RAVEN SGS. OFM 10-19-83

DIALLATE TOX CHAPTER

2/4/83

Toxicology

Received
2/4/83
SPRD

Diallate

Pesticide Registration Standard

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Diallate

Pesticide Registration Standard

Data Request Rationale

a. Postitive findings

1) Neurotoxicity: Unexplained neural lesions were observed in acute studies using hens. Correspondence between dose size and severity of lesions was not shown. Such lack of dose response relationship is not typical of lesions related to "delayed neurotoxicity" such as found after organophosphate type poisoning. Therefore a 90-day subchronic neurotoxicity study in mammals is needed to clarify this potential hazard. This study could be combined with a mammalian study.

2) Feeding studies: We have no valid chronic feeding study. A 90-day feeding study using dogs (Kay, J. H., 1959, MRID 00050252) was considered in relationship to granting tolerances of 0.050 on certain crops. Since that time this study has been declared invalid by both the Canadian Government and EPA. Two chronic feeding studies, one in rat and one in dog, are needed for future registrations or reregistrations which require a tolerance.

3) Oncogenicity: Oncogenicity data have been extensively reviewed by the Cancer Assessment Group of EPA (GS-0098-001). Pertinent data and risk assessment are presented in EPA's Position Document No. 4. Risk was based on a 16-18 month hamster oncogenicity study submitted by Monsanto (Serdy, et al., 1979; GS-0098-015). Risk assessment presentations in PD-4 starts on page 21.

Slope: 5.8×10^{-5} ug/kg/day
TMRC: 0.0089 mg/person/day

Risk = slope x exposure in ug/kg/day
$= (5.8 \times 10^{-5})(1.48 \times 10^{-1}) = 8.3 \times 10^{-6}$

The major hazard from use of diallate is considered to be to the farm worker who applies the pesticide. Label restrictions require the wearing of protective clothing by the person who mixes or applies diallate.

More testing is not needed.

4) Mutagenicity: Studies presented in EPA's RPAR Position Document No. 4 (GS-0098-010) furnished multitest evidence that diallate is mutagenic.

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These studies qualitatively indicated point-mutational activity both in bacterial species and in mammalian cell cultures in vitro, chromosome breakage and rearrangement in plants and mammalian cells, and DNA damage or impairment of DNA repair in lower plants and mammalian cells. In general, positive results were found in tests on mammalian cells only when metabolic actiivty was provided through the introduction of rodent liver extracts.

Valid whole animal assays are lacking for diallate. Such studies are required before any assessment of risk to human or animal population can be attempted.

See specific suggestions under Data Gaps heading.

b. Data Waiver rational.

1) Subchronic dermal toxicity: This study is waived because of low dermal toxicity demonstrated by the acute dermal study.

2) 90-day inhalation: This study is deferred until an acute study has been received and evaluated.

c. Special Testing

None needed unless indicated by studies not yet received.

d. Data Gaps

We do not have valid studies for the following test areas:

1) Acute inhalation.
2) Dermal sensitization.
3) Neurotoxicity in mammals.
4) Long term feeding studies, rat and dog.
5) Teratogenicity, rat and another species.
6) Mutagenicity.

It is suggested that, as a minimum, the following short-term tests be performed (1) Rodent in vivo cytogenetics or micronucleus test, (2) Dominant Lethal, rodent. If either (or both) of these are positive, consultation with the Agency is strongly advised. This should be done while preparing for mutagenicity testing more directly pertinent to quantitiative risk assessment (such as a Specific Locus or Heritable Translocation Test, each to be performed in the mouse).

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DIALLATE

Data Reviews

Acute Oral Toxicity

Braun, W. G. and Rinehart, W. E. 1979 (MRID 00086378) after performing a range finding study and fasting animals 18 hours, groups (5 male and 5 female rats in each) were dosed orally at 500, 700, 1000, 1400, and 2000 mg/kg, using technical diallate, 92.1%.

Common in-life signs of affect observed during this study included: ataxia, convulsions, muscle tremors, red and clear nasal discharges, clear oral discharge, urinary staining of the abdomen, piloerection, lethargy, fecal staining of the abdomen and alopecia.

The oral LD_{50} for Diallate (technical) for rats was estimated to be 1050 mg/kg with 95% confidence limits of 900 to 1200 mg/kg (MRID 00086378).

Acute Oral Toxicity Category: III

Core: Minimum

Younger, FM, 1959 (MRID 00086441) performed an acute oral toxicity study using 3 male and 2 female dogs per dose level except at the highest dose level for which 2 males and 1 female were used. The dose levels were 398, 501, 631, and 794 mg/kg.

The oral LD_{50} for dogs was estimated to be 510 milligrams per kilogram with lower and upper limits of 415 to 610 milligrams per kilograms.

Survival time ranged from six hours to two days with most deaths occurring in less than twenty-four hours.

There was obvious weakness in one to two hours followed by retching and tremors. Emesis was observed in six of the dogs several hours after dosing, but inasmuch as four of them succumbed, it was felt that most of the dose had already been absorbed or had reached the duodenum. The survivors were weak for several days and appetite returned slowly.

At autopsy, there was renal congestion, tissue edema, and pulmonary hyperemia observed by macroscopic examination.

Additional testing is not required.

Supplemental information only.

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Harrison, W. A., 1976 (MRID 00056737) determined cholinesterase inhibition in plasma, red blood cells, and brains in groups (5 males and 5 females per group) in rats which after fasting 16 hours were given either 800 or 1600 mg/kg diallate by gavage. The purity of the test compound was not stated. A group of 10 males and 10 females were used as untreated controls. After 3 hours blood samples were taken from orbital sinuses. No cholinesterase inhibition was demonstrated.

Additional testing is not required.

Core: Acceptable

Acute Dermal Toxicity

Aulette, C. S. and Rinehart, W. E., 1979 (MRID 00086379) performed an acute dermal toxicity study using four rabbits per group (2 males with abraded skin and 2 females with unabraded skin.

Mortality: Dose levels and mortality were as follows:

Dose Level (mg a.i./kg)	Mortality
5,600	0/4
8,000	1/4
11,300	4/4
16,000	4/4

The dermal LD_{50} for Diallate (technical) estimated from the above data is 8700 mg a.i./kg with 95% confidence limits of 7000 to 1040 mg a.i./kg.

Signs commonly observed at all dose levels included motor activity decrease, ataxia, coarse tremors, and clear nasal discharge. Less frequently observed signs inlcuded convulsions, thinness, piloerection, soft stool/fecal staining, hyperpnea, prostration, general poor condition, and the inability to stand on forelegs.

At 5600 mg a.i./kg both abraded animals exhibited well-defined erythema at the 24-hour dermal observation and both nonabraded animals exhibited very slight erythema. There was no correlation between the presence or lack of abrasions and dermal reactions at the other dose levels; dermal reactions became more severe at higher dose levels.

Acute Dermal Toxicity Category: III

Core: Minimum

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Younger, PM, 1959 (MRID 00086440) performed an acute dermal toxicity study.

The undiluted compound was applied in varying amounts to the closely clipped, intact skin of New Zealand white rabbits.

The treated areas were covered with plastic strips and the animals placed in wooden stocks for twenty-four hours, after which time they were assigned to individual cages.

Observations were made for toxic symptoms and the viscera of the animals that succumbed was examined macroscopically. The data are shown in the following Table I.

Sample Applied Undiluted

TABLE 1

Animal Number — Sex	Weight Kg	Dose Mg./Kg	Weight Change 5 Days Later Kg	Fate
1 - Female	2.3	1000	- 0.3	Survived
2 - Male	2.1	1500	- 0.5	Survived
3 - Male	2.6	2000	- 0.4	Survived
4 - Female	2.1	2500	- 0.4	Died--5 Days
5 - Female	2.3	3000	- 0.6	Died--5 Days
6 - Female	2.[illegible]	4000	-----	Died--3 Days

The LD_{50} by Skin Absorption in rabbits was greater than 2000 milligrams per kilogram and less than 2500 milligrams per kilogram.

Toxic symptoms included rapid loss of appetite and weakness followed by paralysis in all animals. The survivors were the least severely affected and all three appeared to have recovered from the paralytic symptoms in about three weeks.

At autopsy no specific abnormalities were observed macroscopically.

Acute Dermal Category Indicated: III

Core: Supplemental

Acute inhalation toxicity

No valid acute inhalation study is available for diallate. A study using rats and dogs was done by Kay, 1960 (MRID 00086438). This study was determined to be inadequate for regulatory use as an acute inhalation study but it did raise questions concerning the neurological effects of diallate inhalations. A valid acute inhalation study is needed.

Before starting an acute inhalation study consultation with the Agency in regards to observations for neurotoxic signs and preparation of nervous tissues for microscopic examination is advised.

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Acute Delayed Neurotoxicity

Ten hens were administered 312 mg/kg twice daily for three consecutive days. The dosing procedure was repeated starting on the 21st day (Kaplinger, 1977; GS-0098-004). Positive (TOCP) and negative controls were used. Focal and multifocal axonal degeneration with and without degeneration of the adjacent myelin sheath were reported to be treatment-related in two of the ten treated hens and in none of the negative controls. Marsh's disease was present. The clinical neurological signs suggested possibility of acute effects rather than typical organophosphorus-like delayed neurotoxicity.

In another study diallate was administered to hens at dose levels 10, 20, 40, 80, 160 and 320 mg/kg which were administered twice daily for three consecutive days (Phillips, 1977; MRID 00046243). Twenty days following the initial dose, all surviving birds were again given the same dose regimen. Controls were dosed with 0.32 gm/kg corn oil and positive controls received 500 mg/kg TOCP on day 0.

All positive controls exhibited lesions typically associated with delayed neurotoxicity (Phillips, 1977; MRID 00046243). No such lesions were found in the negative controls.

Two test birds, one in the 40 mg/kg and one in the 160 mg/kg group showed focal lesions of axonal degenertion and demyelination in the sciatic nerve. While these lesions were described as morphologically indistinguishable from those observed in the positive control birds, the affected birds showed no clinical signs which could be characterized as delayed neurotoxicity prior to sacrifice. No dose response relationship was established nor were there any explanation for the absence of lesions at the highest dose.

Note that the highest dose was nearly the same as in the Kaplinger study in which two of ten hens had neural lesions attributed to treatment.

These two neurotoxicity studies did not furnish quantitatively useful information. They do suggest a causal relationship between treatment and lesions. Clarification and quantitation are needed. They could be made with results from a subchronic neurotoxicity study in a mammalian species (rat). Consultation with the Agency is advised before starting a neurotoxicity study.

Primary Dermal Irritation

Heenehan, P. F. et al., 1979 (MRID00086381) performed a dermal irritation study using 92.1% technical diallate. Six rabbits were used. One side was abraded and the other side of each was not abraded. 0.5 ml was placed on each side beneath 1"x1" squares of surgical gauze (8 single layers thick) which was secured and wrapped with plastic, secured with masking tape. The exposure was for 24 hours. Observations were made and recorded at 24 and 72 hours. Moderate primary dermal irritation was reported.

Dermal Irritation Category: III

Core: Minimum

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Younger FM, 1959 (MRID00086440) performed a primary dermal irritation study.

The undiluted compound was applied to the clipped, intact skin of albino rabbits and removed after twenty-four hours with soap and warm water. The application was covered with plastic strips to retard evaporation.

The sample was applied undiluted.

Observations were made over a period of several days for irritation.

No inflamation was apparent after one hour. Slight to well defined erythema with no edema developed within four hours. Overnight there was well defined redness with very slight to slight edema. Inflammation was more severe at the perimeter of the application than in the center.

Removal of the material caused disappearance of the irritation in two out of three animals by the fifth day and in all animals by the seventh day.

Acute Dermal Irritation Category Indicated: III

Supplemental value only.

Primary Eye Irritation

Beenehan et al., 1979 (MRID00086380) performed an eye irritation study using technical diallate. One tenth ml was placed in one eye of each of six rabbits. The other eye served as control. Eyes were not rinsed. Irritation was scored on days 1, 2, 3, 4, and 7 or until irritation free for two consecutive observations. Slight redness or chemosis was observed in some but all were clear by the fourth day.

Irritation Category: III

Core: Minimum

Younger, FM, 1959 (MRID 00086440) performed an eye irritation study.

A 0.1 milliliter undiluted sample was placed in the conjuctival sac of the right eye of each of three albino rabbits and obeservations made over a period of several days for inflammation.

The eyes were rinsed with warm isotonic saline solution after twenty-four hours.

There was moderate immediate discomfort shown by the animals. Slight edema, moderate erythema, copious lacrimation, and slight iris congestion were observed. The details of the iris remained clearly visible. Congestion and swelling increased somewhat in four hours. Slight improvement was noted overnight.

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Following the twenty-four hour rinse, inflammation reduced to zero by the fifth day in one animal and only very slight to slight erythema was still present on the two remaining animals.

The compound was classed as a moderate eye irritant.

Eye Irritation Category: III

Supplemental value only.

Sensitization

No study on dermal sensitization was found.

90-day feeding

Kay, J. H., 1959 (MRID 00050252) performed a 90-day feeding study using dogs. This study has been declared invalid by both the Canadian government and the Environmental Protection Agency. It is included for historical value. Although the results of this study were considered when tolerances of 0.05 ppm in certain crops were formulated, this study is not adequate for reregistration. Appropriate long-term studies are required for use on food crops.

Subchronic Dermal Toxicity

We have no subchronic dermal toxicity study. Hazleton, 1 day, 15 rats. [illegible] TOXICITY.

Subchronic Inhalation Toxicity

We have no subchronic inhalation study. This study is deferred until after results from the acute inhalation toxicity has been evalutated.

Subchronic neurotoxicity

We have no subchronic neurotoxicity study. Questions raised by the acute studies need clarification, which should be furnished by a subchronic neurotoxicity study using rats. Consultation with the Agency should precede further neurotoxicity testing.

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Chronic Feeding

A two-year chronic feeding study (Keplinger, 1976, IBT No. 622-05250; GS-0098-005) was declared to be invalid as a chronic feeding study. This study is mentioned here for its possible historical value.

We also have a long-term hamster feeding study (Serdy et al., 1979, EPA Accession Number 241087-9; GS-0098-0015.) This study was used by the cancer assessment group to assess oncogenic hazard and more discussion is included elsewhere in this standard. This study was carefully reviewed by Roger Gardner, 1980, (GS-0098-007) as a chronic study and will only briefly be commented on here. Each group consisted of 50 hamsters of each sex. There were two negative control groups. Treated groups received diets containing 200, 600, and 2000 ppm. Males were treated 548 days and females 507 days. Mortality was high (75% males and 86% of females). The number of histopathologic observations was small (less than 10 per group). Oncogenic effects are discussed in the appropriate section of this report. There were no oncogenic or other chronic effects observed at the 200 ppm level.

The Serdy hamster study is not adequate for supporting a food use tolerance. Two long-term studies are needed.

Oncogenicity

Oncogenicity data have been extensively reviewed by the Cancer Assessment Group of the Environmental Protection Agency. Pertinent data and risk assessment are presented in EPA's Position Document No. 4, March 30, 1982 (GS-0098-010). Risk evaluations were based on a 16-18 month hamster oncogenicity study submitted by Monsanto (Serdy et al., 1979; GS-0098-015). Only this study will be referred to here.

In this study (Serdy et al., 1979; GS-0098-015), Syrian gold hamsters were fed diets containing diallate for their entire lifetimes. Animals of both sexes were divided into two control groups and three treatment groups. The test animals received diets containing 200 ppm, 600 ppm, and 2000 ppm of diallate. Gross pathological examinations revealed cutaneous, pigmented masses in the mid- and high-dose (600 ppm and 2000 ppm, respectively) groups. Histopathological examination identified both benign and malignant lesions among these masses.

In evaluating oncogenic potency, the Monsanto Company corrected for early mortality among test and control animals, adjusting the denominator of tumor incidence figures by using the number of animals surviving beyond week 28, when the first melanoma was observed. The agency's Carcinogen Assessment Group (CAG; GS-0098-001) used a more conservative approach, counting only those animals that were examined histopathologically in the denominator. As a result the CAG's slope estimate is about six times greater than the company estimate (EPA PD-4; GS-0098-010). The following Table 2 summarizes the results of CAG's calculations for incidence of skin lesions.

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TABLE 2

Incidence[a] of Benign/Malignant Dermal Melanomas In Hamsters
(Per Animal Examined Histologically)

Dose (ppm)	Male	Female
0	0/29	1/20
200	0/10	0/5
600	3/9	4/7[b]
2000	16/30[c]	10/16

Source: Chen and Haberman, 1981, as quoted in EPA's Position Document No. 4 (GS-0098-010).

[a] Adjusted for early mortality among both test and control animals.
[b] One animal had both benign and malignant tumors.
[c] 2 animals had both benign and malignant tumors.

The Monsanto report characterizes the hamster results as suggestive of compound-related carcinogenic effects. The agency agreed and finds that the results are consistant with previous studies that indicate that diallate is oncogenic in mammalian species (Innes et al., 1969; GS-0098-011; Ulland, et al., 1973; MRID 05012720; Keplinger, 1976; GS-0098-005; EPA's Diallate Position Document No. 4, March 30, 1982 (GS-0098-010).

Mutagenicity

IN PD 2/3 (GS-0098-009) and PD 4 (GS-0098-010), the Agency presented multitest evidence of diallate's mutagenicity. That conclusion was based on eleven studies which evaluated various indicators of diallate's potential for mutagenic activity in several species of microorganism and in cultured mammalian cells. These studies qualitatively indicated point-mutational activity both in bacterial species and in mammalian cells cultures in vitro, chromosome breakage and rearrangement in plants and mammalian cells, and DNA damage or impairment of DNA repair in lower plants and mammalian cells. In general, positive results were found in tests on mammalian cells only when metabolic activity was provided through the introduction of rodent liver extracts. The following Table 5 from PD 4 summarizes these positive effects found in the studies reviewed.

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TABLE 3

Positive Mutagenicity Studies Reviewed for PD 2/3 and PD 4

Effect/Indicator	Organism	Reference
GENE MUTATION		
Bacterial	Salmonella [a]	Carere (1978) (MRID 05011869)
	Streptomyces [a]	Carere (1978) (MRID 05011869)
	Salmonella [a]	DeLorenzo (1978) (MRID 05012173)
	Salmonella [a]	Rosen (1980) (GS-0098-014)
	Salmonella [a]	Douglas (1981) (GS-0098-006)
	Salmonella [a]	Schuphan (1979) (GS-0098-016)
	Salmonella [a]	Sikka and Florczyk (1978) (MRID 05011877)
Yeast	Aspergillus [a]	Morpurgo (1977) (MRID 05018683)
Mammalian (cells in culture)	L5178Y-TK +/- [a]	Simmon (1978) (GS-0098-017)
	L5178Y-TK +/- [a]	Brusick (1977) (GS-0098-002)
CHROMOSOMAL ABERRATION		
Plants	(Species Not Specified in Summary Reviewed by EPA)	Douglas (1981) (GS-0098-006)
Mammalian (cells in culture)	Chinse Hamster [a]	Douglas (1981) (GS-0098-006)
DNA DAMAGE OR REPAIR		
Yeast	Aspergillus	Morpurgo (1977) (05018683)
Mammalian (cells in culture)	Rat Thymocytes [a]	Rocchi (1980) (GS-0098-013)
	Chinese Hamster [a]	Douglas (1981) (GS-0098-006)

Source: Mauer in the Agency's Diallate Position Document No. 4, dated March 30, 1982 (GS-0098-010).

[a] Positive effects found only in presence of mammalian metabolic activation system (S-9).

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Valid whole animal assays are lacking for diallate. Such studies are required before any assessment of risk to human or animal population can be attempted. It is suggested, that as a minimum, the following short-term tests be performed (1) Rodent in vivo cytogenetics or micronucleus test, (2) Dominant Lethal, rodent. If either (or both) of these are positive, consultation with the Agency is strongly advised. This should be done while preparsing for mutogenicity testing more directly pertinent to quantitative risk assessment (such as a Specific Locus or a Heritable Translocation Test, each to be performed in the mouse).

Three-Generations Reproduction

Salamon et al., 1979 (MRID 00028956) fed Avadex(R) herbicide (diallate technical) to groups of male and female rats at dietary concentrations of 0, 10, 30 and 100 ppm. No treatment-related effects were observed in parental behavior, mortality, body weight gain, organ weight, organ-to-body weight ratios, or gross histopathology in groups of rats fed up to 100 ppm diallate. No consistent, statistically significant differences were observed between the control and treatment groups in the number of progeny born and weaned or their survival and body weight gains. All progeny had a normal external appearance and exhibited no abnormal behavioral signs. Histopathologic examination of selected control and high-dose F3b weanlings revealed no lesions attributable to compound administration.

On several occasions, mating and fertility indices for each of the three treatment levels were lower than those for control animals for a given mating. However, control animals showed variability in those indices and there was an absence of consistent dose-related trend among treated groups. Other parameters of reproductive performance compared favorably with the control animals.

Additional testing is not required.

The no-observed-effect-level was 100 ppm, the highest dose tested.

Teratogenicity

We have no valid teratogenicity study.

Metabolism

A metabolic fate study of diallate in rats was performed by Cliffe and Watkins, 1961 (MRID 00073505). During 24 hours after oral ingestion of 200 mg/kg of 14C labeled diallate, 43% of the radioactivity was excreted in the urine, 10% in the feces and less than 5% in the expired CO_2.

In a similar study during 48 hours after ingesting 143 mg/kg 61% was found in the urine, 19% in the feces and 8% in CO_2. These results suggest adequate elimination after a single oral exposure.

No additional testing is required at this time.

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