UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

May 30, 2008

MEMORANDUM

Subject:	Name of Pesticide Product:	GF-1848
	EPA Reg. No. /File Symbol:	62719-LIE
	DP Barcode:	D343726
	Decision No.:	380789
	Action Code:	R02.4
	PC Codes:	128968 (Fluroxypyr-1-methyl heptyl ester)
		129108 (Florasulam)
		108702 (Pyroxsulam)

From: Byron T. Backus, Ph.D., Toxicologist
Technical Review Branch
Registration Division (7505P)

Byron T. Backus 5-30-2008 Hasta

To: James Stone/Joanne Miller, RM 23
Herbicide Branch
Registration Division (7505P)

Registrant: DOW AGROSCIENCES LLC

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
129108: Florasulam	0.20%
128968 Fluroxypyr 1-methylheptyl ester	11.57%
108702 Pyroxsulam	1.20%
Other Ingredient(s):	87.03%
TOTAL	100.00%

ACTION REQUESTED: The Risk Manager requests:

"Review acute studies for application for registration. New ai and safener have to be approved before this product can be registered."

BACKGROUND:

The material received for review includes 5 acute toxicity studies with their corresponding study profile templates. The package also contains a cover letter from the registrant dated June 21, 2007, a label for the proposed product, and a basic CSF.

COMMENTS AND RECOMMENDATIONS:

1. An Agency contractor, Oak Ridge National Laboratory, conducted the primary review of the 5 acute toxicity studies which were included in this submission. TRB performed the secondary review and made changes as necessary. It was noted that an acute inhalation toxicity study (in MRID 47166905) for GF-1848 had been sent to the Agency, but a copy of this study was not sent to ORNL for review. This study was cited by the registrant to support another registration (see the memorandum dated Dec. 10, 2007 from M. Hashim to D. Morgan); however, there is no record that this study has been previously reviewed. TRB has now done the primary review on this study (DER included in this memorandum).

2. The Skin Sensitization (Mouse Local Lymph Node Assay) in MRID 47166908 is classified as unacceptable. Although it does satisfy the guideline requirements for a skin sensitization study (OECD 429), the procedure "was never validated for mixtures by the assay creators." It has undergone a validation series through ICCVAM and found not to be an acceptable method for mixtures. Since a positive response was elicited in this study, the product can be labeled as a sensitizer (it has most conservative statement no further testing would be required.).

3. The remaining 5 acute toxicity studies have been reviewed and have all been classified as acceptable. These studies, together with labeling indicating that this product is a sensitizer, satisfy the acute toxicity data requirements for the registration of 62719-LIE.

4. Based on the results of the submitted acute toxicity studies and the classification of this proposed product as a positive dermal sensitizer, the following is the acute toxicity profile for EPA File Symbol 62719-LIE [GF-1848]. The indicated signal word is WARNING, based on the results from the eye irritation study:

Acute oral toxicity	III	Acceptable	MRID 47166903
Acute dermal toxicity	IV	Acceptable	MRID 47166904
Acute inhalation toxicity	IV	Acceptable	MRID 47166905
Primary eye irritation	II	Acceptable	MRID 47166906
Primary dermal irritation	III	Acceptable	MRID 47166907
Dermal sensitization (LLNA)	Positive	Unacceptable[1]	MRID 47166908

[1]Labeling indicating this product is a sensitizer satisfies the dermal sensitization data requirement for the registration of 62719-LIE.

5. Based on the acute toxicity profile for 62719-LIE [GF-1848] given above, as well as information from the CSF and proposed labeling, the following would be its precautionary and first aid labeling, as obtained from the TRB Label Review System:

PRODUCT ID #: **062719-00582**

PRODUCT NAME: **GF-1848**

PRECAUTIONARY STATEMENTS

SIGNAL WORD: **WARNING**

SPANISH SIGNAL WORD: AVISO

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle.
(If you do not understand the label, find someone to explain it to you in detail.)

Hazards to Humans and Domestic Animals:

Contains Petroleum Distillate.

Causes substantial but temporary eye injury. Harmful if swallowed. Avoid contact with skin or clothing. Do not get in eyes or on clothing. Wear protective eyewear (goggles, face shield, or safety glasses). Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco. Remove and wash contaminated clothing before reuse. Wear long-sleeved shirt and long pants, socks, shoes, and chemical-resistant gloves (such as Barrier Laminate, Butyl Rubber, Nitrile Rubber, Viton, Barrier Laminate, Viton, Selection Category F, G). If the Selection Category F, G gloves do not provide adequate protection for this product, the registrant should indicate a specific glove category from the EPA chemical resistance glove selection chart that will provide adequate protection.

Prolonged or frequently repeated skin contact may cause allergic reactions in some individuals.

First Aid:

If in eyes:
-Hold eye open and rinse slowly and gently with water for 15-20 minutes.
-Remove contact lenses, if present, after the first 5 minutes, then continue rinsing.
-Call a poison control center or doctor for treatment advice.

If swallowed:
-Call a poison control center or doctor immediately for treatment advice.
-Have person sip a glass of water if able to swallow.
-Do not induce vomiting unless told to by a poison control center or doctor.
-Do not give anything to an unconscious person.

If on skin:
-Take off contaminated clothing.
-Rinse skin immediately with plenty of water for 15-20 minutes.
-Call a poison control center or doctor for treatment advice.

NOTE TO PHYSICIAN: May pose an aspiration pneumonia hazard. Contains petroleum distillate. NOTE TO PHYSICIAN: Note to PM/CRM/Registrant: The proposed label should contain a "Note to Physician". The following statements are suggested types of information that may be included, if applicable: - technical

information on symptomatology; - use of supportive treatments to maintain life functions; - medicine that will counteract the specific physiological effects of the pesticide; - company telephone number to specific medical personnel who can provide specialized medical advice.

Have the product container or label with you when calling a poison control center or doctor or going for treatment. You may also contact 1-800-xxx-xxxx for emergency medical treatment information.

6. CSF (dated June 13, 2007) for the proposed product should also be reviewed and accepted by the TRB Chemistry Team.

DATA EVALUATION RECORD

FLUROXYPYR, FLORASULAM, PYROXSULAM (GF-1848)

STUDY TYPE: ACUTE ORAL TOXICITY - RAT [OPPTS 870.1100; OECD 425]
ACUTE DERMAL TOXICITY - RAT [OPPTS 870.1200; OECD 402]
ACUTE EYE IRRITATION - RABBIT [OPPTS 870.2400; OECD 405]
ACUTE DERMAL IRRITATION - RABBIT [OPPTS 870.2500; OECD 404]
SKIN SENSITIZATION (LOCAL LYMPH NODE ASSAY) - MOUSE [OECD 429]

MRID 47166903, 47166904, 47166906, 47166907, and 47166908

Prepared for
Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 1-18

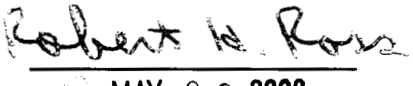
Primary Reviewer:
Susan Chang, M.S.

Signature: 
Date: MAY 08 2008

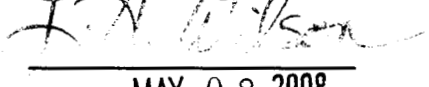
Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.

Signature: 
Date: MAY 08 2008

Robert H. Ross, M.S., Group Leader

Signature: 
Date: MAY 08 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: 
Date: MAY 08 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

STUDY TYPE: Acute Oral Toxicity – Rat; OPPTS 870.1100; OECD 425

TEST MATERIAL: GF-1848 (11.8% w/w Fluroxypyr meptyl, 0.21% w/w Florasulam, 1.2% w/w Pyroxsulam, and 3.6% w/w Cloquintocet mexyl; Lot No. E-2154-74; dark brown liquid, specific gravity 1.05 g/mL, emulsible in water, soluble in ethanol and acetone, dispersible in corn oil and mineral oil)

CITATION: Durando, J. (2007) GF-1848 – Acute Oral Toxicity Up and Down Procedure in Rats. Study Number 21426. Eurofins|Product Safety Laboratories, 2394 US Highway 130, Dayton, NJ 08810. April 20, 2007. MRID 47166903.

SPONSOR: The Dow Chemical Company, Midland, MI 48674 for Dow Agrosciences LLC, Indianapolis, IN 46268

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 47166903), nine fasted, young adult female Fischer 344 rats (age: 10-11 weeks; body weight: 116-154 g; source: Harlan, Indianapolis, In) were given a single dose of GF-1848 (11.8% w/w Fluroxypyr meptyl, 0.21% w/w Florasulam, 1.2% w/w Pyroxsulam, and 3.6% w/w Cloquintocet mexyl; Lot No. E-2154-74) as received at concentrations of 175 (1 rat), 550 (1 rat), 1750 (3 rats), or 5000 (4 rats) mg/kg bw by gavage and observed for 14 days.

All 5000 mg/kg animals were euthanized in extremis or dead by day 1 or 2. All other animals survived the study. The 175 mg/kg animal appeared active and healthy throughout the study. The 550 and 1750 mg/kg surviving animals were hypoactive and/or had reduced fecal volume and/or hunched posture on day 1 or up to day 3 with recovery by day 4. They were active and healthy thereafter. All survivors gained weight throughout the study. The decedents had hypoactivity, piloerection, hunched posture, shallow respiration, facial staining, prone posture, reduced fecal volume and/or were moribund prior to death. Stomachs blackened or filled with light yellow, creamy liquid and/or black intestines were noted from the decedents. No gross abnormalities were noted from any surviving animal at necropsy.

Females Estimated LD_{50} = 3129 mg/kg bw (Based on an assumed sigma of 0.5). Approximate 95% confidence interval is 1750 to 5000 mg/kg bw.

GF-1848 is in EPA Toxicity Category III.

This study is classified as acceptable. It does satisfy the guideline requirements for an acute oral study (OPPTS 870.1100; OECD 425) in the rat.

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

RESULTS and DISCUSSION:

One rat was initially dosed at 5000 mg/kg. Because this rat died, a Main Test was conducted.

AOT425statpgm (Version: 1.0) Test Results and Recommendations
Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program

Date/Time: Monday, March 31, 2008, 2:47:41 PM
Data file name: work.dat
Last modified: 3/31/2008 2:47:39 PM

Test/Substance: GF-1848
Test type: Main Test
Limit dose (mg/kg): 5000
Assumed LD_{50} (mg/kg): Default
Assumed sigma (mg/kg): 0.5

Recommended dose progression: 5000, 1750, 550, 175, 55, 17.5, 5.5, 1.75

DATA:

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	1	175	O	O
2	2	550	O	O
3	3	1750	O	O
4	4	5000	X	X
5	5	1750	O	O
6	6	5000	X	X
7	7	1750	O	O
8	8	5000	X	X

(X = Died, O = Survived)

Dose Recommendation: The main test is complete.

Stopping criteria met: 5 reversals in 6 tests. LR criterion.

SUMMARY OF LONG-TERM RESULTS:

Dose	O	X	Total
175	1	0	1
550	1	0	1
1750	3	0	3
5000	0	3	3
All Doses	5	3	8

Statistical Estimate based on long term outcomes:
Estimated LD_{50} = 3129 (Based on an assumed sigma of 0.5).
Approximate 95% confidence interval is 1750 to 5000.

Animals were dosed as follows:

Animal Number	Sex	Dose Level (mg/kg)	Long-Term Outcome
3101[a]	F	5000	D
3102	F	175	S
3103	F	550	S
3104	F	1750	S
3105	F	5000	D
3106	F	1750	S
3107	F	5000	D
3108	F	1750	S
3109	F	5000	D

S = Survival, D = Death
[a] Dosed for limit test, due to mortality of this animal, main test was conducted.

A. Mortality: All 5000 mg/kg animals were euthanized or dead on day 1 or 2. All other animals survived the study.

B. Clinical observations: The 175 mg/kg animal appeared active and healthy throughout the study. The 550 and 1750 mg/kg surviving animals were hypoactive and/or had reduced fecal volume and/or hunched posture on day 1 or up to day 3 with recovery by day 4 and were active and healthy thereafter. The decedents had hypoactivity, piloerection, hunched posture, shallow respiration, facial staining, prone posture, reduced fecal volume and/or were moribund prior to death.

C. Gross necropsy: Stomachs blackened or filled with light yellow, creamy liquid and/or black intestines were noted from the decedents. No gross abnormalities were noted from any surviving animal.

D. Reviewer's conclusions: This reviewer agrees with the study author that the acute oral LD_{50} = 3129 mg/kg bw (Based on an assumed sigma of 0.5), with an approximate 95% confidence interval is 1750 to 5000 mg/kg bw. GF-1848 is in EPA Toxicity Category III for oral toxicity.

STUDY TYPE: Acute Dermal Toxicity – Rat; OPPTS 870.1200; OECD 402

TEST MATERIAL: GF-1848 (11.8% w/w Fluroxypyr meptyl, 0.21% w/w Florasulam, 1.2% w/w Pyroxsulam, and 3.6% w/w Cloquintocet mexyl; Lot No. E-2154-74; dark brown liquid, specific gravity 1.05 g/mL, emulsible in water, soluble in ethanol and acetone, dispersible in corn oil and mineral oil)

CITATION: Durando, J. (2007) GF-1848 – Acute Dermal Toxicity Study in Rats – Limit Test. Study Number 21427. Eurofins/Product Safety Laboratories, 2394 US Highway 130, Dayton, NJ 08810. April 20, 2007. MRID 47166904.

SPONSOR: The Dow Chemical Company, Midland, MI 48674 for Dow AgroSciences LLC, Indianapolis, IN 46268

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 47166904), five male and five female young adult Fischer 344 rats (age: 9 weeks; body weight: males: 177-190 g and females: 135-150 g; source: Harlan, Indianapolis, IN) were dermally exposed for 24 hours on an area of 6 in^2 (approximately 10% of the total body surface area) on the clipped dorsal trunk to 5000 mg/kg bw GF-1848 (11.8% w/w Fluroxypyr meptyl, 0.21% w/w Florasulam, 1.2% w/w Pyroxsulam, and 3.6% w/w Cloquintocet mexyl; Lot No. E-2154-74) as received. The test material was applied evenly over the dose area and covered with a gauze pad. The gauze and the trunk were wrapped with Durapore tape. The animals were observed for 14 days.

All animals survived and gained weight during the study, although one of the females had a slight (1 g) weight loss between days 0 and 7, and two additional females had only slight (1 g) weight gains in this period. All females had more substantial weight gains (9-14 g) from day 7 to 14. Erythema, edema, and desquamation were noted on the dose sites of all animals on days 1 and/or up to day 9. Eschar was noted on one male and all females on days 3 up to 9. Thereafter, the animals were active and healthy. All other animals appeared active and healthy throughout the study. No gross abnormalities were noted at necropsy.

LD_{50} Males > 5000 mg/kg bw
LD_{50} Females > 5000 mg/kg bw
LD_{50} Combined > 5000 mg/kg bw

GF-1848 is in EPA Toxicity Category IV for dermal toxicity.

This study is classified as acceptable. It does satisfy the guideline requirements for an acute dermal study (OPPTS 870.1200; OECD 402) in the rat.

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

RESULTS and DISCUSSION:

Dose (mg/kg bw)	Mortality/Number Tested		
	Males	Females	Combined
5000	0/5	0/5	0/10

A. **Mortality:** All animals survived the study.

B. **Clinical observations:** Erythema, edema, and desquamation were noted on the dose sites of all animals on days 1 and/or up to day 9. Eschar was noted on one male and all females on days 3 up to 9. Thereafter, the animals were active and healthy. One female had a slight weight loss (1 g) during the first week and two other females had only slight (1 g) weight gains in this period. All females had more substantial weight gains (9-14 g) from day 7 to 14.

C. **Gross necropsy:** No gross abnormalities were noted at necropsy.

D. **Reviewer's conclusions:** This reviewer agrees with the study author that the acute dermal $LD_{50} > 5000$ mg/kg; this formulation is in Toxicity Category IV for dermal toxicity.

STUDY TYPE: Acute Inhalation Toxicity – Rat; OPPTS 870.1300; OECD 403

TEST MATERIAL: GF-1848 (11.8% w/w [124 g/L] Fluroxypyr meptyl, 0.21% w/w [2.2 g/L] Florasulam, 1.2% w/w [13 g/L] Pyroxsulam, and 3.6% w/w [38 g/L] Cloquintocet mexyl; Lot No. E-2154-74; a dark brown liquid).

SYNONYMS: A mixture of N-(5,7-dimethoxy(1,2,4)triazolo(1,5-a)pyrimidin-2-yl)2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide (XDE-742), N-(2,6-difluorophenyl)8-fluoro-5-methoxy-(1,2,4)triazolo(1,5-c)pyrimidine-2-sulfonamide (Florasulam), ((4-amino-3,5-dichloro-2-pyridinyl)oxy)-acetic acid methyl ester (Fluroxypyr-meptyl), and ((5-chloro-8-quinolinyl)oxy)-acetic acid-1-methylhexyl ester (Cloquintocet-mexyl)

CITATION: Krieger, S. and B. Radtke (2007) GF-1848: Acute Liquid Aerosol Inhalation Toxicity Study in F344/DUCRL Rats. Study Number 071002. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI 48674. May 2, 2007. MRID 47166905.

Radtke, B. (2007) Study Profile Template (SPT) for GF-1848: Acute Liquid Aerosol Inhalation Toxicity Study in F344/DUCRL Rats. Study Number 071002.SPT. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI 48674. May 2, 2007. MRID 47166911.

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 47166905 and 47166911), five male and five female young adult F344/DuCrl rats (age: 9 weeks; body weight: males: 190.0-195.8 g and females: 136.6-146.0 g; source: Charles River Laboratories, Inc., Kingston, NY) were exposed by nose-only inhalation to GF-1848 (11.8% w/w [124 g/L] Fluroxypyr meptyl, 0.21% w/w [2.2 g/L] Florasulam, 1.2% w/w [13 g/L] Pyroxsulam, and 3.6% w/w [38 g/L] Cloquintocet mexyl) for 4 hours at an time-weighted average (TWA) concentration of 2.46 mg/L (the highest attainable respirable chamber concentration). The animals were observed for 14 days. The MMAD was 3.34 and 3.52 µm at 57 minutes and 167 minutes into the exposure, respectively, and the GSD 2.31 and 2.16, respectively.

All animals survived the study. Effects noted during the 4-hour exposure were soiling of the haircoat in one male and two females. Post-exposure signs included slow, noisy, labored respiration with or without mouth breathing, decreased feces, dehydration, and perineal, perioral, abdominal and/or extensive body soiling. All rats appeared normal by day 6. All rats had lost weight from their preexposure values at 24 and 72 hours after exposure, but all showed weight gains at 7 and 14 days after exposure.

No visible lesions were noted in any animal at necropsy.

LC_{50} Males > 2.46 mg/L
LC_{50} Females > 2.46 mg/L
LC_{50} Combined > 2.46 mg/L

GF-1848 is in EPA Toxicity Category IV for inhalation toxicity.

This study is classified as acceptable. It does satisfy the guideline requirements for an acute inhalation study (OPPTS 870.1300; OECD 403) in the rat.

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

RESULTS and DISCUSSION:

Nominal Conc. (mg/L)	Gravimetric Conc. (mg/L)	MMAD µm	GSD	Mortality/Number Tested		
				Males	Females	Combined
8.21	2.48	3.34, 3.52	2.31, 2.16	0/5	0/5	0/10

Test Atmosphere / Chamber Description: The exposure atmosphere was generated by metering the test material with a FMI pump (Fluid Metering, Inc., Oyster Bay, NY) into a stainless steel ¼-J spray nozzle (Spraying Systems Co., Wheaton, IL). The test material was mixed with humidified compressed air within the spray nozzle and the aerosol was sprayed into the inhalation chamber. Since the formulation contained materials of varying vapor pressures, the test material was not recycled.

Gravimetric Conc. (mg/L):	**2.46 (the highest attainable respirable chamber concentration)**
Chamber Volume (L):	**42**
Total Airflow (L/min):	**30.0**
Temperature	**21 ± 0°C**
Relative Humidity	**18 ± 0%**
Time to equilibrium (T_{99})	**6.4 minutes**

Test atmosphere concentration: During exposure, gravimetric samples were collected six times from the breathing zone of the animals, using pre-weighed polytetrafluoroethylene filters. After each sampling, the filter was reweighed to obtain the total weight of the particles. Filters were air-dried to a stable weight and the time-weighed average exposure concentration was calculated from the net dry weight gravimetric analysis and the percent solids in the total formulation. The nominal concentration was calculated based on the amount of test material fed into the generation system divided by the total chamber airflow during the exposure period.

Particle size determination: Particle size was determined twice using a mercer multi-stage cascade impactor. The mass median aerodynamic diameter and geometric standard deviation were determined for each sample.

A. **Mortality:** All animals survived the study.

B. **Clinical observations:** Two females and one male had soiling of the haircoat during exposure. Post-exposure signs included slow, noisy, labored respiration with or without mouth breathing, decreased feces, dehydration, and perineal, perioral, abdominal and/or extensive body soiling. All rats appeared normal by day 6. All rats had lost weight from their preexposure values at 24 and 72 hours after exposure, but all showed weight gains at 7 and 14 days after exposure.

C. **Gross necropsy:** No visible lesions were noted in any animal at necropsy.

D. **Reviewer's conclusions:** This reviewer agrees with the study author that the acute inhalation $LC_{50} > 2.46$ µg/L. GF-1848 is in EPA Toxicity Category IV for acute inhalation toxicity.

STUDY TYPE: Primary Eye Irritation – Rabbit; OPPTS 870.2400; OECD 405

TEST MATERIAL: GF-1848 (11.8% w/w Fluroxypyr meptyl, 0.21% w/w Florasulam, 1.2% w/w Pyroxsulam, and 3.6% w/w Cloquintocet mexyl; Lot No. E-2154-74; dark brown liquid, specific gravity 1.05 g/mL, emulsible in water, soluble in ethanol and acetone, dispersible in corn oil and mineral oil)

CITATION: Durando, J. (2007) GF-1848 – Acute Eye Irritation Study in Rabbits. Study Number 21428. Eurofins|Product Safety Laboratories, 2394 US Highway 130, Dayton, NJ 08810. April 3, 2007. MRID 47166906.

SPONSOR: The Dow Chemical Company, Midland, MI 48674 for Dow AgroSciences LLC, Indianapolis, IN 46268

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 47166906), 0.1 mL of undiluted GF-1848 (11.8% w/w Fluroxypyr meptyl, 0.21% w/w Florasulam, 1.2% w/w Pyroxsulam, and 3.6% w/w Cloquintocet mexyl; Lot No. E-2154-74; pH not reported) was instilled into the conjunctival sac of the right eye of three male young adult New Zealand White rabbits (source: Robinson Services, Inc., Clemmons, NC). The untreated eye served as a control. The animals were observed for 72 hours and on days 4, 7, 10, 14, and 17.

Corneal opacity was noted in 3/3 rabbits from 24 hours through day 4 after test material instillation with clearance in one rabbit by day 7, in another rabbit by day 14, and in the third rabbit by day 17. Iritis was noted in 3/3 rabbits at one through 24 hours after test material instillation with clearance in one rabbit by 48 hours and in two rabbits by day 7. Positive conjunctival irritation was noted in 3/3 rabbits from one through 24 hours after test material instillation with clearance in one rabbit by 48 hours, in another rabbit by day 4, in the third rabbit by day 7. The highest maximum mean total score was 29.0, recorded 24 hours after test material instillation.

In this study, the formulation was moderately irritating. GF-1848 is in EPA Toxicity Category II for primary eye irritation.

This study is classified as acceptable. It does satisfy the guideline requirements 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.

RESULTS and DISCUSSION:

	Number "positive"/Number treated								
	Hours				Days				
Observations	**1**	**24**	**48**	**72**	**4**	**7**	**10**	**14**	**17**
Corneal Opacity	0/3	3/3	3/3	3/3	3/3	2/3	2/3	1/3	0/3
Iritis	3/3	3/3	2/3	2/3	2/3	0/3	0/3	0/3	0/3
Conjunctivae:									
Redness*	3/3	3/3	2/3	2/3	1/3	0/3	0/3	0/3	0/3
Chemosis*	1/3	2/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Discharge**	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

* Score of 2 or more required to be considered "positive"
** Discharge is not a positive effect according to the grading scale

A. **Observations:** Corneal opacity was noted in 3/3 rabbits from 24 hours through day 4 after test material instillation with clearance in one rabbit by day 7, in another rabbit by day 14, and in the third rabbit by day 17. Iritis was noted in 3/3 rabbits from one through 24 hours after test material instillation with clearance in one rabbit by 48 hours and in two rabbits by day 7. Positive conjunctival irritation was noted in 3/3 rabbits from one through 24 hours after test material instillation with clearance in one rabbit by 48 hours, in another rabbit by day 4, and in the third rabbit by day 7.

B. **Results:** GF-1848 was moderately irritating. The highest maximum mean total score was 29.0, recorded 24 hours after test material instillation.

C. **Reviewer's conclusions:** This reviewer agrees with the study author that the test material caused corneal opacity, iritis, and conjunctival irritation with clearance by day 17. This reviewer classifies the test material as moderately irritating. GF-1848 is in EPA Toxicity Category II for eye irritation.

STUDY TYPE: Primary Dermal Irritation – Rabbit; OPPTS 870.2500; OECD 404

TEST MATERIAL: GF-1848 (11.8% w/w Fluroxypyr meptyl, 0.21% w/w Florasulam, 1.2% w/w Pyroxsulam, and 3.6% w/w Cloquintocet mexyl; Lot No. E-2154-74; dark brown liquid, specific gravity 1.05 g/mL, emulsible in water, soluble in ethanol and acetone, dispersible in corn oil and mineral oil)

CITATION: Durando, J. (2007) GF-1848 – Primary Skin Irritation Study in Rabbits. Study Number 21429. Eurofins|Product Safety Laboratories, 2394 US Highway 130, Dayton, NJ 08810. April 3, 2007. MRID 47166907.

SPONSOR: The Dow Chemical Company, Midland, MI 48674 for Dow AgroSciences LLC, Indianapolis, IN 46268

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 47166907), three male young adult New Zealand White rabbits (source: Robinson Services, Inc., Clemmons, NC) were dermally exposed to 0.5 mL of undiluted GF-1848 (11.8% w/w Fluroxypyr meptyl, 0.21% w/w Florasulam, 1.2% w/w Pyroxsulam, and 3.6% w/w Cloquintocet mexyl; Lot No. E-2154-74; pH not reported) for 4 hours on a 6 cm^2 area of the clipped dorsal skin that was covered with a gauze patch. The patch and trunk were wrapped with semi-occlusive Micropore tape. Elizabethan collars were placed on the rabbits. The animals were observed and irritation was scored at 1, 24, 48, and 72 hours after patch removal and on days 7, 10, and 14.

Well defined (score of 2) erythema was noted in 2/3 rabbits 30-60 minutes through day 7 after patch removal with reduction to very slight (score of 1) erythema by day 10 through day 14. Well defined erythema was noted in 1/3 rabbits 30-60 minutes through 24 hours after patch removal with intensification to moderate (score of 3) erythema by 48 through 72 hours, with reduction to well defined by day 7, and further reduction to very slight erythema by day 10 through day 14. Slight (score of 2) edema was noted in 3/3 rabbits 30-60 minutes after patch removal with reduction to very slight (score of 1) edema on one rabbit by day 7 through day 14 and on two rabbits by day 10 and with clearance by day 14. Desquamation was noted on all rabbits by day 9 through 14.

In this study, the formulation was moderately irritating based on the Primary Irritation Index (PII) of 4.15. GF-1848 is in EPA Toxicity Category III for primary dermal irritation.

This study is classified as acceptable. It does satisfy the guideline requirements 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.

RESULTS and DISCUSSION:

Animal Number	Sex	Hours				Days		
		1	24	48	72	7	10	14
3501	M	2/2[a]	2/2	2/2	2/2	2/2	1/1	1/0
3502	M	2/2	2/2	3/2	3/2	2/2	1/1	1/0
3503	M	2/2	2/2	2/2	2/2	2/1	1/1	1/1
Severity of Irritation – Mean Score		4.0	4.0	4.3	4.3	3.7	2.0	1.3

[a] Erythema/edema

A. **Observations:** Well defined (score of 2) erythema was noted in 2/3 rabbits 30-60 minutes through day 7 after patch removal with reduction to very slight (score of 1) erythema by day 10 through day 14. Well defined erythema was noted in 1/3 rabbits 30-60 minutes through 24 hours after patch removal with intensification to moderate (score of 3) erythema by 48 through 72 hours, with reduction to well defined by day 7, and with reduction to very slight erythema by day 10 through day 14. Slight (score of 2) edema was noted in 3/3 rabbits 30-60 minutes after patch removal with reduction to very slight (score of 1) edema in one rabbit by day 7 through day 14 and in two rabbits by day 10 and with clearance by day 14. Desquamation was noted on all rabbits by day 9 through 14.

B. **Results:** GF-1848 was moderately irritating. The Primary Irritation Index (PII) is 4.15 (calculated by the reviewer).

C. **Reviewer's conclusions:** This reviewer agrees with the study author that the test material caused moderate to severe erythema, slight edema, and desquamation with dermal irritation persistence through day 14. This reviewer classifies the test material as moderately irritating. GF-1848 is in EPA Toxicity Category III for dermal irritation.

STUDY TYPE: Skin Sensitization (Local Lymph Node Assay) – mouse; OECD 429

TEST MATERIAL: GF-1848 (11.8% w/w Fluroxypyr meptyl, 0.21% w/w Florasulam, 1.2% w/w Pyroxsulam, and 3.6% w/w Cloquintocet mexyl; Lot No. E-2154-74; dark brown liquid, specific gravity 1.05 g/mL, emulsible in water, soluble in ethanol and acetone, dispersible in corn oil and mineral oil).

SYNONYMS: A mixture of N-(5,7-dimethoxy(1,2,4)triazolo(1,5-a)pyrimidin-2-yl)2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide (XDE-742), N-(2,6-difluorophenyl)8-fluoro-5-methoxy-(1,2,4)triazolo(1,5-c)pyrimidine-2-sulfonamide (Florasulam), ((4-amino-3,5-dichloro-2-pyridinyl)oxy)-acetic acid methyl ester (Fluroxypyr-meptyl), and ((5-chloro-8-quinolinyl)oxy)-acetic acid-1-methylhexyl ester (Cloquintocet-mexyl)

CITATION: Woolhiser, M., C. Wiescinski, and L. Sosinski (2007) GF-1848 – Local Lymph Node Assay in CBA/J Mice. Study ID 061198. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI 48674. April 5, 2007. MRID 47166908.

SPONSOR: The Dow Chemical Company, Midland, MI 48674 for Dow AgroSciences LLC, Indianapolis, IN 46268

EXECUTIVE SUMMARY: In a local lymph node assay study (MRID 47166908) with GF-1848 (11.8% w/w Fluroxypyr meptyl, 0.21% w/w Florasulam, 1.2% w/w Pyroxsulam, and 3.6% w/w Cloquintocet mexyl), six female CBA/J mice/group received 25 µL of the test material administered topically to the dorsal surface of each ear once daily for 3 consecutive days at 0 (negative control: 1% L92), 1, 5, or 25% test material in 1% L92, respectively. Six female mice were treated with 30% v/v HCA in 1% L92 as a positive control. On day 6, the mice were injected intravenously with 20 µCi of ^{3}H-thymidine/mouse via the lateral tail vein. ^{3}H-thymidine incorporation into the auricular lymph nodes was measured and expressed as the number of disintegrations per minute (dpm). A stimulation index (SI) was derived for each group by dividing the mean dpm by the mean dpm of the vehicle control group. A positive response was indicated by an SI of ≥3.0.

One of the three tested concentrations of the test material exceeded the SI of 3: 1.8, 2.8, and 5.7 for the 1% test material group, 5% test material group, and 25% test material group, respectively. SI's of < 3.0 were observed for the negative control and a mean SI of 7.8 was observed for the positive control. The concentration that would cause a 3-fold increase in proliferation (EC_3) was calculated to be 6.4% which is consistent with a moderate dermal sensitization potential as described by an expert ECETOC panel (Technical Report No. 87, 2003).

Based on the results of this study, GF-1848 did produce a dermal sensitization response in mice.

This study is classified as unacceptable. Although it does satisfy the guideline requirements for a skin sensitization study (OECD 429), however, the procedure "was never validated for mixtures by the assay creators. It is undergoing a validation step through ICCVAM - which is a lead NIEHS group of all gov't agency." Since the study has the most conservative statement, no further testing would be required.

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

PROCEDURE:

MATERIALS AND METHODS:

A. **Vehicle and positive control**: The block copolymer Pluronic L92 surfactant (1% w/v) in water was the vehicle. The positive control was α–hexylcinnamaldehyde (HCA) diluted to 30% v/v in 1% L92.

B. **Treatment preparation and administration**: The dermal sensitization potential of the test material was examined using the local lymph node assay (LLNA). GF-2153 was combined with 1% L92 to obtain concentrations of 1%, 5%, and 25%. Six female CBA/J mice/group received twenty-five µL of the test material administered topically to the dorsal surface of each mouse ear once daily for 3 consecutive days at 0 (negative control: 1% L92), 1, 5, or 25% test material in 1% L92, respectively. Six female mice were treated with 30% v/v HCA in 1% L92 as a positive control. On day 6, the mice were injected intravenously with 20 µCi of ^{3}H-thymidine/mouse via the lateral tail vein. Approximately 5 hours after the injection, the animals were killed. The auricular lymph nodes were excised and placed in phosphate buffered saline (PBS), washed, and single cell suspensions were prepared. The cells were suspended in 5% TCA for approximately 18 hours. The suspended precipitates were centrifuged and the pellet was reconstituted in 1 mL of 5% TCA and transferred to a scintillation vial containing 10 mL of Aquasol-2 scintillation fluid. ^{3}H-thymidine incorporation was measured in a beta counter and expressed as the number of disintegrations per minute (dpm). Determination of radioactivity was performed individually on each animal. A stimulation index (SI) was derived for each group by dividing the mean dpm by the mean dpm of the vehicle control group. A positive response was indicated by an SI of ≥3.0. The EC_3 value is determined by interpolating between two values one above and one below the SI value of 3 (statistics and calculations).

RESULTS AND DISCUSSION:

A. Disintegrations per Minute (group means):

Concentration %	Animal Number	Individual Animal DPM	Group Mean DPM	Stimulation Index (SI)#
Vehicle (1% L92)	7337	1086.9	820.50±262.97	1.0±0.3
	7338	747.95		
	7339	4257.8[a]		
	7340	914.42		
	7341	950.69		
	7342	402.60		
1% GF-1848	7349	1747.3	1481.2±620.61	1.8±0.8
	7350	631.34		
	7351	2125.8		
	7352	769.99		
	7353	1811.3		
	7354	1801.2		
5% GF-1848	7355	1918.4	2316.0±400.75	2.8±0.5
	7356	2468.0		
	7357	2735.9		
	7358	1720.5		
	7359	2559.1		
	7360	2494.3		
25% GF-1848	7361	7927.6	4646.4*±1832.8	5.7±2.2
	7362	5088.6		
	7363	4356.6		
	7364	2442.4		
	7365	3771.5		
	7366	4291.3		
30% HCA	7343	8383.9	6393.3*±1578.6	7.8±1.9
	7344	8337.8		
	7345	6133.0		
	7346	5395.9		
	7347	4868.9		
	7348	5240.0		

#SI = Group Mean DPM ÷ Vehicle Control Mean DPM

[a] Statistical outlier not included in calculation

ND not determined; animals moribund after 1 or 2 days of dosing

* statistically different from control mean by Dunnett's test, alpha = 0.05

B. Stimulation Index:

Sample Description Test or Control	Vehicle	Low	Medium	High	Positive Control
Stimulation Index	1.0	1.8	2.8	5.7	7.8

C. Reviewer's conclusion: This reviewer agrees with the study author that the test material was a dermal sensitizer. However, the local lymph node assay procedure "was never validated

for mixtures by the assay creators. It is undergoing a validation step through ICCVAM - which is a lead NIEHS group of all gov't agency."

D. **Reference**: ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals). Contact Sensitization: Classification According to Potency, Technical Report No. 87. Brussels, April 2003 (ISSN-0773-8072-87).

1. **DP BARCODE:** DP343726
2. **PC CODE:** 128968, 129108, and 108702
3. **CURRENT DATE:** April 7, 2008
4. **TEST MATERIAL:** GF-1848 (11.8% w/w Fluroxypyr meptyl, 0.21% w/w Florasulam, 1.2% w/w Pyroxsulam, and 3.6% w/w Cloquintocet mexyl; Lot No. E-2154-74; dark brown liquid, emulsible in water, soluble in ethanol and acetone, dispersible in corn oil and mineral oil)

Study/Species/Lab Study # / Date	MRID	Results	Tox. Cat.	Core Grade
Acute oral toxicity/rat Eurofins/Product Safety Laboratories 21426/April 20, 2007	47166903	Females Estimated LD_{50} = 3129 mg/kg bw (Based on an assumed sigma of 0.5).Approximate 95% confidence interval is 1750 to 5000 mg/kg bw	III	A
Acute dermal toxicity/rat Eurofins/Product Safety Laboratories 21427/April 20, 2007	47166904	LD_{50} Males > 5000 mg/kg bw LD_{50} Females > 5000 mg/kg bw LD_{50} Combined > 5000 mg/kg bw	IV	A
Acute inhalation toxicity/rat Toxicology and Environmental Research and Consulting, Dow Chemical Co. 071002/May 2, 2007	47166905	LC_{50} Males > 2.46 mg/L LC_{50} Females>2.46 mg/L LC_{50}Combined>2.46mg/L	IV	A
Primary eye irritation/rabbit Eurofins/Product Safety Laboratories 21428/April 3, 2007	47166906	2/3 rabbit eyes positive for corneal opacity on day 10; all eyes clear by day 17.	II	A
Primary dermal irritation/rabbit Eurofins/Product Safety Laboratories 21429/April 3, 2007	47166907	Moderately irritating	III	A
Dermal sensitization/LLNA (mouse) Toxicology and Environmental Research and Consulting, The Dow Chemical Company 061198/April 5, 2007	47166908	Sensitizing	-	U

Core Grade Key: A = Acceptable, S = Supplementary, U = Unacceptable, W = Waived