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

OFFICE OF PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

TXR: **0054614**

DATE: January 31, 2007

SUBJECT: **Mefluidide: Toxicology Section** for the Reregistration Eligibility Decision Document (RED)
Reregistration Case #: 2370
PC Code: 114001
DP Code D334513

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Action Requested:

Prepare a toxicology section for mefluidide re-registration eligibility decision.

4.0 HAZARD CHARACTERIZATION/ASSESSMENT

4.1 Hazard characterization

Mefluidide has shown low acute toxicity by the oral, dermal and inhalation routes (Toxicity Category III and IV). It was a weak eye or dermal irritant (Toxicity Category III and IV). However, the precursor of mefluidide (S-15733) caused eye irritation (Toxicity Category II). Mefluidide did not cause dermal sensitization in the guinea pig. In rats and rabbits, critical effects of acute oral toxicity occurring at doses of 100 mg/kg/day and above were tremors, hunched posture, salivation, reduced body weight and body weight gain.

Mefluidide and its diethanolamine salt subchronic and chronic toxicity are manifested by decreased body weight and body weight gain in several species tested (rats, rabbits and dogs). Dogs are most sensitive to these effects, which occur at doses as low as 15 mg/kg/day in diets fed for one year. In addition, dogs fed mefluidide for one year exhibited chronic cortical nephrosis at doses of 150 mg/kg/day. Increased incidence of liver hyperplastic nodules in both sexes was observed in mice fed mefluidide at doses of 270 mg/kg/day and higher, but there was no oncogenic response in mice at doses as high as 900 mg/kg/day. Rats fed mefluidide at doses up to 300 mg/kg/day did not exhibit any carcinogenic response either. Based on lack of carcinogenic response in both rats and mice, mefluidide is considered as not likely to be carcinogenic to humans.

Mefluidide exhibited a negative response in various genotoxicity screening assays (bacterial reverse mutation, *in vitro* mouse lymphoma gene mutation, *in vitro* mammalian chromosome aberration, *in vivo* sister chromatid exchange, unscheduled DNA synthesis).

Mefluidide and its DEA salt were not dermally toxic when tested in rabbits at limit doses of 1000 mg/kg/day for 21 days. Effects were limited to slight erythema at the application site at the 1000 mg/kg/day dose.

Mefluidide or its DEA salt has not been tested for subacute or subchronic inhalation toxicity. However, both of them are in category IV for acute inhalation toxicity.

Developmental effects of Mefluidide in rats included increased number of early resorptions and mean postimplantation loss. These effects were observed at the same dose that caused maternal toxicity indicating there was no increased susceptibility to fetuses. The maternal toxicity included tremors, decreased body weight, weight gain and mortality. In rabbit, the LOAEL/NOAEL for developmental toxicity were above the highest dose tested (60 mg/kg/day). In the 2-generation reproduction toxicity study, the offspring toxicity was characterized by decreased body weights in both sexes and both litters in all generations. The reproductive LOAEL was not observed (NOAEL = 346/604 mg/kg bw/day).

The toxicology profile of mefluidide does not indicate a potential concern for estrogens, androgen and/or thyroid mediated toxicity.

Mefluidide was almost completely absorbed following oral ingestion (approximately 96%) and rapidly eliminated within 24 hours. A majority of dose was eliminated in urine (86-89 %) and remainder in feces after a single oral dose in 24 hours. Residue consisted of mefluidide (97%) and 2 unidentified metabolites (1.2% and 0.5%) and unidentified polar material (0.7%). Excretion of the radioactivity in expired air was not detected. The chemical is unlikely to accumulate in body since it was excreted almost completely within 24 hrs and steadily declined thereafter.

The toxicology profile of mefluidide and its DEA salt is adequate for the purposes of hazard and dose response assessment.

4.2 Hazard considerations For Women and Children

4.2.1. Adequacy of the Toxicity Database

The toxicology database for mefluidide is considered adequate. The following acceptable studies are available:

- Developmental toxicity studies in rats
- Developmental toxicity studies in rabbits
- Two-generation reproduction study in rats

4.2. 2. Evidence of Neurotoxicity

Acute and subchronic neurotoxicity studies were not performed. Clinical signs of neurotoxicity (such as tremors, ataxia, atonia, decreased limb tone, salivation) were seen in several studies (14-day oral in rabbit at or above 200 mg/kg/day, demyelination study in chickens at 1000 mg/kg/day and two developmental toxicity studies in rats at 115 mg/kg/day. Edema and swelling with myelin loss in sciatic nerve was observed in a dermal toxicity study in rabbits at doses of 720 mg/kg and above. However, these effects were not seen in an additional dermal test of similar duration using a 58.2% mefluidide formulation or diethanolamine salt of mefluidide 28.8%.

4.2.3. Developmental Toxicity Study Conclusions

Developmental Toxicity Study - Rabbits:

In a developmental toxicity study (MRIDs 00047139 and 00047138), technical MBR 12325 (Lot #9) in 4% gum acacia was administered to 16-20 New Zealand White rabbits/dose group via gavage at dose levels of 0, 15, 30, or 60 mg/kg bw/day from gestation days (GD) 6-18.

There were no treatment-related effects on survival, clinical signs, body weight, food consumption, or cesarean parameters.

The maternal LOAEL was not observed. The maternal NOAEL is 60 mg/kg bw/day (the highest dose tested).

There were no effects of treatment on the numbers of litters, live fetuses, dead fetuses, or resorptions, or on fetal body weights, sex ratio, or post-implantation loss. There were no treatment-related external, visceral, or skeletal variations or malformations.

The developmental LOAEL was not observed. The developmental NOAEL is 60 mg/kg bw/day (the highest dose tested).

This developmental toxicity study in rabbits has a number of deficiencies: a LOAEL was not observed; test material purity was not provided; no information on dose formulation preparation or storage was provided; and no analyses of homogeneity, stability, or concentration were reported. However, when combined with the 14-day oral gavage study in rabbits (MRID 00047138), where a LOAEL of <100 mg/kg bw/day based on mortality and tremor was

established, this developmental toxicity study is considered acceptable and satisfies the guideline for a developmental toxicity study (OPPTS 870.3700b; OECD 414) in rabbits.

Developmental Toxicity Study - Rats:

In a developmental toxicity study (MRID 42026102), Diethanolamine salt of Mefluidide (28.78% a.i. Lot # JB0624) in distilled water was administered to pregnant Sprague Dawley Crl:CD BR VAF/Plus (25/dose) by gavage at dose levels of 0, 50, 200 or 400 mg/kg bw/day (adjusted doses for 100 % purity were 0, 14, 58, or 115 mg/kg/day, respectively) from days 6 through 15 of gestation.

Animals were checked daily for clinical signs, mortality. Body weights were measured on gestation day 0, 6, 9, 12, 16 and 20. Unscheduled deaths, scheduled sacrifice and c-sections were subjected to gross necropsy examination. Each fetus was examined for external/visceral/skeletal anomalies, sexed and then weighed.

Evidence of maternal toxicity included transient clinical signs (tremors, dark material around the nose, few feces, urine stain and reddish vaginal discharge), decreased body weight gain (11-61%), decreased food consumption and mortality (2/25 females found dead on GD 11 and 16) observed at the 400 mg/kg/day levels. At the 400 mg/kg dose, the clinical signs of toxicity appeared within 2 days after dosing in few animals, and after few days of dosing in some others and more than half of the animals at this dose were free from clinical signs of toxicity. No external malformations or developmental variations were observed associated with any fetus. Fetal toxicity was manifested by increase in the number of early resorptions which resulted in increase in mean postimplantation loss at 400 mg/kg/day dose.

After adjusting to the pure active ingredient, the maternal NOAEL is 58 mg/kg/day and the LOAEL is 115 mg/kg/day based on clinical signs (tremors, dark material around the nose, urine stain and reddish vaginal discharge), decreased body weight gain, decreased food consumption and mortality (2/25 females). The developmental toxicity NOAEL is also 58 mg/kg/day, the LOAEL is 115 mg/kg/day based on increase in the number of early resorptions and increase in mean postimplantation loss.

This developmental toxicity study is classified **acceptable/Guideline** and it does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rat.

4.2.4. Reproductive Toxicity Study

In a three-generation reproduction study (MRID 00082748), MBR 12325 (Mefluidide; 93% a.i., Lot #25) was administered in the diet to 20 male and 40 female Charles River CD® rats/dose group at dose levels of 0, 600, 1800, or 6000 ppm (equivalent to Males/Females - 0/0, 34/60, 102/183, and 346/604 mg/kg bw/day). When approximately 100 days old, the P generation animals were mated (1 male: 2 females) for up to 15 days to produce the F1a litter. Following weaning of the F1a litters, 50 F1a offspring/sex/dose were selected for a 2-year chronic feeding study, and the remaining F1a offspring were discarded. The P generation was reduced to 10 males/20 females per dose group. After a 10-day post-weaning rest period, these P animals were mated again to produce the F1b litter. Upon weaning, 10 male and 20 female F1b offspring/dose

group were selected to be parents of the F2 generation. This study design was continued for three generations with 2 litters per generation.

There were no effects on food consumption, organ weights, gross pathology, or histopathology.

Numerous absolute and relative (to bw) organ weights in the 6000 ppm parents were significantly ($p<0.05$) different from the controls, however, none of these differences were corroborated by any macroscopic or microscopic findings indicating these decreases were most likely not related to treatment. Thus, it is likely that they were attributable to decreased body weights at this dose.

The only deaths included one 6000 ppm F1 female, one 6000 ppm F2 male, and one 1800 ppm F2 female. It was stated that macroscopic and microscopic findings in these animals were unremarkable. Therefore, these deaths were considered incidental and were not treatment related.

At 6000 ppm, body weights were decreased by 1-8% in males and 1-12% in females throughout the study in the P generation, attaining significance ($p<0.05$) at Week 18 in the males and Weeks 8, 18, 19, and 27 in the females. In the F1 generation at this dose, body weights were decreased throughout the study in the males (decr. 13-21%) and females (decr. 10-21%), attaining significance ($p<0.01$) at Weeks 27, 37, and 56 in both sexes. Similarly in the F2 generation, body weights were decreased throughout the study in the 6000 ppm males (decr. 14-21%) and females (decr. 11-23%), attaining significance ($p<0.01$) at Weeks 57, 66, and 85 in both sexes.

At 1800 ppm, only minor and infrequent decreases in body weights were noted. There were no treatment-related findings at 600 ppm.

The parental systemic LOAEL is 6000 ppm (346/604 mg/kg bw/day in males/females), based on decreased body weights in both sexes in all generations. The parental systemic NOAEL is 1800 ppm (102/183 mg/kg bw/day in males/females).

There were no effects of treatment on post-natal survival (i.e., viability and lactation) indices in the pups at any dose. There were no treatment-related findings at 600 or 1800 ppm.

At 6000 ppm, body weights were decreased by up to 27% compared to controls throughout the post-natal period in both litters in each generation (i.e., F1a, F1b, F2a, F2b, F3a, and F3b litters). These decreases attained significance in both sexes at PND 21.

The offspring LOAEL is 6000 ppm (346/604 mg/kg bw/day in males/females), based on decreased body weights in both sexes and both litters in all generations. The offspring NOAEL is 1800 ppm (102/183 mg/kg bw/day in males/females).

There were no effects of treatment on male or female fertility indices or gestation survival index.

The reproductive LOAEL was not observed. The reproductive NOAEL is 6000 ppm (346/604 mg/kg bw/day in males/females).

This study is **acceptable/guideline** and satisfies the guideline requirement for a three-generation reproductive study (OPPTS 870.3800; OECD 416) in rats.

4.2.5. Additional Information from Literature sources

There was no published information on this subject.

4.2.6. Pre-and/or Postnatal Toxicity

4.2.6.1. Determination of Susceptibility:

There is no evidence of increased pre-natal susceptibility for mefluidide from *in utero* exposure in rats. Developmental effects of mefluidide in rats included increased number of early resorptions and mean postimplantation loss. These effects were observed at the same dose that caused maternal toxicity indicating there was no increased susceptibility to the fetuses. The maternal toxicity included tremors, decreased body weight, weight gain and mortality. In rabbit, the NOAEL for developmental toxicity were above the highest dose tested (60 mg/kg/day). In the 3-generation reproduction toxicity study, no treatment related reproductive effects were reported. The offspring toxicity (decreased pup body weights) was observed at the highest dose tested (346 mg/kg/day) that also produced maternal toxicity indicating there was no increased post-natal susceptibility for the mefluidide.

4.2.6.2 Degree of Concern analysis and Residual Uncertainities for Pre- and /or Post –natal Susceptibility

There is no evidence of increased pre- or post-natal susceptibility in the developmental study or in the multi-generation reproduction study in rat. Although the LOAEL/NOAEL for developmental toxicity in the rabbits were not established, the concern is low for the increased susceptibility to the rabbit fetuses since the developmental effects were not seen at the highest dose tested (60 mg/kg/day) which is above the developmental NOAEL in rat (58 mg/kg/day) and well above (40X) the dose that is used to establish chronic RfD (1.5 mg/kg/day). Therefore, there is no residual uncertainty for pre- and/or post natal susceptibility.

4.3. Hazard Identification and Toxicity Endpoint Selection

4.3.1. Acute Reference Dose (aRfD)

Females age 13-49 : Acute dietary endpoint for child bearing females (females 13+ years old) was determined from the developmental toxicity study in rat (MRID 42026102). A NOAEL of 58 mg/kg/day was derived based on developmental toxicity (increased number of early resorptions and mean post-implantation loss) at a LOAEL of 115 mg/kg/day. An UF of 100X (10-fold for inter-species extrapolation, 10-fold for intra-species variability) was applied to the NOAEL of 58 mg/kg/day to derive the aRfD.

Acute RfD (Females 13-50 years old) = $\frac{58 \text{ mg/kg (NOAEL)}}{100 \text{ (UF)}}$ = **0.58 mg/kg**

Acute Reference Dose (aRfD) - General Population

The acute RfD for the general population including infants and children was determined from the developmental toxicity study in rat (MRID 42026102). A NOAEL of 58 mg/kg/day was derived based on maternal toxicity (clinical signs: tremors) at a LOAEL of 115 mg/kg/day. An UF of 100X (10- fold for inter-species extrapolation, 10-fold for intra-species variability) was applied. The selected endpoint of toxicity is appropriate for this exposure since clinical signs of toxicity occurred within two days of dosing.

Acute RfD (general population) = $\frac{58 \text{ mg/kg (NOAEL)}}{100 \text{ (UF)}}$ = **0.58 mg/kg**

4.3.2. Chronic Reference Dose (cRfD)

The cRfD of 0.015 mg/kg/day was determined on the basis of the Chronic Oral Feeding study in dogs (MRID 00132995). A NOAEL of 1.5 mg/kg/day was selected based on chronic toxicity (decreased body weight (15%) and body weight gain (50%) in the males) occurring at a LOAEL of 15.0 mg/kg/day. This was the most sensitive endpoint. An UF of 100X (10-fold for interspecies extrapolation, 10-fold for intraspecies variability) was applied to the NOAEL of 1.5 mg/kg/day to derive the cRfD to give and RfD of 0.015 mg/kg/day.

4.3.3. Incidental Oral Exposure (Short-and Intermediate-term durations: 1 day – 6 months)

Points of departure for these scenarios were based on the rat developmental study (MRID 42026102). NOAEL = 58 mg/kg bw/day, LOAEL = 115 mg/kg bw/day based on mortality and clinical signs. These data were also supported by the rabbit developmental study (NOAEL = 60 mg/kg bw/day) and rabbit 14 day oral study (LOAEL = 100 mg/kg bw/day based on mortality). The level of concern for residential exposure is for MOEs = 100 and for occupational exposure is for MOEs =100.

4.3.4. Dermal Absorption Factor

A dermal penetration study is not available. A dermal absorption factor is derived by extrapolation from the rabbit 21-day dermal (MRID 41972901) and rabbit 14 day oral (MRID 00082073) studies. The dermal systemic NOAEL in the 21-day study is 1000 mg/kg/day based on minor increases in liver enzymes. In the 14 day rabbit oral study(MRID 00047138), the LOAEL is less than 100 mg/kg/day based on mortality and clinical signs (tremors) and the NOAEL is <100 mg/kg/day, therefore, the calculated dermal absorption factor would at the most be (100/1000) x 100 = 10%.

4.3.5. Dermal Exposure (Short and Intermediate: (1-30 days and 30 d-180 days)

Three subacute (21-day) dermal toxicity studies were considered. Only one study (MRID 00082073) with 24% active ingredient showed toxic effects. The other two more recent studies (MRID 42029601 and 41972901) showed no systemic effects at the limit dose. These effects were not seen in additional GLP dermal tests of similar duration using a 58.2% mefluidide formulation (MRID 42029601) or diethanolamine salt of mefluidide 28.8% (MRID 41972901). The risk assessment team determined that no quantitative dermal assessment is needed due to the following:

1) Two 21-day dermal toxicity studies with rabbits indicated no dermal systemic toxicity at 1000 mg/kg/day (the highest dose tested),

2) The rat developmental study indicated no developmental concern (developmental NAOEL equals to maternal NOAEL),

3) The acute dermal toxicity of mefluidide, where the acute dermal LD50 is >4000 mg/kg, it is not a skin irritant and is not a dermal sensitizer.

4.3.6. Inhalation (Short- and Intermediate-Term)

Endpoint for this scenario was determined from the rat developmental study. NOAEL = 58 mg/kg bw/day, LOAEL = 115 mg/kg bw/day based on mortality and clinical sings. These data were also supported by the rabbit developmental study (NOAEL = 60 mg/kg bw/day) and rabbit 14 day oral study (LOAEL = 100 mg/kg bw/day based on mortality). Since oral study was selected for inhalation exposure assessment an inhalation-absorption factor of 100% oral equivalent should be used.

4.3.7. Margins of Exposure

These are summarized in the following table:

Route Duration	Short-Term (1-30 Days)	Intermediate-Term (1 - 6 Months)
Occupational (Worker) Exposure		
Dermal	NA	NA
Inhalation	100	100
Residential (Non-Dietary) Exposure		
Oral	100	100
Dermal	NA	NA
Inhalation	100	100

4.3.8. Classification of Carcinogenic Potential

Mefluidide was negative for carcinogenicity in mouse (MRID 00082747) and rat (MRID 00061930 7 00082737) bioassays. It was also evaluated for genotoxicity in several tests and found negative. It is unlikely that mefluidide will pose a cancer risk to humans.

Table 4.3. Summary of Toxicological Dose and Endpoints for Mefluidide and its salt (114001, 114002, 114003) Used in Human Risk Assessment

Exposure Scenario	Point of Departure	Uncertainty Factors	Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary (general population)	**NOAEL = 58 mg/kg/day**	**UF_A = 10X** **UF_H = 10X**	**Acute RfD =** **Maternal NOAEL** **UncertaintyFactor** **= 0.58 mg/kg /day**	MRID 42026102 Developmental toxicity - rat; LOAEL= 115.0 mg/kg/day based on mortality(within 5 days of dosing) and clinical signs (within 2 days of dosing), and the NOAEL of 58 mg/kg/day.
Acute Dietary (Females 13+)	**NOAEL = 58 mg/kg/day**	**UF_A = 10X** **UF_H = 10X**	**Acute RfD =** **Develop. NOAEL** **UncertaintyFactor** **= 0.58 mg/kg**	MRID 42026102 Developmental toxicity - rat; LOAEL= 115.0 mg/kg/day based on increased number of early resorptions and mean postimplantation loss. NOAEL = 58 mg/kg/day
Chronic Dietary (All populations)	**NOAEL = 1.5 mg/kg/day**	**UF_A = 10X** **UF_H = 10X**	**Chronic RfD =** **NOAEL** **Uncertainty Factor** **= 0.015 mg/kg/day**	MRID 00132995 Chronic Oral Feeding - dog; LOAEL= 15.0 mg/kg/day based on decreased body weight (15%) and body weight gain (50%) in the males at 15 mg/kg/day.
Short-Term Incidental Oral (1-30 days) Intermediate-Term Incidental Oral (1- 6 months)	**NOAEL = 58 mg/kg/day**	**UF_A = 10X** **UF_H = 10X**	Residential LOC for MOE = 100	MRID 42026102 Developmental toxicity - rat; NOAEL = 58 mg/kg bw/day, LOAEL = 115 mg/kg bw/day based on mortality and clinical sings. These data were also supported by the rabbit developmental study (MRID 00047139) (NOAEL = 60 mg/kg bw/day) and rabbit 14 day oral study (LOAEL = 100 mg/kg bw/day based on mortality).
Short-Term Dermal (1 to 30 days) Intermediate-Term Dermal (1 to 6 months)	Dermal NOAEL = 1000 mg/kg/day	**UF_A = 10X** **UF_H = 10X**	No quantitative dermal assessment is needed.	Three subacute (21-day) dermal toxicity studies were considered. The risk assessment team determined that no quantitative dermal assessment is needed due to the following: 1) Two 21-day dermal toxicity studies with rabbits indicated no dermal systemic toxicity at 1000 mg/kg/day (the highest dose tested), 2) the rat developmental study indicated no developmental concern (developmental NOAEL

Table 4.3. Summary of Toxicological Dose and Endpoints for Mefluidide and its salt (114001, 114002, 114003) Used in Human Risk Assessment				
Exposure Scenario	**Point of Departure**	**Uncertainty Factors**	**Level of Concern for Risk Assessment**	**Study and Toxicological Effects**
				= maternal NOAEL), 3) acute toxicity of mefluidide, where acute dermal LD50 is >4000 mg/kg, not a skin irritant and is not a dermal sensitizer.
Short-Term Inhalation (1 to 30 days) Intermediate-Term Inhalation (1 to 6 months)	Oral NOAEL = 58 mg/kg/day (inhalation-absorption rate = 100% oral equivalent)	**UF_A = 10X** **UF_H = 10X**	Residential LOC for MOE = 100; Occupational LOC for MOE = 100	MRID 42026102 Developmental toxicity - rat; NOAEL = 58 mg/kg bw/day, LOAEL = 115 mg/kg bw/day based on mortality and clinical sings. These data were also supported by the rabbit developmental study (NOAEL = 60 mg/kg bw/day) and rabbit 14 day oral study (LOAEL = 100 mg/kg bw/day based on mortality).
Cancer	Mefluidide was negative for carcinogenicity in mouse (MRID 00082747) and rat (MRID 00061930 7 00082737) bioassays. It was also evaluated for genotoxicity in several tests and found negative.			

Point of Departure (POD) = a data point or an estimated point that is derived from observed dose-response data and used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures. UF = uncertainty factor, UF_A = extrapolation from animal to human (intraspecies), UF_H = potential variation in sensitivity among members of the human population (interspecies), NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, RfD = reference dose (a = acute, c = chronic), MOE = margin of exposure, LOC = level of concern, NA = Not Applicable. Safety Factor = UF = 100.

4.4. Cumulative Risk Characterization/Assessment

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to Mefluidide and its salts. Mefluidide does not appear to produce a toxic metabolite produced by other substances. For the purposes of this action, therefore, EPA has not assumed that mefluidide has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative/.

Appendix A: Toxicology Assessment

A.1 Toxicology Data Requirements

The requirements (40 CFR 158.340) for food and non food use for Mefluidie are in Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

Test	Technical	
	Required	**Satisfied**
870.1100 Acute Oral Toxicity	yes	yes
870.1200 Acute Dermal Toxicity	yes	yes
870.1300 Acute Inhalation Toxicity	yes	yes
870.2400 Primary Eye Irritation	yes	yes
870.2500 Primary Dermal Irritation	yes	yes
870.2600 Dermal Sensitization	yes	no
870.3100 Oral Subchronic (rodent)	yes	yes
870.3150 Oral Subchronic (nonrodent)	yes	yes
870.3200 21-Day Dermal	yes	yes
870.3250 90-Day Dermal	-	-
870.3465 90-Day Inhalation	-	-
870.3700a Developmental Toxicity (rodent)	yes	yes
870.3700b Developmental Toxicity (nonrodent)	yes	yes
870.3800 Reproduction	yes	yes
870.4100a Chronic Toxicity (rodent)	yes	yes
870.4100b Chronic Toxicity (nonrodent)	yes	yes
870.4200a Oncogenicity (rat)	yes	yes
870.4200b Oncogenicity (mouse)	yes	yes
870.4300 Chronic/Oncogenicity	yes	yes
870.5100 Mutagenicity—Gene Mutation - bacterial	yes	yes
870.5300 Mutagenicity—Gene Mutation - mammalian	yes	yes
870.5375 Mutagenicity—Structural Chromosomal Aberrations	yes	yes
870.5550 Mutagenicity—Unscheduled DNA synthesis	yes	yes
870.6100a Acute Delayed Neurotox. (hen)	no	-
870.6100b 90-Day Neurotoxicity (hen)	no	-
870.6200a Acute Neurotox. Screening Battery (rat)	no	-
870.6200b 90-Day Neuro. Screening Battery (rat)	no	-
870.6300 Develop. Neuro	no	-
870.7485 General Metabolism	yes	yes*
870.7600 Dermal Penetration	-	
Special Studies for Ocular Effects		
Acute Oral (rat)	no	-
Subchronic Oral (rat)	no	-
Six-month Oral (dog)	no	-

* Non-guideline study

A.2. Toxicology Profile

Table A.2.1. Acute Toxicity of Mefluidide and its salts (114001, 114002, 114003)

Guideline No.	Study Type	MRID	Results (LD_{50}/LC_{50})	Toxicity Category
870.1100 (81-1)	Acute Oral (female rat) Mefluidide tech	>4000 mg/kg MRID 00047118		III
870.1100 (81-1)	Acute Oral (mouse) Mefluidide tech	1920.2 mg/kg MRID 00047117		III
870.1100 (81-1)	Acute Oral (mouse) Mefluidide tech	829.8 mg/kg MRID 00047116		III
870.1100 (81-1)	Acute Oral (dog) Mefluidide tech	Not established MRID 00049627; emesis precluded evaluation at 100, 500, 2000 mg/kg doses		III
870.1200 (81-2)	Acute Dermal (female rabbit) Mefluidide tech	>4000 mg/kg MRID 00047122 & 00049628 & 00083817		IV
870.1300 (81-3)	Acute inhalation – rat DEA salt of Mefluidide	>5.2 mg/L MRID 41888801		IV
870.1300 (81-3)	Acute inhalation – rat Mefluidide tech.	>5.4 mg/L MRID 41964601		IV
870.2400 (81-4)	Primary Eye Irritation (rabbit) Mefluidide tech	minimal irritation MRID 00047126, 00049630		III
870.2400 (81-4)	Primary Eye Irritation (rabbit) DEA Mefluidide	minimal irritation MRID43481203		III
870.2500 (81-5)	Primary Skin Irritation (rabbit), Mefluidide tech	Not a dermal irritant MRID 00047124, 00049629, 00083819		IV
87.2600 (81-6)	Dermal Sensitization (guinea pig), Mefluidide	Not a dermal sensitizer MRID 41887701		N/A
87.2600 (81-6)	Dermal Sensitization (guinea pig), Mefluidide	Not a dermal sensitizer MRID 00082076		N/A

Table A.2.2. Toxicity Profile of Mefluidide and its salts (114001, 114002, 114003)		
Guideline No./ Study type	**MRID No.(year)/Doses/ classification**	**Results**
Non-guideline 21-day Oral - dog	00047137, (1975) 0, 1000, 3000, 10000 ppm Vistar tech, 93% a.i./d (0, 25, 75, 250 mg/kg/d) One dog/sex/dose range finding **Acceptable/non-guideline**	**LOAEL** = not established. **NOAEL** > 250 mg/kg/d,
Non-guideline 5-week - mouse	00082072, (1976) 0, 1800, 6000 ppm Vistar tech, 93% a.i./d (0, 270, 900 mg/kg/d) (Dietary 5/sex/dose) range finding **Acceptable/non-guideline**	**LOAEL** = not established **NOAEL** = 900 mg/kg/d,
None-guideline 28-Day oral dietary [rat]	00047135, (1973), 0, 1000, 3000 or 10000 ppm Vistar tech, 93% a.i. (0, 100, 300, 1000 mg/kg/d) (Dietary 5 rats/sex/dose) range finding **Acceptable/non-guideline**	**LOAE**L = not established. **NOAEL** > 1000 mg/kg/d,
870.3100 (82-1a) 90-Day oral dietary [rat]	00047136, (1975), 0, 300, 1000 or 6000 ppm Vistar tech, 93% a.i. (0, 15, 50, 300 mg/kg/d) (10 rats/sex/dose) 00047140 (1975) 0, 300, 1000, 3000 ppm (0, 15, 50, 150 mg/kg/day). (10 females/dose) **Acceptable/Guideline**	**LOAEL** = 300 mg/kg/d, based on decreased body weight, body weight gain and food consumption in the females. **NOAEL** = 150 mg/kg/d (in conjunction with MRID # 00047140),
870.3150 82-1(b) 90-Day oral dietary [dog]	00047141, **(IBT Study**, 1977), 0, 300, 1000 or 6000 ppm Vistar tech, 93% a.i. (0, 7.5, 25, 150 mg/kg/d) (4/sex/dose) **Unacceptable/guideline** (LOAEL was not observed)	**LOAE**L = not established. **NOAEL** = 150 mg/kg/d.
870.3200 82-2 21-Day Dermal toxicity - rabbit	00082073, (1977) 0, 1, 3, 10 ml of 2S formulation/kg/day (Formulation containing 24% a.i., equivalent to 0, 240, 720, or 2400 mg mefluidide/kg/day) (4 rabbits/sex/dose) **Acceptable/Non-guideline** (NOAEL was not observed)	**Dermal LOAEL** = 240 mg/kg/day, based on irritation, inflammation and necrosis at test sites. **Systemic LOAEL** = 240 mg/kg/day, based on clinical chemistry (increased alkaline phosphatase and alanine aminotransferase) and organ weights (decreased spleen weight in females and increased liver weights in males). Edema and swelling with myelin loss in sciatic nerve was seen in 720 and 2400 mg/kg/day dose group. Dehydration observed at 2400 mg/kg/day dose. **Dermal and systemic NOAELs** were not established.

Table A.2.2. Toxicity Profile of Mefluidide and its salts (114001, 114002, 114003)		
Guideline No./ Study type	**MRID No.(year)/Doses/ classification**	**Results**
870.3200 82-2 21-Day Dermal toxicity - rabbit	42029601 (1991) 0, 100, 500 or 1000 mg a.i./kg/d Mefluidide 58.2% a.i. (5/rabbits/sex/dose) **Acceptable/guideline**	Dermal toxicity **LOAEL** = 1000 mg/kg/day based on erythema at the test site. Dermal toxicity **NOAEL** = 500 mg/kg/day Systemic toxicity **LOAEL** was not established. Minor hematological and clinical chemistry findings at 1000 mg/kg/day dose reported, that were within normal biological variation and did not correlate to histopathological findings. Systemic toxicity **NOAEL** = 1000 mg/kg/day
870.3200 82-2 21-Day Dermal toxicity - rabbit	41972901 (1991) 0, 100, 500 or 1000 mg a.i./kg/d Mefluidide DEA salt 28.78% a.i. (5/rabbits/sex/dose) **Acceptable/ guideline**	Dermal and systemic toxicity **LOAEL** was not established. Minor incidences of erythema at 500 mg and 1000 mg/kg/day dose. Increased liver weight (absolute and relative) noted at 1000 mg/kg dose but no correlating histopathological findings. Minor statistical increases in liver enzymes AST and ALT. Dermal and systemic **NOAEL** = 1000 mg/kg/day
Non-guideline 1-year feeding (Rat)	00132993, (1981) 0, 60, 200, 600 ppm Vistar tech, 93% a.i. (0, 3, 10, 30 mg/kg/d) (20 rats/sex/dose) Addendum to 2-year feeding study. **Acceptable/non-guideline**	**LOAEL** = not established **NOAEL** = 30 mg/kg/d,
870.4100b 83-1b Chronic Oral Feeding [dog]	00132995, (1982) 0, 60, 600, 6000 ppm Vistar tech, 93% a.i. (0, 1.5, 15, 150 mg/kg/d) (6 dogs/sex/dose) **Acceptable/guideline**	**LOAEL** = 15 mg/kg/d, based on decreased body weight (15%) and body weight gain (50%) in the males. Chronic cortical nephrosis was observed at 150 mg/kg/day dose. **NOAEL** = 1.5 mg/kg/d,
870.4100b 83-2b Carcinogenicity Dietary [mouse]	00082747, (1979) 0, 600, 1800, 6000 ppm Vistar tech, 93% a.i. (0, 90, 270, 900 mg/kg/d) (60 mice/sex/dose) **Acceptable/guideline**	**LOAEL** = 270 mg/kg/day, based on increased incidence of liver hyperplastic nodules in both sexes. **NOAEL** = 90 mg/kg/day. No oncogenicity up to and including the highest dose tested.
870.4300 83-5 2-year feeding/carcinogenicity [rat]	00061930, 00082737 (1979) 0, 600, 1800, 6000 ppm Vistar tech, 93% a.i. (0, 30, 90, 300 mg/kg/d) (50 rats/sex/dose) **Acceptable/guideline**	**LOAEL** = 30 mg/kg/d, based on body weight loss. **NOAEL** < 30 mg/kg/d, No oncogenicity up to and including the highest dose tested.
870.3700a 83-3(a) Developmental Toxicity, gavage [rat]	00132992, (1981) 0, 15, 30, 60 mg/kg/d **Unacceptable/guideline** (LOAEL was not observed)	**Maternal LOAEL** = not established. **Maternal NOAEL** > 60 mg/kg/day, **Developmental NOAEL** > 60 mg/kg/day, **Developmental LOAEL** = not established.

Table A.2.2. Toxicity Profile of Mefluidide and its salts (114001, 114002, 114003)		
Guideline No./ Study type	**MRID No.(year)/Doses/ classification**	**Results**
Non-guideline Range Finding Developmental Toxicity , gavage[rat]	42026101, (1991) 0, 100, 200, 400, 600or 800 mg **diethanolamine salt of mefluidide** (28.78%)/kg/day Doses adjusted for 100 % purity were 0, 29, 58, 115, 173, 230 mg/kg/day (6 female rats/dose) **Acceptable/non-guideline**	**Maternal LOAEL**: 115 mg a.i./kg/day based on clinical signs (tremors, hunched posture, and salivation), maternal body weight gain and food consumption. **Maternal NOAEL**: 58 mg a.i./kg/day; **Developmental LOAEL**: 230 mg a.i. /kg/day based on significantly decreased fetal body weight. **Developmental NOAEL**: 173 mg a.i. /kg/day, The dosage levels of 0, 50, 200 and 400 mg of the 28.78% formulation/kg/day were selected for the definitive developmental study.
870.3700a 83-3(a) Developmental Toxicity, gavage [rat]	42026102, (1991) 0, 50, 200 400 mg **diethanolamine salt of mefluidide** (28.78%)/kg/d (25 females/dose) Doses adjusted for 100 % purity were 0, 14, 58, or 115 mg/kg/day. **Acceptable/guideline**	**Maternal LOAEL** = 115 mg a.i./kg/day based on mortality, clinical signs (tremors, stained nose, urine and vaginal discharge), decreased body weight and weight gain. **Maternal NOAEL** = 58 mg a.i./kg/day, **Developmental LOAEL** = 115 mg a.i./kg/day based on increased number of early resorptions and mean post-implantation loss. **Developmental NOAEL**: 58mg a.i./kg/day
Non-guideline 14-Day Oral gavage [rabbit]	00047138, (1975) 0, 100, 200, 400, 800 mg/kg/d Vistar tech, 93% a.i. 4 females/dose range finding **Acceptable/non-guideline**	**LOAEL** = < 100 mg/kg/day (females), based on mortality (1/3 deaths) at 100 mg/kg/d. Tremors and 100% mortality were noted at the levels of 200 mg/kg/d and above. Histopathology not reported. **NOAEL**: not established,
870.3700b 83-3(b) Developmental Toxicity, gavage [rabbit]	00047139, (1975) 0, 15, 30, 60 mg technical MBR 12325/kg/d (purity not reported). Unacceptable by itself, however, if combined with the 14-day oral study (00047138), it is acceptable.	**Maternal LOAEL** = not established. **Maternal NOAEL** = 60 mg/kg/day, **Developmental LOAE**L = not established. **Developmental NOAEL** = 60 mg/kg/day,
870.3800 (83-4) 3-generation reproduction [rat]	00082748, (1979) 0, 600, 1800, 6000 ppm, 93% a.i. (M/F: 0/0, 34/60, 102/183, 346/604 mg/kg/d) **Acceptable/guideline**	**The parental systemic LOAEL** = 346/604 mg/kg bw/day (M/F), based on decreased body weights. **The parental systemic NOAEL** = 102/183 mg/kg bw/day in males/females. **The offspring LOAEL** = 346/604 mg/kg bw/day in males/females, based on decreased body weights in both sexes and both litters in all generations. **The offspring NOAEL** = 102/183 mg/kg bw/day in males/females. **The reproductive LOAEL** was not observed. **The reproductive NOAEL** = 346/604 mg/kg bw/day in males/females.
870.5100	00132996, (1983)	No reverse mutations were noted in any of 8 tester

Table A.2.2. Toxicity Profile of Mefluidide and its salts (114001, 114002, 114003)		
Guideline No./ Study type	**MRID No.(year)/Doses/ classification**	**Results**
84-2 Bacterial reverse mutation	EL-565 (Lily compound 151065: mefluidide technical) tested at 0.1 – 1000 µg/ml **Acceptable/Guideline**	strains of *Salmonella typhimurium* and two tryptophan autotrophs of *E. coli with or without metabolic activation*
870.5100 84-2 Bacterial reverse mutation	41888804, (1991) 0, 100, 333, 667, 1000, 3330, or 5000 µg/plate **diethanolamine salt of mefluidide (28.78%)**/ **Acceptable/guideline**	DEA mefluidide did not increase the number of histidine revertants per plate in any of the tester strains with or without metabolic activation.
870.5300 84-2 *In-vitro* Mouse lymphoma - gene mutation	00132996, (1983) EL-565 (Lily compound 151065: mefluidide technical)0, 1, 25, 50, 100, 250, 500, 750 or 1000 µg/ml **Acceptable/guideline**	There was no evidence of mutation in the presence or absence of metabolic activation.
870.5375 84-2 *In-vitro* mammalian chromosome aberration test	41888803, (1991) **Diethanolamine salt of mefluidide (28.8%)** 500,- 5010 µg a.i. /ml (without S9 mix) or 500, - 5000 (with S9 mix) **Acceptable/guideline**	No significant increase in structural chromosomal aberration with or without metabolic activation was seen, however, the results were considered equivocal.
§84-2 In-vitro mammalian chromosome aberration test	(1992) concentrations of 1250 to 5000 µg/ml (w S9 mix) or 200-1600 µg/ml (wt S9 mix)	Not mutagenic in Chinese Hamster Ovary cells
870.5550 84-2 Unscheduled DNA Synthesis	41888802, (1991) **Diethanolamine salt of mefluidide (28.8%)** Concentrations of 100, 250, 500, 1000, 2000, 3000 µg/ml in trial 1; 1000, 1500, 2000, 3000, 3500 µg/ml in trial 2. **Acceptable/guideline**	No unscheduled DNA synthesis response in the absence of moderate to severe cytotoxicity.
870.5550 84-2 Unscheduled DNA Synthesis	00132996, (1983) EL-565 (Lily compound 151065: mefluidide technical) Tested at 0.5 to 1000 nmoles/mL	No indication of DNA repair synthesis was observed in cultured rat heapatocytes treated with the test material (EL-565 (Lily compound 151065: mefluidide technical))
870.5915 84-2 *In-vivo* Sister Chromatid Exchange	00132996, (1983) EL-565 (Lily compound 151065: mefluidide technical) (0, 12.5, 25, 50, or 100 mg/kg. **Acceptable/Guideline**	Negative in sister chromatid exchange in *in-vivo* bone marrow of Chinese hamster assay.
870.5915 85-1 Metabolism- male rat	MRID is not known: Steifer, LJ (1978). 3M Company Report Number 852 (1-26-78) Dose: 1 or 10 mg/kg of C-14, labeled mefluidide **Acceptable/None-Guideline**	By 24 hrs of post-treatment, 86-89% of the dose was found in urine with the remainder in the feces. Residue consisted of mefluidide (97%) and 2 unidentified metabolites (1.2% and 0.5%) and unidentified polar material (0.7%).

Table A.2.2. Toxicity Profile of Mefluidide and its salts (114001, 114002, 114003)		
Guideline No./ Study type	**MRID No.(year)/Doses/ classification**	**Results**
870.6200 81-8 Demyelination study – Female Chicken	00082075 (1977) S-12325-2S formulation Single doses at 1000, 3000, 5000, 10,000 and 20,000 mg/kg/day. TOCP as positive control 5-10 hens/dose **Non-Acceptable/Non-Guideline**	LD50 was 8500 mg/kg At 1000 mg/kg/day: clinical signs (hypoactivity, ataxia, tremors, lethargy and dyspnea) that were subsided by 48 hrs following dosing. The test material did not induce delayed neurotoxicity in hens

M = Males; F = Females