4-25-92

MEMORANDUM:

Subject: EPA File Symbol/EPA Reg. No.:62719-EEL

From: Lucy D. Markarian, Biologist 4/9/92
Precautionary Review Section
Registration Support Branch
Registration Division (H7505C)

To: Dennis Edwards, PM 19
Insecticide-Rodenticide Branch
Registration Division (H7505C)

Thru: Thomas C. Ellwanger, Section Head E 4/25/92
Precautionary Review Section
Registration Support Branch
Registration Division (H7505C)

Applicant: DowElanco
Quad IV
9002 Purdue Rd
Indianapolis, IN 46268-1189

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
Chlorpyrifos: O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)phorphorothioate	22.80 %
Inert Ingredient(s):	77.20 %
Total:	100.00 %

BACKGROUND

DowElanco has submitted six studies to support the registration of XRM-5222 under EPA symbol 62719-EEL.
The active ingredient is chlorpyrifos. The inerts consist of [redacted]

RECOMMENDATION

Oral Toxicity- Core minimum
The weights of the females were not all at the desirable weight range even when allowances were made for the differences in strain. There are gaps between dose levels,and not much meaningful data; therefore the calculation and estimation of the LD_{50} levels appear to be an estimation of an estimation. This point was discussed with the author of the test in reference to DowElanco registration under 62719-ERI (MRID 419325-05). The test is accepted on the basis of no mortality at 500 mg/kg and estimated LD_{50} of > 500 mg/kg.

Inhalation Toxicity- supplementary

The weights of almost all females and some of the males are not in the recommended weight range. PRS finds that this has had an influence on the expressed toxicity even when allowances for strain differences are made. The guidelines call for young adult animals. Serious doubts exist about the maturity of male or female Fischer 344 rats under 150 grams. Certainly a 106 gram rat could not be called a young adult. In the past, DowElanco has argued this point by introducing pamphlets from Charles River breeders and quoting from the Practical Guide to Laboratory Animals[1] that puberty is reached between 40 and 60 days. There is no way to ascertain this when using animals that weigh under 150 grams. The surest way is to use animals within the recommended weight ranges. These recommendations are made on the basis of years of experience of a number of authorities at EPA and elsewhere. PRS expects the performing laboratories to follow the guidelines to a reasonable extent. To illustrate the point that the weight of the animals has had influence on the results it is pointed out that at 3.58 mg/L females weighing 106 - 119 g mortality was at 100 %. At the next higher dose level,4.50 mg/L, females weighing 145 - 155 grams showed 20 % less mortality. In the males, at 4.50 mg/L, that weighed 244-276 g showed no mortality and 80 % mortality at the next higher level, 5.64 mg/L, where the weight range was 141-166 g. These effects cannot entirely be attributed to a steep dose-response curve. To carry it somewhat further, weight loss or failure to gain weight was more noticeable in the mature animals and not in the younger and actively growing animals. Even at the

[1] C.F.Williams, Practical Guide to Laboratory Animals, 1976 C.V.Mosby Co.,St. Louis, Mo

highest dose level in the males where 80 % mortality occurred, the surviving male (initial weight 140 g) showed weight gain at termination, as did most of the surviving females in the weight range of 119-155 g.
Additionally, there was too much difference in body weight between the males in the two lower dose levels and the highest dose level.There was 100 g difference between the average weight of the males at 4.5 mg/L and the average weight of the animals at 5.64 mg/L , 257.8 and 157.2, respectively. This is considerably more than the acceptable 20 %.
PRS is of the opinion that by disregarding the recommendation of the guidelines the integrity of the test has been compromised.

It is not explained why an LC_{50} study was necessary when the initial targeted dose level was about 2 mg/L. It would be preferable if a limit test is submitted with animals in the proper weight range and at the highest achievable respirable particle size. The use of the higher levels could not have changed the toxicity category. Had the right weight range of animals been used no further testing would have been necessary. Additionally if an LC_{50} test was to be submitted, the time span in which the test was completed should have been better determined and animals ordered within the same weight range for all levels. Planning and the conduct of a test in a reasonable time frame to keep the integrity of the test intact remains the laboratory's responsibility. This would have eliminated one of the unacceptable aspects of the test.

In the past objections have come from the registrant about the unnecessary use of the animals in testing, and their concern with animal welfare. It appears that these objections are made if convenient. The agency advocates the use of a minimum of animals as long as the results of the tests are definitive. The assessment of the hazards of any pesticide must not depend on equivocal results for any reason.

In another area, but not as important, the use of terminology is found to be misleading. At the observations a symptom listed as mouth breathing is included. This term does not explain clearly what the symptom is. It appears that the animals were gasping, and if this is the case it must be reported as such. If not gasping , then it must be explained what this mouth breathing is and what it signifies.

A new inhalation test must be submitted.

Dermal Irritation - core minimum

The dermal irritation test poses a lot of questions. PRS finds it hard to equate grade 1 erythema and edema with fissuring and Desquamation that persists to 14 or 22 days. It is even hard to visualize 1 erythema and 1 edema at 48 hrs. Furthermore it is questioned why animals showing presumably nonremarkable reactions, (grade 1 erythema and edema) were maintained and evaluated for 23 days. With these in mind, the submitted test is considered core

minimum data in category III toxicity.

Dermal sensitization

The study did not include naive controls. In different circumstances the study would have been down graded for the omission of this group, because the results of the challenge are to be compared to the naive controls. The positive controls do not serve as base for comparison, they just show that the laboratory is capable of inducing sensitization. In this particular case, the test is accepted as guideline data, because in spite of the absence of the naive controls it is unequivocally decided that the test material was not a sensitizer. This is also born out with historical data. PRS recommends the use of naive controls.

LABELING

The signal word is CAUTION
The precautionary statement and the statement of practical treatment on the proposed label are adequate. The label may have to be revised upon the presentation of the requested inhalation test.

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DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (§ 81-1)

Product Manager: 19 **Reviewer:** L. Markarian
MRID No.: 421190-04 **Report Date:** 01/09/92
Testing Facility: DowElanco **Report No.** M-005222-002A
Toxicology Research Laboratory
Author(s): N.M.Berdasco, J.E.Bettjes, B.L.Yano
Species: Rat, Fischer 344
Age: 9 weeks old
Weight: males 162-183 g, females 136-144 g
Source: Charles River Breeding Laboratories Inc., Kingston, Mi
Test Material: XRM-5222 sample reference AGR 284240 (liquid)
Quality Assurance (40 CFR §160.12): Included

Conclusion:

1. **The estimated LD_{50} is** > 500 mg/kg
3. **Tox. Category:** III **Classification:** Core minimum

Procedure (Deviations from §81-1):
Fasted rats were intubated with either test material as received (2000 mg/kg) or as a 10 % suspension in corn oil(1000, 500, 50 mg/kg). Four dose level were used for the males and three for the females. Observations were frequent on the day of administration and daily thereafter. Body weights were recorded at initiation and on days 2,8, and 15.Necropsy was performed on all animals that died. The survivors were anesthetized with methoxyflurane for live gross pathological examination.

Results:

Dosage mg/kg	(Number Killed/Number Tested)		
	Males	Females	Combined
50	0/5	0/5	0/10
500	0/5	0/5	0/10
1000	3/5	5/5	8/10
2000	5/5	-	5/5

Symptoms & Gross Necropsy Findings:

All mortality occurred within 4 days of administration.
At 2000 mg/kg signs of toxicity were decreased activity, labored respiration, salivation, and diarrhea.
At 1000 mg/kg symptoms of toxicity included decreased activity, chromodacryorrhea, chromorhinorrhea,diarrhea,lacrimation, fecal

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soiling of the perineum, labored, and/or rapid respiration, salivation, and tremors.
At 500 mg/kg decreased activity and perineal soiling was observed in the males. In the females, in addition, chromodacryorrhea chromorhinorrhea, labored respiration, lacrimation, salivation, and tremors were observed.
At 50 mg/kg the only adverse sign was perineal soiling.

At necropsy of the decedents at 200 mg/kg erosions and/or ulceration of the glandular gastric mucosae were observed; at 1000 mg/kg dark foci were seen on the stomach, and some did not show formed feces in the intestines and had hemolyzed blood in the digestive tract. Other observed changes were attributed to typical changes in animals dying spontaneously (stains and congestion of viscera). Among the survivors no product related changes were observed at the terminal examination. One female at 50 mg/kg had a distended ovarian bursa.

Dermal Toxicity.
Although the test was not downgraded for washing the animals with soap and water at this time, PRS discourages this practice and would like to see the use of soap in washing dermal rabbits discontinued. This practice may alter the results of the test.

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DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (§81-2)

Product Manager: 18 **Reviewer:** L. Markarian
MRID No.: 421190-05 **Report Date:** 01/09/92
Testing Laboratory: Dow Chemical Co. **Report No.:** M-005222-002D
The Toxicological Research Laboratory
Author(s): N.M.Berdasco, J.E.Bettjes, B.L.Yano
Species: Rabbit, New Zealand White
Weight: 2.7 - 3.1 K
Source: Hazleton Research Products, In.,Kalamazoo, MI
Test Material: XRM-5222 Test sample AGR 284240 (liquid)
Quality Assurance (40 CFR §160.12): Included

Summary:

1. **The estimated LD_{50} is >2000 mg/kg**

3. **Tox. Category:** III **Classification:** Guideline

Procedure (Deviation From §81-2):
The test material was applied as received to the shaved dorsum of the animals and held in contact with the skin with gauze dressing and tape. The trunks of the animals were wrapped in plastic wrap and overwrapped with cloth bandage. At 24 hrs the wrappings were removed and the sites were washed with mild soap and water,rinsed and dried. Collars were placed around the necks to prevent accidental ingestion for 24 hrs. Daily observations were made during the week. Only animal husbandry procedures were carried out at week ends. Body weights were recorded at initiation and on days 2, 8, and 15. Necropsy was performed on all animals.

Results:

Reported Mortality

DOSAGE mg/kg	(NUMBER KILLED/NUMBER TESTED)		
	Males	Females	Combined
2000	0/5	0/5	0/10

Symptoms & Gross Necropsy Findings:

No signs of systemic toxicity were observed during the study. Dermal toxicity was expressed as erythema.edema, and desquamation. However grades of reaction were not indicated.
At necropsy no gross pathology was observed in any rabbit. The report reiterates the presence of scales at the application sites.

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DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (§81-3)

Product Manager: 18 **Reviewer:** L. Markarian
MRID No.: 421190-06 **Report Date:** 01/09/92
Testing Laboratory: Dow Chemical Co. **Report No.:** M-005222-003
The Toxicology Research Laboratory
Author(s): G.J.Bradley, J.E. Bettjes, and J.W. Crissman
Species: Rat, Fischer 344
Weight: **Males 141-276 g, Females 106-155 g**
Source: Charles River Laboratories, Inc.,Kingston, NY
Test Material: XRM-5222 (AGR# 284240)
Quality Assurance (40 CFR §160.12): Included

Summary:

1. LC_{50} **(mg/kg):** **Males** = Estimated 5.25 mg/L
Females = Estimated 3.14 mg/L
Combined =

2. **Mean Concentration:** 2.30, 2.59, 3.58, 4.50, 5.66 mg/L

4. **Tox. Category:** **Classification:** Supplementary

Procedure (Deviation From §81-3):

Nose only exposures were in a 42 L cylindrical (30 cm diameter x 60 cm high) dynamic flow chamber. Air flow was maintained at 30 lpm.

The chamber atmosphere was generated by metering the test material with a FMI pump (Fluid metering, Oyster BAy, NY) into a stainless steel 1/4 J spray nozzle. The test material was mixed with compressed air in the nozzle and discharged into the chamber. 30 lpm of dry compressed air was used to aerosolize the particles.

Chamber concentrations were measured gravimetrically at a minimum of three times during exposure by sampling from the breathing zone at the rate of 3 lpm for 1.5 - 3 minutes.

Particle size analysis was made using a six stage Cascade Impactor (Sierra model 266) from the breathing zone at 3 lpm for 4 minutes at the 2.30 mg/L, 1.5 or 3.0 minutes for the 4.5 mg/L, and for 2 minutes for all other exposures.

Air flow, temperature and humidity were monitored continuously and recorded at 30 minute intervals.

The animals were examined prior to exposure. This included ophthalmic examination with penlight. Observations were made during the exposure and daily during work days for the two week post exposure period.On week ends monitoring was limited to animal husbandry procedures. Necropsy was performed on all animals. Sacrificed animals were subjected to eye examinations using microscope slide and fluorescent lighting.

Results:

Reported Mortality

Exposure Concentration mg/L	(NUMBER KILLED/NUMBER TESTED)		
	Males	Females	Combined
2.30	0/5	0/5	0/10
2.59	-	1/5	1/5
3.58	-	5/5	5/5
4.50	0/5	4/5	4/10
5.63	4/5	-	4/5

EXPOSURE CONDITIONS

Average Gravimetric Concentration	**2.30 mg/L**	**2.59 mg/L**	**3.58 mg/L**	**4.50 mg/L**	**5.64 mg/L**
Range	**2.21-2.36**	**2.07-2.79**	**3.15-3.80**	**4.27-4.60**	**5.36-5.99**
Particle size I	**34.1 %**	**30.6 %**	**24.7 %**	**25.3 %**	**21.5 %**
1.3 u and under II	**34.2 %**	**23.9 %**	**25.2 %**	**24.4 %**	**22.3 %**
MMAD um	**1.7**	**1.74**	**2.04**	**2.14**	**2.19**
GSD um	**2.24**	**2.13**	**2.12**	**2.11**	**2.09**
Air Flow LPM *	**30**	**30**	**30**	**30**	**30**
Temperature °C*	**22**	**22**	**22**	**22**	**22**
Humidity % *	**50**	**50**	**50**	**50**	**50**

*** Temperature and humidity were controlled by a system that maintained these parameters at constant level. Air flow was maintained through a calibrated flowmeter.**

Symptoms & Gross Necropsy Findings:

During exposure at all levels soiled fur, red exudate around the nose and mouth breathing was observed. After exposure soiled fur and red exudate around the nose persisted in some of the survivors to 8 days. Weight loss or failure to gain weight is seen at 8 days at all levels levels in the males. At 15 days the average weight of the males at 2.3 mg/L was 3 grams less than the average weight at initiation. At 4.50 mg/L the average weight of the males had dropped 16 grams. The only surviving male at 5.64 mg/L shows a gain of 23 grams over the 14 day period. The surviving females gained

weight after day 8.

At the necropsy of the decedents it was noted that there were facial stains from some clear exudate as well as pulmonary congestion and edema, gastric ulcerations, gastrointestinal gas, dark ingesta and decrease in fat. One male at 5.64 mg/L had urinary calculi and one female at 4.5 mg/L had a pale liver.
All survivors showed no abnormalities at termination.

DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (§81-4)

Product Manager: 19 **Reviewer:** L. Markarian
MRID No.: 421190-07 **Report Date:** 01/09/92
Testing Laboratory: Dow Chemical Company **Report No.:** M0050222-001
The Toxicology Research Laboratory
Author(s): N. M. Bardasco
Species: Rabbit, New Zealand White
Sex: Not specified
Weight: 2.8 - 3.7 kg
Source: Hazleton Research Products
Dosage: 0.1 ml
Test Material: XRM-5222 sample reference GHD-2576-28 (liquid)
Quality Assurance (40 CFR §160.12): included

Summary:

1. **Toxicity Category:** III

2. **Classification:** guideline

Procedure (Deviations From §81-4):

The test material was instilled in the conjunctival sacs of six pre-examined right eyes. The left eyes were used as controls. Examinations were at 1, 24, 48 and 72 hours after instillation. Scoring was according to Draize. The eyes of the last five rabbits were anesthetized with ophthain after the initial rabbit showed discomfort after instillation.

Results:

Observations	(number "positive"/number tested)							
	Hour	Days						
	1	1	2	3	4	7	14	21
Cornea Opacity	0/6	0/6	0/6	0/6				
Iris	6/6	0/6	0/6	0/6				
Conjunctivae								
Redness	0/6	1/6	0/6	0/6				
Chemosis	4/6	0/6	0/6	0/6				
Discharge	6/6	0/6	0/6	0/6				

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DATA REVIEW FOR SKIN IRRITATION TESTING (§81-5)

Product Manager:19 **Reviewer:** L. Markarian
MRID No.:421190-08 **Report Date:**01/09/92
Testing Laboratory: Dow Chemical Company **Report No.:**M-005222-002B
The Toxicology Research Laboratory
Author: N.M. Berdasco
Species: Rabbit, New Zealand White
Age: Not specified
Sex: Two male and four female
Weight:2.7 - 3.2 K
Dosage:0.5 ml
Test Material:XRM-5222 sample reference AGR 284240 (liquid)
Quality Assurance (40 CFR §160.12): Included
Summary:

1. **The Primary Irritation Index** =1.08

2. **Toxicity Category:** III

3. **Classification:**Core minimum

Procedure (Deviations From §81-5):

The test material was applied to the backs of animals under 4 x 4 cm gauze patch held in place with tape. The patch was covered with flannel bandage. At 4 hrs the wrappings were removed and the sites wiped with damp paper towels. The sites were graded at 30 minutes and 24, 48 and 72 hrs and on days 7, 14 and 23 according to Draize.

Results:

Intervals	Min.	Hours			Days		
	30	24	48	72	7	14	23
Erythema							
0	6/6				6/6	6/6	6/6
Grade 1		6/6	6/6	4/6			
Edema							
0	6/6			6/6	6/6	6/6	6/6
grade 1		1/6	2/6				
Fissures		6/6	6/6	6/6*	*	*	*
Desquamation		6/6*	6/6	6/6	6/6	1/6	0/6

* It is not clear when fissures were first noted and when desquamation appeared, neither is it clear how deep the fissures were.

Special Comments:
Drying up of the skin is not generally seen with grade 1 erythema and edema, and fissuring is almost never associated with as nonremarkable reactions as this. More surprising is the fact that this type of initial reaction induced desquamation that lasted for 14 to 22 days.

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DATA REVIEW FOR SKIN SENSITIZATION TESTING (§81-6)

Product Manager:19 **Reviewer:** L. Markarian
MRID No.:421190-09 **Report Date:**01/09/92
Testing Laboratory: Dow chemical Company **Report No.:**M-005222-002E
The Toxicology Research Laboratory
Author(s):N. M. Berdasco
Species: Guinea Pig, Hartley
Weight:363 - 441 g
Source: Charles River Breeding Laboratories, Inc., Kingston MI
Test Material:XRM-5222 sample reference AGR 284240 (liquid)
Positive Control Material:DER 331 epoxy resin in dipropylene glycol monoethyl ether (10 %)
Quality Assurance (40 CFR §160.12): Included

Method: Buehler
Summary:

1. **This Product is not a dermal sensitizer**
2. **Classification:**guideline

Procedure (Deviation From §81-6):

A pretest screening was made to define the induction and elicitation concentrations. Four guinea pigs were tested with 100% and aqueous dilutions of 50, 25, and % in 0.4 ml aliquots at six hour exposures. No irritation was observed at any concentration; therefore 100 % was used for induction and elicitation.

Two groups of test animals were used: one group of ten treated with undiluted test material and the second as positive control, treater with 10 % DER 331 epoxy resin in dipropylene glycol monomethyl ether. The concentration of the positive control was lowered to 7,5 % at the observation of erythema following the second induction. There were no naive controls.There were three inductions of six hour duration applied in 0.4 ml aliquots at one week intervals in Hill Top chambers secured with tape.The animals were not restrained.
Challenge was made two weeks after the last induction at a naive site in the same manner as the inductions. The sites were depilated before challenge applications were evaluated.

Induction sites were evaluated at 24 hrs after removal of the patches. Challenge sites were evaluated at 24 and 48 hrs after removal of the patches. Evaluations were according to Buehler.

Results:
In the test group no reaction was observed at any interval at any site after induction or challenge. In the positive control group Grade one reaction was observed in 3/10 following the first application at 10 % DER 33, But no reaction was observed after reducing the concentration to 7.5 %. At challenge at 24 hrs 5/10 were positive and 10/10 were positive at 48 hrs. The laboratory considers the test material a nonsensitizer.

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Tox Chem No. 219 AA **Current Date 01/09/92**
Laboratory: The Toxicology Research Laboratory, Health and Environmental Sciences
Dow Chemical Company, Midland, MI 48674

S T U D Y	M A T E R I A L	MRID No	R E S U L T S	Tox Cat	CORE GRADE
Oral Toxicity LD_{50} Study in Rats M-005222-002A 3/5/91	XRM-5222 Sample Reference AGR 284240	421190-04	LD_{50} >500mg/kg	III	core minimum
Dermal Toxicity Limit Test in Rabbits M-005222-002D 3/5/91	" " "	421190-05	LD_{50} >2000 mg/kg	III	Guideline
Inhalation Toxicity LC_{50} Study in Rats M-005222-003 4/4/91	" " "	421190-06			Supplementary
Eye Irritation in Rabbits M-005222-001 2/20/91	" " "	421190-07	Eyes clear 72 hrs	III	Guideline
Dermal Irritation in Rabbits M-005222-002B 2/4/91	" " "	421190-08	Slightly Irritating	III	Minimum
Demal Sensitization in Guinea Pigs M-005222-002E 1/11/91	" " "	421190-09	Not Sensitizer		Guideline