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

014169

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

May 16, 2000

MEMORANDUM

SUBJECT: D264641 ALDICARB (098301):
Toxicology Review of A Special Non-Guideline Study:
5 day Dermal Toxicity Study in Rats
MRID 45079704

TO: Monica Alvarez
Review Manager
Special Review Branch
Special Review and Reregistration Division (7508C)

FROM: William F. Sette, Ph.D. William F Sette 5-16-00
Toxicologist
Science Analysis Branch
Health Effects Division (7509C)

THRU: William Burnam, Chief 5/16/00
Science Analysis Branch
Health Effects Division

Attached please find the HED review of this recently submitted study of Aldicarb. HED finds this special non-guideline study unacceptable and the data not sufficient for use in EPA's risk assessment.

014169

Aldicarb, Temik 15 G® grit Special 5 Day Dermal Toxicity Study

EPA Reviewer: William F. Sette, Ph.D. William F Sette
Science Analysis Branch (7509C) 5-16-00

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Science Analysis Branch (7509C)

DATA EVALUATION RECORD 014169

STUDY TYPE: 5 Day Dermal Toxicity Study

DP BARCODE: D264641 SUBMISSION CODE: S577754
P.C.CODE: 098301 TOX. CHEM NO: 011A

TEST MATERIAL (PURITY): Aldicarb, Temik 15G® grit (14.75% aldicarb)

SYNONYMS: 2-methyl-2-(methylthio) priopionaldehyde 0-(methylcarbamoyl) oxime.

CITATION: RW Tyl, WP Ross, CB Myers. Assessment of Cholinesterase Activity in CD® Rats Following Topical Application of Temik 15G® Grit for One Week. Research Triangle Institute, Research Triangle Park, NC. RTI Report No. 65C-07594. March 28, 2000. MRID 45079704. Unpublished.

SPONSOR: Aventis Crop Science, Research Triangle Park, NC 27709

EXECUTIVE SUMMARY:

In this 5 day dermal toxicity study (MRID 45079704), 5 mg/kg of analytical grade aldicarb in deionized water, or Temik 15G® grit (14.75% aldicarb) at levels of 0, 100, 500, or 2000 mg/kg/day was dermally applied to a 1" square area on the backs of 8 albino CD® Sprague-Dawley rats/sex/dose, for 6 hours/day, for 5 days. Body weights and clinical observations were recorded daily, and food consumption measured on Day 1 and Day 5. Blood cholinesterase measures were made on 0.25 ml samples taken 1 hour post-dosing on Day 1 and Day 5. On Day 5 after blood sampling, brain weights and Brain ChE measures were made.

There was one female death in the 5 mg/kg technical aldicarb positive control group. In that 5 mg/kg group, cholinergic signs, including tremors, salivation, lacrimation, lethargy, and prostration, were seen in 1 or 2 females on days 3-5. Tremors were seen in 1 male and 2 females in the 2000 mg/kg Temik group one hour after dosing on days 1-3. Males in the 5 mg/kg aldicarb group showed decreased body weight gain (85%) and body weight decreases (7%) on days 4-5. There were no effects on food consumption, brain weights, or on necropsy(except for the dead female, who showed lacrimation, salivation, dark tar-like ano-genital discharge, darkened uterus,

and autolysis of brain and digestive tract).

5 mg/kg rats showed 52-64% plasma ChEI, 29% RBC ChEI, on day 1, 62-90% plasma ChEI, 27-45% RBC ChEI, and 12-26% brain ChEI on day 5. Females showed greater effects on the brain than males, and greater effects on blood ChEs after five days of exposure.

Rats exposed to Temik at 2.000 mg/kg showed 90-94% plasma ChEI, 46-49% RBC ChEI, and 31% (♂ s) and 51% (♀s) brain ChEI. Little change across the 5 days was seen at this dose, though females showed more brain inhibition than males. At 500 mg/kg Temik exposure caused significant inhibition in RBCs in both males (18-22%) and females (13-19%); and significant inhibition in plasma in males (33-50%), but not females (13-27%). Brain ChEI was slight (4-6%). Inhibition was greater on day 5 in plasma for both sexes. Rats exposed to Temik at 100 mg/kg showed no significant effects on brain or blood ChEI, though the effect on RBCs in females of 12% approached the statistically significant level of 13% seen in females in RBCs at 500 mg/kg .

This non-guideline study should be regarded as unacceptable.

It provides positive control data on dermal exposure to one dose of aldicarb technical, and comparable data on Temik 15G exposure, although the continuing concerns about the adequacy of the exposure preparation limit its utility for risk assessment.

While it provides some interesting data on dermal exposures to both Temik and technical grade aldicarb, because of the unique aspects of the preparation, e.g., limited exposure area, limited wetting, and limited skin contact, it is difficult to compare these results to guideline dermal studies. Because the positive control data were collected on technical grade aldicarb dissolved in water makes comparison to granules (where only skin was slightly moistened) flawed and likely to make differences between technical aldicarb and Temik granules appear greater than they are. Because the positive control data also used a smaller surface area than is called for in guideline studies, they provide a distorted estimate of the relation between these dermal exposures and the oral exposure database in relation to how other materials are compared. The deficiencies in both of the rat studies, and the limited study duration, do not provide sufficient data to remedy the deficiencies in the earlier 21 day rat study and the database related to the dermal toxicity of aldicarb.

COMPLIANCE: GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: TEMIK 15G® grit
Description: light brown granules with a sulfurous odor
Lot/batch # 703058VE
Purity: 13.94 - 14.75%

2. Positive control material: Aldicarb, analytical grade
Description: white crystalline solid, odorless to light sulfur smell
Lot/batch # 8KJ092
Purity: 99.5%
CAS # 116-06-3

3. Test Animals: A total of 42 virgin male and 42 virgin female outbred albino CD® Sprague-Dawley rats [Crl:CD® (SD)Br], 7-10 weeks of age and weighing 200-250g, received from Charles River Laboratory, Raleigh, NC, were subjects.

All animals were housed individually, fed Purina Certified Rodent Diet® and tap water *ad libitum*.

Animal rooms were maintained at 64-79° F and 30-70% relative humidity, with a 12/12 hour light/dark cycle.

B. STUDY DESIGN:
1. In life dates: 10/07-10/29/99
2. Animal Assignment:

8 rats/sex/dose were exposed to 0, 100, 500, or 2000 mg/kg of Temik grit or 5 mg/kg of aldicarb technical in de-ionized water for 6 hours/day for 5 consecutive days. ChE determinations were made in plasma and red blood cells one hour after the 6 hour exposure period on day 1 and day 5, and in whole brain on day 5 after blood measures.

3. Statistical Analyses

Study data included mortalities, clinical observations, body weights, food consumption, and cholinesterase activities. Different statistical procedures were described for continuous and nominal data.

For continuous data, including body weights and cholinesterase data, data that were found by Bartlett's test to have homogeneous variances were subjected to parametric statistical analyses, Analyses of variance and the General Linear Models procedures were used, but not further described. Dunnett's test was used for comparing individual dose groups to controls. t tests were used for analyzing the positive control data. If the variances were not homogeneous, then non-parametric tests were used. Non-parametric tests were the Kruskal-Wallis test to determine overall treatment effects, and the Mann-Whitney U Test for pair-wise comparisons, i.e., Group 2 vs Group 3, if the K-W was found significant. Jonckheere's test was used to identify trends.

Nominal scale measures(present or absent signs)were analyzed by a Chi-Square test, the Cochran-Armitage trend test, and a 2-tailed Fisher's Exact Test if the the overall Chi-Square revealed significant differences, with "appropriate adjustments for multiple comparisons".

C. METHODS:

1. Preparation of Animal Skin: The backs of the rats were clipped and shaved within 24 hours prior to dosing. Male rats were shaved on Sunday, Tuesday, and Thursday; females rats were shaved

the following Sunday and Wednesday. The application site was cleaned with 70% ethanol and a gauze pad at the time of shaving, not just prior to dosing. After each daily exposure, the site was cleaned with distilled water and a gauze pad. Before addition of the granules, the skin was moistened with saline (1 ml on a 2" x 2" 12-ply gauze pad) to "simulate a sheen of sweat".

2. Preparation of Test Substance: The dry Temik grit dose was poured into a self-adhesive (7/16" thick) foam rubber dam measuring 2"x2" with a 1"x1" opening (1.4" x 1/4" for the 2000mg/kg dose), allowing the material to lie as a single layer, and covered with filter paper.

3. Administration of Test Substance The rat was then rolled onto the self-adhesive foam rubber dam, the dam was secured with a Vetwrap® adhesive wrap, and adhesive tape over that, to compress the Vetwrap® and confine the test material in an area in close approximation to the skin.

After the 6 hour exposure period, the wrap, filter paper, and rubber dam were removed, the grit removed, and the site lightly brushed and then rinsed clean with distilled water and blotted dry.

Rats exposed to the technical aldicarb were given 5.0 mg/kg, at a concentration of 3.25mg/ml and a volume of 1.6 ml/kg in deionized water. Prior to dosing, the area was also moistened with a 12 ply gauze wetted with 1 ml of saline "to simulate a sheen of sweat". A 16 gauge, 2 inch curved dosing needle attached to a 1 cc syringe was used to "paint" the dose onto the rat's back. The area was then covered with a 2" x 2" gauze pad (12ply), secured with adhesive wrap, Vetwrap, and adhesive tape over that. After dosing, the wrap was removed, and the area rinsed clean with distilled water and blotted dry.

After dosing, all rats were individually placed in "exposure" cages, plastic cages with litter bedding.

4. Body Weights and Food Consumption: Rats were weighed once during quarantine, the day prior to exposure, each day prior to exposure, and at sacrifice. Rats were examined clinically 0, 3, and 6 hours during exposure and 1 hour after exposure. Individual food consumption was recorded twice, on Monday and Friday during the study.

5. Observations: Observations included:

1. any response with respect to body position, activity, coordination, or gait.

2. Any unusual behavior, such as head flicking.

3. The presence of convulsions, tremors, increased salivation, lacrimation, urination, or defecation, piloerection, mydriasis (enlarged pupils), unusual respiration or vocalization.

4. The site of application was examined and results recorded immediately prior to and after each exposure for signs of erythema, edema, or eschar formation according to the scoring system of Draize(1944, 1959).

6. Cholinesterase Measurements

Blood samples were collected on day 1 and day 5, one hour after exposure.

All blood samples were collected within ±5 minutes of the designated time. 0.25 ml blood

samples were collected from the lateral tail vein with a needle pre-rinsed with EDTA as an anti-coagulant into a 1.5 ml centrifuge tube containing dry EDTA, and placed on ice. The tubes were then centrifuged at 1600x g for 10 mins at 4° C. Plasma was pipetted into vials. RBC pellets were washed twice with cold 0.9% NaCl, diluted 1:1 with Triton X-100 in phosphate buffer and gently mixed. The plasma and RBC samples were frozen quickly in dry ice and stored at -70° C until analyzed. Samples were thawed. RBC samples were diluted again 1:10 for a final dilution factor of 1:20, and were immediately analyzed (10 at a time). Brain was collected at necropsy, weighed and frozen on dry ice.

All analyses (brain and blood) were performed within 1 week after collection. Cholinesterase activity was determined by a modification of the Ellman method. The sample was added to the reagent containing 5,5'-dithio-(2-nitrobenzoic acid) (DTNB) and the substrate acetylthiocholine iodide in sodium phosphate buffer at pH 7.2. The resulting color was measured at 405 nm at 30° C on a Gilford Instruments System 2600 spectrophotometer. The absorbance change/minute was determined from the resulting absorbance plots and activity calculated according to Beer's Law. Results were expressed as mIU/ml for blood, and mIU/g for brain.

7. Gross Necropsy

Gross necropsies were performed on all rats after euthanasia by carbon dioxide. At necropsy, the whole brain, minus the olfactory lobes, was removed, weighed, and frozen on dry ice for subsequent ChE determinations.

II. RESULTS

A. Mortality, Clinical Observations and Gross Necropsy Findings

One female positive control rat died. There were no deaths in rats exposed to Temik.

High dose(2000 mg/kg) and positive control females showed a number of clinical signs associated with cholinergic stimulation. One male rat in the 2000 mg/kg group exhibited tremors on day 2, one hour after dosing.

In 2000 mg/kg females, tremors on days 1-3, an hour after exposure in 1 or 2 rats were reported.

In positive control (5mg/kg) females, tremors were seen 2-3 hours into the exposure on days 4-5 in 2 rats, and 2 females showed lacrimation and salivation 3 hours into the exposure on day 5. Individual rats in this group also showed lacrimation, lethargy, and/or prostration.

No dermal effects were seen in either males or females.

There were no treatment related necropsy findings in males, but in the female that died, signs of lacrimation, salivation,slight autolysis of the brain and digestive tract, black tarlike anogenital discharge, and dark colored uterus were noted.

B. Body Weights, Food Consumption.

Body weight gain data from days 1-5 and body weight at sacrifice are shown in Table 1.

These body weights were made before the daily exposures; thus the weight at sacrifice was after the fifth exposure and reflects its potential impact. The relation among dose groups were not different at sacrifice in comparison to day 5 (pre-exposure).

In male rats exposed to 0, 500, or 2,000 mg/kg of the granular formulation, there were no changes in body weight or body weight gain. While the 43% decrease at 100 mg/kg seems noteworthy, the lack of dose dependent changes at higher doses makes this finding inconclusive. It is also remarkable that their statistical analysis did not flag such a large change. Male positive control rats exposed to 5 mg/kg of aldicarb had statistically significantly decreased body weight gains (-85%) and reduced body weight (-7%) on days 4 and 5. There were no effects seen on food consumption expressed as g/day or g/kg/day seen in either group. There were no effects on brain weights in males.

In females, there were no significant changes in body weight, body weight gain, brain weight, or food consumption.

Table 1. Body weight gain(Days 1-5) and Terminal Body Weights (sacrifice) (g) Mean ± S.E. * $p < 0.05$;** $p<0.01$ student t test.

Dose (mg/kg) TEMIK 15G	0	100	500	2000	5 mg/kg Aldicarb
Males					
Body weight gain day 1-5	12.4±1.6	7.1±1.3 (-43%)	10.4±2.1 (-16%)	12.9±3.8 (+4%)	1.9 ±2.3 * (-85%)
Body weight at sacrifice	322.4±7.2	316.4±5.0	310.2±5.8	316.2±6.9	299.8±6.2 * (-7%)
Females					
Body weight gain day 1-5	-7.3±1.0	-6.1±2.0	-6.1±1.6	-1.7±3.1	-7.4±1.9
Body weight at sacrifice	228.7±4.4	230.3±5.6	226.8±3.8	234.6±5.5	224.3±5.2

C. Cholinesterase Measurements

The ChE findings for male and female rats are shown in Tables 2 and 3, respectively.

Positive control rats dermally exposed to aldicarb at 5 mg/kg, and sampled one hour after dosing on day 1, showed plasma inhibition of 52 %, 64% and RBC inhibition of 29 %, 29% for males and females, respectively, and all were considered statistically significant. On day 5, they showed plasma inhibition of 62%, 90%; RBC inhibition of 27%, 45%; and brain inhibition of 12% and 26%, again for males and females, respectively, and all were considered statistically significant. For females, effects were greater for plasma, and for RBCs, on day 5 in comparison to day 1, and in brain ChEI compared to males. For male rats, there was a slightly greater effect on plasma, and very little change on RBCs between day 1 and day 5.

Rats exposed to 2000 mg/kg of TEMIK showed plasma inhibition between 90-94%, RBC inhibition between 46-49%, and brain inhibition of 31% in males and 51% in females. Little change in blood measures were seen then between the sexes at this dose or across the 5 day dosing period. Females showed considerably more brain ChEI than males, as they had in the positive control group.

Rats exposed to 500 mg/kg of TEMIK on day 1 showed plasma inhibition of 33%, 13%; RBC inhibition of 18%, 13%; on day 5 plasma inhibition was 50%, 27%; and RBC inhibition was 22%, 19% for males and females, respectively,. All of the changes in males were considered statistically significant, but in females only the RBC effects were, with the decreases in plasma of 13% and 27% on days 1 and 5 not reaching statistical significance. Across days, plasma inhibition grew for this group in both males and females. RBC values were slightly increased for both sexes. In this group, brain inhibition at 4% and 6% for males and females were not statistically significant.

Rats exposed to 100 mg/kg of TEMIK showed no effects in brain, and only slight effects in blood ChEs. Plasma was inhibited 9% on day 1 and 15% on day 5 in males, while RBCs in females on day 1 were inhibited 12% which approached the statistically significant level of 13% RBC ChEI seen in the 500 mg/kg group. Overall, 100 mg/kg is an NOAEL for ChEI in this study.

TABLE 2. Cholinesterase Data for Male Rats (+ 1 hour after exposure) (mU/ml) Mean ± S.E.
N=7-8

Dose (mg/kg) TEMIK 15G	0	100	500	2000	5 mg/kg Aldicarb
Plasma					
Day 1	361 ±40	330 ±37 -9%	241* ±17 -33%	36*** ±8 -90%	172▲▲ ±28 -52%
Day 5	339 ±36	288 ±26 -15%	170○○○ ±24 -50%	27○○○ ±4 -92%	128▲▲▲ ±13 -62%
RBCs					
Day 1	903 ±38	915 ±64	743* ±33 -18%	480*** ±10 -47%	640▲▲▲ ± 39 - 29%
Day 5	1032 ±39	1023 ±39	809*** ±38 -22%	560*** ±20 -46%	756▲▲▲ ±26 -27%
Brain					
Day 5	6651 ±228	6835 ±191	6271 ±134 -6%	4557*** ±293 -31%	5875▲ ±173 -12%

* p< 0.05; ***p< 0.001, Dunnett's Test
▲p<0.05, ▲▲p<0.01, ▲▲▲p<0.001, t-test
○○○ p< 0.001, Mann-Whitney U Test

TABLE 3. Cholinesterase Data for Female Rats (+ 1 hour after exposure) (mU/ml) Mean ± S.E.
N = 7-8

Dose (mg/kg) TEMIK 15G	0	100	500	2000	5 mg/kg Aldicarb
Plasma					
Day 1	1195 ±147	1238 ±112	1043 ±108 -13%	99*** ±22 -92%	426*** ±80 -64%
Day 5	1134 ±142	1152 ±100	826 ±111 -27%	70°°° ±8 -94%	114*** ±48 -90%
RBCs					
Day 1	1005 ±39	880 ±55 -12%	872* ±24 -13%	534*** ±21 -47%	716*** ±48 -29%
Day 5	992 ±36	943 ±47	805** ±30 -19%	503*** ±24 49%	544*** ± 34 -45%
Brain					
Day 5	6983 ±203	6979 ± 93	6726 ± 155 -4%	3387°°° ± 484 -51%	5145 ▲ ±552 -26%

* p< 0.05; ***p< 0.001, Dunnett's Test
▲p<0.05, ▲▲p<0.01, ▲▲▲p<0.001, t-test
°°° p< 0.001, Mann-Whitney U Test

III. **DISCUSSION**

This 5 day study was run by the registrant after EPA concluded that the 21 day dermal toxicity study was unacceptable and not upgradable. This study uses the same preparation as in the earlier 21 day dermal toxicity study, and thus is subject to the same concerns about the adequacy of this preparation previously described, including the small size of the surface area exposed, the limited wetting of the test area and test substance, and the somewhat limited nature of the contact of the granular material with the skin. Further, 5 days would not seem sufficiently long, since a 21 day study is the standard, and no compelling rationale for a shorter period was suggested or seems warranted. No time course data was provided for the positive control measures. Thus, there is no data to establish when the time of peak effect for dermal exposure might be. Brain measures were not made on day 1 as well as on day 5 for either Temik or aldicarb exposure.

The data from the Temik exposures in this 5 day study may be compared to the data from the earlier 21 day study (although it should be noted that in the main earlier study, elizabethan collars were used). This 5 day study used a much higher top dose (2000 mg/kg), which caused some clinical signs in one or two animals, and considerable ChEI in blood and brain. This study also seems to find an NOAEL of 100 mg/kg (notwithstanding the marginal inhibition of 12% in RBCs in females at 100 mg/kg). In contrast, the 21 day study found decreases in body weight gain late in the study at 100 mg/kg, although it also found no ChEI at 100 mg/kg.

There were a number of indications in this study that effects on day 5 were greater than on day 1, suggesting some cumulative effect of daily dermal exposure and that extending the dosing for another 2 weeks might have yielded a different NOAEL. In the 500 mg/kg group, inhibition on plasma and RBCs was greater on day 5 than on day 1 in males and females. In the positive control group, females showed greater effects on blood ChEI (both plasma and RBC) on day 5 than day 1, and males showed decreased body weight gain and body weights based on changes on days 4 and 5.

The positive control group exposed to 5 mg/kg of aldicarb technical in water provides a basis for attempting comparisons to other oral toxicity data on aldicarb or to other dermal toxicity studies. Both these comparisons are flawed.

Comparison to the granular data, ironically, is confounded by dissolving the technical aldicarb in de-ionized water. So the comparison then is from dry granules to technical aldicarb in liquid, which would likely underestimate the risk from the granules.

Comparisons to orally administered aldicarb in water are flawed by the fact that the study falls short of the guidelines as noted above. Thus, relative to chemicals with guideline dermal toxicity studies, or relative to oral toxicity studies, where the exent of exposure does not share these limitations, the dermal potency may appear relatively less.

Another concern for this study is that the statistical analyses seem to provide results that seem inconsistent with our general experience (less than expected significance found). In the body weight data, a 43% decrease in body weight gain in males comparing 7.1±1.3 g in the treated rats to 12.4 ±1.6 g in control rats was not tagged as statistically significant by a student t test. Whether it was adjusted for multiple comparisons in some way is not clear, but seems likely. In our experience with this widely used endpoint for determining the adequacy of dosing in chronic studies,

10% or greater reductions in body weight gain are typically found to be statistically significant and regarded as biologically significant as well. While in this experiment, this effect has been discounted because of its lack of dose dependence, it calls into question the statistical analysis used and the limited reporting of its apparently remarkable conservative nature.

In a second example, in females given 500 mg/kg, up to 27% plasma ChEI was not found to be statistically significant, despite standard errors on the order of 10% of the means for both controls and the dose group.

In conclusion, this 5 day dermal toxicity study provides some interesting data on dermal exposures to both Temik and technical grade aldicarb, but because of the unique aspects of the preparation, it is difficult to compare these results to guideline studies, or to reach any firm conclusions regarding the quantitative extent of dermal absorption. It does not provide sufficient data to remedy the deficiencies in the existing studies and database related to the potential dermal toxicity of aldicarb or Temik.

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Chemical:	**Aldicarb**
PC Code:	**098301**
HED File Code	**13000 Tox Reviews**
Memo Date:	**05/16/2000**
File ID:	**TX014169**
Accession Number:	**412-01-0121**

HED Records Reference Center
02/12/2001