.66
200	1.38 ± 0.59	2.22 ± 0.66	0.09 ± 0.04	0.15 ± 0.06	0.03 ± 0.01	3.87 ± 1.13#
1200	1.82 ± 1.15* (↑53%)	2.18 ± 0.67	0.10 ± 0.04	0.16 ± 0.07	0.03 ± 0.01	4.30 ± 1.71

G: giga (10^9); a: Clotting assay, thromboplastin from rabbit brain tissue, results as ratio of normal activity; *: significant at 5%; **: significant at 1%; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 354-368 of the study report. No standard deviation values were provided in the study report and thus were calculated by the reviewer. Weeks 13, 26 and 52, n = 20. Weeks 78 and 104, n = 50.

2. Blood chemistry findings

The following changes after 52 weeks were considered to be related to treatment with fluensulfone:

- Statistically significantly increased cholesterol concentration (53%) in males at 1200 ppm;

- Statistically significantly increased triglyceride concentration in males (59%) and females (33%) at 1200 ppm;

- Statistically significantly increased phospholipid concentration in males (44%) and females (26%) at 1200 ppm;

- Statistically significantly increased Aspartate aminotransferase (ASAT) concentration in males at 1200 ppm (29%);

- Statistically significantly decreased Aspartate aminotransferase (ASAT) concentration in females at 1200 ppm (23%);

- Decreased Alanine aminotransferase (ALAT) concentration in females at 200 and 1200 ppm (19 and 29%, respectively), statistically significant at 1200 ppm;

- Statistically significantly increased Lactate dehydrogenase (LDH) concentration in males at 1200 ppm (65%);

- Statistically significantly increased Alkaline phosphatase (ALP) in males at 1200 ppm (22%);

- Statistically significantly increased Creatine kinase (CK) in males at 1200 ppm (61%);

- A dose-related and statistically significant increase of the sodium (Na) concentration in all treated male groups (1-4 %), and females at 200 and 1200 ppm (2-5%);

- Increased potassium (K) concentration in males at 1200 ppm (12%);

- Statistically significantly increased chloride (Cl) concentration in females at 200 and 1200 ppm (2-4%) and in all male dose groups (1-3%);

- Statistically significantly increased calcium (Ca) concentration in males at 200 and 1200 ppm (2 and 7% respectively) and all treated female groups (3-5%);

- Statistically significantly increased phosphorus (P) concentration in males at 200 and 1200 ppm (10 and 11%, respectively);

- A dose-related increase of the total protein concentration in all treated male groups (3-12%), attaining statistical significance at 1200 ppm;

- Statistically significantly increased albumin concentration in males at 1200 ppm (8%);

- Statistically significantly increased globulin concentration in all male dose groups (6-18%).

In most of the parameters similar changes were also recorded after 13 and 26 weeks.

Changes in several other parameters achieving statistical significance were considered unrelated to treatment since they lacked a dose-relationship and were clearly within the historical control range.

Table 11: Selected clinical chemistry parameters – Male rats (n = 20)

Parameter	Study Week	Dose (ppm)			
		0	30	200	1200
Cholesterol (mmol/L)	13	2.15 ± 0.25	2.06 ± 0.31	2.29 ± 0.48	2.86 ± 0.51** (↑33%)
	26	2.47 ± 0.35	2.49 ± 0.38	2.60 ± 0.55	3.50 ± 0.58** (↑42%)
	52	2.50 ± 0.38	2.76 ± 0.56	2.82 ± 0.68	3.83 ± 0.77** (↑53%)
Triglycerides (mmol/L)	13	0.33 ± 0.09	0.33 ± 0.11	0.36 ± 0.18	0.47 ± 0.28* (↑42%)
	26	0.39 ± 0.10	0.43 ± 0.13	0.46 ± 0.18	0.66 ± 0.34** (↑69%)
	52	0.66 ± 0.22	0.82 ± 0.33	0.72 ± 0.33	1.05 ± 0.50** (↑59%)
Phospholipids (mmol/L)	13	2.06 ± 0.22	2.06 ± 0.26	2.18 ± 0.32	2.70 ± 0.40** (↑31%)
	26	1.83 ± 0.22	1.88 ± 0.24	1.93 ± 0.30	2.57 ± 0.39** (↑40%)
	52	1.83 ± 0.24	2.05 ± 0.35	2.05 ± 0.32	2.63 ± 0.50** (↑44%)
ASAT (U/L)	13	82.1 ± 11.7	82.4 ± 15.9	79.8 ± 18.9	87.0 ± 14.4
	26	78.8 ± 11.9	80.1 ± 10.3	89.5 ± 31.8	89.9 ± 14.9 (↑14%)
	52	70.9 ± 10.6	77.6 ± 12.0	79.7 ± 16.4	91.8 ± 17.8** (↑29%)
ALAT (U/L)	13	36.8 ± 7.4	36.3 ± 8.9	38.1 ± 8.5	34.8 ± 7.1
	26	32.8 ± 6.3	33.9 ± 4.2	34.8 ± 6.1	36.0 ± 5.7
	52	30.7 ± 5.9	34.5 ± 5.0	31.5 ± 3.6	31.8 ± 8.0
LDH (U/L)	13	173.9 ± 82.6	107.0 ± 21.5**	114.8 ± 19.5**	165.6 ± 45.7
	26	166.2 ± 51.9	162.6 ± 42.8	192.6 ± 166.3	269.4 ± 76.1** (↑62%)
	52	109.7 ± 21.8	126.8 ± 28.4	123.3 ± 27.7	181.3 ± 40.8** (↑65%)
ALP (U/L)	13	64.6 ± 18.3	59.6 ± 9.7	59.4 ± 6.5	65.3 ± 13.1
	26	46.4 ± 12.9	45.6 ± 7.0	46.6 ± 5.7	52.2 ± 9.7 (↑13%)
	52	46.7 ± 11.1	48.6 ± 7.6	49.3 ± 9.3	57.0 ± 13.2** (↑22%)
CK (U/L)	13	171.9 ± 60.3	138.1 ± 30.2	128.5 ± 39.5	195.4 ± 120.4
	26	165.3 ± 92.1	132.1 ± 45.2	154.0 ± 78.8	168.6 ± 51.3
	52	91.4 ± 27.4	97.0 ± 25.2	97.8 ± 26.4	146.7 ± 64.6** (↑61%)
Na (mmol/L)	13	151.8 ± 7.7	148.9 ± 4.1	148.5 ± 2.9	149.9 ± 5.4
	26	145.5 ± 0.9	145.6 ± 0.8	147.0 ± 1.4** (↑1%)	149.2 ± 1.0** (↑3%)
	52	146.2 ± 1.2	147.2 ±0.9* (↑1%)	149.2 ± 1.5** (↑2%)	152.0 ± 1.7** (↑4%)
K (mmol/L)	13	4.01 ± 0.41	3.91 ± 0.20	3.90 ± 0.19	4.05 ± 0.31
	26	3.81 ± 0.23	3.79 ± 0.21	3.88 ± 0.29	4.11 ± 0.28** (↑8%)
	52	3.83 ± 0.19	3.88 ± 0.20	3.93 ± 0.23	4.27 ± 0.34** (↑12%)
Cl (mmol/L)	13	108.4 ± 5.6	107.2 ± 3.1	106.6 ± 2.1	105.5 ± 3.7* (↓3%)
	26	102.7 ± 0.9	102.9 ± 0.9	103.5 ± 1.8	103.0 ± 1.2
	52	102.6 ±	104 ± 1.0** (↑1%)	106.1 ± 1.6** (↑3%)	105.3 ± 1.3** (↑3%)
Ca (mmol/L)	13	2.77 ± 0.07	2.76 ± 0.08	2.74 ± 0.07	2.83 ± 0.06
	26	2.75 ± 0.08	2.76 ± 0.05	2.74 ± 0.09	2.89 ± 0.06** (↑5%)
	52	2.74 ± 0.06	2.77 ± 0.06	2.80 ± 0.05** (↑2%)	2.94 ± 0.07** (↑7%)
P (mmol/L)	13	2.01 ± 0.17	2.01 ± 0.16	2.03 ± 0.20	2.18 ±** (↑8%)
	26	1.76 ± 0.15	1.82 ± 0.16	1.74 ± 0.19	1.98 ± 0.15** (↑12%)
	52	1.41 ± 0.17	1.47 ± 0.15	1.55 ± 0.14* (↑10%)	1.57 ± 0.16** (↑11%)
Protein (g/L)	13	74.44 ± 5.90	72.43 ± 4.07	71.34 ± 2.71	75.45 ± 4.82
	26	73.87 ± 2.28	74.27 ± 2.79	74.57 ± 2.65	80.53 ± 3.16**
	52	73.96 ± 1.80	75.88 ± 2.32* (↑3%)	76.64 ± 2.23** (↑4%)	82.91 ± 2.68** (↑12%)

Parameter	Study Week	Dose (ppm)			
		0	30	200	1200
Albumin (g/L)	13	45.45 ± 2.79	44.27 ± 1.60	43.88 ± 1.33*	46.30 ± 1.97
	26	42.84 ± 1.35	42.81 ± 1.53	43.37 ± 1.52	46.02 ± 1.20** (↑7%)
	52	42.59 ± 1.41	42.40 ± 1.43	43.48 ± 1.53	46.00 ± 1.50** (↑8%)
Globulin (g/L)	13	28.99 ± 3.61	28.17 ± 3.13	27.46 ± 1.98	29.04 ± 3.30
	26	31.03 ± 1.78	31.46 ± 2.26	31.20 ± 2.04	34.52 ± 2.73** (↑11%)
	52	31.37 ± 1.83	33.48 ± 2.06** (↑7%)	33.16 ± 2.09* (↑6%)	36.91 ± 2.52** (↑18%)

m: milli (10^{-3}); mol: mole; U: unit; g: gram; *: significant at 5%; **: significant at 1%; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 372-377 of the study report. No standard deviation values were provided in the study report and were calculated by the reviewer.

Table 12: Selected clinical chemistry parameters – Female rats (n = 20)

Parameter	Study Week	Dose (ppm)			
		0	30	200	1200
Cholesterol (mmol/L)	13	1.88 ± 0.34	1.64 ± 0.36	2.02 ± 0.47	2.16 ± 0.44
	26	2.40 ± 0.45	2.02 ± 0.44	2.58 ± 0.69	2.53 ± 0.50
	52	2.25 ± 0.53	2.11 ± 0.63	2.63 ± 0.60	2.67 ± 0.59
Triglycerides (mmol/L)	13	0.24 ± 0.05	0.24 ± 0.06	0.27 ± 0.07	0.26 ± 0.06
	26	0.32 ± 0.07	0.33 ± 0.07	0.35 ± 0.10	0.32 ± 0.06
	52	0.30 ± 0.08	0.33 ± 0.10	0.35 ± 0.08	0.40 ± 0.14** (↑33%)
Phospholipids (mmol/L)	13	2.19 ± 0.35	2.09 ± 0.36	2.32 ± 0.45	2.51 ± 0.41* (↑15%)
	26	2.29 ± 0.34	2.00 ± 0.37	2.41 ± 0.52	2.35 ± 0.41
	52	1.96 ± 0.37	1.97 ± 0.49	2.21 ± 0.60	2.47 ± 0.39** (↑26%)
ASAT (U/L)	13	90.6 ± 19.0	79.0 ± 12.6*	84.3 ± 15.3	79.6 ± 11.5 (↓12%)
	26	94.0 ± 13.9	90.9 ± 38.7	85.6 ± 14.2	75.7 ± 6.0* (↓19%)
	52	116.2 ± 39.4	101.5 ± 25.6	105.3 ± 28.4	89.3 ± 25.7* (↓23%)
ALAT (U/L)	13	34.4 ± 9.0	29.2 ± 10.8	27.4 ± 3.4* (↓20%)	28.1 ± 4.0* (↓18%)
	26	30.7 ± 7.7	27.6 ± 8.1	28.1 ± 4.5 (↓8%)	25.3 ± 3.7* (↓18%)
	52	40.4 ± 11.9	38.5 ± 12.0	32.9 ± 9.6 (↓19%)	28.6 ± 6.0** (↓29%)
LDH (U/L)	13	125.0 ± 24.0	115.9 ± 23.2	121.0 ± 24.1	157.8 ± 55.5* (↑26%)
	26	193.6 ± 89.4	189.9 ± 139.1	143.4 ± 38.7	179.6 ± 80.5
	52	131.3 ± 52.5	126.6 ± 39.5	160.6 ± 110.7	149.9 ± 83.1
ALP (U/L)	13	31.3 ± 8.2	27.9 ± 6.4	28.0 ± 7.3	34.7 ± 9.4
	26	19.7 ± 4.9	17.4 ± 4.1	17.0 ± 4.2	19.4 ± 4.9
	52	17.8 ± 5.6	17.0 ± 3.6	18.1 ± 4.2	18.5 ± 4.3
CK (U/L)	13	132.7 ± 39.7	140.0 ± 68.1	138.9 ± 57.2	153.3 ± 60.8 (↑15%)
	26	136.0 ± 49.9	116.1 ± 48.4	120.4 ± 39.9	135.8 ± 57.8
	52	108.5 ± 94.9	97.6 ± 55.9	123.8 ± 68.7	124.6 ± 81.3
Na (mmol/L)	13	148.8 ± 2.5	144.9 ± 2.0**	146.7 ± 1.4**	148.9 ± 1.1
	26	148.1 ± 1.8	143.1 ± 1.1**	144.9 ± 1.5**	146.4 ± 1.1**
	52	144.7 ± 1.4	145.0 ± 0.8	147.2 ± 1.7** (↑2%)	151.5 ± 1.3** (↑5%)
K (mmol/L)	13	3.48 ± 0.32	3.26 ± 0.28	3.34 ± 0.30	3.52 ± 0.31
	26	3.19 ± 0.29	3.29 ± 0.40	3.29 ± 0.27	3.43 ± 0.24
	52	3.44 ± 0.32	3.48 ± 0.31	3.53 ± 0.25	3.51 ± 0.38

Parameter	Study Week	Dose (ppm)			
		0	30	200	1200
Cl (mmol/L)	13	108.0 ± 2.2	105.5 ± 1.7**	106.2 ± 1.8**	107.1 ± 1.4
	26	104.8 ± 1.7	102.3 ± 1.5**	103.2 ± 1.6**	103.9 ± 1.3
	52	102.6 ± 1.8	102.8 ± 1.1	104.8 ± 2.1** (↑2%)	106.5 ± 1.4** (↑4%)
Ca (mmol/L)	13	2.77 ± 0.09	2.66 ± 0.11**	2.68 ± 0.07**	2.70 ± 0.09
	26	2.82 ± 0.10	2.76 ± 0.08	2.78 ± 0.06	2.79 ± 0.06
	52	2.73 ± 0.07	2.80 ± 0.10* (↑3%)	2.80 ± 0.08* (↑3%)	2.87 ± 0.09** (↑5%)
P (mmol/L)	13	1.41 ± 0.22	1.46 ± 0.23	1.61 ± 0.32*	1.57 ± 0.21
	26	1.47 ± 0.36	1.45 ± 0.28	1.44 ± 0.22	1.36 ± 0.26
	52	1.32 ± 0.29	1.35 ± 0.18	1.40 ± 0.14	1.40 ± 0.28
Protein (g/L)	13	77.51 ± 3.62	77.28 ± 3.96	76.55 ± 3.93	77.51 ± 3.16
	26	81.37 ± 3.92	78.29 ± 3.44*	78.39 ± 4.16*	78.46 ± 3.52*
	52	78.12 ± 3.08	79.17 ± 3.96	79.38 ± 3.40	80.39 ± 3.23
Albumin (g/L)	13	51.50 ± 3.26	52.18 ± 2.35	50.05 ± 3..07	49.95 ± 2.96
	26	52.31 ± 3.20	51.52 ± 2.18	50.64 ± 2.97	49.87 ± 2.82*
	52	48.89 ± 2.82	50.49 ± 2.36	49.51 ± 2.50	50.85 ± 2.44
Globulin (g/L)	13	25.95 ± 1.50	24.98 ± 1.92	26.50 ± 2.29	27.56 ± 1.09*
	26	29.06 ± 1.75	26.77 ± 1.89**	27.75 ± 2.24	28.59 ± 1.47
	52	29.23 ± 2.10	28.68 ± 2.40	30.26 ± 2.16	29.54 ± 2.36

m: milli (10^{-3}); mol: mole; U: unit; g: gram; *: significant at 5%; **: significant at 1%; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 372-377 of the study report. No standard deviation values were provided in the study report and were calculated by the reviewer.

G. URINALYSIS

The levels of urinary ketones in males at 1200 ppm were statistically significantly increased at 13 and 26 weeks; the increase at 52 weeks was not statistically significant (Table 13). Ketone levels were also increased in high dose females at weeks 26 and 52, although these changes were not statistically significant. Levels of leukocytes were increased in all male dose groups at week 13, 26 and 52. Changes at the low and mid doses were not considered to be treatment-related due to low control values (compared to historical controls), lack of dose response and/or high standard deviations. No treatment-related changes in leukocytes were noted in females. Levels of leukocytes appeared to be increased at week 52 in 1200 ppm females; however, the standard deviation was notably high due to one outlier. Without the outlier value, the mean leukocyte count would be 6.6 (± 11.3).

Table 13: Urinalysis – mean ± standard deviation of selected parameters (n = 20)

Males			Females		
Week 13	**Ketones** mmol/l	**Leukocytes** per µl	**Week 13**	**Ketones** mmol/l	**Leukocytes** per µl
Historical Control: age 13-18 wks (n = 869)	0.5 ± 0.6	24 ± 27	Historical Control: age 13-18 wks (n = 915)	0.2 ± 0.3	4 ± 10
Group 1	0.3 ± 0.38	13 ± 12.8	Group 1	0.1 ± 0.15	3 ± 7.7
Group 2	0.2 ± 0.26	31 ± 37.1 (↑138%)	Group 2	0.1 ± 0.18	5 ± 10.3
Group 3	0.6 ± 0.59	36* ± 33.9 (↑177%)	Group 3	0.1 ± 0.22	5 ± 10.3
Group 4	1.0** ± 1.1 (↑233%)	49** ± 39.3 (↑277%)	Group 4	0.1 ± 0.18	8 ± 11.8
Week 26			**Week 26**		
Historical Control: age 19-40 wks (n = 1600)	0.6 ± 0.6	28 ± 41	Historical Control: age 19-40 wks (n = 1630)	0.2 ± 0.3	6 ± 21
Group 1	0.6 ± 0.65	21 ± 21.9	Group 1	0.3 ± 0.37	11± 12.8
Group 2	0.8 ± 0.62	66 ± 109.5 (↑214%)	Group 2	0.3 ± 0.25	11 ± 23.6
Group 3	1.1 ±0.65	45 ± 37.7 (↑114%)	Group 3	0.3 ± 0.38	18 ± 30.5
Group 4	2.1** ± 1.8 (↑250%)	63* ± 43.3 (↑200%)	Group 4	0.4 ± 0.52 (↑33%)	5 ± 10.3
Week 52			**Week 52**		
Historical Control: age 41-70 wks (n = 259)	0.5 ± 0.4	102 ± 156	Historical Control: age 41-70 wks (n = 261)	0.3 ± 0.3	58 ± 121
Group 1	1.4 ± 1.3	78 ± 106.7	Group 1	0.3 ± 0.39	14 ± 24.6
Group 2	0.8 ± 0.63	124 ± 170.9 (↑59%)	Group 2	0.3 ± 0.38	9 ± 12.2
Group 3	1.0 ± 0.55	114 ± 169.8 (↑46%)	Group 3	0.3 ± 0.47	18 ± 30.5
Group 4	2.3 ± 1.9 (↑64%)	146 ± 156.1 (↑87%)	Group 4	0.4 ± 0.36 (↑33%)	31 ± 110.9

Data obtained from pages 383 – 386 of the study report. ( ) = % difference from control, calculated by the reviewer. *: significant at 5%; **: significant at 1%; standard deviations calculated by PMRA reviewer.

H. SACRIFICE AND PATHOLOGY

1. Organ Weights

After 52 weeks, the absolute and relative weights of livers, kidneys and adrenals were increased in males at 1200 ppm (Table 14). The kidney to body weight ratio was increased (9%) and the kidney to brain weight ratio was slightly increased (4%) in females at 1200 ppm (Table 15). The increased heart to body weight ratio in males at 1200 ppm was considered to be due to the changes in body weights.

After 104 weeks, the absolute and relative weights of livers were increased in females at 1200 ppm (Table 17). Absolute and relative kidney weights in females at 1200 ppm were also increased (only relative to body weight was statistically significant). Decreases in absolute and relative spleen and adrenal weights were recorded at all dose levels in male groups but were not considered to be treatment related due to lack of dose response (Table 16). In males at 1200 ppm (body weight-) relative brain, heart, liver and kidney weights were increased. These increases were considered to be due to lower body weights recorded in this group.

Table 14: Selected organ weights at week 52 – Male rats (Mean ± SD)

Organ	0 ppm n = 20	30 ppm n = 20	200 ppm n = 20	1200 ppm n = 20
Body weight	595.8 ± 63.9	595.0 ± 65.1	592.4 ± 68.6	560.8 ± 50.0 (↓6%)
Brain Abs.	2.20 ± 0.09	2.23 ± 0.09	2.19 ± 0.10	2.19 ± 0.07
Rel. to body wt (%)	0.37 ± 0.04	0.38 ± 0.04	0.37 ± 0.04	0.39 ± 0.03
Heart Abs.	1.25 ± 0.15	1.31 ± 0.16	1.28 ± 0.15	1.32 ± 0.16
Rel. to body wt (%)	0.21 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.24 ± 0.02** (↑14%)
Rel. to brain wt (%)	56.67 ± 5.73	58.78 ± 5.85	58.19 ± 5.81	60.38 ± 6.86
Liver Abs.	14.34 ± 1.51	15.55 ± 2.24	14.60 ± 2.39	16.03 ± 2.05* (↑12%)
Rel. to body wt (%)	2.42 ± 0.30	2.61 ± 0.20	2.46 ± 0.23	2.86 ± 0.30** (↑18%)
Rel. to brain wt (%)	651.91 ± 65.49	698.72 ± 100.33	664.62 ± 90.93	732.47 ± 88.63** (↑12%)
Kidneys Abs.	2.48 ± 0.23	2.61 ± 0.30	2.56 ± 0.34	2.95 ± 0.36** (↑19%)
Rel. to body wt (%)	0.42 ± 0.06	0.44 ± 0.03	0.43 ± 0.04	0.53 ± 0.05** (↑26%)
Rel. to brain wt (%)	112.67 ± 9.87	117.31 ± 12.28	116.51 ± 12.66	134.91 ± 15.13** (↑20%)
Adrenals Abs.	0.056 ± 0.007	0.058 ± 0.010	0.057 ± 0.009	0.064 ± 0.010* (↑14%)
Rel. to body wt (%)	0.010 ± 0.002	0.010 ± 0.002	0.010 ± 0.002	0.011 ± 0.002** (↑10%)
Rel. to brain wt (%)	2.554 ± 0.295	2.595 ± 0.428	2.610 ± 0.420	2.908 ± 0.458* (↑14%)
Spleen Abs.	1.11 ± 0.17	1.07 ± 0.26	1.06 ± 0.19	1.06 ± 0.16
Rel. to body wt (%)	0.19 ± 0.03	0.18 ± 0.03	0.18 ± 0.03	0.19 ± 0.02
Rel. to brain wt (%)	50.34 ± 7.81	47.97 ± 11.01	48.15 ± 8.15	48.43 ± 7.37

* and **: Dunnett-test based on pooled variance significance at 5% and 1%, respectively; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 388 to 399 of the study report.

Table 15: Selected organ weights at week 52 – Female rats (Mean ± SD)

Organ	0 ppm n = 18	30 ppm n = 20	200 ppm n = 20	1200 ppm n = 20
Body weight	310.4 ± 33.4	319.9 ± 45.0	306.9 ± 48.5	287.3 ± 32.7 (↓7%)
Brain Abs.	2.05 ± 0.08	2.02 ± 0.09	2.02 ± 0.08	1.98 ± 0.07*
Rel. to body wt (%)	0.67 ± 0.07	0.64 ± 0.08	0.67 ± 0.09	0.70 ± 0.07
Heart Abs.	0.88 ± 0.07	0.84 ± 0.07	0.85 ± 0.09	0.85 ± 0.06
Rel. to body wt (%)	0.28 ± 0.02	0.26 ± 0.03*	0.28 ± 0.02	0.30 ± 0.02
Rel. to brain wt (%)	42.75 ± 3.02	41.41 ± 2.81	42.02 ± 3.93	42.82 ± 2.94
Liver Abs.	8.95 ± 0.80	8.68 ± 0.93	8.79 ± 1.35	8.70 ± 0.95
Rel. to body wt (%)	2.89 ± 0.18	2.73 ± 0.22	2.88 ± 0.27	3.04 ± 0.28
Rel. to brain wt (%)	436.03 ± 37.14	429.86 ± 44.72	435.61 ± 65.14	438.52 ± 43.67
Kidneys Abs.	1.71 ± 0.18	1.64 ± 0.14	1.65 ± 0.22	1.72 ± 0.15
Rel. to body wt (%)	0.55 ± 0.05	0.52 ± 0.07	0.54 ± 0.06	0.60 ± 0.07* (↑9%)
Rel. to brain wt (%)	83.41 ± 8.64	81.20 ± 5.80	81.96 ± 10.44	86.69 ± 8.56 (↑4%)
Adrenals Abs.	0.073 ± 0.011	0.064 ± 0.012*	0.065 ± 0.011	0.066 ± 0.009
Rel. to body wt (%)	0.024 ± 0.004	0.020 ± 0.004*	0.021 ± 0.005	0.023 ± 0.003
Rel. to brain wt (%)	3.551 ± 0.514	3.146 ± 0.574	3.206 ± 0.541	3.327 ± 0.462
Spleen Abs.	0.79 ± 0.16	0.77 ± 0.12	0.77 ± 0.15	0.80 ± 0.62
Rel. to body wt (%)	0.26 ± 0.06	0.25 ± 0.05	0.25 ± 0.04	0.27 ± 0.18
Rel. to brain wt (%)	38.57 ± 7.12	38.12 ± 5.25	38.19 ± 7.45	40.43 ± 30.78

* and **: Dunnett-test based on pooled variance significance at 5% and 1%, respectively; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 388 to 399 of the study report.

Table 16: Selected organ weights at week 104 – Male rats (Mean ± SD)

Organ	0 ppm n = 39	30 ppm n = 42	200 ppm n = 35	1200 ppm n = 38
Body weight	688.8 ± 82.2	674.7 ± 86.1	651.3 ± 90.7	623.4 ± 96.8** (↓9%)
Brain Abs.	2.26 ± 0.09	2.24 ± 0.11	2.24 ± 0.12	2.24 ± 0.10
Rel. to body wt (%)	0.33 ± 0.04	0.34 ± 0.05	0.35 ± 0.05	0.37 ± 0.06** (↑12%)
Heart Abs.	1.42 ± 0.21	1.35 ± 0.15	1.36 ± 0.17	1.42 ± 0.16
Rel. to body wt (%)	0.21 ± 0.03	0.20 ± 0.04	0.21 ± 0.02	0.23 ± 0.04** (↑10%)
Rel. to brain wt (%)	62.67 ± 8.57	60.45 ± 7.27	60.87 ± 6.94	63.16 ± 7.29
Liver Abs.	18.12 ± 3.40	17.12 ± 2.32	16.56 ± 2.88*	18.40 ± 2.55
Rel. to body wt (%)	2.63 ± 0.33	2.56 ± 0.39	2.55 ± 0.27	2.98 ± 0.38** (↑13%)
Rel. to brain wt (%)	799.93 ± 136.77	765.04 ± 109.42	740.53 ± 124.31	821.27 ± 117.29
Kidneys Abs.	3.35 ± 1.18	3.25 ± 1.06	3.06 ± 0.54	3.46 ± 0.42
Rel. to body wt (%)	0.48 ± 0.13	0.49 ± 0.20	0.47 ± 0.05	0.56 ± 0.09* (↑17%)
Rel. to brain wt (%)	147.81 ± 48.86	145.47 ± 49.03	136.90 ± 23.79	154.35 ± 19.20
Adrenals Abs.	0.137 ± 0.428	0.071 ± 0.027 (↓48%)	0.066 ± 0.012 (↓52%)	0.087 ± 0.118 (↓37%)
Rel. to body wt (%)	0.020 ± 0.065	0.011 ± 0.005 (↓45%)	0.010 ± 0.002 (↓50%)	0.014 ± 0.017 (↓30%)
Rel. to brain wt (%)	5.996 ± 18.583	3.189 ± 1.295 (↓47%)	2.978 ± 0.566 (↓50%)	3.852 ± 5.010 (↓36%)
Spleen Abs.	1.72 ± 0.64	1.45 ± 0.41* (↓16%)	1.36 ± 0.33** (↓21%)	1.41 ± 0.44* (↓18%)
Rel. to body wt (%)	0.25 ± 0.09	0.21 ± 0.06 (↓16%)	0.21 ± 0.04* (↓16%)	0.23 ± 0.06 (↓8%)
Rel. to brain wt (%)	76.30 ± 29.45	64.41 ± 17.82* (↓16%)	60.93 ± 14.48** (↓20%)	62.97 ± 19.49* (↓17%)

* and **: Dunnett-test based on pooled variance significance at 5% and 1%, respectively. ( ) = % different from control, calculated by the reviewer. Data obtained from pages 400 to 411 of the study report.

Table 17: Selected organ weights at week 104 – Female rats (Mean ± SD)

Organ	0 ppm n = 36	30 ppm n = 36	200 ppm n = 33	1200 ppm n = 38
Body weight	386.4 ± 58.5	399.8 ± 58.8	387.2 ± 64.6	368.6 ± 59.4 (↓5%)
Brain Abs.	2.06 ± 0.15	2.06 ± 0.09	2.01 ± 0.09	2.04 ± 0.10
Rel. to body wt (%)	0.54 ± 0.09	0.52 ± 0.08	0.53 ± 0.07	0.57 ± 0.09
Heart Abs.	0.97 ± 0.12	0.96 ± 0.09	0.97 ± 0.10	0.97 ± 0.11
Rel. to body wt (%)	0.25 ± 0.03	0.24 ± 0.03	0.25 ± 0.03	0.27 ± 0.03
Rel. to brain wt (%)	47.14 ± 5.92	46.76 ± 4.06	48.10 ± 4.19	47.68 ± 5.91
Liver Abs.	10.48 ± 1.76	10.64 ± 1.84	10.57 ± 1.70	11.81 ± 2.43* (↑13%)
Rel. to body wt (%)	2.72 ± 0.29	2.67 ± 0.31	2.75 ± 0.35	3.21 ± 0.42** (↑18%)
Rel. to brain wt (%)	511.98 ± 89.37	516.53 ± 88.76	524.40 ± 74.42	579.47 ± 127.8* (↑13%)
Kidneys Abs.	2.03 ± 0.25	2.06 ± 0.26	2.06 ± 0.24	2.15 ± 0.29 (↑6%)
Rel. to body wt (%)	0.53 ± 0.07	0.52 ± 0.08	0.54 ± 0.07	0.59 ± 0.07** (↑11%)
Rel. to brain wt (%)	98.95 ± 11.85	100.18 ± 12.36	102.45 ± 10.35	105.38 ± 14.98 (↑7%)
Adrenals Abs.	0.069 ± 0.016	0.071 ± 0.014	0.069 ± 0.015	0.067 ± 0.012
Rel. to body wt (%)	0.018 ± 0.005	0.018 ± 0.004	0.018 ± 0.005	0.018 ± 0.004
Rel. to brain wt (%)	3.370 ± 0.774	3.432 ± 0.721	3.448 ± 0.706	3.268 ± 0.648
Spleen Abs.	0.91 ± 0.18	0.95 ± 0.26	0.95 ± 0.23	0.95 ± 0.24
Rel. to body wt (%)	0.24 ± 0.04	0.24 ± 0.06	0.25 ± 0.07	0.26 ± 0.04
Rel. to brain wt (%)	44.17 ± 8.53	46.24 ± 12.28	46.95 ± 10.87	46.37 ± 12.46

* and **: Dunnett-test based on pooled variance significance at 5% and 1%, respectively; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 400 to 411 of the study report.

2. Gross and Histopathology

No gross lesions attributed to the treatment were noted at the interim kill after 52 weeks of dosing. All gross lesions were within the range of normal background alterations.

After 104 weeks (terminal sacrifice), an increased number of focus/foci in the lungs were recorded in animals at 1200 ppm (statistically significant in males) (Table 18). Slight increases in incidences of foci and cysts in livers of females at 1200 ppm, and foci in the prostate of males at 200 and 1200 ppm were also noted.

All remaining findings recorded were regularly distributed among control and treated groups, and were considered to be within the range of normal background lesions, which may be seen in rats of the used strain and age.

Table 18: Incidence of selected macroscopic findings after 104 weeks

Findings	0 ppm n = 50		30 ppm n = 50		200 ppm n = 50		1200 ppm n = 50	
	M	F	M	F	M	F	M	F
Lung: Focus/foci	3 [6%]	4 [8%]	5 [10%]	8 [16%]	5 [10%]	9 [18%]	15** [30%]	11 [22%]
Liver: Focus/foci	7 [14%]	0 [0%]	9 [18%]	2 [4%]	6 [12%]	3 [6%]	10 [20%]	4 [8%]
Cysts	0 [0%]	3 [6%]	1 [2%]	1 [2%]	1 [2%]	1 [2%]	1 [2%]	8 [16%]
Prostate: Focus/foci	1 [2%]		3 [6%]		5 [10%]		5 [10%]	

** significant p <0.001 (Fisher's Exact). Data obtained from pages 419 – 431 and 1733 of the study report. [ ] = % incidence, calculated by the reviewer.

There were no treatment-related tumors recorded. All neoplastic findings were within the range of normal background lesions which may be recorded in animals of the used strain and age.

Non-neoplastic lesions were noted in the esophagus at sacrifice after both 52 and 104 weeks, and in the lungs, kidneys and pharynx after 104 weeks only.

After 52 weeks, hyperkeratosis of the esophagus was recorded at a minimal severity grade in animals of both sexes at 1200 ppm (Table 19). Statistical significance was recognized in both males and females by the Fisher's Exact (males $p<0.01$; females $p<0.05$) and Armitage trend test ($p<0.01$) for both sexes.

After 104 weeks, an increased incidence of hyperkeratosis was recorded in both males and females at 200 and 1200 ppm. Statistical significance was recognized in both males and females (Fisher's Exact $p<0.01$) at 1200 ppm only and there was a positive trend in both sexes by the Armitage trend test ($p<0.01$). A dose-related increase in severity was not observed.

Table 19: Incidence / mean severity grade of hyperkeratosis of the esophagus

	0 ppm		30 ppm		200 ppm		1200 ppm	
52 weeks (N=20)								
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Grade 1	-	-	-	-	-	-	12**	6*
Total affected	0	0	0	0	0	0	12	6
Mean severity	-	-	-	-	-	-	1.0	1.0
104 weeks (N=48-50)								
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Grade 1	3	1	2	3	7	6	20	14
Grade 2	-	1	-	-	-	2	-	5
Grade 3	-	-	-	-	-	-	1	1
Total affected	3	2	2	3	7	8	21**	20**
Mean severity	1.0	1.5	1.0	1.0	1.0	1.3	1.1	1.4

For details regarding tests for statistical significance, see the text above. Data obtained from pages 47, 1735, and 1744-1745 of the study report.

There were no microscopic findings in the lungs after 52 weeks of treatment. After 104 weeks, there was an increase in incidence of chronic interstitial inflammation in the lungs recorded in both males and females at 1200 ppm and females at 200 ppm, which was only statistically significant in the high dose male group as compared to the control group by Fisher's Exact pairwise comparison ($p<0.01$) (Table 20). Statistical significance was recognized in both males and females by Armitage trend test ($p<0.01$). The chronic interstitial inflammation was characterized by focal/multifocal changes consisting of interstitial or intra-alveolar inflammatory cells associated with hypertrophied reactive type II pneumocytes. These lesions were associated with foamy intra-alveolar macrophages, which were slightly increased in mean severity grade when compared to the lungs of control male and female rats. The lesions were non-hyperplastic.

Table 20: Incidence and severity grade of microscopic findings in the lungs (104 weeks)

Severity	0 ppm		30 ppm		200 ppm		1200 ppm	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Chronic interstitial inflammation								
Grade 1	1	3	3	1	1	4	3	7
Grade 2	1	-	1	-	1	3	10	-
Grade 3	-	-	-	-	-	-	-	2
Total affected	2	3	4	1	2	**7**	**13****	**9**
Mean severity	1.5	1.0	1.3	1.0	1.5	1.4	1.8	1.4
Alveolar macrophages								
Grade 1	29	30	21	23	10	26	17	24
Grade 2	5	1	4	10	15	13	14	6
Grade 3	-	-	1	-	1	-	1	3
Total affected	34	31	26	33	26	39	32	33
Mean severity	1.1	1.0	1.2	1.3	1.7	1.3	1.5	1.4

N=49-50. For details regarding tests for statistical significance, see the text above. Data obtained from pages 46-47 and 1734, 1735, and 1744-1745 of the study report.

At 104 weeks, slight increases were observed in chronic nephropathy, tubular basophilia and mononuclear infiltrates in the kidneys and mononuclear infiltrates in the pharynx of females treated at 1200 ppm as compared to controls (Table 21). These tissues were not examined in all animals from the low- and mid-dose groups.

Table 21: Incidence of non-neoplastic lesions in the kidney and pharynx (104 weeks)

Findings	0 ppm		30 ppm		200 ppm		1200 ppm	
	M	F	M	F	M	F	M	F
Kidney –	**n = 50**	**n = 50**	**n = 13**	**n = 16**	**n = 17**	**n = 16**	**n = 50**	**n = 50**
Chronic nephropathy	41 (82%)	8 (16%)	6 (46%)	3 (19%)	8 (47%)	4 (25%)	38 (76%)	12 (24%)
Tubular basophilia	6 (12%)	11 (22%)	2 (15%)	3 (19%)	2 (12%)	5 (31%)	9 (18%)	19 (38%)
Mononuclear foci	4 (8%)	14 (28%)	4 (31%)	6 (38%)	3 (18%)	5 (32%)	5 (10%)	21 (42%)
Pharynx –	**n = 38**	**n =48**	**--**	**n = 11**	**--**	**n = 14**	**n = 42**	**n = 46**
Mononuclear infiltrates	8 (21%)	7 (15%)	--	1 (9%)	--	2 (14%)	4 (10%)	14 (30%)

Data obtained from pages 1721 – 1783. ( ) = % incidence, calculated by the reviewer.

3. Additional Investigations

3.1 Liver Enzyme Determinations

Slight, non- significant decreases in hepatic alanine aminotransferase (ALAT) activity were observed in males at all dose levels, but were not considered to be related to treatment due to the lack of dose response. Decreases in 7-ethoxy-resorufin O-dealkylase (CYP1A1) activities were noted in females (Table 22), reaching statistical significance at 30 and 1200 ppm, but not at 200 ppm; as the response was not dose-dependent, these decreases were not considered to be related to treatment. The phase II enzymes uridine diphosphoglucuronosyl-transferase (UDPGT) and glutathione S-transferase (GST) were induced in females at 200 ppm and in both sexes at 1200 ppm, and epoxide hydrolase (mEH) was induced in all male and female dose groups. These enzymes were more significantly induced in females than males.

Table 22: Selected liver enzyme activities (Mean ± SD; % difference from of control)

Parameter	0 ppm		30 ppm		200 ppm		1200 ppm	
	M	F	M	F	M	F	M	F
ALAT (U/g liver)	93.0 ± 32.2	47.2 ± 10.4	71.7 ± 20.4 (↓23%)	38.6 ± 9.3	69.8 ± 8.8 (↓25%)	46.3 ± 7.4	71.7 ± 18.8 (↓23%)	42.4 ± 2.4
CYP1A1 (nmol/min/ g liver)	2.21 ± 1.30	3.46 ± 1.78	1.91 ± 1.41 (↓14%)	0.89 ± 0.23 (↓74%)	2.52 ± 0.51 (↑14%)	2.22 ± 1.64 (↓36%)	3.94 ± 3.44 (↑78%)	1.13 ± 0.47 (↓67%)*
GST (nmol/min/ g liver)	38.7 ± 6.5	26.6 ± 5.3	40.7 ± 3.7	30.3 ± 3.5	44.2 ± 2.5	38.0 ± 9.0 (↑43%)*	53.3 ± 6.0 (↑38%)**	40.7 ± 2.4 (↑54%)**
UDPGT (µmol/min/ g liver)	1.44 ± 0.25	0.91 ± 0.23	1.47 ± 0.42	0.98 ± 0.21	1.62 ± 0.21	1.27 ± 0.65 (↑40%)	2.36 ± 0.61 (↑64%)*	1.67 ± 0.35 (↑84%)*
mEH (nmol/min/ g liver)	177 ± 21.4	43.6 ± 15.8	242 ± 80.8 (↑37%)	54.9 ± 16.6 (↑26%)	215 ± 42.2 (↑22%)	92.5 ± 29.6 (↑112%)**	351 ± 43.6 (↑98%)**	97.3 ± 27.3 (↑123%)**

ALAT: alanine aminotransferase in 100g supernatants; GST: cytosolic glutathione S-transferase activity; UDPGT: microsomal uridine diphospho-glucuronosyl transferase activity; mEH: microsomal epoxide hydrolase activity; * and **: significantly different from control (Dunnett's t-test) at 5% and 1%, respectively. Data obtained from pages 3512-3539 of the study report. n = 5. () = % difference from control, calculated by the reviewer.

3.2 Fluoride Determination

Treatment with fluensulfone for 52 weeks resulted in statistically significant increases of fluoride content of ashes from bones and teeth of male and female rats receiving 200 or 1200 ppm fluensulfone in the diet, compared to controls (Table 23). Comparatively slight increases in fluoride content from bones were noted in males and females at 30 ppm. No increase of the fluoride content in ashes from teeth was recorded in animals treated with 30 ppm, compared to controls.

After 104 weeks of dosing, statistically significant increases of fluoride content in ashes from bones and teeth of male and female rats receiving 200 or 1200 ppm, and from bones only in rats receiving 30 ppm, compared to controls, were recorded. Comparatively slight increases in fluoride content from teeth were noted in males and females at 30 ppm.

Table 23: Fluoride content – ppm (Mean ± Relative SD %)

Parameter	0 ppm		30 ppm		200 ppm		1200 ppm	
	M	F	M	F	M	F	M	F
52 weeks								
Bones	577.1 ± 8.0	579.8 ± 6.7	647.2 ± 4.4 (↑12%)	743.9 ± 8.6 (↑28%)	1682 ± 9.3* (↑192%)	1896 ± 9.9* (↑227%)	5582 ± 8.2* (↑867%)	5316 ± 7.1* (↑817%)
Teeth	93.48 ± 13.7	123.5 ± 9.8	90.12 ± 15.2	99.75 ± 9.2	519.6 ± 9.4* (↑456%)	640.2 ± 14.0* (↑418%)	1854 ± 19.9* (↑1883%)	2006 ± 13.7* (↑1524%)
104 weeks								
Bones	398.7 ± 15.4	642.4 ± 7.9	720.2 ± 11.5* (↑81%)	945.0 ± 6.9* (↑47%)	2072 ± 11.9* (↑420%)	2560 ± 7.5* (↑299%)	6567 ± 3.6* (↑1547%)	6258 ± 5.0* (↑874%)
Teeth	135.3 ± 34.1	126.0 ± 9.7	162.9 ± 17.5 (↑20%)	209.2 ± 17.9 (↑66%)	484.4 ± 13.9* (↑258%)	567.5 ± 11.6* (↑350%)	1740 ± 16.0* (↑1186%)	1959 ± 8.8* (↑1455%)

*: significantly different from control (Dunnett's t-test) at 5%. Data obtained from pages 3571-3578 of the study report. ( ) = % different from control, calculated by the reviewer.

III. APPLICANT'S CONCLUSIONS

The NOAEL (No Observed Adverse Effect Level) for chronic toxicity was established at 200 ppm (11 mg/kg bw/day in males and 13.1 mg/kg bw/day in females) based on hematological findings, hyperkeratosis of the esophagus and decreased body weights and body weight gains at 1200 ppm. The effects on electrolytes, in some cases affecting all dose levels in a single gender (sodium in males, calcium in females) and the increase in protein concentration in males at all dose levels were not considered to be adverse due to the absence of other correlating findings.

The NOAEL for chronic treatment was established at 30 ppm (1.4 mg/kg bw/day in males and 1.7 mg/kg bw/day in females) based on chronic interstitial inflammation in the lungs of females, decreased body weight and body weight gain in males, and decreased hemoglobin concentration and hemoglobin concentration distribution width in females at 200 ppm.

Fluensulfone showed no carcinogenic potential in rats.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments**: This study in is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for a combined chronic/carcinogenicity study (OPPTS 870.4300; OECD 453) in rats.

C. **Conclusions**: For the current study, the Applicant established separate NOAELs for chronic toxicity and chronic treatment; however, this is not common practice for the regulatory authorities. In this chronic/oncogenicity study, the reviewer is in agreement with establishing the NOAEL at 200 ppm (9.6 mg/kg bw/day in males and 11.6 mg/kg bw/day in females) based on a LOAEL of 1200 ppm (57.7/69.9 mg/kg/day, M/F).. The increased incidence and severity of interstitial inflammation in the lungs of female rats at 200 and 1200 ppm were not considered treatment related. Interstitial inflammatory cells are a common observation in older rats, and ancillary pathologic changes associated with cell injury (e.g. pneumocyte hyperplasia or necrosis) were not observed here. However, there was a statistically significant increase in the incidence (2/50 in controls and 13/50 at 1200 ppm) and increased severity (1.5/4 in controls and 1.8/4 at 1200 ppm) of chronic interstitial inflammation at 1200 ppm in males that was considered treatment related. Additional adverse, treatment-related effects were noted in high dose animals, including hematological and clinical chemistry findings, and histopathological findings in the esophagus. In both males and females at 52 and 104 weeks, statistically significant, dose-related increases in fluoride levels compared to controls in the bone (192 to 1547%) and teeth (258 to 1883%) were observed at 200 and 1200 ppm; slight increases (12 to 88%) were seen in bone and teeth at 30 ppm. However, no structural changes related to fluoride deposition were reported in either tissue. The 11%/15% decrease in body weight/weight gain in 1200 ppm males and 6%/15% decrease in 1200 ppm females indicate that MTD (maximum tolerated dose) was reached at this this dose level. No oncogenic potential was noted in the study.

OCS does not consider that the decreases in male body weight and body weight gain at 200 ppm are of a sufficient magnitude to be considered adverse i.e. both up to 7% max over the study duration with decreases of ≤5% at study termination. OCS considers that the NOAEL in males is 200 ppm (9.6 mg/kg bw/day) based on the magnitude of the decreases seen in male body weight (11%) and body weight gain (21%), hematology and clinical chemistry findings, effects on liver, kidney and adrenal weight, and histopathological changes in the lungs and esophagus.

D. **Deficiencies**: None.

Tier II Summary (PMRA #2181218, MRID 48574766) revised by the regulatory authorities, April 2013

Note that the reviewing agencies have different conclusions with respect to the interpretation of the lung tumors in female mice (see section IV – Evaluation, Summary and Conclusions).

Study Type Carcinogenicity study in the mouse

IIA 5.5.3 – MRID 48574766, PMRA 2181218

Report:	Kaiser St. (2011) MCW-2 TECH: 78-Weeks Oncogenicity (Feeding) Study in CD-1 Mice. Harlan Laboratories Ltd., Switzerland; unpublished report No. B80190, dated 31 August 2011. Sponsor reference No. R-23354 Dates of experimental work: (in-life phase) 26 February 2008 to 4 September 2009
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines	OECD No. 451, EEC Method B.32, and OPPTS 870.4200 Deviations: none
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In an oral oncogenicity study (MRID 48574766; PMRA 2181218), 4 groups of 50 male and 50 female CD-1 mice (allocation A) were treated for 78 weeks with 0, 30, 200 or 1200 ppm of fluensulfone (MCW-2 tech., 96.7-98.65%, Batch No. 36372130-291-PF1) in their feed. Additionally, 6 males and 6 females (allocation B) were exposed to the same dietary concentrations and used for liver enzyme determination after 13 weeks of treatment. The average daily intakes of test material were 0, 4.2, 27.6 or 152.3 mg/kg bw/day for males and 0, 6.4, 39.0 or 188.4 mg/kg bw/day for females.

After 13 weeks of treatment, fluensulfone caused a slight decrease of hepatic alanine aminotransferase (ALAT) activity in females from the 1200 ppm dose group, but no change in ALAT activity was recorded after 78 weeks of treatment. In addition, after 13 weeks a weak increase of microsomal cytochrome P450 content was recorded, accompanied by a slight increase

in the activity of CYP4A-1 and CYP1A-1 in females only, and slight to moderate inductions of phase II enzymes uridine diphosphoglucuronosyl-transferase, glutathione S-transferase and epoxide hydrolase, mainly in females. Most of these changes occurred at 200 ppm and above, but were considered to be adaptive; none of these changes were considered adverse.

Treatment of CD-1 mice with fluensulfone at dietary concentrations of 30, 200 or 1200 ppm for up to 78 weeks had no effect on survival, clinical signs, or presence of palpable nodules and masses. Absolute food consumption (g/animal/day) was statistically significantly reduced at most weighing intervals in both sexes at 1200 ppm. The overall mean for animals treated with 1200 ppm compared to controls was 17% lower in males and 24% lower in females. In males, relative food consumption (g/kg bw/day) was not affected by the treatment. However, in females, relative food consumption was statistically significantly reduced at most weighing intervals at 1200 ppm and the overall mean over treatment was reduced by 21%. Cumulative body weight gain was decreased by 13% and 37% for males at 200 and 1200 ppm, respectively, and by 30% for females at 1200 ppm.

Absolute mean body weight and body weight gain were statistically significantly decreased in a dose-dependent manner in males at 200 and 1200 ppm (statistically significant from week 5 through 54 and throughout the study, respectively), and in females at 1200 ppm (statistically significant from week 2 onward for most measurements). The mean body weights at the end of the treatment period were reduced by 6% and 17% in the males of the mid and high dose groups, and by 12% in the females of the high dose group.

After 52 weeks, hematology revealed a slightly decreased absolute red blood cell counts in mice of both sexes at 1200 ppm (5% below controls) and decreased total white blood cells count in females only, accompanied by reductions in absolute neutrophil, eosinophil, lymphocyte and monocyte counts (19-25%). Total white blood cells and absolute neutrophil and eosinophil counts were also reduced in females at 200 ppm (19-43%). None of these changes was still present at terminal sacrifice after 78 weeks. Slight increases in serum ALAT and ASAT were noted in females from the 200 and 1200 ppm dose groups at week 78, and increases in SDH were noted in both sexes at 200 and 1200 ppm at 78 weeks (these parameters were not assessed at week 52).

Absolute and relative (to brain weight) prostate weights were observed in males in the 200 and 1200 ppm dose groups. Changes in other organ weights at 1200 ppm (increased relative kidney, adrenal gland, epididymal, testes, heart, and brain weight in males; decreased absolute kidney weight in females, and increased relative liver weight in females) were likely secondary to the effect of fluensulfone exposure on body weight.

Non-neoplastic lesions consisted of a statistically significantly increased incidence of bronchiolization (i.e. a change from flattened epithelium to cuboidal epithelium) in the lung of males and females treated at 200 and 1200 ppm (from control to high dose respectively, 1, 0, 24 and 31 in males and 5, 7, 43 and 48 in females; n = 50 all groups). Transmission electron

microscopy (TEM) analysis revealed hypertrophy of the epithelium of the terminal bronchioles affecting mostly the non-ciliated Clara cells as well as the directly surrounding ciliated cells.

The LOAEL was established at 200 ppm (27.35 and 38.96 mg/kg bw/day in males and females, respectively), based on decreased body weight/weight gain in males and an increased incidence of lung bronchiolization in males and females. The NOAEL was established at 30 ppm (4.2 and 6.4 mg/kg bw/day in males and females, respectively).

An increased incidence in nodules in the lung of females treated at 200 and 1200 ppm was recorded at terminal necropsy. The incidence of affected animals was 1, 6, 11 and 13 in the control, 30, 200 and 1200 ppm groups, respectively. Microscopically, the incidence of neoplastic lesions in the lungs was increased in females treated at 200 and 1200 ppm. The neoplastic lesions consisted of statistically significantly increased alveolar/bronchiolar adenomas in females treated at 200 and 1200 ppm, compared to control females (4, 8, 28 and 18%, control to high dose). The number of alveolar/bronchiolar carcinomas in females treated at 1200 ppm did not attain statistical significance using the Fisher's exact test, while it was positive with the Peto trend test (4, 2, 2 and 8%, control to high dose). The latency to tumor onset was also reduced for the carcinomas detected at 1200 ppm. The incidence of carcinoma, but not adenoma, was within historical control range. Combined tumor incidence was 8, 10, 30 and 26%, control to high dose (historical control values not provided). Males did not show a treatment-related increase.

Dosing was considered adequate based on decreased body weight/weight gain and an increased incidence of lung bronchiolization in both sexes.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement for an oral carcinogenicity study in the mouse (OECD 451; OPPTS 870.4200).

I. MATERIAL AND METHODS

1. Test Material	MCW-2 TECH
Description:	Solid, yellow
Lot/Batch:	36372130-291-PF1
Purity:	96.7-98.65%
CAS#:	318290-98-1
Stability:	Stable until May 2010
2. Vehicle	Provimi Kliba Nafag 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland)
3. Test Animals	
Species	Mouse
Strain	CD-1 SPF
Age	Approximately 5 weeks at delivery
Weight	Males: 19.6 to 29.7 g; Females: 16.8 to 24.1 g at start of acclimation
Source	Charles River Germany, Sulzfeld, Germany
Acclimation period	6 days
Diet	Pelleted standard Provimi Kliba Nafag 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum*
Water	Community tap water from Itingen *ad libitum*
Housing	Individually in type 2 Makrolon cages with wire mesh tops and standardized softwood bedding (‘Lignocel’ Schill AG, Switzerland)
4. Environmental conditions	
Temperature	22 ± 3°C. Values outside of this range occurred occasionally, usually following room cleaning, and were considered not to have any influence on the study.
Humidity	30-70%. Values outside of this range occurred occasionally, usually following room cleaning, and were considered not to have any influence on the study.

Air change 10-15 air changes per hour

Photoperiod Light cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN

1. In-life dates 20 February 2008 to 4 September 2009 (treatment initiated 26 February)

2. Animal Assignment and treatment

The animals were assigned to groups using a computer-generated random algorithm (Table 1). After randomization three animals were exchanged with reserve animals to minimize differences in mean body weights between groups. Note that although 8 animals were assigned to the allocation B groups, only 6 animals underwent examination for liver enzymes.

Table 1: Study Design for a Carcinogenicity study in Mice

Allocation		Number of animals	0 ppm	30 ppm	200 ppm	1200 ppm
		Daily intake (mg/kg bw/day)				
Males	**A**	50	0	4.24	27.35	152.31
	B	8	0	nc	nc	nc
Females	**A**	50	0	6.43	38.96	188.44
	B	8	0	nc	nc	nc

Data obtained from pages 275 and 280 of the study report.
A: Oncogenicity animals; sacrificed at 78 weeks.
B: Animals for liver enzyme determinations; sacrificed at 13 weeks.
nc: daily intake not calculated separately for allocation B animals.

3. Dose Selection Rationale

Doses were selected based on the results of the 90-day dietary study in mice (study R-23151; MRID 4574753; PMRA 2181205). In that study, fluensulfone was administered in the diet at 0, 60, 300, and 1500 ppm (equivalent to average daily intakes of 0/0, 11/18, 51/68 and 228/252 mg/kg bw/day in males/females, respectively). Slight changes in hematology, blood biochemistry and food consumption were noted at 300 ppm, with more effects (e.g. decreased body weight, liver pathology) observed at 1500 ppm. The NOAEL was established at 60 ppm.

4. Diet Preparation and Analysis

Fresh batches of the feed pellets for the study were prepared weekly for the first two weeks, and then every two weeks thereafter.

Fluensulfone was warmed in the original container in a water bath up to maximum of approximately 40 °C until the test item was fluid. Thereafter, the whole amount of fluid test item was transferred into a glass beaker (wrapped with aluminum foil) and mixed using a magnetic stirrer on a heating plate (maximum approx. 40 °C). The test item was divided into three aliquots.

The required amount of fluensulfone was weighed into tared glass beakers (wrapped with aluminum foil) and kept at a maximum of approximately 40 °C on a heating plate until use for feed preparation. The test item was mixed with microgranulated feed for each dose group. An appropriate amount of water was added to aid pelleting. The pellets were dried with air for approximately 24 to 96 hours before storage.

Control feed for the animals of group 1 was prepared similarly, but without test item.

Feed preparations were stored at room temperature (20±5 °C) in disposable paper bags until use. They were determined to be stable for up to 3 weeks in previous studies.

Concentration and homogeneity of the actual test item batch in the feed were determined before the start of treatment and at weeks 1, 3, 5, 7, 11, 25, 37, 51 and 63.

Analyses were performed using an HPLC-method, previously developed at the performing laboratory. The diet samples were stored deep-frozen (-20°C ± 5°C) until analysis. The test item was used as analytical standard.

5. Statistics

The following methods were used to analyze food consumption, body weight, clinical laboratory data, organ weights and ratios as well as macroscopic findings:

- If the variables were assumed to follow a normal distribution, the Dunnett-test (many to one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups with control groups for each sex.
- The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data could not be assumed to follow a normal distribution.
- The Fisher’s exact test.

The statistical tests used for the analysis of microscopic findings are indicated in the relevant data tables in this review.

C. METHODS

1. Observations

Observations for viability and mortality were recorded twice daily. General cage-side observations were recorded once daily during acclimatization and once daily during the treatment period. Detailed clinical observations including palpation for tissue masses were

performed weekly during acclimatization and treatment periods.

2. Bodyweight

Body weights were recorded weekly during acclimatization and treatment weeks 1 to 14, and once every four weeks thereafter.

3. Food consumption

Food consumption was recorded weekly during acclimatization and treatment weeks 1 to 14, and once every four weeks thereafter.

4. Hematology and Clinical chemistry

Blood samples were drawn from the retro-orbital plexus, using a micro-hematocrit glass capillary tube, from all animals (allocation A) under light isoflurane anesthesia. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms at week 52 and after 78 weeks. At week 52, only hematology parameters (erythrocyte count, total and differential leukocyte count) were analyzed whereas after week 78 clinical biochemistry parameters (i.e. aspartate aminotransferase, alanine aminotransferase and sorbitol dehydrogenase) were determined from the 6 surviving animals with the lowest animal number of each group and sex. Hematology parameters were determined from the remaining animals at week 78.

Blood samples were also taken from a total of 13 moribund animals prior to sacrifice.

Blood smears were prepared for all animals (except those used for clinical biochemistry) at weeks 52 and 78.

5. Sacrifice and pathology

Allocation B animals were terminated after 13 weeks of treatment; allocation A animals after at least 78 weeks of treatment.

All animals found dead, moribund or sacrificed *in extremis* were necropsied.

All allocation B animals were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination. The liver was sampled for liver enzyme determination (see below).

All allocation A animals were weighed and necropsied. All animals surviving to the end of the treatment period and all moribund animals were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination. Descriptions of all macroscopic abnormalities were recorded.

Livers of all animals from which blood for clinical biochemistry was taken after week 78 were cut into two equal pieces. One piece was used for histopathology, the other was used for liver enzyme determination.

Samples of a complete list of tissues and organs were collected from all animals at necropsy and, unless otherwise indicated, fixed in neutral phosphate buffered 4% formaldehyde solution. Additional tissues (such as ear tattoo) were retained in accordance with standard operating procedures but were not processed or examined further.

From allocation A animals, adrenal glands, brain, epididymides, heart including auricles, kidneys, liver (with gall bladder), ovaries, prostate gland, spleen, testes and uterus were weighed before fixation. Relative organ weights were calculated on the basis of the body weight and brain weight.

Slides of all organs and tissues of the control and high-dose groups and all gross lesions from all animals were examined by the study pathologist (including adrenal gland, aorta, bone, bone marrow, brain, cecum, colon, duodenum, epididymides, esophagus, eyes with optic nerve, Harderian gland, heart, ileum, jejunum, kidneys, larynx, liver, lung, lymph nodes, mammary gland, nasal cavity, ovaries, pancreas, pharynx, pituitary gland, prostate gland, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, vagina, all gross lesions and tissue masses). Organ and tissue samples taken from animals which died spontaneously or which were killed *in extremis* were evaluated similarly to those organs taken from animals of the high-dose group. Due to test item-related morphologic changes detected in the lungs of high-dose animals, lungs were also processed and examined from the mid- and low-dose group.

For investigation on the nature of the bronchiolization, the lungs of two control (nos. 33 and 253) and two high dose animals (nos. 218 and 419) were analyzed by transmission electron microscopy.

6. Liver Enzyme Determination

At scheduled necropsy after 13 weeks, the liver was sampled from all allocation B animals. After 78 weeks, half of the liver of all animals from which blood for clinical biochemistry was taken. Livers from the first 6 allocation B animals per sex and group, sampled after week 13, were homogenized, subcellular fractions were prepared and the following enzyme activities determined:

- Protein content of subcellular fractions
- Microsomal cytochrome P450 content (differential spectroscopy)
- CYP1A1 (microsomal 7-ethoxyresorufin O-dealkylation)
- CYP1A2 (microsomal 7-methoxyresorufin O-dealkylation)
- CYP2B1 (microsomal 7-pentoxyresorufin O-dealkylation)

• CYP3A (microsomal testosterone 6β-hydroxylation)

• CYP4A1 (microsomal lauric acid 12-hydroxylation)

• Cytosolic glutathione S-transferase (GST, glutathione conjugation of 1-chloro-2,4-dinitrobenzol)

• Microsomal uridine diphosphoglucuronosyl-transferase (UDPGT, glucuronidation of 3-methyl-2-nitrophenol)

• Microsomal epoxide hydrolase (hydrolysis of styrene oxide)

• Cytosolic alanine aminotransferase (ALAT, transamination of ketoglutarate)

• Peroxisomal beta Oxidation

Portions of liver from 6 allocation A animals per sex and group, sampled after week 78, were homogenized, subcellular fractions were prepared and the following parameter was determined:

• Cytosolic alanine aminotransferase (ALAT, transamination of ketoglutarate)

II. RESULTS AND DISCUSSION

A. ANALYSIS OF FEED PREPARATIONS

The identity of fluensulfone was confirmed by its retention time which was similar to that measured in the working standards. The test item content in 93 out of 99 samples met the required content limit of ±20% with reference to the nominal content. The three samples of the 30 ppm diet prepared on 21-Feb-2008 were slightly above 120% of the nominal concentration and the variation from the mean was 0.6%. The 30 ppm diet prepared on 28-Feb-2008 had 74%, 182% and 77% of the nominal content at the top, middle and bottom, respectively, with a mean recovery of 111% and maximum variation from the mean of 64%. The six samples outside the limit range were all from the first two dose preparations. In addition, the homogenous distribution of fluensulfone in the preparations is considered appropriate because the result of 32 out of 33 dose-preparations deviated by not more than 8.5% (<15%) from the corresponding mean.

The absence of test item in the control diet was confirmed.

In conclusion, the results obtained confirm the accurate preparation and use of fluensulfone and the control feed during the conduct of this study. Diet samples were found to contain acceptable concentrations and to be homogenously prepared.

Stability testing carried out in in the 2-year dietary study in the rat (Study Report R-23353), using the same test item and the same feed, showed that fluensulfone was stable in feed

preparation stored at 20 ± 5°C for at least three weeks.

B. OBSERVATIONS

1. Clinical signs

There were no treatment-related clinical signs.

No test-item-related increase in the incidence of palpable masses occurred.

The masses noted were not distinguishable between treated groups and controls with respect to their onset, incidence or location. Most frequently, small masses were recorded in males in the genital region.

2. Mortality

Mortality was not affected by treatment with fluensulfone (Table 2). The most common cause of death was amyloidosis, followed by systemic neoplasia; there was no dose-related increase in any particular cause of death.

Table 2: Mortality data (allocation A animals only) (N=50 per group)

Dose (ppm)	Spontaneous death		Killed *in extremis*		Planned necropsy	
	Males	Females	Males	Females	Males	Females
0	10 (20%)	6 (12%)	4 (8%)	1 (2%)	36 (72%)	43 (86%)
30	6 (12%)	9 (18%)	2 (4%)	4 (8%)	42 (84%)	37 (74%)
200	3 (6%)	12 (24%)	1 (2%)	2 (4%)	46 (92%)	36 (72%)
1200	8 (16%)	8 (16%)	1 (2%)	6 (12%)	41 (82%)	36 (72%)

Data obtained from page 33 of the study report.

C. BODYWEIGHT AND BODYWEIGHT GAINS

Mean body weight and body weight gain were decreased in a dose-dependent manner at 200 ppm (males only) and 1200 ppm. Males were more sensitive than females.

Mean body weight of males treated at 1200 ppm was statistically significantly reduced from test day 1 onwards (body weights were measured approximately 4 hours after treatment start on day 1) throughout the whole treatment period (from 3 to 20 % lower than controls). This may have been due to slightly lower body weights (3% below controls) in the 1200 ppm males at the start of the study. However, since mean cumulative body weight gain was also significantly reduced in both sexes (see below) and females also had lower body weights through a large part of the study, the changes in male body weights after the first few weeks were judged to have been due to treatment. Males treated at 200 ppm showed statistically significantly reduced mean body weight from week 5 (day 36) through week 54 (day 379) in the range of 3 to 8% from the control mean.

Mean body weight was also affected in females treated at 1200 ppm, where a statistically significant decrease could be observed in most measurements from week 2 onwards (with decreases of up to 12% relative to controls); all body weight measurements from week 38 to study termination were statistically significantly lower than controls.

The mean body weights at the end of the treatment period were reduced by 6% and 17% in the males of the mid and high dose groups, and by 12% in the females of the high dose group.

Body weight gain of males treated at 1200 ppm was statistically significantly reduced from week 4 onwards throughout the treatment period (18-47% lower than controls). In males treated at 200 ppm, body weight gain was also reduced (5-18% lower than controls), although not always achieving statistical significance.

In females, body weight gain was reduced at 1200 ppm only, and statistical significance was achieved for most time points from week 16 onwards (10-31% lower than controls).

Overall body weight gain in males at 200 ppm and 1200 ppm was 54g and 39g, respectively, compared to 62g in the controls (compared to starting body weight). Overall body weight gain in females at 1200 ppm was 49g, compared to 70g in the controls (compared to starting body weight). When compared to controls, the overall body weight gain in males from the 200 and 1200 ppm dose groups was decreased 13% and 37%, and the overall body weight gain in females from the 1200 ppm dose group was decreased 30% (Table 3).

Table 3: Selected mean ± SD body weights (g) and body weight gains (g)

Day	Control	30 ppm	200 ppm	1200 ppm
Males				
Initial BW	29.7 ± 1.4	29.3 ± 1.7	29.3 ± 1.5	28.7 ± 1.7** (↓3%)
BW Wk 5	36.0 ± 2.3	35.1 ± 2.2	34.7 ± 2.5** (↓4%)	33.2 ± 2.0** (↓8%)
BW Wk 54	47.6 ± 7.1	46.1 ± 6.6	44.0 ± 6.2* (↓8%)	37.9 ± 3.6** (↓20%)
Final BW	47.7 ± 5.9	48.3 ± 6.7	45.0 ± 6.4 (↓6%)	39.8 ± 4.2** (↓17%)
BWG Wk 1	6.7 ± 2.8	6.7 ± 3.6	6.7 ± 7.0	4.7 ± 4.8 (↓30%)
BWG Wk 1-13	35 ± 13	34 ± 13	30 ± 17	24 ± 7** (↓31%)
Overall BWG	62 ± 21	66 ± 23	54 ± 23 (↓13%)	39 ± 14** (↓37%)
Females				
Initial BW	21.8 ± 1.2	21.5 ± 1.2	21.7 ± 1.2	21.6 ± 1.3
Final BW	36.7 ± 5.0	36.6 ± 6.7	38.0 ± 6.2	32.3 ± 3.6** (↓12%)
BWG Wk 1	7.0 ± 4.3	6.9 ± 6.1	8.1 ± 3.8	4.9 ± 4.1* (↓30%)
BWG Wk 1-13	30 ± 11	32 ± 13	36 ± 12*	32 ± 8
Overall BWG	70 ± 25	70 ± 31	74 ± 26	49 ± 13** (↓30%)

Data obtained from pages 282-302 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * $p < 0.05$; ** $p < 0.01$

D. FOOD CONSUMPTION

Absolute food consumption (g/animal/day) was statistically significantly reduced at most weighing intervals in both sexes treated at 1200 ppm (data not presented). The overall mean was 17% lower in males and 24% lower in females, compared to controls.

In males, relative food consumption (g/kg bw/day) was not affected by treatment. However, in females relative food consumption was statistically significantly reduced at most weighing intervals at 1200 ppm and the overall mean over the treatment duration was reduced by 20% compared to controls.

E. HEMATOLOGY AND CLINICAL CHEMISTRY

After 52 weeks, hematology analysis revealed slightly decreased absolute red blood cell counts in mice of both sexes at 1200 ppm and decreased total white blood cells count in females only, accompanied by reductions in absolute neutrophil, eosinophil, basophil, lymphocyte (not statistically significant) and monocyte counts (Table 4). Absolute neutrophil and eosinophil counts were also statistically significantly reduced in females at 200 ppm. Non-statistically significant reductions in total white blood cells and in monocyte counts were noted in females from the 200 ppm group as well. None of these changes was still present at terminal sacrifice after 78 weeks.

Table 4. Mean hematology parameters

Parameter	0 ppm	30 ppm	200 ppm	1200 ppm
Males - 52 Weeks				
RBC (10^{12}/L)	8.85	8.73	8.47	8.40 (↓5%)*
WBC (10^9/L)	6.79	6.15	5.46	6.92
Diff. WBC - Neutrophils (10^9/L)	2.28	1.50	1.40	2.14
- Eosinophils (10^9/L)	0.28	0.27	0.20	0.21
- Basophils (10^9/L)	0.04	0.03	0.02	0.03
- Lymphocytes (10^9/L)	3.86	4.09	3.63	4.27
- Monocytes (10^9/L)	0.12	0.14	0.13	0.11
- Leukocytes (10^9/L)	0.21	0.12	0.08	0.16
Males - 78 Weeks				
RBC (10^{12}/L)	8.58	8.25	8.14	8.41
WBC (10^9/L)	6.34	6.36	5.91	7.27
Diff. WBC - Neutrophils (10^9/L)	1.87	1.92	1.69	2.21
- Eosinophils (10^9/L)	0.27	0.23	0.18	0.26
- Basophils (10^9/L)	0.04	0.05	0.03	0.04
- Lymphocytes (10^9/L)	3.94	3.90	3.80	4.47
- Monocytes (10^9/L)	0.12	0.12	0.10	0.14
- Leukocytes (10^9/L)	0.10	0.15	0.12	0.14
Females – 52 Weeks				
RBC (10^{12}/L)	8.33	8.35	8.23	7.83** (↓6%)
WBC (10^9/L)	4.85	4.38	3.91 (↓19%)	3.56** (↓26%)
Diff. WBC - Neutrophils (10^9/L)	1.46	1.28	0.93 (↓36%)**	0.90** (↓38%)
- Eosinophils (10^9/L)	0.21	0.15	0.12 (↓43%)**	0.10** (↓52%)
- Basophils (10^9/L)	0.04	0.04	0.04	0.03** (↓25%)
- Lymphocytes (10^9/L)	2.95	2.73	2.65	2.40 (↓19%)
- Monocytes (10^9/L)	0.09	0.09	0.06 (↓33%)	0.06* (↓33%)
- Leukocytes (10^9/L)	0.11	0.09	0.12	0.08
Females - 78 Weeks				
RBC (10^{12}/L)	8.18	8.15	7.89	8.01
WBC (10^9/L)	4.30	4.30	4.65	4.54
Diff. WBC - Neutrophils (10^9/L)	1.51	1.46	1.58	1.42
- Eosinophils (10^9/L)	0.17	0.15	0.21	0.15
- Basophils (10^9/L)	0.04	0.04	0.04	0.03
- Lymphocytes (10^9/L)	2.41	2.46	2.64	2.75
- Monocytes (10^9/L)	0.09	0.09	0.08	0.09
- Leukocytes (10^9/L)	0.10	0.11	0.11	0.11

Data obtained from pages 305-308 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). Standard deviations were not provided in the study report.

* $p < 0.05$; ** $p < 0.01$

Marked increases were noted in sorbitol dehydrogenase (SDH) for males and females from the 200 and 1200 ppm dose groups, and in aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) in females from the 200 and 1200 ppm dose groups when compared to controls (Table 5). These increases did not reach statistical significance nor were they of a magnitude that would be considered adverse.

Table 5. Mean clinical chemistry parameters at 78 weeks

Parameter	0 ppm	30 ppm	200 ppm	1200 ppm
Males				
ASAT (U/L)	107.7	93.7	82.5	104.4
ALAT (U/L)	97.4	51.7	45.5	75.4
SDH (U/L)	9.0	11.2	21.1 (↑134%)	27.7 (↑208%)
Females				
ASAT (U/L)	98.7	96.9	132.0 (↑34%)	160.4 (↑62%)
ALAT (UL)	29.4	40.6	54.3 (↑85%)	61.5 (↑109%)
SDH (UL)	12.9	14.9	26.1 (↑102%)	24.8 (↑92%)

Data obtained from pages 310-311 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). Standard deviations were not provided in the study report. Historical control values – mean of 18 studies from April 2002 to November 2006 – ASAT in females from 71 weeks = 166.3 ± 136.3 U/L; ALAT in females from 71 weeks = 65.2 ± 53.3 U/L.

F. SACRIFICE AND PATHOLOGY

1. Organ Weights

In males treated with fluensulfone, dose-dependent, statistically significant decreases in absolute prostate weight, absolute brain weight and prostate to brain weight ratio were observed (Table 6a). In addition, a statistically significant decrease in absolute kidney weight, as well as a statistically significant increase in liver to body weight ratio, was recorded in females at 1200 ppm (Table 6b). These changes in organ weights did not correlate with any histopathological findings and were therefore not considered to be of toxicological relevance.

Statistically significantly increased organ to body weight ratios in males at 1200 ppm for brain, heart, kidneys, epididymides and testes were observed. A non-statistically significant increase in adrenal gland to body weight ratio was also observed in males at this dose. These findings are likely due to the reduced absolute body weight in this group, and in the absence of histological correlates, are not considered to be of any toxicological relevance.

Reductions in spleen weight (absolute, relative to body weight and relative to brain weight)

were also noted in males from the 1200 ppm dose group when compared to controls. These changes did not reach statistical significance, and there was a high degree of variability in spleen weight measurements in all dose groups. Furthermore, there were no pathological correlates indicating a toxic effect on the spleen, and the incidence of enlarged spleen was decreased in males in the 200 and 1200 ppm dose groups (incidence of 10, 10, 5, and 6 in the 0, 30, 200 and 1200 ppm dose groups, respectively). Therefore, these changes in spleen weight are not considered to be related to treatment.

Table 6a. Selected mean (± SD) absolute and relative organ weights in male mice

Parameter	0 ppm	30 ppm	200 ppm	1200 ppm
Final body weight (g)	45.9 ± 6.1	46.1 ± 6.1	43.3 ± 6.0	38.8 ± 4.0** (↓15%)
Brain - absolute (g)	0.515 ± 0.020	0.508 ± 0.025	0.507 ± 0.025	0.501 ± 0.023* (↓3%)
- rel. to bw (%)	1.142 ± 0.157	1.119 ± 0.145	1.193 ± 0.163	1.303 ± 0.132** (↑14%)
Heart - absolute (g)	0.248 ± 0.024	0.255 ± 0.031	0.251 ± 0.034	0.234 ± 0.030 (↓6%)
- rel. to bw (%)	0.545 ± 0.063	0.559 ± 0.071	0.591 ± 0.141	0.607 ± 0.078* (↑11%)
Liver - absolute (g)	2.34 ± 0.72	2.25 ± 0.54	2.15 ± .35	2.14 ± 0.30
- rel. to bw (%)	5.15 ± 1.55	4.94 ± 1.22	4.98 ± 0.63	5.53 ± 0.52
Kidney - absolute (g)	0.794 ± 0.157	0.796 ± 0.165	0.761 ± 0.129	0.740 ± 0.090
- rel. to bw (%)	1.740 ± 0.303	1.747 ± 0.387	1.772 ± 0.289	1.915 ± 0.192 (↑10%)*
Adrenals - absolute (g)	0.007 ± 0.002	0.007 ± 0.003	0.007 ± 0.002	0.009 ± 0.011
- rel. to bw (%)	0.015 ± 0.005	0.016 ± 0.006	0.015 ± 0.006	0.023 ± 0.029 (↑53%)
Spleen - absolute (g)	0.167 ± 0.202	0.168 ± 0.138	0.139 ± 0.111	0.112 ± 0.048 (↓33%)
- rel. to bw (%)	0.368 ± 0.415	0.371 ± 0.294	0.323 ± 0.278	0.291 ± 0.126 (↓21%)
- rel. to brain wt (%)	32.8 ± 41.1	33.4 ± 28.0	27.3 ± 21.4	22.6 ± 10.1 (↓31%)
Epidid. - absolute (g)	0.136 ± 0.017	0.134 ± 0.024	0.130 ± 0.019	0.132 ± 0.017
- rel. to bw (%)	0.300 ± 0.045	0.292 ± 0.046	0.302 ± 0.046	0.342 ± 0.048** (↑14%)
Testes - absolute (g)	0.239 ± 0.032	0.229 ± 0.035	0.219 ± 0.038	0.239 ± 0.048
- rel. to bw (%)	0.528 ± 0.097	0.504 ± 0.095	0.515 ± 0.105	0.623 ± 0.140** (↑18%)
Prostate - absolute (g)	0.125 ± 0.032	0.119 ± 0.023	0.106 ± 0.025** (↓15%)	0.098 ± 0.018** (↓22%)
- rel. to bw (%)	0.275 ± 0.072	0.260 ± 0.051	0.246 ± 0.055 (↓10%)	0.256 ± 0.052 (↓7%)
- rel. to brain wt (%)	24.2 ± 5.9	23.4 ± 4.5	20.9 ± 5.2** (↓14%)	19.7 ± 3.4** (↓19%)

Data obtained from pages 313-318 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * $p < 0.05$; ** $p < 0.01$

Table 6b. Selected mean (± SD) absolute and relative organ weights in female mice

Parameter	0 ppm	30 ppm	200 ppm	1200 ppm
Final body weight (g)	34.7 ± 4.2	34.6 ± 6.6	35.7 ± 5.5	31.6 ± 3.7*
Brain - absolute (g)	0.527 ± 0.034	0.537 ± 0.048	0.533 ± 0.032	0.509 ± 0.027
- rel. to bw (%)	1.540 ± 0.198	1.604 ± 0.343	1.530 ± 0.263	1.631 ± 0.189
Heart - absolute (g)	0.197 ± 0.028	0.200 ± 0.023	0.199 ± 0.021	0.185 ± 0.020
- rel. to bw (%)	0.571 ± 0.076	0.594 ± 0.104	0.571 ± 0.124	0.588 ± 0.052
Liver - absolute (g)	1.75 ± 0.42	1.71 ± 0.35	1.78 ± 0.33	1.79 ± 0.37
- rel. to bw (%)	5.06 ± 1.06	4.99 ± 0.75	5.01 ± 0.70	5.62 ± 0.69** (↑11%)
Kidneys - absolute (g)	0.487 ± 0.085	0.469 ± 0.051	0.471 ± 0.057	0.442 ± 0.065** (↓9%)
- rel. to bw (%)	1.413 ± 0.234	1.384 ± 0.197	1.341 ± 0.201	1.403 ± 0.134
Adrenals - absolute (g)	0.012 ± 0.003	0.012 ± 0.003	0.012 ± 0.003	0.011 ± 0.002
- rel. to bw (%)	0.034 ± 0.008	0.035 ± 0.009	0.034 ± 0.009	0.036 ± 0.008
Spleen - absolute (g)	0.188 ± 0.127	0.219 ± 0.158	0.179 ± 0.091	0.217 ± 0.318
- rel. to bw (%)	0.546 ± 0.372	0.628 ± 0.422	0.524 ± 0.322	0.654 ± 0.828
Uterus - absolute (g)	0.696 ± 0.480	0.715 ± 0.510	0.785 ± 0.649	0.552 ± 0.367
- rel. to bw (%)	2.073 ± 1.524	2.113 ± 1.427	2.317 ± 2.157	1.724 ± 1.018
Ovaries - absolute (g)	0.285 ± 0.428	0.365 ± 0.558	0.239 ± 0.860	0.488 ± 1.338
- rel. to bw (%)	0.851 ± 1.322	1.065 ± 1.602	0.703 ± 2.583	1.300 ± 3.190

Data obtained from pages 319-321 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * $p < 0.05$; ** $p < 0.01$

2. Gross and Histopathology

Macroscopically, an increase in nodules in the lung of females at 200 and 1200 ppm was recorded. The incidence was 1, 6, 11** and 13** in the 0, 30, 200 and 1200 ppm groups, respectively (**: $p<0.01$; N=50).

At microscopic examination both non-neoplastic and neoplastic lesions were observed in the lungs of treated animals.

Non-neoplastic

An increase in the incidence and severity of bronchiolization was seen in the lung of males and females at 200 and 1200 ppm. Morphologically this finding mainly consisted of a change from flattened epithelium to cuboidal epithelium, or hypertrophy of the epithelium (Clara cells), lining the terminal bronchioles. In the highest dose, this change extended to the adjacent alveolar walls. The diagnosis was confirmed by using transmission electron microscopy (TEM) analysis which revealed hypertrophy of the epithelium of the terminal bronchioles affecting mostly the non-ciliated Clara cells as well as the surrounding ciliated

cells. These cells were arranged in few layers giving rise to a pseudo-stratified epithelium extending occasionally to the respiratory bronchioles and alveolar ducts.

Table 7: Incidence / mean severity grade of bronchiolization in lungs

	Male				Female[##]			
Dose (ppm)	**0**	**30**	**200**	**1200**	**0**	**30**	**200**	**1200**
No. examined	50	50	50	50	50	50	50	50
Bronchiolization	1	0	24**	31**	5	7	43**	48**
Mean severity grade	1.0	-	1.3	1.6	1.0	1.0	1.8	2.6

Data obtained from page 38 of the study report.
[##] $p<0.05$ using Armitage Trend Test
** $p<0.01$ using Fisher's Exact Test (One-Sided)

The remaining non-neoplastic findings recorded were within the range of normal background lesions observed in animals of the used strain and age.

Neoplastic

In the lungs, alveolar/bronchiolar adenomas were increased in females at 200 and 1200 ppm; although statistically significant by the Fisher's exact test, a clear dose-relationship was not evident. The incidence in females at 200 ppm and in both sexes at 1200 ppm exceeded the laboratory maximum historical control incidence of 14% in males and 6% in females. Alveolar/bronchiolar carcinomas in females at 1200 ppm were statistically significantly positive by the Peto trend test but not by the Fisher's exact test. Their incidence was slightly below the maximum historical control incidence reported for this strain of mice in the performing laboratory (up to 10% reported in control females versus 8% seen at 1200 ppm). In males, no statistically significant increases in tumors were observed. The incidence of alveolar/bronchiolar adenomas was outside the historical control range at 30 and 1200 ppm. Males did not show an increased incidence of carcinoma with treatment, but levels in controls were high compared to historical controls.

The onset (chronological occurrence) of adenomas and carcinomas in the female mice was reduced at 200 and 1200 ppm (Table 9), when compared to controls and females at 30 ppm, with the earliest onset observed at 1200 ppm (from week 50 on). In males there were no differences among control and treated groups in the onset of lung tumors.

Control females had a comparatively low incidence of both adenomas and carcinomas compared to control males, but the historical background incidence of each tumor type was also lower in females than in males. The combined incidence of adenoma and carcinoma was increased at 200 and 1200 ppm. In treated males, the combined incidence of

alveolar/bronchiolar adenomas and carcinomas was comparable between control and high dose animals and the incidence of each tumor type was also high among all dose groups including controls, relative to historical control values.

Table 8: Incidence of alveolar/bronchiolar tumors

	Males					Females				
Dose (ppm)	**0**	**30**	**200**	**1200**	**HC Data**	**0**	**30**	**200**	**1200**	**HC Data**
No. examined	50	50	50	50	266	50	50	50	50	265
Adenoma	7 (14%)	9	5	12 (24%)	18 (7%) (0-14%)	2 (4%)	4	14** (28%)	9* (18%)	9 (3%) (0-6%)
Carcinoma	8 (16%)	3	3	4 (8%)	22 (8%) (4-12%)	2 (4%)	1	1	4[#] (8%)	9 (3%) (0-10%)
Adenoma & carcinoma combined	15 (30%)	12	8	16 (32%)	Not given	4 (8%)	5	15	13[#] (26%)	Not given

Data obtained from pages 39 and 2469 of the study report.
*/** p<0.05 and p<0.01, respectively, using Fisher's Exact Test (one-sided and pairwise);
[#] p<0.05 using Peto Trend Test

Table 9. Individual onset (by study week) of alveolar/bronchiolar tumors

Tumor type	0 ppm	30 ppm	200 ppm	1200 ppm
Males				
Adenoma	62, 74, 79 (2), 80 (3)	52, 79 (6), 80 (2)	46, 80 (4)	73, 79 (4), 80 (7)
Carcinoma	62, 77, 79, 80 (5)	79 (2), 80	46, 79, 80	79 (3), 80
Median week onset	77	77	71	79
Females				
Adenoma	79 (2)	79 (2), 80 (2)	67, 76 (2), 79 (8), 80 (3)	56, 66, 70, 79 (3), 80 (3)
Carcinoma	79 (2)	79	79	50, 66, 79, 80
Median week onset	79	79	78	73

Data obtained from page 1327 of the study report. Numbers in parentheses represent the number of decedent or sacrificed animals with tumor onset for that week.

With the Peto trend test, a few other tumors attained a level of statistical significance; however considering their single or very low occurrence they were not considered to be of any

toxicological significance. A hemangiosarcoma of the ovaries (n=1) and uterus (n=1) was seen in individual females at 1200 ppm. However considering the very low incidence, and as stated by the study authors that hemangiosarcomas are rather common tumors in mice, it was regarded as a spontaneous occurrence and not related to the treatment. A higher incidence of hepatocellular adenomas was seen in males at 30 (n=7) and 200 ppm (n=6). The incidence of this benign tumor at 1200 ppm (n=1) was lower than in controls (n=3). Therefore, considering the absence of a dose response, they were regarded as incidental.

Some other tumors occurred in several organs/systems, but did not attain statistical significance and all were within the range of the biological variation that occurs in mice of this age. The organs most commonly affected by neoplasia were the hemolymphoreticular system, liver, Harderian gland and pituitary gland (females). In male mice, the incidence of malignant lymphomas was decreased at 200 and 1200 ppm when compared to controls. However the decrease, affecting males only, was within the biological variation reported for this strain of mice (Charles River monography on spontaneous neoplastic lesions in the Crl:CD1 (ICR) mouse, March 2010).

All remaining organs were infrequently affected by neoplasia and tumors were randomly distributed among control and treated groups. Some rare tumors were observed as single occurrences, namely a brain oligodendroglioma in a control female, a hemangioma in the testis of a control male, a rectal adenocarcinoma in a single high dose female, and a tubular cell carcinoma of the kidney in single males of the 0, 200 and 1200 ppm dose groups. However none of them was considered related to treatment.

G. LIVER ENZYME DETERMINATION

Fluensulfone caused a slight inhibition of hepatic ALAT activity in females treated at 1200 ppm for 13 weeks, but not for 78 weeks (Table 10). In addition, a weak increase of microsomal cytochrome P450 content, accompanied by slight increases of the activity of CYP4A-1 (lauric acid 12-hydroxylase) and CYP1A1 (7-ethoxyresorufin O-dealkylation) were observed in females only at 200 and 1200 ppm. The phase II enzymes uridine diphosphoglucuronosyl-transferase, glutathione S-transferase and epoxide hydrolase were slightly to moderately induced, with females being more susceptible than males.

There was no dose-related change in the activity of microsomal 7-methoxyresorufin O-dealkylase, 7-pentoxyresorufin O-dealkylase, 6 ß-testosterone hydroxylase, or peroxisomal ß-oxidation.

The results exclude a potent induction of liver enzymes by fluensulfone. Increases in a few phase I activities, observed in females only and mainly at the highest dose level, indicate that fluensulfone exerts a minor non-adverse activity, and no peroxisome proliferation can be

expected. Induction of some phase II activities, observed in both sexes generally at the highest dose level, can be considered an adaptive response to the constant load on the liver from a xenobiotic, Therefore, these effects were considered not to be adverse.

Table 10: Selected liver enzyme activities (Mean ± SD; % difference from control)

Parameter	0 ppm		30 ppm		200 ppm		1200 ppm	
	M	F	M	F	M	F	M	F
ALAT (U/g) Week 13	177 ± 29	236 ± 57	207 ± 28	218 ± 15	187 ± 28	211 ± 31	177 ± 20	**126 ± 16** (↓47%)**
ALAT (U/g) Week 78	136 ± 25	160 ± 30	148 ± 18	151 ± 13	165 ± 24	161 ± 17	135 ± 29	136 ± 29
P450 (nmol/g)	26.2 ± 6	16.7 ± 3	25.6 ± 3	17.7 ± 2	28.1 ± 5	**21.7 ± 4*** (↑30%)**	29.9 ± 3	**22.8 ± 3** (↑37%)**
EROD (nmol/min/g)	2.5 ± 0.6	1.6 ± 0.4	2.3 ± 0.5	1.9 ± 0.6	2.8 ± 0.6	**2.1 ± 0.9 (↑36%)**	3.1 ± 1.0	**2.6 ± 0.4* (↑63%)**
LA12OH (nmol/min/g)	12.6 ± 3	9.2 ± 3	14.4 ± 4	10.6 ± 4	10.1 ± 3	**15.6 ± 5 (↑68%)**	15.0 ± 5	**20.1 ± 6* (↑117%)**
GST (nmol/min/g)	288 ± 51	92.5 ± 15	300 ± 30	87.6 ± 23	323 ± 46	**145 ± 39* (↑57%)**	**440 ± 35** (↑53%)**	**368 ±101** (↑298%)**
UDPGT (µmol/min/g)	1.2 ± 0.4	1.1 ± 0.4	1.3 ± 0.2	1.1 ± 0.2	1.4 ± 0.1	1.3 ± 0.2	1.5 ± 0.1	**1.6 ± 0.2* (↑45%)**
mEH (nmol/min/g)	144 ± 20	62 ± 12	161 ± 41	**91 ± 24* (↑48%)**	**213 ± 42** (↑48%)**	**131 ± 31** (↑113%)**	**297 ± 34** (↑106%)**	**249 ± 36** (↑304%)**

Data obtained from pages 2503-2508 of the study report.
M: males; F: females; ALAT: alanine aminotransferase in 100g supernatants; P450: microsomal cytochrome P450 content; EROD: microsomal 7-ethoxyresorufin O-dealkylase; LA12OH: microsomal lauric acid 12-hydroxylase activity; GST: cytosolic glutathione S-transferase activity; UDPGT: microsomal uridine diphospho-glucuronosyl transferase activity; mEH: microsomal epoxide hydrolase activity
* and **: significantly different from control (Dunnett's t-test) at 5% and 1%, respectively

III. CONCLUSIONS

Due to effects on body weights in both sexes as well as neoplastic and non-neoplastic lesions in the lungs of females at 200 and 1200 ppm, the NOAEL was established at 30 ppm (4.2 and 6.4 mg/kg bw/day in males and females, respectively).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: Overall, the regulatory authorities agree with the majority of the conclusions reached by the study investigators and concur with the systemic toxicity LOAEL and NOAEL of 1200 and 200 ppm, respectively.

The study investigators concluded that the increase in alveolar/bronchiolar carcinomas in females from the 1200 ppm dose group were not treatment-related since the incidence fell within the range of control incidence in previous oncogenicity studies. However, due to the significant increase in adenoma exceeding historical controls at 200 and 1200 ppm, the statistically significant trend noted for carcinomas as well as for adenomas and carcinomas combined, and the shortened latency to onset for carcinomas at 1200 ppm in females, the relationship between the increased incidence of these tumors and exposure to fluensulfone cannot be ruled out.

OCS notes that while the onset of tumor formation in female CD-1 mice at 1200 ppm was reduced (largely due to onset of an adenoma at 56 weeks [i.e. in 1/9 animals] and a carcinoma at 50 and 66 weeks [i.e. in 2/4 animals]) the incidence of carcinoma was still within the historical control range. Also, although the incidence of adenomas in females was outside the historical control range at 200 and 1200 ppm (28% and 18% respectively) and statistically significant by the Fisher exact test a dose response relationship was not evident. Overall, OCS considers that based on the available data, fluensulfone exhibited a weak carcinogenic potential in female mice.

C. Conclusions: This carcinogenicity study in the mouse is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for a carcinogenicity study (OPPTS 870.4200; OECD 451) in mice.

D. Deficiencies: Standard deviations for hematology and clinical chemistry analyses were not provided in the study report. This omission does not alter the conclusions or acceptability of the study. No other deficiencies were noted.

Tier II Summary (PMRA #2181211 and PMRA #2181210, MRID 48574759 and MRID 48575015) revised by the regulatory authorities, April 2013 (note: the Tier II Summary included only an Executive Summary; the remainder of the review was prepared by the regulatory authorities)

Study Type In-vitro genotoxicity testing - Bacterial assay for reverse gene mutation

IIA 5.4.1– MRID 48574759 and MRID 48575015; PMRA 2181211 and 2181210

Report: Herbold B. (2002a)

BYI 01921 *Salmonella*/microsome test plate incorporation method.

Bayer HealthCare, Rodents and Genotoxicity, Germany; unpublished report No. PH 32046, dated 27 May 2002. Study No. T7070901, Sponsor reference No. R-23135

Dates of experimental work: 13 to 21 February 2002

AND

Herbold B. (2002b)

BYI 01921 Brief report of screen with *Salmonella typhimurium* TA 100 strain.

Bayer HealthCare, Rodents and Genotoxicity, Germany. unpublished report no. T6074774, dated 13 August 2002; Sponsor reference No. R-23159

Dates of experimental work: 9 August 2002

Sponsor Makhteshim Chemical Works Ltd., Beer Sheva, Israel

Guidelines: OECD 471, Commission Directive 2000/32/EC B.13/14 and OPPTS 870.5100

Deviations: The second experiment and the additional test (Herbold B. 2002b) were performed at concentrations higher than the maximum of 5000 µg/plate recommended by the guidelines (6000 and 5200 µg/plate, respectively). In addition, the second experiment and the additional test were performed only in *Salmonella typhimurium* strain TA100 and the additional test was performed only without S9 mix using a single plate per concentration.

GLP/Compliance: Herbold B. (2002a): Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Herbold B. (2002b): No. Signed and dated GLP and Quality Assurance statements were not provided. A signed and dated Data Confidentiality statement was provided.

Executive summary:

In a bacterial reverse gene mutation test conducted according to the Ames method (MRID 48574759 and PMRA 2181211, main study; MRID 48575015 and PMRA 2181210, additional test), fluensulfone (BYI 09121; main study 98.8-98.9%, Batch NLL6692-5; additional test 98.4%, Batch No. NLL6692-13.1) in dimethylsulfoxide (DMSO) was tested up to 5000 µg/plate in a first experiment, in triplicate, on *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA102 and TA100 using the plate incorporation method, both in the presence and absence of S9 mix. In a second experiment, fluensulfone in DMSO was tested up to 6000 µg/plate, in triplicate, on *Salmonella typhimurium* strain TA100 only, for which a weak positive response was obtained in the first experiment, in the presence and absence of S9 mix.

In an additional test, fluensulfone from a different batch (NLL6692-13.1) was tested up to 5200 µg/plate on *Salmonella typhimurium* strain TA100, only in the absence of S9 mix.

In the main study, there was no indication of a bacteriotoxic effect at doses up to and including 500 µg/plate. No inhibition of growth was noted as well. Higher doses had only a weak, strain-specific bacteriotoxic effect.

Only one of the five tested strains (TA100) revealed a weak, dose-related increase in mutant counts to well above those of negative controls. The findings for this strain of *Salmonella typhimurium* were confirmed in a further repeat test. The lowest effective dose was 4000 µg/plate. Positive findings were obtained with and without S9 mix, the effects being reduced in the presence of metabolic activation S9 mix. All increases were less than two-fold above the vehicle controls.

Because of the weakness of the mutagenic effect observed, it was postulated that this effect could have been related to the presence of either the test compound or by-product(s).

Another batch of fluensulfone (NLL6692-13.1) was therefore tested, using one trial (single plates per test concentration) without S9 mix only, in *Salmonella typhimurium* TA100 at concentrations up to 5200 µg/plate. A weak bacteriotoxic effect was observed at 5200 µg per plate but no genotoxic effect was detected.

Fluensulfone was not mutagenic either in the absence or in the presence of metabolic activation, up to a concentration of 5000 µg/plate, when tested on *Salmonella typhimurium* TA1535, TA1537, TA98 and TA102 strains. A weak positive response was obtained in *Salmonella typhimurium* strain TA100, both in the presence and in the absence of metabolic activation in two independent experiments carried out up to 6000 µg/plate.

The result without metabolic activation was not confirmed in any additional tests carried out by either Bayer Crop Science or Makhteshim Chemical Works (up to 5200 µg/plate), using different more representative batches of fluensulfone.

This study is classified as **acceptable/guideline (fully reliable)** and satisfies the requirement for mutagenicity (bacterial reverse gene mutation) data (OECD 471; OPPTS 870.5100).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: BYI 01921 (fluensulfone)

Description: Main test: brown aqueous liquid

Additional test: aqueous yellow liquid

Lot/Batch: Main test: NLL6692-5

Additional test: NLL6692-13.1

Purity: Main test: 98.8-98.9%

Additional test: 98.4%

CAS#: 318290-98-1

Stability: Main test: Stable until July 8, 2002 (stored at room temperature protected from light)

Additional test: Stable until 21 December 2002

2. Control materials:

Negative: DMSO

Positive:

Non-activation (-S9): TA1535: sodium azide (Na-azide) (SERVA); 10 µg/plate in DMSO

TA100 : nitrofurantoin (NF) (Sigma); 0.2 µg/plate in DMSO

TA1537 and TA98: 4-nitro-1,2-phenylene-diamine (4-NPDA) (Merck-Schuchardt); 10 µg/plate in DMSO for TA1537, 0.5 µg/plate in DMSO for TA98

TA102: mitomycin C (MMC) (Fluka); 0.2 µg/plate in deionized water

Activation (+S9): All strains: 2-aminoanthracene (2-AA) (Aldrich); 3 µg/plate in DMSO

3. Activation: S9-mix prepared from Sprague-Dawley male rats, induced by i.p. injection of 500 mg/kg bw of Aroclor 1254 dissolved in corn oil.

Prior to first use, the metabolizing capacity of each batch of S9-mix was confirmed by using reference mutagens.

The protein concentration in the S9 preparation was 24.6 mg/mL.

4. Test organism: *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and TA 102 obtained from Prof. Bruce Ames.

5. Test concentrations:

(a) Preliminary cytotoxicity (range- Tester strains were *Salmonella typhimurium* TA98 and TA100. Concentrations tested were 3.16, 10.0, 31.6, 100, 316, 1000, 2500

finding) assay:	and 5000 μg/plate.
(b) Mutation assays:	Main test: 16, 50, 158, 500, 1581 and 5000 μg/plate Additional test: 375, 750, 1300, 2600, and 5200 μg/plate (in DMSO)

B. TEST PERFORMANCE

1. Test procedure

The plate incorporation method was used, both in the presence and absence of S9-mix. For each strain and dose, three plates were used. The plates were incubated for 48 hours at 37°C.

2. Evaluation criteria

2a. Criteria of Validity

A test was considered acceptable for each strain if:

- the negative controls were within the expected range, as defined by published data and/or the laboratory's own historical data,

- the positive controls showed sufficient effects, as defined by the laboratory's experience, and

- titer determinations demonstrated sufficient bacterial density in the suspension.

A trial was accepted if it showed mutagenic activity of the test compound even if the last two criteria were not met.

2b. Evaluation of Mutagenicity

A reproducible and dose-related increase in mutant colonies of at least one strain was considered to be a positive result. For TA1535, TA100 and TA98, this increase must be about twice that of negative controls, whereas for TA1537 at least a threefold increase should be reached. For TA102 an increase of about 100 mutants should be reached.

II. RESULTS AND DISCUSSION

MUTATION ASSAY

Results are reported in the Table 1. There was no indication of a bacteriotoxic effect at doses up to and including 500 μg/plate. The fluensulfone-treated plates consistently produced results for total bacteria counts comparable to the negative controls. No inhibition of growth was noted. Higher doses demonstrated a weak, strain-specific bacteriotoxic effect.

Strain TA100 showed a dose-related increase in mutant counts in the presence and absence of S9 mix, which was confirmed in a repeat test. The lowest effective dose was 4000 μg/plate.

No biologically relevant increases in revertant colony numbers of any of the other tester strains were observed following treatment with fluensulfone at any concentration, neither in the absence nor in the presence of metabolic activation.

The reference mutagens induced a distinct increase in revertant colonies indicating the validity of the experiments.

Table 1: Summary of Main Test (with repeat test for strain TA100)

Test Material	Mean ± SD revertants per plate									
	TA98		TA100		TA102		TA1535		TA1537	
Fluensulfone [µg/plate]	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
Main test										
Control	48±6	59±4	161±10	172±13	187±6	252±11	16±3	10±3	6±1	8±1
16	50±5	58±8	157±12	164±10	196±15	252±14	17±6	9±2	7±3	6±3
50	50±1	54±7	157±10	164±20	186±6	247±13	17±6	7±1	6±3	7±2
158	44±4	60±8	162±16	166±16	159±7	249±23	15±2	10±2	5±2	7±3
500	51±8	51±13	173±8	167±12	161±20	238±6	12±1	7±1	6±2	6±4
1581	45±14	52±5	177±1	165±10	151±6	211±7	20±3	9±2	5±2	5±1
5000	26±8	41±7	**274±18**	211±25	32±5	93±3	9±3	6±0	2±2	2±2
Positive controls										
Na-azide							786±19			
NF			300±24							
4-NPDA	139±15								63±11	
MMC					578±42					
2-AA		1213±118		1551±38		504±45		135±36		303±41
Repeat test										
Control			144±12	172±8						
1000			165±7	168±13						
2000			176±15	156±27						
3000			198±5	168±2						
4000			**263±12**	205±20						
5000			**269±40**	210±3						
6000			**275±45**	242±38						
Positive controls										
NF			328±45							
2-AA				1527±43						
Additional test										
Control			138							
375			137							
750			116							
1300			126							
2600			103							
5200			80							
Positive controls										
NF			312							

Data obtained from pages 37-42 and 47 of the study report. Only one plate was used in the additional test with TA100.

III. CONCLUSIONS

The bacterial reverse mutation test, employing doses of up to 5000 µg/plate, showed fluensulfone to produce bacteriotoxic effects, starting at 1581 µg/plate.

Fluensulfone was not mutagenic either in the absence or in presence of metabolic activation, up to the concentration of 5000 µg/plate, when tested on *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA102. A weak positive response was obtained in *Salmonella typhimurium* strain TA100, both in the presence and in the absence of metabolic activation in two independent experiments carried out up to 6000 µg/plate. This response was observed at more than one dose level, but in all cases the increase was less than two-fold of the vehicle control. It is also noted that

the vehicle control values in these assays were slightly above the in-lab historical control range for DMSO in strain TA100 (70-101 mean revertants per plate) provided in the study report.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the requirement for mutagenicity (bacterial reverse gene mutation) data (OECD 471; OPPTS 870.5100).

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: Limited information was provided with the additional test conducted with Batch NLL 6692-13. Also, in the additional test, only one plate was tested.

Tier II Summary (PMRA #2181213, MRID 48574760) revised by the regulatory authorities, April 2013

Study Type In-vitro genotoxicity testing - Bacterial assay for reverse gene mutation

IIA 5.4.1 - MRID 48574760, PMRA 2181213

Report: Sokolowski A. (2008)

Salmonella typhimurium and *Escherichia coli* reverse mutation assay with MCW-2 tech.

RCC-CCR, Germany: unpublished report No. 1176200, dated 1 August 2008. Sponsor reference No. R-23352

Dates of experimental work: 9 to 23 May 2008

Sponsor Makhteshim Agan Group, Beer-Sheva, Israel

Guidelines: OECD 471, Commission Directive 2000/32/EC B.13/14 and Japanese guidelines (Kanpoan No 287 – Environmental Protection Agency; Eisei No. 127 – Ministry of Health and Welfare and Heisei 09/10/31 Kikioku No. 2 – Ministry of International Trade & Industry)

Deviations: None

GLP/Compliance: Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary:

In a bacterial reverse gene mutation test (MRID 48574760, PMRA 2181213), fluensulfone (MCW-2 tech., 96.2%, Batch No. 36372130-291-PF1) dissolved in DMSO was tested in triplicate on *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and on *Escherichia coli* strain WP2 uvrA in two independent experiments up to 5000 µg/plate, both in the presence and absence of S9-mix, using the plate incorporation method in the first one, and the pre-incubation method in the second one. For each experiment, positive controls were tested to validate bacterial strains and to confirm the activity of S9-mix. No precipitation of test material occurred. Normal background growth was observed up to 5000 µg/plate with and without metabolic activation in the first experiment while in the second experiment reduced background growth was observed with and without metabolic activation at the higher concentrations, with variation according to strain. No reduction in the number of revertants occurred in the first experiment. In the second experiment reductions of background growth were observed at 5000 µg/plate without metabolic activation in strain TA1537 and with metabolic activation in strains TA1535 and TA1537.

In two experiments with each test strain, no substantial increase in the number of revertant colonies was observed at any dose level, either in the absence or in the presence of metabolic activation. The substances used as positive controls showed a distinct increase in induced revertant colonies.

Fluensulfone was not mutagenic either in the absence or in the presence of metabolic activation, up to a concentration of 5000 µg/plate, when tested on *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and on *Escherichia coli* strain WP2 uvrA, in two independent

experiments, according to the Ames assay.

This study is classified as acceptable/guideline (fully reliable) and satisfies the requirement for mutagenicity (bacterial reverse gene mutation) data (OCED 471; OPPTS 870.5265).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	MCW-2 TECH (fluensulfone)
Description:	Not stated
Batch No.:	36372130-291-PF1
Purity:	96.2%
CAS#:	318290-98-1
Stability of Test Item:	Stable until 13 December 2009 (stored under refrigeration wrapped in foil)
2. Control materials:	
Negative:	Negative (untreated) and Solvent control (DMSO)
Positive:	
Non-activation (-S9):	TA1535 and TA100: sodium azide (NaN_3) (Serva); 10 μg/plate dissolved in deionized water
	TA1537 and TA98: 4-nitro-o-phenylene-diamine (4-NOPD) (Sigma); 10 μg/plate (TA98) or 50 μg/plate (TA1537) dissolved in DMSO
	E. coli WP2 uvrA: Methyl methane sulfonate (MMS) (Merck-Schuchardt); 3 μL/plate dissolved in deionized water
Activation (+S9)	2-aminoanthracene (2-AA) (Sigma); 2.5 μg/plate (*S. typhimurium* strains) or 10 μg/plate (*E. coli* strain) dissolved in DMSO
3. Test system	
Species:	*Salmonella typhimurium* and *Escherichia coli*
Strain:	TA1535, TA1537, TA98 and TA100 and WP2 uvrA
Source:	Trinova Biochem GmbH, Germany
4. Test concentrations:	Pre-experiment/Experiment I: 3, 10, 33, 100, 333, 1000, 2500, 5000 μg/plate
	Experiment II: 33, 100, 333, 1000, 2500, 5000 μg/plate
5. Metabolic activation system:	S9 fraction prepared from livers of Wistar HanIbm male rats (8 -12 weeks old, 220 – 320 g) induced by Phenobarbital i.p. at 80 mg/kg bw and β-naphthoflavone per oral for three consecutive days. Each

batch of S9 mix (S9 fraction in cofactor solution) is routinely tested with 2-aminoanthracene and benzo(a)pyrene. The protein concentration was 32.5 mg/mL (lot R 010208) in the pre-experiment/first experiment, and 31.4 mg/mL (lot R 290208) in the second experiment.

B. STUDY DESIGN AND METHODS

1. Dates of experiments: The first experiment was carried out on 9 May 2008, and the second one on 20 May 2008.

2. Acceptability of the assay

The assay is considered acceptable if it meets the following criteria:

- regular background growth in the negative and solvent control

- the spontaneous reversion rates in the negative and solvent control are in the range of the performing laboratory historical data

- the positive control substances should produce a significant increase in mutant colony frequencies

3. Data evaluation

A test item is considered a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA98, TA100 and WP2 uvrA) or three times (strains TA1535 and TA1537) the colony count of the corresponding solvent control is observed.

A dose-dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproducible in an independent experiment.

A dose-dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remains within the historical control range of negative and solvent controls such an increase is not considered biologically relevant.

II. RESULTS AND DISCUSSION

A. FINDINGS

The pre-experiment was carried out in the same manner as a main experiment (i.e. plate incorporation method, all tester strains, 3 plates per concentration, 8 concentrations per strain); as there were evaluable plates (>0 colonies) for 5 concentrations or more, the pre-experiment was reported as the main first experiment.

No precipitation and no toxic effects (i.e. no reduction in the number of revertants) occurred. Normal background growth was observed up to 5000 µg/plate with and without metabolic activation.

No substantial increase in the number of revertant colonies was observed at any dose level, either in the absence or in the presence of metabolic activation (Table 1). There was also no tendency for higher mutation rates with increasing concentrations in the range below the generally acknowledged level of biological significance.

Table 1: Summary of Experiment I (plate incorporation method)

Fluensulfone [µg/plate]	TA98		TA100		TA1535		TA1537		*E. coli* WP2 uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0 (untreated)	33±6	30 ± 3	129±6[BM]	144±6[BM]	12±2	18±3	12±3	13±1	56±2	65±6
0 (DMSO)	35±7	35 ± 2	116±11[BM]	137±9[BM]	18±4	17±2	14±4	17±4	52±7	69±6
3	32±3	33 ± 4	118±10[BM]	143±5[BM]	20±2	17±2	12±4	16±5	51±12	67±3
10	34±6	37 ± 6	115±11[BM]	138±8[BM]	17±3	18±3	15±2	11±2	57±5	60±4
33	33±6	38 ± 7	122±7[BM]	138±7[BM]	19±3	16±1	14±4	11±2	64±3	69±17
100	33±4	34 ± 1	114±14[BM]	141±6[BM]	15±1	19±3	12±4	15±4	59±10	68±2
333	30±5	39 ± 5	119±6[BM]	144±6[BM]	12±2	22±2	10±2	14±1	48±7	66±2
1000	31±4	45 ± 2	108±9[BM]	135±6[BM]	12±3	17±3	10±2	14±6	44±4	79±6
2500	30±8	40 ± 5	102±7[BM]	135±7[BM]	18±4	19±3	11±2	15±0	42±2	55±11
5000	29±1	34 ± 5	104±10[BM]	135±9[BM]	14±2	11±4	12±2	17±2	35±10	35±3
Positive controls										
4-NOPD	403±23						132±22			
2-AA		1303±55		2054±25		278±10		138±9		241±8
NaN_3			2266±98		2013±42					
MMS									1484±81	

[B]: extensive bacterial growth; [M]: manual count
Data obtained from page 21 of the study report.

In the second experiment (Table 2), using the pre-incubation method, reduced background growth was observed without metabolic activation at 5000 µg/plate in strains TA1537 and TA98, and from 1000 up to 5000 µg/plate in strains TA100 and WP2 uvrA. In the presence of metabolic activation, reduced background growth was observed at 5000 µg/plate in strains TA1535, TA1537 and TA98, and from 2500 up to 5000 µg/plate in strains TA100 and WP2 uvrA. Toxic effects (reduction in the number of revertants below the indication factor of 0.5) were observed at 5000 µg/plate in strain TA1537 without metabolic activation, and in strains TA1535 and TA1537 with metabolic activation. No substantial increase in the number of revertant colonies was observed at any dose level, either in the absence or in the presence of metabolic activation. There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged level of biological relevance.

Table 2: Summary of Experiment II (pre-incubation method)

Fluensulfone [µg/plate]	TA98		TA100		TA1535		TA1537		*E. coli* WP2 uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0 (untreated)	33±8	37±1	131±9	140±18	12±3	15±7	11±4	16±6	38±1	51±2
0 (DMSO)	27±2	39±4	118±13	116±3	12±4	18±3	13±4	18±7	38±5	54±15
33	22±2	39±4	101±16	108±11	12±5	16±2	14±2	17±5	41±4	53±13
100	28±2	35±5	113±3	114±11	15±3	18±3	14±3	14±6	35±2	52±14
333	26±8	42±7	109±5	114±1	13±4	18±4	14±1	17±3	44±6	52±5
1000	22±1	36±2	66±8[R]	115±14	14±2	19±2	11±3	17±4	27±4[R]	47±6
2500	23±10	31±2	69±6[R]	90±10[R]	11±1	10±4	14±1	18±5	26±5[R]	39±7[R]
5000	21±5[R]	21±3[RM]	66±5[R]	70±4[R]	12±1	5±2[RM]	3±3[R]	5±2[RM]	23±8[R]	34±5[R]
Positive controls										
4-NOPD	445±22						118±10			
2-AA		1424±60		1461±64		224±15		181±8		334±29
NaN_3			2121±59		1764±43					
MMS									417±51	

[R]: reduced background growth; [M]: manual count
Data obtained from page 22 of the study report.

III. CONCLUSIONS

Fluensulfone was not mutagenic either in the absence or in the presence of metabolic activation, up to a concentration of 5000 µg/plate, when tested on *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and on *Escherichia coli* strain WP2 uvrA, in two independent experiments, according to the Ames assay.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the requirement for mutagenicity (bacterial reverse gene mutation) data (OECD 471; OPPTS 870.5100).

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: No deficiencies were identified.

Tier II Summary **(PMRA #2181212, MRID 48574761)** revised by the regulatory authorities, April 2013

Study Type In-vitro genotoxicity testing - Bacterial assay for reverse gene mutation

IIA 5.4.1– MRID 48574761, PMRA 2181212

Report:	Donath C. (2011) Reverse Mutation Assay using Bacteria (*Salmonella typhimurium* and *Escherichia coli*) with MCW-2 TECH. BSL Bioservice Scientific Laboratories GmbH, Germany; unpublished report No. 110567, dated 5 April 2011. Sponsor reference No. R-23356 Dates of experimental work: 17 February to 7 March 2011
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 471, EEC method B.13/14 and OPPTS 870.5100 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary:

In a bacterial reverse gene mutation test (MRID 48574761, PMRA 2181212), fluensulfone (MCW-2 tech., 96.8%, Batch No. 411-33-1) in DMSO was tested in triplicate on *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and on *Escherichia coli* strain WP2 uvrA in two independent experiments up to 5000 µg/plate, both in the presence and absence of S9-mix, using the plate incorporation method in the first one, and the pre-incubation method in the second one. For each experiment, positive controls were tested to validate bacterial strains and to confirm the activity of S9-mix.

No precipitation occurred. Reduced background growth was observed in the first experiment at 2500 µg/plate and above without metabolic activation in *Salmonella* strains TA98 and TA100. In the second experiment without metabolic activation, reduced background growth was observed in *Salmonella* strain TA100 at 316 µg/plate and above, in *Salmonella* strain TA98 and *E. coli* WP2 uvrA at 2500 µg/plate and above and in *Salmonella* strains TA1535 and TA1537 at 1000 µg/plate and above. In the second experiment with metabolic activation, reduced background growth was observed in *Salmonella* strain TA100 at 2500 µg/plate and above, and in *Salmonella* strains TA1535 and TA1537 and *E. coli* WP2 uvrA at 5000 µg/plate.

In two experiments with each test strain, no substantial increase in the number of revertant colonies was observed at any dose level, either in the absence or presence of metabolic activation. The substances used as positive controls showed a distinct increase in induced revertant colonies.

Fluensulfone was not mutagenic either in the absence or in the presence of metabolic activation, up to the concentration of 5000 µg/plate, when tested on *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and on *Escherichia coli* strain WP2 uvrA, in two independent reverse mutation assay experiments (Ames Assay).

This study is classified as acceptable/guideline (fully reliable) and satisfies the requirement for mutagenicity (bacterial reverse gene mutation) data (OECD 471; OPPTS 870.5100).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 TECH (fluensulfone)

Description: Yellowish solid

Lot/Batch: 411-33-1

Purity: 96.8%

CAS#: 318290-98-1

Stability: Stable until 23 January 2013. Stored under refrigeration protected from light.

2. Control materials:

Negative: Negative (bidistilled water) and Solvent control (DMSO)

Positive:

Non-activation (-S9): TA1535 and TA100: sodium azide (NaN_3) (Sigma); 10 µg/plate dissolved in bidistilled water

TA1537 and TA98: 4-nitro-o-phenylene-diamine (4-NOPD) (Fluka); 10 µg/plate (TA98) or 40 µg/plate (TA1537) dissolved in DMSO

E. coli: Methyl methane sulfonate (MMS) (Sigma); 1 µL/plate dissolved in bidistilled water

Activation (+S9): 2-aminoanthracene (2-AA) (Aldrich); 2.5 µg/plate (*S. typhimurium* strains) or 10 µg/plate (*E. coli* strain) dissolved in DMSO

3. Activation: S9 fraction prepared from livers of Wistar male rats, induced by administration of 80 mg/kg bw/day Phenobarbital and 100 mg/kg bw/day β-Naphthoflavone p.o. for three consecutive days.

Each batch of S9 mix (S9 fraction:cofactor mix 5:9.5 v/v) is routinely tested with 2-AA, cyclophosphamide and benzo(a)pyrene.

The protein concentration in the S9 preparation was 36 mg/mL.

4. Test organism: *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 obtained from Moltox Inc., USA and *Escherichia coli* WP2 uvrA obtained from DSMZ Sales, Germany

5. Test concentrations:

(a) Preliminary Tester strains were *Salmonella typhimurium* TA98 and TA100.

cytotoxicity (range-finding) assay:	Concentrations tested were 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 μg/plate with or without S9 under the same conditions as the main study. As the results obtained met the validity criteria, these were reported as a part of the main Experiment I.
(b) Mutation assays:	Experiment I: 10.0, 31.6, 100, 316, 1000, 2500, 5000 μg/plate Experiment II: 3.16, 10.0, 31.6, 100, 316, 1000, 2500, 5000μg/plate

B. TEST PERFORMANCE

1. Test procedure

The plate incorporation method was used in the pre-experiment/first experiment, and the pre-incubation method in the second independent experiment, both in the presence and absence of S9-mix. For each strain and dose level, three plates were used, except for certain strains and dose levels in Experiment I in which case two plates were used due to contamination of one plate. Plates were incubated for at least 48 hours at 37°C.

2. Data recording

The colonies were counted using a ProtoCOL counter (Meintrup DWS Laborgeräte GmbH, Germany). Tester strains with a low spontaneous mutant frequency like TA1535 and TA1537 were counted manually.

3. Evaluation criteria

3a. Criteria of Validity

A test is considered acceptable for each strain if:

- the bacteria demonstrate their typical response to ampicillin (TA98 and TA100)

- the control plates with and without S9 mix show mean values of the spontaneous reversion frequency within historical control ranges

- corresponding background growth on both negative control and test plates is observed

- the positive controls show a distinct enhancement of revertant rates over the control plate.

3b. Evaluation of Mutagenicity

The Mutation Factor is calculated by dividing the mean value of the revertant counts through the mean values of the solvent control (the exact and not the rounded values are used for calculation).

A test item is considered as mutagenic if:

- a clear and dose-related increase in the number of revertants occurs and/or

- a biologically relevant positive response for at least one dose group occurs in at least one tester strain with or without metabolic activation.

A biologically relevant increase is described as follows:

- if in strains TA98, TA100 and WP2 uvrA the number of reversions is at least twice as high

- if in strains TA1535 and TA1537 the number of reversions is at least three times higher as

compared to the reversion rate of the solvent control.

According to the OECD guidelines, the biological relevance of the result is the criterion for the interpretation of results, a statistical evaluation of the result is not regarded as necessary.

A test item producing neither a dose-related increase in the number of revertants nor a reproducible biologically relevant positive response at any of the dose groups is considered to be non-mutagenic in this system.

II. RESULTS AND DISCUSSION

MUTATION ASSAY

Results are reported in Table 1 (Experiment I) and Table 2 (Experiment II). No precipitation of the test item was observed. Toxic effects were noted at the higher concentration in two tester strains in Experiment I and in all tester strains in Experiment II.

No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with fluensulfone at any concentration, neither in the absence nor in the presence of metabolic activation in Experiment I and II.

The reference mutagens induced a distinct increase in revertant colonies indicating the validity of the experiments.

Table 1: Summary of Experiment I (plate incorporation method)

Fluensulfone [µg/plate]	TA98		TA100		TA1535		TA1537		WP2 uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0 (water)	23±4	26±9	147±9	132±12	11±1	6±3	11±3	5±3	50±7	65±12
0 (DMSO)	27±1	23±4	138±17	134±2	10±2	9±3	10±2	5±2	42±15	50±3
10	26±7	23±6	142±9	131±12	15±4	6±1	11±1	8±4	36±10	47±12
31.6	25±6	29±7	132±24	136±14	13±4	8±1	11±2	6±2	35±5	50±4
100	28±5	24±3	129±11	130±1	13±3	11±4	9±1	10±2	44±9	47±5
316	33±9	27±1	137±11	144±10	15±8	7±2	7±1	4±3	37±3	45±10
1000	29±2	34±2	129±6	147±9	15±4	14±2	9±3	7±4	38±3	52±2
2500	19±10[B]	32±1	103±2[B]	127±10	20±3	7±2	4±1	7±3	25±6	37±3
5000	12±2[B]	25±3	110±8[B]	104±13	14±4	8±3	6±3	5±1	17±5	22±5
Positive controls										
4-NOPD	355±49						89±5			
2-AA		3375± 111		2974± 162		61±2		231±9		379±49
NaN_3			1773±143		1555±62					
MMS									462±31	

B: background lawn reduced
Data obtained from pages 28-32 of the study report.

Table 2: Summary of Experiment II (pre-incubation method)

Fluensulfone [µg/plate]	TA98		TA100		TA1535		TA1537		WP2 uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0 (water)	26±5	30±6	155±13	99±2	10±2	9±2	7±1	11±3	65±19	56±2
0 (DMSO)	25±7	22±6	115±3	85±6	7±3	8±2	8±2	7±1	41±8	54±3
3.16	27±4	41±9	125±10	90±12	11±1	8±2	15±4	6±2	48±1	50±6
10	28±8	31±1	110±5	99±4	9±2	6±1	8±4	9±3	45±5	59±8
31.6	27±9	35±5	104±15	82±11	9±1	8±2	15±0	6±2	48±1	53±8
100	21±3	38±2	103±6	98±1	9±3	9±1	9±2	10±1	54±13	64±7
316	29±2	43±11	97±8[B]	132±17	7±3	8±4	12±2	10±3	35±2	55±4[c]
1000	23±1	40±9	46±9[B]	108±2	6±3[B]	10±1	4±2[B]	9±3	36±13	58±8
2500	22±9[B,c]	35±8[c]	54±3[B]	81±23[B]	3±1[B]	5±1	5±2[B]	10±1	27±8[B]	58±7
5000	18±4[B,c]	26±3	32±3[B]	71±8[B]	5±3[B]	3±1[B]	5±1[B]	9±4[B]	26±3[B]	31±8[B]
Positive controls										
4-NOPD	479±35						93±11			
2-AA		2976±262		1313± 133		58±33		329±16		247±20
NaN_3			1080± 34		1051± 181					
MMS									641±22	

B: background lawn reduced; c: one replicate contaminated
Data obtained from pages 33-37 of the study report.

III. CONCLUSIONS

Fluensulfone was not mutagenic either in the absence or in the presence of metabolic activation, up to the concentration of 5000 µg/plate, when tested on *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and on *Escherichia coli* strain WP2 uvrA, in two independent reverse mutation assay experiments (Ames Assay).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the requirement mutagenicity (bacterial reverse gene mutation) data (OECD 471; OPPTS 870.5100).

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: The study report summary indicates that the E. coli strain had cytotoxicity in Experiment I, but this is not indicated in the data table. This omission does not affect the conclusions of the study. No other deficiencies were identified.

Tier II Summary (PMRA #2181238, MRID 48574784) revised by the regulatory authorities, April 2013

Study Type In-vitro genotoxicity testing - Bacterial assay for gene mutation - Metabolite #3625

IIA 5.8 - MRID 48574784, PMRA 2181238

Report:	Sokolowski A. (2010) Salmonella typhimurium and Escherichia coli Reverse mutation assay with MCW-2 Metabolite #3625 Harlan Cytotest Cell Research GmbH, Germany; unpublished report No. 1317202, dated 5 July 2010. Sponsor reference No. R-23469 Dates of experimental work: 27 April to 10 May 2010
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 471 (1997), EEC method B.13/B.14 and OPPTS 870.5100 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In a bacterial gene mutation test (MRID 48574784, PMRA 2181238), fluensulfone metabolite thiazole sulfonic acid (MCW-2 metabolite #3625, 93.6%, Batch No. 231PAL071) in DMSO was tested at concentrations ranging between 3 and 5000 µg/plate (adjusted for purity), in triplicate, on *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and on *Escherichia coli* strain WP2 uvrA. The plate incorporation method was used in the pre-experiment/first experiment, and the pre-incubation method was used in the second independent experiment, both in the presence and absence of S9-mix prepared from Phenobarbital and β-napthoflavone-induced Wistar male rats. For each experiment, positive controls were tested in parallel.

Normal background growth and no toxic effects, evidenced as a reduction in the number of revertants, were observed in treated plates up to and including 5000 µg/plate both with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the five tester strains were observed following treatment with thiazole sulfonic acid at any concentration, neither in the presence nor in the absence of metabolic activation in both experiments. The reference mutagens induced a distinct increase in revertant colonies confirming the validity of the experiments.

Under the conditions of this assay, thiazole sulfonic acid was not mutagenic either in the absence or in presence of metabolic activation, up to concentration of 5000 µg/plate when tested on *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and on *Escherichia coli* strain WP2 uvrA, in two independent experiments, according to Ames.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 metabolite #3625 (thiazole sulfonic acid)

Chemical name: 5-chloro-thiazole-2-sulfonic acid sodium salt

Description: White solid

Lot/Batch: 231PAL071

Purity: 93.6% (doses adjusted for purity)

CAS#: Not available

Stability: Stable until 3 November 2011

2. Control materials:

Negative: Concurrent untreated and solvent control (DMSO)

Positive:

Non-activation (-S9): TA1535 and TA100: sodium azide (NaN_3) (purity at least 99%, Serva)

TA1537 and TA98: 4-nitro-o-phenylene-diamine (4-NOPD) (purity >99.9%, Sigma)

WP2 uvrA: methyl methane sulfonate (MMS) (purity 99%, Merck)

Activation (+S9): 2-aminoanthracene (2-AA) (purity 97.5%, Sigma)

3. Activation: S9-mix prepared from Wistar male rats, induced by administration of 80 mg/kg bw/day Phenobarbital i.p. and 100 mg/kg bw/day β-Naphthoflavone p.o. for three consecutive days.

Each batch of S9 mix is routinely tested with 2-AA and benzo(a)pyrene.

The protein concentration in the S9 preparation was 34.3 mg/mL in the pre-experiment/experiment I, and 33.1 mg/mL in experiment II.

4. Test organism: *Salmonella typhimurium* and *Escherichia coli* strains TA1535, TA1537, TA98 and TA100 and WP2 uvrA obtained from Trinova Biochem GmbH, Germany

5. Test concentrations:

(a) Preliminary cytotoxicity assay: As there were evaluable plates at five or more concentrations in all strains used, the pre-experiment was reported as main experiment 1.

(b) Mutation assays: Experiment I: 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate

Experiment II: 33, 100, 333, 1000, 2500 and 5000 µg/plate

B. TEST PERFORMANCE

1. Test procedure

The plate incorporation method was used in the pre-experiment/first experiment, and the pre-incubation method in the second independent experiment, both in presence and absence of S9-mix. For each strain and dose level, three plates were used. Plates were incubated for at least 48 hours at 37°C.

2. Data recording

The colonies were counted using the Petri Viewer Mk2 (Perceptive Instruments Ltd, UK) with the software program Ames Study manager.

3. Evaluation criteria

3a. Criteria of Validity

A test is considered by the conducting laboratory to be acceptable if:

- regular background growth is observed in the negative and solvent controls,

- the spontaneous reversion rates in the negative and solvent control are in the range of the historical data, and

- the positive control substances produce a significant increase in mutant colony frequencies.

3b. Evaluation of Mutagenicity

A test item is considered by the conducting laboratory as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (TA98, TA100 and WP2 uvrA) or three times (TA1535 and TA1537) the colony count of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of mutagenic potential if reproduced in an independent second experiment. However, whenever the colony count remains within the historical control range of negative and solvent controls such an increase is not considered by the conducting laboratory to be biologically relevant.

II. RESULTS AND DISCUSSION

MUTATION ASSAY

Normal background growth and no toxic effects, evidenced as a reduction in the number of revertants, were observed in treated plates up to and including 5000 µg/plate both with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the five tester strains were observed following treatment with MCW-2 metabolite #3625 at any concentration, neither in the presence nor in the absence of metabolic activation in both experiments. The reference mutagens induced a distinct increase in revertant colonies confirming the validity of the experiments.

Tables 1 and 2 summarize results of the 1st and 2nd experiments, respectively.

Table 1: Summary of Pre-experiment/Experiment I (plate incorporation method)

Metabolite #3625 [µg/plate]	TA98		TA100		TA1535		TA1537		WP2 uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
1st experiment (plate incorporation method)										
0 (untreated)	27 ± 1	37 ± 12	148 ± 6	161 ± 51	11 ± 2	22 ± 1	11 ± 7	13 ± 4	42 ± 9	65 ± 8
0 (DMSO)	30 ± 11	37 ± 11	164 ± 8	168 ± 6	15 ± 5	21 ± 4	11 ± 4	13 ± 6	49 ± 15	60 ± 14
3	32 ± 11	44 ± 7	148 ± 10	166 ± 16	16 ± 4	16 ± 3	10 ± 2	12 ± 3	45 ± 8	58 ± 5
10	27 ± 1	41 ± 6	149 ± 14	170 ± 13	15 ± 6	20 ± 4	13 ± 4	11 ± 4	55 ± 12	58 ± 6
33	28 ± 6	36 ± 6	160 ± 7	176 ± 3	19 ± 3	23 ± 1	10 ± 3	15 ± 2	47 ± 5	65 ± 5
100	35 ± 3	45 ± 5	149 ± 12	161 ± 23	17 ± 8	25 ± 4	7 ± 2	14 ± 3	51 ± 11	62 ± 5
333	27 ± 6	36 ± 5	151 ± 26	167 ± 12	16 ± 6	19 ± 3	9 ± 3	11 ± 2	55 ± 5	57 ± 12
1000	28 ± 7	36 ± 10	154 ± 7	158 ± 19	17 ± 4	17 ± 8	8 ± 1	15 ± 2	53 ± 2	52 ± 5
2500	29 ± 2	46 ± 8	148 ± 7	170 ± 5	18 ± 4	23 ± 2	10 ± 2	14 ± 3	47 ± 5	66 ± 3
5000	33 ± 5	44 ± 8	149 ± 5	173 ± 18	13 ± 2	22 ± 2	7 ± 2	17 ± 2	49 ± 13	75 ± 3
Positive controls										
4-NOPD	304 ± 14						58 ± 3			
2-AA		4241 ± 130		4165 ± 472		506 ± 41		678 ± 56		389 ± 34
NaN_3			2365 ± 149		2242 ± 52					
MMS									898 ± 80	

Data obtained from page 22 of the study report. Values represent the mean ± S.D. revertant colony counts.

Table 2: Summary of Experiment II (pre-incubation method)

Metabolite #3625 [µg/plate]	TA98		TA100		TA1535		TA1537		WP2 uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
2nd experiment (pre-incubation method)										
0 (untreated)	24 ± 5	35 ± 6	132 ± 8	142 ± 7	15 ± 3	22 ± 5	13 ± 4	12 ± 4	56 ± 6	56 ± 9
0 (DMSO)	20 ± 2	38 ± 2	118 ± 10	130 ± 8	11 ± 2	19 ± 3	11 ± 4	11 ± 1	49 ± 13	51 ± 5
33	22 ± 2	34 ± 0	105 ± 16	136 ± 8	12 ± 2	18 ± 5	10 ± 2	14 ± 1	49 ± 5	51 ± 9
100	21 ± 3	34 ± 4	109 ± 14	138 ± 2	12 ± 0	22 ± 1	12 ± 5	12 ± 3	44 ± 7	59 ± 6
333	23 ± 4	36 ± 3	116 ± 9	132 ± 10	9 ± 3	21 ± 3	9 ± 2	12 ± 4	51 ± 4	51 ± 4
1000	21 ± 1	35 ± 2	111 ± 7	133 ± 10	11 ± 4	19 ± 3	9 ± 1	12 ± 3	45 ± 10	52 ± 13
2500	19 ± 2	31 ± 4	116 ± 12	129 ± 12	13 ± 3	18 ± 5	12 ± 2	9 ± 2	50 ± 6	45 ± 12
5000	24 ± 3	36 ± 5	112 ± 7	131 ± 8	12 ± 3	17 ± 3	7 ± 1	9 ± 1	38 ± 4	61 ± 3
Positive controls										
4-NOPD	334 ± 20						75 ± 7			
2-AA		3244 ± 216		3578 ± 88		393 ± 24		565 ± 28		376 ± 6
NaN_3			2072 ± 82		1806 ± 20					
MMS									351 ± 2	

Data obtained from page 23 of the study report. Values represent the mean ± S.D. revertant colony counts.

III. CONCLUSIONS

Under the conditions of this assay, thiazole sulfonic acid (metabolite #3625) was not mutagenic either in the absence or in presence of metabolic activation, up to concentration of 5000 µg/plate when tested on *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and on *Escherichia coli* strain WP2 uvrA, in two independent experiments, according to Ames.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the requirement for (OPPTS 870.5100; OECD 471) for mutagenicity (bacterial reverse gene mutation) data.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: No deficiencies were identified.

Tier II Summary **(PMRA #2181239, MRID 48574788)** revised by the regulatory authorities, April 2013

Study Type In-vitro genotoxicity testing - Bacterial assay for reverse gene mutation - Metabolite #3626

IIA 5.8 – MRID 48574788, PMRA 2181239

Report:	Sokolowski A. (2010) Salmonella typhimurium and Escherichia coli Reverse mutation assay with MCW-2 Metabolite #3626 Harlan Cytotest Cell Research GmbH, Germany; unpublished report No. 1317203, dated 23 August 2010. Sponsor reference No. R-23473 Dates of experimental work: 19 April to 13 July 2010
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 471 (1997), EEC method B.13/B.14 and OPPTS 870.5100 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In a bacterial reverse gene mutation test (MRID 48574788, PMRA 2181239), fluensulfone metabolite methyl sulfone (MCW-2 metabolite #3626, 98.1%, Batch No. 231PAL021) in DMSO was tested at concentrations ranging between 3 and 5000 μg/plate, in triplicate, on *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and on *Escherichia coli* strain WP2 uvrA. The plate incorporation method was used in the pre-experiment/first experiment, and the pre-incubation method was used in the second independent experiment, both in presence and absence of S9-mix prepared from Phenobarbital and β-napthoflavone-induced Wistar male rats. Strains TA100 and WP2 uvrA were repeated without metabolic activation in the pre-incubation assay (reported as experiment IIa). For each experiment, positive controls were tested in parallel.

Normal background growth and no toxic effects, evidenced as a reduction in the number of revertants, were observed in treated plates up to and including 5000 μg/plate both with and without metabolic activation.

A slight but dose-dependent increase in revertant colony numbers was observed following treatment with methyl sulfone in the absence of metabolic activation in strain TA100 in experiments I and II (just exceeding the threshold of 2-fold control level in pre-incubation experiment II and IIa but within the laboratory's historical control range for experiment IIa, and not exceeding the threshold of 2-fold in the plate incorporation experiment I). In strain WP2 uvrA a tendency towards higher mutation frequency was observed only in experiment II in the absence of metabolic activation. A confirmatory experiment (experiment IIa) was performed with strain TA 100 and WP2 uvrA in the absence of metabolic activation to confirm the findings. In this experiment the mutagenic effect in strain TA 100 was reproduced at higher concentrations, while

the tendency in strain WP2 uvrA was not observed and is therefore considered an isolated finding.

Methyl sulfone did induce gene mutations in the genome of *Salmonella typhimurium* strain TA100 in the absence of metabolic activation. It should be noted that the other tester strains specific for base pair changes reacted either not at all (TA1535), or not in a reproducible way (*E. coli* WP2 uvrA). Addition of a metabolic activation system (rat liver S9) abrogates the mutagenic response, and no evidence for frame shift mutations was observed (TA1537, TA98).

Therefore, fluensulfone metabolite #3626 (methyl sulfone) is considered to be weakly mutagenic in this *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 metabolite #3626 (methyl sulfone)

Chemical name: 5-chloro-2-methylsulfonyl thiazole

Description: Brown, waxy solid

Lot/Batch: 231PAL021

Purity: 98.1%

CAS#: Not available

Stability: Stable until 2 January 2011

2. Control materials:

Negative: Concurrent untreated and solvent control (DSMO)

Positive:

Non-activation (-S9): TA1535 and TA100: sodium azide (NaN_3) (purity at least 99%, Serva)

TA1537 and TA98: 4-nitro-o-phenylene-diamine (4-NOPD) (purity >99.9%, Sigma)

WP2 uvrA: Methyl methane sulfonate (MMS) (purity 99%, Merck)

Activation (+S9): 2-aminoanthracene (2-AA) (purity 97.5%, Sigma)

3. Activation: S9-mix prepared from Wistar male rats, induced by administration of 80 mg/kg bw/day Phenobarbital i.p. and 100 mg/kg bw/day β-Naphthoflavone p.o. for three consecutive days.

Each batch of S9 mix is routinely tested with 2-AA and benzo(a)pyrene.

The protein concentration in the S9 preparation was 34.3 mg/mL in the pre-experiment/experiment I, and 33.1 mg/mL in experiment II.

4. Test organism: *Salmonella typhimurium* and *Escherichia coli* strains TA1535, TA1537, TA98 and TA100 and WP2 uvrA obtained from Trinova Biochem GmbH, Germany

5. Test concentrations:

(a) Preliminary cytotoxicity assay: As there were evaluable plates at five or more concentrations in all strains used, the pre-experiment was reported as the main experiment I.

(b) Mutation assays: Experiment I: 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate

Experiment II: 33, 100, 333, 1000, 2500 and 5000 µg/plate

Experiment IIa: 100, 333, 1000, 2500, 3750 and 5000 µg/plate

B. TEST PERFORMANCE

1. Test procedure

The plate incorporation method was used in the pre-experiment/first experiment, and the pre-incubation method in the second independent experiments, both in presence and absence of S9-mix. For each strain and dose level, three plates were used. Plates were incubated for at least 48 hours at 37°C.

2. Data recording

The colonies were counted using the Petri Viewer Mk2 (Perceptive Instruments Ltd, UK) with the software program Ames Study manager.

3. Evaluation criteria

3a. Criteria of Validity

A test is considered acceptable by the conducting laboratory if:

- regular background growth is observed in the negative and solvent controls,

- the spontaneous reversion rates in the negative and solvent control are in the range of the historical data, and

- the positive control substances produce a significant increase in mutant colony frequencies.

3b. Evaluation of Mutagenicity

A test item is considered as a mutagen by the conducting laboratory if a biologically relevant increase in the number of revertants exceeding the threshold of twice (TA98, TA100 and WP2 uvrA) or three times (TA1535 and TA1537) the colony count of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of mutagenic potential if reproduced in an independent second experiment. However, whenever the colony count remains within the historical control range of negative and solvent controls such increase is not considered by the conducting laboratory to be biologically relevant.

II. RESULTS AND DISCUSSION

MUTATION ASSAY

Normal background growth and no toxic effects, evidenced as a reduction in the number of revertants, were observed in treated plates up to and including 5000 µg/plate both with and without metabolic activation.

MCW metabolite #3626 was non-mutagenic in all tester strains in the presence of metabolic activation. In strain TA100 a slight but dose-dependent increase in revertant colony numbers was observed following treatment with MCW-2 metabolite #3626. In the absence of metabolic activation, the threshold of two times the number of the corresponding solvent control was not reached in experiment I but was exceeded at 5000 µg/plate in experiment II. In strain WP2 uvrA an increase in revertant colony numbers was observed but the required threshold of twice the number of the corresponding control was not reached. To verify this result a confirmatory experiment was performed with strain TA100 and WP2 uvrA as pre-incubation assay with a closer concentration range at higher concentrations. Again in strain TA100 the threshold of two times the number of the corresponding solvent control was exceeded at 5000 µg/plate in this experiment, but not at 3750 µg/plate. In strain WP2 uvrA no increase in revertant colony numbers was observed. All remaining strains did not show any mutagenic effects.

Tables 1, 2 and 3 summarize results of the 1st, 2nd and confirmatory (IIa) experiments, respectively.

Table 1: Summary of Pre-experiment/Experiment I (plate incorporation method)

Metabolite #3626 [µg/plate]	TA98		TA100		TA1535		TA1537		WP2 uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
1st experiment (plate incorporation method)										
0 (untreated)	27 ± 8	40 ± 3	134 ± 10	171 ± 10	12 ± 4	20 ± 10	9 ± 2	17 ± 5	50 ± 11	65 ± 7
0 (DMSO)	31 ± 4	40 ± 8	144 ± 3	151 ± 15	17 ± 4	25 ± 4	12 ± 3	14 ± 6	43 ± 4	56 ± 6
3	27 ± 1	37 ± 6	146 ± 16	157 ± 3	18 ± 2	22 ± 6	11 ± 4	15 ± 2	49 ± 4	60 ± 6
10	27 ± 1	35 ± 2	146 ± 10	154 ± 13	16 ± 4	25 ± 6	12 ± 4	17 ± 3	40 ± 12	57 ± 8
33	30 ± 4	35 ± 2	146 ± 12	170 ± 9	17 ± 5	18 ± 5	11 ± 5	16 ± 4	43 ± 9	58 ± 4
100	25 ± 5	36 ± 8	152 ± 5	171 ± 27	13 ± 6	21 ± 2	15 ± 2	13 ± 4	46 ± 8	58 ± 3
333	29 ± 5	35 ± 4	163 ± 16	161 ± 11	18 ± 5	25 ± 6	11 ± 2	16 ± 1	42 ± 6	64 ± 2
1000	29 ± 4	35 ± 2	167 ± 22	149 ± 7	19 ± 4	23 ± 1	9 ± 1	16 ± 6	48 ± 1	63 ± 7
2500	34 ± 8	37 ± 4	218 ± 17	163 ± 5	13 ± 6	22 ± 4	14 ± 5	17 ± 2	49 ± 5	80 ± 4
5000	36 ± 1	38 ± 10	**279 ± 19**	164 ± 4	23 ± 4	22 ± 1	11 ± 3	12 ± 4	66 ± 1	78 ± 10
Positive controls										
4-NOPD	276 ± 17						74 ± 12			
2-AA		3166 ± 227		3147 ± 120		485 ± 56		379 ± 11		279 ± 27
NaN_3			2231 ± 57		2006 ± 96					
MMS									999 ± 67	

Values in bold: above historical control data. Data obtained from page 22 of the study report. Values represent the mean ± S.D. revertant colony counts.

Table 2: Summary of Experiment II (pre-incubation method)

Metabolite #3626 [µg/plate]	TA98		TA100		TA1535		TA1537		WP2 uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
2nd experiment (pre-incubation method)										
0 (untreated)	24 ± 4	46 ± 17	183 ± 23	194 ± 16	21 ± 2	12 ± 4	12 ± 3	8 ± 2	55 ± 8	65 ± 8
0 (DMSO)	26 ± 9	27 ± 7	140 ± 16	145 ± 16	13 ± 3	22 ± 6	11 ± 4	9 ± 4	42 ± 4	63 ± 11
33	27 ± 3	37 ± 11	141 ± 6	162 ± 18	12 ± 2	20 ± 3	12 ± 2	9 ± 1	49 ± 5	69 ± 5
100	21 ± 5	33 ± 6	132 ± 17	144 ± 8	11 ± 1	15 ± 2	13 ± 1	10 ± 0	47 ± 1	69 ± 13
333	30 ± 3	29 ± 8	142 ± 5	150 ± 12	14 ± 2	19 ± 6	10 ± 4	12 ± 4	57 ± 11	56 ± 8
1000	31 ± 3	34 ± 6	161 ± 10	178 ± 6	13 ± 1	21 ± 2	8 ± 4	13 ± 6	65 ± 8	52 ± 3
2500	28 ± 4	32 ± 10	**228 ± 27**	172 ± 5	14 ± 2	19 ± 2	12 ± 3	10 ± 3	**76 ± 12**	68 ± 9
5000	29 ± 2	38 ± 8	**364 ± 57***	177 ± 13	13 ± 4	18 ± 3	13 ± 2	8 ± 5	**80 ± 1**	65 ± 8
Positive controls										
4-NOPD	302 ± 57						59 ± 5			
2-AA		1769 ± 375		2756 ± 628		388 ± 24		512 ± 42		324 ± 28
NaN_3			1617 ± 51		1704 ± 42					
MMS									416 ± 11	
Historical control range - untreated	14-59	16-84	85-226	94-239	7-36	8-55	5-27	7-31	34-80	32-87
Historical control range - solvent	15-52	16-59	94-218	91-241	8-38	10-41	6-27	7-33	33-76	34-82

Values in bold: above historical control data; *: above the threshold of two times the corresponding solvent control. Data obtained from page 23 and 25 of the study report. Values represent the mean ± S.D. revertant colony counts except for historical control values, which represent the minimum and maximum revertant colony counts from approximately 550 experiments for S. typhimurium strains and approximately 300 experiments for WP2 uvrA conducted from January to December 2009.

Table 3: Summary of results of confirmatory experiment (Experiment IIa - pre-incubation method)

Metabolite #3626 [µg/plate]	TA100 -S9	WP2 uvrA -S9
Experiment IIa - pre-incubation method		
0 (untreated)	129 ± 4	48 ± 4
0 (DMSO)	98 ± 2	45 ± 6
100	99 ± 7	48 ± 9
333	113 ± 9	44 ± 5
1000	123 ± 3	51 ± 4
2500	133 ± 20	42 ± 6
3750	174 ± 16	50 ± 4
5000	203 ± 2*	51 ± 4
Positive controls		
NaN_3	1306 ± 97	
MMS		478 ± 23

*: above the threshold of two times the corresponding solvent control. Data obtained from page 24 of the study report. Values represent the mean ± S.D. revertant colony counts.

III. CONCLUSIONS

Under the conditions of this assay, methyl sulfone did induce gene mutations in the genome of *Salmonella typhimurium* strain TA100 in the absence of metabolic activation, in the absence of a corresponding response in the other strains used in this test.

Therefore, fluensulfone metabolite #3626 (methyl sulfone) is considered to be weakly mutagenic in this *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the requirement (OPPTS 870.5100; OECD 471) for mutagenicity (bacterial reverse gene mutation) data.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: No deficiencies were identified.

Tier II Summary **(PMRA #2181240, MRID 48574793)** revised by the regulatory authorities, April 2013

Study Type: In-vitro genotoxicity testing - Bacterial assay for reverse gene mutation - Metabolite #3627

IIA 5.8 – MRID48574793, PMRA 2181240

Report:	Sokolowski A. (2010) Salmonella typhimurium and Escherichia coli Reverse mutation assay with MCW-2 Metabolite #3627 Harlan Cytotest Cell Research GmbH, Germany; unpublished report No. 1317201, dated 25 June 2010. Sponsor reference No. R-23465 Dates of experimental work: 4 to 15 February 2010
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 471 (1997), EEC method B.13/B.14 and OPPTS 870.5100 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In a bacterial reverse gene mutation test (MRID 48574793, PMRA 2181240), fluensulfone metabolite butene sulfonic acid (MCW-2 metabolite #3627, 99.5%, Batch No. 215PAL44) in deionised water was tested at concentrations ranging between 3 and 5000 µg/plate, in triplicate, on *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and on *Escherichia coli* strain WP2 uvrA. The plate incorporation method was used in the pre-experiment/first experiment, and the pre-incubation method in the second independent experiment, both in presence and absence of S9-mix prepared from Phenobarbital and β-napthoflavone-induced Wistar male rats. For each experiment, positive controls were tested in parallel.

Normal background growth and no toxic effects, evidenced as a reduction in the number of revertants, were observed in treated plates up to and including 5000 µg/plate both with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the five tester strains were observed following treatment with butene sulfonic acid at any concentration, neither in the presence nor in the absence of metabolic activation in both experiments. The reference mutagens induced a distinct increase in revertant colonies confirming the validity of the experiments.

Under the conditions of this assay, butene sulfonic acid was not mutagenic either in the absence or in the presence of metabolic activation, up to a concentration of 5000 µg/plate when tested on *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and on *Escherichia coli* strain WP2 uvrA, in two independent reverse mutation (Ames Assay) experiments.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 metabolite #3627 (butane sulfonic acid)

Chemical name: 3,4,4-trifluorobutene-1-sulfonic acid, sodium salt

Description: White solid

Lot/Batch: 215PAL44

Purity: 99.5%

CAS#: Not available

Stability: Stable until 21 December 2010

2. Control materials:

Negative: Concurrent untreated and solvent control (deionized water)

Positive:

Non-activation (-S9): TA1535 and TA100: sodium azide (NaN_3) (purity at least 99%, Serva)

TA1537 and TA98: 4-nitro-o-phenylene-diamine (4-NOPD) (purity >99.9%, Sigma)

WP2 uvrA: Methyl methane sulfonate (MMS) (purity 99%, Merck)

Activation (+S9): 2-aminoanthracene (2-AA) (purity 97.5%, Sigma)

3. Activation: S9-mix prepared from Wistar male rats, induced by administration of 80 mg/kg bw/day Phenobarbital i.p. and β-Naphthoflavone p.o. for three consecutive days.

Each batch of S9 mix is routinely tested with 2-AA and benzo(a)pyrene. The protein concentration in the S9 preparation was 34.3 mg/mL in both experiments.

4. Test organism: *Salmonella typhimurium* and *Escherichia coli* strains TA1535, TA1537, TA98 and TA100 and WP2 uvrA obtained from Trinova Biochem GmbH, Germany

5. Test concentrations:

(a) Preliminary cytotoxicity assay: As there were evaluable plates at five or more concentrations in all strains used, the pre-experiment was reported as main experiment I.

(b) Mutation assays: Experiment I: 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate
Experiment II: 33, 100, 333, 1000, 2500 and 5000 µg/plate

B. TEST PERFORMANCE

1. Test procedure

The plate incorporation method was used in the pre-experiment/first experiment, and the pre-incubation method in the second independent experiment, both in presence and absence of S9-mix. For each strain and dose level, three plates were used. Plates were incubated for at least 48 hours at 37°C.

2. Data recording

The colonies were counted using the Petri Viewer Mk2 (Perceptive Instruments Ltd, UK) with the software program Ames Study manager.

3. Evaluation criteria

3a. Criteria of Validity

A test is considered acceptable by the conducting laboratory if:

- regular background growth is observed in the negative and solvent controls,

- the spontaneous reversion rates in the negative and solvent control are in the range of the historical data, and

- the positive control substances produce a significant increase in mutant colony frequencies.

3b. Evaluation of Mutagenicity

A test item is considered as a mutagen by the conducting laboratory if a biologically relevant increase in the number of revertants exceeding the threshold of twice (TA98, TA100 and WP2 uvrA) or three times (TA1535 and TA1537) the colony count of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of mutagenic potential if reproduced in an independent second experiment. However, whenever the colony count remains within the historical control range of negative and solvent controls such an increase is not considered by the conducting laboratory to be biologically relevant.

II. RESULTS AND DISCUSSION

MUTATION ASSAY

Normal background growth and no toxic effects, evidenced as a reduction in the number of revertants, were observed in treated plates up to and including 5000 µg/plate both with and without metabolic activation. In experiment I, a slight reduction in the number of revertants was observed in strain TA1535 at 2500 µg/plate (factor 0.4) in the absence of metabolic activation. This single effect was judged to be caused by biological fluctuations in the rather low number of colonies and does not represent a true toxic effect.

No substantial increase in revertant colony numbers of any of the five tester strains were observed following treatment with MCW-2 metabolite #3627 at any concentration, neither in the presence nor in the absence of metabolic activation in both experiments. The reference mutagens induced a distinct increase in revertant colonies confirming the validity of the experiments.

Tables 1 and 2 summarize results of the 1st and 2nd experiments, respectively.

Table 1: Summary of Experiment I (plate incorporation method)

Metabolite #3627 [µg/plate]	TA98		TA100		TA1535		TA1537		WP2 uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
1st experiment (plate incorporation method)										
0 (untreated)	30 ± 6	45 ± 6	112 ± 24	109 ± 5	10 ± 4	19 ± 3	14 ± 6	28 ± 11	47 ± 3	65 ± 10
0 (deionised water)	31 ± 9	51 ± 3	116 ± 9	126 ± 7	16 ± 1	19 ± 2	16 ± 5	25 ± 12	52 ± 7	64 ± 2
3	38 ± 5	40 ± 5	117 ± 21	119 ± 13	16 ± 5	23 ± 4	12 ± 3	26 ± 12	49 ± 10	67 ± 5
10	32 ± 2	47 ± 15	124 ± 14	133 ± 11	18 ± 5	19 ± 4	17 ± 6	28 ± 1	59 ± 5	79 ± 14
33	39 ± 7	63 ± 12	104 ± 10	135 ± 13	15 ± 2	20 ± 7	15 ± 2	20 ± 6	55 ± 6	71 ± 2
100	29 ± 4	43 ± 3	140 ± 3	149 ± 15	17 ± 4	19 ± 9	14 ± 5	18 ± 2	52 ± 4	64 ± 11
333	36 ± 14	43 ± 6	100 ± 6	107 ± 15	16 ± 1	16 ± 1	13 ± 3	27 ± 6	52 ± 7	60 ± 6
1000	33 ± 2	48 ± 11	112 ± 8	131 ± 10	9 ± 5	24 ± 5	16 ± 5	29 ± 3	47 ± 6	70 ± 3
2500	36 ± 5	51 ± 4	115 ± 3	128 ± 9	7 ± 2	17 ± 4	14 ± 2	24 ± 2	50 ± 7	69 ± 7
5000	37 ± 9	46 ± 6	122 ± 10	127 ± 10	11 ± 2	23 ± 3	13 ± 4	18 ± 5	57 ± 9	64 ± 13
Positive controls										
4-NOPD	388 ± 14						107 ± 5			
2-AA		2936 ± 124		2754 ± 42		305 ± 8		415 ± 2		525 ± 44
NaN_3			1900 ± 96		1731 ± 29					
MMS									648 ± 51	

Data obtained from page 22 of the study report. Values represent the mean ± S.D. revertant colony counts.

Table 2: Summary of Experiment II (pre-incubation method)

Metabolite #3627 [µg/plate]	TA98		TA100		TA1535		TA1537		WP2 uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
2nd experiment (pre-incubation method)										
0 (untreated)	26 ± 5	43 ± 1	112 ± 1	116 ± 11	15 ± 11	18 ± 9	9 ± 2	19 ± 8	47 ± 7	62 ± 10
0 (deionised water)	23 ± 1	43 ± 4	116 ± 8	117 ± 13	15 ± 6	20 ± 2	10 ± 3	20 ± 6	45 ± 6	74 ± 4
33	23 ± 1	40 ± 5	107 ± 7	115 ± 16	15 ± 6	16 ± 6	10 ± 2	19 ± 3	53 ± 4	71 ± 9
100	21 ± 1	42 ± 4	127 ± 5	120 ± 18	13 ± 3	21 ± 6	12 ± 3	17 ± 6	48 ± 7	70 ± 16
333	24 ± 4	37 ± 4	112 ± 16	140 ± 10	15 ± 6	21 ± 2	12 ± 3	19 ± 7	46 ± 8	70 ± 4
1000	27 ± 0	39 ± 6	109 ± 13	119 ± 19	16 ± 2	22 ± 8	11 ± 3	18 ± 6	53 ± 13	62 ± 13
2500	26 ± 3	42 ± 5	113 ± 2	126 ± 7	16 ± 3	17 ± 1	12 ± 2	21 ± 1	45 ± 3	65 ± 6
5000	28 ± 9	30 ± 3	110 ± 16	123 ± 18	11 ± 3	22 ± 3	14 ± 5	16 ± 5	49 ± 6	65 ± 11
Positive controls										
4-NOPD	307 ± 24						117 ± 24			
2-AA		2107 ± 76		2202 ± 109		383 ± 18		345 ± 22		384 ± 51
NaN_3			2013 ± 31		1879 ± 52					
MMS									302 ± 28	

Data obtained from page 23 of the study report. Values represent the mean ± S.D. revertant colony counts.

III. CONCLUSIONS

Under the conditions of this assay, butene sulfonic acid was not mutagenic either in the absence or in presence of metabolic activation, up to and including a concentration of 5000 µg/plate when tested on *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and on *Escherichia coli* strain WP2 uvrA, in two independent reverse mutation (Ames Assay) experiments.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the requirement (OPPTS 870.5100; OECD 471) for mutagenicity (bacterial reverse gene mutation) data.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: No deficiencies were identified.

Tier II Summary (PMRA #2181215, MRID 48574763) revised by the regulatory authorities, April 2013

Study Type *In vitro* genotoxicity testing – Test for gene mutation in mammalian cells

IIA 5.4.3 – MRID 48574763, PMRA 2181215

Report:	Herbold B. (2003) BYI 01921 V79/HPRT-test *in vitro* for the detection of induced forward mutations. Bayer HealthCare, Molecular and Genetic Toxicology, Germany; unpublished report No. AT00357, dated 9 April 2003. Study No. T4071484, Sponsor reference No. R-23147 Dates of experimental work: 25 October to 4 December 2002
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 476, 2000/32/EC B.17 and OPPTS 870.5300
Deviations:	None
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary:

In a mammalian gene mutation test (MRID 48574763, PMRA 2181215), fluensulfone (BYI 01921, MCW-2 TECH, 98.4%, Batch No. NLL6692-13.1) was evaluated for point mutagenic effects at the hypoxanthine-guanine phosphoribosyl transferase locus (forward mutation assay) in V79 cell cultures. V79 cells were exposed to the test substance dissolved in dimethylsulfoxide (DMSO) both in the presence and absence of metabolic activation in two independent experiments. Following a preliminary range finding test, the main assays were carried out at concentrations ranging from 24 to 72 μg/mL without S9 mix and from 20 to 80 μg/mL in the presence of metabolic activation.

No precipitation was noted. Clear cytotoxic effects were observed both with and without metabolic activation. No biologically relevant increases in mutant frequencies were recorded in any of the experiments.

The positive controls ethyl methanesulfonate (EMS) and dimethyl-benzanthracene (DMBA) had a marked mutagenic effect, as was seen by a biologically relevant increase in mutant frequencies as compared to the corresponding negative controls and thus demonstrated the sensitivity of the test system and the activity of the S9 mix.

Fluensulfone was considered to be non-mutagenic in the V79/HPRT forward mutation assay, both with and without metabolic activation.

This study is classified as acceptable/guideline (fully reliable) and satisfies the requirement for (OECD 476; OPPTS 870.5300) for in vitro mutagenicity (mammalian forward gene mutation) data.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BYI 01921 (MCW-2 tech)

Description: Solid crystalline amber colored mass

Batch No.: NLL6692-13.1

Purity: 98.4%

CAS#: 318290-98-1

Expiry date: 21 December 2002

Stability of Test Item: A stability test in the solvent (DMSO) did not reveal significant degradation (<10%) of the active ingredient over a 24-hour period. Stored at room temperature.

2. Control materials: Negative (untreated) and Solvent Control (DMSO)

Positive controls: ethyl methanesulfonate (EMS - Merck, batch 40606721) undiluted (900 µg/mL final concentration in culture) in the absence of metabolic activation; dimethylbenzanthracene (DMBA - Sigma, batch 86H0330) dissolved in DMSO (20 µg/mL final concentration in culture) in the presence of metabolic activation

3. Test system

Cells: Chinese Hamster Lung cells - V79

Clone: Not stated

Source: Originally obtained from Prof. G. Speit, University of Ulm, Germany, and then maintained in the cell bank of the testing facility

Cell cultures: V79 cell stocks were stored in liquid nitrogen. Laboratory cultures were maintained in plastic tissue culture vessels at 37°C in a humidified atmosphere containing approximately 5% CO_2. Exponential growth of cell cultures was maintained by subculturing at least twice a week. No evidence of mycoplasma contamination was detected at a routine check performed on 5 December 2002 while karyotype stability was confirmed on 27 September 2002. To keep the number of spontaneous 6-thioguanine (6-TG) resistant mutants at a low level, cell cultures were subcloned by plating about 1000 cells per culture vessel at least every two weeks. If necessary, the spontaneous frequency of HPRT-mutants was additionally reduced by supplementing the culture medium with thymidine (9 µg/mL), hypoxanthine (10 µg/mL), glycine (22.5 µg/mL) and methotrexate (0.3 ug/mL). A 6-TG sensitive subclone was then used for the HPRT-test. Cells were maintained in hypoxanthine-free Eagle's Minimal Essential Medium.

4. Metabolic activation: The S9 fraction was isolated in-house from the livers of Aroclor 1254 induced male Sprague Dawley rats. The used S9 fraction was derived from the preparation dated 27 March 2001 (protein content 24.8 mg per mL).

5. Test concentrations Pre-Test: 0.63, 1.25, 2.5, 5, 10, 20, 40, 80, 160 µg/mL (with and without metabolic activation)

Main Test: 24, 32, 40, 48, 56, 64, 72 µg/mL without metabolic activation; 20, 30, 40, 50, 60, 70, 80 µg/mL with metabolic activation

B. STUDY DESIGN AND METHODS

1. Dates of experiments Experiments were performed between 25 October and 4 December 2002

2. Test performance

To determine cytotoxicity, exponentially growing V79 cells were plated in culture medium. After attachment (16-24 hours later), cells were exposed to a range of concentrations of the test substance for 5 hours in medium containing 2% fetal calf serum (FCS). Cell monolayers were washed with phosphate-buffered saline (PBS), trypsinized and replated in culture medium at a density of 200 cells into each of three Petri dishes. The dishes were incubated for 6 to 8 days to allow colony development. Thereafter, colonies were fixed with 95% methanol, stained with Giemsa and counted. If not interfered with by precipitation or coloration, colonies were counted with an automatic counter.

For the main assay, exponentially growing V79 cells were plated in culture medium. After attachment (16-24 hours later), cells were exposed to a range of concentrations of the test substance for 5 hours in medium with reduced serum content (2%). The procedure for the nonactivated and activated assays was identical except for substitution of 1 mL S9 mix for 1 mL culture medium during the exposure period. Cell monolayers were washed with PBS, trypsinized and replated in culture medium at a density of 200 cells into each of three Petri dishes. The dishes were incubated (normally for 6 days) to allow colony development. Cells were then incubated to permit growth and expression of induced mutations. Cells were subcultured (normally after 3 days) by re-seeding cells in flasks. At the end of the expression period (normally 6 days), cultures were re-seeded in Petri dishes at 3×10^5 cells per dish (8 dishes per culture) in culture medium without hypoxanthine but containing 10 µg/mL 6-TG for selection of mutants. In addition, 200 cells per dish were seeded in culture medium to determine the absolute cloning efficiency for each concentration. After incubation for 6 to 8 days, the colonies were fixed, stained with Giemsa and counted to determine the number of 6-TG resistant colonies in the mutation assay dishes and the number of colonies in the cloning efficiency dishes. Two trials were performed with and without metabolic activation.

3. Acceptance criteria

- The mean mutant frequency of the vehicle controls should not exceed 25×10^{-6} cells.
- The mutant frequency of the two cultures of the vehicle and/or the negative control should differ only to an acceptable extent. As a rule of thumb, the difference of mutant frequencies should not be greater than 5×10^{-6}.

- The positive control should induce an average mutant frequency of at least three times that of the vehicle control in the presence and absence of metabolic activation.
- If not limited by the solubility of the test substance in the vehicle, the highest concentration should induce cytotoxicity of about 80 to 90% or should be a concentration where precipitation occurs in the medium. The survival at the lowest concentration should be in the range of the negative control.
- For the calculation of an acceptable mutant frequency at least 5 dishes per culture should be available and relative survival to treatment, relative population growth and absolute cloning efficiency should be 10% or greater.

However, these criteria may be overruled by good scientific judgment.

4. Classification criteria

- Mutant frequencies will be used for assessment only if at least 5 dishes per culture were available and relative survival to treatment, relative population growth and absolute cloning efficiency were 10% or greater.
- A trial will be considered positive if a concentration-related and reproducible increase in mutant frequencies is observed. To be relevant, the increase in mutant frequencies should be at least two to three times that of the highest negative or vehicle control value observed in the respective trial. If this result can be reproduced in a second trial, the test substance is considered to be mutagenic.
- Despite these criteria, a positive result will only be considered relevant if no significant change in osmolality compared to the vehicle control can be observed. Otherwise, non-physiological culture conditions may be the reason for the positive result.
- A test substance will be judged as equivocal if there is no concentration-related increase in mutation frequencies but if one or more concentrations induce a reproducible and biologically relevant increase in mutant frequencies in all trials.
- An assay will be considered negative if no reproducible and relevant increases of mutant frequencies are observed.

However, these criteria may be overruled by good scientific judgment.

5. Statistics

All acceptable groups were included in a weighted analysis of variance followed by pairwise comparisons to the vehicle control on a nominal significance level of a = 0.05 using the Dunnett test. The regression analysis part was performed on the basis of the actual concentrations thereby omitting the positive, negative and vehicle controls. If there was a significant concentration-related increase of the mutant frequency (α= 0.05) in the main analysis, the highest concentration was dropped and the analysis was repeated. This procedure was repeated until $p > 0.05$. In that way eliminated concentrations were flagged correspondingly.

II. RESULTS AND DISCUSSION

A. FINDINGS

A preliminary cytotoxicity test was conducted without and with metabolic activation using concentrations ranging from 0.63 µg/mL to 160 µg/mL. Concentrations of up to 5000 µg/mL did not change the pH in the medium of the pretest. The osmolality in the medium of the pre-test was not changed by concentrations of up to 1500 µg/mL. Precipitation in the culture medium was not observed. Cytotoxic effects were observed at 80 µg/mL and above both with and without S9 mix (Table 1).

Due to these findings, fluensulfone was tested in the mutation experiments in concentrations ranging from 24 µg/mL to 72 µg/mL without metabolic activation and from 20 µg/mL to 80 µg/mL with metabolic activation.

Table 1: Pre-experiments for cytotoxicity

Fluensulfone Concentration (µg/mL)	Mean colony number ± SD[a]	Absolute cloning efficiency %	Vehicle control %
Without S9 mix			
DMSO[b]	168.0 ± 10.4	84.0	100.0
0.63	124.3 ± 11.5	62.2	74.0
1.25	132.0 ± 9.5	66.0	78.6
2.5	122.0 ± 11.4	61.0	72.6
5	123.0 ± 10.8	61.5	73.2
10	117.0 ± 11.4	58.5	69.6
20	140.3 ± 15.0	70.2	83.5
40	88.7 ± 3.2	44.3	52.8
80	1.7 ± 1.2	0.8	1.0
160	0.0	0.0	0.0
With S9 mix			
DMSO[b]	141.7 ± 2.1	70.8	100.0
0.63	111.0[c]	55.5	78.4
1.25	146.3 ± 14.4	73.2	103.3
2.5	148.3 ± 4.0	74.2	104.7
5	158.0 ± 12.7[d]	79.0	111.5
10	131.0 ± 10.1	65.5	92.5
20	157.7 ± 10.7	78.8	111.3
40	125.3 ± 4.9	62.7	88.5
80	13.7 ± 6.0	6.8	9.6
160	0.0	0.0	0.0

[a]: mean and standard deviation of 3 plates;
[b]: culture medium with 1% (or less) vehicle ± S9 mix;
[c]: two dishes lost due to contamination;
[d]: one dish lost due to contamination.
Data obtained from page 31-32 of the study report.

For the two trials performed without metabolic activation (Table 2), the mutant frequencies of the negative controls and of the vehicle controls were all within the normal range. The positive control EMS induced clear mutagenic and statistically significant effects in all trials (level of significance was not specified).

In the treated cultures, concentration-related decreases were observed both in relative survival to treatment and relative population growth.

There was no increase in mutant frequency with exposure to fluensulfone in the absence of metabolic activation.

Table 2: Results of the experiments without S9 mix

Fluensulfone conc. (µg/mL)	First experiment				Second experiment			
	Survival (% of vehicle control)	Total mutant colonies[a]	Absolute cloning efficiency %[b]	Mutant frequency x 10^{-6}	Survival (% of vehicle control)	Total mutant colonies[a]	Absolute cloning efficiency %[b]	Mutant frequency x 10^{-6}
Negative control	81.1	4	25.2 ± 6.0	6.6	102.9	6	62.7 ± 2.3	4.0
	104.0	1	26.2 ± 3.5	1.6	100.2	8	71.0 ± 7.2	4.7
Vehicle control	100.0	4	23.3 ± 5.5	7.1	100.0	6	63.3 ± 6.5	3.9
	100.0	4	23.5 ± 3.1	7.1	100.0	5	52.0 ± 6.1	4.0
EMS 900	32.1	446	24.8 ± 2.9	748.3	17.2	729	34.5 ± 0.9	880.4
	36.6	526	27.5 ± 3.0	797.0	32.3	630	37.5 ± 7.4	700.0
24	84.4	3	26.7 ± 2.8	4.7	75.8	3	50.5 ± 2.2	2.5
	92.2	4	41.7 ± 2.5	4.0	107.2	6	61.8 ± 1.3	4.0
32	59.9	1	45.8 ± 3.6	0.9	108.9	3	58.5 ± 12.4	2.1
	67.7	3	26.5 ± 5.6	4.7	68.2	4	52.3 ± 3.6	3.2
40	36.0	3	33.0 ± 3.3	3.8	51.9	2	36.8 ± 2.3	2.3
	49.1	4	25.8 ± 0.8	6.5	65.9	4	41.0 ± 3.5	4.1
48	17.1	3	50.8 ± 3.3	2.5	41.9	4	62.2 ± 3.8	2.7
	18.9	8	38.2 ± 0.8	8.7	15.4	3	49.2 ± 4.6	2.5
56	11.7	4	39.2 ± 3.2	4.3	13.2	5	58.3 ± 4.9	3.6
	9.3	3	34.7 ± 3.1	3.6	11.7	3	62.5 ± 1.3	2.0
64	4.1	8	55.2 ± 11.3	6.0	2.6	0	62.0 ± 5.0	0.0
	6.2	1	61.8 ± 5.4	0.7	9.0	4	57.3 ± 3.5	2.9
72	0.8	1	68.3 ± 4.9	0.6	2.9	2	45.2 ± 4.6	1.8
	0.6	0	53.3 ± 7.5	0.0	1.2	2	46.3 ± 4.3	1.8

[a]: 3 x 10^5 cells seeded per plate; total for 8 plates;
[b]: 200 cells seeded per plate
Data obtained from page 33-34 of the study report.

For the two trials performed with metabolic activation, the mutant frequencies of the negative controls and of the vehicle controls were all within the normal range (Table 3). The positive control DMBA induced a clear mutagenic and statistically significant effect in all trials.

In the treated cultures, concentration-related decreases were observed both in relative survival to treatment and relative population growth.

There was no increase in mutant frequency with exposure to fluensulfone in the presence of metabolic activation.

Table 3: Results of the experiments with S9 mix

Fluensulfone conc. (µg/mL)	First experiment				Second experiment			
	Survival (% of vehicle control)	Total mutant colonies[a]	Absolute cloning efficiency %[b]	Mutant frequency x 10^{-6}	Survival (% of vehicle control)	Total mutant colonies[a]	Absolute cloning efficiency %[b]	Mutant frequency x 10^{-6}
Negative control	108.4	1	49.8 ± 11.8	0.8	153.6	2	65.5 ± 3.5	1.3
	80.3	6	68.2 ± 2.4	4.2	105.5	6	75.5 ± 13.8	3.3
Vehicle control	100.0	4	60.0 ± 3.5	2.8	100.0	4	67.5 ± 4.8	2.5
	100.0	3	66.0 ± 9.2	1.9	100.0	6	70.8 ± 9.9	3.5
DMBA 20	50.5	167	49.8 ± 0.6	139.6	1.3	-	-	-
	43.6	227	68.0 ± 1.5	139.1	5.3	209	66.5 ± 7.3	131.0
20	72.0	0	52.5 ± 8.7	0.0	95.1	6	63.5 ± 1.8	3.9
	70.4	1	58.8 ± 5.3	0.7	89.5	3	64.5 ± 3.9	1.9
30	71.8	3	64.0 ± 4.3	2.0	107.2	5	73.8 ± 2.5	2.8
	72.2	8	69.8 ± 5.8	4.8	96.6	8	83.3 ± 5.1	4.0
40	63.7	2	49.3 ± 9.2	1.7	80.7	1	70.7 ± 8.4	0.6
	85.1	4	42.0 ± 7.2	4.0	93.5	4	90.8 ± 9.2	1.8
50	88.2	4	53.8 ± 2.6	3.1	51.5	5	78.7 ± 12.4	2.6
	82.1	1	57.5 ± 1.5	0.7	39.7	1	71.3 ± 8.3	0.6
60	51.8	1	49.3 ± 4.8	0.8	41.8	0	63.0 ± 7.0	0.0
	32.1	5	51.7 ± 2.8	4.0	37.4	7	73.3 ± 6.8	4.0
70	26.9	3	43.7 ± 3.3	2.9	22.2	7	70.8 ± 2.5	4.1
	25.0	4	56.2 ± 11.5	3.0	12.5	5	63.0 ± 3.0	3.3
80	7.1	0	52.3 ± 3.9	0.0	7.2	1	76.7 ± 6.3	0.5
	8.5	6	60.0 ± 13.5	4.2	8.1	0	58.0 ± 4.6	0.0

[a]: 3 x 10^5 cells seeded per plate; total for 8 plates;
[b]: 200 cells seeded per plate
-: not cloned due to cytotoxicity
Data obtained from pages 35-36 of the study report.

III. CONCLUSIONS

Fluensulfone was considered to be non-mutagenic in the V79/HPRT forward mutation assay, both with and without metabolic activation.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the requirement (OECD 476; OPPTS 870.5300) for in vitro mutagenicity (mammalian forward gene mutation) data.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: Some footnotes to the tables of the original report are missing. No other deficiencies were identified.

Tier II Summary (PMRA #2181228, MRID 48574789) revised by the regulatory authorities, April 2013

Study Type: *In vitro* genotoxicity testing – Test for gene mutation in mammalian cells – Metabolite #3626

IIA 5.8 – MRID 48574789, PMRA 2181228

Report:	Hall C. (2011) Gene Mutation Assay in Chinese Hamster V79 Cells *In Vitro* (*V79* / HPRT) with MCW-2 Metabolite #3626 Harlan CCR, Germany; unpublished report No. 1388400, dated 14 June 2011. Page 14 amended and substituted in the original report; amendment date 1 March 2012. Sponsor reference No. R-23464 Dates of experimental work: 13 January to 5 April 2011
Sponsor:	Makhteshim Chemical Works, Beer-Sheva, Israel
Guidelines:	OECD 476, EEC method B.17 and OPPTS 870.5300 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary:

In an *in vitro* mammalian cell forward gene mutation assay (MRID 48574789; PMRA 2181228), the potential of fluensulfone metabolite methyl sulfone (MCW-2 metabolite #3626, 98.7%, Batch No. 231PAL052) to induce gene mutations at the HPRT locus in V79 cells of the Chinese hamster was investigated.

The assay was performed in 2 independent experiments, using 2 parallel cultures each. The first main experiment was performed with and without liver microsomal activation and a treatment period of 4 hours. The second experiment was performed with a treatment time of 4 hours with and 24 hours without metabolic activation.

The highest concentration in the pre-experiment (2000 µg/mL) dissolved in DMSO was equal to a molar concentration of about 10 mM, the maximum test concentration defined in OECD guideline 476. Doses were corrected for purity of 98.7%.

The dose range of the 2 main experiments was limited by cytotoxicity of the test item. Without S9 mix, relevant cytotoxicity represented as reduction of relative cloning efficiency or relative cell density to 10 – 20% was observed after 4 hours of treatment with 40 µg/mL and after 24 hours treatment with 87.5 µg/mL. In the presence of metabolic activation, cytotoxicity occurred after treatment at 900 µg/mL and above.

In the first experiment, concentrations ranged between 3.8 and 60 µg/mL without S9 mix and between 37.5 and 1000 µg/mL with S9 mix. The mutation rate was determined at concentrations of 15, 30, 40, 50 and 60 µg/mL without S9 mix, and at 150, 300, 600, 800 and 1000 µg/mL with S9 mix.

In the second experiment, concentrations ranged between 5.5 and 175 µg/mL without S9 mix and 87.5 and 1000 µg/mL with S9 mix. The mutation rate was determined at concentrations of 21.9, 43.8, 87.5, 131.3 and 175 µg/mL without S9 mix and at 350, 700, 800, 900 and 1000 µg/mL with S9 mix.

No substantial and reproducible dose dependent increase of the mutation frequency was observed in both main experiments.

Appropriate reference mutagens, used as positive controls, induced a distinct increase in mutant colonies

Under the conditions of this assay, methyl sulfone did not induce gene mutations at the HPRT locus in V79 cells.

This study is classified as acceptable/guideline (fully reliable) and satisfies the requirement (OPPTS 870.5300, OECD 476) for in vitro mutagenicity (mammalian forward gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: MCW-2 metabolite #3626 (methyl sulfone)

Chemical name: 5-chloro-2-methylsulfonyl thiazole

Description: Not given

Lot/Batch: 231PAL052

Purity: 98.7%

CAS#: Not available

Stability: Stable until February 2013, stored at room temperature

2. Control Materials:

Negative: Dimethylsulfoxide (DMSO, Merck, Germany, purity 99.9%)

Positive:

Nonactivation (-S9): Ethylmethane sulfonate (EMS, Acros Organics, Belgium, purity not given)

Activation (+S9): 7,12-dimethylbenz(a)anthracene (DMBA, Sigma Chemie GmbH, Germany, purity not given)

3. Activation: S9 fractions were prepared from livers of 8 – 12 week old male Wistar rats (Hsd Cpb: WU, weight approx. 220 – 320 g, Harlan Laboratories B.V., The Netherlands) induced by intraperitoneal applications of 80 mg/kg bw phenobarbital (Desitin, Germany) and by peroral administration of 80 mg/kg bw β-naphthoflavone (Sigma-Aldrich Chemie GmbH, Germany) each, on 3 consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by

dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at –80 °C. Small numbers of the ampoules were kept at –20 °C for up to one week. Each batch of S9 mix was routinely tested with 2-aminoanthracene as well as benzo(a)pyrene.

The protein concentration of the S9 preparation was 35.2 mg/mL (Lot. No.: 291010) in Experiments I and II.

The S9 mix was prepared by mixing an appropriate quantity of thawed S9 supernatant with S9 cofactor solution to result in a final protein concentration of 0.75 mg/mL in the cultures. Cofactors were added to the S9 supernatant to reach the following concentrations in the S9 mix: 8 mM $MgCl_2$, 22 mM KCl, 5 mM glucose-6-phosphate, 4mM NADP in 100 mM sodium-phosphate-buffer (pH 7.4).

4. Test cells:

V79 cell line (supplied by Laboratory for Mutagenicity Testing; Technical University, 64287 Darmstadt, Germany), stored in liquid nitrogen in the cell bank of Harlan CCR. Before freezing, the level of spontaneous mutants was depressed by treatment with HAT-medium. Each batch was screened for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency.

Thawed stock cultures were propagated at 37°C in 80 cm^2 plastic flasks. About $5x10^5$ cells were seeded into each flask with 15 mL of MEM (minimal essential medium) containing Hank's salts supplemented with 10% fetal bovine serum (FBS), neomycin (5 µg/mL) and amphotericin B (1%). The cells were sub-cultured twice weekly at 37°C in a 1.5% carbon dioxide atmosphere (98.5% air).

5. Culture medium:

For seeding and treatment of the cell cultures the complete culture medium was MEM (minimal essential medium) containing Hank's salts, neomycin (5 µg/mL) and amphotericin B (1%).

For the selection of mutant cells the complete medium was supplemented with 11 µg/mL 6-thioguanine. All cultures were incubated at 37°C in a humidified atmosphere with 1.5 % CO_2 (98.5% air).

Two days after sub-cultivation stock cultures were trypsinized at 37°C for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium with 10 % FBS and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.2 % in Ca-Mg-free salt solution (Trypsin).

The Ca-Mg-free salt solution had the following constituents (per litre): NaCl 8000 mg, KCl 200 mg, KH_2PO_4 200 mg, Na_2HPO_4 150 mg. Prior to the trypsin treatment the cells were rinsed with Ca-Mg-free salt solution containing 200 mg/L EDTA (ethylene diamine tetraacetic acid). Approximately $1.5x10^6$ (single culture) and $5x10^2$ cells (in duplicate) were seeded in plastic culture flasks.

6. Locus examined: HPRT (hypoxanthine-guanine phosphoribosyl transferase)

7. Test concentrations:

(a) Preliminary cytotoxicity (range finding) assay: In the range finding pre-experiment, concentrations between 15.6 and 2000 μg/mL dissolved in DMSO were used to evaluate toxicity in the presence (4 hours treatment) and absence (4 hours and 24 hours treatment) of metabolic activation. The top dose corresponded to approximately 10 mM with respect to the purity of the test item.

(b) Mutation assay I: Concentrations in bold were evaluated for mutation rate (doses were corrected for purity)

3.8, 7.5, **15, 30, 40, 50 and 60** μg/mL without S9 mix (4 hours)

37.5, 75, **150, 300, 600, 800 and 1000** μg/mL with S9 mix (4 hours)

(b) Mutation assay II: Concentrations in bold were evaluated for mutation rate (doses were corrected for purity)

5.5, 10.9, **21.9, 43.8, 87.5, 131.3 and 175** μg/mL without S9 mix (24 hours)

87.5, 175, **350, 700, 800, 900 and 1000** μg/mL with S9 mix (4 hours)

B. TEST PERFORMANCE

The cells were grown for 24 hours prior to treatment. After 24 hours the medium was replaced with serum-free medium containing the test item, either without S9 mix or with 50 μL/mL S9 mix. Concurrent solvent and positive controls were treated in parallel. After 4 hours this medium was replaced with complete medium following two washing steps with "saline G". In the second experiment the cells were exposed to the test item for 24 hours in complete medium, supplemented with 10% FBS, in the absence of metabolic activation. The "saline G" solution had the following constituents (per litre): NaCl 8000 mg, KCl 400 mg, Glucose 1100 mg, Na_2HPO_4 x 2 H_2O 192 mg, KH_2PO_4 150 mg. The pH was adjusted to 7.2.

1. Test procedure

The colonies used to determine the cloning efficiency (survival) were fixed and stained approximately 7 days after treatment. Four (Exp. II & Exp. I without S9 mix) or 3 days (Exp. I with S9 mix) after treatment 1.5 x 10^6 cells per experimental point were sub-cultivated in 175 cm^2 flasks containing 30 mL medium. Following the expression time of 7

days, five 80 cm² cell culture flasks were seeded with about 3 x 10^5 to 5 x 10^5 cells each in medium containing 6-thioguanine (6-TG). Two additional 25 cm² flasks were seeded with approximately 500 cells each in non-selective medium to determine the viability.

The cultures were incubated at 37°C in a humidified atmosphere with 1.5% CO_2 for about 8 days. The colonies were stained with 10% methylene blue in 0.01% KOH solution. The stained colonies with more than 50 cells were counted. When there was doubt, the colony size was checked with a preparation microscope.

2. Statistics

A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend was judged as significant whenever the p-value (probability value) was below 0.05. However, both biological and statistical significance were considered together.

3. Evaluation criteria

The assay was considered acceptable by the conducting laboratory if it met the following criteria:

- the numbers of mutant colonies per 10^6 cells found in the solvent controls fell within the laboratory historical control data range;

- the positive control substances produced a significant increase in mutant colony frequencies; and

- the cloning efficiency II (absolute value) of the solvent control exceeded 50%.

A test item is classified as positive by the testing laboratory if it induces either a concentration-related increase of the mutant frequency or a reproducible and positive response at one of the test points.

A test item producing neither a concentration-related increase of the mutant frequency nor a reproducible positive response at any of the test points is considered by the testing laboratory as non-mutagenic in this system.

A positive response is described by the testing laboratory as follows:

A test item is classified as mutagenic if it reproducibly induces a mutation frequency that is 3 times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.

The test item is classified as mutagenic if there is a reproducible concentration-related increase of the mutation frequency. Such evaluation may be considered also when a 3-fold increase of the mutant frequency is not observed.

However, in a case by case evaluation this decision depends on the level of the corresponding solvent control data. If there is by chance a low spontaneous mutation rate within the laboratory's historical control data range, a concentration-related increase of the mutations within this range has to be discussed. The variability of the mutation rates of solvent controls within all experiments of this study was also taken into consideration.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY

No precipitation of methyl sulfone was observed up to the maximum concentration in all experiments.

Results of the pre-test for toxicity are reported in Table 1. Relevant cytotoxic effects indicated by a relative cell efficiency of below 20% were noted at 31.3 µg/mL and above following 4 hours of treatment without metabolic activation. In the presence of S9 mix, cytotoxicity was observed at 1000 µg/mL. Following 24 hours of treatment without metabolic activation, cytotoxic effects were noted beginning at 62.5 µg/mL and up to the highest tested concentration (2000 µg/mL).

The test medium was checked for precipitation or phase separation at the end of each treatment period (4 or 24 hours) prior to removal of the test item. There were no signs for either event and there was no relevant shift in pH and osmolarity of the medium even in the stock solution of the test item.

Based on the results of the pre-experiment, the concentrations for the main experiments were selected. A series of concentrations spaced by a factor of 2 was placed into the lower range. Narrower spacing was used at high concentrations to cover the toxic range more closely.

Table 1: Toxicity data

Test group	Conc. (µg/mL)	S9 mix	Duration of treatment (hrs)	Number of colonies per flask found			CE% absolute	CE% relative
				Culture I	Culture II	Mean		
DMSO	-	-	4	492	499	496	95.7	100.0
Fluensulfone Metabolite #3626	15.6	-		413	401	407	78.6	82.1
	31.3	-		52	117	85	16.3	17.1
	62.5	-		2	1	2	0.3	0.3
	125	-		0	0	0	0.0	0.0
	250	-		0	0	0	0.0	0.0
	500	-		0	0	0	0.0	0.0
	1000	-		0	0	0	0.0	0.0
	2000	-		0	0	0	0.0	0.0
DMSO	-	+	4	499	506	503	97.0	100.0
Fluensulfone Metabolite #3626	15.6	+		492	495	494	95.3	98.2
	31.3	+		485	479	482	93.1	95.9
	62.5	+		492	471	482	93.0	95.8
	125	+		473	482	478	92.2	95.0
	250	+		450	464	457	88.2	90.9
	500	+		394	412	403	77.8	80.2
	1000	+		2	1	2	0.3	0.3
	2000	+		0	0	0	0.0	0.0
DMSO	-	-	24	512	496	504	97.3	100.0
Fluensulfone Metabolite #3626	15.6	-		464	485	475	91.6	94.1
	31.3	-		421	415	418	80.7	82.9
	62.5	-		59	63	61	11.8	12.1
	125	-		0	0	0	0.0	0.0
	250	-		0	0	0	0.0	0.0
	500	-		0	0	0	0.0	0.0
	1000	-		0	0	0	0.0	0.0
	2000	-		0	0	0	0.0	0.0

Data obtained from page 27 of the study report. For each culture, 518 cells were seeded. CE% absolute: cloning efficiency, absolute = (mean number of colonies per flask divided by the number of cells seeded) x 100; CE% relative: cloning efficiency, relative = (mean number of colonies per flask divided by mean number of colonies per flask of the corresponding control) x 100

B. MUTATION ASSAYS

The maximum concentration of the test item in the main experiments was limited by cytotoxic effects. In all experiments, the cloning efficiency I and/or the cell density was reduced to approximately 10 – 20% of the respective solvent control cell cultures at the higher concentrations tested. However, due to the strong test item induced cytotoxic effects the values varied to some extent in the two cultures treated within the same concentration and were sometimes even lower than 10%.

In Experiment I relevant cytotoxic effects occurred at 40 µg/mL (10.9% cloning efficiency I in culture I) and 50 µg/mL (7.9% cloning efficiency I in culture II) up to the maximum concentration of 60 µg/mL without metabolic activation. In the presence of S9 mix cytotoxicity was observed at a concentration of 1000 µg/mL (1.9 and 1.4% cloning efficiency I) in Experiment I and at 900 µg/mL (13.8 and 22.5% cloning efficiency I) and above in Experiment II. After 24 hours of treatment without metabolic activation in Experiment II, cytotoxicity could be observed beginning at 87.5 µg/mL in both cultures (8.6 and 15.4% cloning efficiency I).

No relevant and reproducible increase in mutant colony numbers/10^6 cells was observed in the main experiments up to the maximum concentration, although several mutation frequencies exceeded the historical control data range in both experiments (e.g., at 150 µg/mL in Experiment I – Culture I in the presence of metabolic activation, the solvent control in Experiment II – Culture I in the absence of metabolic activation, at 21.9 µg/mL in Experiment II – Culture II in the absence of metabolic activation). *[The historical control data provided included values for studies conducted from 2008 to 2009 with several solvent controls combined (medium, water, DMSO, ethanol and THF). For 42 assays conducted without metabolic activation for 4 hours, the range of mutant colonies per 10^6 cells was 3.4 to 31.7 (mean ± S.D. of 14.0 ± 6.6). For 42 assays conducted with metabolic activation for 4 hours, the range of mutant colonies per 10^6 cells was 3.0 to 33.2 (mean ± S.D. of 15.2 ± 6.5). For 34 assays conducted without metabolic activation for 24 hours, the range of mutant colonies per 10^6 cells was 3.9 to 31.5 (mean ± S.D. of 15.3 ± 6.7)]*. As these incidental observations of mutant frequencies above historical values were not reproducible in the respective parallel culture and the induction factor was generally below 3.0, they were not considered to be biologically relevant.

In culture II of the first experiment in the absence of S9 mix, the induction factor twice exceeded the threshold of 3.0. This was due to a rather low mutation frequency in the respective solvent control. As the results did not follow a dose-response relationship, this finding was not considered to be biologically relevant. A similar observation was made at 900 µg/mL in the first culture of the second experiment in the presence of metabolic activation. The induction factor was 4.5 but the mutation frequency remained within the historical range of solvent controls. Both the induction factor and the historical range of solvent controls were exceeded at the maximum concentration of 175 µg/mL in the second culture of the second experiment without metabolic activation. However, the isolated increase was judged as an irreproducible artifact since no comparable increase was noted in the parallel culture under identical conditions. This concentration also resulted in poor cell survival.

A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequency. No significant dose-dependent trend in the mutation frequency indicated by a probability value of <0.05 was determined in the main experiments.

In both experiments of this study (with and without S9 mix) the range of the mutation rate of the solvent controls was from 4.4 up to 40.1 mutants per 10^6 cells; the range of the groups treated with the test item was from 7.1 up to 45.5 mutants per 10^6 cells. In Experiment II the value of 40.1 mutant colonies/10^6 cells in the solvent control in the absence of S9 mix, culture I slightly exceeded the historical data range of solvent controls (3.9 – 31.5 colonies per 10^6 cells). However, this effect was judged as irrelevant since it was very minor and the corresponding solvent control of the respective parallel culture remained well within the range of historical controls.

EMS (150 µg/mL) and DMBA (1.1 µg/mL) were used as positive controls and showed a distinct increase in induced mutant colonies.

Results are summarized in Table 2.

Table 2: Summary of Results

Test group	Conc. (µg/mL)	S9 mix	Rel. clon. eff. I (%)	Rel. cell. density (%)	Rel. clon. eff. II (%)	Mutant colonies / 10^6 cells	Induction factor
Experiment I / 4 hrs treatment – Culture I							
DMSO	-	-	100.0	100.0	100.0	13.3	1.0
EMS	150	-	104.2	100.4	71.6	123.0	9.3
Fluensulfone Metabolite #3626	3.8	-	103.2	Culture not continued[#]			
	7.5	-	85.8	Culture not continued[#]			
	15	-	66.2	66.0	85.9	13.2	1.0
	30	-	34.2	93.0	88.6	19.1	1.4
	40	-	10.9	91.4	79.3	19.3	1.5
	50	-	3.5	83.5	81.2	11.6	0.9
	60	-	0.8	83.5	82.6	17.2	1.3
Experiment I / 4 hrs treatment - Culture II							
DMSO	-	-	100.0	100.0	100.0	4.4	1.0
EMS	150	-	103.6	80.2	92.6	123.6	27.9
Fluensulfone Metabolite #3626	3.8	-	107.9	Culture not continued[#]			
	7.5	-	84.2	Culture not continued[#]			
	15	-	104.7	76.9	93.7	7.1	1.6
	30	-	52.9	91.6	104.5	12.8	2.9
	40	-	33.5	81.8	94.2	31.7	7.2
	50	-	7.9	75.5	105.7	12.9	2.9
	60	-	1.7	88.9	100.5	18.3	4.1
Experiment I / 4 hrs treatment – Culture I							
DMSO	-	+	100.0	100.0	100.0	17.9	1.0
DMBA	1.1	+	32.8	38.7	76.6	520.4	29.0
Fluensulfone Metabolite #3626	37.5	+	103.5	Culture not continued[#]			
	75	+	108.8	Culture not continued[#]			
	150	+	97.4	100.5	78.6	45.5	2.5
	300	+	101.7	78.3	77.3	25.2	1.4
	600	+	94.6	102.2	76.1	25.4	1.4
	800	+	27.2	71.7	65.0	18.5	1.0
	1000	+	1.9	29.1	62.1	35.9	2.0
Experiment I / 4 hrs treatment - Culture II							
DMSO	-	+	100.0	100.0	100.0	21.3	1.0
DMBA	1.1	+	35.5	20.6	83.6	725.1	34.1
Fluensulfone Metabolite #3626	37.5	+	89.5	Culture not continued[#]			
	75	+	95.3	Culture not continued[#]			
	150	+	96.5	72.5	93.8	29.5	1.4
	300	+	97.2	110.1	101.7	30.7	1.4
	600	+	99.1	95.4	93.8	41.9	2.0
	800	+	27.0	82.8	87.9	41.2	1.9
	1000	+	1.4	45.3	85.8	24.9	1.2
Experiment II / 24 hrs treatment – S9 mix, 4 hrs treatment +S9 mix – Culture I							
DMSO	-	-	100.0	100.0	100.0	40.1	1.0
EMS	150	-	88.4	71.3	91.8	395.5	9.9

Test group	Conc. (µg/mL)	S9 mix	Rel. clon. eff. I (%)	Rel. cell. density (%)	Rel. clon. eff. II (%)	Mutant colonies / 10^6 cells	Induction factor
Fluensulfone Metabolite #3626	5.5	-	96.4	Culture not continued[#]			
	10.9	-	95.8	Culture not continued[#]			
	21.9	-	97.8	89.5	93.9	23.6	0.6
	43.8	-	93.4	82.7	94.9	20.5	0.5
	87.5	-	8.6	79.3	91.2	11.1	0.3
	131.3	-	0.0	82.6	128.6	9.3	0.2
	175	-	0.0	79.2	88.0	22.3	0.6
Experiment II / 24 hrs treatment – S9 mix, 4 hrs treatment +S9 mix - Culture II							
DMSO	-	-	100.0	100.0	100.0	11.3	1.0
EMS	150	-	94.4	95.3	91.9	299.0	26.4
Fluensulfone Metabolite #3626	5.5	-	97.3	Culture not continued[#]			
	10.9	-	100.8	Culture not continued[#]			
	21.9	-	82.1	91.7	89.4	39.8	3.5
	43.8	-	91.3	98.7	91.6	21.7	1.9
	87.5	-	15.4	109.6	99.7	22.6	2.0
	131.3	-	1.4	94.0	90.0	8.8	0.8
	175	-	0.0	89.5	83.4	36.9	3.3
Experiment II / 24 hrs treatment – S9 mix, 4 hrs treatment +S9 mix - Culture I							
DMSO		+	100.0	100.0	100.0	6.7	1.0
DMBA	1.1	+	29.9	62.3	81.9	789.7	118.6
Fluensulfone Metabolite #3626	87.5	+	97.9	Culture not continued[#]			
	175	+	96.5	Culture not continued[#]			
	350	+	92.9	86.3	72.7	12.8	1.9
	700	+	74.0	90.2	103.4	15.9	2.4
	800	+	61.7	100.5	93.3	16.0	2.4
	900	+	13.8	84.9	102.2	29.8	4.5
	1000	+	0.0	92.5	103.4	13.2	2.0
Experiment II / 24 hrs treatment – S9 mix, 4 hrs treatment +S9 mix - Culture II							
DMSO		+	100.0	100.0	100.0	25.6	1.0
DMBA	1.1	+	27.2	61.6	61.3	1266.5	49.6
Fluensulfone Metabolite #3626	87.5	+	100.1	Culture not continued[#]			
	175	+	116.1	Culture not continued[#]			
	350	+	98.3	105.0	97.9	18.7	0.7
	700	+	72.2	102.8	83.1	21.1	0.8
	800	+	57.2	104.0	114.2	12.5	0.5
	900	+	22.5	87.9	91.6	19.1	0.7
	1000	+	2.3	109.4	109.8	27.0	1.1

Data obtained from page 26 of the study report. [#] culture was not continued since a minimum of only four analyzable concentrations is required. Rel. clon. eff. I %: survival = (mean number of colonies per flask divided by the mean number of colonies per flask of the corresponding control) x 100; Rel. cell. density %: cell density % of control = (cell density at 1st subcultivation divided by the cell density at 1st subcultivation of the corresponding control) x 100; Rel. clon. eff. II %: viability = (cloning efficiency II absolute divided by the cloning efficiency II absolute of the corresponding control) x 100 [cloning efficiency II absolute = mean number of colonies per flask divided by the number of cells seeded]; Mutant colonies / 10^6 cells: mean number of mutant colonies per flask found after plating in TG medium x 10^6 divided by the mean number of survived cells; Induction factor: mutant colonies x 10^6 cells / mutant colonies per 10^6 cells of the corresponding solvent control

III. Conclusions

Under the conditions of this assay, methyl sulfone did not induce gene mutations at the HPRT locus in V79 cells.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for in vitro mutagenicity (mammalian forward gene mutation) data.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: No deficiencies were identified.

Tier II Summary (PMRA #2181214, MRID 48574762) revised by the regulatory authorities, April 2013

Study Type *In vitro* genotoxicity testing – Test for clastogenicity in mammalian cells

IIA 5.4.2 – MRID 48574762, PMRA 2181214

Report:	Flügge C. (2010) *In vitro* assessment of the clastogenic activity of MCW-2 technical in cultured human peripheral lymphocytes LTP, Germany; unpublished report No. 24556, dated 7 June 2010. Sponsor reference No. R-23372 Dates of experimental work: 23 July to 8 October 2009.
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 473, OPPTS 870.5375, EC Regulation No. 440/2008 method B.10, ICH Guideline S2B
Deviations:	None
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary:

In an *in vitro* chromosome aberration assay (MRID 48574762, PMRA 2181214), duplicate cultures of human lymphocytes were treated with fluensulfone (MCW-2 tech., 96.5%, Batch No. 36372130-291-PF1) dissolved in dimethylsulfoxide (DMSO) at concentrations of 0, 62.5, 125, 250, 500, or 1000 µg/mL both in the presence and absence of metabolic activation (S9 mix). The test was carried out employing two exposure times without S9 mix: 4 and 24 hours, and one exposure time with S9 mix: 4 hours. The experiment with S9 mix was carried out twice. The harvesting time was 24 hours following exposure.

Cytotoxicity was noted at a concentration of 1000 µg/mL in the experiments without and with metabolic activation (4-hour exposure) or 500 µg/mL in the experiment without metabolic activation (24-hour exposure).

In the experiments without S9 mix, the mean incidence of chromosomal aberrations (excluding gaps) was within the normal range of the solvent control from 125 to 500 µg/mL (4-hour exposure) or 62.5 to 500 µg/mL (24-hour exposure). Only at the pronounced cytotoxic concentration of 1000 µg/mL (4-hour exposure, only 26 of 200 metaphases could be evaluated) a significant increase to 11.5% was noted in the number of aberrations. In the experiments with S9 mix, the mean incidence of chromosomal aberrations (excluding gaps) was within the normal range of the solvent control from 125 to 500 µg/mL. Only at the pronounced cytotoxic concentration of 1000 µg/mL in both experiments with metabolic activation (only 67 or 34 of 200 metaphases could be evaluated) were significant increases to 17.9% or 8.8% noted in the number of aberrations.

It is known that high cytotoxicity causes artefacts in the form of aberrations in *in vitro* chromosomal tests. Hence, the increases at the concentration of 1000 µg/mL were considered as artefacts and not test item-related.

No test item-related polyploidy or endoreduplication was noted in the experiments without or with metabolic activation.

Based on the results of this test, fluensulfone tested up to cytotoxic concentrations in the absence and in the presence of metabolic activation employing two exposure times without S9 and one exposure time with S9 revealed no indications of genotoxic properties with respect to chromosomal or chromatid damage. In the same test, Mitomycin C and cyclophosphamide induced significant damage.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OECD 473; OPPTS 870.5375) for *in vitro* cytogenetic mutagenicity data.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	MCW-2 technical (fluensulfone)
Description:	Yellow crystalline solid (according to the sponsor), brown solid (as identified at the testing facility)
Batch No.:	36372130-291-PF1
Purity:	96.5%
CAS#:	318290-98-1
Expiry date:	3 January 2010
2. Control materials:	Vehicle: Dimethylsulfoxide – DMSO Positive control: Mitomycin C (Mito - batch no. 098K1681; Sigma-Aldrich Chemie, Germany) in the absence of metabolic activation (0.1 and 0.2 μg/mL); cyclophosphamide (CPA – batch no. 097K1311; Sigma-Aldrich Chemie, Germany) in the presence of metabolic activation (10 and 20 μg/mL)
3. Test system	
Cells:	Human peripheral lymphocytes
Source:	Human peripheral blood was obtained by venipuncture from healthy donors known to be without any medication and collected in heparinised vessels.
Cell cultures:	Small innocula of whole blood (0.5 mL) were added to tubes containing 5 mL of complete culture medium. The tubes were sealed and incubated at 37°C for 48 hours with occasional shaking to prevent clumping.
4. Test concentrations	62.5, 125, 250, 500, 1000 μg/mL with and without metabolic activation for both the 4-hour and 24-hour exposures
5. Metabolic activation:	S9-mix prepared from Aroclor 1254 induced male rats. The S9 mix was freshly prepared on the day of the test.

B. STUDY DESIGN AND METHODS

1. Dates of experiments The experiments were conducted between September 28 and October 8, 2009.

2. Test procedure

In a preliminary experiment, cytotoxicity was noted from concentrations of 1000 µg/mL or greater in the experiments without and with metabolic activation (24-hour or 4-hour exposure, respectively). Hence, the top concentration employed in the main study was 1000 µg/mL in the experiments without and with metabolic activation.

In the main study, tubes were centrifuged after 48 hours of culture in complete medium, and the cell pellet resuspended to 4.5 mL (for S9 mix addition) or 5.0 mL with treatment medium including test item at the final concentrations.

S9 mix (0.5 mL) was added to the appropriate cultures. Treatments were added at a volume 50 µL. During treatment, the tubes were incubated for 4 hours in a shaking water bath at 37°C.

After this period, the tubes were centrifuged and the cells were washed with 5 mL treatment medium to remove the test item and S9 mix. After a further centrifugation, the cell pellet was resuspended in 5 mL of complete medium and returned to the incubator for a further 20 hours. The incubation procedure took place in the dark.

In a second set of experiments, a continuous treatment of 24 hours without metabolic activation was carried out, and the 4-hour treatment with metabolic activation was repeated.

All cultures were run in duplicate using blood from a different donor.

Two hours before termination, the cell division was arrested by the addition of 0.5 mL of a 10 µg/mL solution of the spindle poison colcemid to each culture. The tubes were capped and left to incubate for a further two hours. The cells were harvested by low speed centrifugation (80 - 100 x g) and the pellets of cells thus collected were resuspended in hypotonic potassium chloride solution (0.56%) and later fixed in freshly prepared methanol:glacial acetic acid fixative (4:1, v/v).

For slide preparation, the tubes were centrifuged, the fixative removed and the cell pellet resuspended in a few drops of 60% acetic acid. Single drops of the cell suspension were spread on clean, grease-free glass slides, and the slides were left to air-dry. Two slides were made from each culture, stained for 30 minutes in Giemsa stain (1:10 in WEISE's buffer pH 6.8), washed in buffer and left to air-dry on a hot plate (approx. 50°C).

Permanent slides were made using CONSUL MOUNT7 mountant after clearing in xylene.

3. Acceptance criteria

The study was conducted in accordance with OECD test guideline 473; however, the acceptance criteria were not specified.

4. Evaluation of results

The slides were examined under low power (x 100) and those areas judged to be of sufficient technical quality were located and examined under high power (x 1000, oil immersion objective).

For each treatment and culture 100 metaphases were examined, if possible. In addition, the total number of gaps was recorded in 100 metaphases for each culture.

Metaphases which differed from the normal diploid complement (46) were excluded from evaluation. However, test item-related variations of the normal chromosome number were noted (polyploidy / endoreduplication).

To examine the toxicity of the test item, 1000 cells were scored and the mitotic index was calculated as the percentage of cells in metaphase.

The test item is judged to have mutagenic properties with respect to chromosomal or chromatid change, if the following criteria are fulfilled:

- the number of chromosomal aberrations is significantly (at $p \leq 0.05$) increased compared with the solvent control
- the increase observed is concentration-dependent
- both duplicate cultures lead to similar results
- the increase should not occur in the severely cytotoxic range (mitotic index <0.25), as it is known that high cytotoxicity causes artefacts in the form of aberrations in *in vitro* chromosomal aberration tests.
- there is a reproducible increase in the number of cells with chromosomal aberrations.

5. Statistics

The assessment was carried out by a comparison of the number of chromosome aberrations of the treated samples with those of the solvent control, using the Fisher exact test ($p \leq 0.05$).

II. RESULTS AND DISCUSSION

A. FINDINGS

Concentrations to be tested in the main study were chosen on the basis of results obtained in a pre-experiment for cytotoxicity (Table 1), in which pronounced to complete cytotoxicity and/or slight hemolysis were noted from concentrations of 1000 µg/mL or greater in the experiment without metabolic activation (24-hour exposure) or 2500 µg/mL or greater in the experiment with metabolic activation (4-hour exposure).

Table 1: Pre-test for toxicity

Fluensulfone Concentration (µg/mL)	S9 mix	Metaphases / 1000 cells	Mitotic index (%)
24-hour exposure			
0	-	45	1.00
10	-	46	1.02
25	-	34	0.76
100	-	66	1.47
250	-	48	1.07
1000	-	0	0.00
2500*	-	0	0.00
5000*	-	0	0.00
4-hour exposure			
0	+	62	1.00
10	+	84	1.35
25	+	72	1.16
100	+	50	0.81
250	+	91	1.47
1000	+	25	0.40
2500*	+	0	0.00
5000*	+	0	0.00

*: Slight hemolysis. Data obtained from page 27 of the study report.

In the main study, the following concentrations were not evaluated, as the study investigators indicated that that they would provide no further information:

- 62.5 µg fluensulfone/mL (in the experiments without and with metabolic activation, 4-hour exposure)
- 1000 µg fluensulfone/mL (in the experiment without metabolic activation, 24-hour exposure)
- 0.2 µg Mitomycin C/mL (in the experiment without metabolic activation, 4-hour and 24-

hour exposure)

- 20 µg cyclophosphamide/mL (in the experiments with metabolic activation, 4-hour exposure)

In the main study, cytotoxic effects were observed without metabolic activation at 1000 µg/mL after 4 hours of treatment and at 500 µg/mL and above after 24 hours of treatment (Table 2). With S9 mix, cytotoxic effects were observed at 1000 µg/mL (Table 3).

In the absence of metabolic activation, the mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with fluensulfone at concentrations from 125 to 500 µg/mL medium (4-hour exposure) or 62.5 to 500 µg/mL (24-hour exposure) ranged from 0% to 3.5%. The results obtained are considered to be within the normal range of the solvent control where a mean incidence of chromosomal aberrations (excluding gaps) of 1.5% was observed after a 4-hour and 24-hour exposure. The range of aberrations in historical control data from 2007 to 2009 was 0 - 4%.

Only at the pronounced cytotoxic concentration of 1000 µg/mL medium (4-hour exposure, only 26 of 200 metaphases could be evaluated) was a significant increase to 11.5% noted in the number of aberrations (excluding gaps). It is known that high cytotoxicity causes artefacts in the form of aberrations in in vitro chromosomal tests. Hence, the increase in aberrations at the concentration of 1000 µg fluensulfone/mL medium (4-hour exposure) is considered an artefact and not test item-related.

Table 2: Summary of experiments without metabolic activation

Fluensulfone Concentration (µg/mL)	Mitotic index (%)	Number of metaphases scored	% of cells with aberrations (excluding gaps)	% of cell with aberrations (including gaps)	% of cells with gaps
1st experiment – 4 hour exposure					
DMSO	1.00	200	1.5	3.5	2.0
125	1.13	200	0.5[a]	2.0[a]	1.5
250	1.30	200	0.5	4.0	3.5
500	1.14	200	3.0	5.0	2.0
1000[b]	0.23	26[c]	11.5*	15.4	3.8
Mito 0.1	0.84	200	10.0*	17.0	7.0
2nd experiment – 24 hour exposure					
DMSO	1.00	200	1.5	8.0	6.5
62.5	1.18	200	1.5	4.5	3.0
125	1.48	200	2.5	6.5	4.0
250	0.99	200	3.5	8.0	6.0
500	0.26	10[c]	0.0	20	20
Mito 0.1	0.90	200	10.0*	29	19

[a]: tetraploidy excluded from evaluation;
[b]: slight hemolysis
[c]: no more metaphases of sufficient quality for evaluation due to cytotoxicity
*: significantly different from negative control ($p < 0.05$)
Mito: mitomycin C
Data obtained from pages 28, 30 and 33 of the study report.

In the presence of metabolic activation, the mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with fluensulfone at concentrations from 125 to 500 µg/mL medium in the first and second experiment ranged from 1.5% to 3.5%. The results obtained are considered to be within the normal range of the solvent control where a mean incidence of chromosomal aberrations (excluding gaps) of 2.0% or 1.5% was observed after a 4-hour exposure in the first and second experiments, respectively. The range of aberrations in historical control data from 2007 to 2009 was 0 - 4%.

Only at the pronounced cytotoxic concentration of 1000 µg/mL medium in both experiments with metabolic activation (only 67 or 34 of 200 metaphases could be evaluated) were significant increases to 17.9% or 8.8% noted in the number of aberrations. It is known that high cytotoxicity causes artefacts in the form of aberrations in in vitro chromosomal tests. Hence, the increases at the concentration of 1000 µg fluensulfone/mL medium (4-hour exposure) are considered an artefact and not test item-related.

Table 3: Summary of experiments with metabolic activation

Fluensulfone Concentration (µg/mL)	Mitotic index (%)	Number of metaphases scored	% of cells with aberrations (excluding gaps)	% of cell with aberrations (including gaps)	% of cells with gaps
1st experiment – 4 hour exposure					
DMSO	1.00	200	2.0	6.5	4.5
125	1.30	200	1.5	4.5	3.0
250	1.14	200	2.0[a]	3.0	1.0
500	1.06	200	3.5	7.5	4.0
1000[b]	0.50	67[c]	**17.9*** [d]	31.3	13.4
Cyclo 10	0.54	200	**20*** [d]	30.5	10.5
2nd experiment – 4 hour exposure					
DMSO	1.00	200	1.5	7.0	5.5
125	1.17	200	2.0	8.5	6.5
250	1.37	200	1.5	5.0	3.5
500	1.26	200	3.5	10.0	6.5
1000[b]	0.20	34[c]	8.8*	20.6	11.8
Cyclo 10	0.75	200	10.5*	28.5	18.0

[a]: tetraploidy excluded from evaluation;
[b]: slight hemolysis
[c]: no more metaphases of sufficient quality for evaluation due to cytotoxicity
[d]: For the % cells with aberrations (excluding gaps) for the first experiment, page 29 of the study report indicated a value of 10.4% for the 1000 µg/mL concentration and 16.5% for the positive control. However, from the individual values reported on page 36 for the two duplicate cultures, these values should read 17.9% and 20% for the 1000 µg/mL and positive control cultures, respectively.
*: significantly different from negative control ($p < 0.05$)
Cyclo: cyclophosphamide
Data obtained from page 29, 36 and 39 of the study report.

III. CONCLUSIONS

Based on the results of this test, fluensulfone tested up to cytotoxic concentrations in the absence and in the presence of metabolic activation in human lymphocytes employing two exposure times without S9 and one exposure time with S9 revealed no indications of genotoxic properties with respect to chromosomal or chromatid damage. In the same test, positive controls Mitomycin C and Cyclophosphamide, induced significant damage.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the requirement for (OECD 473; OPPTS 870.5375) in human lymphocytes.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: No deficiencies were identified.

Tier II Summary **(PMRA #2181229, MRID 48574785)** revised by the regulatory authorities, April 2013

Study Type *In vitro* genotoxicity testing – Test for clastogenicity in mammalian cells – Metabolite #3625

IIA 5.8 - MRID 48574785, PMRA 2181229

Report:	Hall C. (2010) *In vitro* chromosome aberration test in Chinese hamster *V79* cells with MCW-2 Metabolite #3625 Harlan Cytotest Cell Research GmbH, Germany; unpublished report No. 1345801, dated 22 December 2010. Sponsor reference No. R-23471 Dates of experimental work: 16 June to 18 August 2010
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 473, EEC method B.10 and OPPTS 870.5375 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In an *in vitro* chromosome aberration assay (MRID 48574785, PMRA 2181229), duplicate cultures of Chinese hamster V79 cells were treated for 4 hours with fluensulfone metabolite thiazole sulfonic acid (MCW-2 metabolite #3625, 93.6%, Batch No. 231PAL071) (dissolved in deionised water) both in the presence and the absence of metabolic activation (Experiment I) or for 4 hours with metabolic activation and 18 hours without metabolic activation (Experiment II). Chromosomes were prepared 18 hours after the start of treatment, and at least 100 metaphases per culture were scored for structural chromosomal aberrations. Concentrations of 592.5, 1185 and 2370 µg/mL (corrected for purity) both without and with metabolic activation were evaluated, with the highest concentration corresponding to the highest recommended concentration of 10 mM.

In the absence and presence of S9 mix, neither cytotoxic nor clastogenic activity was observed up to and including the maximal concentration required by the OECD guideline.

No relevant evidence of an increase in polyploidy metaphases was noted after treatment with fluensulfone metabolite #3625 as compared to the control cultures.

Appropriate mutagens used as positive controls induced statistically significant increases in cells with structural chromosome aberrations.

Under the conditions of this assay, thiazole sulfonic acid did not induce structural chromosomal aberrations and showed no clastogenic effects when tested in Chinese hamster V79 cell line.

This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement (OPPTS 870.5375; OECD 473) for in vitro genotoxicity (chromosomal aberration) data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 metabolite #3625 (thiazole sulfonic acid)

Chemical name: 5-chloro-thiazole-2-sulfonic acid sodium salt

Description: White solid

Lot/Batch: 231PAL071

Purity: 93.6% (dose calculation adjusted for purity)

CAS#: Not available

Stability: Stable until 11 March 2011, stored at room temperature

2. Control materials:

Negative: Concurrent solvent control (deionised water)

Positive:

Non-activation (-S9): Ethyl methane sulfonate (EMS) (purity >98%, Acros Organic, Belgium)

Activation (+S9): Cyclophosphamide (CPA) (purity >98%, Sigma-Aldrich)

3. Activation: S9 fraction prepared by Harlan CCR from livers of Wistar male rats, induced by administration of 80 mg/kg bw/day Phenobarbital i.p. and 100 mg/kg bw/day β-Naphthoflavone p.o. for three consecutive days.

Each batch of S9 mix is routinely tested with 2-AA and benzo(a)pyrene.

The protein concentration in the S9 preparation was 35.0 mg/mL in the pre-experiment/experiment I, and 33.7 mg/mL in experiment II.

4. Test system:

Cells: V79

Source: Cell bank of Harlan RCC, Germany

Cell cultures: Thawed stock cultures were propagated at 37°C in 80 cm^2 plastic flasks. About 5 x 10^5 cells per flask were seeded in 15 mL of cell culture medium supplemented with 10% (v/v) fetal bovine serum (FBS). The cells were sub-cultured twice a week. The cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % carbon dioxide (98.5% air).

5. Test concentrations:

(a) Preliminary cytotoxicity assay: Concentrations between 148.1 and 2370.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity

(b) Mutation assays:

Since the cultures in the pre-experiment for cytotoxicity fulfilled the requirements for cytogenetic evaluation, the preliminary test was designated Experiment I: 9.3, 18.5, 37.0, 74.1, 148.1, 296.3, **592.5, 1185.0** and **2370.0** µg/mL (concentrations in bold evaluated for chromosome aberrations)

Experiment II: 9.3, 18.5, 37.0, 74.1, 148.1, 296.3, **592.5, 1185.0** and **2370.0** µg/mL (without S9 mix) and 148.1, 296.3, **592.5, 1185.0** and **2370.0** µg/mL (with S9 mix) (concentrations in bold evaluated for chromosome aberrations)

B. TEST PERFORMANCE

1. Test procedure

Exponentially growing stock cultures more than 50% confluent were rinsed with Ca-Mg-free salt solution containing 8000 mg/L NaCl, 200 mg/L KCl, 200 mg/L KH_2PO_4 and 150 mg/L Na_2HPO_4. Afterwards the cells were treated with trypsin-EDTA-solution at 37°C for approx. 5 minutes. Then, by adding complete culture medium including 10% (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.5% (w/v) in Ca-Mg-free salt solution. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1 x 10^4 to 6 x 10^4 cells were seeded with regard to the preparation time.

4-hour exposure

The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL culture medium were added (S9 protein concentration in assay medium 0.75 mg/mL).

Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4. The cells were then cultured in complete medium containing 10% (v/v) FBS for the remaining culture time of 14 hours.

18-hour exposure

The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10% (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.

All cultures were incubated at 37°C in a humidified atmosphere with 1.5% CO_2 (98.5 % air)

Colcemid was added to the culture medium (0.2 µg/mL) 15.5 hours after the start of the treatment. The cells were treated, 2.5 hours later, on the slides in the chambers with hypotonic solution (0.4% KCl) for 20 minutes at 37°C. After incubation in the hypotonic solution the cells were fixed with a mixture of methanol and glacial acetic acid (3:1 parts, respectively). After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

The evaluation of cytotoxicity indicated by reduced cell numbers was made after the preparation of the cultures on spread slides. The cell numbers were determined microscopically by counting 10 defined fields per coded slide. The cell number of the treatment groups is given in percentage compared to the respective solvent control.

Evaluation of the cultures was performed according to the OECD guideline using NIKON microscopes with 100x objectives. Breaks, fragments, deletions, exchanges, and chromosome disintegrations were recorded as structural chromosome aberrations. Gaps were recorded as well but not included in the calculation of the aberration rates. At least 100 well spread metaphases per culture were evaluated for cytogenetic damage on coded slides. Only metaphases with characteristic chromosome numbers of 22 ± 1 were included in the analysis. To describe a cytotoxic effect the mitotic index (% cells in mitosis) was determined.

2. Acceptability of the test

The chromosome aberration test was considered acceptable by the conducting laboratory, if:

a) The number of structural aberrations found in the solvent controls falls within the range of the laboratory's historical control data, and

b) The positive control substances produce significant increases in the number of cells with structural chromosome aberrations, which are within the range of the laboratory's historical control data.

3. Evaluation criteria

A test item is classified as non-clastogenic by the conducting laboratory if:

− the number of induced structural chromosome aberrations in all evaluated dose groups is in the range of the laboratory's historical control data, and

− no significant increase of the number of structural chromosome aberrations is observed.

A test item is classified as clastogenic by the conducting laboratory if:

- the number of induced structural chromosome aberrations is not in the range of the Laboratory's historical control data; and

- either a concentration-related or a significant increase of the number of structural chromosome aberrations is observed.

Statistical significance was confirmed by means of the Fisher's exact test. However, both biological and statistical significance should be considered together. If the criteria mentioned above for the test item are not clearly met, the classification with regard to the historical data and the biological relevance is discussed and/or a confirmatory experiment is performed.

Although the inclusion of structural chromosome aberrations is the purpose of this study, it is important to include the polyploids and endoreduplications. The following criterion is considered by the conducting laboratory to be valid:

- A test item can be classified as aneugenic if the number of induced numerical aberrations is not in the range of the laboratory's historical control data.

II. RESULTS AND DISCUSSION

No precipitation and no influence on pH value or osmolarity were noted. No toxic effects (i.e. reduction of mitotic index and/or reduced cell number) were observed following treatment up to the highest required concentration.

Both in the absence and presence of S9 mix, no treatment-related, statistically significant or biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. The aberration rates of the cells after treatment with the test item (0.5 - 2.8% aberrant cells, excluding gaps) were within the range of the solvent control values (0.5 - 3.5% aberrant cells, excluding gaps) and within the range of the laboratory's historical control data (0.0 - 4.0% aberrant cells, excluding gaps).

No relevant evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the controls.

In both experiments, either EMS (500.0 or 1000.0 µg/mL) or CPA (1.4 µg/mL) were used as positive controls and showed distinct increases in the number of cells with structural chromosome aberrations.

Results are summarized in Table 1.

Table 1: Summary of results

Experiment	Preparation interval (hours)	Conc. (µg/mL)	Cell number (% of control)	Mitotic index (% of control)	Aberrant cells		
					Including gaps[a]	Excluding gaps[a]	With exchanges
Exposure period 4 hours, without S9 mix							
I	18	Deionised water	100.0	100.0	2.5	2.0	0.5
		EMS 1000	Not determined	52.3	32.0	31.0*	21.5
		592.5	99.0	90.1	1.0	1.0	1.0
		1185.0	95.8	88.2	1.5	0.5	0.5
		2370.0	91.0	99.7	1.0	1.0	0.5
Exposure period 18 hours, without S9 mix							
II	18	Deionised water	100.0	100.0	1.0	0.5	0.0
		EMS 500	Not determined	80.2	16.5	16.0*	9.0
		592.5	85.1	95.7	2.5	2.0	1.0
		1185.0	90.6	115.1	1.5	1.5	0.0
		2370.0	83.6	114.7	0.0	0.0	0.0
Exposure period 4 hours, with S9 mix							
I	18	Deionised water 10% (v/v)	100.0	100.0	1.5	1.5	0.5
		CPA 1.4	Not determined	53.5	13.0	13.0*	3.5
		592.5	98.4	106.5	0.5	0.5	0.0
		1185.0	98.4	103.5	2.0	2.0	1.0
		2370.0	100.0	107.8	1.5	1.5	0.0
II	18	Deionised water	100.0	100.0	3.5	3.5	1.5
		CPA 1.4	Not determined	52.9	17.0	14.0*	2.5
		592.5	83.1	97.9	1.0	1.0	0.0
		1185.0	108.8	94.3	3.0	2.5	1.0
		2370.0[#]	110.0	103.2	2.8	2.8	0.5

[a]: inclusive cells carrying exchanges; *: statistically significantly different compared to the control (level of significance not indicated); [#]: 200 metaphase cells per culture evaluated; EMS: ethyl methane sulfonate; CPA: cyclophosphamide. Data obtained from page 24 of the study report.

III. CONCLUSIONS

Under the conditions of this assay, thiazole sulfonic acid did not induce structural chromosomal aberrations and showed no clastogenic effects when tested in Chinese hamster V79 cell line.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement (OPPTS 870.5375; OECD 473) for in vitro genotoxicity (chromosomal aberration) data.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: No deficiencies were identified.

Tier II Summary (PMRA #2181230, MRID 48574794) revised by the regulatory authorities, April 2013

Study Type: *In vitro* genotoxicity testing – Test for clastogenicity in mammalian cells – Metabolite #3627

IIA 5.8 – MRID 48574794, PMRA 2181230

Report:	Hall C. (2010) *In vitro* chromosome aberration test in Chinese hamster *V79* cells with MCW-2 Metabolite #3627 Harlan Cytotest Cell Research GmbH, Germany; unpublished report No. 1328701, dated 12 July 2010. Sponsor reference No. R-23467 Dates of experimental work: 17 March to 12 May 2010
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 473, EEC method B.10 and OPPTS 870.5375 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In an *in vitro* chromosome aberration assay (MRID 48574794, PMRA 2181230), duplicate cultures of Chinese hamster V79 cells were treated for 4 hours with fluensulfone metabolite butene sulfonic acid (MCW-2 metabolite #3627, 99.5%, Batch No. 215PAL44) (dissolved in deionised water) both in the presence and the absence of metabolic activation (Experiment I) or for 4 hours with metabolic activation and 18 hours without metabolic activation (Experiment II). Chromosomes were prepared 18 hours after the start of treatment, in duplicate cultures of each experiment, and at least 100 metaphases per culture were scored for structural chromosomal aberrations, with the exception of the positive control in Experiment I without metabolic activation where only 50 metaphases were evaluated. Concentrations of 532.8, 1065.5 and 2131 µg/mL both without and with metabolic activation were evaluated, with the highest concentration corresponding to the highest recommended concentration of 10 mM.

In the absence and presence of S9 mix, neither cytotoxic nor clastogenic activity was observed up to and including the maximal concentration required by the OECD guideline.

No relevant evidence of an increase in polyploid metaphases was noted after treatment with butene sulfonic acid as compared to the control cultures.

Appropriate mutagens used as positive controls induced statistically significant increases in cells with structural chromosome aberrations.

Under the conditions of this assay, butene sulfonic acid did not induce structural chromosomal aberrations and showed no clastogenic effects when tested in the Chinese hamster V79 cell line.

This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement (OPPTS 870.5375; OECD 473) for in vitro genotoxicity (chromosomal aberration) data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 metabolite #3627 (butane sulfonic acid)

Chemical name: 3,4,4-trifluorobutene-1-sulfonic acid, sodium salt

Description: White solid

Lot/Batch: 215PAL44

Purity: 99.5%

CAS#: Not available

Stability: Stable until 21 December 2010, stored at room temperature

2. Control materials:

Negative: Concurrent solvent control (deionised water)

Positive:

Non-activation (-S9): Ethyl methane sulfonate (EMS) (purity >98%, Acros Organic, Belgium)

Activation (+S9): Cyclophosphamide (CPA) (purity >98%, Sigma-Aldrich)

3. Activation: S9 fraction prepared from livers of Wistar male rats, induced by administration of 80 mg/kg bw/day phenobarbital i.p. and 100 mg/kg bw/day β-naphthoflavone p.o. for three consecutive days.

Each batch of S9 mix is routinely tested with 2-AA and benzo(a)pyrene.

The protein concentration in the S9 preparation was 35.0 mg/mL in the pre-experiment/experiment I, and 34.4 mg/mL in experiment II.

4. Test system:

Cells: V79

Source: Cell bank of Harlan CCR, Germany

Cell cultures: Thawed stock cultures were propagated at 37°C in 80 cm^2 plastic flasks. About 5 x 10^5 cells per flask were seeded in 15 mL of cell culture medium supplemented with 10% (v/v) fetal bovine serum (FBS). The cells were sub-cultured twice a week. The cell cultures were incubated at 37°C in a humidified atmosphere with 1.5 % carbon dioxide (98.5% air).

5. Test concentrations:

(a) Preliminary cytotoxicity assay: Concentrations between 148.1 and 2131.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity

(b) Mutation assays: Since the cultures in the pre-experiment for cytotoxicity fulfilled the requirements for cytogenetic evaluation, the preliminary test was designated Experiment I: 8.3, 16.6, 33.3, 66.6, 133.2, 266.4, **532.8, 1065.5** and **2131.0** µg/mL (concentrations in bold evaluated for chromosome aberration)

Experiment II: 8.3, 16.6, 33.3, 66.6, 133.2, 266.4, **532.8, 1065.5** and **2131.0** µg/mL (without S9 mix) and 133.2, 266.4, **532.8, 1065.5** and **2131.0** µg/mL (with S9 mix) (concentrations in bold evaluated for chromosome aberration)

B. TEST PERFORMANCE

1. Test procedure

Exponentially growing stock cultures more than 50% confluent were rinsed with Ca-Mg-free salt solution containing 8000 mg/L NaCl, 200 mg/L KCl, 200 mg/L KH_2PO_4 and 150 mg/L Na_2HPO_4. Afterwards the cells were treated with trypsin-EDTA-solution at 37°C for approx. 5 minutes. Then, by adding complete culture medium including 10% (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.5% (w/v) in Ca-Mg-free salt solution. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1 x 10^4 to 6 x 10^4 cells were seeded with regard to the preparation time.

4-hour exposure

The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix (S9 fraction in cofactor solution) per mL culture medium were added.

Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4. The cells were then cultured in complete medium containing 10% (v/v) FBS for the remaining culture time of 14 hours.

18-hour exposure

The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10% (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.

All cultures were incubated at 37°C in a humidified atmosphere with 1.5% CO_2 (98.5 % air)

Colcemid was added to the culture medium (0.2 µg/mL) 15.5 hours after the start of the treatment. The cells were treated, 2.5 hours later, on the slides in the chambers with hypotonic solution (0.4% KCl) for 20 minutes at 37°C. After incubation in the hypotonic

solution the cells were fixed with a mixture of methanol and glacial acetic acid (3:1 parts, respectively). After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

The evaluation of cytotoxicity indicated by reduced cell numbers was made after the preparation of the cultures on spread slides. The cell numbers were determined microscopically by counting 10 defined fields per coded slide. The cell number of the treatment groups is given in percentage compared to the respective solvent control.

Evaluation of the cultures was performed according to the OECD guideline using NIKON microscopes with 100x objectives. Breaks, fragments, deletions, exchanges, and chromosome disintegrations were recorded as structural chromosome aberrations. Gaps were recorded as well but not included in the calculation of the aberration rates. At least 100 well spread metaphases per culture were evaluated for cytogenetic damage on coded slides. Only metaphases with characteristic chromosome numbers of 22 ± 1 were included in the analysis. To describe a cytotoxic effect the mitotic index (% cells in mitosis) was determined.

2. Acceptability of the test

The chromosome aberration test was considered acceptable by the conducting laboratory, if:

a) The number of structural aberrations found in the solvent controls falls within the range of the laboratory's historical control data, and

b) The positive control substances produce significant increases in the number of cells with structural chromosome aberrations, which are within the range of the laboratory's historical control data.

3. Evaluation criteria

A test item is classified as non-clastogenic by the conducting laboratory if:

− the number of induced structural chromosome aberrations in all evaluated dose groups is in the range of the laboratory's historical control data, and

− no significant increase of the number of structural chromosome aberrations is observed.

A test item is classified as clastogenic by the conducting laboratory if:

− the number of induced structural chromosome aberrations is not in the range of the Laboratory's historical control data,

− either a concentration-related or a significant increase of the number of structural chromosome aberrations is observed.

Statistical significance was confirmed by means of the Fisher's exact test. However, both biological and statistical significance should be considered together. If the criteria mentioned above for the test item are not clearly met, the classification with regard to the historical data and the biological relevance is discussed and/or a confirmatory experiment is performed.

Although the inclusion of the structural chromosome aberrations is the purpose of this study, it is important to include the polyploids and endoreduplications. The following criterion is considered by the conducting laboratory to be valid:

− A test item can be classified as aneugenic if the number of induced numerical aberrations is not in the range of the laboratory's historical control data.

II. RESULTS AND DISCUSSION

No precipitation and no influence on pH value or osmolarity were noted. No toxic effects (i.e. reduction of mitotic index and/or reduced cell number) were observed following treatment up to the highest required concentration.

Both in the absence and presence of S9 mix, no treatment-related, statistically significant or biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. The aberration rates of the cells after treatment with butene sulfonic acid (0.5 - 3.8% aberrant cells, excluding gaps) were close to the range of the solvent control values (2.0 – 3.0% aberrant cells, excluding gaps) and within the range of the laboratory's historical control data (0.0 - 4.0% aberrant cells, excluding gaps).

No relevant evidence of an increase in polyploid metaphases was noticed after treatment with butene sulfonic acid (2.6 – 4.9%) as compared to the controls (3.2-4.3%). In Experiment I, in the absence and presence of S9 mix slight increases in the rate of endomitotic metaphases were found. Since no increases in the rate of endomitotic metaphases were found in Experiment II, these observations were regarded as being not biologically relevant.

In both experiments, either EMS (500.0 or 1000.0 µg/mL) or CPA (1.4 µg/mL) were used as positive controls and showed distinct increases in the number of cells with structural chromosome aberrations.

Results are summarized in Table 1.

Table 1: Summary of results

Exp	Prepar-ation interval (hours)	Conc. (µg/mL)	Polyploid cells (%)	Endomitotic cells (%)	Cell No. (% of control)	Mitotic index (% of control)	Aberrant cells		
							Incl. gaps[a]	Excl. gaps[a]	With exchanges
Exposure period 4 hours, without S9 mix									
I	18	Water	3.7	0.1	100.0	100.0	2.0	2.0	1.0
		EMS[#]	Not determined	Not determined	Not tested	92.7	44.0	42.0*	34.0
		532.8	3.6	0.0	113.2	115.7	0.5	0.5	0.5
		1065.5[##]	3.7	0.0	99.4	110.1	4.0	3.8	1.8
		2131.0[##]	4.2	0.6	99.4	95.8	3.8	3.8	2.0
Exposure period 18 hours, without S9 mix									
II	18	Water	3.7	0.0	100.0	100.0	2.5	2.5	0.0
		EMS	4.3	0.0	Not tested	82.8	14.0	14.0*	8.5
		532.8	4.2	0.0	94.0	102.3	1.0	1.0	0.0
		1065.5	2.9	0.0	73.4	112.0	1.5	1.5	0.0
		2131.0	2.8	0.0	108.5	105.7	1.5	1.5	0.0
Exposure period 4 hours, with S9 mix									
I	18	Water	4.3	0.2	100.0	100.0	2.5	2.0	0.5
		CPA	Not determined	Not determined	Not tested	98.1	18.5	18.5*	6.0
		532.8	4.5	0.2	89.2	112.7	2.0	1.5	0.0
		1065.5	4.9	0.1	80.9	115.5	1.5	1.5	0.5
		2131.0	4.8	0.1	78.8	122.0	2.0	2.0	0.5
II	18	Water	3.2	0.0	100.0	100.0	3.5	3.0	1.5
		CPA	2.4	0.0	Not tested	69.6	27.5	24.5*	11.5
		532.8	3.2	0.0	91.5	112.3	2.0	2.0	0.5
		1065.5	2.6	0.0	86.5	119.0	2.5	2.0	1.0
		2131.0	2.8	0.0	84.4	109.0	3.5	3.5	1.5

[a]: inclusive cells carrying exchanges; *: statistically significantly different compared to the control (level of significance not indicated); [#]: 50 metaphase cells per culture evaluated; [##]: 200 metaphase cells per culture evaluated; EMS: ethyl methane sulfonate; CPA: cyclophosphamide. Data obtained from pages 24-25 of the study report.

III. CONCLUSIONS

Under the conditions of this assay, butene sulfonic acid did not induce structural chromosomal aberrations and showed no clastogenic effects when tested in the Chinese hamster V79 cell line.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement (OPPTS 870.5375; OECD 473) for in vitro genotoxicity (chromosomal aberration) data.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: No deficiencies were identified.

Tier II Summary **(PMRA #2181216, MRID 48574764)** revised by the regulatory authorities, April 2013

Study Type *In vivo* genotoxicity testing (somatic cells) – Metaphase analysis in rodent bone marrow or micronucleus test in rodents

IIA 5.4.4 – MRID 48574764, PMRA 2181216

Report:	Herbold B. (2003) BYI01921 Micronucleus test on the male mouse. Bayer HealthCare, Molecular and Genetic Toxicology, Germany; unpublished report No. AT00208, dated 15 January 2003. Study No. T3071483, Sponsor reference No. R-23143 Dates of experimental work: 22 October to 8 November 2002
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 474, 2000/32/EC B.12 and OPPTS 870.5395
Deviations:	Clinical signs are mentioned but not reported for individual animals. This deviation did not affect the outcome of the study.
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary:

In an *in vivo* micronucleus study (MRID 48574764, PMRA 2181216), fluensulfone (BYI 01921, MCW-2 tech., 98.4%, Batch No. NLL-6692-13-1) suspended in 0.5% aqueous Cremophor was administered to male Hsd/Win: NMRI mice (5/dose) twice by intraperitoneal injection at doses of 75, 150 and 300 mg/kg bw, separated by 24 hours. Males of the positive control received a single intraperitoneal treatment with 20 mg/kg bw cyclophosphamide. Bone marrow slides from femurs were prepared 24 hours after the last administration. Selection of dose levels and testing of males only was based on results of a preliminary experiment.

Symptoms of toxicity (apathy, roughened fur, loss of weight, spasm, difficulty in breathing, slitted eyes and closed eyes) were observed in treated mice after administration until terminal sacrifice, starting at 75 mg/kg bw. These symptoms demonstrate relevant systemic exposure. However, all males survived until the end of the test and no altered ratio between polychromatic and normochromatic erythrocytes was recorded.

After two intraperitoneal treatments of males with doses up to and including 300 mg/kg bw, no indications of a clastogenic effect of fluensulfone were found as there was no dose-related increase in polychromatic erythrocytes with micronuclei.

Cyclophosphamide, the positive control, had a clear clastogenic effect, shown by the biologically relevant increase in polychromatic erythrocytes with micronuclei. The ratio of polychromatic to normochromatic erythrocytes was not altered.

Fluensulfone administered by intraperitoneal route twice up to 300 mg/kg bw showed no clastogenic effects in this *in vivo* test.

This study is classified as **acceptable/guideline (fully reliable)** and satisfies the requirement for (OECD 474; OPPTS 870.5395) for in vivo cytogenetic mutagenicity data.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BYI 01921 (MCW-2 tech. or fluensulfone)
Description:	Light-brown crystalline molten mass
Batch No.:	NLL6692-13.1
Purity:	98.4%
CAS#:	318290-98-1
Expiry date:	21 December 2002; stored at room temperature
Stability of Test Item:	A stability test in the solvent (Cremophor) did not reveal significant degradation of the active ingredient (no change in active ingredient content after storage for 4 hours at room temperature).
2. Control materials:	Vehicle: 0.5% aqueous Cremophor (Fluka, batch 398261/1 34099) Positive control: Cyclophosphamide (CPA - in the form of Endoxan 100 mg injection vials of dry substance, ASTA Medica AG, batch 9M642) in deionised water
3. Test animals	
Species:	Mouse (male)
Strain:	Hsd/Win: NMRI
Age:	6 to 12 weeks
Weight at dosing:	38-44 g
Source:	Harlan Winkelmann GmbH, Germany
Acclimation period:	At least 5 days
Diet:	Fixed formula feed 3883 (10 mm cubes), produced according to specification by Provimi Kliba SA, Switzerland, available *ad libitum*
Water:	Tap water provided in polycarbonate bottles of 300 mL, available *ad libitum*
Housing:	Singly in type I cages, with bedding of soft wood granules (type BK8/15 - J. Rettenmaier & Söhne, Germany)

4. Environmental conditions

Temperature:	22.5 °C (setting: 22 ± 1.5 °C)
Humidity:	Mean 41-49% (setting: 40 to 70%)
Air changes:	About ten times per hour
Photoperiod:	Twelve hours of electrical lighting daily (6:00 hours to 18:00 hours, about 500 lux)

B. STUDY DESIGN AND METHODS

1. In life dates: 22 October to 8 November 2002

2. Dose selection rationale:

The doses selected were based on the results of a pilot test, in which groups of three mice/sex received two intraperitoneal injections separated by 24 hours of fluensulfone at 200, 400 (males only), 500 and 1000 mg/kg bw. At 200 mg/kg bw and above, several signs of toxicity were noted in males and/or females (e.g. apathy, uncoordinated movement, roughened fur, loss of weight, staggering gait, spasm, twitching, difficulty breathing, slitted eyes, closed eyes, white tears). Deaths were observed at 400 mg/kg bw (1 male), 500 mg/kg bw (3 males and 1 female), and 1000 mg/kg bw (3 males and 3 females). Based on these findings, a dose of 300 mg/kg bw was selected as the maximum tolerable dose for males. The study investigators noted that as there were no substantial differences between sexes with respect to toxicity, females were not used in the main study.

3. Animal assignment and treatment:

Mice were divided into groups of 5/dose by a randomization plan.

Fluensulfone was administered twice by intraperitoneal injection, separated by 24 hours, whereas cyclophosphamide was administered only once. Fluensulfone was administered at doses of 75, 150, and 300 mg/kg bw, while cyclophosphamide was administered at a dose of 20 mg/kg bw, based on the animal's weight that day.

Animals of a replacement group were treated twice with 300 mg/kg bw and would have only been used for substitution of animals which died in the group treated with the same dose of the test substance.

Fluensulfone was suspended in 0.5% aqueous Cremophor emulsion, using a microdismembrator for 5 minutes, and formed white turbid suspensions. The suspensions were stirred with a magnetic mixer during administration and injected intraperitoneally.

Cyclophosphamide was dissolved in deionised water and administered in the same way. The negative control received 0.5% aqueous Cremophor emulsion by the same method. In all of the groups, the administered volume was 10 mL/kg bw.

All animals were sacrificed 24 hours after the last treatment.

4. Preparation of specimens

At least one intact femur was prepared from each sacrificed animal. The bone marrow was extracted into fetal calf serum and then centrifuged at approximately 1000 rpm for five minutes. Smears of the suspension were prepared on slides that were then dried overnight and stained automatically with an Ames Hema-Tek slide stainer (Miles Company), destained with methanol, rinsed with deionized water, and left to dry.

5. Acceptance Criteria

An assay is considered acceptable if the figures of negative and positive controls are within the expected range, in accordance with the laboratory's experience and/or the available literature data.

6. Evaluation of results

Coded slides were evaluated using a light microscope at a magnification of about 1000. Micronuclei appear as stained chromatin particles in the anucleated erythrocytes and can be distinguished from artifacts by varying the focus.

Typically, 2000 polychromatic erythrocytes were counted per animal. The incidence of cells with micronuclei was established by scanning the slides in a meandering pattern.

The number of normochromatic erythrocytes per 2000 polychromatic erythrocytes was recorded, and the number of normochromatic erythrocytes showing micronuclei was also established.

A test was considered positive if there was a relevant and significant increase in the number of polychromatic erythrocytes showing micronuclei in comparison to the negative controls. A test was considered negative if there was no relevant or significant increase in the rate of micronucleated polychromatic erythrocytes. A test was also considered negative if there was a significant increase in the rate which, according to the laboratory's experience, was within the range of historical negative controls. A test was considered equivocal if there was an increase of micronucleated polychromatic erythrocytes above the range of attached historical negative controls, provided the increase was not significant and the result of the negative control was not closely related to the data of the respective treatment group.

7. Statistics

The treated group(s) with the highest mean (provided this superceded the negative control mean) and the positive control were checked by Wilcoxon's nonparametric rank sum test with respect to the number of polychromatic erythrocytes having micronuclei and the number of normochromatic erythrocytes. A variation was considered statistically significant if its error probability was below 5% and the treatment group figure was higher than that of the negative control.

II. RESULTS AND DISCUSSION

A. FINDINGS

Symptoms of toxicity (apathy, roughened fur, loss of weight, spasm, difficulty breathing, slitted eyes and closed eyes) were observed in treated mice after administration and to terminal sacrifice, starting at 75 mg/kg bw. These symptoms demonstrate relevant systemic exposure; however, all males survived until the end of the test and no altered ratio between polychromatic and normochromatic erythrocytes was recorded.

After two intraperitoneal treatments of males with doses up to and including 300 mg/kg bw, no indications of a clastogenic effect of fluensulfone were found, as there was no increase in polychromatic erythrocytes with micronuclei (Table 1).

Cyclophosphamide, the positive control, had a clear clastogenic effect, shown by the biologically relevant increase in polychromatic erythrocytes with micronuclei. The ratio of polychromatic to normochromatic erythrocytes was not altered, demonstrating that an alteration of the ratio of polychromatic to normochromatic erythrocytes is not necessary for the induction of micronuclei.

Table 1: Summary of results (mean ± standard deviation of 5 mice/group)

Experimental group	Number of evaluated PCE	Number of NCE per 2000 PCE	MNNCE per 2000 NCE	MNPCE per 2000 PCE
Negative control	10,000	1845 ± 863	3.4 ± 0.8	3.4 ± 1.8
Fluensulfone 2 x 75 mg/kg bw	10,000	2327 ± 1032	1.9 ± 1.3	6.0 ± 2.0
Fluensulfone 2 x 150 mg/kg bw	10,000	2662 ± 897	4.3 ± 2.6	3.8 ± 2.6
Fluensulfone 2 x 300 mg/kg bw	10,000	2551 ± 850	2.1 ± 1.1	3.8 ± 1.1
CPA 20 mg/kg bw	10,000	1053 ± 209	3.8 ± 1.8	22.8* ± 8.1

PCE: polychromatic erythrocytes; NCE: normochromatic erythrocytes; MNNCE: micronucleated NCE; MNPCE micronucleated PCE; CPA = cyclophosphamide
* $p < 0.01$ in non-parametric Wilcoxon ranking test
Data obtained from page 45 of the study report.

III. CONCLUSIONS

Fluensulfone administered by intraperitoneal route twice, separated by 24 hours, up to 300 mg/kg showed no clastogenic effects in this *in vivo* test. Animals were tested at maximally tolerated doses resulting in systemic toxicity, although no evidence of toxicity to the bone marrow was observed.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable (fully reliable) and satisfies the requirement for Test Guideline OPPTS 870.5395; OECD 474 for in vivo cytogenetic mutagenicity data.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: Individual clinical signs were not reported. No other deficiencies were identified.

Tier II Summary **(PMRA #2181236, MRID 48574786)** revised by the regulatory authorities, April 2013

Study Type: In vivo micronucleus assay – metabolite #3625

IIA 5.8 - MRID 48574786, PMRA 2181236

Report:	Merker M. (2011) Micronucleus assay in bone marrow cells of the rat with MCW-2 METABOLITE #3625 Harlan Cytotest Cell Research GmbH, Germany; unpublished report No. 1345802, dated 6 July 2011. Sponsor reference No. R-23472 Dates of experimental work: 12 July to 11 August 2010
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 474, EC method B.12 and OPPTS 870.5395 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary:

This *in vivo* micronucleus assay (MRID 48574786, PMRA 2181236) was performed to investigate the potential of fluensulfone metabolite thiazole sulfonic acid (MCW-2 metabolite #3625, 93.6%, Batch No. 231PAL080) to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the rat.

Seven male rats per test group were evaluated for the occurrence of micronuclei. At least 2000 PCEs per animal were scored. To describe a cytotoxic effect due to the treatment with the test item, the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and reported as the number of PCEs per 2000 erythrocytes.

The following dose levels were investigated (single oral doses in sterile water):

- 24 hour preparation interval: 500, 1000 and 2000 mg/kg bw
- 48 hour preparation interval: 2000 mg/kg bw

The high dose (2000 mg/kg bw) was estimated to be suitable by a pre-experiment in which similar toxic signs (i.e. ruffled fur) were noted in males and females treated at the limit dose of 2000 mg/kg bw. As there were no differences in the toxic response of the two sexes, males only were used in the main study. In the main study, treatment at 2000 mg/kg bw caused reduction of spontaneous activity in some of the treated animals up to 4 hours post dosing, and ruffled fur in all animals up to 24 hours post dosing. Similar signs were noted in rats treated at 1000 mg/kg bw while reduction of spontaneous activity was limited to 1 hour post dosing for those receiving 500 mg/kg bw.

The mean number of PCEs was comparable among all groups.

In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any dose level at 24 or 48 hours after administration of the test item.

Cyclophosphamide administered orally at 20 mg/kg bw was used as positive control and showed a substantial increase in induced micronucleus frequency.

Under the conditions of this assay, thiazole sulfonic acid was considered to be non-genotoxic in this micronucleus assay.

This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for in vivo cytogenetic mutagenicity data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material:	MCW-2 metabolite #3625 (thiazole sulfonic acid)
Chemical name:	5-chloro-thiazole-2-sulfonic acid sodium salt
Description:	White solid
Lot/Batch:	231PAL080
Purity:	93.6% (dose calculation adjusted for purity)
CAS#:	Not available
Stability:	Stable until 25 March 2011, stored at room temperature
2. Vehicle / positive control:	Sterile water / Cyclophosphamide (CPA)
3. Test animals:	
Species:	Rat
Strain:	Wistar - males
Age:	At dosing: 8-12 weeks
Weight at dosing:	Mean ± S.D: 169.1 ± 6.9 g
Source:	Harlan Laboratories B.V., The Netherlands
Acclimation period:	Minimum 5 days
Diet:	Pelleted standard diet (Harlan Laboratories B.V., The Netherlands) *ad libitum*
Water:	Tap water *ad libitum*
Housing:	In groups in Makrolon type-3 or 4 cages with wire mesh tops and granulated softwood bedding (Rettenmaier & Söhne GmbH & Co. KG, Germany)

4. Environmental conditions:

Temperature:	20 - 25°C
Humidity:	45 - 80%
Air changes:	Not indicated
Photoperiod:	Light cycle of 12 hours light and 12 hours dark

B. STUDY DESIGN AND METHODS:

1. In life dates: 12 July to 11 August 2010

2. Animals assignment and treatment

A preliminary study on acute toxicity was performed with two animals/sex dosed with 2000 mg/kg bw of metabolite #3625 under identical conditions as the mutagenicity study. The only sign noted was ruffled fur observed in both males and females at 1, 2-4, 6, 24, 30 and 48 hours post-dose. Since no sex specific differences concerning signs of toxicity were observed, the main study was performed using males only.

In the main study, animals were randomly assigned to the test groups.

Fluensulfone metabolite #3625 in sterile water was administered by oral gavage as a single oral dose at 2000, 1000 and 500 mg/kg bw to groups of 7 male Wistar rats/dose. Similar sized groups received the vehicle or the positive control substance once orally. The individual volume to be administered (10 mL/kg bw) was adjusted to the animal's body weight. Sampling and preparation of bone marrow smears were carried out at 24 hours after treatment and, for the higher dose only, at 48 hours after treatment. Sacrifice at 24 hours included vehicle and positive (cyclophosphamide) controls. The animals of all dose groups (except the positive control) were examined for acute toxic symptoms at intervals of approximately 1, 2 - 4, 6, 24 and 48 hours after administration of the test item.

3. Preparation of bone marrow and evaluation of cells

The animals were sacrificed using CO_2 followed by exsanguination. The femora were removed, the epiphyses were cut off and the marrow was flushed out with fetal calf serum using a syringe. The nucleated cells were separated from the erythrocytes using the method of Romagna. Briefly, the cell suspensions were passed through a column consisting of α-Cellulose and Cellulose. The columns were then washed with Hank´s buffered saline. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 minutes and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald/Giemsa. Cover slips were then mounted. At least one slide was made from each bone marrow sample.

Evaluation of the slides was performed using NIKON microscopes with 100x oil immersion objectives. 2000 polychromatic erythrocytes (PCE) were analyzed for micronuclei per animal. To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes. The analysis was performed with coded slides, the analyst blinded to treatment.

4. Acceptance criteria

The study was considered by the study investigators to be valid as the following criteria were met:

- at least 5 animals per sex and test group could be evaluated,

- PCE to erythrocyte ratio was not less than 20% of the negative control, and

- the positive control showed a statistically significant and biologically relevant increase of micronucleated PCEs compared to the vehicle control.

5. Evaluation of results

A test item is classified by the study investigators as mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group. Statistical methods (nonparametric Mann-Whitney test) were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results.

A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes is considered by the study investigators to be non-mutagenic in this system.

II. RESULTS AND DISCUSSION

A. FINDINGS

Treatment at 2000 mg/kg bw caused reduction of spontaneous activity in some of the treated animals up to 4 hours post dosing, and ruffled fur in all animals up to 24 hours post dosing. Similar signs were noted in rats treated at 1000 mg/kg bw while reduction of spontaneous activity was limited to 1 hour post dosing for those receiving 500 mg/kg bw.

The number of PCEs was comparable among groups. In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any dose level at 24 hours after administration of the test item, or at the high dose at the 48-hour time point (Table 1).

Cyclophosphamide administered orally at 20 mg/kg bw was used as positive control and showed a substantial increase in induced micronucleus frequency.

Table 1: Summary of a micronucleus test in rats

Test group	Dosage (mg/kg bw)	Sampling time (hours)	Mean ± S.D. PCEs with micronuclei (%)	Range (micronucleated cells/2000 PCEs)	Mean ± S.D. PCE per 2000 erythrocytes
Corn oil	0	24	0.143 ± 0.101	0-6	1305 ± 213
Fluensulfone metabolite #3625	500		0.107 ± 0.061	1-4	1276 ± 177
	1000		0.186 ± 0.107	1-8	1298 ± 93
	2000		0.221 ± 0.180	1-11	1302 ± 183
Cyclophosphamide	20		2.057 ± 0.935*	19-71	1058 ± 198
Fluensulfone metabolite #3625	2000	48	0.129 ± 0.118	0-7	1301 ± 182

Data obtained from page 21 of the study report. Standard deviations were not provided in the report and thus were calculated by the reviewer. * Statistically significantly different from the vehicle control group ($p<0.05$).

III. CONCLUSIONS

Under the conditions of this assay, metabolite #3525 (thiazole sulfonic acid) was considered to be non-genotoxic in this micronucleus assay at the maximum guideline recommended dose level of 2000 mg/kg bw. Systemic toxicity to the test animals, but not direct cytotoxicity to the target (bone marrow), was observed.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for in vivo cytogenetic mutagenicity data.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: Individual clinical signs were not reported. No other deficiencies were identified.

Tier II Summary **(PMRA #21812137, MRID 48574791)** revised by the regulatory authorities, April 2013

Study Type: In vivo micronucleus assay – metabolite #3626

IIA 5.8 – MRID 48574791, PMRA 21812137

Report:	Merker M. (2011) Micronucleus assay in bone marrow cells of the rat with MCW-2 METABOLITE #3626 Harlan Cytotest Cell Research GmbH, Germany; unpublished report No. 1345902, dated 6 July 2011. Sponsor reference No. R-23476 Dates of experimental work: 21 June to 26 July 2010
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 474, EC method B.12 and OPPTS 870.5395 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary

This in vivo micronucleus assay (MRID 48574791, PMRA 21812137) was performed to investigate the potential for fluensulfone metabolite methyl sulfone (MCW-2 metabolite #3626, 98.8%, Batch No. 231PAL052) to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the rat.

Seven male rats per test group were evaluated for the occurrence of micronuclei. At least 2000 PCEs per animal were scored. To describe a cytotoxic effect due to the treatment with the test item, the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and reported as the number of PCEs per 2000 erythrocytes.

The following dose levels were investigated (single oral doses in corn oil):

- 24 hour preparation interval: 125, 250 and 500 mg/kg bw
- 48 hour preparation interval: 500 mg/kg bw

The high dose (500 mg/kg bw) was estimated to be suitable based on a pre-experiment in which toxic signs (i.e. reduction of spontaneous activity, abdominal position and ruffled fur) were noted in males and females treated at 500 mg/kg bw. Treatment at 1000 mg/kg bw as well as 750 mg/kg bw caused the death or required the premature sacrifice of most or all animals. As there were no differences in the toxic response of the two sexes, males only were used in the main study. In the main study, treatment at 500 mg/kg bw caused reduction of spontaneous activity and abdominal position up to 4 hours post dosing, and ruffled fur up to 24 hours post dosing.

After treatment at 500 mg/kg bw the number of PCEs was decreased in 4 of the 7 treated animals at the 48-hour sacrifice, thus indicating a slight cytotoxic effect in the bone marrow and

bioavailability of methyl sulfone. No statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei was noted.

In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any dose level at 24 hours after administration of methyl sulfone. Additionally, all recorded values for the treated animals were near to the vehicle control and within the historical vehicle control data range.

Cyclophosphamide administered orally at 20 mg/kg bw was used as positive control and showed a substantial increase ($p \leq 0.05$) in induced micronucleus frequency.

Under the conditions of this assay, methyl sulfone was considered to be non-genotoxic in this micronucleus assay.

This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for in vivo cytogenetic mutagenicity data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material:	MCW-2 metabolite #3626 (methyl sulfone)
Chemical name:	5-chloro-2-methylsulfonyl thiazole
Description:	Brown, waxy solid
Lot/Batch:	231PAL052
Purity:	98.8%
CAS#:	Not available
Stability:	Stable until 17 February 2011, stored at room temperature
2. Vehicle / positive control:	Corn oil / Cyclophosphamide (CPA)
3. Test animals:	
Species:	Rat
Strain:	Wistar - males
Age:	At dosing: 8-12 weeks
Weight at dosing:	Mean ± S.D.: 189.9 ± 6.2 g
Source:	Harlan Laboratories B.V., The Netherlands
Acclimation period:	Minimum 5 days
Diet:	Pelleted standard diet (Harlan Laboratories B.V., The Netherlands) *ad libitum*
Water:	Tap water *ad libitum*

Housing: In groups in Makrolon type-3 or 4 cages with wire mesh tops and granulated softwood bedding (Rettenmaier & Söhne GmbH & Co. KG, Germany)

4. Environmental conditions:

Temperature: 20 - 25°C

Humidity: 45-75%

Air changes: Not indicated

Photoperiod: Light cycle of 12 hours light and 12 hours dark

B. STUDY DESIGN AND METHODS:

1. In life dates: 21 June to 26 July 2010

2. Animals assignment and treatment

A preliminary study on acute toxicity was performed with two animals/sex dosed with 500, 750 or 1000 mg/kg bw of metabolite #3626 under identical conditions as the mutagenicity study. Animals were observed at 1, 2-4, 6, 24, 30 and 48 hours post-dosing. Clinical signs noted at 500 mg/kg bw starting 2-4 hours post-dosing included reduced spontaneous activity (persisting to 48 hours post-dose) and abdominal position (persisting to 6 hours post-dose) as well as ruffled fur starting 24 hours post-dose and persisting to 48 hours post-dose. At 750 mg/kg bw, reduced spontaneous activity and abdominal position were noted at 1 hour post-dose and persisting for up to 30 hours in one male. Ruffled fur was also observed from 6 to 28 hours post-dose. At 6 hours post-dose, one female was sacrificed due to severe clinical signs and the other female was found dead. At 1000 mg/kg bw, both females were sacrificed due to severe clinical signs at 6 hours post-dose, and one male was euthanized and the other male died within 24 hours of dosing. Clinical signs noted at 1000 mg/kg bw included reduced spontaneous activity from 1 to 6 hours post-dose and abdominal position from 2 to 6 hours post-dose. Based on these results, a dose of 500 mg/kg bw was chosen as the highest dose for the main experiment. Since no sex specific differences concerning signs of toxicity were observed, the main study was performed using males only.

In the main study, animals were randomly assigned to the test groups.

Fluensulfone metabolite #3626 in corn oil was administered by oral gavage as a single oral dose at 125, 250 and 500 mg/kg bw to groups of 7 male Wistar rats. Similar sized groups received the vehicle or the positive control substance once orally. The individual volume administered (10 mL/kg bw) was adjusted to the animal's body weight. Sampling and preparation of bone marrow smears were carried out at 24 hours after treatment and, for the higher dose only, at 48 hours after treatment. Sacrifice at 24 hours included vehicle and positive (cyclophosphamide) controls. The animals of all dose groups (except the positive control) were examined for acute toxic symptoms at intervals of approximately 1, 2-4, 6, 24 and 48 hours after administration of the test item.

3. Preparation of bone marrow and evaluation of cells

The animals were sacrificed using CO_2 followed by exsanguination. The femora were removed, the epiphyses were cut off and the marrow was flushed out with fetal calf serum

using a syringe. The nucleated cells were separated from the erythrocytes using the method of Romagna. Briefly, the cell suspensions were passed through a column consisting of α-Cellulose and Cellulose. The columns were then washed with Hank´s buffered saline. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 minutes and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald/Giemsa. Cover slips were then mounted. At least one slide was made from each bone marrow sample.

Evaluation of the slides was performed using NIKON microscopes with 100x oil immersion objectives. 2000 polychromatic erythrocytes (PCE) were analysed for micronuclei per animal. To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes. The analysis was performed with coded slides, the analyst blinded to treatment.

4. Acceptance criteria

The study was considered valid by the study investigators as the following criteria were met:

- at least 5 animals per sex and test group could be evaluated,

- PCE to erythrocyte ratio was not less than 20 % of the negative control, and

- the positive control showed a statistically significant and biologically relevant increase of micronucleated PCEs compared to the vehicle control.

5. Evaluation of results

A test item is classified as mutagenic by the conducting laboratory if it induces either a dose-related increase or a statistically significant positive response for at least one of the test point.

A test item producing neither a dose-related increase in the number of micronucleated polychromatic erythrocytes nor a statistically significant positive response at any of the test points is considered by the conducting laboratory to be non-mutagenic in this system.

This can be confirmed by means of the nonparametric Mann-Whitney test, if necessary. However, both biological and statistical significance should be considered together.

II. RESULTS AND DISCUSSION

A. FINDINGS

Treatment at 500 mg/kg bw caused reduction of spontaneous activity and abdominal position up to 4 hours post dosing, and ruffled fur up to 24 hours post dosing in all animals.

The number of PCEs was decreased in 4 of the 7 animals treated at 500 mg/kg bw at the 48 hours sacrifice, thus indicating a slight cytotoxic effect in the bone marrow and bioavailability of methyl sulfone.

In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any dose

level at 24 hours after administration of the test item, or at the high dose at the 48-hour time point (Table 1). Additionally, all recorded values for the treated animals were near to the vehicle control and within the historical vehicle control data range (0.01-0.32% micronucleated cells).

Cyclophosphamide administered orally at 20 mg/kg bw was used as positive control and showed a substantial increase in induced micronucleus frequency ($p \leq 0.05$).

Table 1: Summary of a micronucleus test in rats

Test group	Dosage (mg/kg bw)	Sampling time (hours)	Mean ± S.D. PCEs with micronuclei (%)	Range (micronucleated cells/2000 PCEs)	Mean ± S.D. PCE per 2000 erythrocytes
Corn oil	0	24	0.186 ± 0.180	1-11	1140 ± 74
Fluensulfone metabolite #3626	125		0.243 ± 0.154	1-11	1134 ± 117
	250		0.229 ± 0.099	2-8	1103 ± 150
	500		0.200 ± 0.112	2-8	1099 ± 115
Cyclophosphamide	20		2.814 ± 0.894*	32-78	1073 ± 124
Fluensulfone metabolite #3626	500	48	0.164 ± 0.103	1-7	1001 ± 140

Data obtained from page 22 of the study report. Standard deviations were not provided in the report and thus were calculated by the reviewer. * Statistically significantly different from the vehicle control ($p<0.05$).

III. CONCLUSIONS

Under the conditions of this assay, methyl sulfone (metabolite #3626) was considered to be non-genotoxic in this micronucleus assay at the maximum guideline recommended dose level of 500 mg/kg bw. Evidence of systemic toxicity to the rats and target (bone marrow cell) cytotoxicity were observed at this dose.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for in vivo cytogenetic mutagenicity data.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: Individual clinical signs were not reported. The rationale to use males only was that no sex specific differences were noted in the preliminary toxicity test. While males and females generally displayed similar clinical signs, females appeared to be more sensitive in terms of viability. However, the use of males only is not considered to have a significant impact on the study results. No other deficiencies were identified.

Tier II Summary **(PMRA #2181235, MRID 48574795)** revised by the regulatory authorities, April 2013

Study Type: In vivo micronucleus assay – metabolite #3627

IIA 5.8 – MRID48574795, PMRA 2181235

Report:	Merker M. (2010) Micronucleus assay in bone marrow cells of the rat with MCW-2 METABOLITE #3627 Harlan Cytotest Cell Research GmbH, Germany; unpublished report No. 1328702, dated 21 October 2010. Sponsor reference No. R-23468 Dates of experimental work: 7 April to 27 May 2010
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 474, EC method B.12 and OPPTS 870.5395 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary:

This in vivo micronucleus assay (MRID 48574795, PMRA 2181235) was performed to investigate the potential for fluensulfone metabolite butene sulfonic acid (MCW-2 metabolite #3627, 99.5%, Batch No. 215PAL44) to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the rat.

The following dose levels were investigated (single oral doses in sterile water):

- 24 hour preparation interval: 500, 1000 and 2000 mg/kg bw
- 48 hour preparation interval: 2000 mg/kg bw

The high dose (2000 mg/kg bw) was estimated to be suitable based on a pre-experiment in which toxic signs (i.e. reduction of spontaneous activity and ruffled fur) were noted in males and females treated at 1000 mg/kg bw. As there were no differences in the toxic response of the two sexes, males only were used in the main study. In the main study, treatment at 2000 mg/kg bw caused ruffled fur up to 24 hours post dosing; the same sign was also noted in animals treated at 1000 mg/kg bw up to 6 hours after dosing.

Seven male rats per test group were evaluated for the occurrence of micronuclei. At least 2000 PCEs per animal were scored. To describe a cytotoxic effect due to the treatment with butene sulfonic acid, the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and reported as the number of PCEs per 2000 erythrocytes.

After treatment with the test item at 2000 mg/kg bw, the number of PCEs was only decreased in a single animal, while not substantially decreased as compared to the mean value of PCEs of the vehicle control in all other animals. Thus, butene sulfonic acid did not exert cytotoxic effects in the bone marrow.

In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any dose level at 24 hours after administration of the test item.

A statistically significant increase in micronucleated cells was observed at the highest dose level at 48 hours. The mean value was within the negative historical control range, as were the individual values in the treated group. The statistical significance was due to a single animal (the same which showed a decreased number of PCEs) with a relatively high incidence of micronucleated cells and relatively reduced number of PCEs. This may have been due to threshold cytotoxic effects of the high dose in the 48-hour preparation interval. If this value is excluded from the statistics, no significant increase compared to the controls is observed. Thus this result is considered not to be biologically relevant.

Cyclophosphamide administered orally at 20 mg/kg bw was used as positive control and showed a substantial increase in induced micronucleus frequency.

Under the conditions of this assay, butene sulfonic acid was considered to be non-genotoxic in this micronucleus assay.

This study is classified as **acceptable/guideline (fully reliable)** and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for in vivo cytogenetic mutagenicity data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material:	MCW-2 metabolite #3627 (butene sulfonic acid)
Chemical name:	3,4,4-trifluorobutene-1-sulfonic acid, sodium salt
Description:	White solid
Lot/Batch:	215PAL44
Purity:	99.5%
CAS#:	Not available
Stability:	Stable until 21 December 2010, stored at room temperature
2. Vehicle / positive control:	Sterile water / Cyclophosphamide (CPA)
3. Test animals:	
Species:	Rat
Strain:	Wistar - males
Age:	At dosing: 8-12 weeks
Weight at dosing:	Mean ± S.D.: 184.9 ± 8.0 g
Source:	Harlan Laboratories B.V., The Netherlands

Acclimation period:	Minimum 5 days
Diet:	Pelleted standard diet (Harlan Laboratories B.V., The Netherlands) *ad libitum*
Water:	Tap water *ad libitum*
Housing:	In groups in Makrolon type-3 or 4 cages with wire mesh tops and granulated softwood bedding (Rettenmaier & Söhne GmbH & Co. KG, Germany)

4. Environmental conditions:

Temperature:	22 ± 2°C
Humidity:	25-65%
Air changes:	Not indicated
Photoperiod:	Light cycle of 12 hours light and 12 hours dark

B. STUDY DESIGN AND METHODS:

1. In life dates: 7 April to 27 May 2010

2. Animals assignment and treatment

A preliminary study on acute toxicity was performed with two animals/sex dosed with 100, 1000 or 2000 mg/kg bw of metabolite #3627 under identical conditions as the mutagenicity study, with clinical evaluation between 1 and 48 hours post-dosing. At 100 mg/kg bw, ruffled fur was observed in both sexes from 6 to 48 hours post-dose. At 1000 mg/kg bw, reduced spontaneous activity and ruffled fur were noted in both sexes from 2 hours up to 6 or 24 hours post-dose. At 2000 mg/kg bw, reduced spontaneous activity was noted from 1 to 6 hours post-dose, and ruffled fur was recorded from 2 to 28 hours post-dose in both sexes. Based on these results, 2000 mg/kg bw was selected as the highest dose for the main study. Since no sex specific differences concerning signs of toxicity were observed, the main study was performed using males only.

In the main study, animals were randomly assigned to the test groups.

Butene sulfonic acid in sterile water was administered by oral gavage as a single oral dose at 500, 1000, or 2000 mg/kg bw to groups of 7 male Wistar rats. Similar sized groups received the vehicle or the positive control substance once orally. The individual volume administered (10 mL/kg bw) was adjusted to the animal's body weight. Sampling and preparation of bone marrow smears were carried out at 24 hours after treatment in all dose groups and, for the higher dose only, at 48 hours after treatment. Sacrifice at 24 hours included vehicle and positive (cyclophosphamide) controls. The animals of all dose groups (except the positive control) were examined for acute toxic symptoms at intervals of approximately 1, 2-4, 6, 24 and 48 hours after administration of the test item.

3. Preparation of bone marrow and evaluation of cells

The animals were sacrificed using CO_2 followed by exsanguination. The femora were removed, the epiphyses were cut off and the marrow was flushed out with fetal calf serum using a syringe. The nucleated cells were separated from the erythrocytes using the method

of Romagna. Briefly, the cell suspensions were passed through a column consisting of α-Cellulose and Cellulose. The columns were then washed with Hank´s buffered saline. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 minutes and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald/Giemsa. Cover slips were then mounted. At least one slide was made from each bone marrow sample.

Evaluation of the slides was performed using NIKON microscopes with 100x oil immersion objectives. 2000 polychromatic erythrocytes (PCE) were analyzed for micronuclei per animal. To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes. The analysis was performed with coded slides, the analyst blinded to treatment.

4. Acceptance criteria

The study was considered valid by the study investigators as the following criteria were met:

- at least 5 animals per sex and test group could be evaluated,

- PCE to erythrocyte ratio was not to be less than 20 % of the negative control, and

- the positive control showed a statistically significant and biologically relevant increase of micronucleated PCEs compared to the vehicle control.

5. Evaluation of results

A test item is classified as mutagenic by the conducting laboratory if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group. Statistical methods (nonparametric Mann-Whitney test) were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results.

A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes is considered by the conducting laboratory to be non-mutagenic in this system.

II. RESULTS AND DISCUSSION

A. FINDINGS

Treatment at 2000 mg/kg bw caused ruffled fur up to 24 hours post dosing; the same sign was also noted in animals treated at 1000 mg/kg bw up to 6 hours after dosing.

After treatment with the test item the number of PCEs was only decreased in a single animal dosed with 2000 mg/kg bw of metabolite #3627 (animal no. 37) at the 48-hour sampling (this animal had 928 PCEs per 2000 erythrocytes versus 1078-1313 in the other treated animals). The number of PCEs in the other treated animals was not substantially decreased as compared to the mean value of PCEs of the vehicle control (mean of 1310, range of 1233-1390). Thus, butene sulfonic acid did not exert cytotoxic effects in the bone marrow.

In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any dose

level at 24 hours after administration of butene sulfonic acid (Table 1).

A statistically significant increase in micronucleated cells with the highest dose was observed at 48 hours. The mean value was within the negative historical control range, as were the individual values in the treated group. The statistical significance was due to a single animal (animal no. 37, the same which showed decreased number of PCEs) with a relatively high incidence of micronucleated cells (10) and relatively reduced number of PCEs (928 or 71% of mean vehicle control). This may have been due to threshold cytotoxic effects of the high dose for the 48-hour preparation interval. If this value is excluded from the statistics (mean ± S.D. = 0.233 ± 0.103% cells with micronuclei), no statistically significant increase at the 5% probability level compared to the controls is observed (p=0.0659). The incidence of micronucleated cells in the other animals of this group ranged from 2 to 7. Thus this result is considered not to be biologically relevant.

Cyclophosphamide administered orally at 20 mg/kg bw was used as positive control and showed a substantial increase in induced micronucleus frequency.

Table 1: Summary of a micronucleus test in rats

Test group	Dosage (mg/kg bw)	Sampling time (hours)	Mean ± S.D. PCEs with micronuclei (%)	Range (micronucleated cells/2000 PCEs)	Mean ± S.D. PCE per 2000 erythrocytes
Sterile water	0	24	0.129 ± 0.122	1 – 7	1310 ± 56
Fluensulfone metabolite #3627	500		0.193 ± 0.127	1 – 9	1274 ± 99
	1000		0.171 ± 0.125	0 – 8	1394 ± 64
	2000		0.157 ± 0.089	1 – 6	1366 ± 113
Cyclophosphamide	20		3.193 ± 1.092*	37 – 98	1215 ± 124
Fluensulfone metabolite #3627	2000	48	0.271 ± 0.138*	2 – 10	1184 ± 139
Range of historical control mean values (at 48 hours)			0.14-0.32		

Data obtained from page 21 of the study report. Standard deviations were not provided in the report and thus were calculated by the reviewer. * Statistically significantly different from the vehicle control ($p<0.05$).

III. CONCLUSIONS

Under the conditions of this assay, butene sulfonic acid was considered to be non-genotoxic in this micronucleus assay at the maximum guideline recommended dose level of 2000 mg/kg bw.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for in vivo cytogenetic mutagenicity data.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: Individual clinical signs were not reported. No other deficiencies were identified.

Tier II Summary (PMRA #2181231, MRID 48574790) revised by the regulatory authorities, April 2013

Study Type: *In vivo* genotoxicity testing – Unscheduled DNA synthesis – Metabolite #3626

IIA 5.8 – MRID 48574790, PMRA 2181231

Report:	Merker M. (2011) In vivo unscheduled DNA synthesis in rat hepatocytes with MCW-2 METABOLITE #3626 Harlan Cytotest Cell Research GmbH, Germany; unpublished report No. 1345901, dated 6 July 2011. Page 13 amended and substituted in the original report; amendment date 29 March 2012. Sponsor reference No. R-23475 Dates of experimental work: 21 June to 9 September 2010
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 486, EC method B.39 and OPPTS 870.5550 Deviations: slight deviations of relative humidity and temperature in the animal room occurred for some hours during the study. These deviations did not affect the validity of the study.
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary:

This study (MRID 48574790, PMRA 2181231) was performed to investigate the potential of fluensulfone metabolite methyl sulfone (MCW-2 metabolite #3626) to induce DNA repair (unscheduled DNA synthesis or UDS) in the hepatocytes of rats.

Groups of 4 male Wistar rats received a single oral dose of methyl sulfone (fluensulfone metabolite #3626, 98.8%, Lot No. 231PAL052) in corn oil (10 mL/kg bw) at 250 and 500 mg/kg bw. Four and 16 hours after treatment the animals were anaesthetised and their livers were perfused. Primary hepatocytes cultures were established and exposed for 4 hours to methyl-^{3}H-thymidine (^{3}HTdR), which is incorporated if UDS occurs. Autoradiographs were then prepared and evaluated for UDS induction. Vehicle and positive control groups were run in parallel.

As one rat treated at the high dose died, and perfusion of one rat of the low dose failed, only 3 animals per treated group were evaluated at 4 hours.

The viability of the hepatocytes was not affected by the *in vivo* treatment with methyl sulfone.

None of the tested dose levels revealed UDS induction in the hepatocytes of the treated animals as compared to the corresponding vehicle controls.

Treatment with the positive control substances revealed distinct increases in the number of

nuclear and net grain counts.

Under the conditions of this assay, methyl sulfone did not induce DNA-damage leading to increased repair synthesis in the hepatocytes of the treated rats.

This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement (OPPTS 870.5550; OECD 486) for other genotoxicity data.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 metabolite #3626 (methyl sulfone)

Chemical name:	5-chloro-2-methylsulfonyl thiazole
Description:	Brown, waxy solid
Lot/Batch:	231PAL052
Purity:	98.8%
CAS#:	Not available
Stability:	Stable until 17 February 2011 (stored at room temperature)

2. Control materials:

Negative:	Vehicle: corn oil
Positive:	4-hour preparation interval: N,N´-dimethylhydrazine-dihydrochloride (DMH; sym.), purity >99%, 80 mg/kg p.o. in 0.9 % NaCl solution at 10 mL/kg bw
	16-hour preparation interval: 2-acetylaminofluorene (2-AAF), purity >99%, 100 mg/kg p.o. in dimethylsulfoxide/polyethylene glycol 400 (1+9) at 10 mL/kg bw

3. Test animals:

Species:	Rat
Strain:	Wistar - males
Age:	8-12 weeks
Weight at dosing:	Mean ± S.D: 223.8 ± 15.4 g
Source:	Harlan Laboratories B.V., The Netherlands
Acclimation period:	Minimum 5 days
Diet:	Pelleted standard diet (Harlan Laboratories B.V., The Netherlands) *ad libitum*
Water:	Tap water *ad libitum*
Housing:	In groups in Makrolon type-3 or 4 cages with wire mesh tops

and granulated softwood bedding (Rettenmaier & Söhne GmbH & Co. KG, Germany)

4. Environmental conditions:

Temperature:	20 - 25°C
Humidity:	45-75%
Air changes:	Not indicated
Photoperiod:	Light cycle of 12 hours light and 12 hours dark
Number of animals per dose:	4 per time point

4. Test concentrations

(a) Range finding (toxicity) test	500, 750 and 1000 mg/kg bw
(b) UDS assay:	250 and 500 mg/kg bw

B. TEST PERFORMANCE

Four male rats were assigned to each test group (i.e. negative, low dose, high dose and positive control) and each sacrifice time point. The animals were identified by their cage number and tail tags.

The animals were weighed prior to dosing and received fluensulfone metabolite #3626, the vehicle only or the positive control once. All animals were examined for acute toxic symptoms at intervals of approximately 1, 2 and 4 hours after dosing for the 4-hour sacrifice time point, and 1, 2-4, and 16 hours after dosing for the 16-hour sacrifice time point.

At the appropriate time points, the animals were anaesthetised and their livers perfused as described below.

1. Test procedure

After anaesthetizing the rats with 46% Ketamin (Ketavet 100, Pharmacia GmbH, Germany), 23% Xylazin (Rompun 2%, Bayer HealthCare, Germany) and 31% Midazolan (Dormicum, Hoffmann LaRoche, Germany) (approx. 2 mL/kg bw) the liver was perfused through the vena portae at first with Hanks' balanced salt solution (HBSS, Invitrogen, Germany) containing EGTA and in the next step with HBSS supplemented with collagenase (0.05 % (w/v), Roche Diagnostics, Germany) adjusted to pH 7.4 and maintained at 37 °C.

The isolated hepatocytes were washed twice with HBSS. The crude cell suspension was filtered through a stainless steel mesh (94 µm) to yield a single cell suspension. The quality of the performed perfusion was determined by the trypan blue dye exclusion method for cell viability. In addition, the number of cells was determined.

The washed hepatocytes were centrifuged and transferred into Williams medium E (WME, Invitrogen, Germany) supplemented with Hepes, L-Glutamine, Penicillin, Insulin, Streptomycin and Fetal calf serum (FCS) adjusted to pH 7.6.

At least three cultures were established from each animal. Aliquots of 2.5 mL with freshly isolated hepatocytes in complete culture medium (2.0×10^5 viable cells/mL) were added to 35 mm six-well dishes (Greiner, Germany) containing one 25 mm round plastic coverslip (Thermanox, Nunc, Germany) per well coated with gelatine.

After an attachment period of approximately 1.5 hours in a 95% air/5% CO_2 humidified incubator at 37 °C, the culture medium was discarded. Then the cell layer was rinsed once with PBS to remove non-adherent cells. Subsequently, ^{3}HTdR (5 µCi/mL, specific activity 20 Ci/mmol; New England Nuclear, Germany) in 2.0 mL culture medium (WME, 1 % (v/v) FCS) was added to the cultures. After a labelling time of 4 hours the cells were washed twice with WME supplemented with 1 % (v/v) FCS and 0.25 mM unlabelled thymidine. Cultures were incubated overnight using the same medium. To prepare for autoradiography the medium was replaced by a hypotonic solution of 1% (w/v) sodium citrate for 10 minutes to swell the nuclei for better grain detection. The cells on the coverslips were then fixed by three washes in methanol:acetic acid (3+1 v/v) for 20 minutes each, rinsed with 96% (v/v) ethanol, and air-dried.

The cover slips were mounted with the side carrying the cells up on glass slides and coated with KODAK NTB (Tecnomara, Germany) photographic emulsion in the dark. The coated slides were stored in light-proof boxes in the presence of a drying agent for 14 days (except the reserve slides that were stored for 7 days) at 4°C. The photographic emulsion was then developed with Ilford Phenisol (Ilford Imaging GmbH, Germany) at room temperature, fixed in Rapid Fixer (Ilford Imaging GmbH, Germany) and stained with hematoxylin/eosin.

Evaluation was performed microscopically on coded slides using NIKON microscopes with oil immersion objectives. The cells for scoring were randomly selected according to a fixed scheme. The number of silver grains in the nuclear area was counted automatically using the Sorcerer UDS device version 2.0 DT3152 (Perceptive Instruments). In addition, the number of grains of the most heavily labelled nuclear-sized cytoplasm area adjacent to the nucleus was counted. At least two slides per animal and 50 cells per slide were evaluated. Heavily radio-labelled cells undergoing replicative DNA synthesis were excluded from counting.

At least three animals per group were evaluated as described above.

2. Statistics

A statistical evaluation of the results was not necessary as the number of net grain counts of the groups treated with the test item were in the range of the corresponding controls.

3. Evaluation criteria

Nuclear and net grain counts are estimated together. Increased net grains should be based on enhanced nuclear grain counts rather than on decreased cytoplasmic grain counts.

A test item is classified by the conducting laboratory as positive if the mean number of net

grains is higher than five per nucleus at one of the test points.

A group average between 0 and 5 net grains is considered by the conducting laboratory to be a marginal response. A dose-related increase in nuclear and net grains and/or a substantial shift of the percentage distribution of the nuclear grain counts to higher values provide additional information to confirm a positive response with less than 5 net grains.

Statistical significance may give further evidence for a positive evaluation. Statistical significance can be evaluated by means of the non-parametric Mann-Whitney test.

A test item producing net grains not greater than 0 at any one of the test points is considered non-effective in this system. However, the primary point of consideration is the biological relevance of the results.

II. RESULTS AND DISCUSSION

A. RANGE FINDING (TOXICITY) TEST

The high dose (500 mg/kg bw) was estimated to be suitable based on a pre-experiment in which similar toxic signs (i.e. reduction of spontaneous activity, abdominal position and ruffled fur) were noted in males and females treated at 500 mg/kg bw. Treatment at 1000 mg/kg bw as well as 750 mg/kg bw had caused the death or required the premature sacrifice of most or all animals. As there were no differences in the toxic response of the two sexes, males only were used in the main study.

B. UDS ASSAY

One rat of the 500 mg/kg bw group for the 4-hour time point died within 4 hours of dosing. Reduction of spontaneous activity and ruffled fur were noted in animals treated with both 250 and 500 mg/kg bw. Clinical signs are summarised in Table 1.

Table 1: Clinical signs observed following treatment with fluensulfone metabolite #3626

Clinical sign	Hours Post-Treatment					
	1	2	4	1	2	4
	250 mg/kg bw			500 mg/kg bw		
4-hour time point						
Reduced spontaneous activity	4	3	2	4	4	3
Ruffled fur	4	4	4	4	4	3
Apathy	0	0	0	0	3	2
Death	0	0	0	0	0	1
16-hour time point						
Reduced spontaneous activity	3	3	2	4	4	4
Ruffled fur	3	3	4	4	4	4

Data obtained from page 24 of the study report.

The viability of the hepatocytes was not substantially affected by the *in vivo* treatment with fluensulfone metabolite #3626 at any dose levels or time point. The range of cell viability among all treated cultures was 70-90% and no association with dose level was observed. The interindividual variations obtained for the numbers and the viabilities of the isolated hepatocytes were in the range of the laboratory's historical control data.

Fluensulfone metabolite #3626 did not induce UDS in the hepatocytes of the treated animals as compared to the current vehicle controls. Neither the nuclear grains nor the resulting net grains were distinctly enhanced due to the *in vivo* treatment of the animals for 4 hours or 16 hours. Therefore, the net grain values obtained after treatment with Fluensulfone metabolite #3626 were consistently negative and within the historical vehicle control data range (-14.3 to -2.5 net grains for 84 studies conducted from 2000 to 2008 using various vehicles).

In addition, no substantial shift to higher values was obtained in the percentage of cells in repair.

Treatment with the positive control substances revealed distinct increases in the number of nuclear and net grain counts that were within or slightly exceeding available historical control values.

Results are summarized in Tables 2 and 3.

Table 2: Results at 4-hour time point (Standard deviation shown for each animal is the deviation between the 100 analyzed cells; Mean ± SD of at least 3 animals)

Test group	Animal No.	Mean Nuclear Grain Count	Mean Cytoplasmic Grain Count	Mean Net Grain Count	Mean Nuclear Grains of Cells in Repair	% Cells in Repair
Vehicle control (Corn oil)	1	7.96 ± 8.50	11.46 ± 10.50	-3.50 ± 5.30	7.00 ± 1.58	5
	2	5.56 ± 4.53	7.31 ± 4.24	-1.75 ± 4.75	9.14 ± 3.34	7
	3	10.23 ± 7.61	15.99 ± 11.30	-5.76 ± 7.24	5.67 ± 0.82	6
	4	9.02 ± 6.14	15.68 ± 11.51	-6.66 ± 7.26	6.00 ± 0.00	2
Mean		**8.19 ± 1.98**	**12.61 ± 4.09**	**-4.42 ± 2.22**	**6.95 ± 1.57**	**5**
Metabolite #3626 250 mg/kg bw	5	Perfusion failed				
	6	8.87 ± 4.97	14.63 ± 6.80	-5.76 ± 5.21	0.00 ± 0.00	0
	7	14.54 ± 7.35	20.26 ± 8.47	-5.72 ± 7.25	6.29 ± 1.80	7
	8	7.58 ± 6.17	14.34 ± 8.86	-6.76 ± 5.56	0.00 ± 0.00	0
Mean		**10.33 ± 3.70**	**16.41 ± 3.34**	**-6.08 ± 0.59**	**2.10 ± 3.63**	**2**
Metabolite #3626 500 mg/kg bw	9	12.13 ± 8.09	17.77 ± 9.12	-5.64 ± 6.15	7.83 ± 2.14	6
	10	11.82 ± 5.23	21.53 ± 7.85	-9.71 ± 7.80	6.67 ± 1.15	3
	11	Animal died				
	12	10.52 ± 6.42	14.06 ± 8.47	-3.54 ± 7.62	7.46 ± 2.26	13
Mean		**11.49 ± 0.85**	**17.79 ± 3.74**	**-6.30 ± 3.14**	**7.32 ± 0.60**	**7**
Positive control (DMH 80 mg/kg bw)	13	66.91 ± 47.22	28.00 ± 18.70	38.91 ± 34.95	47.01 ± 32.90	83
	14	65.40 ± 37.46	23.52 ± 11.54	41.88 ± 32.53	45.60 ± 31.23	92
	15	60.56 ± 16.95	20.86 ± 8.24	39.70 ± 15.83	40.10 ± 15.40	99
	16	73.77 ± 40.37	22.06 ± 18.22	51.71 ± 43.49	57.92 ± 41.28	90
Mean		**66.66 ± 5.46**	**23.61 ± 3.12**	**43.05 ± 5.91**	**47.66 ± 7.46**	**91**

Data obtained from pages 13 and 26 of the study report.

Table 3: Results at 16-hour time point (Standard deviation shown for each animal is the deviation between the 100 analyzed cells; Mean ± SD of at least 3 animals)

Test group	Animal No.	Mean Nuclear Grain Count	Mean Cytoplasmic Grain Count	Mean Net Grain Count	Mean Nuclear Grains of Cells in Repair	% Cells in Repair
Vehicle control (Corn oil)	17	16.53 ± 9.27	24.68 ± 9.63	-8.15 ± 8.82	7.89 ± 2.67	9
	18	8.52 ± 5.98	13.67 ± 8.22	-5.15 ± 6.65	6.33 ± 1.53	3
	19	10.75 ± 5.23	16.21 ± 7.21	-5.46 ± 6.74	7.67 ± 2.52	3
	20	16.72 ± 10.41	29.32 ± 14.45	-12.60 ± 10.88	18.00 ± 0.00	1
Mean		**13.13 ± 4.14**	**20.97 ± 7.29**	**-7.84 ± 3.45**	**9.97 ± 5.40**	**4**
Metabolite #3626 250 mg/kg bw	21	17.88 ± 8.63	25.22 ± 8.84	-7.34 ± 7.73	9.33 ± 3.08	6
	22	21.44 ± 13.81	28.77 ± 14.15	-7.33 ± 9.17	9.10 ± 3.98	10
	23	18.21 ± 9.17	28.84 ± 10.49	-10.63 ± 7.94	6.75 ± 1.26	4
	24	12.48 ± 6.08	19.11 ± 6.82	-6.63 ± 5.91	6.25 ± 1.89	4
Mean		**17.50 ± 3.71**	**25.48 ± 4.57**	**-7.98 ± 1.80**	**7.86 ± 1.58**	**6**
Metabolite #3626 500 mg/kg bw	25	23.55 ± 10.58	32.77 ± 14.89	-9.22 ± 9.82	8.60 ± 2.79	5
	26	15.37 ± 5.94	21.39 ± 8.15	-6.02 ± 5.61	5.00 ± 0.00	1
	27	11.47 ± 4.49	20.85 ± 8.23	-9.38 ± 7.12	5.00 ± 0.00	1
	28	19.62 ± 12.19	28.21 ± 14.45	-8.59 ± 8.84	11.25 ± 5.32	4
Mean		**17.50 ± 5.23**	**25.81 ± 5.73**	**-8.30 ± 1.56**	**7.46 ± 3.04**	**3**
Positive control (2-AAF 100 mg/kg bw)	29	49.66 ± 28.38	29.65 ± 17.23	20.01 ± 15.78	23.65 ± 14.51	84
	30	24.71 ± 13.84	22.25 ± 13.02	2.46 ± 8.38	10.32 ± 6.75	37
	31	32.44 ± 17.61	23.62 ± 11.40	8.82 ± 9.07	14.31 ± 7.19	61
	32	21.19 ± 10.09	10.14 ± 4.60	11.05 ± 10.08	15.80 ± 8.12	70
Mean		**32.00 ± 12.68**	**21.41 ± 8.18**	**10.59 ± 7.26**	**16.02 ± 5.59**	**63**

Data obtained from pages 13 and 27 of the study report.

III. CONCLUSIONS

Under the conditions of this assay, the methyl sulfone metabolite of fluensulfone did not induce DNA-damage leading to increased repair synthesis in the hepatocytes of rats treated by oral gavage at 250 or 500 mg/kg bw.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement (OPPTS 870.5550; OECD 486) for other genotoxicity data.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: No deficiencies were identified.

Tier II Summary **(PMRA #2181226, MRID 48574773)** revised by the regulatory authorities, April 2013

Study Type: Acute neurotoxicity – rat

IIA 5.7.1 – MRID 48574773, PMRA 2181226

Report:	Sommer E.W. (2010) MCW-2 Tech. Acute Oral Neurotoxicity (Gavage) Study in Rats Harlan Laboratories Ltd., Switzerland; unpublished report No. C56895, dated 8 April 2010. Sponsor reference No. R-23375 Dates of experimental work: (in-life dates) 26 August 2009 to 17 September 2009
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 424, US EPA OPPTS 870.6200, JMAFF Notification Nos. 12 NohSan No. 8147, EEC method B.43 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Executive Summary:

In an acute neurotoxicity study (MRID 48574773, PMRA 2181226), groups of 10 male and 10 female 6-week old Wistar rats were administered single oral doses of 0 (control), 100, 400 and 1200 mg/kg bw of fluensulfone (MCW-2 tech., 98.65%, Batch No. 36372130-291-PF1) in polyethylene glycol (PEG) 300 and observed for 14 days.

Detailed clinical observations comprising of open field evaluation of clinical signs and a functional observational battery (FOB) were performed on days 1, 7 and 14. Motor activity was assessed over a period of 30 minutes after each FOB evaluation. At the end of the scheduled observation period, 5 rats/sex/group were perfusion-fixed *in situ*. Selected nervous system tissues were dissected, processed and examined histologically.

One female dosed at 1200 mg/kg bw died within 24 hours after dose administration of an unknown cause.

Administration of a single oral gavage dose of fluensulfone to Wistar rats at doses of 100, 400 and 1200 mg/kg bw produced reversible effects on motor activity and FOB parameters. Signs of general systemic toxicity in FOB parameters such as reduced activity and decreased rearing activity, reduced righting response, and salivation were observed only on day 1 (the day of dosing). Reduced activity and decreased rearing activity in 2 or 3 males and 1 female, together with a reduced righting response in 4 males and 1 female were observed at 100 mg/kg bw although to a much lesser degree than at 400 mg/kg bw and 1200 mg/kg bw. Locomotor activity comprising total distance, center time and rearing activity was dose dependently reduced on day 1 in males and females at 400 mg/kg bw and 1200 mg/kg bw and in females at 100 mg/kg bw. The locomotor effects in the 100 mg/kg group females included reductions for total distance, center time and rearing activity. At 1 to 4 hours post-dose on day 1, a lower body temperature was recorded in males and females at 400 mg/kg bw and 1200 mg/kg bw, which could be attributed to

an effect on the autonomous function.

Body weights were not affected by treatment. On day 8, the mean body weight gain was lower in males and females treated at 1200 mg/kg bw. Food consumption was dose dependently reduced in males at 1200 mg/kg bw and in females at 400 and 1200 mg/kg on days 1 to 8 with statistical significance attained in females at 1200 mg/kg bw only.

No test item-related effects on absolute and relative brain weights were evident in males or females. There were no treatment-related findings in the nervous system during macroscopic and microscopic pathology examinations.

The LOAEL is 100 mg/kg bw in males and females, based on reduced locomotor activity in females, and decreased spontaneous activity, decreased rearing and impaired righting response in both sexes at 100 mg/g bw. A NOAEL was not identified as effects were observed at the lowest dose tested.

The acute neurotoxicity study in the rat is classified as **acceptable/guideline** (fully reliable) and satisfies the guideline requirement for an acute neurotoxicity study (OPPTS 870.6200) in rats.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 tech. (fluensulfone)

Description:	Yellow solid
Lot/Batch:	36372130-291-PF1
Purity:	98.65% (GC-assay)
CAS#:	318290-98-1
Stability:	Stable until 30 November 2009

2. Vehicle: Polyethylene glycol 300 (PEG 300)

3. Test animals:

Species:	Rat
Strain:	HanRcc: WIST(SPF) – males and females
Age:	At dosing: approx. 6 weeks
Weight at dosing:	Males: 152 – 160 g; females: 123 – 128 g (mean values)
Source:	Harlan Laboratories B.V., The Netherlands
Acclimation period:	7 - 8 days
Diet:	Pelleted standard Provimi Kliba Nafag 3433 rat maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum*
Water:	Community tap water from Itingen *ad libitum*
Housing:	Individually in Makrolon type-3 cages with wire mesh tops and standard softwood bedding ('Lignocel' J. Rettenmaier & Söhne

GmbH & CoKG, Germany, imported by Provimi Kliba SA, Switzerland)

4. Environmental conditions:

Temperature:	19-25°C
Humidity:	30-70%
Air changes:	10-15 air changes per hour
Photoperiod:	Light cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS:

1. In life dates: 26 August to 17 September 2009

2. Animals assignment and treatment

Animals were assigned to groups using a computer-generated algorithm. Each group consisted of 10 rats per sex, subdivided into two subgroups of 5 animals per sex, with every second animal selected for perfusion fixation.

The animals were not fasted prior to dose administration. Each dose (0, 100, 400 or 1200 mg/kg bw) was administered once, by oral gavage, at the constant volume of 5 mL/kg bw.

3. Preparation and analysis of the test substance

Fluensulfone was warmed in the original container in a water bath up to maximum 40°C until it became fluid. Thereafter, the whole amount of liquid test item was transferred into a glass beaker (wrapped with aluminum foil) and mixed using a magnetic stirrer on a heating plate (maximum 40°C). The required amount of fluensulfone was weighed using a precision balance into a tared glass beaker (wrapped with aluminum foil) and kept at maximum 40°C, the vehicle was added and the dose formulation was mixed using a magnetic stirrer. Separate formulations were prepared from the aliquots for each concentration.

Samples for the determination of concentration and homogeneity of the dose formulations were taken before the first dose administration. Three samples of approximately 2 mL were taken from the top, middle and bottom of each formulation and transferred into flat bottomed flasks. Storage stability of formulations was determined in additional samples stored for 4 hours at room temperature.

The achieved concentrations of fluensulfone were in the range of 83.8% to 100.7% of the nominal concentration. In addition, the homogenous distribution of fluensulfone in the dosing formulations was demonstrated by the fact that single results found did not deviate more than 5.4% from the corresponding mean value. The test item was found to be stable in four out of six stability samples when kept for 4 hours at room temperature due to recoveries that were within 5% of the time-zero mean. The other two samples were found to be 48.9% higher and 14.6% lower than the time-zero mean.

4. Dose selection rationale

The dose levels were selected based on a preliminary acute neurotoxicity study (Report C57413, MRID 48574772, PMRA 2181225). All animals dosed with 2000 mg/kg bw died within 24 hours of dosing. Clinical signs noted at 2000 mg/kg bw included salivation, somnolence, decreased activity, prostration and ruffled fur. All animals of lower dose groups (dosed with up to 1000 mg/kg bw) survived the 8-day observation period without any signs of toxicity. The time to peak effect was determined to be 5 hours post-dose. In the current study, the dose of 1200 mg/kg bw was selected for the high dose as the limit dose of 2000 mg/kg bw was considered to be excessive, the low dose of 100 mg/kg bw was selected as it corresponded to the low dose level in the preliminary study and was predicted to be a NOAEL, and the mid dose level of 400 mg/kg bw was chosen to provide an even spacing of doses and to investigate the dose-response relationship of any effects that were observed. In the preliminary study report, the study investigators concluded that the time to peak effect was approximately 5 hours. In the current study report, it is stated that the clinical signs noted in the preliminary study reached a peak effect at 2 to 5 hours after dose administration. In the current study, animals were assessed between 1 and 4 hours post-dosing. In the preliminary study, some effects (e.g. prostration in females, hunched posture in both sexes, and ruffled fur in females) were not observed until 4 hours or later after dosing. Despite the shortening of the observation period to 1 to 4 hours post-dose in relation to the identified time of peak effect at 5 hours in the preliminary study, several clinical effects were observed in this study, thus indicating that the time of observation was appropriate.

5. Statistics

The following methods were used to analyze the functional observational battery (FOB) parameters and locomotor activity, body weight, organ weights and organ to body weight ratios, and food consumption:

• If the variables were assumed to follow a normal distribution, the Dunnett-test (many to one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups with control groups for each sex.

• Macropathology incidence data were analyzed using Fisher’s Exact Test.

Means and standard deviations were calculated.

C. METHODS

1. Observations

Observations for viability and mortality were recorded twice daily. General cage-side observations were recorded once during acclimatization and once daily thereafter.

Detailed clinical observations, in each animal’s home cage, in a standard arena and in hand, were performed in random sequence once on the day of dosing (day 1) and once weekly thereafter. Table 1 summarizes the parameters assessed for each animal.

Table 1: Summary of parameters for detailed clinical observations

Categories	Score	Parameter	D	P	W
APPEARANCE	1-3	Piloerection	X	X	X
	1-3	Salivation	X	X	X
	present	Hunched posture	X	X	X
MOTOR	1-3	Ataxia	X	X	X
	1-3	Tremor / twitching	X	X	X
	present	Prostration	X	X	X
	present	Circling		X	X
	1-3	Spasm		X	X
BEHAVIOR	1-3	Hyperactivity	X	X	X
	1-3	Somnolence	X	X	X
	1-3	Increased exploration		X	X
	1-3	Reduced grooming		X	X
	1-3	Vocalization		X	X
RESPIRATION	present	Dyspnea	X	X	X
	present	Tachypnea	X	X	X
	x	Bradypnea	X	X	X
REFLEXES	absent	Blink		X	X
	absent	Pinna		X	X
	absent	Iridic light reflex		X	X
	absent	Push-off (hind leg)		X	X
	absent	Pain response		X	X
	absent	Startle / hearing		X	X
MISCELLANEOUS	1-3	Lacrimation		X	X
	present	Limbs cyanotic		X	X
	present	Mydriasis		X	X
	present	Miosis		X	X
	present	Exophthalmos		X	X
	1-3	Reduced muscle tone		X	X

D: daily cage-side; P: pre-test; W: weekly; X: measured; 1-3: severity of finding noted

2. Functional Observation Battery

All animals were evaluated with an FOB (including grip strength of fore- and hind paws, landing foot splay, rectal body temperature and Preyer's reflex) at acclimatization, on day 1 (approximately 1 to 4 hours after dosing, i.e. at approximately the time of peak effect), and on days 7 and 14. For the conduct of the FOB, animals were randomized and the cage labels covered with the corresponding FOB number in order to make experimenters unaware of the animal's treatment group. Animals were observed in their home cage, during handling and

in an open field. Observations were conducted over the functional domains of CNS activity, CNS excitation and sensorimotor, autonomic and physiological functions.

Functional examinations included tests for:

Sensorimotor functions: approach, touch, vision, audition, pain, vestibular

Autonomic functions: pupillary reflex, body temperature

Sensorimotor coordination: grip strength (fore- and hind paw) and landing foot splay.

A contemporary positive control study (MRID 48149001, PMRA 2257060) was performed with acrylamide at the laboratory demonstrating the adequacy of these methods to detect neurotoxicity.

3. Motor Activity

Motor activity was assessed after conduct of the FOB at acclimatization, on day 1 (approximately 1 to 4 hours after dosing), and on days 7 and 14 using an automated open field device (TRUSCAN, Coulbourn Instruments, Allentown, USA). This device had been shown to detect increases as well as decreases in locomotor activity. The test boxes (40 x 40 x 40 cm) were made of transparent plexiglass. Horizontal activity was monitored by 16 infrared beams per side that cross the box at 3 cm above the floor. To register vertical activity, a single row of 16 photobeams was mounted at an approximate height of ¾ of the rat's body length. Motor activity was recorded over 30 minutes and stored on a personal computer at 3-minute intervals. Activity measurements were performed between 8:00 am and 3:00 pm. The test room had the same environmental conditions as the animal room (illuminated), with background noise provided by the fan of the personal computer. Animals were allocated to the different runs and test boxes by means of a Latin square design so that treatment groups were balanced across test boxes and time. Males and females were tested on separate days. The following parameters were evaluated:

Horizontal activity: total distance (in cm)

Vertical activity: number of rearings (counts)

Other parameters: time spent in central quadrant (center time, in sec.)

4. Body weight and food consumption

Body weights and food consumption were recorded once during acclimatization and on days 1, 8 and 15.

5. Pathology

All animals not selected for perfusion fixation were sacrificed by CO_2 asphyxiation without any further examinations. The animal that died during the study was necropsied, assessed by gross pathology and the organs were preserved in 4% formaldehyde solution, but not processed for microscopic examination.

After the 14-day observation period, the animals selected for perfusion fixation were subjected to deep anesthesia by intraperitoneal injection of pentobarbital (approximately 200 mg/kg bw; Esconarkon, Streuli Pharma SA, Switzerland) and sacrificed by perfusion fixation *in situ* with neutrally buffered 4% formaldehyde solution after a brief rinse with 1 mL of 50 IU heparin and 0.9% saline buffer. The animals fixed by perfusion fixation were kept *in situ* in a plastic

recipient containing neutrally buffered 4% formaldehyde for approximately one week until post-fixation.

The peripheral nerve tissues foreseen for plastic embedding were post-fixed with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (at pH 7.4) for approximately 2 hours at 5 ± 3°C. Rinsing with 0.1 M sodium phosphate buffer was done 3 times for approximately 10 minutes each. Furthermore, the control and high dose groups were post-fixed with 2% osmium tetraoxide and 1.8% potassium hexacyanoferrate II trihydrate ($C_6FeK_4N_6$ x $3H_2O$) in 0.1 M sodium phosphate buffer for approximately 2 hours at room temperature (20 ± 5 °C). Afterwards, the tissues were rinsed three times for approximately 30 min. each in 0.1 M sodium phosphate buffer.

The animals fixed by perfusion were necropsied, and the visible organs or organ sections were assessed by gross pathology as accurately as possible following perfusion fixation.

The brain (with olfactory bulb) was weighed after its removal, before any further processing. The terminal body weight was used for determination of the relative brain weight.

The brain (remaining material after trimming), spinal cord (sections from cervical and lumbar cord), and all gross lesions were preserved in neutrally buffered 4% formaldehyde solution.

The remaining animal body was stored in neutrally buffered 4% formaldehyde solution.

Samples of the following tissues and organs were collected from all perfusion fixed animals at necropsy and, unless otherwise indicated, post-fixed in neutrally buffered 4% formaldehyde solution:

Brain: forebrain, midbrain, cerebellum and pons, upper brain stem, medulla oblongata

Spinal cord**:** cervical segment (C4-C7), thoracic segment (T1-T4), lumbar segment (L4-L5)

Spinal nerve root: dorsal (cervical & lumbar level), ventral (cervical & lumbar level)

Sciatic nerve: proximal, below sciatic notch

Tibial nerve: proximal & distal

Dorsal root ganglion: spinal ganglion, cervical & lumbar level

Eyes/optic nerves (fixed in Davidson's solution)

Gasserian ganglion

Gastrocnemius muscle

The following organ specimens, summarized in Tables 2 and 3, were removed after perfusion, processed histotechnologically and examined by the Study Pathologist:

Table 2: Expoxide embedded samples of peripheral nervous system

Organ sample	Dose level (mg/kg bw)			
	0	**100**	**400**	**1200**
Dorsal root ganglion, (min. 2 of C4–C7) (L)	T5	P5	P5	T5
Dorsal root, longitudinal (min. 2 of C4-C7) (L)	T5	P5	P5	T5
Ventral root, longitudinal (min. 2 of C4-C7) (L)	T5	P5	P5	T5
Dorsal root ganglion, (min. 2 of L4-L5) (L)	T5	P5	P5	T5
Dorsal root, longitudinal (min. 2 of L4-L5) (L)	T5	P5	P5	T5
Ventral root, longitudinal (min. 1 of L4-L5) (L)	T5	P5	P5	T5
Proximal sciatic nerve below sciatic notch (T)	T5	P5	P5	T5
Proximal tibial nerve (at knee) (T)	T5	P5	P5	T5
Distal tibial nerve (at lower leg) (T)	T5	P5	P5	T5

(L): longitudinal section (T): transversal section; T: plastic embedded (epoxy resin), semithin sectioning and staining with 1% toluidine blue; P: storage of fixed specimen in neutrally buffered 4% formaldehyde solution; 5: all perfused animals per sex and per group

Table 3: Paraffin embedding, sectioning, staining and preservation

Organ sample	Dose level (mg/kg bw)			
	0	**100**	**400**	**1200**
Central nervous system				
Forebrain	A5	F5	F5	A5
Cerebrum, including section through the hippocampus	A5	F5	F5	A5
Midbrain with occipital and temporal lobe	A5	F5	F5	A5
Pons	A5	F5	F5	A5
Cerebellum	A5	F5	F5	A5
Medulla oblongata	A5	F5	F5	A5
Eyes with retina and optical nerve (L)	A5	F5	F5	A5
Cervical cord (C4–C7) (T,L)	A5	F5	F5	A5
Thoracic cord (T1-T4) (T,L)	A5	F5	F5	A5
Lumbar cord (min. L4–L5) (T,L)	A5	F5	F5	A5
Peripheral nervous system				
Gasserian ganglia with nerve (L)	A5	F5	F5	A5
Gastrocnemius muscle (T,L)	A5	F5	F5	A5
Proximal sciatic nerve below sciatic notch (L)	A5	F5	F5	A5
Proximal tibial nerve (at knee) (L)	A5	F5	F5	A5
Distal tibial nerve (at lower leg) (L)	A5	F5	F5	A5
All gross lesions	A2	A2	A2	A2

(L): longitudinal section; (T): transversal section; A: paraffin embedded, sectioning and staining with hematoxylin-eosin (HE); F: preservation in neutrally buffered 4% formaldehyde solution; 5: all perfused animals per sex and per group; 2: all affected animals from the group fixed by perfusion

These HE-stained paraffin sections were examined by light microscopy and assessed.

II. RESULTS AND DISCUSSION

A. MORTALITY

One female treated at 1200 mg/kg bw died spontaneously within 24 hours after dose administration. The cause of death remained unclear. All remaining animals survived to scheduled sacrifice.

B. CLINICAL OBSERVATIONS

B.1 General cage-side observations

On the day of dosing (1-4 hours post-dose), decreased activity was recorded in one male and one female treated at 1200 mg/kg bw, and from day 1 through 4 in one female treated at 400 mg/kg bw. Furthermore, ruffled fur was observed on day 1 in an additional female treated at 400 mg/kg bw and in the same female showing reduced activity and another one treated at 1200 mg/kg bw. These findings were attributed to effects associated with general systemic toxicity caused by treatment.

No further general clinical signs were observed in any of the remaining animals during the two week observation period.

B.2 Detailed clinical observations

No treatment-related effects were noted at any of the detailed clinical observations performed on days 1, 8 and 15.

B.3 Functional Observation Battery

Observations noted during FOB and attributed to treatment were mainly limited to animals treated at 400 and/or 1200 mg/kg bw, at approximately 1 to 4 hours post dosing, on the day of dosing only. These included increased incidences of reduced activity, decreased rearing, reduced righting response, hunched posture, salivation, piloerection and skin cold to touch. These findings were transient as there was no evidence of treatment-related findings subsequent to day 1 of the study. Some males treated at 100 mg/kg bw exhibited reduced activity (3/10), decreased rearing (2/10) and a reduced righting response (4/10) on day 1, and these signs were also noted in one female (1/10) each at this interval. Males from the 100 mg/kg bw dose group also exhibited piloerection (3/10).

The only observation noted in FOBs conducted on days 7 and 14 was the occurrence of soft feces (degree 1) in a single male treated at 100 mg/kg bw on day 7 only. This was considered as a chance effect because no similar observations were noted in any other animals.

Table 4 reports FOB findings noted on day 1.

Table 4: Incidences of observations (score) noted during FOB assessments at 1 to 4 hours post-dose

Day 1	0 mg/kg bw		100 mg/kg bw		400 mg/kg bw		1200 mg/kg bw	
Finding (maximum possible score)	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Soft feces (2)	0	0	0	0	0	1 (1)	1 (1) 1 (2)	4 (1)
Skin cold to touch (1)	0	0	0	0	0	0	2 (1)	0
Reduced activity (2)	0	0	3 (1)	1 (1)	6 (1)	4 (1) 2 (2)	2 (1) 6 (2)	5 (1) 2 (2)
Decreased rearing (2)	0	0	2 (1)	1 (1)	4 (1)	6 (1) 2 (2)	1 (1) 5 (2)	5 (1) 3 (2)
Piloerection (2)	0	0	3 (1)	0	2 (1)	2 (1)	4 (1) 4 (2)	6 (1)
Righting response reduced (1)	0	0	4 (1)	1 (1)	7 (1)	7 (1)	7 (1)	8 (1)
Hunched posture (2)	0	0	0	0	0	0	2 (1)	0
Salivation (2)	0	0	0	0	1 (1)	0	0	1 (1)

N = 10 rats/sex/group. Data obtained from page 31 of the study report.

Lower mean body temperatures were noted at 1 to 4 hours post-dose on day 1 in males and females at 400 mg/kg bw and 1200 mg/kg bw (Table 5). These decreases were attributed to treatment and possibly indicative of an autonomic effect. There were no effects on body temperature on days 7 and 14.

Table 5. Mean (± S.D.) body temperature (°C)

Day 1	0 mg/kg bw	100 mg/kg bw	400 mg/kg bw	1200 mg/kg bw
Males	37.9 ± 0.5	37.9 ± 0.3	37.0 ± 0.6*	36.4 ± 1.1**
Females	37.8 ± 0.2	37.6 ± 0.4	36.5 ± 1.0**	36.7 ± 0.9**

N = 10 rats/sex/group. Data obtained from pages 186-187 of the study report. * Statistically significantly different from controls ($p<0.05$). ** Statistically significantly different from controls ($p<0.01$).

Grip strength and landing foot splay measurements were not affected by treatment.

B.4 Locomotor activity

At approximately 1 to 4 hours post-dose on day 1, locomotor activity comprising total distance, center time and rearing activity was dose dependently reduced in males and females treated at 400 and 1200 mg/kg bw (Table 6). The decreases in total distance, center time and rearing activity in both sexes at 400 and 1200 mg/kg were clearly outside the

reference ranges. With the exception of the mean center time for males from the 400 mg/kg bw dose group, all of these findings were statistically significantly different from the concurrent control value. At 100 mg/kg bw, females showed statistically significant reductions in total distance, shortened center time, and reduced rearing activity when compared to concurrent controls (the mean center time and rearing activity were also below the reference ranges).

On day 1, males and females treated at 400 and 1200 mg/kg bw showed a good correlation between reduced activity in the FOB and reduced total distance in locomotor activity and also between decreased rearing in the FOB and reduced rearing activity in locomotor activity testing. In contrast, males at 100 mg/kg bw revealed a more pronounced reduced activity in the FOB and had also a higher reduction in rearing activity in the FOB than females, whereas females at 100 mg/kg bw showed a higher reduction in the locomotor parameters for total distance and rearing activity than males. These effects on the animals treated at 100, 400 and 1200 mg/kg bw were rapidly reversible.

There were no effects on total distance, center time and rearing activity on day 7 that were considered attributable to treatment.

On day 14, total distance, center time and rearing activity in females at 1200 mg/kg bw were lower than controls, and they were slightly outside the reference range. Since no corresponding decrease in total distance in males and no related effects on day 7 had occurred, these effects were not considered to be attributable to treatment.

Table 6: Summary (mean ± S.D.) of locomotor activity

Parameter	Sex	Dose (mg/kg bw)				Historical Controls (range of means)[#]
		0	100	400	1200	
Day 1						
Total distance (cm)	Males	2488 ± 513	2565 ± 853	1419 ± 529** (↓43%)	1016 ± 680** (↓59%)	2806 ± 821 (2488-3164)
	Females	2615 ± 812	1939 ± 614* (↓26%)	1505 ± 442** (↓42%)	1088 ± 525** (↓58%)	3104 ± 1083 (1922-4102)
Center time (seconds)	Males	124 ± 111	107 ± 51	67 ± 45 (↓46%)	27 ± 29** (↓78%)	193 ± 163 (124-269)
	Females	124 ± 79	68 ± 39* (↓45%)	51 ± 27** (↓59%)	29 ± 26** (↓77%)	235 ± 160 (115-418)
Rearing activity (counts)	Males	48 ± 27	43 ± 17	20 ± 16** (↓58%)	11 ± 13** (↓77%)	60 ± 32 (48-75)
	Females	48 ± 20	27 ± 21** (↓44%)	17 ± 8** (↓65%)	9 ± 7** (↓81%)	77 ± 41 (46-116)
Day 7						
Total distance (cm)	Males	3048 ± 896	3018 ± 1077	2692 ± 943	2871 ± 879	2881 ± 880 (2600-3315)
	Females	2591 ± 871	2792 ± 1204	2623 ± 1296	2302 ± 725	3389 ± 998 (2591-3844)
Center time (seconds)	Males	234 ± 140	243 ± 200	203 ± 115	169 ± 89 (↓28%)	287 ± 200 (132-350)
	Females	160 ± 116	198 ± 143	187 ± 159	125 ± 77 (↓22%)	339 ± 220 (160-462)
Rearing activity (counts)	Males	64 ± 32	62 ± 31	69 ± 39	59 ± 30	87 ± 36 (48-91)
	Females	46 ± 25	48 ± 31	49 ± 32	42 ± 19	90 ± 46 (46-125)
Day 14						
Total distance (cm)	Males	2898 ± 810	3038 ± 758	2910 ± 803	2627 ± 918	3347 ± 815 (2899-3815)
	Females	3519 ± 937	3497 ± 695	3238 ± 1332	2596 ± 777 (↓26%)	3715 ± 1037 (3268-4350)
Center time (seconds)	Males	222 ± 115	286 ± 144	292 ± 111	186 ± 101	401 ± 205 (222-607)
	Females	302 ± 171	328 ± 204	253 ± 137	189 ± 86 (↓37%)	417 ± 222 (302-540)
Rearing activity (counts)	Males	60 ± 27	53 ± 20	67 ± 23	51 ± 21	87 ± 36 (60-102)
	Females	83 ± 26	81 ± 34	71 ± 39	50 ± 24 (↓40%)	103 ± 44 (83-129)

N = 10 rats/sex/group. Data obtained from pages 215-250 and 922 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).* Statistically significantly different from controls ($p<0.05$); ** Statistically significantly different from controls ($p<0.01$). # Historical control data from 8 studies conducted from 2004 to 2009.

C. BODY WEIGHT

Body weights were not affected by treatment with fluensulfone (Table 7). Mean body weight gains were lower in males and females treated at 1200 mg/kg bw at day 8.

Table 7. Mean (± S.D.) body weights and body weight gains

Day	Sex	Dose (mg/kg bw)			
		0	100	400	1200
Body Weight (g)					
1	M	152 ± 8	160 ± 10	153 ± 7	156 ± 8
	F	123 ± 8	127 ± 8	125 ± 6	128 ± 8
8	M	195 ± 14	206 ± 12	194 ± 9	192 ± 10
	F	147 ± 11	147 ± 10	144 ± 11	145 ± 11
15	M	234 ± 17	249 ± 13	237 ± 11	235 ± 14
	F	164 ± 12	164 ± 11	164 ± 11	165 ± 17
Body Weight Gain (% of initial body weight)					
8	M	28.3 ± 3.9	28.4 ± 1.1	26.8 ± 2.6	23.2 ± 3.4** (↓18%)
	F	19.1 ± 4.8	16.0 ± 5.4	15.0 ± 5.0	13.0 ± 4.2* (↓32%)
15	M	54.0 ± 5.6	55.4 ± 3.2	55.1 ± 5.1	51.2 ± 5.6
	F	32.9 ± 5.7	29.6 ± 3.8	30.7 ± 5.1	29.0 ± 7.1

N = 10 rats/sex/group. Data obtained from pages 258-262 of the study report. * Statistically significantly different from controls ($p<0.05$); ** Statistically significantly different from controls ($p<0.01$). Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

D. FOOD CONSUMPTION

Food consumption (g/animal/day) was dose-dependently reduced in males at 1200 mg/kg bw (5% relative to controls) and females at 400 (8% relative to controls) and 1200 (15% relative to controls) mg/kg bw on days 1 to 8, with statistical significance attained in females at 1200 mg/kg bw only (data not shown). A statistically significant increase in food consumption was noted in males treated at 100 mg/kg bw during days 8 to 15. This was considered to be unrelated to treatment in the absence of any effects in the mid and high dose groups on days 8 to 15.

The overall means of food consumption was only slightly reduced (5% relative to controls) in females treated at 400 and 1200 mg/kg bw without attaining any statistical significance.

Relative food consumption (g/kg bw/day) was dose-dependently and statistically significantly reduced (6-7%) in females at 400 and 1200 mg/kg bw during days 1 to 8.

E. PATHOLOGY

No treatment-related changes were detected at gross pathology, or histological examination.

No test item-related effects on absolute and relative brain weights were evident in males or females (Table 8).

Table 8. Mean (± S.D.) absolute and relative brain weight

Sex	Dose (mg/kg bw)			
	0	100	400	1200
Absolute brain weight (g)				
M	1.77 ± 0.15	1.80 ± 0.12	1.74 ± 0.07	1.76 ± 0.07
F	1.60 ± 0.12	1.65 ± 0.09	1.59 ± 0.11	1.70 ± 0.08
Brain weight/body weight ratio (%)				
M	0.77 ± 0.04	0.73 ± 0.04	0.75 ± 0.06	0.76 ± 0.04
F	0.96 ± 0.05	1.03 ± 0.06	1.00 ± 0.08	1.02 ± 0.08

N = 10 rats/sex/group. Data obtained from pages 264-267 of the study report.

There were no neurohistopathological lesions present that could be attributed to treatment with fluensulfone (Table 9). Microscopic examination of the neuronal organs and tissues revealed intact nerve cells and nerve fibers, as well as the supporting cells, blood vessels and connecting tissue elements.

Table 9. Summary of microscopic findings

Lesion	Dose (mg/kg bw)			
	0	100	400	1200
Males				
Gasserian ganglia – single fiber degeneration	1/5	-	-	0/5
Eyes – retinal degeneration	1/5	-	-	0/5
Proximal sciatic nerve – single fiber degeneration	0/5	-	-	2/5
Females				
Proximal tibial nerve – single fiber degeneration	0/5	-	-	1/5

N = 5 rats/sex/group. Data obtained from page 935 of the study report. Values represent the number of animals with the lesion/the number of animals examined.

III. CONCLUSIONS

One female dosed at 1200 mg/kg bw died within 24 hours after dose administration of an unknown cause.

Body weights were not affected by treatment whereas food consumption was dose dependently reduced in males at 1200 mg/kg bw and females at 400 and 1200 mg/kg bw on days 1 to 8, with statistical significance attained in females at 1200 mg/kg bw only. Mean body weight gains were lower in males and females treated at 1200 mg/kg bw at day 8.

Administration of a single oral gavage dose of fluensulfone to Wistar rats at doses of 100, 400 and 1200 mg/kg bw, produced reversible effects on motor activity and FOB parameters. Signs of general systemic toxicity in FOB parameters such as reduced activity and decreased rearing activity, reduced righting response, and salivation were observed only on day 1. Reduced activity and decreased rearing activity in 2 or 3 males and 1 female, together with a reduced righting response in 4 males and 1 female were observed at 100 mg/kg bw although to a much lesser degree than at 400 mg/kg bw and 1200 mg/kg bw. Locomotor activity comprising total distance, center time and rearing activity was dose dependently reduced on day 1 in males and females at 400 and 1200 mg/kg bw and in females at 100 mg/kg bw. The locomotor effects in the 100 mg/kg bw group females included reductions of 26% for total distance, and 45% or 44% for center time and rearing activity, respectively. At 1 to 4 hours post-dose on day 1, a lower body temperature was recorded in males and females at 400 and 1200 mg/kg bw, which could be attributed to an effect on the autonomous function. The changes on the animals in the 100, 400 and 1200 mg/kg bw dose groups were rapidly reversible.

A decreased activity was recorded in the daily clinical observations from day 1 through 4 in one female at 400 mg/kg bw. However, one week after dosing no clinical and/or behavioral signs of toxicity were evident in any of the rats.

No test item-related effects on absolute and relative brain weights were evident in males or females and there were no treatment-related findings in the central and peripheral nervous system during macroscopic and microscopic pathology examinations.

The LOAEL is 100 mg/kg bw in males and females, based on reduced locomotor activity in females, and decreased spontaneous activity, decreased rearing and impaired righting response in both sexes at 100 mg/kg bw. A NOAEL was not identified as effects were observed at the lowest dose tested.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: The study investigators indicated that food consumption was reduced in males from the 400 mg/kg bw dose group. The reviewer does not consider the food

consumption in males at this dose level to be related to treatment given the very low magnitude of the change relative to controls (<2%). Furthermore, the study investigators noted that body weight gain was reduced in males and females from the 1200 mg/kg bw dose group on days 8 and 15. The reviewer acknowledges the reduction in body weight gain in these animals relative to controls on day 8, but does not agree that there was a reduction in body weight gain at day 15 based on the small change from control and the lack of a dose response.

A positive control study (RCC Study Number 852323, MRID 48149001, PMRA 2257060) conducted in 2004 was provided to demonstrate the laboratory's ability to elicit and detect changes in neurotoxicity parameters. In that study, acrylamide was administered orally by gavage to male and female Wistar rats for 28 days. Effects noted in the study included hind leg paresis, decreased spontaneous activity, impaired pain response, uncoordinated movement, dyspnea, impaired iridic light reflex, no push-off (hind legs), reduced muscle tone, reduced grip strength, decreased body temperature, decreased locomotor activity (reductions in total distance, center time and rearings), and neuronal chromatolysis in the dorsal root ganglia and Gasserian ganglia, degeneration of nerve fibers (in the dorsal and ventral spinal nerve roots and the sciatic, tibial and plantar peripheral nerves and terminal intramuscular nerve branches in the gastrocnemius muscle), and the presence of necrotic Purkinje cells in the cerebellum.

The study investigators concluded that the LOAEL for general toxicity and the NOAEL for neurotoxicity was 100 mg/kg bw. The study investigators argued that the effects observed at 100 mg/kg bw were indications of general systemic effects due to the bolus dose. However, the reviewer does not agree with this assessment, as the effects noted at 100 mg/kg bw were similar to those noted at 400 mg/kg bw, which the study investigators attributed to a neurotoxic effect. The effects were also similar in nature to those observed in the positive control study conducted with acrylamide.

The study is classified as acceptable/guideline (fully reliable) and satisfies the requirement for an acute neurotoxicity study in rats (OPPTS 870.6200).

C. Conclusions: The LOAEL is 100 mg/kg bw in males and females, based on reduced locomotor activity in females, and decreased spontaneous activity, decreased rearing and impaired righting response in both sexes at 100 mg/g bw. A NOAEL was not identified as effects were observed at the lowest dose tested.

D. Deficiencies: Measurements for eye prominence and palperbral closure were missing from the functional observation battery. These omissions do not alter the conclusions or acceptability of the study. No other deficiencies were noted.

Tier II Summary **(PMRA #2181227, MRID 48574774)** revised by the regulatory authorities, April 2013

Study Type: Subchronic neurotoxicity – rat – 90 day

IIA 5.7.4 – MRID 48574774, PMRA 2181227

Report:	Sommer E.W. (2011) MCW-2 TECH: 13-Week Neurotoxicity (Feeding) Study in Rats. Harlan Laboratories Ltd., Switzerland; unpublished report No. C95347, dated 15 June 2011, and amended on 7 October 2011. Sponsor reference No. R-23336 Dates of experimental work: 25 June 2010 to 15 June 2011
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD No. 424, EEC Method B.43, OPPTS 870.6200, and Japanese MAFF (12-NohSan no. 8147, 2000). Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary:

In a subchronic dietary neurotoxicity study (MRID 48574774, PMRA 2181227), groups of 12 male and 12 female RccHan:WIST (SPF) rats received fluensulfone (MCW-2 tech., 95.7%, Batch No. 36372130-291-PF1) in their diet at concentrations of 0 (control), 100, 500 and 2500 ppm (equivalent to 0/0, 6/7, 31/34, and 153/162 mg/kg bw/day in males/females) for at least 90 days.

Functional observational batteries (FOBs) including quantitative assessments of landing foot splay, sensory perception and muscle weakness (grip strength), were performed in all animals in a randomized order once prior to first administration, and during weeks 2, 5, 9 and 13. Locomotor activities were assessed after each FOB evaluation. At the end of the treatment period, 5 rats/sex/group were perfusion-fixed *in situ*. Selected nervous system tissues were dissected, processed and examined microscopically.

All animals scheduled for neurohistological examination survived the scheduled study period. Treatment-related effects included lower food consumption and body weight gain in males and females at 2500 ppm, as well as reduced body weight in males.

Detailed clinical observations and FOB evaluations of clinical symptoms revealed no treatment-related effects of fluensulfone. Locomotor activity (total distance, time in center arena and rearing counts) was significantly lower in males at 2500 ppm during week 5. Males in this dose group also exhibited reduced grip strength at week 13, and a slight reduction in absolute brain weight. These treatment-related effects were attributed to systemic toxicity, and not to neurotoxicity. There were no treatment-related effects on neuropathology.

The NOAEL was 500 ppm (equivalent to 31 mg/kg bw/day for males and 34 mg/kg bw/day for females) and the LOAEL was 2500 ppm (equivalent to 153 mg/kg bw/day for males and 162

mg/kg bw/day for females) based on decreased food consumption and body weight gain in both sexes and decreased body weight in males.

The subchronic neurotoxicity study in the rat is classified as **acceptable/guideline (fully reliable)** and satisfies the guideline requirement for a subchronic neurotoxicity study (OECD 424, OPPTS 870.6200b) in rats.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: MCW-2 tech. (fluensulfone)

Description:	Yellow solid
Lot/Batch:	36372130-291-PF1
Purity:	95.7%
CAS#:	318290-98-1
Stability:	Stable until 30 November 2010

2. Vehicle: Provimi Kliba Nafag 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland)

3. Test animals:

Species:	Rat
Strain:	HanRcc: WIST(SPF) – males and females
Age:	At start of dosing: approx. 7 weeks
Weight at acclimation:	Males: 132.3 to 151.7 g (mean: 142.1 g); females: 100.6 to 119.3 g (mean: 111.0 g)
Source:	Harlan Laboratories B.V., The Netherlands
Acclimation period:	7 - 8 days
Diet:	Pelleted standard Provimi Kliba Nafag 3433 rat/mouse maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum*
Water:	Community tap water from Itingen *ad libitum*
Housing:	In groups of 3 in Makrolon type-4 cages but individually during FOB and locomotor activity measurements in Makrolon type-3 cages with wire mesh tops and standard softwood bedding ('Lignocel' J. Rettenmaier & Söhne GmbH & CoKG, Germany, imported by Provimi Kliba SA, Switzerland) including paper enrichment (Enviro-dri from Lillico, Biotechnology, UK)

4. Environmental conditions:

Temperature:	22±3 °C
Humidity:	30-70% (75% and 72% in two consecutive days, no impact on the

study)

Air changes: 10-15 air changes per hour

Photoperiod: Light cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS

1. In life dates: 29 June to 6 October 2010

2. Animal assignment and treatment:

A computer-generated random algorithm was used to assign the animals to the groups. Each group consisted of 12 males and 12 females, subdivided into subgroups A (not planned for perfusion fixation) and B (5 out of the 6 animals planned for perfusion fixation).

Animals were treated for a total of 92 days at nominal dose levels of 0, 100, 500 or 2500 ppm of test material in the diet.

3. Dose selection rationale:

The doses selected were based on the results of the 28-day (Study R-23146, MRID 48575023, PMRA 2181203) and 90-day (Study R-23150, MIRD 48574754, PMRA 2181206) dietary studies conducted with fluensulfone in Wistar rats that revealed decreases in body weights and food consumption, and changes in clinical chemistry parameters at 500 and 2000 ppm.

4. Preparation and analysis of the test diets

Fresh batches of the feed pellets for the study were prepared every two weeks.

Fluensulfone was warmed in the original container in a water bath up to a maximum of approximately 40°C until the test item became liquid. The liquid fluensulfone was then transferred into a glass beaker (wrapped with aluminum foil) and mixed using a magnetic stirrer on a heating plate (kept at maximum of approximately 40 °C). The test item was divided into aliquots in brown glass containers for the bi-weekly preparations.

The required amount of fluensulfone was weighed into tared glass beakers (wrapped with aluminum foil) and kept at maximum of approximately 40°C on a heating plate until use for feed preparation. The test substance was mixed with macrogranulated feed and pellets were prepared. Water (approximately max. 1:10 volume/weight ratio) was added to aid pelleting. The pellets were dried with warm air for approximately 48 hours before storage.

Feed for the animals of the control group was prepared identically, but without test item.

Analysis by HPLC of the first diet preparation was performed prior to the first administration and of diets prepared on 3 other occasions.

5. Statistics

The following methods were used to analyze the functional observational battery (FOB) parameters and locomotor activity, body weight, organ weights and organ to body weight ratios, and food consumption:

- If the variables were assumed to follow a normal distribution, the Dunnett-test (many to one t-test) based on a pooled variance estimate was applied for the comparison of the

treated groups with control groups for each sex.

• The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data could not be assumed to follow a normal distribution.

• The Fisher's exact test was applied for necropsy, ophthalmoscopy and histopathological findings.

Means and standard deviations were calculated.

C. METHODS

1. Observations

Observations for viability and mortality were recorded twice daily. General cage-side observations were recorded once during acclimation and once daily during the treatment period.

Detailed clinical observations, in each animal's home cage, in a standard arena and in hand, were performed in random sequence once before commencement of administration and once weekly thereafter. Table 1 summarizes the parameters examined for each animal.

Table 1: Summary of parameters for detailed clinical observation

Categories	Score	Parameter	D	P	W
APPEARANCE	1-3	Piloerection	X	X	X
	1-3	Salivation	X	X	X
	present	Hunched posture	X	X	X
MOTOR	1-3	Ataxia	X	X	X
	1-3	Tremor / twitching	X	X	X
	present	Prostration	X	X	X
	present	Circling		X	X
	1-3	Spasm		X	X
BEHAVIOR	1-3	Hyperactivity	X	X	X
	1-3	Somnolence	X	X	X
	1-3	Increased exploration		X	X
	1-3	Reduced grooming		X	X
	1-3	Vocalization		X	X
RESPIRATION	present	Dyspnea	X	X	X
	present	Tachypnea	X	X	X
	present	Bradypnea	X	X	X
REFLEXES	Absent	Blink		X	X
	Absent	Pinna		X	X
	Absent	Iridic light reflex		X	X
	Absent	Push-off (hind leg)		X	X
	Absent	Pain response		X	X
	Absent	Startle / hearing		X	X
MISCELLANEOUS	1-3	Lacrimation		X	X
	present	Limbs cyanotic		X	X
	present	Mydriasis		X	X
	present	Miosis		X	X
	present	Exophthalmos		X	X
	1-3	Reduced muscle tone		X	X

D: daily cage-side; P: pre-test; W: weekly; X: observed; 1-3: severity of finding noted

2. Functional Observation Battery

All animals were evaluated with an FOB (including quantitative assessments of landing foot splay, sensory perception and muscle weakness) once during the acclimation period, and at weeks 2 (day 8), 5 (day 29), 9 (day 57) and 13 (day 84). For the conduct of the FOB, animals were randomized and the cage labels covered with the corresponding FOB number in order to make experimenters unaware of the animal's treatment group. Animals were observed in their

home cage, during handling and in an open field. Observations were conducted over the functional domains of CNS activity, CNS excitation and sensorimotor, autonomic and physiological functions.

Functional examinations included tests for:

Sensorimotor functions: approach, touch, vision, audition, pain, vestibular

Autonomic functions: pupillary reflex, body temperature

Sensorimotor coordination: grip strength (fore- and hind paw) and landing foot splay.

A contemporary positive control study (MRID 48149001, PMRA 2257060) was performed with acrylamide at the laboratory demonstrating the adequacy of these methods to detect neurotoxicity.

3. Motor Activity

Motor activity was assessed after conduct of the FOB once during the acclimation period and at weeks 2, 5, 9 and 13 using an automated open field device (TRUSCAN, Coulbourn Instruments, USA). This device had been shown to detect increases as well as decreases in locomotor activity. The test boxes (40 x 40 x 40 cm) were made of transparent plexiglass. Horizontal activity was monitored by 16 infrared beams per side that cross the box at 3 cm above the floor. To register vertical activity, a single row of 16 photobeams was mounted at an approximate height of ¾ of the rat's body length. Motor activity was recorded over 30 minutes and stored on a personal computer at 3-minute intervals. Activity measurements were performed between 8:00 am and 3:00 pm. The test room had the same environmental conditions as the animal room (illuminated), with background noise provided by the fan of the personal computer. Animals were allocated to the different runs and test boxes by means of a Latin square design so that treatment groups were balanced across test boxes and time. Males and females were tested on separate days. The following parameters were evaluated:

Horizontal activity: total distance (in cm)

Vertical activity: number of rearings (counts)

Other parameters: time spent in central quadrant (center time, in sec.)

4. Body weight and food consumption

Body weights were recorded in all animals once during the acclimation period and on day 1 and weekly thereafter.

The food consumption was monitored twice a week throughout the study and reported bi-weekly during weeks 1 to 13.

5. Ophthalmoscopy

Ophthalmoscopic examination was performed in all animals once during the acclimation period. At week 13, the eyes of animals from the control and 2500 ppm dose groups were examined. As there were no treatment-related findings noted in the 2500 ppm dose group, the eyes of animals from the low and mid dose groups were not examined.

6. Pathology

All animals not selected for perfusion fixation were sacrificed by deep isoflurane anesthesia,

decapitated and discarded without any further examinations. The animal that died during the study was necropsied and assessed by gross pathology.

At the end of the treatment period, the 5 animals/sex/dose selected for perfusion fixation were sacrificed by intraperitoneal injection of pentobarbital (Eutha 77®, Provet AG, Switzerland; about 400 mg/kg bw) and subjected to perfusion fixation *in situ* with 1 mL of 50 IU heparin followed by 0.9% saline buffer as the rinsing solution. For fixation a 4% formaldehyde solution was used. After perfusion fixation, the brain with the optic nerve and olfactory bulb was removed and post-fixed. All organs and tissues that were not needed for further processing were removed and discarded. The animals fixed by perfusion were kept *in situ* in a plastic container containing 4% formaldehyde for approximately 24 hours until post-fixation was performed. The peripheral nerve tissues foreseen for plastic embedding were post-fixed with 4% paraformaldehyde, 5% glutaraldehyde in 0.1 M sodium phosphate buffered saline (at pH 7.4) for approximately 2 hours at 5 ± 3 °C. Rinsing with 0.1 M sodium phosphate buffered saline was done 3 times for approximately 10 minutes each. Furthermore, the control and high dose groups were post-fixed with 2% osmium tetraoxide and 1.8% potassium hexacyanoferrate II trihydrate ($C_6FeK_4N_6$ x $3H_2O$) in 0.1 M sodium phosphate buffer for approximately 2 hours at room temperature (20 ± 5 °C).

The animals fixed by perfusion were necropsied and the visible organs or organ sections were assessed by gross pathology as accurately as possible following perfusion fixation.

The brain (with olfactory bulb) was weighed after post-fixation for approximately 24 hours. The terminal body weight was used for determination of the relative brain weight.

The brain (remaining material after trimming), spinal cord (sections from cervical and lumbar cord), and all gross lesions were preserved in neutral buffered 4% formaldehyde solution.

The remaining animal body was stored in neutral buffered 4% formaldehyde solution.

Samples of the following tissues and organs were collected from all perfusion fixed animals at necropsy and post-fixed in neutral buffered 4% formaldehyde solution:

Brain: forebrain, midbrain, cerebellum and pons, upper brain stem, medulla oblongata, hippocampus

Spinal cord**:** cervical segment (C4-C7) and lumbar segment (L4-L5)

Spinal nerve root: dorsal (cervical & lumbar) and ventral (cervical & lumbar) levels

Sciatic nerve: proximal, below sciatic notch

Tibial nerve at knee: (proximal & distal) and gastrocnemius branches

Dorsal root ganglion: spinal ganglion, cervical & lumbar level

Eyes with optic nerves (fixed in Davidson's solution)

Gasserian ganglion

Gastrocnemius muscle

The organ specimens listed in Tables 2 and 3 were removed after perfusion, processed histologically and examined by the Study Pathologist.

Table 2: Epoxide embedded samples of peripheral nervous system

Organ sample*	Dose level (ppm)			
	0	**100**	**500**	**2500**
Dorsal root ganglion, (min. 2 of C4–C7) (L)	T5	T5	T5	T5
Dorsal root, longitudinal (min. 2 of C4-C7) (L)	T5	T5	T5	T5
Ventral root, longitudinal (min. 2 of C4-C7) (L)	T5	T5	T5	T5
Dorsal root ganglion, (min. 2 of L4-L5) (L)	T5	T5	T5	T5
Dorsal root, longitudinal (min. 2 of L4-L5) (L)	T5	T5	T5	T5
Ventral root, longitudinal (min. 1 of L4-L5) (L)	T5	T5	T5	T5
Proximal sciatic nerve below sciatic notch (T)	T5	T5	T5	T5
Proximal tibial nerve (at knee) (T)	T5	T5	T5	T5
Distal tibial nerve (at lower leg) (T)	T5	T5	T5	T5

*: the left component was taken and processed; the right component was taken and stored in 4% formaldehyde solution. (L): longitudinal section; (T): transversal section; T: plastic embedded (epoxy resin), semithin sectioning and staining with 1% toluidine blue; 5: all perfused animals per sex and per group

Table 3: Paraffin embedding, sectioning, staining and preservation

Organ sample	Dose level (ppm)			
	0	**100**	**500**	**2500**
Central nervous system				
Frontal lobe	A5	A5	A5	A5
Parietal lobe with diencephalon	A5	A5	A5	A5
Midbrain (hippocampus) with occipital & temporal lobe	A5	A5	A5	A5
Pons	A5	A5	A5	A5
Cerebellum	A5	A5	A5	A5
Medulla oblongata	A5	A5	A5	A5
Eyes with retina and optical nerve (L)	A5	A5	A5	A5
Cervical cord (C4–C7) (T,L)	A5	A5	A5	A5
Lumbar cord (min. L4–L5) (T,L)	A5	A5	A5	A5
Peripheral nervous system				
Gasserian ganglia with nerve (L)	A5	A5	A5	A5
Gastrocnemius muscle (T,L)	A5	A5	A5	A5
Proximal sciatic nerve below sciatic notch (L)	A5	A5	A5	A5
Proximal tibial nerve (at knee) (L)	A5	A5	A5	A5
Distal tibial nerve (at lower leg) (L)	A5	A5	A5	A5
All gross lesions	A2	A2	A2	A2

(L): longitudinal section; (T): transversal section; A: paraffin embedded, sectioning and staining with hematoxylin-eosin (HE); 5: all perfused animals per sex and per group; 2: all affected animals from the group fixed by perfusion

II. RESULTS AND DISCUSSION

A. FINDINGS

1. Actual achieved intakes of test substance

The actual achieved intakes (means of means over the treatment period) of the test substance were as follows:

Dietary level (ppm)	Test material intake (mg/kg bw/day)	
	Males	**Females**
100	6.04	6.73
500	30.70	33.53
2500	152.89	162.19

Data obtained from pages 283 and 287 of the study report.

The content of fluensulfone in 47 out of a total of 51 diet samples ranged from 82.2% to 106.0%. The content of four feed samples of the high dose group exceeded this range by up to 5.6%. The absence of fluensulfone in the control diet was confirmed.

Diet preparations were considered to be homogeneous as the mean concentration determined from the individual concentrations obtained from the top, middle, and bottom of each dose formulation did not deviate more than 7.6% from the overall mean.

The stability of fluensulfone was tested in samples taken 28 days after preparation and stored at room temperature and frozen at -20 ± 5°C. The variation values, except two, were found to be <10% from the time-zero value. The variation value for one sample (room temperature for 2500 ppm diet preparation) was 10.5% (below the mean time-zero value), the other sample that was out of range was the -20°C, 100 ppm diet sample, which varied from the mean by 70.3% (above the mean time-zero value).

2. Mortality and Clinical signs

All animals scheduled for neurohistological examination survived the scheduled study period.

One female receiving the 500 ppm test diet died spontaneously on day 87 without displaying any clinical signs before its unscheduled death. Macropathological findings indicated a hemorrhage in the right ventricle of the cerebrum as the possible cause of death. This finding was not considered to be test item-related as it occurred in a single animal of the mid dose group.

Results of daily in-cage observations, weekly clinical examinations and observations in the open field revealed no treatment-related findings.

3. FOBs and Motor activity assessments

No treatment-related clinical observations were present during the FOB evaluations performed during weeks -1, 2, 5, 9 and 13.

Reduced activity and decreased rearing activity or reduced righting response, respectively, were noted in two males at 100 ppm, and reduced activity and reduced righting response were present in one male at 2500 ppm during week 2 only. The incidence of these signs was considered to be too low to be toxicologically relevant. In addition, there was no dose-relationship present and similar signs were not noted in the corresponding female groups. In addition, reduced activity and reduced righting response were recorded in one male of the control group and reduced activity and decreased rearing activity were noted in one male at 100 ppm during week 5 only. These findings were not considered to be treatment-related due to their low incidence, occurrence in the control group, and lack of dose-relationship. No clinical observations were noted in females at weeks 2 or 5.

No clinical observations were recorded in any males or females of any dose groups during the FOB evaluations performed during weeks 9 and 13. No effects were noted at any time points on landing foot splay measurements and mean body temperature. There was a slight decrease (not statistically significant) in fore- and hindlimb grip strength in males from the 2500 ppm dose group at week 13 only (Table 4), which may have been related to the reduced body weights noted in these animals.

Table 4. Mean (± S.D.) grip strength

Parameter	Study Week	Dose (ppm)			
		0	100	500	2500
Males					
Forelimb grip strength (kg)	-1	0.52 ± 0.09	0.51 ± 0.09	0.59 ± 0.11	0.49 ± 0.08
	2	0.58 ± 0.11	0.57 ± 0.12	0.60 ± 0.16	0.61 ± 0.13
	5	0.81 ± 0.19	0.83 ± 0.14	0.79 ± 0.16	0.85 ± 0.11
	9	0.65 ± 0.11	0.74 ± 0.18	0.72 ± 0.24	0.76 ± 0.26
	13	1.34 ± 0.28	1.33 ± 0.44	1.31 ± 0.25	1.12 ± 0.57 (↓16%)
Hindlimb grip strength (kg)	-1	0.30 ± 0.09	0.28 ± 0.05	0.29 ± 0.06	0.30 ± 0.08
	2	0.33 ± 0.05	0.35 ± 0.05	0.35 ± 0.05	0.34 ± 0.09
	5	0.61 ± 0.10	0.62 ± 0.09	0.63 ± 0.10	0.62 ± 0.06
	9	0.47 ± 0.13	0.52 ± 0.10	0.47 ± 0.07	0.50 ± 0.12
	13	0.72 ± 0.18	0.66 ± 0.14	0.63 ± 0.12	0.59 ± 0.21 (↓18%)
Females					
Forelimb grip strength (kg)	-1	0.50 ± 0.09	0.52 ± 0.12	0.55 ± 0.13	0.48 ± 0.14
	2	0.49 ± 0.18	0.50 ± 0.13	0.47 ± 0.16	0.45 ± 0.14
	5	0.50 ± 0.19	0.61 ± 0.16	0.53 ± 0.24	0.56 ± 0.16
	9	0.59 ± 0.14	0.53 ± 0.10	0.52 ± 0.12	0.52 ± 0.09
	13	1.04 ± 0.24	1.00 ± 0.16	1.12 ± 0.23	1.19 ± 0.23
Hindlimb grip strength (kg)	-1	0.28 ± 0.05	0.29 ± 0.06	0.30 ± 0.06	0.27 ± 0.05
	2	0.31 ± 0.06	0.28 ± 0.04	0.30 ± 0.06	0.28 ± 0.03
	5	0.49 ± 0.12	0.48 ± 0.07	0.42 ± 0.08	0.45 ± 0.07
	9	0.44 ± 0.06	0.43 ± 0.07	0.44 ± 0.09	0.40 ± 0.07
	13	0.59 ± 0.08	0.53 ± 0.12	0.53 ± 0.10	0.61 ± 0.12

N=12/sex/dose. Data obtained from pages 160-169 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

4. Locomotor Activity

Males in the 2500 ppm dose group exhibited reduced center time (not statistically significant) at week 2, and both males and females from the 500 and 2500 ppm dose groups demonstrated decreased total rearing counts (not statistically significant) at week 13 (Table 5). In the absence of any other indications of effects on locomotor activity at this time point, this finding is considered to be incidental and unrelated to treatment. Males in the 2500 ppm dose group also demonstrated reduced total distance, center time and total rearing counts at week 5, with the differences from control reaching statistical significance for total distance and rearing activity.

These effects are possibly a consequence of the reduced body weight in these animals.

Table 5. Summary (mean ± S.D.) of locomotor activity

Parameter	Sex	Dose (ppm)			
		0	100	500	2500
Week 2					
Total distance (cm)	M	2754 ± 751	2423 ± 488	2562 ± 787	2442 ± 694
	F	3240 ± 456	3093 ± 587	3005 ± 505	3128 ± 951
Center time (seconds)	M	320 ± 204	212 ± 68	284 ± 183	195 ± 105 (↓39%)
	F	355 ± 120	306 ± 159	246 ± 115	307 ± 270
Rearing activity (counts)	M	70 ± 28	67 ± 20	66 ± 31	72 ± 29
	F	92 ± 23	78 ± 21	77 ± 21	80 ± 39
Week 5					
Total distance (cm)	M	3755 ± 623	3375 ± 612	3398 ± 776	2973 ± 925* (↓21%)
	F	3708 ± 791	3664 ± 692	3650 ± 871	3604 ± 963
Center time (seconds)	M	595 ± 261	536 ± 146	554 ± 215	441 ± 266 (↓26%)
	F	459 ± 135	422 ± 178	350 ± 194	383 ± 223
Rearing activity (counts)	M	86 ± 29	71 ± 26	76 ± 34	54 ± 28* (↓37%)
	F	99 ± 32	92 ± 26	85 ± 39	86 ± 37
Week 9					
Total distance (cm)	M	3517 ± 822	2844 ± 508	3102 ± 779	3027 ± 822
	F	3540 ± 886	3253 ± 696	3473 ± 1145	3436 ± 828
Center time (seconds)	M	740 ± 261	601 ± 184	696 ± 272	677 ± 384
	F	543 ± 180	418 ± 258	495 ± 307	465 ± 255
Rearing activity (counts)	M	53 ± 22	45 ± 16	49 ± 28	48 ± 23
	F	75 ± 29	59 ± 30	64 ± 33	65 ± 31
Week 13					
Total distance (cm)	M	2856 ± 693	2817 ± 467	2545 ± 899	2562 ± 567
	F	3258 ± 749	3172 ± 712	3080 ± 844	3029 ± 630
Center time (seconds)	M	560 ± 200	571 ± 173	575 ± 204	487 ± 255
	F	443 ± 163	364 ± 164	325 ± 192	360 ± 188
Rearing activity (counts)	M	41 ± 12	37 ± 17	32 ± 21 (↓22%)	31 ± 11 (↓24%)
	F	66 ± 26	61 ± 20	55 ± 24 (↓17%)	50 ± 18 (↓24%)

N=12/sex/dose. Data obtained from pages 197-256 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). *Statistically significantly

different from the control mean ($p<0.05$).

5. Body weight and food consumption

At 2500 ppm, mean body weights were generally lower in males (6-16% decreased compared to control values), achieving statistical significance from day 8 through 91, but were similar to controls in females throughout the study (Table 6). Mean cumulative body weight gain was also statistically significantly lower in males beginning on day 8 and continuing throughout the study whereas in females it was statistically significantly lower on day 8 only. The overall cumulative body weight change values of the 2500 ppm males and females were 73% and 86% of controls, respectively.

Body weight gain was also slightly lower in males at 100 and 500 ppm on a few occasions, which were considered to be incidental and unrelated to treatment, with overall body weight gain comparable to control value at the end of the treatment period.

Table 6. Mean (± S.D.) body weight and body weight gain

Study Day	Sex	Dose (ppm)			
		0	100	500	2500
Body Weight (g)					
Day 1	M	195 ± 8	194 ± 8	194 ± 4	193 ± 6
	F	133 ± 3	140 ± 20	139 ± 11	139 ± 4
Day 8	M	236 ± 13	237 ± 9	237 ± 5	222 ± 8** (↓6%)
	F	158 ± 9	156 ± 9	156 ± 9	153 ± 8
Day 36	M	355 ± 26	342 ± 18	342 ± 12	306 ± 19** (↓14%)
	F	204 ± 12	206 ± 15	204 ± 18	201 ± 19
Day 64	M	422 ± 36	407 ± 25	401 ± 18	355 ± 26** (↓16%)
	F	228 ± 14	230 ± 17	231 ± 19	229 ± 25
Day 91	M	464 ± 41	443 ± 29	434 ± 22	390 ± 30** (↓16%)
	F	245 ± 18	247 ± 16	247 ± 23	240 ± 26
Body Weight Gain (% of initial body weight)					
Day 8	M	21 ± 7	22 ± 1	22 ± 1	15 ± 2**
	F	19 ± 7	13 ± 11	12 ± 9	10 ± 4*
Day 36	M	82 ± 12	76 ± 7	76 ± 4	58 ± 8**
	F	53 ± 9	48 ± 15	47 ± 13	45 ± 11
Day 64	M	117 ± 20	110 ± 10	106 ± 7	84 ± 14**
	F	71 ± 10	66 ± 16	67 ± 14	64 ± 14
Day 91	M	139 ± 23	129 ± 12	124 ± 10	102 ± 15**
	F	84 ± 14	78 ± 18	78 ± 18	72 ± 15

Data obtained from pages 289-301 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * Statistically significantly different from

controls ($p<0.05$). ** Statistically significantly different from controls ($p<0.01$).

Food consumption (g/animal/day) was lower in males (33% relative to controls) and females (37% relative to controls) at 2500 ppm during the first three days of treatment, possibly due to a palatability problem. Food consumption continued to be lower throughout the study in males at 2500 ppm (up to 24% lower than controls). Food consumption was slightly lower in females during weeks 5 and 11 (up to 27% lower than controls). The overall mean food consumption in the 2500 ppm males was decreased by 14% and in the 2500 ppm females by only 6%.

At 100 and 500 ppm, on a few occasions there were slight effects on mean food consumption in males which were considered to be incidental and unrelated to treatment.

6. Ophthalmoscopic Examinations

Ophthalmoscopic examination revealed no treatment-related findings.

7. Pathology

A slightly lower mean absolute brain weight was recorded in males at 2500 ppm (Table 7). This was likely related to the lower body weight at the end of treatment, as supported by the fact that brain weight relative to body weight did not show any difference compared to the control value.

Table 7. Mean (± S.D.) absolute and relative brain weight

Sex	Dose (ppm)			
	0	100	500	2500
Terminal body weight (g)				
M	474 ± 45	430 ± 33	431 ± 11	398 ± 21** (↓16%)
F	240 ± 14	244 ± 20	249 ± 21	240 ± 13
Absolute brain weight (g)				
M	2.34 ± 0.05	2.29 ± 0.06	2.27 ± 0.06	2.21 ± 0.11* (↓5%)
F	2.08 ± 0.04	2.15 ± 0.08	2.10 ± 0.04	2.12 ± 0.03
Brain weight/body weight ratio (%)				
M	0.50 ± 0.06	0.54 ± 0.05	0.53 ± 0.02	0.56 ± 0.03
F	0.87 ± 0.06	0.88 ± 0.07	0.85 ± 0.08	0.89 ± 0.05

N = 5 rats/sex/group. Data obtained from pages 309-312 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer). * Statistically significantly different from controls ($p<0.05$); ** Statistically significantly different from controls ($p<0.01$).

There were no treatment-related gross lesions recorded at necropsy. Macroscopic inspection of selected neuronal organs and tissues revealed no treatment-related findings.

No fluensulfone-related microscopic changes were observed (Table 8). Microscopic examination of the neuronal organs and tissues revealed intact nerve cells and nerve fibers, as well as the supporting cells, blood vessels and connecting tissue elements.

Table 9. Summary of microscopic findings

Lesion	Dose (ppm)			
	0	100	500	2500
Males				
Proximal sciatic nerve (longitudinal section) – single fiber degeneration	0/5	0/5	0/5	1/5
Proximal sciatic nerve (transverse section) – single fiber degeneration	0/5	1/5	0/5	0/5
Proximal tibial nerve (longitudinal section) – single fiber degeneration	0/5	0/5	0/5	1/5
Proximal tibial nerve (transverse section) – single fiber degeneration	1/5	0/5	0/5	1/5
Eyes – hyperkeratosis	0/5	0/5	1/5	0/5
Females				
Proximal sciatic nerve (longitudinal section) – single fiber degeneration	1/5	1/5	0/5	0/5
Proximal tibial nerve (longitudinal section) – single fiber degeneration	0/5	0/5	0/5	1/4
Eyes – corneal degeneration	1/5	1/5	0/5	0/5

Data obtained from page 908 of the study report. Values represent the number of animals with the lesion/the number of animals examined.

III. CONCLUSIONS

Treatment-related effects included lower food consumption and body weight gain in males and females at 2500 ppm, as well as lower body weight in males at this dose level.

Detailed clinical observations and FOB evaluations of clinical symptoms revealed no treatment-related effects of fluensulfone. Locomotor activity (total distance, time in center arena and rearing counts) was significantly lower in males at 2500 ppm during week 5. Males in this dose group also exhibited reduced grip strength at week 13, and a slight reduction in absolute brain weight. These treatment-related effects were attributed to systemic toxicity, and not to neurotoxicity. There were no treatment-related effects on neuropathology.

Therefore, the NOAEL was 500 ppm (equivalent to 31 mg/kg bw/day for males and 34 mg/kg bw/day for females), based on systemic effects noted at the LOAEL of 2500 ppm (equivalent to 153 mg/kg bw/day for males and 162 mg/kg bw/day for females).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: The study investigators indicated that there was no effect of treatment on grip strength; whereas the reviewer identified an apparent treatment-related decrease in both fore- and hindlimb grip strength in males dosed with the 2500 ppm test diet at week 13 (albeit likely secondary to the reduced body weight noted in these animals).

The study investigators also discounted the reduced locomotor activity in males from the 2500 ppm dose group at week 5 as being unrelated to treatment because the incidence of these signs was considered to be too low to be toxicologically relevant, there was no dose relationship present, and similar signs were not noted in the corresponding female groups. However, the reviewer could not discount these findings as potentially related to treatment, although they are possibly a consequence of the reduced growth of these animals.

A positive control study (RCC Study Number 852323, MIRD 48149001, PMRA 2257060) conducted in 2004 was provided to demonstrate the laboratory's ability to elicit and detect changes in neurotoxicity parameters. In that study, acrylamide was administered orally by gavage to male and female Wistar rats for 28 days. Effects noted in the study included hind leg paresis, decreased spontaneous activity, impaired pain response, uncoordinated movement, dyspnea, impaired iridic light reflex, no push-off (hind legs), reduced muscle tone, reduced grip strength, decreased body temperature, decreased locomotor activity (reductions in total distance, center time and rearings), and neuronal chromatolysis in the dorsal root ganglia and Gasserian ganglia, degeneration of nerve fibers (in the dorsal and ventral spinal nerve roots and the sciatic, tibial and plantar peripheral nerves and terminal intramuscular nerve branches in the gastrocnemius muscle), and the presence of necrotic Purkinje cells in the cerebellum.

The study is classified as acceptable/guideline (fully reliable) as a subchronic neurotoxicity study in rats (870.6200b; OECD 424).

C. Conclusions: The reviewer agrees with the study author's determination of the NOAEL for systemic toxicity at 500 ppm. The effects observed in this study at the LOAEL of 2500 ppm are indicative of systemic toxicity and not neurotoxicity.

D. Deficiencies: Measurements for eye prominence and palpebral closure were missing from the functional observation battery. These omissions do not alter the conclusions or acceptability of the study. No other deficiencies were noted.

Tier II Summary (PMRA #2181184, MRID 48574742) revised by the regulatory authorities, April 2013

Study Type: Toxicokinetic studies – Single dose, oral route, in rats

IIA 5.1.1 – MRID 48574742, PMRA 2181184

Report	Quistad G.B., Kovatchev A. and La Mar J. (2011) The Metabolism and Excretion of [^{14}C] MCW-2 in the Rat Upon Administration of Single Oral High and Low Doses PTRL West, Inc. (preparation and analysis of test substances) and Pacific BioLabs (in-life phase), California, USA, unpublished report Laboratory Project No. PTRL WEST 1789W (PBL study No. 10B0104G-X01G) dated 2 February 2011 and amended 29 September 2011. Sponsor reference No. R-23410 Dates of experimental work: (in-life phase) 21 April to 21 June 2010
Sponsor	Makhteshim Chemical Works, Ltd., Beer Sheva, Israel
Guidelines	Japanese MAFF guidelines (12-Nousan-No. 8147, Part 2-3-1), US EPA OPPTS 870.7485 European Union Commission Directive 87/302/EEC (18 November 1987), as modified by the Council Directive 67/548/EEC Deviations: none
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. The study was conducted according to GLP standards with one exception: preparation of the KOH traps was not documented in a notebook, but did this did not affect the data since subsequently the trapping medium was verified as pH > 14 and would have trapped $^{14}CO_2$.

Executive Summary

In a metabolism study (MRID 48574742, PMRA 2181184), the metabolism and excretion of [^{14}C]fluensulfone ([thiazole-^{14}C]MCW-2, 98.7-99.3%, TJBIOS-NB21-33 and [butene-^{14}C]MCW-2, 96.8-98.8%, TJBIOS-NB18-44), given as single oral doses at a high and a low dose level for each label, was determined in male and female rats.

[^{14}C]Fluensulfone, prepared as a suspension in polyethylene glycol 300, was administered in single oral low (nominal dose of 5 mg/kg bw; actual doses of 4.6-5.4 mg/kg bw) or high (nominal dose of 500 mg/kg bw; actual doses of 450-483 mg/kg bw) doses to groups of 4 male and 4 female Wistar rats. Rats were housed in individual glass metabolism cages equipped for collection of $^{14}CO_2$, urine and feces over 120 hours, and were sacrificed thereafter. Metabolites in urine and feces were identified by comparison to reference standards or by liquid chromatography with mass spectrometric detection (LC/MS). The radiochemical purity of the dose suspensions were measured by reverse phase HPLC before and after administration, and all measurements indicated a radiochemical purity of at least 96.8% and stability during the time of

dose administration.

Fluensulfone administered orally to rats is rapidly metabolized. The recovery of radioactivity for the low and high dose butene and thiazole labels was 91.3-97.3% of the administered dose. The disposition and excretion of radiolabel for males and females were generally similar. There was no indication that the metabolic elimination processes were saturated by the 100-fold increase in dose level, as levels of radiolabel in urine, cage wash, feces, carcass, and gastro-intestinal (GI tract) were similar. Urine was the major route of excretion for both radiolabel positions and the only source of metabolites at ≥5% of the administered radiolabel for both radiolabel positions. The cage wash contained relatively high levels of radiolabeled metabolites and analysis showed that those metabolites in cage wash and urine were similar; thus indicating that the cage wash mainly recovered urine. Low amounts of thiazole sulfonic acid were found in feces, but no other fecal metabolites were ≥5% of the administered dose.

For the low-dose thiazole radiolabel, 84-86% of the administered radiolabel was excreted in urine (including in cage wash), 8-11% in feces, 2% remained in the carcass and GI tract, and 0.4-0.6% was exhaled as CO_2. The major urinary metabolite was a thiazole mercapturate (38-40% of the administered dose) with lesser levels of thiazole glucuronides (24-27%), and thiazole sulfonic acid (3-5%).

For the high-dose thiazole radiolabel, 85-88% of the administered radiolabel was excreted in urine (including cage wash), 5-7% in feces, 1% remained in the carcass and GI tract, and approximately 0.07% was exhaled as CO_2. The major urinary metabolite was a thiazole mercapturate (49-54% of the administered dose) with lesser levels of thiazole glucuronides (20-22%), and thiazole sulfonic acid (1-3%).

For the low-dose butene radiolabel, 75-76% of the administered radiolabel was excreted in urine (including cage wash), 10-13% in feces, 2-3% remained in the carcass and GI tract, and 4% was exhaled as CO_2. The major urinary metabolite was a butene sulfinic acid (32-35% of administered dose) with lesser levels of butene sulfonic acid (4-5%).

For the high-dose butene radiolabel, 81-83% of the administered radiolabel was excreted in urine (including cage wash), 6-11% in feces, 1% remained in the carcass and GI tract, and 1-2% was exhaled as CO_2. The major urinary metabolite was a butene sulfinic acid (53-57% of administered dose) with lesser levels of butene sulfonic acid (4%).

This metabolism study in the rat is classified as acceptable/guideline (fully reliable) and, in combination with other metabolism studies conducted with fluensulfone (Lab report number R-23411/ MRID 48574741/ PMRA 2181185, R-23412/ MRID 48574743/ PMRA 2181186, and R-23389/ MRID 48574744/ PMRA 2181187), satisfies the guideline requirement for a metabolism study (OPPTS 870.7485; OECD 417) in rats.

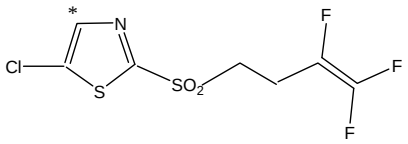
I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Radiolabelled substance **[Ring-^{14}C]fluensulfone or [Thiazole-4-^{14}C]fluensulfone**

(termed as [thiazole-^{14}C]MCW-2)



* Indicates position of ^{14}C label

Description: Not given

Lot/Batch: TJBIOS-NB21-33

Specific Activity: 56.7 mCi/mmol

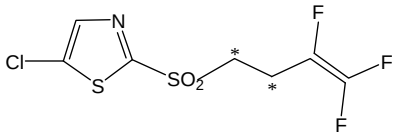
Radiochemical Purity: 98.7-99.3% (prior to dosing)

CAS#: 318290-98-1

Stability: Confirmed after dosing

Radiolabelled substance **[Butene-^{14}C]fluensulfone or [trifluorobutene-1,2-^{14}C] fluensulfone**

(termed as [butene-^{14}C]MCW-2)



* Indicates position of ^{14}C label

Description: Not given

Lot/Batch: TJBIOS-NB18-44

Specific Activity: 57.7 mCi/mmol

Radiochemical Purity: 96.8-98.8% (prior to dosing)

CAS#: 318290-98-1

Stability: Confirmed after dosing

Unlabelled substance Fluensulfone (MCW-2)

Description: Not given

Lot/Batch:	411-12
Purity:	99.9%
CAS#:	318290-98-1
Stability:	Stable for the duration of the study
Reference substances	Thiazole sulfonic acid (5-chloro-thiazole-2-sulfonic acid sodium salt), Lot No. 381-174-00, purity 90.9%, expiry date December 2011, supplied by Makhteshim Chemical Works, Ltd. Methyl sulfone (thiazole, 5-chloro-2-(methylsulfonyl)), Lot No. 381-173-00, purity 97.0%, expiry date December 2011, supplied by Makhteshim Chemical Works, Ltd. Butene sulfonic acid MAN-2 (3,4,4-trifluoro-butene-1-sulfonic acid, sodium salt), Lot No. 215PAL2, purity 99.1%, expiry date 10 September 2010, supplied by PharmAgra Labs MAN-03 (3-sulfo-propionic acid, disodium salt), Lot No. 215PAL8, purity 97.8%, expiry date 10 September 2010, supplied by PharmAgra Labs
2. Vehicle	Polyethylene glycol (PEG 300)

3. Test Animals

Species	Rat
Strain	Wistar [Han:WIST(SPF)]
Age	Approx. 7 weeks
Weight	Males: 173-203 g; females: 142-160 g
Source	Charles River Laboratories, Hollister, CA, USA
Acclimation period	5 days
Diet	Certified Laboratory Rodent Diet *ad libitum*
Water	Fresh potable drinking water *ad libitum* via a sipper tube
Housing	Individually in rodent cages containing absorbent shavings as bedding during acclimation; individually in glass metabolism cages after dosing and to sacrifice
Husbandry	Controlled environment with a temperature of 18-26°C, humidity at 50 ± 20% and a light/dark cycle of 12 hours

4. Preparation of Dose solutions

The [^{14}C]fluensulfone was isotopically diluted with non-radioactive fluensulfone. Radioactive and non-radioactive fluensulfone were dissolved in ethyl acetate. Each test solution was mixed thoroughly and the ethyl acetate was evaporated under a gentle stream of nitrogen in the presence of polyethylene glycol (PEG 300) (20.0 mL). The suspension doses were vortexed and sonicated to achieve homogeneity.

Aliquots of each test suspension (measured by weight) were removed for liquid scintillation counting (LSC) before dose administration. These measurements were used to determine the concentration of [^{14}C]fluensulfone in the suspensions and the homogeneity of the preparations. Small quantities of each suspension were taken after dose administration and diluted with methanol for HPLC analysis. These analyses served to determine the radiochemical purity.

B. STUDY DESIGN AND METHODS

Groups of 4 male and 4 female Wistar rats received a single oral dose of [^{14}C]fluensulfone (nominally at 5 or 500 mg/kg bw), labelled either on the thiazole ring or on the butene moiety. Rats were housed individually in glass metabolism cages. Urine, feces, cage wash, and CO_2 were collected at specified time intervals (Table 1).

Table 1: Sample collection times

Sample	Collection interval (hours after dose administration)
CO_2 Traps	0-24, 24-48, 48-72
Urine	0-6, 6-24, 24-48, 48-72, 72-96, 96-120
Feces	0-24, 24-48, 48-72, 72-96, 96-120
Cage wash	0-6, 6-24, 24-48, 48-72, 72-96, 96-120
Carcass	At sacrifice (120)
Gastro-intestinal tract (with contents)	At sacrifice (120)

Urine and feces were continuously collected over wet ice in glass metabolism cages designed for separate collection of urine and feces. The conical collecting surfaces of the metabolism cages were washed with water (approximately 100 mL) whenever the urine collection vessels were emptied. Urine, feces and cage wash samples were transferred to frozen storage upon collection.

Two CO_2 traps (10% KOH) were connected in series and samples were collected at specified intervals from the glass metabolism cages. Samples were kept at room temperature upon collection.

The complete gastrointestinal (GI) tract (with contents) was removed from the animals at sacrifice (120 hours after dosing) and transferred to frozen storage.

The total amounts of urine, cage wash, and feces were determined gravimetrically. Subsamples for measurement of total radioactivity taken from urine, feces, cage wash, blood and plasma were measured by weight. Subsamples taken from CO_2 traps were measured by volume.

Radioactivity content in liquid samples was determined by liquid scintillation counting (LSC). Portions of feces, prepared as a homogeneous paste by adding weighed amounts of water, were weighed and analyzed by LSC after combustion and trapping of resulting $^{14}CO_2$. Portions (approximately 0.5 g) of carcass or GI tract solubilised for 5-9 days in 25% (w/w) KOH were mixed with 3 mL of methanol, and then analyzed by LSC.

Metabolites in urine and feces were identified by comparison to reference standards or by LC/MS.

II. RESULTS AND DISCUSSION

Dose suspensions and actual administered dosages

The data indicated that the dose suspensions had an acceptable degree of homogeneity (relative standard deviation of 0.3-2.2% measured by replicate LSC analysis of weighed subsamples) and radiochemical purity (96.8-99.3 % before dosing and 96.5-99.4% after dosing).

The target doses were 5 and 500 mg/kg bw. The mean achieved administered dosages are summarised in Table 2 below. All dose groups were dosed at 90-108% of the target dose.

Table 2: Summary of administered dosages (mg/kg bw)

[thiazole-^{14}C]fluensulfone					
Animal number (low dose; high dose)		Low dose		High dose	
Male	Female	Male	Female	Male	Female
1; 9	5; 13	5.4	5.3	479	499
2; 10	6; 14	5.3	5.3	469	470
3; 11	7; 15	5.4	5.4	474	483
4; 12	8; 16	5.3	5.4	483	478
Mean ± SD		**5.4 ± 0.06**	**5.4 ± 0.06**	**476 ± 6**	**483 ± 12**
[butene-^{14}C]fluensulfone					
Animal number (low dose; high dose)		Low dose		High dose	
17; 25	21; 29	4.6	4.6	451	464
18; 26	22; 30	4.6	4.6	442	470
19; 27	23; 31	4.5	4.7	447	461
20; 28	24; 32	4.7	4.7	458	459
Mean ± SD		**4.6 ± 0.08**	**4.7 ± 0.06**	**450 ± 7**	**464 ± 5**

Data obtained from page 48 of the study report.

EXCRETION

Radioactivity balance

In general, there were no major differences between males and females in the amount of radioactivity recovered from various matrices (Table 3). The total recovery of radiolabel for the low and high dose butene and thiazole labels was 91.3-97.3% of the administered dose.

Radiolabel exhaled as $^{14}CO_2$ represented 0.36-0.55% and 0.06-0.07% of the administered dose for the thiazole label (low and high dose, respectively). There was more exhalation of $^{14}CO_2$ for the butene radiolabel than for the thiazole label, as radiolabel exhaled as $^{14}CO_2$ represented 4.0-4.4% and 1.3-1.9% of the administered dose for the butene label (low and high dose, respectively). Most of the administered radiolabel was excreted in the urine for both radiolabels (63.2-77.8% of administered dose for the low dose and 71.7-76.0% for the high dose). The cage wash contributed 6.6-14.2% of the administered dose. Rats sacrificed after 5 days contained only 0.9-2.6% and 0.06-0.27% of the administered radiolabel in the carcass and the contents of the GI tract, respectively. Thus, radiolabel from fluensulfone is readily eliminated by rats for both labels.

Table 3: Summary of radioactivity balance (% of administered radioactivity)

	[thiazole-^{14}C]fluensulfone				[butene-^{14}C]fluensulfone			
	Low dose 5 mg/kg bw		High dose 500 mg/kg bw		Low dose 5 mg/kg bw		High dose 500 mg/kg bw	
	Male	Female	Male	Female	Male	Female	Male	Female
CO_2 traps	0.36 ± 0.13	0.55 ± 0.13	0.06 ± 0.03	0.07 ± 0.05	4.0 ± 1.8	4.4 ± 1.7	1.9 ± 1.0	1.3 ± 0.7
Urine	77.8 ± 1.6	71.7 ± 9.9	76.0 ± 7.6	76.0 ± 9.6	63.8 ± 4.5	63.2 ± 6.0	71.7 ± 8.1	71.8 ± 4.9
Cage wash	6.6 ± 0.9	14.2 ± 4.0	9.1 ± 3.3	12.2 ± 5.2	11.9 ± 2.2	11.9 ± 6.6	8.9 ± 3.7	10.9 ± 3.2
Feces	11.0 ± 1.1	8.3 ± 0.7	7.2 ± 0.9	5.1 ± 1.3	13.3 ± 1.2	10.0 ± 1.1	11.4 ± 3.0	6.3 ± 1.5
Carcass	1.5 ± 0.3	1.8 ± 0.1	1.2 ± 0.1	1.2 ± 0.3	2.6 ± 0.8	1.6 ± 0.2	1.4 ± 0.4	0.9 ± 0.4
GI tract	0.07 ± 0.01	0.08 ± 0.02	0.06 ± 0.01	0.08 ± 0.02	0.27 ± 0.21	0.15 ± 0.02	0.09 ± 0.01	0.07 ± 0.02
Total	**97.3**	**96.6**	**93.6**	**94.7**	**95.9**	**91.3**	**95.4**	**91.3**

Data obtained from page 49 of the study report. Values represent the mean ± SD.

METABOLISM

[thiazole-^{14}C]fluensulfone – Low dose (5 mg/kg bw)

In urine collected from male rats, a total of 71.7% of the administered dose was identified as urinary metabolites. The main metabolites were identified as thiazole mercapturate (39.9% of administered dose), thiazole glucuronide MW327-I (19.5%), thiazole glucuronide MW327-II (7.0%), and thiazole sulfonic acid (5.3%). Thiazole glucuronide MW327-I and MW327-II represent the α- and ß-isomers, respectively, at the 1-position of the glucuronide moiety. No other single metabolite was >5% of the administered radiolabel.

In urine collected from female rats, a total of 65.6% of the administered dose was identified as urinary metabolites. The main metabolites were identified as thiazole mercapturate (38.0% of the administered dose), thiazole glucuronide MW327-I (14.9%), thiazole glucuronide MW327-II (9.3%), and thiazole sulfonic acid (3.4%). No other single metabolite was >5% of the administered radiolabel.

In cage wash collected from female rats, identified metabolites were thiazole mercapturate (6.6% applied dose), thiazole glucuronide MW327-I (2.6%), thiazole glucuronide MW327-II (1.6%), and thiazole sulfonic acid (0.5%). No other single metabolite was >1% of the administered radiolabel. The profile of metabolites in cage wash is similar to that in urine. Taking the results from the analysis of both urine and cage wash from female rats, a total of 76.9% of the administered dose was identified as urinary metabolites. Cage wash from male rats was not analyzed, but it is expected to be similar to that of females.

Analysis of the fecal extract for male rats identified thiazole sulfonic acid (0.5% of the administered radiolabel) and three major peaks (2.5%, 2.0% and 1.2% of the administered radiolabel). The maximum of any other single metabolite was 0.8% of the administered radiolabel. The post-extraction solid (PES) contained 3.3% of the administered radiolabel.

Analysis of the fecal extract for female rats revealed thiazole sulfonic acid (0.4% of the administered radiolabel) and three major peaks (1.9%, 1.7% and 1.3% of the administered radiolabel). The maximum of any other single metabolite was 0.5% of the administered radiolabel. The PES contained 2.0% of the administered radiolabel.

[thiazole-^{14}C]fluensulfone – High dose (500 mg/kg bw)

In urine collected from male rats, a total of 78.3% of the administered dose was identified as urinary metabolites. The main metabolites were identified as thiazole mercapturate (48.7% of the administered dose), thiazole glucuronide MW327-I (12.0%), thiazole glucuronide MW327-II (10.0%), and thiazole sulfonic acid (3.1%). No other single metabolite was >1.7% of the administered radiolabel.

In urine collected from female rats, a total of 74.6% of the administered dose was identified in the urine. The main metabolites were identified as thiazole mercapturate (53.3% of the administered dose), thiazole glucuronides MW327-I and MW327-II together (20.0%), and thiazole sulfonic acid (1.3%). No other single metabolite was >2.0% of the administered radiolabel.

Analysis of cage wash from female rats revealed thiazole mercapturate (8.7% of the administered dose), thiazole glucuronide MW327-I (0.6%), thiazole glucuronide MW327-II (0.9%), and thiazole sulfonic acid (0.2%). No other single metabolite was >0.6% of the administered radiolabel. The profile of metabolites in cage wash was similar to that in urine. Taking the results from the analysis of both urine and cage wash, a total of 85.0% of the administered dose was identified as urinary metabolites. Cage wash from male rats was not analyzed, but it is expected to be similar to that of females.

Analysis of the fecal extracts for male rats identified thiazole sulfonic acid (0.1% of the administered radiolabel) and two major peaks (1.3%, and 1.0% of the administered radiolabel). The maximum of any other single metabolite was 0.6% of the administered radiolabel. The PES contained 2.2% of the administered radiolabel.

Analysis of the fecal extracts collected from female rats identified thiazole sulfonic acid (0.1% of the administered radiolabel) and three major peaks (1.3%, 0.8% and 0.4% of the administered radiolabel). The maximum of any other single metabolite was 0.3% of the administered radiolabel. The PES contained 1.5% of the administered radiolabel.

[butene-^{14}C]fluensulfone – Low dose (5 mg/kg bw)

In male rats, a total of 39.4% of the administered dose was identified as urinary metabolites. Identified metabolites were butene sulfinic acid (35.3% of administered radiolabel) and butene sulfonic acid (4.1%). No other single metabolite was >4.4% of the administered radiolabel.

In female rats, a total of 37.1% of the administered dose was identified as urinary metabolites. Identified metabolites were butene sulfinic acid (32.3% of the administered dose) and butene sulfonic acid (4.8%). No other single metabolite was >4.2% of the administered radiolabel.

Analysis of cage wash collected from female rats revealed butene sulfinic acid (5.2% of the administered dose) and butene sulfonic acid (0.7%). No other single metabolite was >1.2% of the administered radiolabel. The profile of metabolites in cage wash is similar to that in urine. Taking the results from the analysis of both urine and cage wash, a total of 43.0% of the administered dose was identified as urinary metabolites. Cage wash from male rats was not analyzed, but it is expected to be similar to that of females. Analysis of the fecal extracts collected from male rats identified several metabolite regions but none was >2% of the administered radiolabel. The PES contained 3.9% of the administered radiolabel.

Analysis of the fecal extracts collected from female rats identified several metabolite regions but none was >1.5% of the administered radiolabel. The PES contained 2.8% of the administered radiolabel.

[butene-^{14}C]fluensulfone – High dose (500 mg/kg bw)

In male rats, a total of 57.0% of the administered dose was identified as urinary metabolites. The main metabolites were identified as butene sulfinic acid (53.4% of the administered dose) and butene sulfonic acid (3.6%). No other single metabolite was >2.4% of the administered radiolabel.

In female rats, a total of 60.3% of the administered dose was identified as urinary metabolites. The main metabolites were identified as butene sulfinic acid (56.5% of the administered dose) and butene sulfonic acid (3.8%). No other single metabolite was >1.8% of the administered radiolabel.

Analysis of cage wash collected from female rats revealed butene sulfinic acid (8.2% of the administered dose) and butene sulfonic acid (0.8%). No other single metabolite was >0.3% of the administered radiolabel. The profile of metabolites in cage wash was similar to that in urine. Taking the results from the analysis of both urine and cage wash, a total of 69.3% of the administered dose was identified as urinary metabolites. Cage wash from male rats was not analyzed, but it is expected to be similar to that of females.

Analysis of the fecal extracts collected from male rats identified several metabolite regions but none was >1.5% of the administered radiolabel. The PES contained 2.7% of the administered radiolabel.

Analysis of the fecal extract collected from female rats identified several metabolite regions but none was >0.8% of the administered radiolabel. The PES contained 1.7% of the administered radiolabel.

A summary of the urinary metabolites is given in Table 4.

Table 4: Summary of urinary metabolites[a]

Metabolite		Average % of administered dose			
		Low dose (5 mg/kg bw)		High dose (500 mg/kg bw)	
		Male	Female	Male	Female
[thiazole-^{14}C]fluensulfone					
Thiazole mercapturate	Urine Cage wash	39.9 NA	38.0 6.6	48.7 NA	53.3 8.7
	Total	--	44.6	--	62.5
Thiazole glucuronide MW 327-I	Urine Cage wash	19.5 NA	14.9 2.6	12.0 NA	(20.0)* 0.6
	Total	--	17.5	--	*
Thiazole glucuronide MW 327-II	Urine Cage wash	7.0 NA	9.3 1.6	10.0 NA	(20.0)* 0.9
	Total	--	10.9	--	*
Thiazole sulfonic acid	Urine Cage wash	5.3 NA	3.4 0.5	3.1 NA	1.3 0.2
	Total	--	3.9	--	1.5
Max. other	Urine Cage wash	2.9 NA	3.2 0.7	1.7 NA	2.0 0.6
[butene-^{14}C]fluensulfone					
Butene sulfinic acid	Urine Cage wash	35.3 NA	32.3 5.2	53.4 NA	56.5 8.2
	Total	--	37.5	--	64.7
Butene sulfonic acid	Urine Cage wash	4.1 NA	4.8 0.7	3.6 NA	3.8 0.8
	Total	--	5.5	--	4.6
HPLC solvent front	Urine Cage wash	7.8 NA	6.4 1.2	4.2 NA	2.4 0.3
Max. other	Urine Cage wash	4.4 NA	4.2 0.8	2.4 NA	1.8 0.3

[a] Data obtained from pages 50-52, 55-57, 60-62, and 65-67 of the study report. Values for urine represent mean % of administered dose from sum of 0-6, 6-24, 24-48 and 48-72 hour individual specimens for each animal. Values for cage wash for the low dose groups (both labels) represent the mean % of administered dose from sum of 0-6 and 6-24 hour individual specimens for each animal. Values for cage wash for the high dose groups (both labels) represent the mean % of administered dose from the sum of the 0-6, 6-24, and 24-48 hour individual specimens for each animal. NA = not analysed. * Urinary values represent thiazole glucuronide MW327-I and MW327-II.

III. CONCLUSIONS

Fluensulfone administered orally to rats at nominal doses of either 5 mg/kg bw or 500 mg/kg bw is rapidly metabolized. The recovery of radioactivity for the low and high dose thiazole and butene labels was 91.3-97.3% of the administered dose for all groups of rats. The disposition and excretion of radiolabel were generally similar between males and females. There was no indication that the metabolic elimination processes were saturated by the 100-fold increase in dose level, as levels of radiolabel in urine, cage wash, feces, carcass, and GI tract were similar between the low and high dose groups.

Urine was the major route of excretion for both radiolabel positions and the only source of metabolites at ≥5% of the administered radiolabel for both radiolabel positions. The cage wash contained relatively high levels of radiolabeled metabolites and analysis showed that those metabolites in cage wash and urine were similar. Low amounts of thiazole sulfonic acid were found in feces, but no other fecal metabolites were ≥5% of the administered dose.

Unmetabolized fluensulfone was not detected as a significant residue in either urine or feces. The study investigators indicated that initially the parent compound probably reacts with glutathione to displace butene sulfinic acid, the major urinary metabolite. This sulfinic acid is unstable to oxidation and is converted to butene sulfonic acid. The glutathione adduct of the thiazole ring is cleaved to the cysteine conjugate that ultimately is acetylated to the mercapturate as a major urinary product. The glutathione adduct is also cleaved to thiazole thiol (not detected) that is either oxidized to thiazole sulfonic acid or conjugated with glucuronic acid to give thiazole glucuronides (MW327-I and MW327-II). The two thiazole glucuronides are probably α- and ß-isomers at anomeric C-1 of the glucuronic acid moiety. The recovery of $^{14}CO_2$ indicates that a portion of both radiolabels is extensively degraded. The proposed metabolic pathway is outlined in Figure 1.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

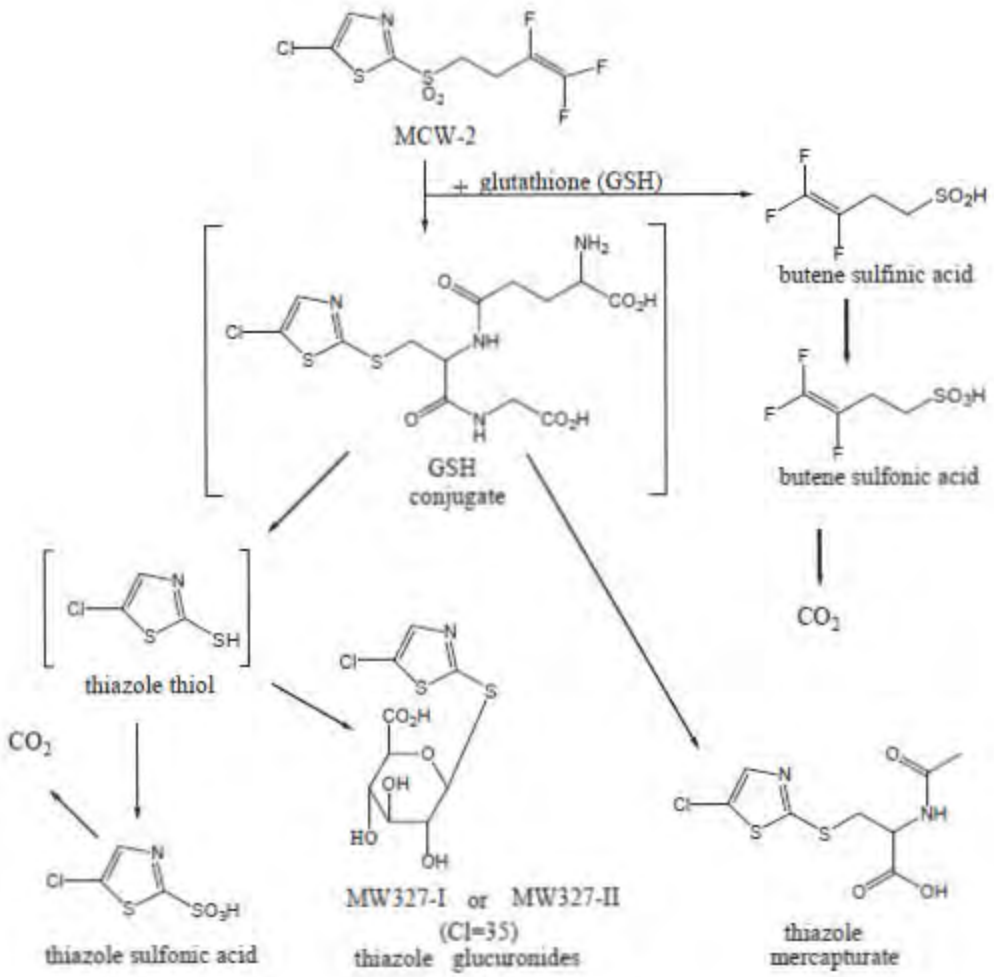
Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This metabolism study in the rat is classified as acceptable/guideline (fully reliable) and, in combination with other metabolism studies conducted with fluensulfone (Lab report number R-23411/ MRID 48574741/ PMRA 2181185, R-23412/ MRID 48574743/ PMRA 2181186, and R-23389/ MRID 48574744/ PMRA 2181187), satisfies the guideline requirement for a metabolism study (OPPTS 870.7485; OECD 417) in rats.

C. Conclusions: The reviewer agrees with the conclusions reached by the study investigators.

D. Deficiencies: No deficiencies were identified.

Figure 1. Proposed metabolic pathway for fluensulfone in rats (obtained from page 22 of the study report)



Tier II Summary (PMRA #2181187, MRID 48574744) revised by the regulatory authorities, April 2013

Study Type Toxicokinetic studies – Repeated dose, oral route, in rats

IIA 5.1.3 – MRID 48574744, PMRA 2181187

Report	Quistad G.B., Kovatchev A. and La Mar J. (2011) The Metabolism, Excretion, and Tissue Distribution of [Thiazole-^{14}C]Fluensulfone (MCW-2) in the Rat Upon Administration of Repeated Oral Doses (5 mg/kg) PTRL West, Inc. and Pacific BioLabs, California, USA, unpublished report Laboratory Project No. PTRL WEST 2134W (PBL study No. 10L0405G-X01G) dated 15 September 2011. Sponsor reference No. R-23389 Dates of experimental work: (in-life phase) 7 February to 01 March 2010
Sponsor	Makhteshim Chemical Works, Ltd., Beer Sheva, Israel
Guidelines	OECD 417 (1984) and US EPA OPPTS 870.7485 Deviations: none
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In a metabolism study (MRID 48574744, PMRA 21881187), the disposition and metabolism of [^{14}C]fluensulfone ([thiazole-^{14}C]MCW-2, 98.5%, TJBIOS-NB21-33, Lot 2466-20) was studied in groups of 4 male and 4 female Wistar rats after 14 repeated daily oral doses with unlabeled fluensulfone, followed by a single oral dose of [^{14}C]fluensulfone 1 day after the last unlabeled dose, with sacrifice 1 and 7 days later (4 rats/sex/dose/sacrifice time). The nominal daily doses were 5 mg/kg bw (actual daily mean 4.9-5.0 mg/kg bw). The study determined the distribution of ^{14}C derived from the test substance in selected tissues and identified the major metabolites of fluensulfone in urine and feces.

Fluensulfone was administered as a suspension in PEG 300 by oral gavage.

The recovery of excreted radiolabel was 94-99% for individual rats. Radiolabel was found mainly in urine (71-83%), feces (9-11%) and the cage wash (6-16%). One day after dosing with radiolabeled fluensulfone, an average of 0.6-0.8% of the administered radiolabel was in liver and 0.1% in kidney with 1.7-2.1% in the carcass (selected tissues removed) and 4.5-4.7% remained in the gastro-intestinal tract. By 7 days after treatment with radiolabeled fluensulfone, an average of 0.1-0.2% of the administered radiolabel remained in liver and 0.03% in kidney. There were no significant sex differences in either elimination or tissue residues.

Analysis of urine showed four major metabolites identified as thiazole sulfonic acid (TSA), glucuronide I, glucuronide II and thiazole mercapturate. Low levels of fluensulfone (0.1-0.2% of the administered radiolabel) were detected in fecal extracts. Comparison to residues from rats treated with a single oral dose from another study (MRID 48574742, PMRA 2181184) showed

similar residues in tissues, urine and feces in rats administered repeated doses in this study.

This metabolism study in the rat is classified as acceptable/guideline (fully reliable) and, in combination with other metabolism studies conducted with fluensulfone (Lab report number R-23410/ MRID 48574742/ PMRA 2181184, R-23411/ MRID 48574741/ PMRA 2181185, and R-23412/ MRID 48574743/ PMRA 2181186), satisfies the guideline requirement for a metabolism study (OPPTS 870.7485; OECD 417) in rats.

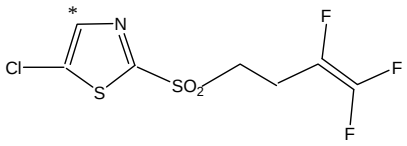
I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Radiolabeled substance

[Ring-^{14}C]fluensulfone or [Thiazole-4-^{14}C]fluensulfone
(termed as [thiazole-^{14}C]MCW-2)



* Indicates position of ^{14}C label

Description:	Not given
Lot/Batch:	TJBIOS-NB21-33; Lot 2466-20 (purified)
Specific Activity:	56.7 mCi/mmol
Radiochemical Purity:	98.5% (prior to dosing)
CAS#:	318290-98-1
Stability:	Confirmed after dosing (98.6% radiochemical purity)
Unlabelled substance	Fluensulfone (MCW-2)
Description:	Not given
Lot/Batch:	411-12
Purity:	99.9%
CAS#:	318290-98-1
Stability:	Stable for the duration of the study
Reference substances	Thiazole sulfonic acid (5-chloro-thiazole-2-sulfonic acid sodium salt), Lot No. 381-174-00, purity 90.9%, expiry date December 2011, supplied by Makhteshim Chemical Works, Ltd. Methyl sulfone (thiazole, 5-chloro-2-(methylsulfonyl)), Lot No. 381-173-00, purity 97.0%, expiry date December 2011, supplied by Makhteshim Chemical Works, Ltd.

2. Vehicle	Polyethylene glycol (PEG 300)
3. Test Animals	
Species	Rat
Strain	Wistar [Han:WIST(SPF)]
Age	Approx. 7 weeks
Weight	Males: 223-238 g; females: 140-160 g
Source	Charles River Laboratories, Hollister, CA, USA
Acclimation period	5 days
Diet	Certified Laboratory Rodent Diet *ad libitum*
Water	Fresh potable drinking water *ad libitum* via a sipper tube
Housing	Individually in rodent cages containing absorbent shavings as bedding during acclimation; non-suspended rodent cages during the administration of non-radioactive fluensulfone; individually in suspended cages (group 1 – sacrifice 24 hours after radiolabelled dose) or individually in glass metabolism cages (group 2 – sacrifice 7 days after radiolabelled dose) after dosing with the radiolabeled test substance until sacrifice
Husbandry	Controlled environment with a temperature of 18-26°C, humidity at 50 ± 20%, at least 10 room air changes per hour and a light/dark cycle of 12 hours

4. Preparation of Dose suspensions

Two non-radioactive dose suspensions were prepared in PEG 300. One dose suspension was used for administration days 1 to 7 and the second for days 8 to 14. Fluensulfone (157.7 mg) was added to a 500-mL Teflon bottle. Polyethylene glycol (354.4 g, PEG 300) was added to the dose containers by weight. The suspension doses were stirred using a magnetic stir bar to achieve homogeneity. After stirring for 1 hour, aliquots were diluted and analyzed by HPLC relative to a standard curve generated from five different concentrations of fluensulfone. Aliquots were also analyzed in the same manner after dosing. The nominal concentration was 0.44 mg fluensulfone/g suspension (0.49 mg/mL). Single oral doses of unlabeled fluensulfone were administered daily via gavage for 14 consecutive days. The dose rate for each animal was based on the body weight of each animal measured at days 1, 4, 7, 10, and 14.

One radioactive suspension was prepared in PEG 300 for the dosing on day 15. The [^{14}C]fluensulfone was isotopically diluted with non-radioactive fluensulfone. Radioactive (5.59 mg) and non-radioactive (14.36 mg) fluensulfone were added to a tube and the solvent was evaporated with a stream of nitrogen. Polyethylene glycol (45.0 g, PEG 300) was added by weight. The suspension dose was vortexed to achieve homogeneity (0.44 mg fluensulfone/g PEG 300). Aliquots of the test suspension (measured by weight) were removed for liquid scintillation counting before dose administration. These measurements were used to determine the concentration of [^{14}C]fluensulfone in the suspension and the homogeneity. Small quantities of the suspension were taken after dose administration and diluted with methanol for HPLC analysis. This analysis served to determine the radiochemical purity.

B. STUDY DESIGN AND METHODS

Groups of 4 male and 4 female Wistar rats received repeated oral doses of non-radiolabelled fluensulfone for 14 consecutive days (nominally at 5 mg/kg bw). On day 15, each animal received a single oral dose of [^{14}C]fluensulfone at a nominal level of 5 mg/kg bw. Group 1 rats were sacrificed 24 hours after receiving the radiolabel dose and Group 2 was sacrificed 168 hours (7 days) after the radiolabel dose. At each terminal sacrifice, blood, bone (femur), heart, lung, spleen, liver, kidney, thyroid, testes/ovaries, muscle (thigh), fat, brain and gastro intestinal tract (including stomach) samples, as well as remaining carcass were taken for measurement of total radioactive residues.

Urine and feces of Group 2 animals were continuously collected over wet ice in glass metabolism cages designed for the separate collection of urine and feces. The conical collecting surfaces of the metabolism cages were washed with water (approximately 100 mL) whenever the urine collection vessels were emptied. The total amounts of urine, cage wash, and feces were determined gravimetrically.

Portions of urine and cage wash were weighed and analyzed by liquid scintillation counting (LSC). Composite samples (0-24 hours) were prepared by adding the same percentage from individual rats for HPLC analysis.

Weighed portions of water were added to the complete feces samples, and the resulting sample mixed to obtain a homogenous paste. Portions were then weighed for combustion analysis/liquid scintillation counting. Composite samples (0-24, 24-48 hours) were prepared by adding the same percentage from individual rats for HPLC analysis. The post-extraction solid (PES) was combusted followed by LSC analysis.

The [^{14}C] content of whole blood and bone was determined by combustion analysis. The sample size was measured by weight, and typically about 100-500 mg samples were used for each combustion.

Tissues were aliquoted into 20 mL glass scintillation vials. Typically duplicate portions (approximately 50 to 100 mg) were weighed and submitted to tissue solubilization/LSC. The tissues included: thyroid, ovaries, fat, muscle, brain, heart, kidney, liver, lung, spleen, uterus, and testes.

Tissue Solubilization Procedure

Weighed portions (ca. 50-200 mg) were mixed with 2 mL tissue solubilizer (Soluene) and digested at ambient temperature (between 4 hours and up to 3 days). After digestion, 0.25 mL glacial acetic acid was added, followed by 15 mL LSC cocktail.

Carcasses and Whole Gastro-Intestinal (GI) Tracts (with Contents)

The carcass or GI tract was weighed and placed in 25% (w/w) KOH (approximately 4 g KOH solution per g of sample). The samples were allowed to digest at ambient temperature for 5-9 days, until the samples had completely dissolved. Portions (approximately 0.5 g) were mixed with 3 mL of methanol, followed by liquid scintillation counting.

Metabolites in urine and feces were identified by comparison to reference standards or by LC/MS.

II. RESULTS AND DISCUSSION

One male rat in Group 1 died before administration of the radiolabel dose. A necropsy did not reveal any obvious cause of death.

Dose suspensions and actual administered dosages

The data indicated that the suspensions had an acceptable degree of homogeneity (relative standard deviation of 0.2-0.5% measured by replicate LSC analysis of weighed subsamples) and radiochemical purity (98.5% before dosing and 98.6% after dosing).

The mean dose of unlabeled fluensulfone administered to Group 1 was 4.9 ± 0.05 mg/kg bw (males) and 5.0 ± 0.06 mg/kg bw (females). The mean dose of unlabeled fluensulfone administered to Group 2 was 4.9 ± 0.06 mg/kg bw (males) and 4.9 ± 0.10 mg/kg bw (females).

The mean dose of [^{14}C]fluensulfone administered to Group 1 was 5.0 ± 0.05 mg/kg bw (males) and 5.0 ± 0.08 mg/kg bw (females). The mean dose [^{14}C]fluensulfone administered to Group 2 was 5.0 ± 0.06 mg/kg bw (males) and 5.0 ± 0.12 mg/kg bw (females).

Radioactivity balance

Results are summarized in Table 1. In general, there were no major differences between males and females. The average recovery of radiolabel for Group 2 was 96.6% and 97.8% of the administered dose (AD) for males and females, respectively.

Most radiolabel was excreted in the urine (an average of 75.7% AD for males and 75.8% AD for females). Feces contained an average of 10.3% and 10.0% AD for males and females, respectively. The average cage wash contributed 9.5-11.4% AD. Rats sacrificed after 7 days contained only 1.1% and 0.5% AD in the carcass and 0.04-0.05% AD in the contents of the gastrointestinal tract.

Table 1: Summary of average radioactivity balance (% of administered dose)

		Group 1 (24 hours after radiolabel dosing)		Group 2 (7 days after radiolabel dosing)	
		Male	**Female**	**Male**	**Female**
Tissues	Liver	0.78	0.58	0.19	0.14
	Kidney	0.12	0.10	0.03	0.03
	Carcass	2.10	1.71	1.08	0.54
Excreta	Feces	NA	NA	10.29	10.01
	Urine	NA	NA	75.69	75.84
	Cage wash	NA	NA	9.49	11.38
	Gastrointestinal tract	4.53	4.7	0.05	0.04
Total		**7.5**	**7.1**	**96.8**	**98.0**

Data obtained from page 46 of the study report.

Tissue distribution

One day after dosing with [^{14}C]fluensulfone (Tables 1 and 2), the highest radiolabeled residues for individual rats were in the gastrointestinal tract (4.53% and 4.7% of the administered dose in males and females, respectively). The highest tissue levels were in liver, kidney, lung, thyroid, spleen and heart. The radiolabel residue in bone, brain, fat and muscle were 9 to 20-fold lower than in liver. The levels of residues were comparable in both sexes, although generally slightly higher in males.

The tissue distribution of radiolabel is similar for rats given a single oral dose of [^{14}C]fluensulfone (5 mg/kg bw with sacrifice at 17 hours, see Study R-23412) and rats given repeated oral doses of unlabeled fluensulfone (5 mg/kg bw) daily for 14 days followed by [^{14}C]fluensulfone (5 mg/kg bw) on day 15 with sacrifice at 24 hours.

Table 2: Concentration of radioactivity in tissues of rats at 24 hours after a single oral administration of [thiazole-^{14}C]fluensulfone (mean ± SD)

Organ/tissue	µg/g		% of administered dose	
	Male	Female	Male	Female
Bone	0.101 ± 0.035	0.069 ± 0.019	NA	NA
Brain	0.053 ± 0.005	0.033 ± 0.007	0.01 ± 0.00	0.01 ± 0.01
Fat	0.065 ± 0.019	0.065 ± 0.018	NA	NA
Heart	0.256 ± 0.053	0.181 ± 0.039	0.02 ± 0.01	0.01 ± 0.01
Kidney	0.711 ± 0.137	0.543 ± 0.121	0.12 ± 0.02	0.10 ± 0.02
Liver	1.062 ± 0.204	0.734 ± 0.119	0.78 ± 0.02	0.59 ± 0.07
Lung	0.579 ± 0.091	0.524 ± 0.127	0.06 ± 0.01	0.06 ± 0.01
Muscle	0.056 ± 0.008	0.044 ± 0.012	NA	NA
Spleen	0.258 ± 0.027	0.187 ± 0.046	0.01 ± 0.00	0.01 ± 0.00
Testes/Ovaries	0.070 ± 0.012	0.100 ± 0.032	0.02 ± 0.01	0.00 ± 0.00
Thyroid	0.852 ± 0.011	0.527 ± 0.166	0.01 ± 0.00	0.01 ± 0.00
Whole blood	0.831 ± 0.209	0.622 ± 0.159	NA	NA
Gastrointestinal contents	2.333 ± 0.594	1.755 ± 0.776	4.53 ± 1.16	4.70 ± 1.97
Carcass	0.143 ± 0.045	0.120 ± 0.026	2.10 ± 0.71	1.71 ± 0.28

Data obtained from page 49 of the study report.

Seven days after dosing with [^{14}C]fluensulfone radiolabel (Table 3), residue levels for individual rats were 2 to 10-fold lower compared to values obtained at 24 hours after dosing.

Table 3: Concentration of radioactivity in tissues of rats at 168 hours after a single oral administration of [thiazole-^{14}C]fluensulfone (mean ± SD)

Organ/tissue	µg/g		% of administered dose	
	Male	Female	Male	Female
Bone	0.037 ± 0.004	0.023 ± 0.003	NA	NA
Brain	0.026 ± 0.005	0.015 ± 0.002	0.00 ± 0.00	0.00 ± 0.00
Fat	0.020 ± 0.007	0.024 ± 0.007	NA	NA
Heart	0.120 ± 0.029	0.073 ± 0.011	0.01 ± 0.00	0.01 ± 0.01
Kidney	0.184 ± 0.040	0.141 ± 0.022	0.03 ± 0.01	0.03 ± 0.01
Liver	0.223 ± 0.030	0.155 ± 0.023	0.19 ± 0.04	0.14 ± 0.02
Lung	0.242 ± 0.026	0.195 ± 0.020	0.03 ± 0.01	0.02 ± 0.01
Muscle	0.025 ± 0.005	0.018 ± 0.004	NA	NA
Spleen	0.147 ± 0.033	0.092 ± 0.013	0.01 ± 0.00	0.00 ± 0.00
Testes/Ovaries	0.022 ± 0.005	0.035 ± 0.007	0.00 ± 0.01	0.00 ± 0.00
Thyroid	0.105 ± 0.046	0.070 ± 0.015	0.00 ± 0.00	0.00 ± 0.00
Whole blood	0.610 ± 0.125	0.375 ± 0.023	NA	NA
Gastrointestinal contents	0.020 ± 0.000	0.018 ± 0.010	0.05 ± 0.01	0.04 ± 0.02
Carcass	0.065 ± 0.010	0.035 ± 0.006	1.08 ± 0.18	0.58 ± 0.07

Data obtained from page 50 of the study report.

Excretion

Urine was the major route for the excretion of radiolabel from [^{14}C]fluensulfone (71-83% AD). The majority of the radioactivity eliminated in the urine was collected in the first 12 hours after dosing (81-96% of total radioactivity recovered in urine; data not shown).

HPLC analysis of the 0-24 hour composite urine was compared to a standard mix and showed 4 major metabolites, including thiazole sulfonic acid (2-3% AD) and three metabolites that were later identified as thiazole mercapturate (39-45% AD), glucuronide I (20-23% AD) and glucuronide II (6-8% AD). Fluensulfone was not found in urine. Cage wash contained 9-11% of the administered radiolabel. HPLC analysis of 0-24 hour composite cage wash showed similar residues to urine. Thiazole sulfonic acid (TSA) in urine was also identified by 2nd method verification via TLC, but methyl sulfone was not evident.

Feces were the secondary route of excretion (9-11% AD). Extractions with acetonitrile/water and acetonitrile removed 68-72% of the total radioactive residue (TRR) corresponding to 2.7-3.9% AD. HPLC analysis showed minimal fluensulfone (0.1-0.2% AD) as well as numerous metabolites in the 0-24 hour fecal extracts from males and females. The distribution of metabolites generally was similar for males and females with the largest single residue accounting for 1.4-2.2% of AD at 24-48 hours.

Purification and analysis of unknown metabolites

Metabolites (from 0-6 hour urine, Rat #13F) were isolated via HPLC. An unresolved mixture of thiazole glucuronides I and II and thiazole mercapturate were identified.

III. CONCLUSIONS

Unlabeled fluensulfone was administered in repeated oral doses for 14 days to male and female rats (4.9 ± 0.05 and 5.0 ± 0.06 mg/kg bw, respectively). [^{14}C]Fluensulfone was administered 1 day after the last unlabeled dose with sacrifice 1 and 7 days later. The amount of radiolabeled residues was determined in the gastrointestinal tract, carcass remains, and 11 tissues.

One day after the radiolabeled dose, liver contained only an average of 0.6-0.8% of the administered radiolabel (0.734-1.062 µg/g). Kidney contained 0.1% of the administered dose (0.543-0.711 µg/g). Similar levels were found in lung (0.06% of administered dose, 0.524-0.279 µg/g) and thyroid (0.01% of administered dose, 0.527-0.852 µg/g). Lower levels were in heart and spleen (0.01-0.02% of administered dose, 0.181-0.258 µg/g). Other tissues contained an average of 0.033-0.101 µg/g.

Seven days after the radiolabeled dose, an average of 0.1-0.2% of the administered radiolabel remained in liver (0.155-0.223 µg/g) and 0.02-0.03% in lung (0.195-0.242 µg/g). Heart, kidney, spleen and thyroid contained averages of 0.070-0.184 µg/g (0-0.03% of administered dose) while other tissues contained lower levels (0.015-0.037 µg/g). Higher amounts of radiolabel in some tissues may result from higher amounts of hemoglobin in those tissues since radiolabel associated with blood is generally high following exposure to fluensulfone.

Radiolabel was excreted mostly in urine (individual values of 70.9-82.8% of the administered dose 7 days after dosing) with secondary excretion in feces (8.9-11.3%). Thiazole sulfonic acid, glucuronide I, glucuronide II, and thiazole mercapturate were the major residues identified in urine. Minor levels of fluensulfone and thiazole methyl sulfone were found in fecal extracts, accounting for 0.1% and 0.3-0.8% of the administered dose, respectively. As the study was performed with [^{14}C-thiazole]fluensulfone only and not [^{14}C-butene]fluensulfone, the presence of sulfinic acid and derivatives was not investigated.

Very little parent compound was detected in feces (0.1% of the administered dose), and no parent compound was detected in urine. Rapid metabolism and elimination of the metabolites resulted in low residues in tissues, including organs of elimination, liver and kidney.

The proposed metabolic pathway for [thiazole-^{14}C]fluensulfone is similar for rats given repeated oral doses compared to rats given single oral doses (Study R-23410).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This metabolism study in the rat is classified as acceptable/guideline (fully reliable) and, in combination with other metabolism studies conducted with fluensulfone (Lab report number R-23410/ MRID 4857472/ PMRA 2181184, R-23411/ MRID 48574741/ PMRA 2181185, and R-23412/ MRID 48574743/ PMRA 2181186), satisfies the guideline requirement for a metabolism study (OPPTS 870.7485; OECD 417) in rats.

C. Conclusions: The reviewer agrees with the conclusions reached by the study investigators.

D. Deficiencies: No justification was provided for testing only the thiazole labelled test material and not the butene labelled test material. No other deficiencies were identified.

Tier II Summary (PMRA #2181185, MRID 48574741) revised by the regulatory authorities, April 2013

Study Type Toxicokinetic studies – Single dose, oral route, in rats

IIA 5.1.1– MRID 48574741, PMRA 2181185

Report	Quistad G.B., Kovatchev A. and La Mar J. (2010) The Pharmacokinetics of [^{14}C]Fluensulfone (MCW-2) in the Rat Upon Administration of Single Oral High and Low Doses PTRL West, Inc. and Pacific BioLabs, California, USA, unpublished report Laboratory Project No. PTRL WEST 1790W (PBL study No. 10174G-X01G) dated 26 July 2010. Sponsor reference No. R-23411 Dates of experimental work: (in-life phase) 26 January to 14 April 2010
Sponsor	Makhteshim Chemical Works, Ltd., Beer Sheva, Israel
Guidelines	Japanese MAFF guidelines (12-Nousan-No. 8147, Part 2-3-1), US EPA Health Effects Test Guidelines OPPTS 870.7485, European Union Commission Directive 87/302/EEC (18 November 1987), as modified by the Council Directive 67/548/EEC Deviations: none
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. The study was conducted in compliance with GLP standards with one exception: reference standards for bovine hemoglobin, rat albumin, glutathione, and mercaptoethanol may not have been characterized under all aspects of GLP.

Executive Summary

In a metabolism study (MRID 48574741, PMRA 2181185), groups of Wistar rats (8/sex/dose/radiolabel) received single nominal oral doses of either 5 mg/kg bw or 500 mg/kg bw of fluensulfone radiolabeled with ^{14}C at two different positions ([thiazole-^{14}C]MCW-2, 100%, TJBIOS-NB21-33 and [butene-^{14}C]MCW-2, 96.5-97.0%, TJBIOS-NB18-44 and TJBIOS-NB21-52), in PEG 300 to determine the pharmacokinetic parameters of fluensulfone. Blood was drawn at 11-12 sampling times via surgically implanted jugular cannulae (up to 336 hours for the thiazole radiolabel and up to 192 hours for the butene radiolabel). The same sampling schedule was used for both low and high doses. Plasma was prepared from portions of the collected blood,

and the total fluensulfone-derived ^{14}C in whole blood and plasma was measured by radiochemical techniques. The eight animals for each dose/sex were arranged in two groups, and blood was sampled from alternative groups during the first 144 hours on test from thiazole label and first 72 hours for the butene label. Therefore, each blood or plasma concentration was the mean from four animals.

The following pharmacokinetic parameters were calculated: Maximum concentration (C_{max}), Time for maximum concentration (T_{max}), Time for decline to ½ T_{max} ($T_{1/2}$), Time for decline to 1/10 T_{max} ($T_{1/10}$), Time from dose to $T_{1/2}$ (DT_{50}), Time from dose to $T_{1/10}$ (DT_{90}), Elimination rate constant (k_{el}), Area under the curve (AUC), and Volume of distribution (V_d(L)).

The absorption of fluensulfone-derived ^{14}C was rapid at both doses. The kinetic parameters for males and females were generally similar for the butene radiolabel, while for the thiazole label, male rats demonstrated a slightly higher absorption based on AUC values. There was no indication that the elimination processes were saturated by the increase in dose from 5 mg/kg bw to 500 mg/kg bw. This is illustrated by the fact that the 100-fold concentration increase in dose resulted in less than a 100-fold increase in the area under the curve (AUC).

Additional work was performed to determine the nature of the radiolabeled residues from [^{14}C]fluensulfone, and possible reaction with glutathione (GSH) and mercaptoethanol.

The amount of radiolabel associated with whole blood increased relative to plasma with time (about 20-fold for thiazole label but only 2-fold for butene label). Fluensulfone reacts with the free thiol moiety of globin (protein of hemoglobin) to form a covalent linkage to the thiazole group and displaces butene sulfinic acid. The removal of radiolabel from thiazole label whole blood seems to follow the removal of derivatized hemoglobin with a DT_{50} =148-216 hours (6-9 days) for the low dose.

This metabolism study in the rat is classified as acceptable/guideline (fully reliable) and, in combination with other metabolism studies conducted with fluensulfone (Lab report number R-23410/ MRID 48574742/ PMRA 2181184, R-23412/ MRID 48574743/ PMRA 2181186, and R-23389/ MRID 48574744/ PMRA 2181187), satisfies the guideline requirement for a metabolism study (OPPTS 870.7485; OECD 417) in rats.

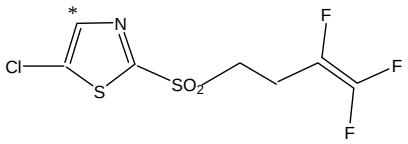
I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

Radiolabelled substance [Ring-^{14}C]fluensulfone or [Thiazole-4-^{14}C]fluensulfone

(termed as [thiazole-^{14}C]MCW-2)



* Indicates position of ^{14}C label

Description: Not given

Lot/Batch: TJBIOS-NB21-33

Specific Activity: 56.7 mCi/mmol

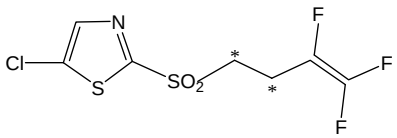
Radiochemical Purity: 100.0% (prior to dosing)

CAS#: 318290-98-1

Stability: Confirmed after dosing

Radiolabelled substance [Butene-^{14}C]fluensulfone or [trifluorobutene-1,2-^{14}C] fluensulfone

(termed as [butene-^{14}C]MCW-2)



* Indicates position of ^{14}C label

Description: Not given

Lot/Batch: TJBIOS-NB18-44 (low dose); TJBIOS-NB21-52 (high dose)

Specific Activity: 57.7 mCi/mmol (low dose); 58.7 mCi/mmol (high dose)

Radiochemical Purity: 96.5-97.0% (prior to dosing)

CAS#:	318290-98-1
Stability:	Confirmed after dosing
Unlabelled substance	Fluensulfone (MCW-2)
Description:	Not given
Lot/Batch:	411-12
Purity:	99.9%
CAS#:	318290-98-1
Stability:	Stable for the duration of the study
2. Vehicle	Polyethylene glycol (PEG 300)
3. Test Animals	
Species	Rat - Jugular vein catheterized at source
Strain	Wistar [Han:WIST(SPF)]
Age	Approx. 7 weeks
Weight	Males: 171-220 g; females: 152-180 g
Source	Charles River Laboratories, Hollister, CA, USA
Acclimation period	Overnight
Diet	Certified Laboratory Rodent Diet *ad libitum*
Water	Tap water *ad libitum*
Housing	Individually in rodent cages
Husbandry	Controlled environment with a temperature of 18-26°C, humidity at 50 ± 20%, a light/dark cycle of 12 hours, and at least 10 room air changes per hour

4. Preparation of Dose solutions

The [^{14}C]fluensulfone was isotopically diluted with non-radioactive fluensulfone. Radioactive and non-radioactive fluensulfone were dissolved in ethyl acetate. Each test solution was mixed thoroughly and the ethyl acetate was evaporated under a gentle stream of nitrogen. The resulting solid was suspended in polyethylene glycol (PEG 300). In order to prevent radiolysis, for the butene radiolabel the PEG 300 was added prior to evaporation of the ethyl acetate. The suspension doses were vortexed and sonicated to achieve homogeneity.

Aliquots of each test suspension (measured by weight) were removed for liquid scintillation counting (LSC) before dose administration. These measurements were used to determine the concentration of [^{14}C] fluensulfone in the suspensions and the homogeneity of the preparations. Small quantities of each suspension were taken after dose administration and diluted with methanol for HPLC analysis. These analyses served to determine the radiochemical purity.

B. STUDY DESIGN AND METHODS

Sixty four rats, approximately seven weeks of age at dosing, were used in this study. The rats were divided into groups such that 8 rats/sex/dose/radiolabel were arranged into two groups, and blood was sampled from 4 rats/sex/dose/radiolabel at alternating time points (see Table 1). The rats were fitted with jugular vein cannulae, and received a single oral dose of [^{14}C]fluensulfone (nominally at 5 or 500 mg/kg bw). Blood was drawn at specified intervals into heparinized tubes up to 336 hours (14 days) after dose administration. Portions of each blood sample were separated into plasma and packed cells by centrifugation. Whole blood and plasma samples were frozen after preparation until analysis and the packed cells were discarded.

Table 1: Sampling times (hours after dosing) (1: low dose; 2: high dose)

Sampling times (hours after dosing)												
	[thiazole-^{14}C]fluensulfone											
Group	0.5	1	2	8	24	48	72	96	144	192	264	336
1A	X		X		X		X		X	X	X	X
1B		X		X		X		X				
2A	X		X		X		X		X	X	X	X
2B		X		X		X		X				
[butene-^{14}C]fluensulfone												
Group	0.5	1	2	4	8	24	48	72	96	144	192	
1A	X		X		X		X					
1B		X		X		X		X	X	X	X	
2A	X		X		X		X					
2B		X		X		X		X	X	X	X	

Data obtained from page 18 of the study report. Each group contained 4 rats/sex.

The [^{14}C] content of whole blood was determined by combustion analysis followed by liquid scintillation counting (LSC). The sample size was measured by weight.

Plasma samples were analyzed by weighing portions into glass scintillation vials. Water (0.5 mL) and scintillation cocktail were added and the [^{14}C] content was measured by LSC. The average sample for LSC was about 50 mg.

Blood Analysis and *In Vitro* Methods

Blood from ^{14}C-fluensulfone treated rats

Whole blood was analyzed by HPLC to determine the nature of the radiolabeled residues from [^{14}C]fluensulfone. Whole blood (previously frozen) was pre-mixed with 5% bleach in water to reduce quenching. Mixed specimens were micro-filtered (0.45 µm) and centrifuged at 14,000 rpm for 5 min and the filtrate analyzed directly by HPLC with a bovine hemoglobin standard.

In vitro rat red blood cells

Rat red blood cells were obtained from a commercial supplier. Cells were lysed by freezing. Lysed blood cells were mixed with radiolabeled fluensulfone (each label in a separate vessel) in 0.1 M NaH_2PO_4 buffer pH 7.4 and incubated at 37°C overnight (about 24 hours). Specimens were placed on ice after 24 hours until analysis by HPLC.

Reactivity of fluensulfone in vitro with bovine hemoglobin

Bovine hemoglobin (about 1.0 mg) was mixed with radiolabeled fluensulfone (about 4-12 µg, each label in a separate vessel) in 0.1 M NaH_2PO_4 buffer (0.5 mL, pH 7.4) and incubated at 37°C overnight. Specimens were placed on ice after 24 hours until analysis by HPLC. Assuming the rate of reaction was dependent on de-protonation of the thiol group of hemoglobin, the above experiment was repeated with 0.1 M NaOH in water in an attempt to increase yield.

Reactivity of fluensulfone with glutathione

Fluensulfone (~100 mg, technical standard) was mixed with glutathione (~50 mg) in dilute NaOH in methanol/water (4:1) and left shaking overnight. The specimen mixture was acidified to pH~4 with 5% formic acid in water and analyzed by HPLC and LC/MS.

In a separate *in vitro* experiment, glutathione (~11 mg) was mixed with 1 µCi from each label of ^{14}C- fluensulfone in separate containers along with 4 mL 0.005 M NaOH in methanol/water (4:1). Both specimens were left shaking overnight and analyzed by HPLC the following day. Each solution was then adjusted to 0.1 M NaOH by diluting with 1 M NaOH in H_2O. Specimens were then reanalyzed by HPLC.

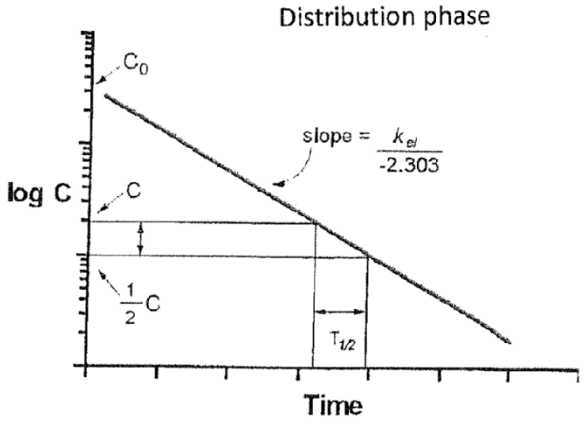
Reactivity of fluensulfone with β-mercaptoethanol

Fluensulfone (~100 mg, technical standard) was mixed with β-mercaptoethanol (~250 mg) in dilute NaOH in methanol/water (4:1) and left shaking overnight. The specimen mixture was acidified to pH~4 with 5% formic acid in water and analyzed by HPLC and LC/MS.

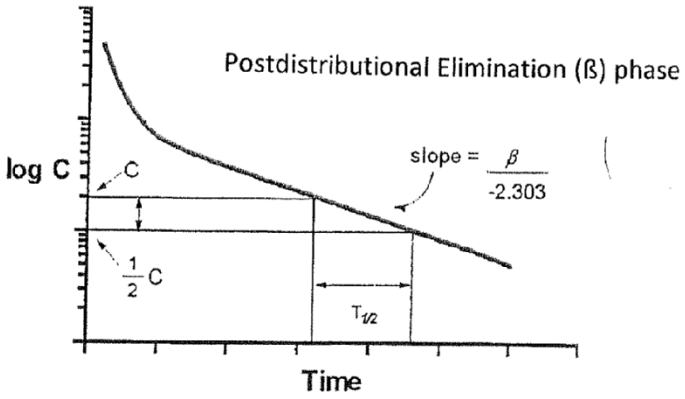
Calculation of Kinetic Parameters

The following mathematical relationships were used calculate kinetic parameters:

TOXICOKINETICS



From Casarett & Doull's Toxicology-The Basic Science of Poisons
6th Edition, 2001, p. 225.



C = concentration (µg/g); T= time (hr)

Ln (C) = slope (T) or log (C)/2.30 = slope (T)

K_{el} = elimination rate constant = -slope

$T_{1/2}$ = kinetic half-life = -ln (2)/slope; $T_{1/10}$ = ln (10)/slope

T_{max} = time to maximum concentration

C_{max} = maximum concentration in whole blood or plasma

DT_{50} = time from dose to decline to 50% of peak concentration = $T_{1/2}$ + time (0 to C_{max})

DT_{90} = time from dose to decline to 10% of peak concentration = $T_{1/10}$ + time (0 to C_{max})

R^2 = square of correlation coefficient in linear regression analysis to calculate k_{el}

V = A/C, V =volume of distribution; A = amount of chemical in body (mg); C = concentration in plasma (mg/L); V = L

The AUC's (area under the concentration-time curves) for the blood and plasma pharmacokinetic data were calculated by the Trapezoidal Rule. The calculations were performed on the mean blood/plasma concentrations (in µg fluensulfone equivalents/g). For a series of n concentrations (C_1 to C_n) measured at times T_1 to T_n, the area under the concentration-time curve was approximated as the sum of the areas of a series of trapezoids, as follows.

The area of the first trapezoid (corresponding to the time from dose administration to the first measurement) was calculated by assuming that the plasma concentration was 0 at T_0.

Area = $(C_1 \text{ X } T_1)/2$.

The area of the remaining segments were calculated by,

Area = $[(C_x + C_{x-1}) \text{ X } (T_x - T_{x-1})]/2$.

The area of the "tail" that occurred after the last measured plasma concentration (C_n) was calculated as,

Area = C_n/k_{elim}, where k_{elim} was the elimination rate constant calculated by linear regression analysis as the slope of the line formed by the time points as x data points and the ln(plasma concentrations) as the y data points.

II. RESULTS AND DISCUSSION

Dose suspensions and actual administered dosages

The data indicated that the suspensions had an acceptable degree of homogeneity and radiochemical purity (96.5-100.0% before dosing and 95.5-100.0% after dosing).

The target doses were 5 and 500 mg/kg bw. The mean achieved administered dosages are summarized in Table 2 below. Based on the mean doses administered to the animals, both dose groups were dosed at 93-104% of the target doses.

Table 2: Summary of administered dosages

[thiazole-^{14}C]fluensulfone					
Animal number (low – high dose)		Low dose (mg/kg bw)		High dose (mg/kg bw)	
Male	Female	Male	Female	Male	Female
1 – 17	5 – 21	4.9	4.8	479	462
2 – 18	6 – 22	5.0	4.8	466	468
3 – 19	7 – 23	4.9	5.0	466	481
4 – 20	8 – 24	4.7	4.9	466	471
9 – 25	13 – 29	4.7	4.9	474	461
10 – 26	14 – 30	4.8	4.8	468	465
11 – 27	15 – 31	4.9	4.8	465	462
12 – 28	16 – 32	4.9	4.9	473	460
Mean ± SD		**4.9 ± 0.1**	**4.9 ± 0.1**	**470 ± 5**	**466 ± 7**
[butene-^{14}C]fluensulfone					
Animal number		Low dose (mg/kg bw)		High dose (mg/kg bw)	
65 – 49	37 – 53	5.0	5.2	484	504
66 – 50	38 – 54	5.1	5.1	471	519
67 – 51	39 – 55	5.0	5.3	463	525
68 – 52	40 – 56	5.1	5.4	467	519
69 – 57	45 – 61	5.1	5.1	447	511
70 – 58	46 – 62	5.2	5.1	464	526
71 – 59	47 – 63	5.0	5.0	468	510
72 – 60	48 - 64	5.1	5.2	456	527
Mean ± SD		**5.1 ± 0.1**	**5.2 ± 0.1**	**465 ± 11**	**518 ± 8**

Data obtained from page 44 of the study report.

In life observations

Most animals survived until scheduled euthanasia. For the animals that died during the testing phase it was determined that their deaths were due to complications of the jugular catheterization combined with the process of collecting blood. This applied to animal numbers 8, 9, 20 and 47, corresponding to one female from the low dose thiazole label group, one male from the low dose thiazole label group, one male from the high dose thiazole label group, and one female from the low dose butene label group.

No clinical signs of toxicity or behavioural abnormalities were observed.

Toxicokinetic parameters

Calculated toxicokinetic parameters are summarized in Table 3.

For the low dose thiazole label, the time for maximum concentration (T_{max}) in plasma was similar between the sexes (2 hours) but was longer in whole blood for males (8 hours) compared to females (2 hours). For the high dose thiazole label, the T_{max} was similar between males and females in whole blood (48 hours) but was longer in plasma for males (8 hours) compared to females (1 hour). For the butene label, males and females demonstrated similar T_{max} values in plasma and whole blood for each dose level (4 hours for the low dose and 24 hours for the high dose).

For the low dose thiazole label, the maximum concentration (C_{max}) was similar in whole blood and plasma for both males and females (1.12-1.58 µg/g). For the high dose thiazole label, C_{max} in whole blood was higher in males (92 µg/g) than in females (75 µg/g) while the C_{max} in plasma was slightly higher in females (49 µg/g) than males (39 µg/g). For the butene label, there were no substantial differences in the C_{max} in the plasma and whole blood for males and females at each dose level (0.97-1.19 µg/g at the low dose, 25-34 µg/g at the high dose).

The half-life of elimination ($T_{1/2}$) from plasma was 14-15 hours for the low dose thiazole label, 20 hours for the low dose butene label, and 24-26 hours for the high dose butene label, and much slower for the high dose thiazole label at 58-67 hours.

The area under the curve (AUC) for whole blood from rats administered the thiazole label was higher in males (306 µg/g at the low dose, 21,445 µg/g at the high dose) than in females (237 µg/g at the low dose, 15,867 µg/g at the high dose), whereas for the butene label, the AUC was not substantially different between the sexes (43-47 µg/g for the low dose, 2038-2142 µg/g for the high dose). Compared to the approximate 100-fold increase in dose between the low and high dose groups, the AUC for the high dose group administered the thiazole label was 67-70 times higher than the AUC for the corresponding low dose group and the AUC for the high dose group administered the butene label was 45-47 times higher than the AUC for the corresponding low dose group.

Based on these results, the study investigators suggested the following tissue collection times for the tissue distribution study: thiazole label (low dose), T_{max} = 2 hours, DT_{50} = 17 hours, and DT_{90} = 51 hours; thiazole label (high dose), T_{max} = 4 hours, DT_{50} = 67 hours, and DT_{90} = 212 hours; butene label (low dose), T_{max} = 4 hours, DT_{50} = 24 hours, and DT_{90} = 70 hours; butene label (high dose), T_{max} = 24 hours, DT_{50} = 49 hours, and DT_{90} = 106 hours.

Table 3: Toxicokinetic parameters

Kinetic parameter	Radiolabel	Dose	Whole blood (♂/♀)	Plasma (♂/♀)
Maximum concentration C_{max} (µg equivalent/g)	Thiazole	Low	1.12 / 1.36	1.21 / 1.58
		High	91.9 / 75.1	39.3 / 49.2
	Butene	Low	0.97 / 0.99	1.19 / 1.19
		High	32.1 / 24.6	34.2 / 28.7
Time for maximum concentration T_{max} (hr)	Thiazole	Low	8 / 2	2 / 2
		High	48 / 48	8 / 1
	Butene	Low	4 / 4	4 / 4
		High	24 / 24	24 / 24
Time for decline to ½ T_{max} $T_{1/2}$ (hr)	Thiazole	Low	208 / 146	15.0 / 14.2
		High	162 / 134	67 / 58.2
	Butene	Low	33.0 / 30.1	19.8 / 19.8
		High	30.1 / 31.5	23.9 / 25.7
Time for decline to 1/10 T_{max} $T_{1/10}$ (hr)	Thiazole	Low	691 / 485	49.9 / 47.1
		High	537 / 445	221 / 193
	Butene	Low	110 / 100	66 / 66
		High	100 / 105	79 / 85
Time from dose to $T_{1/2}$ DT_{50} (hr)	Thiazole	Low	216 / 148	17.0 / 16.2
		High	210 / 182	75 / 59.2
	Butene	Low	37.0 / 34.1	23.8 / 23.8
		High	54.1 / 55.5	47.9 / 49.7
Time from dose to $T_{1/10}$ DT_{90} (hr)	Thiazole	Low	699 / 487	51.9 / 49
		High	585 / 493	229 / 194
	Butene	Low	114 / 104	70 / 70
		High	124 / 129	103 / 109
Elimination rate constant k_{el}	Thiazole	Low	0.003 / 0.005	0.046 / 0.049
		High	0.004 / 0.005	0.010 / 0.012
	Butene	Low	0.021 / 0.023	0.035 / 0.035
		High	0.023 / 0.022	0.029 / 0.027
Area under the curve AUC	Thiazole	Low	306 / 237	40 / 45
		High	21,445 / 15,867	3376 / 3187

Kinetic parameter	Radiolabel	Dose	Whole blood (♂/♀)	Plasma (♂/♀)
	Butene	Low	47 / 43	38 / 37
		High	2142 / 2038	1933 / 1971
Volume of distribution V_d(L) ($T_{1/2}$) (hr)	Thiazole	Low	0.84 / 0.60	0.78 / 0.51
		High	1.00 / 1.01	2.35 / 1.54
	Butene	Low	1.09 / 0.87	0.89 / 0.72
		High	2.86 / 3.49	2.69 / 2.99

Data obtained from pages 45 and 48 of the study report.

Fluensulfone Derivatization of Hemoglobin and Reaction with Thiol Proteins

Whole blood was analyzed by HPLC to determine the nature of the radiolabeled residues from [^{14}C]fluensulfone. For the thiazole label, radiolabel was associated with the globin protein of hemoglobin at 8 hours after dosing. At 48 and 336 hours most of the radiolabel in whole blood was associated with the globin protein. Rat hemoglobin contains three free thiol groups that are proposed to react with the thiazole ring of fluensulfone, releasing derivatized hemoglobin (Hb) and butene sulfinic acid. Rat red blood cells (erythrocytes) that contain hemoglobin have a life-time of 49-55 days. The slow removal of radiolabel from blood with derivatized hemoglobin probably is dependent on the metabolic removal of red blood cells. Lysed red blood cells react in vitro with fluensulfone to give a low yield of derivatized hemoglobin. Bovine hemoglobin reacts in pH 7.4 buffer overnight at room temperature to give a low yield of derivatized hemoglobin. The yield is increased to 14% if the reaction occurs in 0.1 M NaOH (presumably enhanced by ionization of the thiol to a better nucleophile).

Other thiols also react with [thiazole-^{14}C]fluensulfone. Glutathione (GSH) reacts with fluensulfone in 0.1 M NaOH overnight at room temperature to give a GSH adduct in 79% yield. A reference standard for this GSH adduct was synthesized and verified by LC/MS. The other product of fluensulfone reaction with GSH is butene sulfinic acid, verified from [butene-^{14}C]fluensulfone.

Mercaptoethanol was examined as a possible general non-biological thiol reactant with fluensulfone. Mercaptoethanol reacts in a similar manner with [thiazole-^{14}C]fluensulfone to give a derivatized thiazole ring, verified by LC/MS. Likewise, [butene-^{14}C]fluensulfone gives butene sulfinic acid.

III. CONCLUSIONS

Fluensulfone administered orally at nominal doses of either 5 mg/kg bw or 500 mg/kg bw was rapidly absorbed. The kinetic parameters for males and females were generally similar for the butene radiolabel, while for the thiazole label, male rats demonstrated a slightly higher absorption based on AUC values in whole blood. There was no indication that the metabolic elimination processes were saturated by the increase in dose from 5 mg/kg bw to 500 mg/kg bw. This is illustrated by the fact that the 100-fold concentration increase in dose resulted in less than a 100-fold increase in the area under the curve (AUC).

The amount of radiolabel associated with whole blood increased relative to plasma with time (about 20-fold for the thiazole label but only 2-fold for the butene label). Fluensulfone reacts with the free thiol moiety of globin (protein of hemoglobin) to form a covalent linkage to the thiazole group and displaces butene sulfinic acid. The removal of radiolabel from whole blood seems to follow the removal of derivatized hemoglobin with a DT_{50} =148-216 hours (6-9 days) for the low dose.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This metabolism study in the rat is classified as acceptable/guideline (fully reliable) and, in combination with other metabolism studies conducted with fluensulfone (Lab report number R-23410/ MRID 48574742/ PMRA 2181184, R-23412/ MRID 48574743/ PMRA 2181186, and R-23389/ MRID 48574744/ PMRA 2181187), satisfies the guideline requirement for a metabolism study (OPPTS 870.7485; OECD 417) in rats.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions. The study investigators did indicate that the kinetic parameters were generally similar between males and females, while the reviewer has indicated some sex-related differences in these parameters.

D. Deficiencies: No deficiencies were identified.

Tier II Summary (PMRA #2181186, MRID 48574743) revised by the regulatory authorities, April 2013

Study Type Toxicokinetic studies – Single dose, oral route, in rats

IIA 5.1.1 – MRID 48574743, PMRA 2181186

Report	Quistad G.B. and Kovatchev A. (2011) The Tissue Distribution of [^{14}C] MCW-2 in the Rat Upon Administration of Single Oral High and Low Doses PTRL West, Inc. and Pacific BioLabs, California, USA, unpublished report Laboratory Project No. PTRL WEST 1791W (PBL study No. 10B0102G-X01G) dated 23 September 2011. Sponsor reference No. R-23412. Dates of experimental work: (in-life phase) 20 July to 27 August 2010
Sponsor	Makhteshim Chemical Works, Ltd., Beer Sheva, Israel
Guidelines	Japanese MAFF guidelines (12-Nousan-No. 8147, Part 2-3-1), US EPA OPPTS 870.7485, European Union Commission Directive 87/302/EEC (18 November 1987), as modified by the Council Directive 67/548/EEC Deviations: none
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In a metabolism study (MRID 48574743, PMRA 2181186), the tissue distribution of [^{14}C]fluensulfone ([thiazole-^{14}C]MCW-2, 99.0-99.2%, TJBIOS-NB21-33 and [butene-^{14}C]MCW-2, 96.6-96.7%, TJBIOS-NB18-4) was studied in male and female Wistar rats after single oral doses in polyethylene glycol 300 (3 rats/sex/dose/radiolabel). Two nominal dose levels were used, 5 mg/kg bw (low dose) and 500 mg/kg bw (high dose). The study was designed to measure the distribution of test-substance-derived ^{14}C in selected tissues at three sacrifice intervals corresponding to their respective pharmacokinetic parameters of C_{max} (time to peak plasma concentration), DT_{50} (time from dosing to 1/2 C_{max}), and DT_{90} (time from dosing to 1/10 C_{max}) as determined from a pharmacokinetic study (Study R-23411/ MRID 48574741/ PMRA 2181185).

Residues were generally similar between males and females for each of the three sacrifice intervals. For both labels and both dose levels, the gastrointestinal tract, liver and kidney were among the tissues containing the highest radioactivity levels at C_{max} for both sexes. Other tissues

with high levels of radioactivity at C_{max} (as a percentage of the administered dose) included the pancreas (in males administered the low dose of the thiazole label, in females administered the high dose of the thiazole label, and in both sexes administered the low dose of the butene label), the lung (in females administered the high dose of the thiazole label and in both sexes administered the low dose of the butene label), and the thyroid gland (in both sexes administered the high dose of the thiazole label). At DT_{90}, the levels of radioactivity remaining in the tissues accounted for ≤ 3% of the administered dose.

As predicted by the pharmacokinetic study, radioactivity from the thiazole label was slowly eliminated from red blood cells and organs associated with their formation/storage (bone, bone marrow, spleen), consistent with the derivatization of the hemoglobin cysteine thiol. The ratio of residues at the C_{max} compared to the DT_{90} was near the expected 10% for plasma, but for the thiazole label (low and high doses) was >25% for hair and skin, heart, and lung. For the butene label (low and high doses), the ratio of residues at C_{max} compared to DT_{90} was >25% for numerous tissues/organs except those involved with the digestive system. The slow elimination of radioactivity with the thiazole and butene radiolabels from these tissues may reflect metabolism to 1- and 2- carbon fragments with reincorporation in natural products (e.g. protein, fatty acids) with long turnover rates as previously found for goats and hens (MRID 48574801/ PMRA 2181244 and MRID 48574802/ PMRA 2181245).

This metabolism study in the rat is classified as acceptable/guideline (fully reliable) and, in combination with other metabolism studies conducted with fluensulfone (Lab report number R-23410/ MRID 48574742/ PMRA 2181184, R-23411/ MRID 48574741/ PMRA 2181185, and R-23389/ MRID 48574744/ PMRA 2181187), satisfies the guideline requirement for a metabolism study (OPPTS 870.7485; OECD 417) in rats.

I. MATERIAL AND METHODS

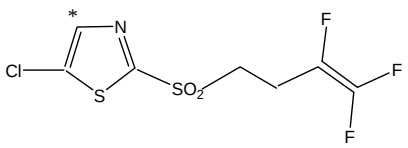
A. MATERIALS

1. Test Material

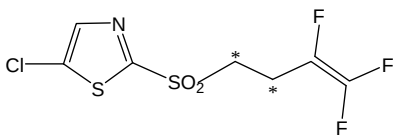
Radiolabelled substance

[Ring-^{14}C]fluensulfone or [Thiazole-4-^{14}C]fluensulfone

(termed as [thiazole-^{14}C]MCW-2)



	* Indicates position of ^{14}C label
Description:	Not given
Lot/Batch:	TJBIOS-NB21-33 (2466-20 purified)
Specific Activity:	56.7 mCi/mmol
Radiochemical Purity:	99.0-99.2% (prior to dosing)
CAS#:	318290-98-1
Stability:	Confirmed after dosing (99.1-99.2% radiochemical purity)
Radiolabelled substance	[Butene-^{14}C]fluensulfone or [trifluorobutene-1,2-^{14}C] fluensulfone (termed as [butene-^{14}C]MCW-2)



	* Indicates position of ^{14}C label
Description:	Not given
Lot/Batch:	TJBIOS-NB18-44 (2466/1-3 purified)
Specific Activity:	57.7 mCi/mmol
Radiochemical Purity:	96.6-96.7% (prior to dosing)
CAS#:	318290-98-1
Stability:	Confirmed after dosing (96.1-96.3% radiochemical purity)
Unlabelled substance	Fluensulfone (MCW-2)
Description:	Not given
Lot/Batch:	411-12
Purity:	99.9%
CAS#:	318290-98-1
Stability:	Stable for the duration of the study
Reference substances	Fluensulfone (MCW-2) (analytical standard), Lot No. 326-115-01, purity 99.12%, expiry date November 2010

2. Vehicle Polyethylene glycol (PEG 300)

3. Test Animals

Species	Rat
Strain	Wistar [Han:WIST(SPF)]
Age	Approx. 7 weeks
Weight	Males: 150-187 g; females: 139-163 g
Source	Charles River Laboratories, Hollister, CA, USA
Acclimation period	5 days
Diet	Certified Laboratory Rodent Diet *ad libitum*
Water	Fresh potable drinking water *ad libitum* via a sipper tube
Housing	Individually in rodent cages containing absorbent shavings as bedding during acclimation; individually in rodent boxes after dosing and to sacrifice
Husbandry	Controlled environment with a temperature of 18-26°C, humidity at 50 ± 20%, at least 10 room air changes per hour and a light/dark cycle of 12 hours

4. Preparation of Dose solutions

The [^{14}C]fluensulfone was isotopically diluted with non-radioactive fluensulfone. Radioactive and non-radioactive fluensulfone were dissolved in ethyl acetate. Each test solution was mixed thoroughly and the ethyl acetate was evaporated under a gentle stream of nitrogen in the presence of polyethylene glycol (PEG 300). The suspension doses were stirred and vortexed to achieve homogeneity.

Aliquots of each test suspension (measured by weight) were removed for liquid scintillation counting (LSC) before dose administration. These measurements were used to determine the concentration of [^{14}C] fluensulfone in the suspensions and the homogeneity of the preparations. Small quantities of each suspension were taken after dose administration and diluted with methanol for HPLC analysis. These analyses served to determine the radiochemical purity.

B. STUDY DESIGN AND METHODS

Groups of 3 male and 3 female Wistar rats per sacrifice interval received a single oral dose of [^{14}C] fluensulfone (nominally at 5 or 500 mg/kg bw), labelled at two different positions (either on the thiazole ring or the butene moiety). After dosing rats were housed individually in rodent boxes and sacrificed at intervals (Table 1) corresponding to the maximum concentration (C_{max}), time to 1/2 C_{max} from dosing (DT_{50}), and time to 1/10 C_{max} from dosing (DT_{90}) of radiolabel in plasma as determined in a previous study of pharmacokinetics (see Report R-23411).

Table 1: Samples collection times

Thiazole	**Sample collection time (hours post-dose)**		
	Interval 1 (C_{max})	**Interval 2 (50% C_{max})**	**Interval 3 (10% C_{max})**
Low dose (5 mg/kg bw)	2	17	51
High dose (500 mg/kg bw)	4	67	212
Butene	**Interval 1 (C_{max})**	**Interval 2 (50% C_{max})**	**Interval 3 (10% C_{max})**
Low dose (5 mg/kg bw)	4	24	70
High dose (500 mg/kg bw)	24	49	106

Data obtained from page 40 of the study report.

Blood was collected from the vena cava. Each whole blood sample was centrifuged to prepare blood plasma and blood cells. The samples were stored frozen until analysis.

The [^{14}C] content of whole blood and blood cells was determined by combustion analysis of duplicate samples. The sample size was measured by weight, and typically about 100 mg samples were used for each combustion.

Plasma samples (about 0.1 g) were analyzed in duplicate. Weighed samples were placed in glass scintillation vials, scintillation cocktail was added, and the [^{14}C] content was measured by liquid scintillation counting.

Large tissues were aliquoted into 20 mL glass scintillation vials. Typically duplicate portions (approximately 50 to 100 mg) were weighed and submitted to tissue solubilization/liquid scintillation counting. The large tissues included salivary gland, thyroid, lymph nodes, ovaries, fat, muscle, brain, heart, kidney, liver, lung, pancreas, spinal cord, spleen, thymus, caecum, large and small intestine, stomach, uterus, prostate gland, hair and skin, uterus, and testes.

Small tissues were weighed and the entire sample usually subjected to tissue solubilization/liquid scintillation counting. These tissues included the bone marrow, pituitary gland, and adrenal gland.

Weighed portions (about 100 mg) of bone and gastrointestinal contents were subjected to combustion analysis/liquid scintillation counting.

Tissue Solubilization Procedure

Weighed portions (ca. 50-100 mg) were mixed with 2 mL tissue solubilizer (Soluene) and digested at ambient temperature (between 4 hours and up to 3 days.) After digestion, 0.25 mL glacial acetic acid was added, followed by 15 mL liquid scintillation counting cocktail.

Carcasses

The carcass was weighed and placed in 25% (w/w) KOH (approximately 4 g KOH solution per g of sample). The samples were allowed to digest at ambient temperature for several days, until the samples had completely dissolved. Aliquots (approximately 200 mg) were mixed with 3 mL of methanol, followed by liquid scintillation counting.

II. RESULTS AND DISCUSSION

Dose suspensions and actual administered dosages

Data indicated that dosing suspensions were homogeneous (relative standard deviations of 3.1-5.6%). The nominal doses were 5 and 500 mg/kg bw. The average actual doses were: 4.7 mg/kg bw (thiazole, low-dose), 515 mg/kg bw (thiazole, high-dose), 4.9 mg/kg bw (butene, low-dose), and 441 mg/kg bw (butene, high dose).

Tissue distribution

Residues were generally similar between males and females for each of the three sampling intervals and for both radiolabel positions.

Thiazole label - Low dose

The tissue distribution of radioactivity following administration of the low dose of the thiazole-labeled test material in males and females is summarized in Tables 2 and 3, respectively. At 2 hours post-dosing, the highest levels of radioactivity (as µg/g) for both sexes (unless otherwise specified) were detected in the gastrointestinal tract (stomach, small intestine, large intestine, caecum), liver, blood (whole blood, blood cells, plasma), thyroid gland, lung and kidney. As a percentage of the administered dose, the highest levels of radioactivity at 2 hours post-dosing were detected in the liver, pancreas (males), gastrointestinal tract, and kidney.

The residue in plasma at the DT_{90} (51 hours) was 8-9% of C_{max} (2 hours) where 10% was expected. The ratio of residues at C_{max} compared to DT_{90} was greater than 25% for whole blood, blood cells and organs associated with hemoglobin production. This is consistent with the derivatization of the hemoglobin cysteine thiol as proposed previously (Report R-23411/ MRID 48574741/ PMRA 2181185). A ratio of C_{max} to DT_{90} of greater than 25% was also found for males and/or females for the adrenal gland, hair and skin, muscle, heart, liver, lung, pituitary gland, uterus, ovary, thymus, and the carcass remains. Radiolabel in bone marrow is susceptible to high variability because of the small sample size. Radiolabel in hair and skin is susceptible to contamination from urine. This slow removal of radiolabel from tissues may reflect: 1) blood remaining in highly perfused organs and 2) the conversion of fluensulfone to small carbon fragments and reincorporation into natural products (e.g. protein) as shown in studies in goats and hens (MRID 48574801/ PMRA 2181244 and MRID 48574802/ PMRA 2181245). Total residual radioactivity in analyzed organs and tissues at DT_{90} accounted for 3.4% and 2.7% of administered dose in males and females, respectively.

Table 2: Distribution of fluensulfone-derived radioactivity in selected tissues of male rats after a single oral low dose, thiazole label (mean ± SD)

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining at 51 hours*
	2 hours	17 hours	51 hours	2 hours	17 hours	51 hours	
Adrenal	1.517 ± 0.259	0.377 ± 0.012	0.289 ± 0.070	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	19
Bone	0.276 ± 0.033	0.094 ± 0.016	0.090 ± 0.020	NA	NA	NA	33
Bone marrow	0.316 ± 0.081	0.230 ± 0.106	0.140 ± 0.023	NA	NA	NA	44
Brain	0.352 ± 0.036	0.046 ± 0.009	0.039 ± 0.004	0.07 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	11
Caecum	1.453 ± 0.298	0.761 ± 0.267	0.174 ± 0.057	0.27 ± 0.15	0.10 ± 0.05	0.03 ± 0.03	12
Fat	1.500 ± 0.301	0.096 ± 0.032	0.062 ± 0.016	NA	NA	NA	4
Hair and skin	0.791 ± 0.113	0.208 ± 0.082	0.242 ± 0.078	NA	NA	NA	31
Heart	0.660 ± 0.048	0.304 ± 0.061	0.268 ± 0.049	0.04 ±0.01	0.02 ± 0.01	0.02 ± 0.01	41
Kidney	3.994 ± 0.613	0.658 ± 0.095	0.547 ± 0.100	0.73 ± 0.04	0.11 ± 0.02	0.10 ± 0.02	14
Large intestine	1.574 ± 0.058	0.258 ± 0.092	0.130 ± 0.014	0.31 ± 0.11	0.03 ± 0.02	0.02 ± 0.01	8
Liver	3.186 ± 0.518	1.179 ± 0.227	0.790 ± 0.121	2.98 ± 0.34	1.29 ± 0.22	0.94 ± 0.22	25
Lung	1.861 ± 0.056	0.824 ± 0.091	0.510 ± 0.078	0.21 ± 0.02	0.10 ± 0.01	0.06 ± 0.01	27
Lymph nodes	1.635 ± 0.092	0.131 ± 0.018	0.098 ± 0.022	NA	NA	NA	6
Muscle	0.347 ± 0.077	0.089 ± 0.011	0074 ± 0.007	NA	NA	NA	21
Pancreas	1.790 ± 0.502	0.257 ± 0.031	0.213 ± 0.038	0.12 ± 0.02	0.02 ± 0.10	0.02 ± 0.00	12
Pituitary gland	0.730 ± 0.140	0.293 ± 0.109	0.186 ± 0.067	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	25
Prostate	1.945 ± 1.005	0.133 ± 0.027	0.108 ± 0.018	0.03 ± 0.02	0.00 ± 0.01	0.00 ± 0.00	6

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining at 51 hours*
	2 hours	17 hours	51 hours	2 hours	17 hours	51 hours	
Salivary gland	0.793 ± 0.092	0.177 ± 0.045	0.139 ± 0.034	0.04 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	18
Small intestine	9.015 ± 1.391	0.384 ± 0.116	0.123 ± 0.031	6.23 ± 1.91	0.21 ± 0.06	0.06 ± 0.01	1
Spinal cord	0.415 ± 0.078	0.057 ± 0.016	0.053 ± 0.018	NA	NA	NA	13
Spleen	0.845 ± 0.235	0.231 ± 0.029	0.202 ± 0.021	0.05 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	24
Stomach	42.186 ± 8.332	0.312 ± 0.051	0.225 ± 0.061	7.62 ± 4.79	0.05 ± 0.02	0.04 ± 0.01	0.5
Testes	0.601 ± 0.035	0.076 ± 0.015	0.059 ± 0.007	0.13 ± 0.02	0.02 ± 0.00	0.01 ± 0.01	10
Thymus	0.392 ± 0.052	0.111 ± 0.013	0.101 ± 0.016	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	26
Thyroid	12.714 ± 14.045	0.980 ± 0.119	0.586 ± 0.031	0.23 ± 0.23	0.02 ± 0.01	0.01 ± 0.00	5
Whole blood	1.458 ± 0.092	0.803 ± 0.146	0.737 ± 0.108	NA	NA	NA	51
Blood cells	1.109 ± 0.184	1.093 ± 0.156	1.171 ± 0.282	NA	NA	NA	106
Plasma	2.136 ± 0.615	0.284 ± 0.033	0.169 ± 0.022	NA	NA	NA	8
Stomach contents	132.962 ± 26.188	0.088 ± 0.026	0.083 ± 0.012	NA	NA	NA	0.06
Small intestine contents	13.205 ± 3.573	0.396 ± 0.331	0.083 ± 0.018	NA	NA	NA	0.6
Caecum contents	2.415 ± 1.587	2.213 ± 0.866	0.145 ± 0.133	NA	NA	NA	6
Large intestine contents	0.390 ± 0.231	4.329 ± 4.537	0.106 ± 0.051	NA	NA	NA	27
Carcass	0.553 ± 0.091	0.150 ± 0.017	0.133 ± 0.021	7.54 ± 1.29	2.18 ± 0.23	2.06 ± 0.33	24

Data obtained from pages 16 and 42-44 of the study report.
*: (µg/g at 51 hours) / (µg/g at 2 hours).

Table 3: Distribution of fluensulfone-derived radioactivity in selected tissues of female rats after a single oral low dose, thiazole label (mean ± SD)

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining after 51 hours*
	2 hours	17 hours	51 hours	2 hours	17 hours	51 hours	
Adrenal	1.071 ± 0.470	0.319 ± 0.111	0.324 ± 0.116	0.01 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	30
Bone	0.189 ± 0.062	0.096 ± 0.013	0.092 ± 0.038	NA	NA	NA	49
Bone marrow	0.344 ± 0.421	0.038 ± 0.033	0.00 ± 0.00	NA	NA	NA	0
Brain	0.200 ± 0.023	0.044 ± 0.005	0.040 ± 0.016	0.04 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	20
Caecum	3.110 ± 0.271	0.754 ± 0.322	0.118 ± 0.048	0.51 ± 0.16	0.08 ± 0.05	0.02 ± 0.01	4
Fat	1.202 ± 0.263	0.078 ± 0.010	0.055 ± 0.020	NA	NA	NA	5
Hair and skin	0.525 ± 0.069	0.257 ± 0.011	0.295 ± 0.154	NA	NA	NA	56
Heart	0.576 ± 0.046	0.286 ± 0.037	0.295 ±0.138	0.04 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	51
Kidney	3.241 ± 0.481	0.680 ± 0.007	0.531 ± 0.169	0.57 ± 0.04	0.12 ± 0.02	0.10 ± 0.03	16
Large intestine	1.174 ± 0.129	0.510 ± 0.208	0.131 ± 0.039	0.16 ± 0.04	0.16 ± 0.03	0.16 ± 0.00	11
Liver	2.285 ± 0.236	0.901 ± 0.022	0.630 ± 0.204	2.08 ± 0.15	0.87 ± 0.03	0.58 ± 0.14	28
Lung	2.163 ± 0.249	0.990 ± 0.094	0.666 ± 0.150	0.26 ± 0.07	0.11 ± 0.01	0.08 ± 0.02	31
Lymph nodes	1.338 ± 0.162	0.098 ± 0.019	0.076 ± 0.029	NA	NA	NA	6
Muscle	0.203 ± 0.031	0.068 ± 0.011	0.059 ± 0.013	NA	NA	NA	29
Pancreas	1.242 ± 0.492	0.267 ± 0.036	0.191 ± 0.059	0.11 ± 0.05	0.03 ± 0.01	0.02 ± 0.01	15
Pituitary gland	0.693 ± 0.142	0.377 ± 0.151	0.244 ± 0.114	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	35
Uterus	0.510 ± 0.223	0.169 ± 0.084	0.186 ± 0.179	0.01 ± 0.00	0.01 ± 0.01	0.00 ± 0.01	36

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining after 51 hours*
	2 hours	17 hours	51 hours	2 hours	17 hours	51 hours	
Salivary gland	0.637 ± 0.058	0.168 ± 0.012	0.127 ± 0.044	0.04 ± 0.01	0.01 ±0.00	0.01 ± 0.01	20
Small intestine	5.601 ± 1.150	0.319 ± 0.029	0.118 ± 0.038	3.21 ± 0.16	0.14 ± 0.04	0.06 ± 0.03	2
Spinal cord	0.198 ± 0.021	0.044 ± 0.001	0.045 ± 0.019	NA	NA	NA	23
Spleen	0.644 ± 0.194	0.260 ± 0.077	0.224 ± 0.076	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.00	35
Stomach	44.586 ± 15.548	0.807 ± 0.658	0.334 ± 0.146	6.87 ± 4.39	0.10 ± 0.08	0.06 ± 0.02	0.7
Ovary	0.755 ± 0.169	0.151 ± 0.014	0.218 ± 0.084	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.01	29
Thymus	0.287 ± 0.026	0.124 ± 0.020	0.103 ± 0.029	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	36
Thyroid	4.376 ± 3.041	0.828 ± 0.119	0.512 ± 0.235	0.09 ± 0.06	0.01 ± 0.00	0.01 ± 0.00	12
Whole blood	1.284 ± 0.169	0.720 ± 0.135	0.911 ± 0.246	NA	NA	NA	71
Blood cells	1.056 ± 0.096	1.024 ± 0.129	1.083 ± 0.436	NA	NA	NA	103
Plasma	1.272 ± 0.081	0.196 ± 0.009	0.109 ± 0.023	NA	NA	NA	9
Stomach contents	104.134 ± 6.275	2.134 ± 3.130	0.091 ± 0.092	NA	NA	NA	0.09
Small intestine contents	8.152 ± 3.006	0.352 ± 0.035	0.070 ± 0.013	NA	NA	NA	0.9
Caecum contents	5.110 ± 0.374	2.100 ± 0.904	0.091 ± 0.007	NA	NA	NA	2
Large intestine contents	0.970 ± 0.763	3.157 ± 0.518	0.131 ± 0.021	NA	NA	NA	14
Carcass	0.330 ± 0.017	0.120 ± 0.017	0.103 ± 0.045	4.70 ± 0.12	1.74 ± 0.27	1.55 ± 0.61	31

Data obtained from pages 17 and 42-44 of the study report.
*: (µg/g at 51 hours) / (µg/g at 2 hours).

Thiazole label - High dose

The tissue distribution of radioactivity following administration of the high dose of the thiazole-labeled test material in males and females is summarized in Tables 4 and 5, respectively. At 4 hours post-dosing, the highest levels of radioactivity (as µg/g) for both sexes (unless otherwise specified) were detected in the gastrointestinal tract, thyroid gland, adrenal gland, lymph nodes, fat, kidney, lung (females), ovaries, liver and pancreas. As a percentage of the administered dose, the highest levels of radioactivity at 4 hours post-dosing were detected in the liver, gastrointestinal tract, thyroid gland, lung (females), pancreas (females), and kidney.

The residue in blood plasma at the DT_{90} (212 hours) was 3-5% of C_{max} (4 hours) where 10% was expected. The ratio of residues at C_{max} compared to DT_{90} was greater than 25% for whole blood, blood cells and organs associated with hemoglobin production. This is consistent with the derivatization of the hemoglobin cysteine thiol as proposed previously (Report R-23411/ MRID 48574741/ PMRA 2181185). A ratio of C_{max} to DT_{90} of greater than 25% was also found for hair and skin, and heart. Male lung also showed 40%, but female lung showed only 5%; however, one 4-hour female lung was similar to the male 4-hour lung values while two 4-hour female lung values were much higher because of likely presence of blood in the subsamples or partial aspiration after gavage dosing. This slow removal of radiolabel from tissues may reflect: 1) blood remaining in highly perfused organs and 2) the conversion of fluensulfone to small carbon fragments and reincorporation into natural products (e.g. protein) as shown in studies in goats and hens (MRID 48574801/ PMRA 2181244 and MRID 48574802/ PMRA 2181245).Total residual radioactivity in analyzed organs and tissues at DT_{90} accounted for 0.84% and 0.45% of administered dose in males and females, respectively.

Table 4: Distribution of fluensulfone-derived radioactivity in selected tissues of male rats after a single oral high dose, thiazole label (mean ± SD)

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining after 212 hours *
	4 hours	67 hours	212 hours	4 hours	67 hours	212 hours	
Adrenal	96.55 ± 35.84	15.94 ± 2.71	6.068 ± 1.120	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6
Bone	7.37 ± 0.12	5.18 ± 0.79	2.387 ± 0.236	NA	NA	NA	32
Bone marrow	9.71 ± 6.30	7.61 ± 5.75	1.020 ± 1.766	NA	NA	NA	11
Brain	10.50 ± 1.86	4.27 ± 0.90	2.532 ± 0.230	0.02 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	24
Caecum	161.82 ± 45.31	15.58 ± 6.51	2.108 ± 0.715	0.16 ± 0.08	0.01 ± 0.01	0.00 ± 0.00	1
Fat	81.20 ± 6.93	3.83 ± 0.85	1.410 ± 0.256	NA	NA	NA	2
Hair and skin	35.64 ± 3.05	31.23 ± 2.92	21.636 ± 1.425	NA	NA	NA	61
Heart	18.85 ± 0.73	18.17 ± 4.07	7.759 ± 1.116	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	41
Kidney	76.33 ± 9.50	32.13 ± 2.82	9.068 ± 1.192	0.13 ± 0.01	0.06 ± 0.01	0.02 ± 0.00	12
Large intestine	52.19 ± 9.73	12.39 ± 5.25	3.227 ± 0.869	0.06 ± 0.02	0.02 ± 0.01	0.00 ± 0.00	6
Liver	67.89 ± 9.45	33.69 ± 5.10	10.160 ± 2.643	0.60 ± 0.12	0.38 ± 0.06	0.11 ± 0.03	15
Lung	33.05 ± 4.21	24.37 ± 4.08	13.078 ± 2.294	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	40
Lymph nodes	94.20 ± 12.10	6.87 ± 0.91	2.228 ± 0.199	NA	NA	NA	2
Muscle	10.15 ± 1.69	4.65 ± 0.45	2.455 ± 0.954	NA	NA	NA	24
Pancreas	62.68 ± 23.20	11.42 ± 0.91	4.236 ± 0.659	0.05 ± 0.02	0.01 ± 0.00	0.00 ± 0.00	7
Pituitary gland	11.11 ± 19.25	8.96 ± 15.51	0.000 ± 0.000	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0
Prostate	35.50 ± 3.51	6.90 ± 0.32	2.024 ± 0.461	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining after 212 hours *
	4 hours	67 hours	212 hours	4 hours	67 hours	212 hours	
Salivary gland	26.54 ± 5.59	7.71 ± 0.44	2.678 ± 0.102	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	10
Small intestine	171.93 ± 98.89	8.06 ± 1.37	2.076 ± 0.578	0.79 ± 0.54	0.04 ± 0.01	0.01 ± 0.00	1
Spinal cord	13.63 ± 1.71	5.02 ± 1.65	3.343 ± 0.671	NA	NA	NA	25
Spleen	37.91 ± 2.44	17.35 ± 1.32	9.254 ± 2.069	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	24
Stomach	8124.89 ± 12350.79	5.78 ± 2.46	0.66 ± 0.576	11.98 ± 18.52	0.01 ± 0.01	0.00 ± 0.00	0
Testes	18.43 ± 4.03	4.06 ± 0.08	1.404 ± 0.325	0.04 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	8
Thymus	14.74 ± 1.92	5.74 ± 0.32	1.868 ± 0.278	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13
Thyroid	729.87 ± 847.95	12.42 ± 0.99	4.393 ± 0.288	0.11 ± 0.12	0.00 ± 0.00	0.00 ± 0.00	0.6
Whole blood	32.07 ± 6.87	74.62 ± 6.68	76.021 ± 2.515	NA	NA	NA	237
Blood cells	34.80 ± 5.81	114.44 ± 12.68	92.256 ± 10.101	NA	NA	NA	265
Plasma	38.89 ± 5.67	15.84 ± 0.98	1.982 ± 0.557	NA	NA	NA	5
Stomach contents	11778.90 ± 3925.07	16.42 ± 22.21	0.000 ± 0.000	NA	NA	NA	0
Small intestine contents	428.79 ± 508.53	7.79 ± 1.32	0.000 ± 0.000	NA	NA	NA	0
Caecum contents	201.07 ± 115.95	35.84 ± 16.18	0.000 ± 0.000	NA	NA	NA	0
Large intestine contents	104.42 ± 87.05	40.64 ± 19.12	0.000 ± 0.000	NA	NA	NA	0
Carcass	20.38 ± 4.17	7.59 ± 0.19	4.433 ± 0.553	2.58 ± 0.56	1.04 ± 0.01	0.67 ± 0.08	22

Data obtained from pages 18 and 45-47 of the study report.*: (µg/g at 212 hours) / (µg/g at 4 hours).

Table 5: Distribution of fluensulfone-derived radioactivity in selected tissues of female rats after a single oral high dose, thiazole label (mean ± SD)

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining at 212 hours *
	4 hours	67 hours	212 hours	4 hours	67 hours	212 hours	
Adrenal	82.02 ± 23.41	13.32 ± 3.28	5.086 ± 0.072	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6
Bone	8.93 ± 2.24	3.89 ± 1.00	1.724 ± 0.294	NA	NA	NA	19
Bone marrow	25.46 ± 7.72	13.23 ± 6.72	4.678 ± 2.395	NA	NA	NA	18
Brain	13.02 ± 0.62	4.34 ± 1.14	2.169 ± 0.189	0.02 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	17
Caecum	68.61 ± 74.12	19.56 ± 17.66	1.890 ± 0.444	0.06 ± 0.04	0.05 ± 0.07	0.00 ± 0.00	3
Fat	116.07 ± 34.08	6.19 ± 2.18	1.971 ± 0.182	NA	NA	NA	2
Hair and skin	39.71 ± 21.06	22.48 ± 4.25	19.147 ± 15.325	NA	NA	NA	48
Heart	22.10 ± 3.29	14.93 ± 2.69	5.927 ± 0.241	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	27
Kidney	88.56 ± 13.54	27.99 ± 1.85	8.645 ± 1.215	0.13 ± 0.02	0.05 ± 0.00	0.02 ± 0.01	10
Large intestine	54.62 ± 19.72	9.48 ± 2.64	3.055 ± 0.385	0.06 ± 0.01	0.01 ± 0.01	0.00 ± 0.01	6
Liver	67.13 ± 20.78	30.50 ± 0.81	8.597 ± 1.282	0.51 ± 0.15	0.31 ± 0.04	0.09 ± 0.02	13
Lung	227.19 ± 159.85	22.88 ± 4.01	12.407 ± 0.626	0.25 ± 0.18	0.03 ± 0.01	0.01 ± 0.01	5
Lymph nodes	94.97 ± 21.15	7.37 ± 2.47	2.525 ± 0.310	NA	NA	NA	3
Muscle	11.38 ± 1.45	3.74 ± 0.51	1.844 ± 0.132	NA	NA	NA	16
Pancreas	139.56 ± 90.45	9.92 ± 3.08	4.019 ± 0.819	0.13 ± 0.10	0.01 ± 0.01	0.00 ± 0.01	3
Pituitary gland	30.76 ± 17.77	10.03 ± 8.82	0.000 ± 0.000	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0
Uterus	41.60 ± 7.56	9.13 ± 1.53	2.416 0.481	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining at 212 hours *
	4 hours	67 hours	212 hours	4 hours	67 hours	212 hours	
Salivary gland	29.20 ± 7.55	8.08 ± 1.91	2.522 ± 0.450	0.02 ± 0.01	0.00 ± 0.01	0.00 ± 0.00	9
Small intestine	161.66 ± 40.69	8.13 ± 2.39	2.039 ± 0.089	0.67 ± 0.17	0.04 ± 0.02	0.01 ± 0.00	1
Spinal cord	15.70 ± 3.06	4.70 ± 1.61	2.401 ± 0.380	NA	NA	NA	15
Spleen	50.56 ± 21.29	18.07 ± 5.75	9.771 ± 1.474	0.02 ± 0.01	0.01 ± 0.00	0.00 ± 0.01	19
Stomach	5224.11 ± 3638.55	17.84 ± 9.99	2.882 ± 0.164	8.15 ± 7.17	0.03 ± 0.01	0.01 ± 0.00	0.06
Ovary	60.39 ± 12.38	7.64 ± 1.69	2.658 ± 0.320	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	4
Thymus	15.57 ± 5.09	5.52 ± 0.29	1.834 ± 0.146	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	12
Thyroid	1978.41 ± 1674.16	10.94 ± 0.78	3.745 ± 0.418	0.21 ± 0.17	0.00 ± 0.00	0.00 ± 0.00	0.2
Whole blood	42.16 ± 4.75	65.10 ± 14.51	43.985 ± 3.532	NA	NA	NA	104
Blood cells	38.46 ± 5.70	125.08 ± 35.95	86.948 ± 11.262	NA	NA	NA	226
Plasma	51.99 ± 10.61	15.71 ± 5.16	1.510 ± 0.236	NA	NA	NA	3
Stomach contents	10064.75 ± 1872.36	14.78 ± 21.07	0.000 ± 0.000	NA	NA	NA	0
Small intest. contents	408.03 ± 45.31	7.86 ± 7.51	0.309 ± 0.022	NA	NA	NA	0.08
Caecum contents	60.64 ± 70.97	21.31 ± 23.67	0.198 ± 0.037	NA	NA	NA	0.3
Large intest. contents	32.38 ± 24.74	32.77 ± 39.47	0.288 ± 0.024	NA	NA	NA	0.9
Carcass	25.74 ± 8.21	6.58 ± 0.43	2.023 ± 0.940	3.38 ± 1.04	0.90 ± 0.06	0.31 ± 0.15	8

Data obtained from pages 19 and 45-47 of the study report. *: (µg/g at 212 hours) / (µg/g at 4 hours). ^: One female 4-hour rat was similar to the male 4-hour rats, but 2 females were much higher

Butene label – Low dose

The tissue distribution of radioactivity following administration of the low dose of the butene-labeled test material in males and females is summarized in Tables 6 and 7, respectively. At 4 hours post-dosing, the highest levels of radioactivity (both as µg/g and a percentage of the administered dose) for both sexes were detected in the gastrointestinal tract, liver, lung, kidney and pancreas.

The residue in plasma at the DT_{90} (70 hours) was 10-12% of C_{max} (4 hours) where 10% was expected. The ratio of residues at C_{max} compared to DT_{90} was greater than 25% for bone, bone marrow, adrenal gland, brain, fat, hair and skin, heart, lung, muscle, pituitary gland, spinal cord, testes, thymus and the carcass remains. This slow removal of radiolabel from tissues may reflect the conversion of fluensulfone to small carbon fragments and reincorporation into natural products (e.g. protein) as proposed in studies in goats and hens (MRID 48574801/ PMRA 2181244 and MRID 48574802/ PMRA 2181245). Total residual radioactivity in analyzed organs and tissues at DT_{90} accounted for 3.4% and 2.6% of the administered dose in males and females, respectively.

Table 6: Distribution of fluensulfone-derived radioactivity in selected tissues of male rats after a single oral low dose, butene label (mean ± SD)

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining after 70 hours *
	4 hours	24 hours	70 hours	4 hours	24 hours	70 hours	
Adrenal	0.918 ± 0.092	0.509 ± 0.057	0.247 ± 0.031	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	27
Bone	0.394 ± 0.035	0.252 ± 0.027	0.143 ± 0.007	NA	NA	NA	36
Bone marrow	0.805 ± 0.018	0.741 ± 0.396	0.353 ± 0.050	NA	NA	NA	44
Brain	0.493 ± 0.069	0.263 ± 0.049	0.098 ± 0.011	0.10 ± 0.02	0.06 ± 0.02	0.02 ± 0.00	20
Caecum	3.490 ± 1.146	0.578 ± 0.163	0.175 ± 0.049	0.62 ± 0.41	0.13 ± 0.06	0.03 ± 0.01	5
Fat	0.382 ± 0.096	0.188 ± 0.002	0.192 ± 0.041	NA	NA	NA	50
Hair and skin	0.457 ± 0.032	0.272 ± 0.018	0.178 ± 0.034	NA	NA	NA	39
Heart	0.545 ± 0.024	0.242 ± 0.008	0.174 ± 0.012	0.04 ± 0.00	0.02 ± 0.00	0.01 ± 0.01	32
Kidney	3.798 ± 0.214	1.026 ± 0.066	0.562 ± 0.014	0.75 ± 0.08	0.18 ± 0.01	0.13 ± 0.00	15
Large intestine	1.489 ± 0.410	0.523 ± 0.116	0.177 ± 0.037	0.17 ± 0.07	0.10 ± 0.06	0.03 ± 0.01	12
Liver	3.868 ± 0.447	1.024 ± 0.156	0.565 ± 0.051	3.33 ± 0.42	1.00 ± 0.21	0.66 ± 0.04	15
Lung	2.078 ± 0.231	1.460 ± 0.094	0.578 ± 0.092	0.25 ± 0.06	0.17 ± 0.02	0.08 ± 0.02	28
Lymph nodes	1.078 ± 0.469	0.292 ± 0.012	0.180 ± 0.010	NA	NA	NA	17
Muscle	0.284 ± 0.032	0.149 ± 0.004	0.110 ± 0.014	NA	NA	NA	39
Pancreas	1.620 ± 0.094	0.419 ± 0.029	0.192 ± 0.010	0.16 ± 0.03	0.04 ± 0.01	0.02 ± 0.01	12
Pituitary gland	1.276 ± 0.311	0.640 ± 0.078	0.236 ± 0.045	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	18
Prostate	1.344 ± 0.566	0.404 ± 0.053	0.191 ± 0.020	0.03 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	14

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining after 70 hours *
	4 hours	24 hours	70 hours	4 hours	24 hours	70 hours	
Salivary gland	0.850 ± 0.026	0.405 ± 0.049	0.162 ± 0.014	0.05 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	19
Small intestine	4.810 ± 1.448	0.594 ± 0.046	0.213 ± 0.046	2.68 ± 0.63	0.28 ± 0.05	0.12 ± 0.01	4
Spinal cord	0.298 ± 0.045	0.176 ± 0.025	0.140 ± 0.008	NA	NA	NA	47
Spleen	0.773 ± 0.031	0.436 ± 0.035	0.197 ± 0.021	0.05 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	25
Stomach	18.773 ± 4.018	0.417 ± 0.056	0.195 ± 0.025	2.95 ± 0.90	0.07 ± 0.01	0.04 ± 0.01	1
Testes	0.293 ± 0.058	0.151 ± 0.037	0.109 ± 0.002	0.06 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	37
Thymus	0.884 ± 0.042	0.958 ± 0.078	0.291 ± 0.035	0.06 ± 0.01	0.08 ± 0.01	0.02 ± 0.00	33
Thyroid	0.738 ± 0.218	0.269 ± 0.012	0.144 ± 0.010	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	20
Whole blood	1.002 ± 0.049	0.353 ± 0.029	0.190 ± 0.010	NA	NA	NA	19
Blood cells	0.818 ± 0.050	0.480 ± 0.040	0.225 ± 0.004	NA	NA	NA	28
Plasma	1.325 ± 0.109	0.392 ± 0.073	0.133 ± 0.004	NA	NA	NA	10
Stomach contents	96.220 ± 12.898	0.045 ± 0.003	0.091 ± 0.106	NA	NA	NA	0.09
Small intestine contents	12.978 ± 0.540	0.343 ± 0.042	0.185 ± 0.159	NA	NA	NA	1.4
Caecum contents	9.363 ± 2.464	0.643 ± 0.241	0.058 ± 0.014	NA	NA	NA	0.6
Large intestine contents	8.010 ± 1.064	0.994 ± 0.510	0.079 ± 0.016	NA	NA	NA	1
Carcass	0.410 ± 0.026	0.237 ± 0.015	0.143 ±0.012	5.48 ± 0.19	3.24 ± 0.15	2.15 ± 0.18	35

Data obtained from pages 20 and 48-50 of the study report. *: (µg/g at 70 hours) / (µg/g at 4 hours)

Table 7: Distribution of fluensulfone-derived radioactivity in selected tissues of female rats after a single oral low dose, butene label (mean ± SD)

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining after 70 hours *
	4 hours	24 hours	70 hours	4 hours	24 hours	70 hours	
Adrenal	0.906 ± 0.049	0.331 ± 0.040	0.212 ± 0.005	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	23
Bone	0.285 ± 0.036	0.175 ± 0.066	0.091 ± 0.022	NA	NA	NA	32
Bone marrow	0.881 ± 0.205	0.815 ± 0.723	0.291 ± 0.023	NA	NA	NA	33
Brain	0.284 ± 0.066	0.123 ± 0.022	0.091 ± 0.005	0.06 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	32
Caecum	3.150 ± 0.618	0.610 ± 0.105	0.222 ± 0.021	0.50 ± 0.10	0.09 ± 0.04	0.03 ± 0.01	7
Fat	0.381 ± 0.022	0.145 ± 0.032	0.106 ± 0.031	NA	NA	NA	28
Hair and skin	0.510 ± 0.094	0.229 ± 0.055	0.126 ± 0.013	NA	NA	NA	25
Heart	0.585 ± 0.037	0.230 ± 0.011	0.166 ± 0.011	0.04 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	28
Kidney	4.141 ± 0.632	1.033 ± 0.085	0.680 ± 0.018	0.67 ± 0.10	0.18 ± 0.01	0.13 ± 0.02	16
Large intestine	1.461 ± 0.540	0.393 ± 0.054	0.184 ± 0.024	0.22 ± 0.11	0.06 ± 0.01	0.02 ± 0.01	13
Liver	3.119 ± 0.211	0.924 ± 0.014	0.543 ± 0.050	2.35 ± 0.19	0.85 ± 0.03	0.48 ± 0.04	17
Lung	3.302 ± 1.054	1.649 ± 0.535	0.878 ± 0.044	0.35 ± 0.10	0.20 ± 0.05	0.10 ± 0.01	27
Lymph nodes	0.947 ± 0.105	0.257 ± 0.034	0.154 ± 0.002	NA	NA	NA	16
Muscle	0.238 ± 0.014	0.089 ± 0.008	0.075 ± 0.005	NA	NA	NA	32
Pancreas	1.756 ± 0.196	0.389 ± 0.039	0.173 ± 0.012	0.16 ± 0.07	0.04 ± 0.01	0.02 ± 0.01	10
Pituitary gland	1.120 ± 0.288	1.326 ± 0.811	0.310 ± 0.048	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	28
Uterus	0.954 ± 0.367	0.228 ± 0.057	0.142 ± 0.021	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	15

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining after 70 hours *
	4 hours	24 hours	70 hours	4 hours	24 hours	70 hours	
Salivary gland	0.824 ± 0.063	0.338 ± 0.023	0.158 ± 0.004	0.05 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	19
Small intestine	6.923 ± 3.034	0.572 ± 0.150	0.229 ± 0.030	3.83 ± 1.52	0.29 ± 0.03	0.09 ± 0.02	3
Spinal cord	0.187 ± 0.143	0.225 ± 0.114	0.098 ± 0.007	NA	NA	NA	52
Spleen	0.863 ± 0.137	0.363 ± 0.040	0.215 ± 0.016	0.04 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	25
Stomach	17.061 ± 2.379	0.300 ± 0.102	0.230 ± 0.089	2.39 ± 0.38	0.04 ± 0.02	0.03 ± 0.01	1
Ovary	0.729 ± 0.087	0.248 ± 0.054	0.142 ± 0.002	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	19
Thymus	1.014 ± 0.166	1.095 ± 0.042	0.338 ± 0.051	0.05 ± 0.01	0.06 ± 0.01	0.02 ± 0.00	33
Thyroid^	0.589 ± 0.069	0.254 ± 0.023	0.133 ± 0.005	0.07 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	23
Whole blood	1.165 ± 0.169	0.407 ± 0.029	0.210 ± 0.007	NA	NA	NA	18
Blood cells	0.924 ± 0.144	0.476 ± 0.045	0.251 ± 0.028	NA	NA	NA	27
Plasma	1.581 ± 0.204	0.382 ± 0.026	0.187 ± 0.018	NA	NA	NA	12
Stomach contents	83.093 ± 31.036	0.119 ± 0.054	0.056 ± 0.042	NA	NA	NA	0.07
Small intestine contents	13.918 ± 3.018	0.384 ± 0.136	0.208 ± 0.164	NA	NA	NA	1.5
Caecum contents	7.305 ± 1.317	0.952 ± 0.379	0.086 ± 0.037	NA	NA	NA	1
Large intestine contents	14.129 ± 4.796	0.394 ± 0.187	0.280 ± 0.146	NA	NA	NA	2
Carcass	0.417 ± 0.093	0.163 ± 0.015	0.110 ± 0.017	5.52 ± 1.17	2.39 ± 0.17	1.64 ± 0.35	26

Data obtained from pages 21 and 48-50 of the study report. *: (µg/g at 70 hours) / (µg/g at 4 hours)
^: based on 2 rats for 4 hours

Butene label – High dose

The tissue distribution of radioactivity following administration of the high dose of the butene-labeled test material in males and females is summarized in Tables 8 and 9, respectively. At 24 hours post-dosing, the highest levels of radioactivity (as µg/g) for both sexes were detected in the gastrointestinal tract, liver, kidney, pancreas, thymus and pituitary gland. As a percentage of the administered dose, the highest levels of radioactivity at 24 hours post-dosing were detected in the liver, gastrointestinal tract, and kidney.

The residue in plasma at the DT_{90} (106 hours) was 17-18% of C_{max} (24 hours) where 10% was expected. The ratio of residues at C_{max} compared to DT_{90} generally was greater than 25% for most tissues/organs except those associated with the digestive system and involved with excretion. This slow removal of radiolabel from tissues may reflect the conversion of fluensulfone to small carbon fragments and reincorporation into natural products (e.g. protein) as proposed in studies in goats and hens (MRID 48574801/ PMRA 2181244 and MRID 48574802/ PMRA 2181245). Total residual radioactivity in analyzed organs and tissues at DT_{90} accounted for 1.5% and 0.9% of administered dose in males and females, respectively.

Table 8: Distribution of fluensulfone-derived radioactivity in selected tissues of male rats after a single oral high dose, butene label (mean ± SD)

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining after 106 hours*
	24 hours	49 hours	106 hours	24 hours	49 hours	106 hours	
Adrenal	27.13 ± 8.87	24.39 ± 2.42	11.46 ± 0.88	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	42
Bone	10.40 ± 2.68	10.51 ± 0.45	5.89 ± 1.22	NA	NA	NA	57
Bone marrow	24.14 ± 5.26	52.63 ± 13.93	0.00 ± 0.00	NA	NA	NA	0
Brain	6.34 ± 1.61	6.36 ± 0.55	4.08 ± 0.60	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	64
Caecum	81.96 ± 18.07	60.52 ± 21.85	8.55 ± 0.79	0.14 ± 0.07	0.11 ± 0.04	0.02 ± 0.01	10
Fat	20.22 ± 7.21	12.25 ± 0.35	6.04 ± 0.68	NA	NA	NA	30
Hair and skin	19.70 ± 8.86	20.27 ± 8.87	9.63 ± 1.32	NA	NA	NA	49
Heart	12.34 ± 3.42	10.59 ± 0.41	6.66 ± 0.11	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	54
Kidney	75.73 ±	46.70 ±	20.50 ±	0.16 ±	0.10 ±	0.05 ±	27

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining after 106 hours*
	24 hours	49 hours	106 hours	24 hours	49 hours	106 hours	
	33.44	5.78	1.92	0.07	0.02	0.00	
Large intestine	259.30 ± 394.43	34.27 ± 8.84	7.74 ± 0.49	0.44 ± 0.68	0.07 ± 0.03	0.01 ± 0.00	3
Liver	69.75 ± 11.30	58.76 ± 6.48	29.18 ± 1.69	0.77 ± 0.10	0.76 ± 0.13	0.33 ± 0.02	42
Lung	23.02 ± 5.07	21.21 ± 1.88	10.23 ± 0.34	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	44
Lymph nodes	22.72 ± 4.83	17.10 ± 0.42	6.69 ± 0.89	NA	NA	NA	29
Muscle	7.58 ± 1.84	7.29 ± 0.35	4.83 ± 0.46	NA	NA	NA	64
Pancreas	40.37 ± 7.30	28.44 ± 0.15	9.06 ± 0.71	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	22
Pituitary gland	34.56 ± 2.52	40.28 ± 5.66	10.47 ± 3.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	30
Prostate	24.22 ± 7.81	27.87 ± 2.60	8.05 ± 0.10	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	33
Salivary gland	21.93 ± 2.51	23.67 ± 1.12	6.71 ± 0.11	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	31
Small intestine	65.67 ± 10.19	38.29 ± 9.49	7.27 ± 0.39	0.40 ± 0.08	0.20 ± 0.06	0.04 ± 0.00	11
Spinal cord	7.13 ± 1.33	8.30 ± 1.05	6.05 ± 0.50	NA	NA	NA	85
Spleen	24.29 ± 4.15	19.61 ± 0.22	8.77 ± 0.57	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	36
Stomach	476.31 ± 486.97	62.16 ± 39.78	8.49 ± 1.02	0.71 ± 0.73	0.11 ± 0.06	0.01 ± 0.01	2
Testes	13.91 ± 1.68	15.26 ± 2.88	4.61 ± 0.55	0.03 ± 0.01	0.04 ± 0.01	0.01 ± 0.00	33
Thymus	40.05 ± 2.90	60.48 ± 7.21	11.41 ± 2.04	0.03 ± 0.00	0.03 ± 0.01	0.01 ± 0.00	28
Thyroid	20.33 ± 6.05	13.55 ± 0.86	5.78 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	28
Whole blood	18.85 ± 6.29	13.27 ± 1.51	5.51 ± 0.58	NA	NA	NA	29
Blood cells	17.96 ±	16.84 ±	9.01 ±	NA	NA	NA	50

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining after 106 hours*
	24 hours	49 hours	106 hours	24 hours	49 hours	106 hours	
	5.13	1.93	0.57				
Plasma	24.72 ± 10.12	13.80 ± 0.63	4.40 ± 0.27	NA	NA	NA	18
Stomach contents	9729.10 ± 5485.49	394.00 ± 339.22	1.26 ± 0.33	NA	NA	NA	0
Small intestine contents	135.32 ± 39.45	47.06 ± 29.92	2.53 ± 0.27	NA	NA	NA	2
Caecum contents	215.06 ± 37.67	142.34 ± 48.72	5.23 ± 3.36	NA	NA	NA	2
Large intestine contents	292.61 ± 77.70	176.83 ± 30.86	9.17 ± 7.53	NA	NA	NA	3
Carcass	10.76 ± 2.57	11.01 ± 0.73	5.60 ± 0.24	1.62 ± 0.39	1.64 ± 0.06	0.91 ± 0.06	52

Data obtained from pages 22 and 51-53 of the study report. *: (µg/g at 106 hours) / (µg/g at 24 hours)

Table 9: Distribution of fluensulfone-derived radioactivity in selected tissues of female rats after a single oral high dose, butene label (mean ± SD)

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining after 106 hours*
	24 hours	49 hours	106 hours	24 hours	49 hours	106 hours	
Adrenal	20.42 ± 3.82	14.92 ± 5.63	7.13 ± 1.85	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	35
Bone	5.76 ± 2.86	5.70 ± 0.76	3.13 ± 0.73	NA	NA	NA	54
Bone marrow	16.33 ± 2.44	19.80 ± 5.34	3.82 ± 3.33	NA	NA	NA	23
Brain	4.53 ± 1.14	4.41 ± 1.06	2.93 ± 0.78	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	65
Caecum	63.37 ± 30.01	43.33 ± 20.94	4.77 ± 1.56	0.11 ± 0.07	0.07 ± 0.03	0.01 ± 0.01	8
Fat	15.90 ± 7.24	10.45 ± 3.91	3.77 ± 0.63	NA	NA	NA	24
Hair and skin	10.73 ± 1.27	10.48 ± 3.46	5.45 ± 1.33	NA	NA	NA	51
Heart	8.70 ± 2.37	7.60 ± 2.05	4.48 ± 0.52	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	51
Kidney	44.15 ± 9.81	37.78 ± 15.16	15.70 ± 3.20	0.09 ± 0.04	0.08 ± 0.02	0.03 ± 0.01	36
Large intestine	28.80 ± 4.80	21.89 ± 7.02	5.25 ± 1.28	0.08 ± 0.06	0.04 ± 0.01	0.01 ± 0.00	18
Liver	37.77 ± 11.09	37.46 ± 8.07	18.24 ± 3.33	0.37 ± 0.12	0.43 ± 0.03	0.19 ± 0.04	48
Lung	17.66 ± 3.26	17.02 ± 4.42	7.72 ± 1.02	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	44
Lymph nodes	15.88 ± 6.31	14.54 ± 7.20	4.36 ± 0.85	NA	NA	NA	27
Muscle	4.53 ± 1.53	4.02 ± 1.24	3.14 ± 1.35	NA	NA	NA	69
Pancreas	34.37 ± 12.13	18.86 ± 6.51	5.71 ± 0.66	0.04 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	17
Pituitary gland	23.24 ± 8.79	29.17 ± 5.53	8.15 ± 3.38	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	35
Uterus	13.85 ± 4.32	9.87 ± 3.52	4.47 ± 1.02	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	32

Organ/tissue	µg equivalents fluensulfone/g			% of dose			% remaining after 106 hours*
	24 hours	49 hours	106 hours	24 hours	49 hours	106 hours	
Salivary gland	14.18 ± 3.81	14.13 ± 4.29	4.64 ± 0.76	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	33
Small intestine	44.42 ± 19.92	24.10 ± 12.92	5.28 ± 1.44	0.19 ± 0.14	0.10 ± 0.03	0.02 ± 0.00	12
Spinal cord	4.67 ± 1.36	5.02 ± 0.75	3.43 ± 0.93	NA	NA	NA	73
Spleen	20.23 ± 1.76	15.68 ± 6.94	6.05 ± 0.94	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	30
Stomach	1348.28 ± 184.62	875.88 ± 1479.77	6.56 ± 1.81	2.10 ± 0.26	1.34 ± 2.24	0.01 ± 0.01	0.5
Ovary	13.16 ± 4.60	11.44 ± 4.77	4.40 ± 1.43	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	33
Thymus	24.35 ± 1.17	30.71 ± 2.83	9.93 ± 1.49	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.01	41
Thyroid	9.17 ± 2.15	9.13 ± 2.86	3.62 ± 0.86	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	39
Whole blood	8.36 ± 1.95	8.30 ± 5.40	4.57 ± 3.02	NA	NA	NA	55
Blood cells	11.01 ± 1.90	11.30 ± 4.75	4.53 ± 0.42	NA	NA	NA	41
Plasma	15.77 ± 6.50	11.15 ± 4.29	2.74 ± 0.59	NA	NA	NA	17
Stomach contents	12568.11 ± 4738.37	1849.90 ± 3119.24	2.52 ± 2.48	NA	NA	NA	0.02
Small intestine contents	85.18 ± 30.36	41.71 ± 42.39	2.78 ± 0.64	NA	NA	NA	3
Caecum contents	130.77 ± 24.59	108.55 ± 73.78	1.98 ± 0.37	NA	NA	NA	1.5
Large intestine contents	184.36 ± 58.78	258.45 ± 178.81	2.97 ± 0.47	NA	NA	NA	1.6
Carcass	7.34 ± 2.31	6.42 ± 2.07	3.65 ± 0.40	1.14 ± 0.35	1.00 ± 0.30	0.58 ± 0.07	50

Data obtained from pages 23 and 51-53 of the study report. *: (µg/g at 106 hours) / (µg/g at 24 hours)

III. CONCLUSIONS

Following single oral administration of fluensulfone (thiazole- and butene-^{14}C) at a low (5 mg/kg bw) or high (500 mg/kg bw) nominal dose to male and female rats, residues in organs and tissues were generally similar in both sexes for each of the three sacrifice intervals (C_{max}, DT_{50} and DT_{90}). For both labels and both dose levels, the gastrointestinal tract, liver and kidney were among the tissues containing the highest radioactivity levels at C_{max} for both sexes. Other tissues with high levels of radioactivity at C_{max} (as a percentage of the administered dose) included the pancreas (in males administered the low dose of the thiazole label, in females administered the high dose of the thiazole label, and in both sexes administered the low dose of the butene label), the lung (in females administered the high dose of the thiazole label and in both sexes administered the low dose of the butene label), and the thyroid gland (in both sexes administered the high dose of the thiazole label). At DT_{90}, the levels of radioactivity remaining in the tissues accounted for ≤ 3% of the administered dose.

As predicted by the pharmacokinetic study, radioactivity from the thiazole label was slowly eliminated from red blood cells, consistent with the derivatization of the hemoglobin cysteine thiol. The ratio of residues at the C_{max} compared to the DT_{90} was near the expected 10% for plasma, but for the thiazole label (low and high doses) was >25% for numerous tissues/organs. For the butene label (low and high doses), the ratio of residues at C_{max} compared to DT_{90} was also >25% for numerous tissues/organs except those involved with the digestive system. The slow elimination of radioactivity with the thiazole and butene labels may reflect metabolism of [^{14}C] fluensulfone to 1- and 2-carbon fragments with reincorporation in natural products (e.g. protein, fatty acids) with long turnover rates as found in studies with goats and hens.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: This metabolism study in the rat is classified as acceptable/guideline (fully reliable) and, in combination with other metabolism studies conducted with fluensulfone (Lab report number R-23410/ MRID 48574742/ PMRA 2181184, R-23411/ MRID 48574741/ PMRA 2181185, and R-23389/ MRID 48574744/ PMRA 2181187), satisfies the guideline requirement for a metabolism study (OPPTS 870.7485; OECD 417) in rats.

C. Conclusions: Overall, the reviewer agrees with the study investigators' conclusions.

D. Deficiencies: No deficiencies were identified.

Tier II Summary (PMRA #2181332, MRID 48574949) revised by the US EPA, March 13, 2014

Study Type: Dermal penetration, *in vivo* in the rat

IIA 5.3.7 - MRID 48574949, PMRA 2181332

Report:	Hassler S. (2011a). MCW-2: Study on the Dermal Penetration of ^{14}C-MCW-2 Formulated as MCW-2 480 EC in Rats. Harlan Laboratories Ltd, Switzerland; unpublished Report C56873, dated 26 September 2011. Sponsor reference No. R-23344. Dates of experimental work: 6 April to 27 June 2011.
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 427, EPA OPPTS 870.7600 and EEC method B.44 Deviations: none
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary

The dermal absorption of fluensulfone formulated as MCW-2 480 EC was determined in rats (MRID 48574949; PMRA #2181332). MCW-2 480 EC (containing ^{14}C-labelled active ingredient) was applied to shorn rat skin at two target dose levels: a low dose (0.1 mg/cm^2) reflecting typical concentration recommended for the use in the field (10 g a.i./L, diluted from the concentrate with water) and a high dose (4.8 mg/cm^2) reflecting the undiluted commercial product (480 g a.i./L). Each group consisted of 4 male Wistar rats. Collection periods were 0-10 hrs, 0-24 hrs, 0-72 hrs and 0-120 hrs for each dose level. Volatile test item was trapped by covering the application site with charcoal paper during and after exposure. The exposure time of 10 hours was chosen as the worst case exposure period of a field worker.

At the end of the 10-hr exposure period, MCW-2 480 EC was removed by washing with mild soap dilution. The dermal absorption of the test item was determined. Furthermore, the amount remaining in/on the skin at the application site after washing and potentially penetrating through the skin was determined at 3 additional time points, 24, 72 and 120 hours after application of the test item in order to estimate the depletion of the dose associated with the application site. The association of the remaining test item in/on the skin at the application site was investigated by skin stripping in order to separate the *stratum corneum* from the epidermis. Urine and feces were collected up to 120 hours after application. Blood and plasma residue levels were also measured up to 120 hours after application.

After dermal exposure to formulated [^{14}C]-MCW-2, 49-56% of the low dose and 16-20% of the high dose were systemically absorbed. Most of the remaining applied test item, i.e. 34-45% of the low dose and 76-78% of the high dose, could be dislodged from the application site at the end of the exposure period. The radioactivity in skin strips I and II represented only ≤0.42% of the administered dose. The radioactivity remaining on/in skin application site was predominantly located in the *stratum corneum.* Only very low amounts of radioactivity were found in lower skin levels, i.e. < 1% of the applied dose. Within 120 hours, there was no apparent increase in

systemic absorption at both dose levels. However, radioactivity recovered at the application site (*stratum corneum* and lower skin levels) decreased with time from 8.0 and 3.5% at 10 hours to 4.0 and 0.4% at 120 hours for the low and high dose, respectively, indicating that the residues in skin were slowly absorbed.

The systemically absorbed test item, a total of 53.50 and 19.57% for the low and high dose, respectively, was excreted mainly in urine. After 5 days, 46.58% of the low dose and 15.73% of the high dose were excreted with the urine. During the same period of time, 4.40% of the low dose and 1.86% of the high dose were excreted with the feces. A remarkable amount of the absorbed test item, i.e. 2.20% of the low dose and 0.66% of the high dose, was still remaining in the animals. The maximum potentially absorbable dose (systemic absorption + skin strips III-VII + remaining treated skin) for the low and high dose groups is 64 (10 h) and 21 (120 h) percent of the administered dose, respectively. There was also a relatively high amount of the total applied dose remaining in the cover and O-ring over the test site due to the volatility of the test material at skin temperature, but the occlusion of the exposed skin results in a higher total recovery and penetration than would occur under typical human exposure conditions, thereby representing a "worst-case" scenario. In summary, MCW-2 formulated as MCW-2 480 EC penetrated at the low and high dose level to a relatively high extent through rat skin.

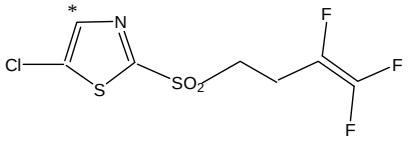
This study is classified as Acceptable/guideline (Fully Reliable) and satisfies the guideline requirement (OECD 427; OPPTS 870.7600) for an *in vivo* dermal absorption study in the rat.

I MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	MCW-2 (Fluensulfone)
Description:	Crystalline solid
Lot/Batch:	a. 36372130-291-PF2 (low dose)
	b. 36372130-291-PF1 (high dose)
Purity:	a. 97.5% (low dose)
	b. 95.92% (high dose)
CAS#:	318290-98-1
Expiry:	a. 27 January 2012 (36372130-291-PF2)
	b. May 2011 (36372130-291-PF1)

Radiolabelled Test Material:	MCW-2 [Ring-U-^{14}C]



* Indicates position of ^{14}C label

Description:	Not given
Batch number:	TJBIOS-NB21-33
Specific activity:	2098 MBq/mmol; 7.185 MBq/mg (when >99% pure)
Radiochemical purity:	83.3% (by TLC). Compound was purified before use in the study
Chemical purity:	Not given
Stability of test compound:	Confirmed after application by radio-HPLC
Blank Formulation:	Blank for MCW-2 480 EC
Lot/Batch:	100304
Expiry:	4 March 2012
2. Test Animals:	
Species:	Rat
Strain:	HanRcc: WIST(SPF) – males
Age:	Approximately 8 weeks
Weight at dosing:	Approximately 250 g
Source:	Harlan Laboratories, B.V., The Netherlands
Acclimation period:	At least 5 days to the laboratory environment including one day to the metabolism cages with a dorsal area of about 25-35 cm^2 shaved. Prior to shaving and separation in metabolism cages the animals were kept in groups in polycarbonate transport cages.
Diet:	Pellets: Harlan Teklad Global rodent 2914C (Composition 3255), Provimi Kliba AG, Switzerland Powder: Kliba 3433, Provimi Kliba AG, Switzerland *ad libitum*
Water:	Tap water *ad libitum*
Housing:	Individually in Open Plexiglas® metabolism cages

4. Environmental conditions:

Temperature:	19.3-20.9°C (low dose groups)
	20.1-21.2°C (high dose groups)
Humidity:	40-44% (low dose groups)
	36-52% (high dose groups)
Air changes:	Not indicated
Photoperiod:	12 hours light/dark cycle

B. STUDY DESIGN

1. Experimental dates: 7 April to 18 April (low dose); 27 April to -9 May 2011 (high dose)

2. Animals assignment and treatment

On receipt, the state of health was examined in all animals, and no unusual appearance or behavior was observed. The animals were assigned to groups 1 and 2, whose target dose levels were 0.1 mg MCW-2/cm^2 and 4.8 mg MCW-2/cm^2, respectively.

Sixteen rats per group were dosed at each dose level. The groups were further divided into subgroups consisting of 4 animals each. The exposure time to the formulated test item was 10 hours for all animals. At the end of the exposure period, the remaining test item was removed from the skin by a washing procedure. Four animals were sacrificed at each of the following time points: 10, 24, 72, and 120 hours after start of treatment. The day prior to application a dorsal area of about 25-35 cm^2 was shaved with an electric clipper taking care not to abrade the skin.

Prior to dosing, a double 'O'-ring, one glued on the top of the other, with an inside area of approximately 10 cm^2 (Ø 36 mm) was glued to the hair clipped skin area using cyanoacrylate (under light inhalation anesthesia). The application solution/emulsion (100 µL) was applied to the skin inside the 'O'-ring using a syringe and spread evenly. In order to prevent uncontrolled loss of the test substance the O-ring was covered with an active charcoal filter paper (Rotilabo®) on the inside and a permeable tape on the outside (non-occlusive conditions). The exact radioactivity administered was determined via weighing the application device before (with administration solution) and after administration (empty). The amount of applied test item was calculated based on the concentration of ^{14}C-MCW-2 in the administration solution.

After an exposure time of 10 hours, the cover and charcoal paper were removed and retained separately for analysis. The remaining test item was removed from the application site by washing 4 times with a mild soap solution followed by a single wash with tap water using soft cotton swabs. The moist skin area was dried with cotton swabs after each washing step. At the end of the washing procedure, a fresh non-occlusive cover tape (with an active charcoal filter paper) was applied to the O-ring.

The upper skin layer, i.e. *stratum corneum* and the fur grown within the duration of the experiment was removed from the application site by 5 consecutive tape strips. Afterwards a

tape with cyanoacrylate adhesive was glued on the top of the skin treated area. After a drying period of about 10 minutes, the tape was snatched off from the application site. This procedure was repeated one to two times until the *stratum corneum* was removed from the application site. These tape strips were individually combined to one specimen.

During hair clipping, dermal application and removal of the unabsorbed dose, animals were maintained under isoflurane anesthesia.

3. Preparation of dosing formulations

The formulations were prepared separately for each dose group. A stock solution was prepared first by dissolving ^{14}C labeled MCW-2 (10 mg) in 10 mL ethyl acetate:hexane (1:3 v:v).

For Group 1, unlabeled test item (93.31 mg) was placed in a flask and an aliquot of the stock solution (5 mL) containing 4.14 mg 14C-labeled MCW-2 (7185 kBq/mg) was added. The labeled and unlabeled test items were totally dissolved. This procedure yielded 14C-labeled test item with a final specific radioactivity of 305 kBq/mg. The solvent was removed under a gentle stream of nitrogen and 150.6 mg blank formulation was added (density of 1.2 g/mL). The formulated test substance was mixed with water (9.77 mL) to achieve a final volume of 10 mL. The final application suspension had a concentration of 9.74 mg MCW-2/mL.

For Group 2, Unlabeled test item (1447.1 mg) was placed in a flask and an aliquot of the stock solution (3.7 mL) containing 3.0 mg 14C-labeled MCW-2 (7185 kBq/mg) was added. The labeled and unlabeled test items were totally dissolved. This procedure yielded 14C-labeled test item with a final specific radioactivity of 14.66 kBq/mg. The solvent was removed by rotary evaporation and 2163.6 mg blank formulation was added resulting in a final volume of 3 mL MCW-2 SC formulation (density 1.2 g/mL). The final application suspension had a concentration of 483.4 mg MCW-2/mL.

The radiochemical purity of the test item in the respective formulation was checked by TLC at the time of the application (April 13, 2011 for the low dose, 97.8% radiochemical purity, and May 4, 2011 for the high dose, 98.0% radiochemical purity).

4. Sample collection and storage

All specimens were individually and separately collected at the stated time points. All volumes and weights were recorded.

4.1 Excreta

Urine: 0-10, 10-24, 24-48, 48-72, 72-96 and 96–120 hours after application.

The vessels used for collecting urine were cooled with solid carbon dioxide.

For the first sampling time points (0-10 and 10-24 hours), the funnel of the cages was rinsed with about 5-10 mL water. The volume of the funnel rinse was added directly to the urine. Apart from transfer into storage vessels, the specimens were kept frozen until analysis.

Feces: 0-10, 10-24, 24-48, 48-72, 72-96 and 96–120 hours after application.

Feces were collected at ambient temperature and kept frozen until analysis.

4.2 Application site

Skin wash: 10 hours after application.

The removed test substance, attached to the cotton swabs, was extracted with 75 mL methanol. The skin wash was stored frozen until analysis.

'O'-ring and cover: after sacrifice at 10 h, 24 h, 72 h, and 120 h after application, the 'O'-ring and used cover tapes were carefully removed from the application site, combined to one specimen, and placed into a flask. Thereafter the specimens were extracted with 50 mL methanol at ambient temperature.

Charcoal papers: 0-10 h and 10-end. Charcoal papers were carefully detached from the cover tapes. Samples were extracted with 50 mL ethyl acetate at ambient temperature.

Tape strips: after sacrifice, at 10 h, 24 h, 72 h, and 120 h after application, the adhesive tapes used for skin stripping were placed into flasks containing 10 - 20 mL tissue solubilizer Solvable.

4.3 Terminal specimens

At the defined time points, i.e. 10 h, 24 h, 72 h, and 120 h after start of exposure, the animals were sacrificed by exsanguination after anesthesia with carbon dioxide in a desiccator. The following specimens were collected:

Skin, treated area: after sacrifice, the skin treated area after skin stripping was carefully removed from the animal and retained for analysis. After collection, the specimens were placed into flasks containing 30 mL tissue solubilizer

Solvable.

Skin, untreated area: a small piece (about 2-3 g) of non-treated skin was excised from the hair clipped area some distance from the application site. After collection the specimens were placed into flasks containing 20 mL tissue solubilizer Solvable.

Blood/plasma: Terminal blood was collected after anesthesia by exsanguination and sampled into heparinized tubes. Aliquots of the blood were separated for radiometry and the remaining blood was centrifuged to prepare plasma. The specimens were stored frozen.

GI tract: the gastrointestinal tract including content was removed from the carcass and retained for analysis. The specimens were stored frozen.

Carcass: after removal of the gastrointestinal tract the remaining carcass was retained for analysis and kept frozen.

Cage wash: at the end of the collection period the cages were rinsed thoroughly with water/ethanol 1/1 (v/v). The specimens were kept at ambient temperature until analysis.

5. Measurement of radioactivity

Liquid specimens (urine, cage wash, skin wash, and 'O'-ring, cover and charcoal paper extract) were added directly into appropriate amounts of scintillation mixture prior to measurement by liquid scintillation counting (LSC). Whole blood was initially solubilised; isopropanol and H_2O_2 were added, and LSC was performed.

Feces, gastro-intestinal tract (including contents) and carcass were homogenized and dissolved with tissue solubilizer prior to LSC.

Tape strips, treated and untreated skin were dissolved with tissue solubilizer prior to LSC.

Thin layer chromatography (TLC) was used for the determination of the stability of ^{14}C MCW-2 in the administration vehicle and during exposure.

6. Data

Mean values and standard deviations were calculated.

The calculated extent of systemic absorption, excretion, total recovery, dislodged dose, and tissue residues were based on duplicate or triplicate measurements. All radioactivity counting of specimens were corrected for background by subtraction to give net "dpm" per specimen.

$$\%\ (\%\ \text{of dose}) = \frac{RA \times T \times 100}{AL \times D}$$

$$\text{ppm}\ (\mu g\ \text{MCW-2 equivalents/g}) = \frac{RA}{AL \times 60 \times SA}$$

$$\text{Mass balance } (\mu g \text{ MCW-2 equivalents/cm}^2) = \frac{\text{Dose } (\mu g/cm^2) \times \% \text{ of Dose}}{100}$$

$$\text{Systemic absorption (\% of dose)} = EX_U + EX_F + Re_T + CW$$

$$\text{Excretion (\% of dose)} = EX_U + EX_F + CW$$

$$\text{Dislodged dose (\% of dose)} = AS_{SW} + AS_O + AS_{CP}$$

$$\text{Dose remaining at the application site (\% of dose)} = AS_{TS} + AS_{SR}$$

$$\text{Total recovery (\% of dose)} = Ex_U + EX_F + Re_T + CW + AS_{SW} + AS_O + AS_{CP} + AS_{TS} + AS_{SR}$$

The limits of determination (LOQ) for tissue residues were calculated as follows:

$$LOQ \text{ (ppm)} = \frac{N_{BG}^{0.5} \times 14 \times 1 \times 10^3}{t_{BG} \times E \times AL \times SA \times 60{,}000} = \frac{N_{BG}^{0.5} \times 0.235}{t_{BG} \times E \times AL \times SA}$$

The penetration rate was calculated for the time of exposure (t = 10 hours)

$$\text{Penetration rate } (\mu g \times cm^{-2} \times h^{-1}) = \frac{\text{Applied dose} \times \%\text{absorption}_{0\text{-}10h}/100}{t_{10h}}$$

Applied dose [µg/cm^2]; % absorption: systemic absorption [% of dose] as determined for the exposure time of 0-10 hours; t: exposure time [h]

Abbreviations

EX_U = Excretion urine (% of dose)

EX_F = Excretion feces (% of dose)

Re_T = Residues in whole blood, skin untreated, gastro-intestinal tract, and carcass (% of dose)

CW = Radioactivity in cage wash (% of dose)

AS_{SW} = Radioactivity in application site skin wash (% of dose)

AS_O = Radioactivity in application site ‘O’-ring and cover (% of dose)

AS_{CP} = Radioactivity in application site charcoal papers (10 h and end) (% of dose)

AS_{TS} = Radioactivity in application site tape strips (% of dose)

AS_{SR} = Radioactivity in application site remaining skin residue (% of dose)

T = Total weight (g) or volume (mL) of specimen

AL = Weight (g) or volume (mL) of an aliquot (portion of specimen assayed)

D = Administered dose to the animal (dpm)

% = Percent of administered dose

RA = Radioactivity in aliquots (dpm – background value) (dpm)

ppm = Concentration of radioactivity in specimen (µg MCW-2 equivalents/g)

SA = Specific activity (kBq/mg)

t_{BG} = Counting time for background specimens (min)

NBG = Counts of background specimens for the counting time of t_{BG} (counts)

LOQ = Limit of determination (ppm)

E = Counting efficiency (cpm/dpm)

II RESULTS AND DISCUSSION

With the exception of a single animal, animals did not gain or lose weight during the study. Weight loss in the single animal was attributed to the discomfort during the experiment, e.g. anesthesia and the glued 'O'-ring on the dorsal area.

The observation of the application site prior to the dermal application, after removal of the applied test item, and at the end of the experiment revealed no visible damage of the skin. Rat number 30 chewed the 'O'-ring between the 48-72 hour collection period; pieces of the 'O'-ring fell into the feces collection pot causing a contamination of the sample. The mean actually administered doses were 0.098 mg/cm^2 and 4.815 mg/cm^2, respectively, for the low and high dose levels. The radiochemical purity of the dosing formulations was determined to be 97.8% and 98.0%, for the low and high dose, respectively.

Results of the study are summarized in Table 1.

Table 1: Summary of *in vivo* dermal absorption (% of dose)

Group	1				2			
Dose level (µg/cm^2)	98.2				4815			
Sacrifice time point (h)	10	24	72	120	10	24	72	120
Urine	42.13± 1.82	43.98± 2.13	42.72± 7.72	46.58± 7.76	7.24± 2.50	13.32± 1.47	16.34± 1.39	15.73± 1.81
Feces	0.08± 0.10	3.38± 0.67	3.53± 0.83	4.40± 1.41	0.19± 0.23	1.05± 0.24	1.82± 0.46	1.86± 0.27
Cage wash	0.62± 0.19	0.43± 0.21	0.47± 0.24	0.31± 0.17	0.26± 0.11	0.15± 0.07	0.20± 0.06	1.31± 1.39
Total excretion	**42.83**± 1.93	**47.79**± 2.31	**46.73**± 8.46	**51.29**± 8.91	**7.69**± 2.74	**14.52**± 1.70	**18.37**± 1.84	**18.90**± 2.56
Residues								
Whole blood	0.55± 0.10	0.37± 0.05	0.34± 0.09	0.37± 0.12	0.15± 0.04	0.09± 0.04	0.12± 0.03	0.12± 0.02
Skin,non-treated area	0.09± 0.02	0.04± <0.01	0.03± <0.01	0.02± 0.01	0.07± 0.08	0.08± 0.16	<0.01± <0.01	<0.01± <0.01
Gastrointestinal tract	5.98± 0.69	0.40± 0.08	0.08± <0.01	0.09± 0.03	1.66± 0.28	0.29± 0.15	0.07± 0.04	0.06± 0.05
Remaining carcass	6.57± 0.86	2.50± 0.29	1.70± 0.37	1.72± 0.41	6.54± 4.78	4.67± 7.73	0.62± 0.10	0.48± 0.11
Subtotal	*13.19*± 0.31	*3.30*± 0.31	*2.15*± 0.46	*2.20*± 0.55	*8.42*± 4.78	*5.13*± 8.03	*0.82*± 0.14	*0.66*± 0.17
Systemic absorption	**56.02**± 3.42	**51.09**± 2.39	**48.88**± 8.92	**53.50**± 9.41	**16.11**± 5.98	**19.65**± 7.98	**19.18**± 1.96	**19.57**± 2.71
Skin stripping I-II	0.32± 0.19	0.21± 0.04	0.22± 0.16	0.16± 0.13	0.42± 0.08	0.17± 0.05	0.08± 0.06	0.04± 0.02
Skin stripping III-VII	7.00± 1.58	6.72± 1.79	3.82± 1.55	3.79± 1.24	2.54± 1.11	0.82± 0.48	0.88± 0.61	0.29± 0.23
Remaining treated skin	0.70± 0.03	0.15± 0.02	0.08± 0.01	0.05±< 0.01	0.50± 0.17	0.17± 0.23	0.04± 0.02	0.02± <0.01
Application site	**8.01**± 1.62	**7.07**± 1.81	**4.13**± 1.49	**3.99**± 1.13	**3.47**± 1.27	**1.15**± 0.70	**1.00**± 0.63	**0.35**± 0.24
Skin wash	14.41± 2.99	19.06± 3.04	20.20± 6.95	18.45± 8.01	58.00± 12.77	60.79± 13.63	58.68± 4.72	61.70± 3.61
Cover and 'O'-ring	6.63± 1.00	5.28± 1.00	5.62± 1.68	5.64± 1.44	19.03± 5.66	14.70± 2.91	15.47± 2.55	12.20± 7.23

Charcoal paper (10h)	13.03± 1.08	15.30± 2.41	16.61±1.10	15.25±2.05	0.72±0.07	0.80±0.08	3.31±5.16	0.96±0.30
Charcoal paper (end)	NA	1.03±0.11	2.84±2.35	1.79±0.60	NA	0.29±0.14	0.68±0.17	0.75±0.13
Dislodged dose	**34.06**± 4.96	**40.67**± 1.26	**45.27**± 9.29	**41.13**± 10.43	**77.76**± 8.29	**76.57**± 10.73	**78.14**± 3.04	**75.61**± 5.81
Total recovery	**98.09**± 1.56	**98.84**± 0.30	**98.27**± 2.23	**98.62**± 0.95	**97.34**± 1.22	**97.37**± 2.50	**98.32**± 0.82	**95.52**± 4.08
Potentially Absorbable Dose	**63.71± 4.03**	**57.96**± 1.37	**52.78**±8.93	**57.33**±1 0.27	**19.16**± 7.12	**20.64**±8 .67	**20.10**± 2.38	**19.88**±2 .60

Data were extracted from Tables 5-12, pp. 38-45 of MRID 48574949 (PMRA#2181332).
NA: Not applicable
Potentially Absorbable Dose = Systemic absorption + skin strips II-VII + remaining treated skin

After 10 hours of exposure, MCW-2 was systemically absorbed to a relatively large extent at the low dose (56.02% of the applied dose) and to a lower extent at the high dose (16.11% of the applied dose).

After 10 hours of exposure, 49-56% of the low dose applied to the skin was systemically absorbed. Most of the remaining applied dose (34-45%) could be dislodged from the application site either washed off after the exposure period (skin wash representing 14 to 20% of the applied dose) or recovered in the extracts of the covers, O-rings and charcoal papers (altogether accounting for 20-25% of the applied dose). Within 120 hours, there was no apparent increase in systemic absorption. However, radioactivity recovered at the application site, tape strips and remaining treated skin after skin stripping, decreased with time from 8% at 10 hours to 4% at 120 hours indicating that the residues in skin will be absorbed very slowly. Most of the radioactivity at the application site was recovered in skin stripping samples (in the *stratum corneum*), i.e. 7.32% at 10 hours, 6.93% at 24 hours, 4.04% at 72 hours and 3.95% at 120 hours. Only low amounts were found in lower skin levels, i.e. ≤ 0.70% of the dose. The first two tape strips were not included in the potentially absorbable dose according to the guidance for dermal absorption studies. The potentially absorbable dose for the low dose was approximately 19%-21% of the applied dose at all time points evaluated. After 10 hours of exposure, most of the high dose applied to the skin (58-62%) could be washed off. An additional 14-20% of the dose was recovered in the extracts of the covers, O-rings and charcoal paper. Thus in total 76-78% of the applied dose could be dislodged from the application site at the end of the exposure. Systemic absorption accounted for 16-20% of the dose. Within 24 hours, there was no apparent increase in systemic absorption. The low amount of radioactivity recovered at the application site, tape strips and remaining treated skin after skin stripping, decreased with time from 3.5% at 10 hours to 0.4% at 120 hours indicating that the residues in skin will be absorbed very slowly. Most of the radioactivity at the application site was recovered in skin stripping samples (in the *stratum corneum*), i.e. 2.96% at 10 hours, 0.99% at 24 hours, 0.96% at 72 hours and 0.33% at 120 hours. Only low amounts were found in lower skin levels, i.e. ≤ 0.50% of the dose. The potentially absorbable dose for the high dose ranged from 53% to 64% at the time points evaluated.

A higher proportion of the residue was captured in the O-ring (12.2-19.03%) in the high dose group compared to the lose dose group (5.28-6.63%) and the opposite was true for the proportion in the charcoal paper (low dose: 13.03-16.61%; high dose: 0.72-3.31%). The registrant provided clarification on the apparent inconsistency between the two dose groups and is summarized here. Due to the occlusive conditions, the amount on the charcoal paper is driven by the equilibrium formed between material at the application site and material in the vapor phase. If saturated, typically one would expect around the same absolute amount (µg) of active substance on both papers. The results of this study support this; 12.8 µg were found for the low dose and 34.7 µg were found for the high dose on the charcoal paper. Thus, the absolute difference between the two groups is much lower than the difference in concentrations used (a factor of 48). The small difference observed in absolute amounts may be related to a greater percentage of penetration observed for the low dose group resulting in less material available on the skin and thus not available to form an equilibrium with the air. In contrast, the majority of the high dose was still located at the application site.

The study author also calculated mass balance. Data are shown below in Table 2. The mass balance demonstrates that within 5 days a maximum of 52.534 µg/cm² and 943.3 µg/cm² were systemically absorbed at the low and high dose level, respectively.

Table 2. Mass balance (µg MCW-2 equivalents/cm^2)

Group	1				2			
Dose level (µg/cm^2)	98.2				4815.3			
Sacrifice time point (h)	10	24	72	120	10	24	72	120
Systemic absorption								
0-10 h	41.452	34.444	31.841	29.705	358.0	373.1	407.2	347.1
10-24 h	-	12.060	11.328	16.946	-	319.1	331.1	314.1
24-48 h	-	-	1.474	1.819	-	-	98.1	82.3
48-72 h	-	-	0.777	0.714	-	-	38.7	41.7
72-96 h	-	-	-	0.503	-	-	-	31.3
96-120 h	-	-	-	0.376	-	-	-	31.5
Subtotal	*41.452*	*46.505*	*45.420*	*50.063*	*358.0*	*692.2*	*875.1*	*848.1*
Cage wash	0.605	0.427	0.464	0.307	12.5	7.2	9.8	63.3
Residues*	12.952	3.242	2.112	2.164	405.5	246.9	39.3	32.0
Total	55.009	50.173	47.996	52.534	776.0	946.3	924.2	943.3
Application site	7.864	6.948	4.052	3.923	167.0	55.2	48.1	16.7
Dislodge dose	33.452	39.940	44.455	40.390	3744.2	3687.3	3762.7	3640.7
Sum	**96.325**	**97.061**	**96.503**	**96.846**	**4687.2**	**4688.8**	**4735.1**	**4600.8**

Data extracted from Table on p. 30 of MRID 48574949 (#2181332)
* comprised of whole blood, non-treated skin, gastrointestinal tract and remaining carcass

The systemically absorbed test item was rapidly excreted mainly via urine. After 5 days, 46.58% of the low dose and 15.73% of the high dose were excreted with the urine whereas 4.40% of the low dose and 1.86% of the high dose were excreted with the feces. These values were somewhat lower (22-24%) than those determined from recovered radioactivity. In the cage wash, additional 0.31% and 1.31% of the applied low and high dose could be recovered, respectively. A portion of the absorbed test item, i.e. 2.20% of the low dose and 0.66% of the high dose, was still remaining in the animals 5 days after start of exposure, i.e. carcass, whole blood, gastrointestinal tract, and non-treated skin.

The skin wash samples were pooled according to dose levels and were analyzed by TLC. For both dose levels, the test item remained essentially unchanged on the application site during the exposure period. All analyzed skin wash pools revealed more than 90% unchanged MCW-2.

III. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency

Secondary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada Health Effects Division and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments:

Reliability Rating: This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for an *in vivo* dermal absorption study (OPPTS 870.7600; OECD 427) in the rat.

C. Conclusions: The maximum potentially absorbable dose (systemic absorption + skin strips III-VII + remaining treated skin; average of all time points) for the low and high dose groups is 64 (10 h) and 21 (120 h) percent of the applied dose, respectively. These results may be used for risk assessment purposes.

It is noted that due to volatility of the test material, a significant fraction of the applied dose (12 to 19%, generally decreasing with increased post-exposure duration) was recovered in the cover + O-ring for the high dose group and therefore this fraction may not have been fully available for penetration. However, the covered skin represents a "worst-case" scenario for dermal absorption since more of the fraction would be retained than for an uncovered test site as would occur with human exposure scenarios.

PMRA and OCS believe that Tape strips I and II should be included as Potentially Absorbable Dose based on the OECD (2011) Guidance Notes on Dermal Absorption because the study author's used cyanoacrylate adhesive. Furthermore, PMRA also believes the first two strips can remove an inconsistent amount of the stratum corneum resulting in unreliable measurements. Therefore, PMRA calculated potentially absorbable doses as 64.03%, 58.17%, 53.00%, and 57.49% for 10, 24, 72, and 120 hour time points, respectively, for the low-dose and 19.59%, 20.80%, 20.20%, and 19.92% for 10, 24, 72, and 120 hour time points, respectively, for the high-dose.

D. Deficiencies: No major deficiencies were identified.

Tier II Summary (PMRA #2181189, MRID 48574779) revised by the regulatory authorities, April 2013

Study Type Immunotoxicity

IIA 5.10.1 - MRID 48574779, PMRA 2181189

Report	Struve P.S. (2011) Immunotoxicity Evaluation of MCW-2 in a 28 Day Dietary Study in CD-1 Female Mice: Evaluation of Anti-Sheep Red Blood Cell (SRBC) Response Burleson Research Technologies, Inc., Morrisville, NC, USA; unpublished Report BRT 20100420, dated 22 February 2011 and amended on 11 April 2011. Sponsor reference No. R-23383 Dates of experimental work: 13 July to 25 August 2010
Sponsor	Makhteshim Agan of North America Inc., Raleigh, NC, USA
Guidelines	OPPTS 870.7800
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In a study evaluating potential immunotoxicity (MRID 48574779, PMRA 2181189), groups of 10 female CD-1 mice received fluensulfone (MCW-2 technical, 96.7%, Batch No. 36372130-291-PF1) in their diet at concentration of 0, 100, 500 or 2500/1500 ppm (equivalent to 0, 17, 86 and 204 mg/kg bw/day, respectively). After one week of treatment, one mouse in the high dose group died and signs of overt toxicity (decreases in body weight, food and water consumption, clinical signs in a few animals) were noted in the other animals of this group. Following 3 days on untreated diet, the dietary concentration for the high dose group was reduced to 1500 ppm and treatment continued for an additional 21 days. The treatment period for all the groups was extended to 32 days in order to complete the planned 28-day treatment period for all of the treated and negative control animals on study. Cyclophosphamide was used as positive control and was administered i.p. at 25 mg/kg bw/day during days 27-31 to a group of 10 female mice.

On Day 27 a single intravenous dose of 0.2 mL of 1 x 10^8 sheep red blood cells (SRBC) in phosphate buffered saline was administered to all surviving animals. Five days after immunization (Day 32), all mice were weighed, euthanized, serum samples were collected from each mouse for anti-SRBC IgM titers, and the spleen and thymus were weighed.

Apart from the effects observed when the highest dose animals were receiving 2500 ppm fluensulfone in their diet, treatment did not affect body weight or food or water consumption in mice at 100 and 500 ppm, and these parameters returned to, or were similar to, vehicle control values in highest dose mice when treated at 1500 ppm.

No effects on absolute or relative spleen or thymus weights were noted in the fluensulfone treated animals. The mean anti-SRBC IgM values for the 100, 500, and 2500/1500 ppm groups were approximately 62 to 70% of control, but were not statistically significantly different from control and no dose-relationship was evident. A high inter-individual variability was noted in all the

treatment groups as well as in the control group. Evaluation of individual animal data of this study did not show any trend or distribution that would demonstrate significant suppression of anti-SRBC IgM response. Absolute and relative spleen and thymus weights were statistically significantly reduced to approximately 75% of vehicle control values for the cyclophosphamide treated mice. Anti-SRBC IgM in positive control animals was also statistically significantly reduced to 11% of the vehicle control mean, thus confirming the sensitivity of the animal model.

The NOAEL was set at 500 ppm (86 mg/kg bw/day), based on clinical signs and body weight effects noted at the LOAEL of 2500/1500 ppm (204 mg/kg bw/day).

This study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in mice.

I. MATERIAL AND METHODS

1. Test Material	MCW-2 Technical Grade (fluensulfone)
Description:	Yellow solid
Lot/Batch:	36372130-291-PF1
Purity:	96.7%
CAS#:	Not available
Stability:	Stable for the duration of the study
2. Vehicle	PMI® LabDiet® (5002 Certified Rodent Diet)
3. Positive control	Cyclophosphamide monohydrate (Sigma Aldrich)
4. Test Animals	
Species	Mouse
Strain	CD-1
Sex	Female
Age	7 weeks
Weight	22.1-26.9 g (mean 24.2 g)
Source	Charles River, Raleigh, NC, USA
Acclimation period	7 days
Diet	PMI® LabDiet® (5002 Certified Rodent Diet) supplied by PMI Nutrition International. Control and treated diets were formulated by Research Diets, Inc. and offered *ad libitum*
Water	Tap water *ad libitum*
Housing	Group-housed (maximum of 5 mice per cage) at receipt and during acclimation Individually in ventilated plastic cages containing bedding material (bed-o'cobs®) during the study

5. Environmental conditions

Temperature	19.3 to 22.2°C
Humidity	34 to 87%
Air change	Not indicated
Photoperiod	Approximately 12-hour day and 12-hour night cycles

B. STUDY DESIGN

1. In-life dates 6 July (animal delivery) to 13 August 2010

2. Animal Assignment and treatment

Ten (10) female mice were randomly assigned to each treatment or control group using JMP® from SAS Institute, Cary, NC, USA.

Diet admixtures were provided *ad libitum* to the mice for 32 consecutive days at 0 (control), 100, and 500 ppm.

The 2500 ppm admixture was provided to mice for the first 7 days of dosing but the unscheduled death of one mouse on Day 8 and a decrease in Day 8 body weights for all other mice in this group necessitated a reduction in fluensulfone concentration. As such, the 2500 ppm treated diet was removed from cages on Day 8, and the surviving high dose animals were provided with control diet (0 ppm) for 3 days. Thereafter a newly prepared diet at 1500 ppm was then provided to these mice for Days 11-32. This resulted in a total duration of administration of treated diet to these animals of 28 days (7 days at 2500 ppm, 3 days at 0 ppm, and 21 days at 1500 ppm).

Animals from the positive control group received cyclophosphamide reconstituted with sterile water for injection once daily for 5 consecutive days (Days 27 to 31) by intraperitoneal injection at a dose of 25 mg/kg bw/day.

3. Dose Selection Rationale

The doses for this study were selected based on the results of a 28-day and 90-day dietary studies in mice, in which liver pathology was induced at a dietary dose level of 2000 ppm after 28 days and decreased body weight gain was observed at a dietary dose level of 1500 ppm after 90-days. The histologic changes noted in the liver at 2000 ppm in the 28-day dietary study were such that it was judged that a dose level of 2500 ppm would not exceed the Maximum Tolerated Dose (MTD).

4. Dose Preparation and Analysis

Preparation and dispensation of the control diet and test substance/dietary admixtures were performed by Research Diets, Inc.

Each initial concentration of diet admixture was prepared by weighing the appropriate quantity of fluensulfone, briefly melting the fluensulfone in a water bath, dissolving the melted fluensulfone in 12 mL ethanol per kilogram of diet (ethanol, Sigma-Aldrich) and then blending as a premix into 5002 Certified Rodent Diet. After blending, each premix was added to the appropriate quantity of 5002 Diet, tap water (150 mL) was added to facilitate pelleting, and the final mixture was blended. For the control diet, the same volume of ethanol was added to untreated diet premix. Each blended final mix diet was pelleted and then allowed to dry at

room temperature for at least 48 hours before shipment. Samples of the pelleted final product were submitted to Eurofins Product Safety Laboratories (EPSL) for homogeneity testing, and fluensulfone concentration determination. Stability was demonstrated in samples not used for treatment, prepared previously without the use of ethanol.

Fluensulfone concentration was below the Limit of Quantitation (LOQ, 5.6 μg/g) in the analyzed control diet that was formulated once for the entire study and administered to the vehicle control and positive control groups. Each of the fluensulfone diet concentrations administered to the study animals (production batches 2 and 3) had fluensulfone diet admixture concentration mean values of ±6% of target with ±1% RSD for samples collected during the middle of the pelleting process. Samples collected from both fluensulfone diet admixture batches administered to the study animals (production batches 2 and 3) had mean homogeneity values of ±11% of target with ±1% RSD for each fluensulfone test concentration. These samples were collected at the beginning, middle, and end of the pelleting process for each batch.

The diets at 100 ppm and 2500 ppm prepared as production batch 1 (not used for dosing) were assayed for six week room temperature stability at Time 0 and then subsequently at Weeks 2, 4, and 6. The values for the 2500 ppm samples were ±3% of Time 0 for the Weeks 2 to 6 sampling periods. The Time 0 measured value for the 100 ppm was 61 ppm, or approximately 39% less than target, whereas the values for the Weeks 2 to 6 sampling periods were up to 54% greater than Time 0. The 100 ppm low Time 0 value could not be explained by formulation or analytical error. This finding was not considered significant for the stability evaluation of fluensulfone because 1) samples from 100 ppm (production batches 2 and 3 that were administered to the study animals) had mean values ±6% of target, 2) stability was demonstrated for the 2500 ppm concentration, and 3) immunotoxicity was not demonstrated for the highest tested concentration in this study.

4. Statistics

A type I error rate of 5% was used for statistical significance. For ANOVA testing, two general linear models were fit: a vehicle versus positive control (cyclophosphamide) model, and a vehicle versus treated groups model. A general linear model for treatment was tested. A Dunnett's test was performed on the least square means to test for treatment differences with control.

Parameters evaluated were: food and water consumption, weekly and terminal body weight, thymus weight, spleen weight, and anti-SRBC IgM.

C. METHODS

1. Observations

The mice were monitored daily by cageside observation for the appearance of local or systemic toxicity. A detailed clinical examination was performed on each mouse prior to starting treatment on Day 1 and thereafter once a week.

2. Bodyweight

All mice assigned to the study were weighed at randomization, on Day 1 (prior to administration of fluensulfone diet admixtures), and then once a week thereafter.

3. Food and water consumption

All study mice were monitored at least once each week for the quantity of food consumed. Mice were monitored at least once each week for the quantity of water consumed, except for mice in the high dose group for which water consumption was not assessed during week 2 due to concerns of excessive water bottle spillage during the multiple changes in diets that occurred only during this week and only for this treatment group.

4. Immunization with SRBC

Pooled sheep red blood cells in Alsever's (Colorado Serum Company) were washed 3 times in PBS and resuspended to a final concentration of 1×10^8 SRBC/mL. Each mouse was immunized with 0.2 mL of this preparation on Day 27.

5. Sacrifice and pathology

Terminal body weights were recorded on Day 32 and the mice were then euthanized with CO_2. Blood was collected in tubes without anticoagulant and spleen and thymus weights were recorded. The carcass, spleen, and thymus were discarded without further evaluation. The blood was allowed to clot at room temperature for approximately 30 minutes. The blood tubes were centrifuged at 2000g for 10 minutes, and serum collected and stored at -20°C until evaluated for anti-SRBC IgM.

Serum samples were evaluated using a commercial ELISA kit (Life Diagnostics). Diluted test samples and standards were added to microwells and incubated for 45 minutes. The wells were washed and HRP-conjugated anti-mouse IgM added to the wells. The microplate was incubated at room temperature for 45 minutes, the wells washed, and the substrate solution added. Color development was stopped after 20 minutes by addition of the stop solution. The optical density was determined spectrophotometrically at 450 nm. All samples and standards were run in duplicate and data analysis was performed using Molecular Devices Softmax Pro software (version 2.2.1).

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs

On Day 8, all surviving mice receiving 2500 ppm had decreased body weights (relative to Day 1 pre-dose body weights) and light colored feces. Two animals had ruffled fur, and one of these also had hunched posture. Thereafter, there were no other clinical signs or abnormalities for any animal on study.

2. Mortality

One mouse at 2500 ppm was found dead on Day 8.

B. BODYWEIGHT AND BODYWEIGHT GAINS

Mean body weight of mice treated at 500 ppm were statistically significantly decreased, compared to controls, at Days 15 and 22 (Table 1). As the other weekly body weights were comparable to control, no decrease in body weight was noted in mice administered the 1500 ppm test diet, and mean body weights at Days 15 and 22 for the 500 ppm dose group were 94-96% of control values, these significant decreases were not considered to be related to treatment.

After 1 week of exposure at 2500 ppm, the mean body weight (Day 8) in these animals had decreased by approximately 15% from the pre-dose value and all of the surviving mice from this group had lost weight. The Day 8 value was also significantly less than control (approximately 80%). Subsequently, weekly mean body weights recovered and were comparable to vehicle control for the remainder of the study after the dose was lowered to 1500 ppm.

There were no fluensulfone-related effects on terminal body weights.

Cyclophosphamide was not administered until Day 27. There were no cyclophosphamide-related changes in weekly or terminal body weights.

Table 1: Mean ± S.E. weekly and terminal body weights (g)

Day	0 ppm	100 ppm	500 ppm	2500/1500 ppm	Positive control
-7	21.9 ± 0.41	22.6 ± 0.40	22.0 ± 0.37	22.2 ± 0.21	22.2 ± 0.40
1	24.4 ± 0.39	24.4 ± 0.42	24.1 ± 0.46	24.2 ± 0.46	24.2 ± 0.49
8	25.7 ± 0.57	25.6 ± 0.42	24.7 ± 0.40	20.5 ± 0.68*** (↓20%)	25.3 ± 0.56
15	26.1 ± 0.60	25.7 ± 0.44	24.6 ± 0.38* (↓6%)	25.4 ± 0.35	25.8 ± 0.46
22	27.0 ± 0.66	27.0 ± 0.38	25.7 ± 0.50* (↓5%)	26.4 ± 0.47	27.0 ± 0.63
29	27.3 ± 0.57	27.9 ± 0.41	26.6 ± 0.51	27.2 ± 0.63	27.8 ± 0.44
Terminal	27.6 ± 0.57	27.9 ± 0.44	26.7 ± 0.51	27.2 ± 0.65	26.9 ± 0.31

* statistically significantly different from the vehicle control ($p<0.05$); ** statistically significantly different from the vehicle control ($p<0.01$); Data obtained from pages 33-34 of the study report.
Values in parentheses represent the percent change from the vehicle control mean (calculated by the reviewer).

C. FOOD AND WATER CONSUMPTION AND TEST SUBSTANCE INTAKES

At the end of Week 1, the mean water and food consumption per mouse at 2500 ppm were significantly decreased compared to the vehicle control (Table 2). These changes were consistent with the large decrease in the mean body weight noted at this dose level. The subsequent reduction in dietary concentrations to 0 ppm (control) for Days 8-11 and then to 1500 ppm for the remainder of the study resulted in a recovery of mean food consumed, albeit

the Week 2 food consumption value was still significantly lower than control. Although the Week 2 water consumption was not monitored due to possible water bottle spillage, the values for Weeks 3 and 4 were slightly greater than control but not statistically significantly different.

Mean food and water consumption values were comparable to the vehicle control for the cyclophosphamide treated mice and the 100 and 500 ppm treatment groups.

In the study report, the dose levels achieved were reported as 120, 600 and 1429 mg/kg bw/day for the 100, 500 and 2500/1000 ppm dose groups, respectively. However, the calculation equation used weekly food consumption values and thus determined a weekly test material intake. Therefore, the values for test material provided in the study report should read 120, 600 and 1429 mg/kg bw/week for the 100, 500 and 2500/1500 ppm dose groups respectively, and the daily intakes should be 17, 86 and 204 mg/kg bw/day, respectively.

Table 2: Mean ± S.E. weekly food and water consumption (gram per mouse)

Week	0 ppm	100 ppm	500 ppm	2500/1500 ppm	Positive control
Food consumption					
1	33.3 ± 0.83	33.8 ± 1.12	30.6 ± 0.99	9.6 ± 1.28*** (↓71%)	33.1 ± 1.18
2	30.5 ± 2.36	30.7 ± 2.35	28.3 ± 1.42	25.2 ± 0.90** (↓17%)	28.1 ± 1.04
3	30.4 ± 0.77	31.9 ± 0.80	30.8 ± 1.57	26.8 ± 1.11	30.9 ± 0.66
4	27.2 ± 0.64	29.1 ± 1.07	30.8 ± 2.53	25.1 ± 1.50	29.4 ± 0.60
Achieved test substance intake (mg/kg bw/day)					
1-4	0	17	86	204	Not applicable
Water consumption					
1	34.6 ± 1.30	33.0 ± 1.02	34.5 ± 1.48	25.3 ± 2.71 *** (↓27%)	35.1 ± 1.88
2	33.0 ± 1.11	33.2 ± 1.15	34.7 ± 1.47	Not calculated	34.5 ± 1.40
3	32.6 ± 1.24	35.0 ± 1.01	35.2 ± 1.26	36.0 ± 2.15	33.7 ± 1.52
4	30.8 ± 1.15	32.8 ± 1.08	34.2 ± 1.74	33.1 ± 2.04	32.5 ± 1.41

* Statistically significantly different from vehicle control ($p<0.05$). ** Statistically significantly different from vehicle control ($p<0.01$). *** Statistically significantly different from vehicle control ($p<0.001$). Data obtained from pages 25, 31, and 32 of the study report. Values in parentheses represent the percent change from the vehicle control mean (calculated by the reviewer).

D. PATHOLOGY AND Anti-SRBC IgM

1. Organ Weights

There were no treatment-related changes in absolute or relative (per 100 g body weight) spleen and thymus weights (Table 3).

For the cyclophosphamide treated animals, absolute and relative spleen and thymus weights were statistically significantly lower than the vehicle control.

Table 3: Mean ± S.E. absolute (g) and relative (g/100 g) organ weights

Organ	0 ppm	100 ppm	500 ppm	2500/1500 ppm	Positive control
Spleen - abs.	0.107 ± 0.004	0.111 ± 0.005	0.103 ± 0.003	0.104 ± 0.009	0.077 ± 0.004*** (↓28%)
- rel.	0.391 ± 0.016	0.397 ± 0.019	0.388 ± 0.014	0.381 ± 0.032	0.285 ± 0.015*** (↓27%)
Thymus - abs.	0.067 ± 0.002	0.064 ± 0.005	0.062 ± 0.003	0.074 ± 0.005	0.050 ± 0.003*** (↓25%)
- rel.	0.242 ± 0.005	0.231 ± 0.016	0.233 ± 0.011	0.272 ± 0.016	0.187 ± 0.011*** (↓23%)

*** Statistically significantly different from the vehicle control mean ($p<0.001$). Data obtained from pages 35-38 of the study report. Values in parentheses represent the percent change from the vehicle control mean (calculated by the reviewer).

2. Anti-SRBC IgM

Anti-SRBC IgM was measured 5 days post-immunization with SRBC. There was no clear evidence of an effect of treatment with fluensulfone on anti-SRBC IgM response (Table 4). The mean values for the 100, 500, and 2500/1500 ppm groups were approximately 62 to 70% of control, but not statistically different and a dose-relationship was not established. A high inter-individual variability was noted in all the treatment groups as well as in the control group. Evaluation of individual animal data of this study did not show any trend or distribution that would demonstrate significant suppression of anti-SRBC IgM response.

Anti-SRBC IgM was statistically significantly reduced in animals treated with cyclophosphamide (11% of control).

Table 4: Anti-SRBC IgM (U/mL) (mean ± S.E. of duplicate samples)

0 ppm	100 ppm	500 ppm	2500/1500 ppm	Positive control
1526.9 ± 371.64	1008.2 ± 214.27 (↓34%)	1036.9 ± 169.65 (↓32%)	917.6 ± 154.52 (↓40%)	167.4 ± 57.55** (↓89%)

** Statistically significantly different from the vehicle control mean ($p<0.01$). Data obtained from page 39 of the study report. Values in parentheses represent the percent change from the vehicle control mean (calculated by the reviewer).

III. CONCLUSIONS

Treatment at 2500 ppm was higher than the MTD in this study, as one animal died and other animals in this group showed reduced body weight, food and water consumption and some clinical signs after exposure for one week. Body weight, food and water consumption as well as terminal body weight for these animals showed recovery following administration of untreated diet for 3 days and exposure at 1500 ppm for the remainder of the study.

Treatment had no effects on absolute and relative spleen and thymus weights, and no treatment-related differences from controls in the anti-SRBC IgM titre were observed.

In the positive control animals, treated i.p. with cyclophosphamide at 25 mg/kg bw/day on days 27-31, statistically significant decreases in absolute and relative spleen and thymus weights, as well as in anti-SRBC IgM titre were recorded, thus demonstrating that appropriate methods were used and the animal system was adequately sensitive.

The NOAEL was 2500/1500 ppm, equivalent to a mean achieved intake of 204 mg/kg bw/day.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments/Conclusions: Overall, the reviewer agrees with the conclusions reached by the study investigators. There was no indication in this study that exposure to fluensulfone in the diet at the concentrations tested elicited changes in anti-SRBC IgM levels.

In the Tier II summary, the achieved test material intake was reported at 17, 86 and 204 mg/kg bw/day for the 100, 500 and 2500/1500 ppm dose groups, respectively. In the study report, the dose levels achieved were reported as 120, 600 and 1429 mg/kg bw/day for the 100, 500 and 2500/1000 ppm dose groups, respectively. The equation used by the study investigators to calculate the test material intake was reported as follows:

> Average mg/kg/day fluensulfone intake = Group Mean Daily Food Consumption per Mouse x Nominal Dose Concentration (1 ppm = 1 mg fluensulfone / 1 kg diet) / Group Mean Body Weight

This equation gives a weekly test material intake as food consumption was recorded over weekly intervals. Therefore, the values for test material provided in the study report should read 120, 600 and 1429 mg/kg bw/week for the 100, 500 and 2500/1500 ppm dose groups respectively. The daily intakes of 17, 86 and 204 mg/kg bw/day reported in the Tier II summary are more accurate.

No enumeration of splenic or peripheral blood total B cells, total T cells, or T cell subpopulations was conducted, but this analysis was conducted in the 28-day dietary study in rats.

This immunotoxicity study is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in mice.

C. Deficiencies: Necropsy findings of animals that were found moribund and euthanized or died during the study should be reported but was not for the animal found dead on day 8. The daily intake of test material was incorrectly reported in the study report. No other deficiencies were identified.

Tier II Summary (PMRA #2181202, MRID 48574752,) revised by the regulatory authorities, April 2013

Study Type: Oral 28-day toxicity

IIA 5.3.1 – MRID 48574752, PMRA 2181202

Report:	Braun L. (2009) MCW-2 Tech. 28-Day Range-Finding Oral (Feeding) Toxicity Study in the Beagle Dog. Harlan Laboratories Ltd, Switzerland; unpublished Report B89087, dated 21 October 2009. Sponsor reference No. R-23349 Dates of experimental work: 19 May to 21 October 2009
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	OECD 409 (1998) and US EPA OPPTS 870.3150 Deviations: none
GLP/Compliance:	Yes. Signed and dated GLP. Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In a 4-week dietary study (MRID 48574752, PMRA 2181202), groups of 2 male and 2 female Beagle dogs received fluensulfone (MCW-2 tech., 96.67%, Lot No. 36372130-291-PF1) in their diet at 0, 50, 200 and 900 ppm (0, 1.9, 7.3, 31 mg/kg bw/day in males; 0, 2.0, 7.9, 30 mg/kg bw/day in females) for 29 days.

Special investigations included thyroid hormone levels in blood, alanine aminotransferase activity in liver tissue and metabolic enzyme activity in liver.

No mortality occurred and no clinical signs were noted during the study. Body weight stasis in males and body weight loss in females was noted at 900 ppm during the treatment period when compared to the pretreatment values or the control group. No treatment-related changes were recorded in ophthalmologic examination, hematological or urinalysis parameters.

Decreased alanine aminotransferase activity in the blood was observed in all treated groups and decreased protein and albumin levels and albumin/globulin ratios were noted in the females at 900 ppm. A tendency of an increase in thyroid stimulating hormone (TSH) values was observed in male and female dogs at 900 ppm when compared to the control values. Generally, no differences in triiodothyronine (T3) or thyroxine (T4) levels were observed in the treated groups when compared to the control values.

Increased absolute and relative liver and thyroid weights were recorded in the males at 900 ppm; however, there was no associated macroscopic or microscopic histopathology. A slight decrease in

absolute heart weight was observed in both sexes from the 900 ppm dose group, which was considered to be a secondary effect to the reduced growth in these animals.

Fluensulfone caused a slight inhibition of hepatic alanine aminotransferase (ALAT) activity in both sexes at 900 ppm. While some hepatic enzymes were elevated in treated animals, the overall results of liver enzyme determinations exclude a potent effect on liver enzymes by fluensulfone.

A NOAEL and LOAEL were not established for this study as it is a range-finding study. Adverse effects were noted at 900 ppm (31 and 30 mg/kg bw/day in male and female dogs, respectively), in the form of slight changes in body weight and organ weight (liver and thyroid) at 900 ppm. No adverse effects were noted at 200 ppm (7.3 and 7.9 mg/kg bw/day in male and female dogs, respectively).

This subchronic toxicity study in the dog is acceptable/non-guideline (reliable with restrictions) as it is a range-finding study.

I. MATERIAL AND METHODS

1. Test Material	MCW-2-tech. (fluensulfone)
Description:	Yellow solid
Lot/Batch:	36372130-291-PF1
Purity:	96.67% (GC-assay)
CAS#:	318290-98-1
Stability:	Stable for the duration of the study
2. Vehicle	Pelleted standard Kliba 3353 dog maintenance diet (Provimi Kliba AG, Switzerland)
3. Test Animals	
Species	Dog
Strain	Pure bred Beagle
Age	6.5 to 7.5 months
Weight	6.2 to 9.9 kg
Source	Harlan Laboratories Ltd., Switzerland
Acclimation period	10 to 20 days
Diet	350 ± 1 g per day and dog of pelleted standard Kliba 3353 dog maintenance diet (Provimi Kliba AG, Switzerland). The appropriate feed preparations were presented to the animals at the same time daily. Any remaining diet was withdrawn

approximately 4 hours after feeding.

Water Tap water *ad libitum*.

Housing Housing in pairs per sex and group, in pens with minimum of 2.0 square meters floor space per dog. Dogs were individually housed during feeding periods and at the discretion of the study director to facilitate recording of clinical signs or performance of special investigations.

4. Environmental conditions

Temperature: 20 ± 3 °C; pen floor temperature was maintained at approximately 21 ± 4 °C

Humidity: 30-70% (transient values above the range for humidity, with a maximum of 79%, occasionally occurred during room cleaning but were considered to have no impact on the study outcome)

Air changes: approx. 10-15 changes per hour

Photoperiod: 12-hour fluorescent light / 12-hour dark cycle. Music was played during the major part of the light period.

B. STUDY DESIGN

1. In-life dates 28 April to 24 June 2008

2. Animal Assignment and treatment

Shortly after arrival the dogs were temporarily allocated to groups by a computer generated random algorithm; the spare animals were allocated to group 5 and assigned numbers 17 and 18. Thereafter, pre-treatment investigations were carried out on all dogs. Shortly before the start of treatment, the data obtained were reviewed. The allocation was adjusted exchanging with the spare male dog due to low body weight. The rejected animals did not take further part in the study after commencement of treatment.

Following a pre-treatment period of one week, dogs received the treated (or control) diets daily for four hours, at approximately 24 hour intervals, for 29 consecutive days, at dose levels of 0, 50, 200 or 900 ppm. Dosing was delayed to allow sufficient time on the days with special investigations.

3. Diet Preparation and Analysis

Treated diets were prepared weekly and stored at room temperature in disposable paper bags by group.

Fluensulfone was warmed in the original container in a water bath up to maximum of approximately 40 °C until the test item was liquid. The liquid test item was then transferred

into a glass beaker (wrapped with aluminum foil) and mixed using a magnetic stirrer on a heating plate (maximum approximately 40 °C). The test item was divided into aliquots in brown glass containers for the weekly preparations. The required amounts of fluensulfone were weighed into tared glass beakers (wrapped with aluminum foil) and kept at maximum approximately 40 °C on a heating plate until use for feed preparation. The test substance was mixed with microgranulated feed, and pellets were prepared. Water (approximately 1:10 v/w) was added to aid pelleting. The pellets were dried with warm air for approximately 48 hours before storage. The pellets for the control group were prepared separately as described above but without test item.

Samples for determination of concentration, homogeneity and stability (14 days) of the dose formulations were taken from the feed preparation for the first week of the administration period. Additionally, samples for determination of concentration (by HPLC coupled to a UV/VIS detector and quantified with the area under the peak) were taken from the feed preparation for the last week of the administration period.

The test item content in all samples was found to be within 89.2% to 96.8% of the nominal content. In addition, the homogenous distribution of fluensulfone in feed was demonstrated as values for individual samples did not deviate by more than 4.2% from the corresponding mean. The test item was considered to be stable in the feed for at least 14 days when kept at room temperature (maximum deviation from the time-zero mean of 3.3%).

4. Statistics

No statistical methods were used due to the small number of animals per group.

C. METHODS

1. Observations

Dogs were inspected twice a day for morbidity and mortality from commencement of the pre-test period. General clinical examinations (in the pen) were made twice daily from commencement of the pre-test period.

Veterinary examinations, including pupil reflex, corneal reflex, and patellar reflex, cardiac and pulmonary auscultation, were performed once during the pre-test and once during week 4.

2. Bodyweight

Body weight was recorded at least once weekly from commencement of the pre-test period, weekly during treatment and before necropsy. All weights were recorded before feeding.

3. Food consumption

The feed intake of each individual dog was determined daily from commencement of the pre-test period. The daily ration was weighed before and after feeding.

4. Water consumption

Not determined.

5. Ophthalmoscopic examination

Observations of the cornea, lens, conjunctiva, sclera, iris and fundus were performed using an ophthalmoscope following the instillation of 0.5% tropicamide on all dogs once during the pre-test period and once during week 4.

6. Hematology and Clinical Chemistry

Blood was collected from the jugular vein of all animals following an overnight fast (dogs were allowed access to water *ad libitum*) once during the pre-test period and at week 4 for the analysis of hematology and clinical chemistry. The following parameters were determined at hematology:

Erythrocyte count, Hemoglobin concentration, Hematocrit, Mean corpuscular volume, Red cell volume distribution width, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration, Hemoglobin concentration distribution width, Platelet count, Reticulocyte count, Reticulocyte maturity index, Total leukocyte count, Differential leukocyte count, Thromboplastin time and Activated partial thromboplastin time

The following parameters were determined at clinical chemistry:

Glucose, Urea, Creatinine, Bilirubin (total), Cholesterol (total), Triglycerides, Phospholipids, Aspartate aminotransferase, Alanine aminotransferase, Glutamate dehydrogenase, Creatine kinase, Alkaline phosphatase, Gamma-glutamyl-transferase, Calcium, Phosphorus, Sodium, Potassium, Chloride, Protein (total), Albumin, Globulin, and Albumin/Globulin Ratio

In addition, blood samples were collected from all animals at the same time as the clinical laboratory investigations for T3, T4, and TSH analyses.

7. Urinalysis

Urine was collected from all animals into a specimen vial using a catheter once during the pre-test period and at week 4. The following parameters were determined:

Color, Appearance, Relative density, pH, Protein, Glucose, Ketones, Urobilinogen, Bilirubin, Erythrocytes, Leukocytes

8. Sacrifice and pathology

All animals were anesthetized by intravenous injection of sodium pentobarbital and sacrificed by exsanguination. All animals were necropsied and descriptions of all macroscopic abnormalities were recorded.

Samples of tissues and organs (Table 1) were collected from all animals at necropsy and fixed, unless otherwise indicated, in neutral phosphate-buffered 4% formaldehyde solution (10% formalin). Selected organs (Table 1) were weighed prior to fixation (paired organs were

weighed separately; organ to terminal body weight ratios and organ to brain weight ratios were determined). Bone marrow smears from the sternum from all animals were taken, stained and retained for possible further investigation.

A liver sample was taken at the scheduled necropsy after 4 weeks from all animals, minced into small pieces and frozen in liquid nitrogen. Liver subcellular fractions were prepared and the following parameters were determined: Protein content of subcellular fractions, Microsomal cytochrome P450 content (differential spectroscopy), CYP1A1 (microsomal 7-ethoxyresorufin O-dealkylation), CYP1A2 (microsomal 7-methoxyresorufin O-dealkylation), CYP2B1 (microsomal pentoxy-resorufin O-dealkylation), CYP2B11 (microsomal 7-benzyloxyresorufin O-dealkylation), CYP3A (microsomal testosterone 6β-hydroxylation), Cytosolic glutathione S-transferase (GST, glutathione conjugation of 1-chloro-2,4-dinitrobenzol), Microsomal uridine diphosphoglucuronosyl-transferase (UDPGT, glucuronidation of 3-methyl-2-nitrophenol), Microsomal epoxide hydrolase (hydrolysis of styrene oxide), and Cytosolic alanine aminotransferase (ALAT, transamination of ketoglutarate).

Table 1: Summary of tissues collected for pathology examination

Organ/tissue	Weighed	Collected	Examined
Adrenal gland	X	X	
Aorta		X	
Bone - sternum, femur including articular surface		X	
Bone marrow - femur		X	
Brain - including sections of medulla/pons, cerebral and cerebellar cortex	X	X	X
Epididymides (fixed in Bouin's solution)	X	X	
Esophagus		X	
Eyes with optic nerve (fixed in Davidson's solution)		X	X
Female mammary gland area, (male mammary gland area)		X	
Gallbladder		X	
Heart	X	X	X
Kidneys	X	X	X
Large intestine - cecum, colon, rectum		X	X
Liver	X	X	X
Lungs, instilled via trachea with formalin at approximately 30 cm H_2O pressure		X	X
Lymph nodes - retropharyngeal, mesenteric, tracheobronchial		X	
Ovaries	X	X	X
Pancreas		X	X

Pituitary gland		X	
Peyer's Patches		X	X
Prostate gland		X	
Salivary glands - mandibular, parotid, (sublingual)		X	
Sciatic nerve		X	
Skeletal muscle		X	
Skin - and subcutaneous tissue		X	
Small intestine - duodenum, jejunum, ileum		X	X
Spinal cord - cervical, midthoracic, lumbar segments		X	
Spleen	X	X	X
Stomach		X	X
Testes (fixed in Bouin's solution)	X	X	X
Thymus	X	X	
Thyroid gland with parathyroid gland	X	X	
Tongue		X	
Trachea		X	
Urinary bladder		X	
Uterus with (vagina), cervix	X	X	
Oviducts		X	
All gross lesions		X	X

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs

No treatment-related clinical signs were noted during the study.

2. Mortality

There were no mortalities during the study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Male dogs receiving 900 ppm did not gain weight (overall body weight gain of 0.1 kg at 900 ppm versus 0.8 kg in the control group; based on a difference of mean values) whereas the females in this group lost weight (0.3 kg based on a difference of mean values) over the treatment period when compared to the values on day 1 (Table 2). At the end of the treatment period, the mean body weight of males and females from the 900 ppm dose group were 93% and 76% of the mean control values, respectively. There were no effects on body weight in animals receiving the 50 or 200 ppm test diets.

Table 2: Mean (± S.D.) body weights (kg)

Day	0 ppm	50 ppm	200 ppm	900 ppm
Male dogs				
Pre-test day 1	8.5 ± 0.3	8.9 ± 0.7	9.2 ± 0.4	8.5 ± 0.7
1	9.0 ± 0.6	9.0 ± 0.5	9.4 ± 0.7	8.6 ± 0.6
8	9.2 ± 0.7	8.9 ± 0.5	9.5 ± 1.0	8.6 ± 0.9
15	9.0 ± 0.5	9.0 ± 0.6	9.6 ± 0.9	8.5 ± 0.6
22	9.1 ± 0.5	9.0 ± 0.3	9.7 ± 1.0	8.5 ± 0.7
29	9.4 ± 0.3	9.2 ± 0.4	10.0 ± 1.2	8.6 ± 0.9
30	9.3 ± 0.4	9.2 ± 0.6	10.0 ± 1.2	8.6 ± 0.9 (↓7%)
Female dogs				
Pre-test day 1	7.7 ± 0.9	7.0 ± 0.5	7.7 ± 1.0	6.6 ± 0.5
1	7.9 ± 0.7	7.2 ± 0.4	7.9 ± 1.0	6.7 ± 0.7
8	7.9 ± 0.4	7.4 ± 0.6	8.1 ± 0.8	6.5 ± 0.7
15	8.1 ± 0.8	7.3 ± 0.4	8.1 ± 0.8	6.5 ± 0.7
22	8.2 ± 0.7	7.7 ± 0.6	8.3 ± 0.7	6.4 ± 0.8
29	8.3 ± 0.8	7.7 ± 0.6	8.3 ± 0.7	6.4 ± 0.9
30	8.4 ± 0.7	7.7 ± 0.5	8.3 ± 0.7	6.4 ± 0.8 (↓24%)

Data obtained from pages 51-52 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

C. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

A slight reduction in mean food consumption was noted in males and females at 900 ppm during the treatment period, compared to the control group (Table 3). However, similar reductions in food consumption when compared to controls were noted during the pre-test period. With only two animals per group, it is difficult to interpret these findings. The mean achieved test item intakes are summarized in Table 4.

Table 3. Mean (overall mean of means) food consumption (g/animal/day)

Day	0 ppm	50 ppm	200 ppm	900 ppm
Male dogs				
Pre-test	349.9	344.9	317.4 (↓9%)	301.9 (↓14%)
Treatment	350.1	350.1	349.2	296.5 (↓15%)
Female dogs				
Pre-test	313.2	283.0	312.4	198.3 (↓37%)
Treatment	322.4	296.6	321.8	212.7 (↓34%)

Data obtained from pages 35-42 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

Table 4: Mean achieved test substance intakes (mg/kg bw/day)

50 ppm		200 ppm		900 ppm	
M	**F**	**M**	**F**	**M**	**F**
1.9	2.0	7.3	7.9	30.9	29.7
Combined: 2.0		Combined: 7.6		Combined: 30.3	

Data obtained from page 28 of the study report.

D. OPHTHALMOSCOPIC AND VETERINARY EXAMINATIONS

Ophthalmoscopic and veterinary examinations did not show any treatment-related effect.

E. BLOOD ANALYSIS

1. Hematological findings

No changes were recorded in hematological parameters that were considered to be related to the treatment. There was an apparent dose-related decrease in absolute reticulocyte counts in males (means of 97, 69, 59, and 43 G/l at the 0, 50, 200 and 900 ppm dose groups,

respectively). However, the values in the control animals (108 and 85 G/L) were relatively high based on historical control values (mean ± S.D. from 294 dogs from studies conducted between 2002 and 2007 of 58 ± 26 G/L). In addition, reticulocyte counts were increased in the 90-day and 1-year dietary studies in the dog conducted with fluensulfone. Therefore, the apparent reductions in reticulocyte counts in males are not considered to be related to treatment.

For other hematological parameters, the intergroup variations fell within historical control ranges at the performing laboratory, differences were present at pre-test, or the values did not show a relationship to dose.

2. Blood chemistry findings

Decreased alanine aminotransferase activity in the blood was recorded after 4 weeks in males and females in all treated groups (Table 5). The changes showed a dose relationship. Decreased protein and albumin values and albumin/globulin ratios were recorded after 4 weeks for female animals at 900 ppm when compared to concurrent controls and pre-test values.

For the remaining clinical chemistry parameters, the intergroup variations fell within historical control ranges at the performing laboratory, differences were present at pre-test, or the values did not show a relationship to dose.

Generally, no differences in T3 and T4 levels were observed in the treated groups when compared to the control values. A tendency toward higher TSH values in male and female dogs at 900 ppm was evident when compared to the control values.

Table 5: Selected clinical chemistry parameters (mean ± SD.)

Parameter	0 ppm	50 ppm	200 ppm	900 ppm
Male dogs				
ALAT (U/L) – *pre-test*	*44.0 ± 17.1*	*45.3 ± 12.7*	*31.9 ± 11.9*	*38.1 ± 7.8*
– week 4	44.4 ± 8.7	18.7 ± 2.2 (↓58%)	10.5 ± 4.0 (↓76%)	6.6 ± 4.2 (↓85%)
Protein (g/L) – *pre-test*	*58.62 ± 3.2*	*56.57 ± 0.9*	*52.48 ± 2.3*	*56.40 ± 6.7*
– week 4	58.11 ± 1.7	59.10 ± 0.06	54.46 ± 1.0	54.30 ± 2.5
Albumin (g/L) – *pre-test*	*28.36 ± 1.9*	*31.67 ± 1.1*	*27.77 ± 0.6*	*31.30 ± 0.3*
– week 4	30.12 ± 0.5	31.03 ± 1.3	28.53 ± 0.7	27.13 ± 1.7
A/G ratio – *pre-test*	*0.96 ± 0.23*	*1.27 ± 0.05*	*1.13 ± 0.05*	*1.29 ± 0.3*
– week 4	1.08 ± 0.03	1.11 ± 0.1	1.10 ± 0.1	1.02 ± 0.2
TSH (ng/mL) – *pre-test*	*0.07 ± 0.04*	*0.07 ± 0.01*	*0.11 ± 0.0*	*0.08 ± 0.05*
– week 4	0.05 ± 0.01	0.08 ± 0.01	0.16 ± 0.10	0.27 ± 0.13 (↑440%)
T3 (ng/dL) – *pre-test*	*88.2 ± 18.9*	*97.2 ± 3.2*	*118.4 ± 32.7*	*73.3 ± 10.4*
– week 4	75.9 ± 8.1	96.1 ± 2.5	108.3 ± 14.5	72.0 ± 14.9
T4 (µg/dL) – *pre-test*	*1.55 ± 0.07*	*1.63 ± 0.18*	*1.93 ± 0.74*	*1.38 ± 0.53*
– week 4	1.18 ± 0.11	1.55 ± 0.28	1.73 ± 0.25	1.38 ± 0.53
Female dogs				
ALAT (U/L) – *pre-test*	*27.5 ± 4.7*	*31.0 ± 3.2*	*34.6 ± 7.3*	*30.8 ± 4.6*
– week 4	33.2 ± 9.6	14.0 ± 7.4 (↓58%)	8.1 ± 1.1 (↓76%)	7.8 ± 0.3 (↓77%)
Protein (g/L) – *pre-test*	*53.92 ± 1.4*	*51.51 ± 0.7*	*53.21 ± 1.0*	*52.69 ± 3.1*
– week 4	54.27 ± 3.1	50.24 ± 0.1	52.13 ± 0.05	47.69 ± 1.7 (↓12%)
Albumin (g/L) – *pre-test*	*31.59 ± 1.6*	*29.39 ± 1.0*	*32.18 ± 0.3*	*30.69 ± 1.0*
– week 4	31.75 ± 3.6	28.83 ± 0.4	31.14 ± 0.3	23.38 ± 1.4 (↓26%)
A/G ratio – *pre-test*	*1.41 ± 0.1*	*1.33 ± 0.08*	*1.53 ± 0.04*	*1.42 ± 0.3*
– week 4	1.41 ± 0.2	1.35 ± 0.06	1.48 ± 0.04	0.97 ± 0.2 (↓31%)
TSH (ng/mL) – *pre-test*	*0.37 ± 0.49*	*0.12 ± 0.04*	*0.16 ± 0.12*	*0.24 ± 0.30*
– week 4	0.17 ± 0.21	0.11 ± 0.01	0.23 ± 0.22	0.70 ± 0.86 (↑312%)
T3 (ng/dL) – *pre-test*	*77.9 ± 3.5*	*99.6 ± 6.9*	*90.0 ± 15.6*	*86.2 ± 2.9*
– week 4	85.6 ± 6.1	102.0 ± 21.9	92.9 ± 4.0	77.0 ± 3.6
T4 (µg/dL) – *pre-test*	*1.20 ± 0.0*	*2.18 ± 0.39*	*1.60 ± 0.14*	*1.38 ± 0.39*
– week 4	1.83 ± 0.32	2.60 ± 0.64	1.30 ± 0.35	1.25 ± 0.14

Data obtained from pages 63-66 and 276-278 of the study report. Standard deviations were calculated by the reviewer from the individual data. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

E. URINALYSIS

There were no treatment-related findings.

F. SACRIFICE AND PATHOLOGY

1. Necropsy findings

There were no gross lesions that could be attributed to treatment with the test item. All gross lesions recorded were considered to be within the range of normal background alterations.

2. Organ weight

A slight increase in absolute and relative liver weight was recorded in males receiving 900 ppm (Table 6). In addition, a slight increase in absolute and relative thyroid weight was recorded in males at 900 ppm. These increases were not seen in the females at 900 ppm. A decrease in absolute heart weight was also noted in both males and females from the 900 ppm dose group, but no corresponding changes in relative heart weight was observed, nor were there any pathological changes noted in heart tissue; therefore, the slight reduction in absolute heart weight is likely a secondary effect of the reduced growth in these animals.

Table 6. Mean (± S.D.) terminal body weight and organ weights

Parameter	0 ppm	50 ppm	200 ppm	900 ppm
Male dogs				
Terminal bw (g)	9343 ± 350	9178 ± 576	10,003 ± 1241	8585 ± 912 (↓8%)
Abs. liver wt (g)	304 ± 5	270 ± 13	310 ± 59	373 ± 22 (↑23%)
Rel. liver wt (%)	3.3 ± 0.2	3.0 ± 0.1	3.1 ± 0.2	4.4 ± 0.2 (↑33%)
Abs. left thyroid wt (g)	0.308 ± 0.047	0.317 ± 0.018	0.407 ± 0.121	0.484 ± 0.081 (↑57%)
Rel. left thyroid wt (%)	0.0033 ± 0.0004	0.0035 ± 0.0004	0.0040 ± 0.0007	0.0057 ± 0.0004 (↑73%)
Abs. right thyroid wt (g)	0.328 ± 0.048	0.330 ± 0.016	0.374 ± 0.071	0.508 ± 0.00 (↑55%)
Rel. right thyroid wt (%)	0.0036 ± 0.0006	0.0036 ± 0.000	0.0038 ± 0.0002	0.0060 ± 0.0006 (↑67%)
Abs. heart wt (g)	84.4 ± 8.0	79.8 ± 1.8	77.0 ± 0.6	67.4 ± 7.5 (↓20%)
Rel. heart wt (%)	0.9 ± 0.0	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.0
Female dogs				
Terminal bw (g)	8425 ± 714	7663 ± 513	8348 ± 711	7420 ± 601 (↓12%)
Abs. liver wt (g)	290 ± 18	244 ± 2	245 ± 0.6	252 ± 24
Rel. liver wt (%)	3.5 ± 0.1	3.2 ± 0.1	3.0 ± 0.2	3.5 ± 0.6
Abs. left thyroid wt (g)	0.321 ± 0.007	0.433 ± 0.158	0.327 ± 0.100	0.301 ± 0.003
Rel. left thyroid wt (%)	0.0038 ± 0.0004	0.0056 ± 0.0017	0.0039 ± 0.0008	0.0041 ± 0.0004
Abs. right thyroid wt (g)	0.328 ± 0.013	0.349 ± 0.115	0.264 ± 0.024	0.320 ± 0.062
Rel. right thyroid wt (%)	0.0040 ± 0.0005	0.0046 ± 0.0012	0.0032 ± 0.0006	0.0044 ± 0.0012
Abs. heart wt (g)	68.1 ± 2.7	61.6 ± 8.2	67.5 ± 6.4	51.0 ± 8.3 (↓25%)
Rel. heart wt (%)	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.1

Data obtained from pages 71-87 of the study report. bw = body weight; wt = weight; Abs. = absolute; Rel. = relative to body weight

3. Liver enzyme determination

There was no effect on the protein content of the 100g supernatants, cytosolic and microsomal fractions in males and females at any dose level (Table 7).

A dose-dependent decrease in hepatic ALAT activities was observed in males and females after treatment at doses of 50, 200 and 900 ppm.

An increase in microsomal cytochrome P450 content was observed in males and females after treatment at doses of 50, 200 and 900 ppm.

No effect was seen on the microsomal phase I enzymes activities 7-ethoxy- (CYP1A1), 7-methoxy- (CYP1A2), and 7-pentoxy- (CYP2B1) resorufin O-dealkylase as well as testosterone 6ß-hydroxylase (CYP3A) in males or females. An increase of 7-benzyloxy-resorufin O-dealkylase (CYP2B11) was obtained in males at 200 and 900 ppm. There was no effect on CYP2B11 activity in females.

There was no effect on the phase II enzyme activities of microsomal uridine diphosphoglucuronosyl-transferase (UDPGT) after treatment. Cytosolic glutathione S-transferase (GST) increased at 200 and 900 ppm in males only. An increase of microsomal epoxide hydrolase (EH) was obtained in males at 200 and 900 ppm and in females at 50, 200 and 900 ppm.

Table 7a: Activities of phase I and phase II enzymes in the liver of male dogs (% of control)

Parameter	0 ppm	50 ppm	200 ppm	900 ppm
Protein content of 100g supernatant (mg/g liver)	100.0	108.2	109.0	102.7
Protein content of cytosolic fractions (mg/g liver)	100.0	98.9	103.9	104.0
Protein content of microsomal fractions (mg/g liver)	100.0	90.5	128.9	135.2
ALAT activity in 100g supernatants (U/g liver)	100.0	80.7	72.6	54.6
Microsomal cytochrome P450 content (nmol/g liver)	100.0	153.1	211.1	270.7
MROD activity (nmol/min/g liver)	100.0	73.7	146.5	153.7
EROD activity (nmol/min/g liver)	100.0	81.1	92.8	85.4
PROD activity (nmol/min/g liver)	100.0	81.9	110.5	134.0
BROD activity (nmol/min/g liver)	100.0	86.0	230.8	396.4
6 ß -OHT activity (nmol/min/g liver)	100.0	77.6	226.1	127.6
GST activity (µmol/min/g liver)	100.0	96.5	189.3	226.7
UDPGT activity (µmol/min/g liver)	100.0	93.5	121.1	144.7
EH activity (µmol/min/g liver)	100.0	95.7	135.3	195.7

Data obtained from pages 357-369 of the study report.

Table 7a: Activities of phase I and phase II enzymes in the liver of female dogs (% of control)

Parameter	0 ppm	50 ppm	200 ppm	900 ppm
Protein content of 100g supernatant (mg/g liver)	100.0	108.0	100.4	89.3
Protein content of cytosolic fractions (mg/g liver)	100.0	109.3	88.0	86.1
Protein content of microsomal fractions (mg/g liver)	100.0	107.2	113.7	103.8
ALAT activity in 100g supernatants (U/g liver)	100.0	80.0	80.8	64.3
Microsomal cytochrome P450 content (nmol/g liver)	100.0	136.7	185.8	166.4
MROD activity (nmol/min/g liver)	100.0	100.1	116.4	80.2
EROD activity (nmol/min/g liver)	100.0	111.8	110.2	52.7
PROD activity (nmol/min/g liver)	100.0	101.2	154.7	118.5
BROD activity (nmol/min/g liver)	100.0	163.0	52.9	97.7
6 ß -OHT activity (nmol/min/g liver)	100.0	70.5	87.0	68.3
GST activity (µmol/min/g liver)	100.0	99.8	92.9	111.6
UDPGT activity (µmol/min/g liver)	100.0	108.4	109.1	113.3
EH activity (µmol/min/g liver)	100.0	115.1	140.3	167.6

Data obtained from pages 357-369 of the study report.

4. Histopathology

There were no microscopic findings considered to be related to treatment with the test item.

III. CONCLUSIONS

At 900 ppm (31 and 30 mg/kg bw/day in male and female dogs, respectively), slight changes body weight and organ weight (liver and thyroid) were observed. No adverse effects were noted at 200 ppm (7.3 and 7.9 mg/kg bw/day in male and female dogs, respectively).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: Overall, the reviewer is in agreement with the conclusions reached by the study investigators. The study investigators did note that there was a slight reduction in mean food consumption noted in males at 900 ppm during the treatment period, compared to the control group. However, comparable reductions in food consumption relative to controls were noted during the pre-test period, and the reviewer does not consider the reduced food consumption in males to be related to treatment. A similar trend was noted in female dogs, who lost weight during the study, which the study investigators did not consider to be related to treatment.

The reviewer also noted a slight reduction in absolute heart weight in both sexes at 900 ppm which the study investigators did not mention, although this reduction is likely a secondary effect of the reduced body weight gain in males and body weight loss in females observed at this dose level.

This subchronic toxicity study in the dog is acceptable/non-guideline (reliable with restrictions) as it is a range-finding study.

C. Conclusions: Alterations in serum and hepatic enzyme levels were observed in this study down to the lowest dose tested (50 ppm). However, the absence of additional indications of hepatic toxicity at the two lowest doses levels, these changes are not considered to be adverse. Additional findings at the high dose of 900 ppm included increased TSH levels in both sexes, and decreased serum protein and albumin levels in females.

D. Deficiencies: No deficiencies were identified.

Tier II Summary **(PMRA #2181190, MRID 48574776)** revised by the regulatory authorities, April 2013

Investigation of possible modes of action leading to decreases in alanine amino transferase (ALAT) activity in dogs

IIA 5.10.2 – MRID 48574776, PMRA 2181190

Report	Braun L. (2011) MCW-2 TECH: 2-Week Oral (Feeding) Mode of Action (Effects on Alanine Aminotransferase Activities - ALAT) Study in the Beagle Dog. Harlan Laboratories Ltd, Switzerland; unpublished Report C59044, dated 14 July 2011. Sponsor reference No. R-23457 Dates of experimental work: 26 November 2009 to 13 July 2011
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines	Not applicable
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

The purpose of this study (MRID 48574776, PMRA 2181190) was to assess other possible modes of action by an investigation of alanine aminotransferase (ALAT) activity, concentrations of pyridoxal 5'-phosphate and ALAT1-protein and ALAT1–messenger RNA expression in Beagle dogs, treated for 2 weeks with fluensulfone (MCW-2 tech., 96.7%, Batch No. 36372130-291-PF1) by the oral (feeding) route.

Two groups of 4 male dogs each received pelleted diet, either without test item (control animals - group 1), or with 500 ppm fluensulfone (treated animals - group 2). The mean test item intake in the animals receiving fluensulfone in the diet was 21.1 mg/kg bw/day.

During pre-test, and on days 7 and 14 of treatment, blood samples were collected for quantification of the enzyme activity of the liver enzymes (leakage markers) aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), sorbitol dehydrogenase (SDH).

Plasma concentrations of the ALAT co-factor, pyridoxal 5'-phosphate, were determined before commencement of treatment and on days 7 and 14 of treatment with fluensulfone

Following completion of the treatment period, samples of liver tissue were taken for mechanistic investigations, including investigations of ALAT1 mRNA expression by PCR, ALAT1 protein expression by Western Blot, ALAT activity in liver tissue, and liver concentrations of pyridoxal 5'-phosphate.

All animals survived the scheduled treatment period. No clinical signs were considered to be related to treatment and all animals appeared in good general health throughout the study. There were no effects on food consumption or body weight. There were no macroscopic or microscopic findings that could be attributed to the treatment.

Decreased plasma ALAT was observed after 2 weeks at 500 ppm. No changes were observed in ASAT or SDH.

ALAT1 mRNA levels were 3.36 times higher in the treated group compared to the control group. Hepatic ALAT1 protein levels were similar in the control and treated groups. After 2 weeks of treatment at 500 ppm, mean hepatic ALAT activity was significantly decreased to 13.6% of the control group mean. A slight decrease in the concentrations of Pyridoxal 5'-phosphate in plasma was recorded, whereas slightly higher hepatic Pyridoxal 5'-phosphate concentrations (attaining statistical significance) were observed in fluensulfone-treated dogs than in control dogs. The amplitude of the effects appears small and does not correlate with the effect pattern (ALAT activity was reduced in both plasma and liver). Thus, it is considered unlikely to be the primary cause of the marked reduction in ALAT activity.

Based on the results of this study, the reduction of the activity of alanine aminotransferase activity by fluensulfone is not mediated by

i) a reduction of ALAT1 mRNA expression

ii) a reduction of ALAT1 protein expression

iii) a decrease of the cofactor Pyridoxal 5'-phosphate

Other mechanisms, such as alteration of post-translational modification might be the cause for the transiently decreased activity of ALAT upon fluensulfone treatment. The increase in ALAT1 mRNA might be indicative of a counter-regulation to the decrease in ALAT activity.

This special study is classified as acceptable/non-guideline (reliable with restrictions) as it does not satisfy any guideline requirement.

I. MATERIAL AND METHODS

1. Test Material MCW-2-TECH (fluensulfone)

Description: Yellow solid

Lot/Batch: 36372130-291-PF1

Purity: 96.7%

CAS#: 318290-98-1

Stability: Stable for the duration of the study

2. Vehicle Pelleted standard Kliba Nafag 3353 dog maintenance diet (Provimi Kliba AG, Switzerland)

3. Analytical Reference Items

3.1 ALAT Reference Standard

Identification: Precipath U

Supplier: Roche, Switzerland

Article:	1065 1265 922
Activity:	19.2 to 307.5 U/L
Stability:	Stable for the duration of the study

3.2 Bioanalytical Reference Item

Identification:	Pyridoxal 5'-phosphate (hydrate) Free base concentrations in ng of Pyridoxal 5'-phosphate per mL plasma or µg of Pyridoxal 5'-phosphate per g liver were used within the report
Supplier:	Sigma-Aldrich, USA
Lot/Batch:	058K0818
Purity:	>99% (by thin layer chromatography - TLC)
Purity for calculations:	98.90%
Stability:	Stable for the duration of the study

3.3 Bioanalytical Internal Standard

Identification:	2-Hydroxy-6-methylpyridine-3-carboxylic acid
Supplier:	Sigma-Aldrich, USA
Lot/Batch:	S40379-218
Purity:	98%
Purity for calculations:	98%
Stability:	Stable for the duration of the study

4. Test Animals

Species	Dog
Strain	Beagle - males
Age	5.5 months at treatment start
Weight	7.3-9.2 kg at treatment start
Source	Harlan France S.A.R.L., France
Acclimation period	Approximately 20 days
Diet	350 ± 1 g of pelleted standard Kliba Nafag 3353 dog maintenance diet (Provimi Kliba AG, Switzerland). Any remaining diet was withdrawn approx. 4 hours after feeding.
Water	Domestic tap water (chlorinated to approximately 0.5 ppm), *ad libitum*, by an automatic watering system.
Housing	Individual or group housing (2 animals/group/sex) in solid floor pens with minimum of 2.0 square meters floor space per dog. Autoclaved softwood bedding was provided and changed at least

once weekly. Dogs were individually housed during feeding periods and following dosing to facilitate recording of clinical signs, for performance of special investigations and/or to avoid aggressive behavior.

5. Environmental conditions

Temperature	22 ± 3 °C
Humidity	30 - 70%
Air change	10 - 15 per hour.
Photoperiod	12-hour fluorescent light / 12-hour dark cycle with at least 8 hours music during the light period.

B. STUDY DESIGN

1. In-life dates 13 November 2009 to 17 December 2009

2. Animal Assignment and treatment

Shortly after arrival the dogs were temporarily allocated to groups by a computer generated random algorithm. Shortly before the start of treatment, the data obtained were reviewed and the allocation was adjusted by exchanging one dog with a spare animal due to a biochemistry outlier and better body weight distribution. Animal numbers 1 to 4 were assigned to Group 1 (control) and animal numbers 5 to 8 to Group 2 (treated – 500 ppm, achieving a mean test item intake of 21.1 mg/kg bw/day).

3. Diet Preparation and Analysis

Diets were prepared once for the 2- week period and stored at room temperature in disposable paper bags.

Fluensulfone was warmed in the original container in a water bath up to a maximum of approximately 40°C until it became liquid. Thereafter, the whole amount of fluid test item was transferred into a glass beaker wrapped with aluminum foil and mixed using a magnetic stirrer on a heating plate (maximum approximately 40 °C). The required amount of fluensulfone was weighed into tared glass beakers (wrapped with aluminum foil) and kept at a maximum of approximately 40 °C on a heating plate until use for feed preparation. Fluensulfone was mixed with microgranulated feed for each dose group and pellets were prepared. Water (approximately 1:10 volume/weight ratio) was added to aid pelleting. The pellets were dried with warm air for approximately 48 hours before storage.

The pellets for the control group were prepared as described above but without test item.

Samples for determination of concentration, homogeneity and stability (14 days) of the dose formulation (500 ppm) were taken from the admixture.

Analyses were performed according to a HPLC-method developed at the performing laboratory in a separate study. The diet samples were stored deep-frozen (-15 °C to -25 °C) until analysis. Fluensulfone was used as analytical standard.

4. Statistics

The following statistical methods were used to analyze body weight, clinical laboratory

parameters and organ weights:

• If the variables could be assumed to follow a normal distribution, the Dunnett-test (many to one t-test) based on a pooled variance estimate was applied for the comparison of the treated group and the control group.

• The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data could not be assumed to follow a normal distribution.

• Liver enzyme assays were analyzed using Mann-Whitney test (2-tailed) for pyridoxal 5'-phosphate or T-test (2-tailed) for liver ALAT.

C. METHODS

1. Clinical observations

Observations for viability were recorded at least twice daily from commencement of the pre-test period.

Clinical signs were recorded at least twice daily from commencement of the pre-test period (pen-side observation) for any change in behavior, reaction to treatment or ill health. A detailed observation was performed once weekly.

2. Bodyweight

The body weight of each animal was recorded before feeding at least once during the pre-test, weekly during treatment and before necropsy.

3. Food consumption

Food consumption was recorded daily from commencement of the pre-test period.

4. Clinical laboratory investigations

Blood samples were collected from all animals during pre-test, in week 1 (day 7) and in week 2 (day 14). The dogs were fasted overnight but allowed access to water *ad libitum*. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms. Blood samples were drawn from the jugular vein into evacuated 4 mL lithium heparin tubes. The samples were protected from light during storage.

Aspartate aminotransferase (ASAT), Alanine aminotransferase (ALAT) and Sorbitol dehydrogenase (SDH) levels were determined.

5. Blood sampling for bioanalytics

On each occasion that blood was sampled for clinical laboratory investigations, additional blood samples were drawn from the jugular vein and collected into 4 mL EDTA-K3 tubes. Following centrifugation, each plasma sample was divided into two aliquots and transferred to plastic (polypropylene) tubes and stored at -80±10°C in the dark until transfer on dry ice for determination of pyridoxal 5'-phosphate. The plasma samples were protected from light during storage.

6. Sacrifice and pathology

All animals were anesthetized by intravenous injection of sodium pentobarbital (Esconarkon®, Veterinaria AG) and killed by exsanguination at the end of the 2-week treatment period. A

description of all macroscopic abnormalities was recorded.

Samples of tissues and organs (Table 1) were collected from all animals at necropsy and fixed, unless otherwise indicated, in neutral phosphate-buffered 4% formaldehyde solution. Bone marrow smears from the sternum from all animals were taken and retained for possible further investigation.

Organs were weighed before fixation. Paired organs were weighed separately. Relative organ weights were calculated on the basis of the body weight and brain weight.

Organ and tissue samples were processed, embedded and cut at a nominal thickness of 4 µm and stained with hematoxylin and eosin. Slides of organs and tissues collected at necropsy, with the exception of some for which no test item related effects were expected, were examined by the study pathologist. An attempt was made to correlate gross observations with microscopic findings. A peer review of the findings was also performed.

Table 1: Summary of tissues collected for pathology examination

Organ/tissue	Weighed	Collected	Examined
Adrenal gland	X	X	X
Aorta		X	X
Bone - sternum, femur including articular surface		X	X
Bone marrow - femur		X	X
Brain - including sections of medulla/pons, cerebral and cerebellar cortex	X	X	X
Epididymides (fixed in Bouin's solution)	X	X	X
Esophagus		X	X
Eyes with optic nerve (fixed in Davidson's solution)		X	X
Female mammary gland area, male mammary gland area		X	X
Gallbladder		X	X
Heart	X	X	X
Kidneys	X	X	X
Large intestine - cecum, colon, rectum		X	X
Larynx		X	
Liver	X	X	X
Lungs, instilled via trachea with formalin at approximately 30 cm H_2O pressure		X	X
Lymph nodes - retropharyngeal, mesenteric		X	X
Pancreas		X	X
Pineal gland		X	X
Pituitary gland		X	X
Peyer's Patches		X	X
Prostate gland		X	X
Salivary glands - mandibular, parotid (sublingual)		X	X
Sciatic nerve		X	X
Skeletal muscle		X	X
Skin - with subcutaneous tissue		X	X

Small intestine - duodenum, jejunum, ileum		X	X
Spinal cord - cervical, midthoracic, lumbar segments		X	X
Spleen	X	X	X
Stomach		X	X
Testes (fixed in Bouin's solution)	X	X	X
Thymus	X	X	X
Thyroid gland with parathyroid gland	X	X	X
Tongue		X	X
Trachea		X	X
Ureter		X	X
Urinary bladder		X	X
Uterus with (vagina), cervix	X	X	X
All gross lesions		X	X

7. Determination of biochemical parameters

Samples of the liver were collected from all animals at the scheduled necropsy for ALAT activity, protein and RNA level and pyridoxal 5'-phosphate determination. The samples were minced into small pieces and four weighed aliquots of 1 - 2 g were placed into labeled plastic bags (study and animal number) and frozen in liquid nitrogen. Three samples were transferred on dry ice to the study scientist for determination of hepatic pyridoxal 5'-phosphate and one sample to another study scientist for determination of liver ALAT activity and stored at -80 ± 10°C until analyses. Additionally, for protein and RNA level determination, two aliquots of 500 mg were placed in RNAlater tissue protect tubes for stabilization in harvested livers. These two samples were stored at -80 ± 10°C until transfer on dry ice for analysis. All samples were protected from light during storage.

All remaining liver tissue of each animal was placed into labeled plastic bags (study and animal number), frozen in liquid nitrogen and transferred on dry ice for storage at -80 ± 10 °C until possible further analysis.

The assays were performed at Harlan Laboratories Ltd. (Füllinsdorf and Itingen) under internal laboratory quality control conditions to assure reliable test results. Liver homogenates were prepared and the following parameters were determined:

- ALAT1 mRNA expression measured by RT-PCR
- ALAT1 protein expression determined by immunoblotting
- Hepatic ALAT activity (transamination of ketoglutarate)

II. RESULTS AND DISCUSSION

A. ANALYSIS OF FEED PREPARATIONS

The results of the diet preparation analysis comprised 89.3% to 90.4% fluensulfone. The distribution of fluensulfone in the diet preparations was homogeneous as no single results deviated more than 0.7% from the corresponding mean.

In addition, fluensulfone was found to be stable in diet when kept 14 days at room temperature

based on the recoveries that were within 92.9% to 93.3% of the nominal concentration and 3.5% of the time-zero (homogeneity) mean.

B. OBSERVATIONS AND PATHOLOGY

1. Mortality and Clinical signs

No mortality occurred during the treatment period and all animals appeared in good general health throughout the treatment period.

The clinical signs observed, such as loose and watery feces, occurred occasionally in animals dosed with 500 ppm fluensulfone in a similar incidence and severity as during the pre-test and therefore were not considered to be related to treatment.

2. Body weight and Food consumption

No effects on body weight and/or food consumption were noted during the treatment period.

3. Pathology

There were no effects on organ weight, macroscopic and microscopic findings. Small testes and epididymides noted at necropsy for 2 control animals were considered to be due to the young age of the animals.

C. CLINICAL BIOCHEMISTRY

Decreased plasma ALAT was observed after 1 week (18% compared to control) and 2 weeks (65% compared to control) at 500 ppm (Table 1). There were no changes observed in plasma ASAT and SDH activities that were considered to be related to treatment.

Table 1: Mean ± S.D. clinical chemistry parameters (% of control)

Group	ASAT (U/L)	ALAT (U/L)	SDH (U/L)
Pre-test			
0 ppm	38.0 ± 8.7	26.9 ± 13.0	21.9 ± 3.6
500 ppm	33.8 ± 5.6 (89%)	43.8 ± 24.6 (163%)	19.5 ± 4.1 (89%)
Week 1 – Day 7			
0 ppm	49.5 ± 26.0	34.1 ± 16.7	20.3 ± 3.8
500 ppm	37.1 ± 4.3 (75%)	24.5 ± 15.1 (72%)	16.7 ± 4.2 (82%)
Week 2 – Day 14			
0 ppm	35.2 ± 3.1	33.5 ± 20.5	22.1 ± 3.0
500 ppm	32.3 ± 5.7 (92%)	11.6 ± 2.1 (35%)	20.7 ± 5.0 (94%)

Data obtained from page 43-45 of the study report.

D. MODE OF ACTION INVESTIGATIONS

1. Concentrations of Pyridoxal 5'-phosphate

Concentrations of Pyridoxal 5'-phosphate in dog plasma and liver were determined using protein precipitation and LC/MS/MS. The pretest-normalized ratios of plasma Pyridoxal 5'-

phosphate were calculated. A slight but non-statistically significant decrease in plasma Pyridoxal 5'-phosphate was seen in the fluensulfone-treated dogs (Table 2). In contrast to plasma, hepatic Pyridoxal 5'-phosphate concentrations were clearly higher in fluensulfone-treated dogs than in control dogs (2-tailed, Mann-Whitney test, p = 0.021).

Table 2: Concentration of Pyridoxal 5'-phosphate in plasma (ng/mL) and in liver at necropsy (µg/g liver) (Mean ± S.D.)

Sample - Time point	0 ppm	500 ppm
Plasma - Pre-test	47 ± 20	38 ± 9 (↓19%)
Plasma - Week 1 (Day 7)	55 ± 21	28 ± 7 (↓49%)
Plasma - Week 2 (Day 14)	59 ± 25	29 ± 9 (↓51%)
Liver - Day 15 (necropsy)	44 ± 11	57 ± 2* (↑29%)

*: $p<0.05$ at Mann-Whitney Test. Data obtained from page 28 of the study report. Values in parentheses represent the percent change from controls (calculated by the reviewer).

2. Liver enzyme determination

2.1 Hepatic ALAT1 mRNA expression

Hepatic ALAT1 mRNA was quantified using oligo nucleotides matching dog ALAT1 cDNA and expressed relative to dog Cyclophilin cDNA as reference. ALAT1 specific signal was detected between Ct = 24.9 to 27.1 in the control group and Ct = 23.2 to 25.9 in the treated group.

This was confirmed in a second test (Ct = 22.8 to 25 in control and Ct = 21.1 to 24.1 in the treated group). The mean normalized Ct values, ΔCt ($Ct_{ALAT} - Ct_{Cyclophylin}$), were 4.76 and 3.95 in the control group and 3.01 and 2.33 in the treated group resulting in ΔΔCt (ΔCt treated - ΔCt control) of -1.75 and -1.62, respectively. Therefore, ALAT1 mRNA expression levels were 3.36 ($2^{-\Delta\Delta Ct}$) times higher in the treated group compared to the control group and this was confirmed in a second test (3.07 times higher in the treated group than in the control group).

Table 3: Mean ALAT1 mRNA expression (ΔCt) (Mean ± SD)

Experiment	0 ppm	500 ppm
No. 1	4.76 ± 0.5	3.01 ± 0.8
No. 2	3.95 ± 0.4	2.33 ± 0.4

Data obtained from page 29 of the study report.

2.2 Hepatic ALAT1 protein levels

Hepatic ALAT1 protein levels, detected by Immunoblotting (polyclonal human anti-ALAT1 antibody) in dog liver homogenates, were similar between the control and treated groups (mean value 128 and 124 intensity/protein concentration in the control and treated groups, respectively).

The supplier Aviva Systems Biology describes that this antibody recognizes the region of the human protein from amino acid 31 (R) to 80 (T), and that this sequence is highly conserved

among the different species including dog. As a control, different amounts of recombinant human ALAT protein (54.5 kDa) were used.

2.3 Hepatic ALAT activity

Hepatic ALAT activities of 99 to 237 (mean ± S.D.: 153.4 ± 59) U/g liver were obtained in control animals. As expected, treatment with 500 ppm fluensulfone for 2 weeks significantly decreased the mean ALAT activity (20.9 ± 19) to 13.6% of the control mean (two-tailed T-test, $p < 0.01$).

Table 4. **Mean ± S.D. hepatic ALAT activity (% of control)**

Group	ALAT (U/µg)
0 ppm	153.4 ± 59.2
500 ppm	20.9 ± 18.6* (13.6%)

*: $p<0.01$, two-tailed T-test. Data obtained from page 211 of the study report. Values in parentheses represent the percent change from controls.

III. CONCLUSIONS

Oral administration of fluensulfone in the diet at a dose of 500 ppm for 2 weeks resulted in decreased plasma and hepatic alanine aminotransferase activity, without any evidence of toxic or adverse effects.

Endogenous Pyridoxal 5'-phosphate determination in both plasma and liver resulted in slightly, non-statistically significantly decreased plasma Pyridoxal 5'-phosphate and slightly, statistically significantly higher hepatic Pyridoxal 5'-phosphate concentrations in the animals treated with 500 ppm fluensulfone compared to the controls. The amplitude of the effects appears small and does not correlate with the effect pattern (ALAT activity was reduced in both plasma and liver). Thus, it is considered unlikely to be the primary cause of the marked reduction in ALAT activity.

ALAT activity was clearly decreased (by 86% in the liver tissue and by 65% in plasma) in the animals treated at 500 ppm compared to the controls. Since the activities of two other typical hepatic leakage markers in plasma, ASAT and SDH, were not affected, direct hepatocyte damage due to treatment with fluensulfone can be excluded to be the cause for the observed changes in ALAT activity.

The decrease was not accompanied by a reduction of ALAT1 mRNA level in dog liver, as determined by RT-PCR, nor by a reduction of the translation of ALAT1, as determined by immunoblotting of ALAT1 protein in the liver. In the dog (as well as in rat, mouse and human) at least two isoforms of ALAT, ALAT1 and ALAT2 have been identified. They show approximately 70% homology and both can catalyze the above mentioned reaction but with different turnover and specificity. ALAT isoenzymes are distributed differentially at the subcellular level. ALAT1 is mainly a cytosol protein, while ALAT2 has mitochondrial localization. Consequently, the tissue distribution of ALAT isoforms are also different,

ALAT1 is highly expressed in liver while ALAT2 is mainly expressed in muscles and fat. However, a specific effect on the expression of other ALAT isoforms might not have been detectable by the ALAT1 mRNA analysis or ALAT1 protein expression.

Based on the results of this study, the reduction of the activity of alanine aminotransferase activity by fluensulfone is not mediated by a decrease of Pyridoxal 5'-phosphate, nor by a reduction of ALAT1-protein or ALAT1-mRNA expression. Other mechanisms, such as alteration of post-translational modification or a specific effect on the expression of other ALAT isoforms, might be the cause for the transiently decreased activity of ALAT upon fluensulfone treatment.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments/Conclusions: This special study is classified as acceptable/non-guideline (reliable with restrictions) as it does not satisfy any guideline requirement.

C. Deficiencies: No deficiencies were identified.

Tier II Summary (PMRA #2181188, MRID 48574778 revised by the regulatory authorities, April 2013

Investigation of possible modes of action leading to decreases in alanine amino transferase (ALAT) activity in dogs

IIA 5.10.2 – MRID 48574778, PMRA 2181188

Report	Sagelsdorff P. (2009) Determination of Hepatic Alanine Aminotransferase (ALAT) in Untreated Dogs. Harlan Laboratories Ltd, Switzerland; unpublished Report C57896, dated 18 December 2009. Sponsor reference No. R-23459 Dates of experimental (laboratory) work: 3 to 4 November 2009
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines	Not applicable
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In this study (MRID 48574778, PMRA 2181188), liver samples from adult untreated male and female dogs were collected between June 2009 and September 2009 in order to provide a historical database for hepatic alanine aminotransferase (ALAT) activity. ALAT activities were measured in homogenates prepared from the respective livers.

In male and female control animals a mean ± S.D. activity of 119.6 ± 23.6 U/g Liver (Range 71.3 to 174.7 U/g Liver, 23 animals) and 126.4 ± 30.6 U/g Liver (Range 88.7 to 186.7 U/g Liver, 14 animals), was obtained, respectively.

This special study is acceptable/nonguideline (reliable with restrictions) as it does not satisfy any guideline requirement.

I. MATERIAL AND METHODS

1. Test Material Not applicable: no substance was tested in this study

2. Vehicle Not applicable

3. Test Samples Deep frozen liver samples from adult untreated male (23 animals) and female (14 animals) dogs from different studies and from stock animals were obtained from Harlan Laboratories Ltd., Switzerland between Jun-2009 and Sep-2009. The samples were kept at -80 °C until preparation of homogenates.

B. STUDY DESIGN

1. In-life dates June to September 2009

2. Statistics

Means and standard deviations were calculated separately for male and female dogs. Range of values for each sex was also given.

C. METHODS

1. Preparation of Liver Homogenates

The frozen liver samples were thawed and homogenized in 10 mM Tris/HCl buffer, pH 7.5, containing 250 mM sucrose to give a 10% (w/v) homogenate. The homogenate was centrifuged for 15 min at 100g at 4 °C. The supernatant was stored at 4° and used the day after homogenization for ALAT determination.

2. Determination of ALAT

ALAT activities were determined using a commercially available kit (AL 1205, Randox, Germany) according to the description from the supplier. The 100g supernatants were diluted with 250 mM sucrose, 10 mM Tris/HCl, pH 7.5, and 20 µL were incubated in 96-well plates with L-alanine, α-oxoglutarate and lactate dehydrogenase in the presence of NADH for 5 min at 37°C (final volume 220 µL). The decrease in NADH was monitored at 340 nm during 5 min. ALAT activities were calculated based on the decrease of the optical density per minute in comparison with an ALAT reference standard (Precipath U, Roche, Switzerland, Standard curve 19.2 to 307.5 U/L).

II. RESULTS AND DISCUSSION

Table 1: Individual hepatic ALAT activities from control male and female Beagle dogs

Male		Female	
Animal number	**Activity (U/g Liver)**	**Animal number**	**Activity (U/g Liver)**
001	124.0	005	110.1
002	122.7	006	88.7
003	82.4	007	186.0
004	109.5	008	151.3
009	108.3	012	107.2
010	78.3	013	133.2
011	71.3	014	95.9
016	126.3	015	142.2
017	134.5	023	186.7
018	121.5	024	119.6
019	166.0	029	112.8
020	125.0	030	119.2
021	111.3	031	117.6
022	135.0	032	99.1
025	123.6		
026	125.7		
027	127.6		
028	129.5		
033	108.9		
034	174.7		
035	114.6		
036	127.6		
037	101.8		
Mean ± SD	**119.6 ± 23.6**	**Mean ± SD**	**126.4 ± 30.6**

Data obtained from pages 15-16 of the study report.

III. CONCLUSIONS

In male and female control animals mean activities of 119.6 ± 23.6 U/g Liver (Range 71.3 to 174.7 U/g Liver, 23 animals) and 126.4 ± 30.6 U/g Liver (Range 88.7 to 186.7 U/g Liver, 14 animals), were obtained, respectively.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments/Conclusions: This special study is acceptable/non-guideline (reliable with restrictions) as it does not satisfy any guideline requirement.

C. Deficiencies: No deficiencies were identified.

Tier II Summary (PMRA #2181191, MRID 48574777) revised by the regulatory authorities, April 2013

Investigation of possible modes of action leading to decreases in alanine amino transferase (ALAT) activity in dogs

IIA 5.10.2 – MRID 48574777, PMRA 2181191

Report:	Sagelsdorff P. (2010) MCW-2 TECH Mode of Action Study on Alanine Aminotransferase (ALAT) in Vitro in Dog Liver Homogenate. Harlan Laboratories Ltd, Switzerland; unpublished Report C58796, dated 30 March 2010. Sponsor reference No. R-23458 Dates of experimental work: 11 December 2009 to 18 January 2010
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines	Not applicable
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In this special study (MRID 48574777, PMRA 2181191), dog liver homogenate from untreated dogs was incubated with fluensulfone (MCW-2 tech., 96.7%, Batch No. 36372130-291-PF1) for different time periods in order to investigate a possible direct inhibition of ALAT activity by fluensulfone and/or its metabolites,. LC-MS/MS determination in the incubation mixtures clearly demonstrated an effective metabolism of fluensulfone.

No inhibition of the ALAT activity was observed following incubation of dog liver homogenates with fluensulfone. Based on the results of this study, the reduction of ALAT activity by fluensulfone is not considered to be caused by a direct inhibition of ALAT by the test item or its metabolites.

This special study is classified as acceptable/non-guideline (reliable with restrictions) as it does not satisfy any guideline requirement.

I. MATERIAL AND METHODS

1. Test Material	MCW-2 TECH
Description:	Not given
Lot/Batch:	36372130-291-PF1
Purity:	96.7%
CAS#:	318290-98-1
Stability:	Stable for the duration of the study

2. Analytical Reference Items

2.1 Human ALAT Reference Standard

Identification:	Precipath U
Supplier:	Roche, Switzerland
Article/Batch:	1065 1265 922 / 180667
Activity:	307.5 U/L
Stability:	Stable for the duration of the study

2.2 Bioanalytical Reference Item

Identification:	MCW-2 Analytical standard
Supplier:	Makhteshim Chemical Works Ltd.
Lot/Batch:	326-115-01
Purity:	99.1%
CAS#:	Not available
Stability:	Stable for the duration of the study

3. Test System Livers from untreated dogs (7-28 months old) were collected by Harlan Laboratories Ltd., Switzerland at different occasions between Jun-2009 and Dec-2009. Upon sacrifice of the animals an aliquot of the liver was excised, snap frozen in liquid nitrogen and stored at -80 °C.

B. STUDY DESIGN

1. In-life dates 11 December 2009 to 18 January 2010

C. METHODS

1. Preparation of Homogenates

The frozen liver samples were thawed and homogenized in 10 mM Tris/HCl buffer, pH 7.5, containing 250 mM sucrose to give a 10% (w/v) homogenate. The homogenate was centrifuged for 15 min at 100*g*. The supernatant (liver homogenate) was used for this *in vitro* study.

2. Incubation

Fluensulfone was dissolved in DMSO to get a stock solution of 400 µM.

An aliquot of this stock solution was incubated with liver homogenates (0-5 mg protein/mL) in a final volume of 0.2 to 1 mL of 0.1 M Tris/HCl, pH 7.4, 5 mM $MgCl_2$ in the presence of an NADPH regenerating system at 37 °C for up to 3 hours (final concentration of MCW-2 tech:20 µM).

3. Determination of ALAT

ALAT activities were determined using a commercially available kit (AL 1205, Randox, Germany) according to the description by the supplier.

Immediately after the incubation, an aliquot was diluted with 250 mM saccharose, 10 mM Tris/HCl, pH 7.5, and incubated with L-alanine, α-oxoglutarate and lactate dehydrogenase in the presence of NADH for 5 min at 37 °C. The decrease of NADH was monitored at 340 nm. ALAT activities were calculated based on the decrease of the optical density per minute in comparison with the ALAT reference standard (Precipath U, Roche, Switzerland, standard curve 19.2 to 307.5 U/L). .

The ALAT activities were expressed as U/L, µmol alanine deamination/min/L. One unit (U) is the enzyme activity needed to convert 1 µmol of substrate (alanine) within 1 min. As the matrix in this study is liver homogenate, the experimental results (U/L) and the protein concentration found after homogenization were used to standardize the results into "activity per gram of total protein obtained after homogenization of the liver" (U/g protein).

4. Protein Determination

Protein contents of liver homogenates were determined with bicinchoninic acid (BCA protein assay, Pierce).

Liver homogenates were solubilized in 1% (w/v) Triton X-100 for 1 hour at room temperature. 10 µL of the solubilized sample was mixed with 200 µL of the bicinchoninic acid reagent and incubated overnight at room temperature. Following incubation, the optical density at 562 nm was recorded on a Molecular Devices Spectramax 190 ELISA reader. Total protein concentrations of the homogenates were calculated on the basis of a standard curve containing bovine serum albumin at concentrations of 0.1-1.0 mg/mL.

5. Bioanalytics

Immediately after incubation, a second aliquot was stopped by addition of 3 volumes of methanol, and proteins were precipitated at 4 °C for 30 min and centrifugation at 13,000 x *g* for 10 min. The supernatant was transferred at 4°C to the laboratory for a determination of the content of non-metabolized fluensulfone by LC-MS/MS.

For preparation of calibration standards (C) and quality control (QC) samples a 1:1 mixture of untreated 100 g supernatant and NADPH regenerating system in TRIS-$MgCl_2$ buffer was used as blank matrix. 50 µL aliquots of the study samples were transferred in a 1.5 mL PP-tube and vortexed for 3 seconds. For preparation of the Cs and QCs 45 µL blank matrix was transferred in a 1.5 mL PP-tube and spiked with 5 µL of the corresponding stock solution. 150 µL of methanol was added to each sample and vortexed for 30 seconds. After 30 min at 5 °C 100 µL of water was added and the tubes were vortexed for 30 seconds. After centrifugation for about 10 min at 4 °C and 17,400 x g, the samples were transferred in a 96-well collection plate for LC-MS/MS measurement. Each analytical assay consisted of solvent standards (STD), C, QC, and study samples. The results of the study samples were calculated using calibration curves. Each series was validated with QC samples (3 concentration levels with at least 2 replicates each) prepared and measured together with the study samples.

The metabolism of fluensulfone was expressed as "% remaining parent" (unmetabolized) compound, as well as U/L, µmol fluensulfone metabolized/min/L. One unit (U) is the enzyme activity needed to convert 1 µmol of substrate (fluensulfone) within 1 min. As the matrix in this study is liver homogenate, the experimental results (U/L) and the protein concentration found after homogenization were used to standardize the results into "activity per gram of total protein obtained after homogenization of the liver" (U/g protein).

II. RESULTS AND DISCUSSION

A. In Vitro Metabolism of fluensulfone

Upon incubation with liver homogenate from male and female dogs in the presence of an NADPH regenerating system, fluensulfone decreased with time and increasing protein concentrations, indicating an effective metabolism of fluensulfone. Following incubation of 19.1 µM fluensulfone with dog liver homogenates for 60 min at concentrations of 0.6 to 5 mg protein/mL, 10-21% (at 0.6 mg protein/mL) to 42-70% (at 3-5 mg/protein/mL) of the test item was metabolized in males and females, respectively (Table 2).

B. ALAT Activity

ALAT activities of 932 to 1194 U/g protein were obtained in the absence of DMSO and fluensulfone (Table 1). The activity was not affected by the presence of 5% DMSO (990-1076 U/g protein) or up to 19.1 µM fluensulfone (854-1513 U/g protein; Table 2).

Upon incubation for up to 1 hour of up to 19.1 µM fluensulfone with dog liver homogenate (up to 5 mg protein/mL), no inhibition of ALAT activity was observed, whereas fluensulfone determination in the incubation mixtures clearly demonstrated an effective metabolism of fluensulfone, and thereby indicated the presence of substantial amounts of fluensulfone metabolites.

Table 1: Control ALAT activity in dog liver homogenates

Sample (protein content)	Incubation time (min)	Incubation with		ALAT activity (U/g total homogenate protein)
		Fluensulfone (µM)	DMSO (%)	
Male A (0.59 mg/mL)	0	0	0	1061
	60	0	0	1194
Female B (0.63 mg/mL)	0	0	0	967
	60	0	0	1162
Male C (2.93 mg/mL)	0	0	0	1017
	60	0	0	932
Female D (3.14 mg/mL)	0	0	0	1010
	60	0	0	973
Male A (0.59 mg/mL)	0	0	5	1076
	60	0	5	1059
Female B (0.63 mg/mL)	0	0	5	991
	60	0	5	990

Data obtained from page 20 of the study report.

Table 2: Metabolism of fluensulfone and ALAT activity in dog liver homogenates

Sample (protein content)	Incubation time (min)	Fluensulfone			ALAT activity	
		µM	% of remaining parent	U/g total homogenate protein	U/g total homogenate protein	% of initial activity
Male A (0.59 mg/mL)	0	21.2	100	n/a	1046	100
	20	19.5	92	0.144	940	90
	40	19.7	93	0.064	964	92
	60	19.0	90	0.062	1048	100
Female B (0.63 mg/mL)	0	24.2	100	n/a	1025	100
	20	19.5	81	0.373	1022	100
	40	21.3	88	0.115	1114	109
	60	19.1	79	0.135	958	93
Male C (2.93 mg/mL)	0	24.4	100	n/a	965	100
	20	14.7	60	0.166	903	94
	40	14.8	60	0.082	863	89
	60	11.9	49	0.071	866	90
Female D (3.14 mg/mL)	0	18.8	100	n/a	854	100
	20	12.4	66	0.102	950	111
	40	10.2	54	0.068	910	107
	60	9.7	52	0.048	930	109
Male E (4.74 mg/mL)	0	22.1	100	n/a	1513	100
	20	17.0	77	0.054	1483	98
	40	16.9	77	0.027	1497	99
	60	12.9	58	0.032	1141	75
Male F (4.94 mg/mL)	0	21.4	100	n/a	952	100
	20	12.0	56	0.095	1004	106
	40	7.6	35	0.070	951	100
	60	6.4	30	0.051	1062	112
Female G (4.47 mg/mL)	0	20.6	100	n/a	1413	100
	20	12.5	61	0.091	1465	104
	40	10.0	49	0.059	1414	100
	60	7.8	38	0.048	1411	100
Female H (3.92 mg/mL)	0	20.7	100	n/a	1340	100
	20	14.3	69	0.082	1274	95
	40	9.4	46	0.072	1374	103
	60	8.8	42	0.051	1521	113

Data obtained from page 19 of the study report.

III. CONCLUSIONS

No inhibition of the ALAT activity was observed following incubation of dog liver homogenates with fluensulfone, under conditions where an effective metabolism of fluensulfone was confirmed by LC-MS/MS.

Based on the results of this study, the reduction of ALAT activity observed following repeated oral treatment with fluensulfone is not considered to be caused by a direct inhibition of ALAT by fluensulfone or its metabolites.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments/Conclusions: No demonstration of reliability of the test method used in this study was provided. It was not established that the concentration of fluensulfone or the incubation time used in this study were sufficient to detect inhibition of ALAT activity in liver homogenates. A positive control for ALAT inhibition should have been used.

It is noted that, based on the pharmacokinetic study in rats (MRID 48574741, PMRA 2181185), the concentrations used in this study (up to 19.1 uM) are similar to the cmax in blood (3.8 uM).

This special study is classified as acceptable/non-guideline (reliable with restrictions) as it does not satisfy any guideline requirement.

C. Deficiencies: No demonstration of reliability of the test method was provided.

Tier II Summary **(PMRA #2181192, MRID 48574775)** revised by the regulatory authorities, April 2013

Investigation of possible modes of action leading to decreases in alanine amino transferase (ALAT) activity in dogs

IIA 5.10.2– MRID 48574775, PMRA 2181192

Report:	Sagelsdorff P. (2009) MCW-2 TECH Mode-of-Action Investigations on Inhibition of Alanine-Aminotransferase. Harlan Laboratories Ltd, Switzerland; unpublished Report C15686, dated 11 November 2009. Sponsor reference No. R-23424 Dates of experimental work: 19 September to 3 November 2008
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines	Not applicable
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

In a special mechanistic study (MRID 48574775, PMRA 2181192), the direct inhibition of liver alanine-aminotransferase (ALAT) activity by fluensulfone was evaluated. In a previous study (Harlan Laboratory Study B89087, MRID 48574752, PMRA 2181202), treatment of dogs for 28 days with 50, 200 and 900 ppm fluensulfone (MCW-2 tech., 96.67%, Batch No. 36372130-PF1) in the diet resulted in a dose-dependent decrease of mean alanine-aminotransferase (ALAT) activity to maximally 15-24% in serum and to 55-64% in liver homogenates. To check if the decrease of ALAT activity might be due to a direct inhibition of the enzyme by MCW-2 or one of its metabolites, or by an inhibition of ALAT gene or protein expression in the liver, serum and liver cytosolic fractions of control and high dose dogs obtained in the 28-day study were used.

Serum and liver cytosolic fractions of control animals were spiked with respective samples from dogs treated at 900 ppm (the highest dose level tested). ALAT activity was determined to show whether serum or cytosolic fractions from fluensulfone-treated animals, considered to contain excess of fluensulfone or relevant metabolites, are able to inhibit the activity of ALAT. In addition, the amount of ALAT in serum and cytosolic fractions was determined for both control and fluensulfone-treated dogs using western blotting to see whether fluensulfone suppresses gene expression at the protein level.

No inhibition of the ALAT activity by cytosol or serum from treated animals was observed. In cytosol and serum samples of control dogs, spiked with samples obtained from dogs treated at 900 ppm, the observed activities corresponded exactly to the theoretically calculated activities.

Immunoblotting revealed very similar levels of ALAT in serum and cytosolic fractions of controls and treated dogs. However, as specificity of the used antibody was not tested against dog ALAT, the results indicating the unlikely decrease of the expression of ALAT protein upon treatment with fluensulfone were considered only provisional and need to be evaluated with care.

It is noted that it is not known how much, if any, fluensulfone or its metabolites was contained in the serum and liver cytosol fractions taken from treated animals and used in this study. Levels of fluensulfone or its metabolites were not determined in serum or liver cytosol.

This special study is classified as acceptable/non-guideline (reliable with restrictions) as it does not satisfy any guideline requirement.

I. MATERIAL AND METHODS

Details of the previous 28-day toxicity study in dogs (B89087, R-23349, MRID 48574752, PMRA 2181202) are reported here below.

1. Test Material	MCW-2 TECH (fluensulfone)
Description:	Yellow solid
Lot/Batch:	36372130-291-PF1
Purity:	96.67% (GC-assay)
CAS#:	318290-98-1
Stability:	Stable for the duration of the study
2. Vehicle	Pelleted standard Kliba 3353 dog maintenance diet (Provimi Kliba AG, Switzerland)
3. Test Animals	
Species	Dog
Strain	Pure bred Beagle
Age	6.5 to 7.5 months
Weight	6.2 to 9.9 kg
Source	Harlan Laboratories Ltd., Switzerland
Acclimation period	10 to 20 days
Diet	350 ± 1 g per day and dog of pelleted standard Kliba 3353 dog maintenance diet (Provimi Kliba AG, Switzerland). The appropriate feed preparations were presented to the animals at the same time daily. Any remaining diet was withdrawn approximately 4 hours after feeding.
Water	Tap water *ad libitum*.
Housing	Housing in pairs per sex and group, in pens with minimum of 2.0 square meters floor space per dog. Dogs were individually housed during feeding periods and at the discretion of the study director to facilitate recording of clinical signs or performance of special investigations.

4. Environmental conditions

Temperature	20 ± 3°C; pen floor temperature was maintained at approximately 21 ± 4°C
Humidity	30-70%
Air change	approx. 10-15 changes per hour
Photoperiod	12-hour fluorescent light / 12-hour dark cycle. Music was played during the major part of the light period.

B. STUDY DESIGN

1. In-life dates 28 April to 24 June 2008 (28-day study); 19 September to 3 November 2008 (experimental work dates for this study)

C. METHODS

1. Determination of ALAT

Serum (undiluted) and liver cytosolic fractions (1:100 diluted with 0.9% NaCl) of the control dogs were spiked with 0.1, 0.2, 0.4, 0.6 and 1 volume of the respective diluted samples from dogs treated at 900 ppm according to the following scheme:

Volume of control sample	Volume of treated sample	Total volume (µL)	Control sample (µL)	Treated sample (µL)
1	0.1	200	181.8	18.2
1	0.2	200	166.7	33.3
1	0.4	200	142.9	57.1
1	0.6	200	125.0	75.0
1	1	200	100.0	100.0

ALAT activity was determined with a Hitachi 917 automatic analyzer using a commercially available kit (Roche, Switzerland).

2. Western Blotting

The expression of the ALAT protein in dog serum and cytosol samples from liver was determined by immunoblotting, using a polyclonal anti-human Alanine Transferase antibody. The antibody is known to cross react with mouse, rat and porcine ALAT. Due to the homology of these proteins with the canine ALAT (>80%), the antibody was theoretically suitable for detection of canine protein.

Cytosol and serum samples were diluted 1:7.5 with Milli-Q-water. NUPAGE LDS sample buffer (4x) from Invitrogen was added (30 µL sample plus 10 µL LDS sample buffer) to get a final protein concentration of about 2 mg/mL. A 28.4 mg/mL stock solution of Porcine heart ALAT (Sigma-Aldrich) was diluted using the same procedure, to a final concentration 0.95 mg/mL. The samples were heated for 4 min at 85 °C and 15 µL of the sample were loaded onto the gel. The samples were separated on a 12% SDS-PAGE (BioRad, Ready Gel Tris-HCl

12% resolving gel, 4% stacking) for approximately 85 min at 100 V. The proteins were wet-transferred onto PVDF (BioRad, Criterion Gel Blotting Sandwiches, PVDF) in Towbinbuffer (0.025 M Tris/HCl, pH 8.6, 0.192 M Glycin, 20% Methanol) for approximately 1 h at 75 V.

The blot was incubated in blocking buffer, TBS (0.05% Tris/HCl, 0.9% NaCl, pH 7.6) containing 0.1% Tween 20 and 5% non fat dry milk at room temperature for 1 h. Following washing with 15 mL of TBS, 0.1% Tween 20, three times for 5 minutes each, the blot was incubated in 10 mL TBS with sheep-polyclonal anti-human alanine transferase antibody (Antibodies-online, ABIN94798, dilution 1:3000 in TBS) with gentle agitation 1 hour at room temperature. The blot was washed three times for 5 min each with 15 mL of 1x TBS, 0.1% Tween 20 and incubated with an alkaline phosphatase labeled donkey anti-sheep IgG antibody (Abcam Ab6901, dilution 1:1000 in 10 mL blocking buffer) with gentle agitation for 1 hour at room temperature. After incubation with the secondary antibody, the blot was washed five times for 5 min each with 15 mL of TBS, 0.1% Tween 20.

The membrane was developed with 20 mL alkaline phosphatase substrate solution (100 mM Tris/HCl, 5 mM $MgCl_2$, 100 mM NaCl, pH 9.5) containing 75 µL 5-bromo-4-chloro-3´-indolxylphosphate p-toluidine salt (5% solution in dimethylformamide), 100 µL nitro-blue tetrazolium chloride (7.7% solution in 70% dimethylformamide) for approximately 5 min.

ALAT on the western blot was determined by densitiometry using a ChemiDoc XRS from BioRad.

II. RESULTS AND DISCUSSION

A. ALAT ACTIVITY

In the control animals, ALAT activities ranged between 214.1 and 353.9 U/L in 1:100 diluted liver cytosol samples (Table 2). Clearly decreased activities of 60.8 to 85.7 U/L were determined in respective samples from animals treated at 900 ppm in the diet for 28 days.

ALAT activities of 20.1 to 46.4 U/L were obtained in serum from the control animals (Table 3). Clearly decreased activities of 12.8 to 19.8 U/L were determined in respective serum samples from animals treated at 900 ppm in the diet for 28 days.

In the control cytosol and serum samples spiked with 0.1 to 1 volume of samples prepared from treated dogs the observed activities corresponded exactly to the expected activities.

No inhibition of the ALAT activity by cytosol or serum from treated animals was observed.

Table 2: ALAT activities in cytosol samples

Sample	Spiking ratio Control + Treated	ALAT activity (U/L)		
		Determined	Expected[a]	% of expected
Dog No. 1 (Control, M) spiked with Dog No. 7 (900 ppm, M)	1 + 0	344.9	344.9	100.0
	1 + 0.1	320.6	319.4	100.4
	1 + 0.2	294.9	298.1	98.9
	1 + 0.4	266.5	264.7	100.7
	1 + 0.6	237.6	239.6	99.1
	1 + 1	201.1	204.6	98.3
	0 + 1	64.2	64.2	100.0
Dog No. 2 (Control, M) spiked with Dog No. 8 (900 ppm, M)	1 + 0	353.9	353.9	100.0
	1 + 0.1	333.2	329.5	101.1
	1 + 0.2	311.8	309.2	100.8
	1 + 0.4	271.1	277.3	97.8
	1 + 0.6	257.8	253.3	101.8
	1 + 1	223.6	219.8	101.7
	0 + 1	85.7	85.7	100.0
Dog No. 9 (Control, F) spiked with Dog No. 15 (900 ppm, F)	1 + 0	214.1	214.1	100.0
	1 + 0.1	199.8	200.2	99.8
	1 + 0.2	186.5	188.6	98.9
	1 + 0.4	170.8	170.3	100.3
	1 + 0.6	155.3	156.6	99.2
	1 + 1	133.8	137.5	97.3
	0 + 1	60.8	60.8	100.0
Dog No. 10 (Control, F) spiked with Dog No. 16 (900 ppm, F)	1 + 0	220.2	220.2	100.0
	1 + 0.1	205.5	207.3	99.1
	1 + 0.2	199.9	196.6	101.7
	1 + 0.4	184.9	179.8	102.8
	1 + 0.6	176.8	167.2	105.8
	1 + 1	154.5	149.5	103.3
	0 + 1	78.8	78.8	100.0

[a]: calculated as: $\frac{V_{cont} \times A_{cont} + V_{treat} \times A_{treat}}{V_{tot}}$

Where: V: volume; A: ALAT activity; cont: sample of control animal; treat: sample of treated animal; tot: total. Data obtained from page 16 of the study report.

Table 3: ALAT activities in serum samples

Sample	Spiking ratio Control + Treated	ALAT activity (U/L)		
		Determined	Expected[a]	% of expected
Dog No. 1 (Control, M) spiked with Dog No. 7 (900 ppm, M)	1 + 0	46.4	46.4	100.0
	1 + 0.1	50.0	43.5	114.9
	1 + 0.2	40.8	41.1	99.2
	1 + 0.4	31.5	37.4	84.3
	1 + 0.6	32.7	34.6	94.6
	1 + 1	31.9	30.6	104.2
	0 + 1	14.8	14.8	100.0
Dog No. 2 (Control, M) spiked with Dog No. 8 (900 ppm, M)	1 + 0	30.2	30.2	100.0
	1 + 0.1	32.8	29.3	112.1
	1 + 0.2	32.0	28.5	112.4
	1 + 0.4	28.6	27.2	105.0
	1 + 0.6	30.7	26.3	116.7
	1 + 1	27.7	25.0	110.8
	0 + 1	19.8	19.8	100.0
Dog No. 9 (Control, F) spiked with Dog No. 15 (900 ppm, F)	1 + 0	20.1	20.1	100.0
	1 + 0.1	22.0	19.4	113.2
	1 + 0.2	20.1	18.9	106.4
	1 + 0.4	21.1	18.0	117.1
	1 + 0.6	18.1	17.4	104.2
	1 + 1	17.9	16.5	108.8
	0 + 1	12.8	12.8	100.0
Dog No. 10 (Control, F) spiked with Dog No. 16 (900 ppm, F)	1 + 0	29.8	29.8	100.0
	1 + 0.1	28.1	28.6	98.3
	1 + 0.2	28.5	27.6	103.3
	1 + 0.4	23.3	26.0	89.6
	1 + 0.6	23.7	24.8	95.5
	1 + 1	20.9	23.2	90.3
	0 + 1	16.5	16.5	100.0

[a]: calculated as: $\frac{V_{cont} \times A_{cont} + V_{treat} \times A_{treat}}{V_{tot}}$

Where: V: volume; A: ALAT activity; cont: sample of control animal; treat: sample of treated animal; tot: total. Data obtained from page 17 of the study report.

B. WESTERN BLOT

The ALAT protein was detected in control samples containing ALAT from porcine heart and in cytosol and serum samples.

As expected a band of 50 kDa could be detected in all samples. The analysis by immunodetection of the cytosolic and serum samples of control and animals treated at 900 ppm showed very similar levels of ALAT.

A densitometric evaluation showed that the ALAT detected quantities were very similar in control and high dose treated animals. However, due the high background and the apparent low cross-specificity of the antibody, the results were considered as provisional and need to be evaluated with care. Relevant images can be found in the original report.

Table 4: Densitometric quantification of ALAT in Western Blots of liver cytosol samples

Sample	Absolute intensity	Protein concentration (mg/mL)	Intensity/concentration
Control dogs			
No. 1 M	n.a.	1.69	n.a.
No. 2 M	2825	1.97	1429
No. 9 F	1694	1.88	899
No. 10 F	1600	1.98	807
Treated dogs			
No. 7 M	n.d.	-	n.d.
No. 8 M	1399	1.86	753
No. 15 F	1663	1.83	910
No. 16 F	2271	1.50	1515

n.a.: not applicable. The lane 5 (Dog No. 1) was partially contaminated with the marker due to overloading of lane 4
n.d.: not determined. No sample available
Data obtained from page 18 of the study report.

Table 5: Densitometric quantification of ALAT in Western Blots of serum samples

Sample	Intensity
Control dogs	
No. 1 M	1133
No. 2 M	1308
No. 9 F	1004
No. 10 F	1287
Treated dogs	
No. 7 M	1063
No. 8 M	1189
No. 15 F	1460
No. 16 F	1714

Data obtained from page 19 of the study report.

III. CONCLUSIONS

Based on the results of this study, the reduction of ALAT activity observed following oral treatment with fluensulfone is not considered to be caused by an inhibition of ALAT by fluensulfone or its metabolites.

Based on the protein expression results, a decrease of the expression of ALAT protein upon treatment with fluensulfone is unlikely, but cannot be excluded.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments/Conclusions: It is noted that it is not known how much, if any, fluensulfone or its metabolites was contained in the serum and liver cytosol fractions taken from treated animals and used in this study. Levels of fluensulfone or its metabolites were not determined in serum or liver cytosol. The time between the last dose and the collection of serum and liver cytosol is also not known. Metabolism and excretion of fluensulfone is rapid in rats based on metabolism studies. It is possible that there was very little fluensulfone or its metabolites present in the blood or cytosol used in this study. It is also noted that a different antibody was used in this study than was used in the 2 week dietary special study (MRID 48574776, PMRA 2181190).

This special study is acceptable/non-guideline (reliable with restrictions) as it does not satisfy any guideline requirement.

C. Deficiencies: No deficiencies were identified.

Tier II Summary **(PMRA #2181220, MRID 48574767)** revised by the regulatory authorities, April 2013

Study Type: Mechanism of action and supporting data

IIA 5.5.4– MRID 48574767, PMRA 2181220

Report:	Kaiser St. (2011) MCW-2 TECH: 3- and 7-Day Oral (Feeding) Mechanistic Lung Toxicity Study in Mice. Harlan Laboratories Ltd, Switzerland; unpublished Report D26983, dated 8 November 2011. Sponsor reference No. R-27479 Dates of experimental work: 8 June to 8 November 2011
Sponsor	Makhteshim Chemical Works Ltd, Beer-Sheva, Israel
Guidelines	Not applicable
GLP/Compliance	Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary

This mechanistic toxicity study (MRID 48574767, PMRA 2181220) was conducted with the purpose to analyze local cell proliferation in the lungs as a basis for assessment of the human relevance of observed lung tumors in mice.

Groups of 10 CD-1 female mice were treated via the diet with 0 ppm or 1200 ppm fluensulfone (MCW-2 tech., 96.17%, Batch No. 36372130-291-PF1) for 3 days or 7 days. An additional 10 females each were administered 1305 ppm Isoniazid (positive control, ≥99%, Batch No. 060M0090) for 3 or 7 days. Approximately 14 and 2 hours prior to necropsy, all animals received an intraperitoneal injection of BrdU for determination of cell proliferation. Lungs, brain, and a piece of the small intestine were collected from all animals at necropsy. The weights of the lungs and brain were recorded. Lungs and intestine were processed for the determination of BrdU positive cells and analyzed histopathologically.

There were no mortalities and no clinical signs. Decreased food consumption was recorded in females treated with 1200 ppm fluensulfone from day 1 to day 2 when compared to the respective controls. Afterwards the food consumption of these animals returned to a level comparable to the respective controls. Animals treated with 1305 ppm Isoniazid showed increased food consumption from days 5 to 8 when compared to the respective controls. A slight loss of body weight was recorded in animals treated with 1305 ppm Isoniazid and 1200 ppm

fluensulfone on day 4. However, a minimal loss of body weight was also recorded in the respective controls. A slight loss of body weight was also recorded in animals treated for 7 days. Analysis of organ weight data showed no effect of treatment either with fluensulfone or Isoniazid.

Labelling in small intestine demonstrated correct administration of BrdU. In animals treated for 3 days, a marked increase in BrdU positive cells was observed in the epithelium of the bronchioles in the lungs of animals treated with 1305 ppm Isoniazid and 1200 ppm fluensulfone. There was no significant difference in the severity and incidence of the change between the two groups treated with Isoniazid and fluensulfone. In animals treated for 7 days, no difference in the BrdU incorporation in the lung was observed among the groups.

Based on the results, female mice reacted to treatment with both fluensulfone and Isoniazid with increased cell proliferation that was detected by BrdU incorporation. The reaction to treatment was transient, with an effect at an early time point (after 3 days) that was not visible at the later time point (after 7 days).

This special study is classified as **acceptable/non-guideline** (reliable with restrictions) as it does not satisfy any guideline requirement.

I. MATERIAL AND METHODS

1. Test Material	MCW-2 TECH
Description:	Yellow solid
Lot/Batch:	36372130-291-PF1
Purity:	96.17%
CAS#:	318290-98-1
Stability:	Stable for the duration of the study (expiry November 30, 2011)
2. Positive control	Isoniazid
Supplier:	Fluka
Lot/Batch:	060M0090
Purity:	≥ 99% (TLC)
Stability:	Stable for the duration of the study (expiry May 20, 2012)
3. Vehicle	Pelleted standard Kliba Nafag 3433 rodent maintenance diet (Provimi Kliba AG, Switzerland)

4. Test Animals

Species	Mouse (female)
Strain	CD-1
Age	5 weeks (at delivery)
Weight	20.0 to 23.9 g (mean 22.0) grams at acclimatization
Source	Charles River Germany
Acclimation period	7 days
Diet	Pelleted standard Kliba Nafag 3433 rodent maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum*
Water	Community tap-water from Itingen *ad libitum* in water bottles
Housing	In groups of 2 in Makrolon type-II cages with wire mesh tops and standard softwood bedding (J. Rettenmaier & Söhne GmbH & Co. KG, Germany, imported by Provimi Kliba AG, Switzerland) including paper enrichment (Enviro-dri from Lillico, Biotechnology, UK)

5. Environmental conditions

Temperature	22 ± 3°C
Humidity	30-70%
Air change	approximately 10-15 changes per hour
Photoperiod	12-hour fluorescent light / 12-hour dark cycle. Music during the light period

B. STUDY DESIGN

1. In-life dates 8 to 21 June 2011

C. METHODS

1. Animal assignment and treatment

Ten animals were assigned to each group using a computer-generated random algorithm. Allocation A animals were treated for 3 consecutive days; allocation B animals were treated for 7 days. Group 1 received the same diet as treated groups, but without any additional substance; group 2 received 1305 ppm Isoniazid (an actual concentration of 2250 ppm was initially planned) in the diet; group 3 received 1200 ppm of fluensulfone in the diet (the same dose level used in the

carcinogenicity study).

2. Feed preparation

Dietary admixtures were prepared once. For the positive control group, Isoniazid was mixed with a small amount of purified water and then microgranulated feed. For the 1200 ppm test group, fluensulfone was warmed up to approximately 40°C until the test item was liquid and mixed with microgranulated feed. An appropriate amount of water was added to each admixture to aid pelleting. The pellets were dried with air for approximately 48 hours before storage. Feed for the control group was prepared similarly, but without test item. The feed preparations were analyzed using and HPLC method. The diet samples were stored deep-frozen until analysis. Concentration and homogeneity of feed preparations were determined in samples taken before the initiation of treatment and at the end of the treatment period.

3. Viability, Body Weights/Food Consumption and Clinical Signs

Animals were checked for viability/mortality twice daily. Clinical signs were observed twice daily on days 1 to 3 and once daily thereafter. Food consumption was measured daily in allocation A animals and twice weekly in allocation B animals. Body weights were taken twice weekly.

4. Bromodeoxyuridine (BrdU) treatment

Approximately 14 and 2 hours prior to necropsy all animals were treated with BrdU (in 1 X DPBS storage buffer) intraperitoneally (100 µL per mouse of a 10 mg/mL solution) for determination of cell proliferation.

5. Pathology

All animals were weighed and necropsied in the morning to assure that the animals were in their activity period and continuously eating until shortly before sacrifice starting approximately 2 hours after the last BrdU injection. Descriptions of all macroscopic abnormalities were recorded. All animals were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination. The animals were necropsied in the following order: 2 animals of group 1, 2 animals of group 2, 2 animals of group 3, 2 animals of group 1, etc. Allocation A and B animals were killed on Days 4 and 8, respectively.

Lungs, brain, a piece of the small intestine, and all gross lesions were collected from all animals at necropsy. The weights of the lungs and brain were recorded on the scheduled dates of necropsy and their organ to terminal body weight ratios as well as organ to brain weight ratios determined.

The lung and jejunum from all animals were processed, embedded and cut at an approximate thickness of 2 - 4 micrometers. Immunohistochemistry for detecting the BrdU signals was performed for the determination of BrdU positive cells in the lung. The jejunum was also immunohistochemically processed and checked for BrdU labeling to confirm correct i.p.

administration.

The lungs from 5 animals with the lower animal numbers of each group were also evaluated on the BrdU labeling index (the number of BrdU positive cells/ 1000 bronchial epithelial cells). BrdU labeling index was calculated by counting at least 500 cells of the bronchial epithelium of representative bronchioles of the respective animals.

6. Statistics

The following statistical methods were used to analyze the food consumption, body weight, macroscopic findings, as well as organ weights and ratios:

• The Dunnett-test (many to one t-test) based on a pooled variance estimate was applied if the variables could be assumed to follow a normal distribution for the comparison of the treated groups and the control groups for each sex.

• The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data could not be assumed to follow a normal distribution.

• Fisher's exact-test.

II. RESULTS AND DISCUSSION

A. Analysis of feed preparations

It was stated that the fluensulfone diet was stable for at least 21 days at room temperature based on results of stability analyses performed within Harlan Laboratories Study B80188 (the two-year dietary study in rats; study report R-23353). For the positive control diet, sufficient stability was determined by analysis at the beginning and end of the study.

The fluensulfone concentrations measured were within the acceptance limit of ±20% of the nominal concentration. Fluensulfone concentrations ranged from 80.7% to 89.1% of the nominal concentration. Concentrations per sample varied not more than 3.1% from the corresponding mean, thus confirming the homogeneity of the dose formulation.

The isoniazid concentrations measured were not within the acceptance limit of ±20% of the nominal concentration. Isoniazid concentrations ranged from 50.5% to 63.9% of the nominal concentration (2250 ppm). Therefore, it was decided to use the actual concentration (1305 ppm) for the presentation of results. A likely cause for the low recovery is reaction of isoniazid with reducing components (sugars, amino acid side chains, etc.) in the feed, favored by the conditions of the pelleting, e.g. pressure, temperature and the water added to aid pelleting.

B. OBSERVATIONS

There were no mortalities during the study, and no clinical signs were noted.

Decreased absolute (g/animal/day; -66%) and relative (g/kg bw/day; -65%) food consumption were recorded in allocation A females treated with 1200 ppm fluensulfone from day 1 to day 2

when compared to the respective controls. Afterwards the food consumption of these animals returned to a level comparable to the respective controls. Allocation B animals treated with 1305 ppm Isoniazid showed an increased absolute (+77%) and relative (+72%; not statistically significant) food consumption from days 5 to 8 when compared to the respective controls.

A slight loss of body weight was recorded in allocation A animals treated with 1305 ppm Isoniazid (-2.0%) and 1200 ppm fluensulfone (-4.5%) on day 4 when compared to their respective body weights on day 1. However, a slight loss of body weight (-1.6%) was also recorded in the respective controls. A slight loss of body weight (-2.0%) was also recorded on day 5 for allocation B animals treated with 1200 ppm fluensulfone.

C. PATHOLOGY

No effects of the treatment with either Isoniazid or fluensulfone on organ weights were recorded. Increased brain to body weight ratios (+9%) in animals treated with Isoniazid or fluensulfone and decreased lung to brain weight ratio (-6%) recorded in animals treated with Isoniazid recorded on day 4 were considered to be effects secondary to the lower body weights in these groups on day 5 (-8% relative to control), from which they recovered by day 8. Increased lung weights relative to the brain (+8%) recorded in animals treated with Isoniazid on day 8 were considered to be an incidental effect caused by slightly lower brain weights (-4%) in the respective group.

No macroscopic findings were noted at scheduled necropsy.

BrdU labeling was detected in the crypt epithelium of the jejunum of all animals and hence, the correct intraperitoneal administration was confirmed.

In allocation A animals (3 days of treatment), a marked increase in BrdU positive cells was observed in the epithelium of the bronchioles in the lungs of animals treated with 1305 ppm Isoniazid and 1200 ppm fluensulfone (Table 1). There was no significant difference in the severity and incidence of the change between the two groups treated with Isoniazid and fluensulfone (Table 2).

In allocation B animals (7 days of treatment), no difference of the BrdU incorporation in the lung was observed among the groups.

Table 1: BrdU labeling Index in bronchiolar epithelium (mean ± SD)

	Group 1 (control)	Group 2 (Isoniazid 1305 ppm)	Group 3 (fluensulfone 1200 ppm)
Allocation A (3 days treatment)			
Number of BrdU positive cells	17 ± 4	82 ± 48** (↑382%)	74 ± 35** (↑335%)
Number of BrdU positive cells per 1000 cells	25 ± 5	119 ± 69** (↑376%)	120 ± 40** (↑380%)
Allocation B (7 days treatment)			
Number of BrdU positive cells	11 ± 6	4 ± 3	9 ± 5
Number of BrdU positive cells per 1000 cells	17 ± 10	7 ± 5	13 ± 4

** Fisher's exact test; significant at 1%. Data obtained from pages 26-27 of the study report. Values in parentheses represent the percent change from the control mean (calculated by the reviewer).

Table 2: Incidence / Mean severity grade of BrdU incorporation in the epithelium of bronchioles

	Group 1 (control)		Group 2 (Isoniazid 1305 ppm)		Group 3 (fluensulfone 1200 ppm)	
Allocation	A	B	A	B	A	B
Number of animals	10	10	10	10	10	10
Increased BrdU incorporation	-	-	7 / 1.3	-	8 / 1.4	-

Grade 1: minimal / very few / very small; Grade 2: slight / few / small; -: no increase in BrdU incorporation; data obtained from page 25 of the study report.

III. CONCLUSIONS

All animals survived the scheduled study period, and no clinical signs were recorded. Only slight effects on food consumption and body weight development were recorded.

There were no gross lesions or effects of the treatment with test or control item on organ weight.

Microscopic examination in the lung revealed an increase in BrdU incorporation in the epithelium of the bronchioles in the animals treated with Isoniazid or fluensulfone for 3 days compared to control animals. There was no significant difference in the severity and incidence of the change between the two groups treated with Isoniazid and fluensulfone.

BrdU incorporation was not increased in the animals treated with the above two items for 7 days compared to the animals which received identical feed without test item for the same duration.

Based on the results, the animals react to treatment with both Isoniazid and fluensulfone with increased cell proliferation that was detected by BrdU incorporation. The reaction to treatment is transient with an effect at an early time point (after 3 days) that was not visible at the later time point (after 7 days).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: The reviewer agrees with the study investigators' conclusions.

This special study is acceptable/non-guideline (reliable with restrictions) as it does not satisfy any guideline requirement.

C. Conclusions: The results of this study demonstrate that dietary exposure to fluensulfone elicits a transient increase in cell proliferation in the lungs of CD-1 mice. These study results will be considered in the evaluation of the applicant's proposed mode of action for lung tumours observed in mice dosed with fluensulfone. It is noted that only one dose was tested in this study, and as such the dose response of the cell proliferation could not be ascertained.

D. Deficiencies: No deficiencies were identified.

Tier II Summary **(PMRA #2181219, MRID 48574768)** revised by the regulatory authorities, April 2013

Study Type Mechanism of action and supporting data

IIA 5.5.4– MRID 48574768, PMRA 2181219

Report:	Jaeger M. (2011) Comparative biotransformation of MCW-2 in human and mice lung microsomes. Harlan CCR, Germany; unpublished Report 1388500, dated 15 December 2011. Sponsor reference No. R-23346 Dates of experimental work: 27 April to 1 August 2011
Sponsor	Makhteshim Chemical Works Ltd., Beer Sheva, Israel
Guidelines	Not applicable
GLP/Compliance	Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided

Executive Summary

The objective of this study (MRID 48574768, PMRA 2181219) was to compare the metabolic activity of lung microsomes of mice and humans towards fluensulfone (MCW-2, 98.6%, Batch No. 031H0722), and to determine the contribution of Cyp 2f2 (mice specific) and CYP 2E1/Cyp 2e1 (expressed in humans and mice) to the metabolism of MCW-2, by co-incubation with and without the selective inhibitors, 4-methyl pyrazole (CYP 2E1/Cyp 2e1) and 5-phenyl-1-pentyne (Cyp 2f2).

The metabolic activity was determined by incubation of lung microsomes with fluensulfone in the presence of an NADH-regenerating system, and analytical determination of remaining (unmetabolized) fluensulfone by LC-MS/MS at different time points. Two concentrations of fluensulfone were tested (2 and 20 µM); however, the highest concentration was found to be too high (only a small percentage was metabolized) and the results obtained were considered only as confirmatory. The metabolism was further investigated by addition of inhibitors of CYP 2E1 and CYP 2f2 (a mouse-specific isoform). Lung microsomes were prepared from 12 female and 12 male untreated mice, and their activity towards fluensulfone was compared with that of lung microsomes pooled from at least 10 different human non-smokers.

The metabolic capability of the microsomal preparations was confirmed by determination of the turnover of a standard substrate, chlorzoxazone, under conditions similar to those used for fluensulfone.

The detected activity towards chlorzoxazone was approximately 450-fold lower in human lung microsomes compared to mouse lung microsomes, and thus in good correlation with the activity differences reported in the literature.

With human lung microsomes, no metabolism of fluensulfone was detectable. In contrast, in mice female and male lung preparations only approximately 10% of the applied fluensulfone (2 µM)

remained after 120 minutes of incubation.

The negative control without NADH regenerating system showed no metabolic activity in humans as well as in both mice genders showing the validity of the assay and indicating an enzymatic degradation of fluensulfone.

The inhibition of (mouse-specific) Cyp 2f2 (with 5-phenyl-1-pentyne) had a more pronounced effect on the degradation of fluensulfone than inhibition of CYP 2E1 (with 4-methyl pyrazole) which was essentially not effective, although the metabolic activity towards fluensulfone was not abolished completely.

Under the conditions of this study, no metabolic activity towards fluensulfone was detectable after incubation with human lung microsomes of non-smokers. In contrast, fluensulfone was extensively metabolized by lung microsomes of female and male mice, and the mice-specific isoenzyme Cyp 2f2 appears to play a major role in the degradation process.

This special study is classified as **acceptable/non-guideline** (reliable with restrictions) as it does not satisfy any guideline requirement.

I. MATERIAL AND METHODS

1. Test Material	MCW-2 Analytical standard
Description:	Not given
Lot/Batch:	326-115-02
Purity:	98.6% by HPLC
CAS#:	318290-98-1
Stability:	Stable for the duration of the study (expiry date 4 November 2012)
2. Internal standard	Chlorpropamide
Supplier:	Not reported
Lot/Batch:	031H0722
Purity:	99.9%
Stability:	Stable for the duration of the study (expiry date September 2011)
3. Substrate	Chlorzoxazone
Supplier:	Not reported
Lot/Batch:	070M1256V
Purity:	100%
Stability:	Stable for the duration of the study (expiry date January 2016)
4. Metabolite	6-hydroxy-chlorzoxazone
Supplier:	Not reported

Lot/Batch:	1385519
Purity:	99.3%
Stability:	Stable for the duration of the study (expiry date January 2016)
5. Inhibitors	a. 4-methylpyrazole hydrochloride (CYP2E1)
	b. 5-phenyl-1-pentyne (Cyp 2f2)
Supplier:	Not reported
Lot/Batch:	a. 109K1145
	b. E07U001
Purity:	a. 97.9%
	b. 99.2%
Stability:	a. Stable for the duration of the study (expiry date September 2011)
	b. Stable for the duration of the study (expiry date January 2016)
6. Vehicles	Methanol (for internal standard, substrate, metabolite and inhibitors)
	Dimethylsulfoxide (for MCW-2)
7. Test System	Lung microsomes
a. Species	Human
Number of donors	At least 10
Sex	Mixed gender
Other specifications	Non-smokers. Ages: 7-63 years, Lot ORP
Supplier	Celsis Invitro technologies, Baltimore, USA
b. Species	Mouse
Strain	ICR (CD-1)
Number and sex	12 for each sex
Age	9-10 weeks
Weight	Males: mean of 38.05 g; females: mean of 28.03 g
Source	Harlan Laboratories, The Netherlands
Microsomes preparation	The animals were sacrificed by carbon dioxide asphyxiation, the lungs excised, snap-frozen in liquid nitrogen and stored at -80°C until microsome preparation.
	The frozen mouse lung tissue was removed from the freezer and weighed. The tissue was immediately cut into small pieces using a scalpel and transferred to plastic vials. The tissue was covered with

ice-cooled HM buffer (approximately 4°C) and was homogenized with an ultraturrax. The homogenates were centrifuged to remove cell debris at 9000xg for 20 min at 4°C. Afterwards the supernatants were transferred to fresh plastic vials and centrifuged at 138000xg at 4°C for 60 min. After centrifugation the sediment was resuspended in fresh HM buffer and the centrifugation was repeated under the same conditions. The sediment was finally resuspended in HM buffer. After removing a small aliquot for the protein determination, the lung microsomal fractions were frozen in liquid nitrogen and stored until use at –80°C.

B. STUDY DESIGN

1. In-life dates 27 April to 1 August 2011

C. METHODS

1. Protein content determination

The protein content of mice lung microsomes was determined for each sex separately. Aliquots were diluted, and the absorption at 595 nm was measured in a microplate reader and compared to that of standard dilution series of bovine serum albumin. Each sample (standard calibration curve and sample dilution) was run in duplicate.

2. Determination of CYP Enzyme Activity towards fluensulfone

Two concentrations of fluensulfone (2 µM and 20 µM corresponding to 0.584 and 5.84 µg/mL, respectively) were incubated with the different microsomal preparations from human lung tissue and mice lung tissue (final protein concentration 1.0 mg/mL and 0.5 mg/mL, respectively) and with or without the specific inhibitors, 4-methyl pyrazole (CYP 2E1, 10 µM) and 5-phenyl-1-pentyne (Cyp 2f2, 5 µM). The total incubation time was 120 minutes; samples were taken at 0, 30, 60, 90 and 120 minutes from all samples. Three replicates were prepared.

3. Sampling

Samples of 75 µL were taken after each mentioned time point from each replicate. The samples were transferred immediately to vials containing 75 µL methanol/chlorpropamide (internal standard, 775 ng/mL chlorpropamide) for all samples (including positive control samples) and stored at -20°C until analysis. The concentration of MCW-2 was determined quantitatively using a LC-MS/MS method.

4. Positive and negative controls

In the main experiment the performance of the assay was checked by using the reference substrate of CYP 2E1, chlorzoxazone. Inhibition with 4-methyl pyrazole (CYP 2E1) and 5-phenyl-1-pentyne (Cyp 2f2) was also determined. 25 µL microsomes (human), 66 µL (female mice) and 55 µL (male mice) microsomes [1.0 mg protein/mL and 0.25 mg protein/mL, respectively] were used.

As negative control MCW-2 was incubated without NADH to determine possible non-enzymatic and non-specific degradation.

5. Evaluation of results

The metabolic stability of fluensulfone in the presence of human and female and male mouse lung

microsomes was determined by measuring the decrease of the test item. The influence of CYP 2E1 and Cyp 2f2 was determined by comparing the activity towards MCW-2 after co-incubation with the selective inhibitors, 4-methyl pyrazole (CYP 2E1) and 5-phenyl-1-pentyne (Cyp 2f2) by comparison with the solvent control.

II. RESULTS AND DISCUSSION

A. PROTEIN CONCENTRATION IN LUNG MICROSOMES

The protein concentration of the human lung microsomes was determined by the supplier to be 20 mg protein/mL.

The protein concentrations of the mice lung microsome preparations were determined to be 1.9 mg protein/mL (female mice) and 2.3 mg protein/mL (male mice).

B. Determination of CYP enzyme activity towards fluensulfone

The activity of CYP 2E1 within each microsomal fraction was determined by the addition of a standard substrate, chlorzoxazone, and measuring 6-hydroxychlorzoxazone (its relevant metabolite) to show that all microsomes contained a measureable amount of CYP 2E1. Additionally the influence of the isoenzymes CYP 2E1 (expressed in human and mice) and Cyp 2f2 (mice-specific) on chlorzoxazone metabolism was evaluated by incubation with the selective inhibitors 4-methyl pyrazole and 5-phenyl-1-pentyne. CYP 2E1 and Cyp 2f2 activities in lung microsomes toward chlorzoxazone are reported in Table 1 and measured amounts of 6-hydroxychlorzoxazone produced in the presence of cytochrome inhibitors are reported in Table 2.

Table 1: CYP 2E1 and Cyp 2f2 activities in lung microsomes toward chlorzoxazone (30 minutes incubation)

Species	Microsomal protein (mg/mL)	Measured 6-hydroxy chlorzoxazone		CYP 2E1 and/or Cyp 2f2 activity (pmol/mg/min)
		(ng/mL)	(nM)	
Human	1.0	1.04	5.60	0.187
	1.0	1.19	6.41	0.214
	1.0	1.38	7.44	0.248
Mean	**1.0**	**1.20**	**6.48**	**0.216**
Mouse, female	0.25	124	668	89.1
	0.25	128	690	92.0
	0.25	108	582	77.6
Mean	**0.25**	**120**	**647**	**86.2**
Mouse, male	0.25	115	620	82.6
	0.25	87.7	473	63.0
	0.25	83.0	447	59.6
Mean	**0.25**	**95.2**	**513**	**68.4**

Data obtained from page 30 of the study report. Data are presented as individual values for each replicate (3 replicates) and the mean of 3 replicates.

Table 2: Amount of 6-hydroxychlorzoxazone produced by CYP 2E1 and Cyp 2f2 (with chlorzoxazone applied) in the presence of specific inhibitors (solvent control set as 100%)

Species	% of 6-hydroxychlorzoxazone compared to corresponding solvent control	
	With 10 µM 4-methyl pyrazole (CYP 2E1 inhibitor)	With 5 µM 5-phenyl-1-pentyne (Cyp 2f2 inhibitor)
Human	49.1	76.5
Mouse, female	84.2	8.63
Mouse, male	72.6	8.29

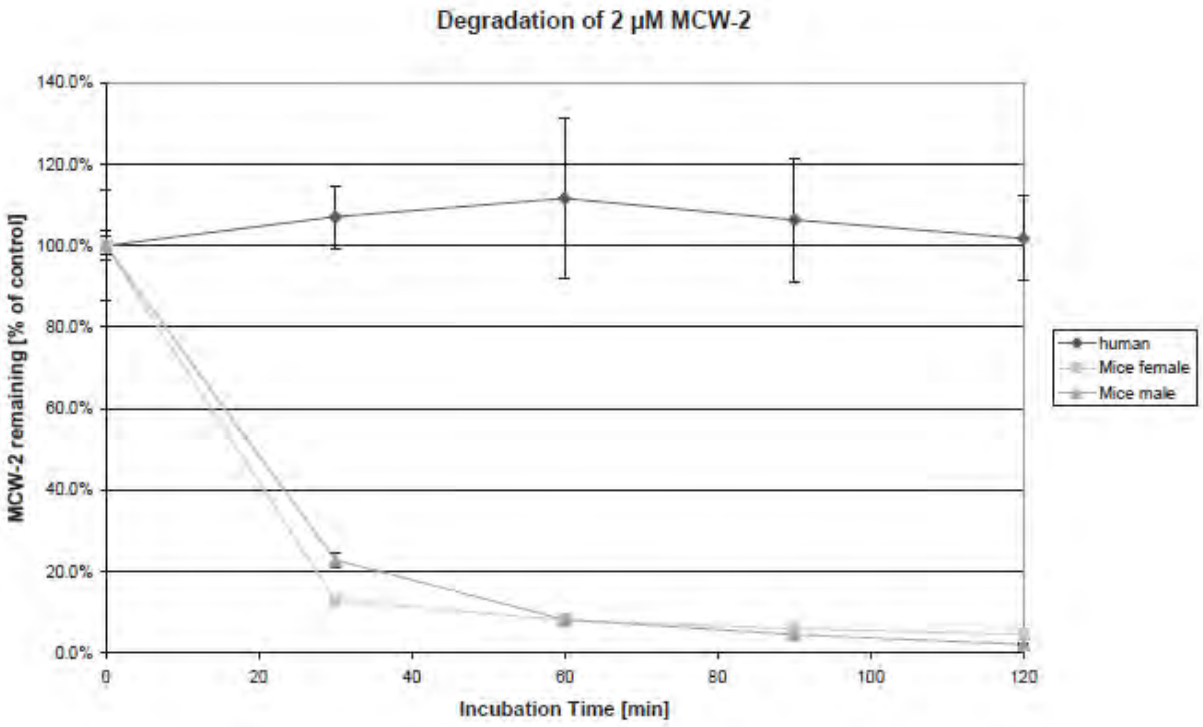
Data obtained from page30 of the study report.

The degradation of fluensulfone was measured after application of two different concentrations, 2 µM and 20 µM, by monitoring the fluensulfone content of the samples taken after 0, 30, 60, 90 and 120 minutes. To detect the influence of the mice specific isoenzyme Cyp 2f2, the degradation of fluensulfone was measured in the presence and absence of the selective inhibitors 4-methyl pyrazole (CYP 2E1, 10 µM) and 5-phenyl-1-pentyne (Cyp 2f2, 5 µM).

The enzyme activity applied within the microsomal fraction was at the maximum velocity with 20 µM, but degradation was not complete. In contrast, with the lower fluensulfone concentration (2 µM) the degradation was nearly complete, in mice at least. Because of this, the effects detectable after incubation with and without specific inhibitors were only obvious with the lower concentration of fluensulfone applied. Therefore, the measured values of the higher concentration were reported, but considered only as supportive and not discussed.

No measurable degradation was detected during the incubation with human lung microsomes over the total incubation time of 120 minutes. In contrast, the incubation with mouse lung microsomes, from female as well as from male mice, led to a conversion of fluensulfone of more than 90% after 120 minutes although only ½ of the human microsomal protein concentration was used (Figure 1)

Figure 1: Time-course of degradation of fluensulfone (2 µM) incubated for 120 minutes with lung microsomes

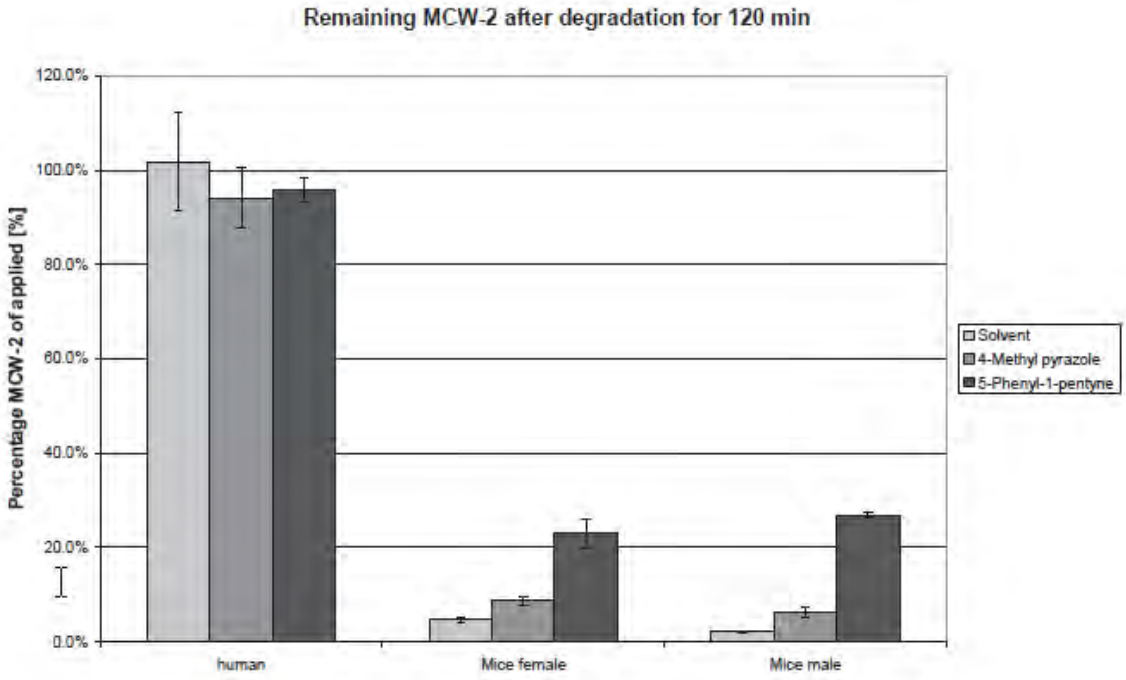


Thereafter fluensulfone (2 µM) was incubated with the lung preparations in the presence and absence of specific inhibitors for CYP 2E1 and Cyp 2f2. The inhibition of Cyp 2f2 had a more pronounced effect on the degradation of fluensulfone although the metabolic activity was not completely abolished. Table 3 reports the remaining amounts of fluensulfone after 120 minutes incubation in the presence of specific inhibitors. A graphical presentation is given in Figure 2.

Table 3: Percentage of remaining of fluensulfone after 120 minutes incubation in the presence of specific inhibitors for CYP 2E1 and Cyp 2f2, compared to correspondent time 0 (taken as 100%)

Species	Solvent control (NADH and DMSO)	% of fluensulfone after 120 min compared to corresponding time 0	
		With 10 µM 4-methyl pyrazole (CYP 2E1 inhibitor)	With 5 µM 5-phenyl-1-pentyne (Cyp 2f2 inhibitor)
Human	101.8	94.1	95.9
Mouse, female	4.6	8.7	23.0
Mouse, male	2.1	6.2	26.9

Figure 2: Remaining fluensulfone after 120 minutes incubation in the presence of specific inhibitors for CYP 2E1 and Cyp 2f2



III. CONCLUSIONS

The study was performed to compare the relevance of the metabolic activity of human and mouse lung tissue towards fluensulfone.

Under the conditions of this study no metabolic activity towards fluensulfone was detectable after incubation with human lung microsomes of non-smokers. In contrast, fluensulfone is metabolized by lung microsomes of female and male mice and it appeared that the mice specific isoenzyme Cyp 2f2 plays a major role in the degradation process.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: The reviewer agrees with the study investigators' conclusions.

This special study is acceptable/guideline (reliable with restrictions) as it does not satisfy any guideline requirement.

C. Conclusions: The results of this study demonstrate that the metabolic activity of mouse lung microsomes toward fluensulfone is much greater than that of human lung microsomes. These study results will be considered in the evaluation of the applicant's proposed mode of action for lung tumours observed in mice dosed with fluensulfone. It is noted that 5-phenyl-1-pentyne is not a very selective inhibitor for CYP2F2, as it has overlapping specificities with other CYP enzymes.

D. Deficiencies: No deficiencies were identified.

Tier II Summary (PMRA #2181330, MRID 48574950) revised by the US EPA, April 25, 2014

Study Type: Comparative dermal absorption, in vitro using rat and human skin

IIA 7.6.1 - MRID 48574950, PMRA 2181330

Report	Hassler S. (2011b) MCW-2: Percutaneous Penetration of ^{14}C-MCW-2 Formulated as MCW-2 480 EC Through Rat and Human Split-thickness Skin Membranes (*in vitro*) Harlan Laboratories Ltd, Switzerland; unpublished Report C56884, dated 27 September 2011. Sponsor reference No. R-23332. Dates of experimental work: 11 April to 12 May 2011. Dates of experimental work: 31 May to 2 December 2011
Sponsor	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines	OECD 428, EPA OPPTS 870.7600 and EEC method B.45 Deviations: none
GLP/Compliance	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive summary:

The comparative percutaneous penetration of fluensulfone, formulated as MCW-2 480 EC (emulsifiable concentrate), was investigated *in vitro* using split-thickness skin membranes from rat and human skin (MRID 48574950; PMRA 2181330).

The skin membranes were set up in flow-through diffusion cells and ^{14}C-fluensulfone, formulated as MCW-2 480 EC, was applied onto the skin membranes at two dose levels: a low dose (0.10 mg/cm^2) reflecting a typical concentration recommended for the use in the field as spray dilution, i.e. 10 g a.i./L (achieved by dilution of the commercial product with water), and a high dose (4.8 mg/cm^2) which represents the concentration of active ingredient in the undiluted commercial product, i.e. 480 g a.i./L. The exposure of the test item was performed under non-occluded conditions over an exposure time of 24 hours. During the exposure period the receptor fluid (ethanol/water 1:1 v/v) was collected in hourly intervals between 0-6 hours and thereafter in 2 hours intervals until the end of the experiments. Volatilized test item was trapped by covering the donor cell with charcoal paper during exposure. At the end of the experiment, the remaining ^{14}C-fluensulfone was removed from the skin surface by rinsing. The skin membranes were removed from the diffusion cell and consecutively stripped until the *stratum corneum* was removed.

The test item was stable on the skin membranes during the exposure period of 24 hours; analysis of skin membrane rinse pools revealed essentially unchanged fluensulfone.

The totally absorbed test item was calculated based on the amount that penetrated through the skin membrane (perfusate), the amount measured in tape strips (including the two first strips because of the study absorption monitoring duration of only 24 hr), and the amount measured in the remaining skin membrane layers after tape stripping. The total potentially absorbable dose was calculated to be 93.5% and 83.99% (low and high dose), and 65.35% and 6.18% (low and high dose) for rat and human skin membranes, respectively.

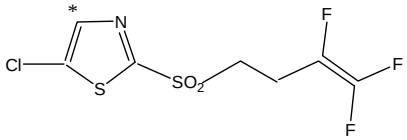
I MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	MCW-2 (Fluensulfone)
Description:	Crystalline solid
CAS number:	318290-98-1
Batch number:	a. 36372130-291-PF2 (low dose) b. 36372130-291-PF1 (high dose)
Purity:	a. 97.5% and b. 95.92%
Expiry of test compound:	a. 27 January 2012 b. 31 May 2011

2. Radiolabelled Test Material:

Description: MCW-2 [Ring-U-^{14}C]



* Indicates position of ^{14}C label

Batch number: TJBIOS-NB21-33

Specific activity: 56.7 mCi/mmol (2098 MBq/mmol; 7.185 MBq/mg when >99% pure)

Radiochemical purity: 83.3% (by TLC). Compound was purified before use in the study

Expiry of test compound: The radiochemical purity was checked at the time of dosing

3. Blank Formulation: Blank for MCW-2 480 EC

Description: Not given

Batch number: 100304

Stability of formulation: 4 March 2012

4. Test system:

Species: Rat [HanRcc: WIST (SPF)]; 4 males, 9 weeks old

Area of skin samples: Dorsal

Species: Human

Area of skin samples: Upper leg dorsal.

B. STUDY DESIGN

1. Experimental dates: 11 April to 12 May 2011

Skin samples: Rats were obtained from Harlan Laboratories, B.V., The Netherlands. The rats were sacrificed by an overdose of carbon dioxide gas. After sacrifice, the fur on the dorsal site was clipped and the full thickness skin excised. The skin was wrapped in aluminum foil and stored at about -18°C.

Human cadaver skin from Caucasian donors (1 male, 64 years old, and 3 females, 52, 75 and 67 years old, respectively) was obtained from the “Institut für Pathologie, Kantonsspital Basel”, Switzerland. Upon receipt the skin was stored at about -18°C until preparation of the skin membranes.

Receptor fluid: Ethanol/water (1:1 v/v). The solubility of the test item in the receptor fluid represented no penetration limiting factor.

Skin preparation: The skin samples were removed from the freezer and allowed to thaw at room temperature. The subcutaneous fat was carefully removed from the full thickness skin and pieces of about 4 x 5 cm were stretched evenly over a cork block, with *stratum corneum* uppermost. Skin sections of about 200 µm thickness were cut off from the top using a dermatome. The skin membranes were wrapped in aluminum foil and stored refrigerated (+5°C) until use.

Flow-through diffusion cell preparation: An automated flow-through cell system was used. Seven flow-through diffusion cells designated cell 1 through cell 7 were placed in one aluminum manifold connected to a water bath to maintain the temperature of the skin membranes at 31 ± 1°C. The diffusion cells of the second part of the system with the same setup were designated cell 8 through cell 14. Each diffusion cell consisted of a donor and receptor chamber. The area of skin membrane exposed to the donor chamber was 0.64 cm^2. The receptor chambers were connected to a multi-channel peristaltic pump. The pump speed was adjusted to about 3 mL/h. During the given time intervals, the effluent from the cell was collected directly into vials on a fraction collector.

Pieces of the skin membranes (approximately 1.8 x 1.8 cm) were cut and mounted in the diffusion cells between the donor and receptor chamber, 7 cells per species each. The cells were placed in the manifolds and connected to the peristaltic pump. For an equilibration period of 0.5 - 1 hour, a saline (0.9% NaCl w/v) was pumped through the receptor chamber at a flow rate of about 3 mL/h.

Barrier integrity assessment: The integrity of the skin membranes was checked by applying 50 µL tritiated water (about 200000 dpm) to the skin membrane surface. The donor chamber was covered with adhesive tape (occluded conditions). The cumulative penetration was determined over 6 hours by collecting hourly fractions. The permeability coefficient (Kp) of each skin membrane was calculated for the 3 - 6 hours interval. Rat skin membranes with $Kp > 3.5 \cdot 10^{-3}$ cm/h and human membranes with $Kp > 2.5 \cdot 10^{-3}$ cm/h were excluded from the subsequent experiment.

After 6 hours, adhesive tape was removed from the donor cell chamber and the cells left open overnight with the saline flowing through the receptor chamber.

Test substance preparation:

The formulations were prepared separately for each dose group. A stock solution was prepared first by dissolving ^{14}C labeled MCW-2 (10 mg) in 10 mL ethyl acetate:hexane (1:3 v:v).

For the low dose, unlabeled test item (93.31 mg) was placed in a flask and an aliquot of the stock solution (5 mL) containing 4.14 mg 14C-labeled MCW-2 (7185 kBq/mg) was added. The labeled and unlabeled test items were totally dissolved. This procedure yielded 14C-labeled test item with a final specific radioactivity of 305 kBq/mg. The solvent was removed under a gentle stream of nitrogen and 150.6 mg blank formulation was added (density of 1.2 g/mL). The formulated test substance was mixed with water (9.77 mL) to achieve a final volume of 10 mL. The final application suspension had an expected concentration of 9.74 mg MCW-2/mL.

For the high dose, unlabeled test item (1447.1 mg) was placed in a flask and an aliquot of the stock solution (3.7 mL) containing 3.0 mg 14C-labeled MCW-2 (7185 kBq/mg) was added. The labeled and unlabeled test items were totally dissolved. This procedure yielded 14C-labeled test item with a final specific radioactivity of 14.66 kBq/mg. The solvent was removed by rotary evaporation and 2163.6 mg blank formulation was added resulting in a final volume of 3 mL MCW-2 SC formulation (density 1.2 g/mL). The final application suspension had an expected concentration of 483.4 mg MCW-2/mL.

The radiochemical purity of the test item in the respective formulation was checked by TLC at the time of the application. The administration emulsions/solutions were used on the same day.

Application to the skin:

Cells with skin membranes of acceptable permeability coefficient (Kp) values in the integrity test were arranged on the manifold and a 6 µL aliquot of the application emulsion/solution was applied to the surface of the skin membranes. In order to prevent uncontrolled loss of volatile test substance the donor chamber was covered with an active charcoal filter paper (Rotilabo®) on the inside and a permeable tape on the outside (non-occlusive conditions). The receptor fluid ethanol/water (1:1 v/v) was delivered at a flow rate of about 3 mL/h during the testing period. The perfusates were collected at ambient temperature in time intervals as follows:

0 - 6 hours: 1 hour intervals (6 intervals)

6 - 24 hours: 2 hours intervals (9 intervals)

Temperature: 31 ± 1°C

Duration of exposure, sampling and terminal procedures:

Exposure lasted 24 hours. Twenty-four hours after application the perfusate sampling was terminated. The skin membrane surface was rinsed 3 times with mild shower gel solution (1% in tap water) followed by one time with 0.5 mL water. All skin membrane rinse fractions were combined according to the individual cells. The skin membranes were removed from the diffusion cell and consecutively stripped until the *stratum corneum* was removed from the skin membrane, i.e.15 tape strips were needed. The first 5 stripping tapes were analysed individually (Tape Strips I-V) and the stripping tape 6 to 10 and 11-15 were combined to a specimen pool of 5 tape strips each. Aliquots of each specimen were measured for radioactivity after solubilization with tissue solubilizer. The skin membranes remaining after stripping were digested in tissue solubilizer and the radioactivity was determined by liquid scintillation counting (LSC). The donor chamber was rinsed with ethanol (donor cell wash).

Data:

Liquid specimens, i.e. perfusate, skin membrane rinse, cell wash and charcoal paper extracts were added directly to scintillation mixture Irgasafe for the measurement of radioactivity. The radioactivity in tape strips and skin membranes was determined after digestion in Solvable tissue solubilizer. Aliquots of the digested specimens were mixed with Irgasafe (ca. 10 mL) prior to LSC. Background values were measured with each specimen sequence using the respective scintillation mixture without any specimens. All specimens were corrected for background by subtraction of appropriate blank values from specimen count rates to give net "dpm" per specimen.

II RESULTS AND DISCUSSION

The mean permeability coefficients (Kp) of tritiated water were 1.63 ± 0.48 and 1.33 ± 0.92 for the low and high dose levels, respectively, for the rat skin. Correspondent values for the human skin were 1.53 ± 0.34 and 1.69 ± 0.51.

The *in vitro* percutaneous penetration of fluensulfone through rat and human skin membranes is summarized in the table below:

Table 1: Summary of MCW-2 480 EC penetration, following topical application to rat and human skin membranes

Dose level	Low dose	High dose
Applied dose (µg/cm^2)	95.3	4792
Applied volume (µL)	6	6
Application area (cm^2)	0.64	0.64
Concentration (mg/mL)	10.2	511

Rat skin membranes				
Penetration (into receptor fluid) within	% of dose	µg/cm^2	% of dose	µg/cm^2
10 h	75.74±5.64	72.21±5.37	26.91±6.44	1289.4±308.5
24 h	87.02±1.90	82.97±1.82	48.82±12.62	2339±604.5
Human skin membranes				
Penetration (into receptor fluid) within	% of dose	µg/cm^2	% of dose	µg/cm^2
10 h	39.41±18.71	37.59±17.83	0.97±0.46	46.3±26.8
24 h	58.54±25.33	55.82±24.15	2.74±1.25	131.2±60.1

Data extracted from Summary Table on p. 26 of MRID 48574950 (PMRA 2181330). ±Standard deviations extracted from Tables 5-8, presented for the 24 h interval, and calculated for the 10 hr interval.

In rats, after application of ^{14}C-fluensulfone at the low dose level, a fast penetration of radioactivity through the skin membrane was observed, accounting to 75.74% within 10 hours, and to 87.02% within 24 hours. At the high dose level (undiluted emulsifiable concentrate MCW-2 480 EC) the portion penetrating through the skin membrane during a 24 hours exposure accounted for 48.82% of the dose.

At both dose levels the penetration through human skin membrane was significantly lower than that in rats. Within 24 hours 58.54% of the low dose and 2.74% of the high dose penetrated through the human skin membrane.

The total potentially absorbable test item was calculated based on the amount penetrated through the skin membrane (perfusate), the amount measured in all tape strips, and the amount measured in the remaining skin membrane layers after tape stripping. The first two tape strips were included in the total calculation of potentially absorbable dose because the duration of monitoring was only 24 hr. Results, together with distribution of radioactivity in the different compartments and total recoveries, are summarized in the table below.

Table 2: Summary of dermal absorption and total recovery of MCW-2 480 EC, following topical application to rat and human skin membranes (percent unless indicated otherwise)

	Rat skin membranes		Human skin membranes	
Dose level	Low	High	Low	High
Applied dose (µg/cm^2)	95.3	4792	95.3	4792
Perfusate (0-24 h)	87.02±1.90	48.82±12.62	58.54±25.33	2.74±1.25
Remaining dose				

Skin rinse	3.65±0.67	6.04±3.77	21.10±16.62	85.12±2.37
Charcoal paper	1.18±0.43	1.09±0.26	8.89±2.60	1.42±0.93
Tape strips				
Tape strip I	0.78±0.27	1.51±1.01	1.98±1.38	1.02±0.66
Tape strip II	0.61±0.22	1.22±1.07	0.91±0.51	0.51±0.35
Subtotal I-II	*1.39*±0.44	*2.73*±2.07	*2.89*±1.87	*1.53*±0.99
Tape strip III	0.56±0.20	0.87±0.70	0.55±0.33	0.30±0.22
Tape strip IV	0.58±0.47	0.75±0.46	0.36±0.28	0.20±0.17
Tape strip V	0.55±0.25	0.63±0.31	0.30±0.22	0.12±0.09
Tape strip VI	1.67±1.06	1.85±1.09	0.65±0.50	0.34±0.28
Tape strip VII	0.57±0.68	0.96±0.43	0.28±0.18	0.11±0.10
Subtotal III-VII	*3.93*±1.86	*5.06*±2.79	*2.14*±1.45	*1.08*±0.80
Total tape strips	5.31±1.61	7.79±4.62	5.03±3.24	2.60±1.75
Skin membrane	1.17±0.94	27.37±12.80	1.78±0.51	0.84±0.50
Donor cell wash	0.24±0.49	2.44±1.18	0.82±0.43	1.07±0.69
Total remaining dose	11.56±2.48	44.74±9.91	37.62±22.48	91.06±2.70
Total potentially absorbed dose*	**93.50**±0.76	**83.99**±5.33	**65.35**±22.20	**6.18**±2.62
Recovery	**98.57**±0.82	**93.99**±4.80	**96.16**±3.61	**93.80**±1.97

Data extracted from Table on p. 28 and Tables 44-47, pp. 13-16 of MRID 48574950, PMRA 2181330.
*Total Potentially Absorbed = perfusate + all tape strips (I-VII) + skin membrane

The skin membrane rinse samples were pooled according to dose levels and species. The radioactivity was analyzed by TLC. For both dose levels and both test systems fluensulfone remained essentially unchanged on the application site during the exposure period. All analyzed skin membrane rinse pools revealed more than 95% unchanged fluensulfone, except for the low dose level on rat skin membranes, which showed 88% unchanged fluensulfone. However the portion in the skin membrane rinse for this group was only 3.65% of the applied dose.

III CONCLUSION

Fluensulfone formulated as MCW-2 480 EC penetrated through rat split-thickness skin membranes at a somewhat higher rate and extent at the low dose and at a significantly higher rate and extent at the high dose than through human split-thickness skin membranes. The potentially absorbable dose was calculated to be 93.50% and 83.99% for low dose rat and high dose rat skin, respectively, and 65.35% and 6.18% for low dose human and high dose human skin, respectively.

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency

Secondary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada Health Effects Division and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments/Conclusions: This study is classified as acceptable/nonguideline (fully reliable). The US EPA does not have a guideline for an *in vitro* dermal absorption study in the rat, but the study appears to have been properly conducted and meets the OECD guidance 428 requirements, and may be used for regulatory purposes. For determination of potentially absorbable dose, the first two skin tape strips were included in the calculation since the duration of the study was only 24 hours.

C. Deficiencies: No major deficiencies were identified.

Tier II Summary **(PMRA #2181225, MRID 48574772)** revised by the regulatory authorities, April 2013

Study Type: Acute neurotoxicity – rat

IIA 5.7.1 – MRID 48574772, PMRA 2181225

Report:	Sommer E.W. (2009) MCW-2 Tech. Acute Oral Neurotoxicity Peak-Effect Study in Rats Harlan Laboratories Ltd., Switzerland; unpublished report No. C57413, dated 30 October 2009. Sponsor reference No. R-23376 Dates of experimental work: 15 July to 3 August 2009
Sponsor:	Makhteshim Chemical Works Ltd., Beer-Sheva, Israel
Guidelines:	Not applicable - preliminary acute toxicity study to assist in the selection of dose levels and time of peak effect for an acute oral neurotoxicity study
GLP/Compliance:	Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

Executive Summary:

In a preliminary acute neurotoxicity study (MRID 48574772, PMRA 2181225) designed to determine the time to peak effect and set dose levels for a subsequent study, groups of 3 male and 3 female Wistar rats were administered single oral doses of 0 (control), 100, 500, 1000 and 2000 mg/kg bw of fluensulfone (MCW-2 tech., 98.65%, Batch No. 36372130-291-PF1) in polyethylene glycol (PEG) 300, and were observed for 8 days post-dosing.

Detailed clinical observations were performed on day 1 at 0.5 hours after dose administration and at hourly intervals for 8 hours, and on days 2 to 8. Body weights and food consumption were measured once during acclimatization and on days 1 and 8. At the end of the observation period, all surviving animals were sacrificed and assessed by gross pathology.

All animals of the high dose group (2000 mg/kg bw) survived the 8-hour observation period but died spontaneously within 24 hours after dose administration after they had developed signs of overt toxicity. All animals of the remaining dose groups survived to terminal sacrifice without showing any signs of toxicity.

Effects observed at 2000 mg/kg bw included a transient occurrence of salivation within 0.5 to 2 hours after dose administration followed by somnolence, decreased activity, prostration, and ruffled fur of variable duration between 2 and 8 hours after dose administration, with a peak effect observed on the day of dosing in animals of both sexes at approximately 5 hours. In addition, in 2 males dosed at 2000 mg/kg bw the push-off reflex was absent. Due to the premature death of all males and females dosed at 2000 mg/kg bw it was not possible to investigate any effect of the test item on body weight and food consumption in this high dose group. Slight body weight reductions were observed in males and females dosed with 1000 mg/kg bw. Reduced body weight gain was noted in males from the 500 mg/kg bw dose group, and in both sexes from the 1000 mg/kg bw dose group. Slight decreases in food consumption were observed in females from the 500 and 1000 mg/kg bw dose groups. No macroscopic findings were recorded in any animals of any dose

groups at necropsy.

A peak systemic effect was observed on the day of dosing in animals of both sexes treated at 2000 mg/kg bw approximately 5 hours after administration, but this dose level had exceeded the maximal tolerated dose (MTD).

This study is a non-guideline study and is therefore classified as acceptable/non-guideline (reliable with restrictions) as a preliminary acute neurotoxicity study in rats.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: MCW-2 tech. (fluensulfone)

Description:	Yellow solid
Lot/Batch:	36372130-291-PF1
Purity:	98.65% (GC-assay)
CAS#:	318290-98-1
Stability:	Stable until 30 November 2009

2. Vehicle: Polyethylene glycol 300 (PEG 300)

3. Test animals:

Species:	Rat
Strain:	HanRcc: WIST(SPF) – males and females
Age:	At dosing: approx. 6 weeks
Weight at dosing:	Males: 159 – 161 g for Groups 1-4, 179 g for Group 5; females: 124 – 133 g for Groups 1-4, 174 g for Group 5 (mean values)
Source:	Harlan Laboratories B.V., The Netherlands
Acclimation period:	7 to 12 days
Diet:	Pelleted standard Provimi Kliba Nafag 3433 rat maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum*
Water:	Community tap water from Itingen *ad libitum* in water bottles
Housing:	In groups of three/sex in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' J. Rettenmaier & Söhne GmbH & CoKG, Germany, imported by Provimi Kliba SA, Switzerland)

4. Environmental conditions:

Temperature:	19-25°C
Humidity:	30-70%
Air changes:	10-15 air changes per hour

Photoperiod: Light cycle of 12 hours light and 12 hours dark, music during the daytime light period

B. STUDY DESIGN AND METHODS:

1. In life dates: 15 July to 3 August 2009

2. Animals assignment and treatment

Animals were assigned to groups using a computer-generated algorithm. Animals 1-3 (males) and 13-15 (females) were assigned to group 1 (control) and treated with the dosing preparation without the test item; animals 4-6 (males) and 16-18 (females) were assigned to group 2 and treated at 100 mg/kg bw; animals 7-9 (males) and 19-21 (females) were assigned to group 3 and treated at 500 mg/kg bw; animals 10-12 (males) and 22-24 (females) were assigned to group 4 and treated at 1000 mg/kg bw; and animals 25-27 (males) and 28-30 (females) were assigned to group 5 and treated at 2000 mg/kg bw.

The dose of 2000 mg/kg bw was selected for the study after doses up to 1000 mg/kg bw were administered without any adverse effects.

The animals were not fasted prior to dose administration. Each dose was administered once, by oral gavage, at the constant volume of 5 mL/kg bw.

3. Preparation of the test substance

Fluensulfone was warmed in the original container in a water bath up to maximum 40°C until it became fluid. Thereafter, the whole amount of liquid test item was transferred into a glass beaker (wrapped with aluminum foil) and mixed using a magnetic stirrer on a heating plate (maximum 40 °C). The required amount of fluensulfone was weighed using a precision balance into a tared glass beaker (wrapped with aluminum foil) and kept at maximum 40°C, the vehicle added (w/v) and the dose formulation mixed using a magnetic stirrer on the heating plate. Separate formulations were prepared from the aliquots for each concentration.

4. Observations

Animals from the 0, 100, 500 and 1000 mg/kg bw dose groups were observed for 8 days following dosing. Due to mortality, animals dosed with 2000 mg/kg bw were only observed for up to 2 days. Animals were observed twice daily for mortality. Detailed clinical observations were conducted at 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hours post-dosing and then daily thereafter. These observations included a scoring of appearance (piloerection, salivation, hunched posture), motor activity (ataxia, tremors/twitching, prostration, circling, spasm), behavior (hyperactivity, somnolence, increased exploration, reduced grooming, vocalization), respiration (dyspnea, tachypnea, bradypnea), reflexes (blink, pinna, iridic light, hind leg push-off, pain response, startle/hearing), and other miscellaneous parameters (lacrimation, limbs cyanotic, mydriasis, miosis, exopthalmos, reduced muscle tone).

Food consumption and body weight were determined for the 0, 100, 500 and 1000 mg/kg bw dose groups on days 1 and 8, and for the 2000 mg/kg bw dose group on day 1 only.

5. Pathology

All animals that died were necropsied, macroscopically inspected, and discarded. All surviving animals were sacrificed on day 9 following intraperitoneal injection of pentobarbital (Esconarkon, Streuli Pharma, Switzerland; approximately 200 mg/kg bw) and discarded after macroscopic examination.

6. Statistics

No statistical method was used.

II. RESULTS AND DISCUSSION

A. MORTALITY

All animals dosed at 2000 mg/kg bw survived up to 8 hours post-dosing but died spontaneously within 24 hours after dose administration. All remaining animals survived to scheduled sacrifice.

B. CLINICAL OBSERVATIONS

On day 1, at 0.5 and 1 hour after dosing, salivation was observed in one female from the 500 mg/kg bw dose group. No further abnormalities were recorded in any animals of the 0, 100, 500 or 1000 mg/kg bw dose groups on the day of dose administration or during the entire observation period.

Therefore, it was decided to introduce an additional high dose group into the study. Five days after the study start another group of animals was dosed once at 2000 mg/kg bw. On day 1, salivation was noted as the first clinical sign present from 0.5 hours up to 2 hours after administration. Shortly thereafter, from 2 or 5 hours through 6 or 8 hours, somnolence, decreased activity, prostration, and ruffled fur were observed in both sexes. In addition, in two males treated at 2000 mg/kg bw the push-off reflex was absent. A systemic peak effect was observed on the day of dosing in males and females at approximately 5 hours after dosing.

C. BODY WEIGHT

No treatment-related effect on body weight development was evident in males and females treated at 100 and 500 mg/kg bw. In males treated at 1000 mg/kg bw there was a slight decrease of 4.9% in mean body weight at the end of the study on day 8. In females of the same group there was a slight decrease of 5.4% in mean body weight at the end of the study on day 8. Due to the premature death within 24 hours after dose administration of all animals treated at 2000 mg/kg bw, the mean body weight was only available on day 1 and body weight development could not be evaluated at study termination.

Body weight gain on day 8 was not different from control in the animals treated at 100 mg/kg bw, but was slightly (15.0%) lower in males treated at 500 mg/kg bw, and also lower (25.6%) in males treated at 1000 mg/kg bw.

Furthermore, body weight gain was minimally (5.2%) lower in females treated at 500 mg/kg bw, and due to the low magnitude of change relative to controls was not considered to be of

any toxicological relevance; however, a more substantial decrease in body weight gain (30.1%) was noted in females treated at 1000 mg/kg bw when compared to controls.

D. FOOD CONSUMPTION

Mean food consumption (g/animal/day) was only slightly affected by treatment with fluensulfone. When compared to controls, mean food consumption was slightly lower (5.0%) in males treated at 100 mg/kg bw, lower (6.0%) in males treated at 500 mg/kg bw, and also slightly lower (5.5%) in males treated at 1000 mg/kg bw. These changes were considered not to be test item-related, because there was no clear dose response effect present. In females treated at 100 mg/kg bw there was a slight decrease (7.0%) in mean food consumption present on day 8, but there was a minimal decrease in food consumption (3.5%) in females treated at 500 mg/kg bw, and a moderate decrease (11.9%) in food consumption in females treated at 1000 mg/kg bw. As no clear dose response effect was present, this effect was considered not to be test item-related or of any toxicological relevance. Due to the premature death of all animals treated at 2000 mg/kg bw, the mean food consumption for this dose group could not be evaluated at study termination.

No dose-related changes in relative food consumption (g/kg bw/day) were observed in males. In females, decreases in relative food consumption were noted at 500 mg/kg bw (5.0%) and at 1000 mg/kg bw (6.9%) when compared to controls. However, due to the low magnitude of deviations from the control group, these changes were not considered to be of any toxicological relevance.

E. NECROPSY

No abnormality was noted at necropsy of any animals.

III. CONCLUSIONS

Fluensulfone-related effects observed in males and females at a dose level of 2000 mg/kg bw included the transient occurrence of salivation with a peak-effect at approximately 1 hour after dose administration. In addition, these animals presented somnolence, decreased activity, prostration and ruffled fur starting from 2 or 5 hours post-dosing. In two males at 2000 mg/kg bw the push-off reflex was absent.

Salivation was also observed at 0.5 and 1 hour after administration in one single female treated at 500 mg/kg bw which was considered as a chance reaction as there was no salivation present in any males and females treated at 1000 mg/kg bw.

Treatment with fluensulfone after a single dose administration by gavage to non-fasted Wistar rats was well tolerated up to dose levels of 1000 mg/kg bw but did produce early mortality and signs of overt toxicity at the dose of 2000 mg/kg bw. Clinical signs observed had their peak effect at approximately five hours after dose administration in males and females treated at 2000 mg/kg bw.

The dose level of 2000 mg/kg bw clearly exceeded the maximal tolerated dose (MTD).

IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. Name of authority: Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. Reviewer's Comments: In the study report and in the Tier II summary, it was indicated that some clinical signs that were noted at 2000 mg/kg bw were observed in only one sex; however, the reviewer noted that all clinical signs noted at this dose level (other than the absent push-off reflex noted in males) occurred in both sexes.

The study investigators indicated that the decrease in body weight in males from the 1000 mg/kg bw dose group was not test-item related, but did not provide an explanation to support this conclusion, and considered a similar change in body weight in females from the same dose group to be treatment-related. The study investigators also considered the decrease in body weight gain in males at 1000 mg/kg bw not to be test item-related due to the low magnitude of the change, and considered the decreases in body weight gain noted in females at 500 and 1000 mg/kg bw not to be toxicologically relevant due to the absence of a dose response effect. The study investigators also concluded that food consumption was not affected by treatment with fluensulfone. The reviewer disagrees with the study investigators' interpretation of the body weight and food consumption findings. The following effects are considered by the reviewer to be potentially related to treatment: the decreases in body weight noted at 1000 mg/kg bw in both sexes, the decreased body weight gain noted in males at 500 mg/kg bw and in both sexes at 1000 mg/kg bw, and the decreased relative food consumption noted in females at 500 and 1000 mg/kg bw.

OCS considers that the low magnitude of the body weight at 1000 mg/kg bw in both sexes and in body weight gain at 500 mg/kg bw in females are not of toxicological relevance. Similarly, OCS considers that the low magnitude of the mean food consumption changes up to and including 1000 mg/kg bw in males and up to and including 500 mg/kg bw in females are not of toxicological relevance. Additionally, OCS does not consider the low magnitude of the changes in relative food consumption to be of toxicological relevance. Consequently, OCS only considers that the decrease in body weight gain in males at 500 mg/kg bw and in females at 1000 mg/kg bw, along with the decrease in mean food consumption in females at 1000 mg/kg bw, to be of toxicological relevance.

This study is a non-guideline study and is therefore classified as acceptable/non-guideline (reliable with restrictions) as a preliminary acute neurotoxicity study in rats.

C. Conclusions: Overall, the reviewer agrees with the study investigators' determination of the peak effect observed at 5 hours post-dose. Although salivation was noted as early at 0.5 to 1 hour post-dosing, the remaining clinical signs were not observed until 2 to 5 hours post-dosing, with most signs present and peaking in severity at 5 hours post-dose.

D. Deficiencies: The dosing formulations were not analyzed for homogeneity, stability or purity. This omission is not expected to significantly impact the study validity.

Study Type: Short-Term Oral (28-day) – Dietary – Rats (TSA Metabolite)

IIA 5.3.1 – MRID 49387601, PMRA 2402076

Report: Takawale, P. (2014)

28-Day Dose Range Finding Dietary Toxicity Study in Wistar Rats with 5-Chlorthiazole-2-Sulfonic Acid.

BSL BIOSERVICE, Germany; unpublished Report No. 122929, dated 03 February 2014. Sponsor Reference No. R-29581.

Dates of experimental work: 20 June 2013 to 20 January 2014

Sponsor/Submitter: Makhteshim Chemical Works Ltd., Israel.

Guidelines: US EPA OPPTS 870.3050, OECD 407 (1995), 67/548/EEC B.7

GLP/Compliance: Signed and dated GLP (see below) and Data Confidentiality statements were provided.

The study was performed in conformity with internal quality assurance regulations, on the basis of OECD GLP regulations, but was not audited by the quality assurance unit. Therefore, no GLP compliance is claimed.

Executive Summary: In a subchronic toxicity study (MRID 49387601, PMRA 2402076), 5-chlorthiazole-2-sulfonic acid (99.38% a.i.) was administered to 3 Wistar rats/sex/dose in the diet at dose levels of 0, 120, 500, 1200, or 12,000 ppm (0/0, 10/12, 41/43, 113/123, or 1194/1779 mg/kg bw/day in males/females) for 28 days. Examinations included clinical signs of toxicity, body weight, food consumption, hematology and clinical chemistry parameters, organ weight measurements, gross pathology, and limited histopathology examinations (liver, kidney, lung, and thymus).

No treatment-related effects were observed in this study up to the highest dose tested. A NOAEL and LOAEL were not established as this was a dose range-finding study with limited investigations.

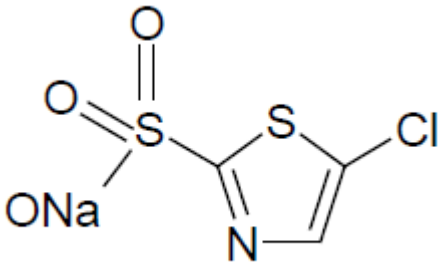
This subchronic toxicity study in the rat is acceptable as a range-finding study, but does not satisfy the guideline requirement (OECD 407; OPPTS 870.3050) for a repeat-dose oral study in rats due to the small group sizes and limited hematology, clinical chemistry and histopathology examinations. However, the number of parameters measured is sufficient to indicate that TSA is

significantly less toxic than fluensulfone in rats.

I. Materials and Methods

A. Materials

Test material: 5-chlorthiazole-2-sulfonic acid (Thiazole sulfonic acid; TSA)
Description: Fine white solid
Lot/Batch #: 316PAL39-2
Purity: 99.38%
CAS #: Not provided
Vehicle: Diet
Structure:



Test species: Rat
Strain: Wistar (Crl:WI(Han))
Age at start: 7 to 8 weeks
Weight at start: Males: 179 to 205 g (mean of 191.67 g); Females: 133 to 154 g (mean of 141.13 g)
Source: Charles River, 97633 Sulzfeld, Germany
Housing: Individually in IVC cages, Type III H, polysulphone cages on Altromin saw fiber bedding
Diet: Powdered special diet for rats and mice (Ssniff Spezialdiaten GmbH, Germany; Art.Nr. SM R/M-H), *ad libitum*
Water: Tap water (sulphur acidified to a pH of approximately 2.8), *ad libitum*
Environmental conditions:
Temperature: 22±3EC
Humidity: 55±10%
Air changes: 10/hr
Photoperiod: 12 hours dark/12 hours light
Acclimation: At least 5 days

B. Study design and methods

1. Animal assignment - Animals were assigned randomly to ensure to the extent possible homogeneous variation in body weight to the test groups noted in Table 1. Since the basal diet was powdered, the animals selected for the study were chosen based on the food spillage during the acclimatization period in order to select animals that did not have an aversion to the powdered basal diet.

Table 1. Study design

Conc. in Diet (ppm)	Dose (mg/kg bw/day)		Number of Animals	
	Male	Female	Male	Female
0	0	0	3	3
120	10	12	3	3
500	41	43	3	3
1200	113	123	3	3
12,000	1194	1779	3	3

2. <u>Diet preparation and analysis</u> – Test item formulated diet was procured directly from the supplier (Ssniff Spezialdiaten GmbH, Germany). Basal diet (powdered special diet for rats and mice, Art.Nr. SM R/M-H) was used for preparation of test item formulated diet. Attempts to pellet the diet were unsuccessful, as test item was lost during the pelleting process (potentially due to binding of the thiazole-moiety to the dietary matrix).

For each diet batch and concentration, a premix was prepared with a smaller sub-portion of the total diet to be prepared for the respective dose group and the total amount of test item to be mixed for the respective dose group. The pre-mix was prepared in a commercial blender. Thereafter, the premix was mixed with the remaining quantity of powdered diet to be prepared for the respective group in a mixer. Fresh diet was prepared once per week during the entire study period.

On the day of mixing of the test diets, approximately 50 gram samples were taken from the top, middle and bottom of the container holding the powdered diet from all four dose groups to confirm homogeneity of mixing with the test material (total of 12 samples).

Concentration verification was performed on diets mixed in weeks 1 and 4. Approximately 50 gram samples were taken from all dose groups for analysis (total of 10 samples).

Investigation of stability was performed as a separate study [*Stability of Thiazole Sulfonic Acid (TSA) in Rodent Feed, PTRL Europe ID P 2987 G*] on samples collected on the day of mixing in the first week of the study (Day 0) and on samples from the same batch of test diet that were stored for 1, 4, 8, and 16 weeks. Results from this study were not provided in the report.

Analysis of homogeneity revealed recoveries from all four dose preparations in the range of 98% to 116% of nominal, with low variability among samples from the top, middle and bottom of the container (the coefficient of variation ranged from 1.7% to 9.0%; calculated by the reviewer). Analysis of concentration indicated actual concentrations in the range of 97% to 115% of nominal. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

3. Statistics – No statistical analyses were conducted by the study investigators.

C. Methods

1. Observations – Animals were inspected at least once a day for general signs of toxicity and twice daily for morbidity and mortality.

2. Body weight – Animals were weighed once prior to assignment to test groups and on study days 1, 3, 8, 14, 22 and 28, as well as on the day of necropsy.

3. Food consumption – Food consumption was measured on study days 1, 3, 8, 15, 22 and 28 for each animal. Food in the food hoppers was additionally checked on a daily basis to ensure that sufficient food was available. Food consumption was reported for days 1-3, 3-8, 8-15, 15-22 and 22-28.

4. Ophthalmoscopic examination – Eyes were not examined.

5. Hematology and clinical chemistry – Blood was collected from the abdominal aorta at the end of the 28-day dosing period following an overnight fast. The checked (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*		Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)*		Mean corpuscular volume (MCV)
	Blood clotting measurements*		Reticulocyte count
X	(Platelet count)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended by OECD 407

b. Clinical chemistry

	ELECTROLYTES		OTHER
	Calcium*	X	Albumin*
	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
	Phosphorus*	X	Total Cholesterol
X	Potassium*		Globulins
X	Sodium*		Glucose*
	ENZYMES		Total bilirubin*
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (ALT/also SGPT)*		
X	Serum aspartate amino-transferase (AST/also SGOT)*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
	Ornithine decarboxylase*		

* Recommended by OECD 407

6. Urinalysis – Urinalysis was not performed.

7. <u>Sacrifice and pathology</u> – On day 29, all surviving animals were sacrificed by exsanguination under deep irreversible anesthesia (ketamine and xylazin) and were subjected to a detailed gross necropsy. The organs marked with a (W) were weighed, and the checked (X) tissues were collected for histological examination. A histological examination was performed on the tissues collected from animals from the control (0 ppm) and high dose (12,000 ppm) groups. As no treatment-related findings were observed at the high dose, histological examination was not extended to tissues from the intermediate dose groups.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta	W	Brain
	Salivary glands	W	Heart*		Periph.nerve
	Esophagus		Bone marrow		Spinal cord (3 levels)
	Stomach		Lymph nodes		Pituitary
	Duodenum	W	Spleen*		Eyes (optic n.)
	Jejunum	W	Thymus		**GLANDULAR**
	Ileum			WX	Adrenal gland*+
	Cecum		**UROGENITAL**		Lacrimal gland
	Colon	WX	Kidneys*+		Mammary gland
	Rectum		Urinary bladder		Parathyroids
WX	Liver*+	W	Testes +		Thyroids
	Gall bladder	W	Epididymides		**OTHER**
	Pancreas	W	Prostate, seminal vesicles with coagulating glands		Bone
	RESPIRATORY	W	Ovaries		Skeletal muscle
X	Trachea	W	Uterus with cervix		Skin
WX	Lung			X	All gross lesions and masses*
	Nose				Target Organs*
	Pharynx				
	Larynx				

* Recommended by OECD 407

II. Results

A. <u>Observations</u>

1. <u>Clinical signs of toxicity</u> – No treatment-related clinical signs of toxicity were observed. Single incidences of alopecia on both forelimbs were observed in one male from each of the 120 and 12,000 ppm dose groups, and one female from the 500 ppm dose group.

2. <u>Mortality</u> – No animals died during the treatment period.

B. Body weight and weight gain – No treatment-related effects on body weight or body weight gain were observed (Table 2).

Table 2. Average body weights (g) and body weight gains (g) during 28 days of treatment

	0 ppm	120 ppm	500 ppm	1200 ppm	12,000 ppm
Males					
Body weight Day 1 (Initial)	190 ± 9.5	190 ± 8.7	192 ± 8.2	193 ± 10.8	193 ± 10.1
Body weight Day 15	268 ± 15.9	264 ± 15.9	273 ± 17.4	269 ± 4.7	266 ± 5.1
Body weight Day 28 (Final)	316 ± 23.7	314 ± 23.7	323 ± 21.6	318 ± 5.3	309 ± 8.1
Overall body weight gain[b]	126	124	131	125	116
Females					
Body weight Day 1 (Initial)	139 ± 5.5	139 ± 5.3	142 ± 10.1	142 ± 10.1	136 ± 2.9
Body weight Day 15	170 ± 2.0	175 ± 9.5	173 ± 12.1	171 ± 11.6	173 ± 16.0
Body weight Day 28 (Final)	186 ± 4.0	194 ± 11.0	192 ± 10.4	189 ± 13.0	195 ± 16.6
Overall body weight gain[b]	47	55	50	47	59

[a] Data obtained from pages 37 and 38 of the study report. N = 3/group.
[b] Overall body weight gain was calculated by the reviewer from the mean body weights on days 1 and 28.

C. Food consumption – An increase in mean food consumption in females from the 12,000 ppm dose group compared to the controls was noted, but was attributed to apparently higher individual food consumption values from one female (animal number 30) throughout the study period. The study investigators noted that this apparently high food consumption was due to an increase in food spillage. In high dose females, the average absolute food consumption was 140 g versus 93 g in control females, while the average relative food consumption was 99 g/kg bw versus 88 g/kg bw in control females (mean values were calculated by the reviewer from the food consumption values on pages 44 and 48-49). Overall, no treatment-related effects on food consumption were observed.

D. Blood analyses

1. Hematology – No treatment-related effects on the hematological parameters investigated in this study were observed (Table 3). However, analysis of these data is hindered by the small sample sizes, the lack of pre-treatment baseline values, and the high variability noted in some measurements.

Table 3. Mean hematological values in male and female rats[a]

Parameter	0 ppm	120 ppm	500 ppm	1200 ppm	12,000 ppm
Males					
White blood cells (10^9/L)	6.65 ± 0.56	7.22 ± 0.89	5.70 ± 2.39	7.25 ± 1.46	7.88 ± 2.73
Red blood cells (10^{12}/L)	8.61 ± 0.09	8.63 ± 0.22	8.43 ± 0.36	8.76 ± 0.55	8.56 ± 0.55
Hemoglobin (g/dL)	15.60 ± 0.46	15.77 ± 0.35	15.13 ± 0.55	15.97 ± 0.76	15.43 ± 0.29
Hematocrit (%)	47.07 ± 1.30	47.80 ± 0.96	46.43 ± 3.87	48.20 ± 1.47	47.00 ± 0.95
Platelets (10^9/L)	788 ± 95	746 ± 19	714 ± 48	740 ± 55	792 ± 166
Females					
White blood cells (10^9/L)	4.87 ± 0.39	4.24 ± 1.48	4.20 ± 0.53	4.13 ± 1.11	3.75 ± 1.17 (↓23%)
Red blood cells (10^{12}/L)	8.68 ± 0.16	7.70 ± 0.04	8.11 ± 0.24	8.44 ± 0.56	8.49 ± 0.29
Hemoglobin (g/dL)	15.77 ± 0.49	14.35 ± 0.07	15.00 ± 0.57	15.43 ± 0.85	15.85 ± 0.21
Hematocrit (%)	46.50 ± 1.45	42.25 ± 0.07	44.45 ± 2.33	45.80 ± 2.87	45.95 ± 0.92
Platelets (10^9/L)	735 ± 97	748 ± 5	772 ± 57	809 ± 21	820 ± 4

[a] Data obtained from pages 50 and 51 of the study report. N=3/group except for females from the 120, 500 and 12,000 ppm dose groups where N=2/group.

2. Clinical chemistry – Decreases in the mean sodium and potassium levels in females from the 12,000 ppm dose group were observed when compared to controls (Table 4). These decreases were attributed to very low values from one high dose female (animal number 28). The study investigators considered these findings to be incidental and unrelated to treatment.

No other treatment-related effects on the clinical chemistry parameters investigated in this study were observed. However, analysis of these data is hindered by the small sample sizes, the lack of pre-treatment baseline values, and the high variability noted in some measurements.

Table 4. Mean clinical chemistry values in male and female rats[a]

Parameter	0 ppm	120 ppm	500 ppm	1200 ppm	12,000 ppm
Males					
Alanine aminotransferase (U/L)	35.40 ± 4.37	34.93 ± 5.80	29.77 ± 4.55	33.63 ± 5.69	34.37 ± 4.94
Aspartate aminotransferase (U/L)	88.80 ± 3.05	91.17 ± 26.03	87.73 ± 19.54	87.60 ± 9.45	91.10 ± 13.07
Alkaline phosphatase (U/L)	131.34 ± 16.93	158.28 ± 8.03	111.14 ± 11.18	129.09 ± 56.92	148.46 ± 15.44
Creatinine (µmol/L)	21.00 ± 1.00	27.33 ± 4.04	25.33 ± 9.71	23.00 ± 5.29	23.33 ± 1.53
Protein (g/L)	59.60 ± 2.31	60.03 ± 2.43	58.37 ± 2.74	57.17 ± 0.90	59.30 ± 2.50
Albumin (g/L)	33.58 ± 1.35	33.43 ± 1.49	31.99 ± 0.96	31.77 ± 0.06	32.73 ± 0.46
Cholesterol (mmol/L)	1.90 ± 0.24	1.76 ± 0.55	1.84 ± 0.61	1.82 ± 0.15	1.77 ± 0.46
Urea (mmol/L)	6.70 ± 1.34	8.43 ± 1.72	7.02 ± 1.17	6.80 ± 2.08	7.32 ± 0.74
Sodium (mmol/L)	134.00 ± 1.00	135.00 ± 1.73	135.33 ± 0.58	135.67 ± 0.58	134.00 ± 1.00
Potassium (mmol/L)	4.18 ± 0.19	3.96 ± 0.15	4.48 ± 0.90	3.91 ± 0.15	3.92 ± 0.04
Females					
Alanine aminotransferase (U/L)	21.60 ± 1.61	28.73 ± 4.69	23.53 ± 2.31	24.90 ± 10.29	27.77 ± 2.02
Aspartate aminotransferase (U/L)	64.07 ± 6.18	70.77 ± 6.27	69.13 ± 11.62	70.43 ± 15.14	81.67 ± 8.88
Alkaline phosphatase (U/L)	69.68 ± 6.86	60.54 ± 7.11	77.06 ± 23.07	87.10 ± 5.97	67.06 ± 16.46
Creatinine (µmol/L)	24.67 ± 1.53	26.00 ± 2.65	28.00 ± 4.36	29.33 ± 5.03	22.00 ± 2.00
Protein (g/L)	59.93 ± 0.55	58.37 ± 1.82	61.03 ± 6.21	57.33 ± 0.64	56.87 ± 2.20
Albumin (g/L)	34.69 ± 0.20	33.03 ± 1.75	34.48 ± 2.78	32.69 ± 0.29	31.89 ± 1.04
Cholesterol (mmol/L)	1.66 ± 0.32	1.03 ± 0.12	1.54 ± 0.42	1.55 ± 0.52	1.49 ± 0.46
Urea (mmol/L)	6.82 ± 0.73	6.85 ± 0.14	6.95 ± 0.64	7.20 ± 1.30	6.21 ± 0.11
Sodium (mmol/L)	131.67 ± 0.58	129.67 ± 0.58	130.67 ± 0.58	132.00 ± 0.00	88.67 ± 72.46
Potassium (mmol/L)	3.72 ± 0.62	4.61 ± 2.07	3.44 ± 0.38	3.27 ± 0.15	2.17 ± 1.79

[a] Data obtained from pages 52 and 53 of the study report. N=3/group.

E. Sacrifice and pathology

1. Organ weight – No treatment-related changes in absolute or relative organ weights were observed (Tables 5a and 5b). However, analysis of these data is hindered by the small sample sizes and the high variability noted in some measurements.

Table 5a. Mean organ weight values in male rats[a]

Parameter	0 ppm	120 ppm	500 ppm	1200 ppm	12,000 ppm
Males					
Liver weight – absolute (g)	8.564 ± 0.399	8.589 ± 1.157	9.157 ± 0.274	8.232 ± 0.159	8.656 ± 0.538
Liver weight – relative (%)	2.923 ± 0.146	2.948 ± 0.225	3.059 ± 0.199	2.760 ± 0.017	3.029 ± 0.083
Spleen weight – absolute (g)	0.712 ± 0.159	0.622 ± 0.007	0.693 ± 0.049	0.729 ± 0.109	0.703 ± 0.026
Spleen weight – relative (%)	0.241 ± 0.037	0.215 ± 0.016	0.232 ± 0.025	0.245 ± 0.041	0.246 ± 0.002
Kidney weight – absolute (g)	2.310 ± 0.166	2.239 ± 0.209	2.395 ± 0.228	2.459 ± 0.139	2.446 ± 0.258
Kidney weight – relative (%)	0.787 ± 0.007	0.770 ± 0.032	0.797 ± 0.017	0.824 ± 0.046	0.855 ± 0.054
Adrenal gland weight – absolute (g)	0.051 ± 0.008	0.059 ± 0.003	0.057 ± 0.013	0.054 ± 0.005	0.058 ± 0.002
Adrenal gland weight – relative (%)	0.018 ± 0.004	0.020 ± 0.001	0.019 ± 0.003	0.018 ± 0.002	0.020 ± 0.001
Testes weight – absolute (g)	3.337 ± 0.145	3.176 ± 0.262	3.233 ± 0.030	3.495 ± 0.192	3.334 ± 0.364
Testes weight – relative (%)	1.139 ± 0.050	1.092 ± 0.042	1.082 ± 0.099	1.173 ± 0.082	1.169 ± 0.135
Epididymides weight – absolute (g)	0.894 ± 0.023	0.849 ± 0.073	0.859 ± 0.037	0.943 ± 0.033	0.888 ± 0.098
Epididymides weight – relative (%)	0.305 ± 0.021	0.292 ± 0.023	0.288 ± 0.036	0.316 ± 0.014	0.311 ± 0.034
Thymus weight – absolute (g)	0.629 ± 0.134	0.504 ± 0.054	0.621 ± 0.031	0.512 ± 0.079	0.482 ± 0.084
Thymus weight – relative (%)	0.214 ± 0.042	0.173 ± 0.008	0.207 ± 0.007	0.172 ± 0.027	0.168 ± 0.025
Heart weight – absolute (g)	0.850 ± 0.043	0.799 ± 0.048	0.859 ± 0.088	0.878 ± 0.071	0.789 ± 0.009
Heart weight – relative (%)	0.290 ± 0.011	0.275 ± 0.005	0.286 ± 0.006	0.295 ± 0.029	0.276 ± 0.009
Brain weight – absolute (g)	1.998 ± 0.226	2.016 ± 0.033	2.012 ± 0.184	2.091 ± 0.105	1.980 ± 0.117
Brain weight – relative (%)	0.679 ± 0.025	0.695 ± 0.038	0.670 ± 0.009	0.702 ± 0.050	0.693 ± 0.012
Lung weight – absolute (g)	1.211 ± 0.184	1.192 ± 0.131	1.226 ± 0.060	1.203 ± 0.045	1.153 ± 0.075
Lung weight – relative (%)	0.411 ± 0.032	0.409 ± 0.020	0.409 ± 0.014	0.403 ± 0.008	0.404 ± 0.032
Prostate & seminal vesicle with coagulating gland weight – absolute (g)	1.515 ± 0.349	1.372 ± 0.055	1.509 ± 0.223	1.646 ± 0.103	1.338 ± 0.107
Prostate & seminal vesicle with coagulating gland weight – absolute (%)	0.512 ± 0.082	0.473 ± 0.015	0.504 ± 0.080	0.552 ± 0.026	0.468 ± 0.020

[a] Data obtained from pages 56 and 58 of the study report. N=3/group.

Table 5b. Mean organ weight values in female rats[a]

Parameter	0 ppm	120 ppm	500 ppm	1200 ppm	12,000 ppm
Females					
Liver weight – absolute (g)	5.146 ± 0.146	5.398 ± 0.625	4.871 ± 0.321	4.829 ± 0.384	4.883 ± 0.551
Liver weight – relative (%)	3.015 ± 0.057	3.019 ± 0.374	2.766 ± 0.059	2.773 ± 0.077	2.720 ± 0.052
Spleen weight – absolute (g)	0.456 ± 0.047	0.443 ± 0.019	0.381 ± 0.067	0.479 ± 0.053	0.463 ± 0.027
Spleen weight – relative (%)	0.267 ± 0.024	0.248 ± 0.007	0.216 ± 0.028	0.275 ± 0.020	0.260 ± 0.031
Kidney weight – absolute (g)	1.448 ± 0.072	1.460 ± 0.061	1.439 ± 0.149	1.404 ± 0.047	1.450 ± 0.099
Kidney weight – relative (%)	0.849 ± 0.053	0.818 ± 0.073	0.817 ± 0.064	0.809 ± 0.069	0.812 ± 0.071
Adrenal gland weight – absolute (g)	0.064 ± 0.007	0.072 ± 0.012	0.061 ± 0.005	0.070 ± 0.021	0.066 ± 0.010
Adrenal gland weight – relative (%)	0.038 ± 0.004	0.040 ± 0.007	0.035 ± 0.003	0.040 ± 0.010	0.037 ± 0.007
Ovarian weight – absolute (g)	0.104 ± 0.006	0.103 ± 0.017	0.094 ± 0.021	0.102 ± 0.022	0.113 ± 0.011
Ovarian weight – relative (%)	0.061 ± 0.003	0.058 ± 0.006	0.053 ± 0.010	0.059 ± 0.013	0.063 ± 0.007
Uterus with cervix weight – absolute (g)	0.495 ± 0.143	0.542 ± 0.128	0.472 ± 0.101	0.429 ± 0.096	0.615 ± 0.244
Uterus with cervix weight – relative (%)	0.291 ± 0.085	0.303 ± 0.069	0.268 ± 0.049	0.245 ± 0.042	0.345 ± 0.146
Thymus weight – absolute (g)	0.454 ± 0.115	0.410 ± 0.081	0.469 ± 0.058	0.477 ± 0.026	0.416 ± 0.076
Thymus weight – relative (%)	0.267 ± 0.071	0.228 ± 0.033	0.266 ± 0.021	0.275 ± 0.017	0.231 ± 0.026
Heart weight – absolute (g)	0.582 ± 0.030	0.614 ± 0.059	0.552 ± 0.042	0.584 ± 0.054	0.601 ± 0.037
Heart weight – relative (%)	0.341 ± 0.012	0.343 ± 0.022	0.314 ± 0.018	0.335 ± 0.014	0.336 ± 0.011
Brain weight – absolute (g)	1.837 ± 0.031	1.959 ± 0.061	1.884 ± 0.035	1.811 ± 0.065	1.821 ± 0.066
Brain weight – relative (%)	1.076 ± 0.002	1.097 ± 0.082	1.071 ± 0.035	1.043 ± 0.065	1.020 ± 0.080
Lung weight – absolute (g)	0.862 ± 0.075	0.933 ± 0.062	0.874 ± 0.008	0.882 ± 0.037	0.881 ± 0.095
Lung weight – relative (%)	0.505 ± 0.035	0.521 ± 0.009	0.498 ± 0.028	0.507 ± 0.006	0.491 ± 0.015

[a] Data obtained from pages 57 and 59 of the study report. N=3/group.

2. <u>Gross pathology</u> – No treatment-related gross pathological changes were observed.

3. <u>Microscopic pathology</u> – No treatment-related histological changes were observed in the lung, liver, kidneys of thymus of high dose animals when compared to controls.

III. Discussion

A. Investigators' conclusions – "Under the conditions of the present study, the repeated dietary exposure of the test item 5-chlorthiazole-2-sulfonic acid to male and female Wistar rats at doses of 120, 500, 1200 and 12,000 ppm for 28 days was not associated with mortality or indications of toxicity."

"Based on the data generated from this dose range finding study, dose levels of 0, 500, 2500 and 12,000 ppm are suggested for the subsequent main study with 5-chlorthiazole-2-sulfonic acid."

B. Reviewer comments – No treatment-related effects were observed in this study up to the highest dose tested. This study is limited due to the small group sizes and reduced number of hematology, clinical chemistry and histopathology examinations. However, the number of parameters measured is sufficient to indicate that TSA is significantly less toxic than fluensulfone in rats.

C. Study deficiencies – This study was designed as a dose range-finding study with limited investigations and was not conducted in compliance with GLP. The group sizes (3/sex) were smaller than recommended in the guidelines (5/sex), and a reduced number of hematology, clinical chemistry and histopathology examinations.

Study Type: Short-Term Oral (28-day) – Dietary – Rats (BSA Metabolite)

IIA 5.3.1 – MRID 49387602, PMRA 2407952

Report: Takawale, P. (2014). 28-Day Dose Range Finding Dietary Toxicity Study in Wistar Rats with 3,4,4-Trifluoro-but-3-ene-1-Sulfonic Acid. BSL BIOSERVICE, Germany; unpublished Report No. 136081, dated 25 March 2014. Sponsor Reference No. R-31447.

Dates of experimental work: 05 December 2013 to 18 March 2014

Sponsor/Submitter: Makhteshim Chemical Works Ltd., Israel.

Guidelines: US EPA OPPTS 870.3050, OECD 407 (1995), 67/548/EEC B.7

GLP/Compliance: Signed and dated GLP (see below) and Data Confidentiality statements were provided.

The study was performed in conformity with internal quality assurance regulations, on the basis of OECD GLP regulations, but was not audited by the quality assurance unit. Therefore, no GLP compliance is claimed.

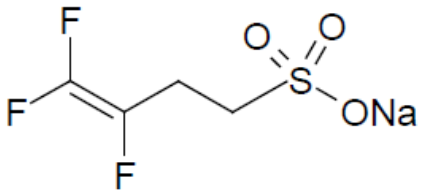
Executive Summary: In a subchronic toxicity study (MRID 49387602, PMRA 2407952), 3,4,4-trifluoro-but-3-ene-1-sulfonic acid (BSA; 93%) was administered to 3 Wistar rats/sex/dose in the diet at dose levels of 0, 100, 500, 1000, or 10,000 ppm (0/0, 6.4/8.6, 30/39, 82/120, or 732/1024 mg/kg bw/day in males/females; doses were corrected for analytical recovery of BSA from the test diet) for 28 days. Examinations included clinical signs of toxicity, body weight, food consumption, hematology and clinical chemistry parameters, organ weight measurements, gross pathology, and limited histopathology examinations (liver, kidney, lung, and thymus).

Aside from marginal (<25%) decreases in mean serum aspartate aminotransferase levels in both sexes and alanine aminotransferase levels in males at the high dose of 10,000 ppm, no treatment-related effects were observed in this study up to the highest dose tested. A NOAEL and LOAEL were not established as this was a dose range-finding study with limited investigations.

This subchronic toxicity study in the rat is acceptable as a range-finding study, but does not satisfy the guideline requirement (OECD 407; OPPTS 870.3050) for a repeat-dose oral study in rats due to the small group sizes and limited hematology, clinical chemistry and histopathology examinations. However, the number of parameters measured is sufficient to indicate that BSA is significantly less toxic than fluensulfone in rats.

I. Materials and Methods

A. Materials

Test material:	3,4,4-trifluoro-but-3-ene-1-sulfonic acid (Butene sulfonic acid; BSA)	
Description:	Fine white solid	
Lot/Batch #:	339PAL34	
Purity:	93%	
CAS #:	Not provided	
Vehicle:	Diet	
Structure:		
Test species:	Rat	
Strain:	Wistar (Crl:WI(Han))	
Age at start:	7 to 8 weeks	
Weight at start:	Males: 231-253 g, mean of 242.33 g; Females: 137-159 g; mean of 146.73 g	
Source:	Charles River, 97633 Sulzfeld, Germany	
Housing:	Individually in IVC cages, Type III H, polysulphone cages on Altromin saw fiber bedding	
Diet:	Pelleted diet prepared from powdered special diet for rat and mice (Art.Nr. SM R/M-H from Ssniff Spezialdiaten, GmbH, Germany), *ad libitum*	
Water:	Tap water (sulphur acidified to a pH of approximately 2.8), *ad libitum*	
Environmental conditions:	**Temperature:**	22±3EC
	Humidity:	55±10%
	Air changes:	10/hr
	Photoperiod:	12 hours dark/12 hours light
Acclimation:	At least 5 days	

B. Study design and methods

1. Animal assignment – Animals were assigned randomly to ensure to the extent possible homogeneous variation in body weight to the test groups noted in Table 1.

Table 1. Study design

Conc. in Diet (ppm)	Dose (mg/kg bw/day)[a]		Number of Animals	
	Male	Female	Male	Female
0	0	0	3	3
100	9 [6]	12 [9]	3	3
500	46 [30]	59 [39]	3	3
1000	93 [82]	135 [120]	3	3
10,000	921 [732]	1288 [1024]	3	3

[a] The dose presented in brackets [] represents the dose corrected for low analytical recovery of the test material from the test diet (correction was based on the average recovery measured in weeks 1 and 4).

2. <u>Diet preparation and analysis</u> – To prepare the test item formulated diet, the test item was ground to a fine powder using a mortar and pestle, weighed, and then mixed with the basal diet (powdered special diet for rat and mice, Art.Nr. SM R/M-H from Ssniff Spezialdiaten, GmbH, Germany). For each batch of diet and concentration, a premix was prepared by mixing 1 kg of diet with the test item for at least 10 minutes at a speed of 120 revolutions per minute in a mixing machine. The premix was then mixed with additional powdered diet (for 20 at 100 revolutions per minute). After mixing, pellets were prepared by adding approximately 125-150 mL water per kg of mixed diet and mixing for 10 minutes and 100 revolutions per minute. The prepared pellets were spread out onto a plate and allowed to dry at room temperature for at least two days. Fresh diet was prepared once a week during the entire study period.

On the day of mixing of the test diets and after preparation of the pellets, approximately 50 gram samples were taken from the top, middle and bottom of the container holding the pelleted diet from all four dose groups to confirm homogeneity of mixing (total of 12 samples).

Concentration verification was performed on pelleted diets mixed in weeks 1 and 4. Approximately 10 to 50 gram samples were taken from all dose groups for analysis (total of 10 samples).

Investigation of stability was performed as a separate GLP study on samples collected on the day of mixing in the first week of the study (Day 0) and then on the same samples stored for 1, 2, 4 and 8 weeks at room temperature [*Stability of 3,4,4-Trifluoro-but-3-ene-1-sulfonic acid (BSA) in Rodent Feed, PTRL Europe ID P 3199 G*].

The recovery of BSA in the prepared test diets ranged from 62% to 94% in the analysis of homogeneity (Table 2). In the determination of the actual concentration of BSA in the prepared test diets during weeks 1 and 4 of the study, actual concentrations were in the range of 64% to 94% of nominal concentration (Table 3).

The study investigators noted that analysis of the test diets revealed that recoveries in the 100 and 500 ppm test diets were lower than acceptable (outside ±20%), which was likely a consequence of slight inhomogeneity of the test item distribution in the diet. The test material intake values were corrected for these low recoveries, based on the average recovery measured in weeks 1 and 4 (71.5%, 66.0%, 88.5%, and 79.5% for the 100, 500, 1000, and 10,000 ppm test diets).

Table 2. Recovery of BSA in test diet – homogeneity analysis[a]

Sample No.	Fraction	Test Diet Concentration (ppm)	Recovery (%)
1	Top	100	62%
2	Middle	100	85%
3	Bottom	100	68%
4	Top	500	84%
5	Middle	500	73%
6	Bottom	500	80%
7	Top	1000	93%
8	Middle	1000	83%
9	Bottom	1000	92%
10	Top	10,000	85%
11	Middle	10,000	94%
12	Bottom	10,000	71%

[a] Data obtained from page 130 of the study report.

Table 3. Recovery of BSA in test diet – concentration analysis[a]

Sample No.	Sampling	Test Diet Concentration (ppm)	Recovery (%)
13	Week 1	0	Not detected
14	Week 1	100	78%
15	Week 1	500	68%
16	Week 1	1000	94%
17	Week 1	10,000	80%
18	Week 4	0	Not detected
19	Week 4	100	65%
20	Week 4	500	64%
21	Week 4	1000	83%
22	Week 4	10,000	79%

[a] Data obtained from page 131 of the study report.

3. Statistics – No statistical analyses were conducted by the study investigators.

C. Methods

1. Observations – Animals were inspected at least once a day for general signs of toxicity and twice daily for morbidity and mortality.

2. Body weight – Animals were weighed once prior to assignment to test groups and on study days 1, 3, 8, 14, 22 and 28, as well as on the day of necropsy.

3. Food consumption and compound intake – Food consumption was measured on study days 1, 3, 8, 15, 22 and 28 for each animal. Food in the food hoppers was additionally checked on a daily basis to ensure that sufficient food was available. Food consumption was reported for days 1-3, 3-8, 8-15, 15-22 and 22-28.

4. Ophthalmoscopic examination – Eyes were not examined.

5. Hematology and clinical chemistry – Blood was collected from the abdominal aorta at the end of the 28-day dosing period following an overnight fast. The checked (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*		Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)*		Mean corpuscular volume (MCV)
	Blood clotting measurements*		Reticulocyte count
X	(Platelet count)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended by OECD 407

b. Clinical chemistry

	ELECTROLYTES		OTHER
	Calcium*	X	Albumin*
	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
	Phosphorus*	X	Total Cholesterol
X	Potassium*		Globulins
X	Sodium*		Glucose*
	ENZYMES		Total bilirubin*
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (ALT/also SGPT)*		
X	Serum aspartate amino-transferase (AST/also SGOT)*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
	Ornithine decarboxylase*		

* Recommended by OECD 407

6. Urinalysis – Urinalysis was not performed.

7. Sacrifice and pathology – On day 29, all surviving animals were sacrificed by exsanguination under deep irreversible anesthesia (ketamine and xylazin) and were subjected to a detailed gross necropsy. The organs marked with a (W) were weighed, and the checked (X) tissues were collected for histological examination. A histological examination was performed on the tissues collected from animals from the control (0 ppm) and high dose (12,000 ppm) groups. As no treatment-related findings were observed at the high dose, histological examination was not extended to tissues from the intermediate dose groups.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta	W	Brain
	Salivary glands	W	Heart*		Periph. nerve
	Esophagus		Bone marrow		Spinal cord (3 levels)
	Stomach		Lymph nodes		Pituitary
	Duodenum	W	Spleen*		Eyes (optic n.)
	Jejunum	W	Thymus		**GLANDULAR**
	Ileum			WX	Adrenal gland*+
	Cecum		**UROGENITAL**		Lacrimal gland
	Colon	WX	Kidneys*+		Mammary gland
	Rectum		Urinary bladder		Parathyroids
WX	Liver*+	W	Testes +		Thyroids
	Gall bladder	W	Epididymides		**OTHER**
	Pancreas	W	Prostate, seminal vesicles with coagulating glands		Bone
	RESPIRATORY	W	Ovaries		Skeletal muscle
X	Trachea	W	Uterus with cervix		Skin
WX	Lung			X	All gross lesions and masses*
	Nose				Target Organs*
	Pharynx				
	Larynx				

* Recommended by OECD 407

II. Results

A. Observations

1. Clinical signs of toxicity – No treatment-related clinical signs of toxicity were observed.

2. Mortality – No animals died during the treatment period.

B. <u>Body weight and weight gain</u> – No treatment-related effects on body weight or body weight gain were observed (Table 4).

Table 4. Average body weights (g) and body weight gains (g) during 28 days of treatment

	0 ppm	100 ppm	500 ppm	1000 ppm	10,000 ppm
Males					
Body weight Day 1 (Initial)	241 ± 8.4	242 ± 4.6	242 ± 5.5	243 ± 7.2	244 ± 8.1
Body weight Day 15	309 ± 12.9	298 ± 7.6	301 ± 9.5	303 ± 4.0	311 ± 7.5
Body weight Day 28 (Final)	352 ± 13.5	341 ± 9.2	343 ± 17.6	344 ± 12.5	358 ± 14.8
Overall body weight gain[b]	111	99	101	101	114
Females					
Body weight Day 1 (Initial)	149 ± 4.9	149 ± 10.3	146 ± 6.7	146 ± 6.9	148 ± 10.1
Body weight Day 15	186 ± 3.8	184 ± 16.3	178 ± 11.7	175 ± 1.0	189 ± 10.8
Body weight Day 28 (Final)	202 ± 9.5	199 ± 16.9	202 ± 15.3	194 ± 3.5	208 ± 11.5
Overall body weight gain[b]	53	50	56	48	60

[a] Data obtained from pages 36 and 37 of the study report. N = 3/group.
[b] Overall body weight gain was calculated by the reviewer from the mean body weights on days 1 and 28.

C. <u>Food consumption</u> – An increase in mean food consumption in females from the 500 ppm dose group compared to the controls was noted, but was attributed to apparently higher individual food consumption values from one female (animal number 24) towards the end of the study period (days 22-28). The study investigators noted that this apparently high food consumption was due to an increase in food spillage. Overall, no treatment-related effects on food consumption were observed.

D. Blood analyses

1. Hematology – No treatment-related effects on the hematological parameters investigated in this study were observed (Table 5). However, analysis of these data is hindered by the small sample sizes, the lack of pre-treatment baseline values, and the high variability noted in some measurements.

Table 5. Mean hematological values in male and female rats[a]

Parameter	0 ppm	100 ppm	500 ppm	1000 ppm	10,000 ppm
Males					
White blood cells (10^9/L)	7.25 ± 2.78	6.97 ± 1.44	6.02 ± 1.99	6.44 ± 0.18	6.50 ± 1.71
Red blood cells (10^{12}/L)	8.85 ± 0.09	9.09 ± 0.30	9.23 ± 0.55	9.39 ± 0.21	8.63 ± 0.50
Hemoglobin (g/dL)	15.97 ± 0.40	16.00 ± 1.05	16.33 ± 0.31	16.23 ± 0.35	15.63 ± 0.91
Hematocrit (%)	46.73 ± 1.17	47.23 ± 3.62	48.07 ± 1.80	48.40 ± 1.05	46.33 ± 3.07
Platelets (10^9/L)	744 ± 92	790 ± 98	736 ± 98	812 ± 25	756 ± 96
Females					
White blood cells (10^9/L)	3.82 ± 0.99	3.60 ± 0.95	3.40 ± 1.09	2.73 ± 0.84	4.10 ± 0.27
Red blood cells (10^{12}/L)	7.96 ± 0.19	8.40 ± 0.19	8.40 ± 0.10	8.40 ± 0.30	8.21 ± 0.15
Hemoglobin (g/dL)	14.97 ± 0.40	15.33 ± 0.25	15.10 ± 0.26	15.83 ± 0.21	15.47 ± 0.21
Hematocrit (%)	43.03 ± 1.15	44.27 ± 0.61	43.60 ± 1.04	45.27 ± 1.00	43.83 ± 1.31
Platelets (10^9/L)	765 ± 141	735 ± 47	787 ± 56	802 ± 81	753 ± 133

[a] Data obtained from pages 49 and 50 of the study report. N=3/group.

2. Clinical chemistry – Marginal decreases in liver enzymes were observed in high dose (10,000 ppm) males (aspartate aminotransferase, 13%; alanine aminotransferase, 16%) and females (aspartate aminotransferase, 24%) when compared with controls (Table 6). These findings were not considered to be adverse. No other treatment-related effects on the clinical chemistry parameters investigated in this study were observed. However, analysis of these data is hindered by the small sample sizes, the lack of pre-treatment baseline values, and the high variability noted in some measurements.

Table 6. Mean clinical chemistry values in male and female rats[a]

Parameter	0 ppm	100 ppm	500 ppm	1000 ppm	10,000 ppm
Males					
Aspartate aminotransferase (U/L)	78.77 ± 5.99	79.50 ± 4.28	78.10 ± 13.26	72.47 ± 7.76	68.23 ± 6.08
Alanine aminotransferase (U/L)	31.30 ± 5.99	33.03 ± 12.88	32.37 ± 3.16	32.97 ± 5.57	26.37 ± 5.25
Alkaline phosphatase (U/L)	143.9 ± 21.1	135.9 ± 21.6	119.6 ± 4.8	145.0 ± 30.1	134.4 ± 35.7
Creatinine (µmol/L)	22.33 ± 2.08	20.67 ± 2.52	19.33 ± 3.21	23.00 ± 1.73	21.33 ± 2.31
Protein (g/L)	55.27 ± 2.32	54.70 ± 2.72	57.00 ± 0.92	59.33 ± 0.25	55.00 ± 1.78
Albumin (g/L)	29.30 ± 2.36	28.95 ± 1.57	30.95 ± 1.07	32.46 ± 0.46	28.95 ± 1.58
Cholesterol (mmol/L)	1.65 ± 0.25	1.59 ± 0.26	1.71 ± 0.17	1.62 ± 0.12	1.61 ± 0.26
Urea (mmol/L)	6.91 ± 0.43	7.06 ± 1.12	6.17 ± 0.41	8.00 ± 0.71	6.39 ± 0.36
Sodium (mmol/L)	118.7 ± 11.6	114.0 ± 2.0	122.0 ± 12.5	128.0 ± 4.0	117.0 ± 6.2
Potassium (mmol/L)	3.28 ± 0.21	3.20 ± 0.07	3.32 ± 0.15	3.72 ± 0.24	3.39 ± 0.08
Females					
Aspartate aminotransferase (U/L)	91.77 ± 13.37	79.23 ± 9.29	81.93 ± 14.30	77.57 ± 12.23	70.20 ± 3.47
Alanine aminotransferase (U/L)	28.00 ± 4.07	24.60 ± 4.65	28.20 ± 4.52	23.87 ± 3.54	30.07 ± 4.62
Alkaline phosphatase (U/L)	100.14 ± 51.91	63.52 ± 11.41	83.17 ± 23.32	59.51 ± 2.84	74.76 ± 3.47
Creatinine (µmol/L)	22.67 ± 3.79	23.33 ± 5.51	21.67 ± 5.13	21.33 ± 1.53	26.67 ± 2.52
Protein (g/L)	57.27 ± 2.32	59.13 ± 4.65	57.13 ± 0.57	59.30 ± 4.25	58.70 ± 1.74
Albumin (g/L)	32.75 ± 2.35	33.98 ± 2.50	33.09 ± 0.55	34.01 ± 2.67	33.53 ± 1.31
Cholesterol (mmol/L)	1.63 ± 0.28	1.83 ± 0.34	1.33 ± 0.13	2.06 ± 0.36	1.42 ± 0.24
Urea (mmol/L)	6.83 ± 0.53	6.32 ± 1.32	5.99 ± 0.92	7.04 ± 0.83	7.58 ± 0.83
Sodium (mmol/L)	131.67 ± 0.58	132.00 ± 1.00	133.33 ± 1.15	132.33 ± 0.58	131.33 ± 0.58
Potassium (mmol/L)	3.14 ± 0.30	3.12 ± 0.12	3.07 ± 0.32	3.40 ± 0.32	3.21 ± 0.25

[a] Data obtained from pages 51 and 52 of the study report. N=3/group.

E. Sacrifice and pathology

1. Organ weight – No treatment-related changes in absolute or relative organ weights were observed (Tables 7a and 7b). However, analysis of these data is hindered by the small sample sizes and the high variability noted in some measurements.

Table 7a. Mean organ weight values in male rats[a]

Parameter	0 ppm	100 ppm	500 ppm	1000 ppm	10,000 ppm
Males					
Liver weight – absolute (g)	9.298 ± 0.461	8.863 ± 0.482	8.933 ± 0.478	9.365 ± 0.331	9.563 ± 0.991
Liver weight – relative (%)	2.806 ± 0.175	2.780 ± 0.045	2.779 ± 0.041	2.924 ± 0.026	2.870 ± 0.244
Spleen weight – absolute (g)	0.812 ± 0.075	0.694 ± 0.029	0.764 ± 0.062	0.755 ± 0.065	0.718 ± 0.039
Spleen weight – relative (%)	0.245 ± 0.017	0.218 ± 0.006	0.238 ± 0.021	0.235 ± 0.011	0.216 ± 0.003
Kidney weight – absolute (g)	2.329 ± 0.099	2.434 ± 0.083	2.466 ± 0.212	2.576 ± 0.071	2.651 ± 0.069
Kidney weight – relative (%)	0.703 ± 0.051	0.764 ± 0.025	0.767 ± 0.043	0.805 ± 0.043	0.796 ± 0.015
Adrenal gland weight – absolute (g)	0.061 ± 0.009	0.065 ± 0.012	0.066 ± 0.009	0.080 ± 0.026	0.074 ± 0.005
Adrenal gland weight – relative (%)	0.018 ± 0.003	0.020 ± 0.005	0.021 ± 0.003	0.025 ± 0.009	0.022 ± 0.003
Testes weight – absolute (g)	3.252 ± 0.203	3.419 ± 0.200	3.573 ± 0.184	3.506 ± 0.217	3.518 ± 0.233
Testes weight – relative (%)	0.981 ± 0.061	1.073 ± 0.038	1.113 ± 0.060	1.098 ± 0.113	1.058 ± 0.098
Epididymides weight – absolute (g)	1.142 ± 0.036	1.188 ± 0.063	1.160 ± 0.024	1.257 ± 0.068	1.180 ± 0.028
Epididymides weight – relative (%)	0.344 ± 0.002	0.373 ± 0.024	0.361 ± 0.019	0.393 ± 0.036	0.355 ± 0.015
Thymus weight – absolute (g)	0.615 ± 0.046	0.550 ± 0.021	0.525 ± 0.072	0.502 ± 0.104	0.502 ± 0.038
Thymus weight – relative (%)	0.185 ± 0.012	0.173 ± 0.013	0.164 ± 0.028	0.157 ± 0.032	0.151 ± 0.009
Heart weight – absolute (g)	1.020 ± 0.049	1.037 ± 0.045	1.003 ± 0.031	1.006 ± 0.081	1.037 ± 0.043
Heart weight – relative (%)	0.308 ± 0.024	0.325 ± 0.011	0.312 ± 0.003	0.314 ± 0.013	0.311 ± 0.003
Brain weight – absolute (g)	1.964 ± 0.052	1.995 ± 0.030	1.996 ± 0.052	2.031 ± 0.056	2.034 ± 0.044
Brain weight – relative (%)	0.593 ± 0.033	0.627 ± 0.035	0.622 ± 0.027	0.634 ± 0.012	0.612 ± 0.033
Lung weight – absolute (g)	1.429 ± 0.035	1.409 ± 0.068	1.541 ± 0.041	1.364 ± 0.110	1.436 ± 0.048
Lung weight – relative (%)	0.431 ± 0.022	0.442 ± 0.004	0.480 ± 0.021	0.426 ± 0.023	0.431 ± 0.015
Prostate & seminal vesicle with coagulating gland weight – absolute (g)	1.908 ± 0.037	1.887 ± 0.091	1.846 ± 0.157	2.108 ± 0.106	1.985 ± 0.165
Prostate & seminal vesicle with coagulating gland weight – absolute (%)	0.575 ± 0.009	0.592 ± 0.027	0.574 ± 0.041	0.659 ± 0.047	0.596 ± 0.034

[a] Data obtained from pages 54 and 56 of the study report. N=3/group.

Table 7b. Mean organ weight values in female rats[a]

Parameter	0 ppm	100 ppm	500 ppm	1000 ppm	10,000 ppm
Females					
Liver weight – absolute (g)	5.753 ± 0.321	5.594 ± 0.718	5.758 ± 0.743	5.398 ± 0.209	6.499 ± 1.008
Liver weight – relative (%)	2.990 ± 0.080	2.951 ± 0.123	3.069 ± 0.654	2.944 ± 0.118	3.236 ± 0.392
Spleen weight – absolute (g)	0.495 ± 0.075	0.513 ± 0.055	0.448 ± 0.037	0.473 ± 0.084	0.541 ± 0.077
Spleen weight – relative (%)	0.257 ± 0.033	0.271 ± 0.001	0.237 ± 0.026	0.258 ± 0.047	0.270 ± 0.039
Kidney weight – absolute (g)	1.579 ± 0.116	1.529 ± 0.178	1.662 ± 0.344	1.466 ± 0.071	1.541 ± 0.175
Kidney weight – relative (%)	0.821 ± 0.038	0.807 ± 0.010	0.877 ± 0.170	0.800 ± 0.040	0.769 ± 0.077
Adrenal gland weight – absolute (g)	0.077 ± 0.008	0.067 ± 0.009	0.064 ± 0.004	0.070 ± 0.005	0.082 ± 0.007
Adrenal gland weight – relative (%)	0.040 ± 0.004	0.035 ± 0.003	0.034 ± 0.003	0.038 ± 0.003	0.041 ± 0.002
Ovarian weight – absolute (g)	0.114 ± 0.010	0.123 ± 0.018	0.096 ± 0.028	0.099 ± 0.022	0.111 ± 0.024
Ovarian weight – relative (%)	0.059 ± 0.003	0.065 ± 0.005	0.051 ± 0.014	0.054 ± 0.012	0.056 ± 0.014
Uterus with cervix weight – absolute (g)	0.703 ± 0.371	0.479 ± 0.041	0.713 ± 0.251	0.563 ± 0.188	0.551 ± 0.147
Uterus with cervix weight – relative (%)	0.362 ± 0.179	0.256 ± 0.041	0.384 ± 0.162	0.307 ± 0.103	0.277 ± 0.086
Thymus weight – absolute (g)	0.456 ± 0.087	0.473 ± 0.177	0.478 ± 0.093	0.440 ± 0.033	0.547 ± 0.057
Thymus weight – relative (%)	0.237 ± 0.040	0.245 ± 0.066	0.252 ± 0.037	0.240 ± 0.018	0.272 ± 0.019
Heart weight – absolute (g)	0.789 ± 0.020	0.732 ± 0.068	0.753 ± 0.092	0.748 ± 0.024	0.796 ± 0.011
Heart weight – relative (%)	0.411 ± 0.011	0.387 ± 0.027	0.397 ± 0.039	0.408 ± 0.014	0.398 ± 0.012
Brain weight – absolute (g)	1.790 ± 0.065	1.773 ± 0.071	1.754 ± 0.072	1.807 ± 0.024	1.808 ± 0.097
Brain weight – relative (%)	0.931 ± 0.005	0.941 ± 0.058	0.930 ± 0.097	0.985 ± 0.013	0.902 ± 0.014
Lung weight – absolute (g)	1.054 ± 0.042	1.021 ± 0.017	1.006 ± 0.058	1.057± 0.046	1.072 ± 0.085
Lung weight – relative (%)	0.548 ± 0.015	0.543 ± 0.052	0.533 ± 0.058	0.577 ± 0.026	0.535 ± 0.035

[a] Data obtained from pages 55 and 57 of the study report. N=3/group.

2. <u>Gross pathology</u> – No treatment-related gross pathological changes were observed.

3. <u>Microscopic pathology</u> – No treatment-related histological changes were observed in the lung, liver, kidneys of thymus of high dose animals when compared to controls.

III. Discussion

A. Investigators' conclusions – "Under the conditions of the present study, the repeated dietary exposure of the test item 3,4,4-Trifluorobut-3-ene-1-sulfonic acid, to male and female Wistar rats at doses of 100, 500, 1000 and 10,000 ppm for 28 days was associated with no signs of toxicity or mortality."

"Based on the data generated from this dose range finding study, dose levels of 0, 440, 2200 and 11,000 ppm are suggested for the subsequent main study with 3,3,4-trifluorobut-3-ene-1-sulfonic acid."

B. Reviewer comments – Aside from marginal (<25%) decreases in mean serum aspartate aminotransferase levels in both sexes and alanine aminotransferase levels in males at the high dose of 10,000 ppm, no treatment-related effects were observed in this study up to the highest dose tested. This study is limited due to the small group sizes and reduced number of hematology, clinical chemistry and histopathology examinations. However, the number of parameters measured is sufficient to indicate that BSA is significantly less toxic than fluensulfone in rats.

C. Study deficiencies – This study was designed as a dose range-finding study with limited investigations and was not conducted in compliance with GLP. The group sizes (3/sex) were smaller than recommended in the guidelines (5/sex), and a reduced number of hematology, clinical chemistry and histopathology examinations.