MICROFICHE

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

013268

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

March 12, 1999

MEMORANDUM

SUBJECT: D249406 ALDICARB (098301):
Toxicology Review of 21 day Dermal Toxicity Study MRID 44636101

TO: Lisa Nisenson
Review Manager
Special Review Branch
Special Review and Reregistration Division (7508C)

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

THRU: William Burnam, Chief
Science Analysis Branch
Health Effects Division

Attached please find the HED review of this recently submitted 21 day dermal toxicity study of aldicarb. HED finds that this study, which attempted to evaluate the dermal toxicity of a granular product in a new protocol, is unacceptable and not upgradabable. This decision was reviewed and confirmed by the HIARC in a meeting on February 18, 1999, and is detailed in a separate memorandum.

EPA Reviewer: William F. Sette, Ph.D. William F. Sette 1-7-99
Science Analysis Branch (7509C)

013268

EPA Secondary Reviewer: Robert P. Zendzian, Ph.D. 1/7/99
Science Analysis Branch (7509C)

DATA EVALUATION RECORD

STUDY TYPE: 21 Day Dermal Toxicity Study OPPTS 870.3200

DP BARCODE: D249406
P.C.CODE: 098301

SUBMISSION CODE: S548540
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. Assessment of Cholinesterase Activity in CD® Rats Following Topical Application of Temik 15G® Grit for Three Weeks. Research Triangle Institute, Research Triangle Park, NC. RTI Report No. 65C-7202. August 14, 1998. MRID 44636101. Unpublished.

SPONSOR: Rhône-Poulenc Ag Company, Research Triangle Park, NC 27709

EXECUTIVE SUMMARY:

In this 21 day dermal toxicity study (MRID 44636101), Temik 15G® grit (14.75% aldicarb) was dermally applied to a 1" square area on the backs of 8 albino CD® Sprague-Dawley rats/sex/dose at levels of 0, 100, 250, or 500 mg/kg/day, for 6 hours/day, 5 days/week, for 3 weeks. In preliminary studies, Temik was applied once at 1000 mg/kg to 3 rats/sex for 6 hours (I), and to 5 male rats at 0, 250, 500, or 1000 mg/kg/day for 6 hours/day for 3 days (II). In II, a positive control group of 5 male rats were given a oral gavage dose of 0.1 mg/kg of aldicarb technical (99.5% a.i.).

There were no deaths or treatment related clinical signs noted in the study. In males, there were significant reductions in overall body weight gain (based on effects on days 15-19) at 100 mg/kg (21%) and 250 mg/kg (27%) but the decrease at 500 mg/kg group(11%) was not significant. There were correlative significant decreases in food consumption for days 15-19 in the 250 mg/kg males,(12.7 %, g/day) but not at 100 mg/kg. In II, plasma and red blood cell (RBC) cholinesterases were significantly decreased at 500 mg/kg (plasma 46-50%; RBCs 14-26%) and 1000 mg/kg (plasma 35-72%; RBC 31-40%). In the definitive study, statistically significant ChEI was seen only in the plasma of males at the mid-dose 250 mg/kg (18-25%). There were no significant effects on brain ChEI in II (9.9% at 1000 mg/kg) or the 21 day study (3.4% at 500 mg/kg).

The LOAEL is 100 mg/kg, based on reduced body weight gain.
The NOAEL < 100 mg/kg.

013268

The study should be regarded as unacceptable, not upgradable based on the inconsistent findings in body weights and ChE inhibition in the definitive study.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:

Description: light brown granules with a sulfurous odor
Lot/batch # 703058VE
Purity: 14.75%
CAS # 116-06-3

2. Postive control material: Aldicarb, analytical grade
Description: white crystalline solid, odorless to light sulfur smell
Lot/batch # 8KJ092
Purity: 99.5%

3. Test Animals: A total of 71 virgin male and 45 virgin female outbred albino CD® Sprague-Dawley rats, 8-10 weeks of age and weighing 200-250g, received from Charles River Laboratory, Raleigh, NC, were used for Preliminary Studies I and II, and the definitive study.

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: May 7 - June 19, 1998

2. Animal Assignment:

For Preliminary Study I (PS I), 3 male and 3 female rats were exposed to 1000 mg/kg of Temik grit for 6 hours.

For Preliminary Study II (PS II), 5 males/dose were exposed to 0, 250, 500, or 1000 mg/kg of Temik grit for 6 hours/day for 3 consecutive days. ChE determinations were made prior to exposure, immediately after the 6 hour exposure, and 1, 2, and 4 hours post-exposure. On day 3 of exposure, samples were taken only 1 hour after exposure, the time of peak effect.

A positive control group of 5 males was given a gavage dose of 0.1 mg/kg of aldicarb technical in deionized water. ChE measures for this group were taken 45 minutes after the dose, the oral time of peak effect.

For the definitive study rats were assigned to the test groups and the ChE measures made as described in Table 1.

TABLE 1: Definitive Study Design

Test Group/Doses	Males	Females	ChE + 1 hour	
0, 100, 250, 500 mg/kg/day	8	8	Days 1,5,8,12, 15,19	

3. Statistical Analyses

Study data included mortalities, clinical observations, body weights, food consumption, and cholinesterase activities.

For continuous data, including body weights and cholinesterase data, nonparametric tests were used to compare treated to control groups; the Kruskal-Wallis test to determine overall treatment effects, and the Mann-Whitney U Test for pairwise comparisons,i.e., Group 2 vs Group 3, if the K-W was found significant. Jonckheere's test was used to identify trends.

For the nominal scale data (mortality, observations), a Chi-Square test for Independence was used to identify differences among groups, and a 2 tailed Fisher's Exact Test was used for post hoc comparisons if the Chi-Square showed significant differences.

C. METHODS:

1. Preparation of Animal Skin: The backs of the rats were clipped and shaved within 24 hours prior to dosing. For Preliminary Studies I & II, rats were shaved once. For the 3.week study, rats were shaved on Sundays, Tuesdays, and Thursdays. 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")foam rubber dam measuring 2"x2" with a 1"x1" opening, 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 close 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 orally to aldicarb in Preliminary Study II were gavaged with 0.1 mg/kg, at a concentration of 0.05mg/ml in deionized water at a volume of 2 ml/kg. A 16 gauge, 2 inch dosing needle attached to a 1 cc syringe delivered the dose.

In the definitive study, rats were fitted with Elizabethan collars after the exposure (1 hour ± 5 mins) until the blood sampling to preclude oral exposure due to grooming of the exposed area.

4. Body Weights and Food Consumption: Rats were weighed once during quarantine, the day prior to exposure, each day of exposure, and at sacrifice. For PS I, rats were examined clinically hourly during exposure, as well as 1 and 2 hours after exposure to determine the time of peak effect. For PS II, rats were observed at 0, 3, and 6 hours during exposure on days 1-3; at 2 hours post-exposure on days 1 and 2, and 1 hour post-exposure on day 3. For the definitive study, observations were made 0, 3, and 6 hours during exposure and 1 hour after exposure.
Food consumption was recorded 2x weekly during the definitive study for days: 1-5; 5-8;8-12;12-15; and 15-19.

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

In PS II, blood samples were collected 5 times during day 1: before exposure, immediately after exposure, and 1,2, and 4 hours post-exposure. On day 3, blood was collected 1 hour after exposure, the time of peak effect. For orally dosed rats, blood samples were taken 45 minutes after the single dose.

In the definitive study, blood samples were collected at the end of exposures on days 1 and 5 of each of the 3 exposure weeks, at 1 hour post-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 rats for PS II and for the definitive study after euthanasia by carbon dioxide.

II. RESULTS

A. Mortality and Observations

PS I. A single 6 hour dermal exposure to 1000 mg/kg produced no deaths or clinical signs in the 3 rats/sex exposed.

PS II. For the 5 male rats exposed to 0, 250, 500, or 1,000 mg/kg for 3 days, there were no deaths or clinical signs at any dose. Positive control males dosed orally at 0.1 mg/kg showed no deaths or clinical signs.

Definitive Study. There were no clinical observations in the males and no treatment related clinical observations in females. There were no deaths.

B. Body Weights, Food Consumption, and Gross Necropsies

In PS II, for the 5 male rats exposed to 0, 250, 500, or 1,000 mg/kg for 3 days, there were no effects on body weight or brain weight. Positive control males dosed orally at 0.1 mg/kg showed no changes in body weight or brain weight. There were no necropsy findings for either group.

Body weight gain and body weights for the 21 day study are shown in Table 2. In males, there were reductions in overall body weight gain attributable to decreases seen on days 15-19. For days 1-19 overall at 100 and 250 mg/kg males decreases were statistically significant at - 21 to -27 %, but not significant in the 500 mg/kg group(-11%). Body weight differences for these groups were -8%, -10%*, and -5% for the 100, 250, and 500 mg/kg groups, respectively, with statistical significance only at the 250 mg/kg dose. Brain weights were unchanged. There were significant decreases in food consumption for days 15-19 in the 250 mg/kg males, expressed either in g/day, (-12.7 %) or relativized to body weight, g/kg/day (- 4.3%) but no significant changes in higher or lower exposure groups.

In female rats, although there were similar decreases in body weight gains in the 100 mg/kg (22%) and 250 mg/kg group (29%), and less decrease at 500 mg/kg (16%), none reached statistical significance. There were also no significant changes in body weight, body weight gain, brain weight, or food consumption among the females.

Table 2. Body weight gain and Terminal Body Weights (g) for Definitive Study
Mean ± S.E. * p<0.05

	0	100 mg/kg	250 mg/kg	500 mg/kg
Males				
Body wt gain d 1-19	107.77±6.42	85.52*±5.38 (-21%)	78.19*±6.29 (-27%)	95.55±9.32 (-11%)
Body wt (day 19)	365.11±9.23	334.94±4.62 (-8%)	327.93*±6.07 (-10%)	349.54±9.54 (-5%)
Females				
Body wt gain d 1-19	34.52 ± 3.26	26.8±4.99 (-22%)	24.5±3.01 (-29%)	29.16±4.92 (-16%)
Body wt (day 19)	258.33±5.72	249.81±5.1 (-3.3%)	244.83±5.67 (-5.2%)	252.96±4.93 (-2%)

C. Cholinesterase Measurements

The ChE findings for PS II are shown in Table 3. After exposure to 500 and 1000 mg/kg, plasma and red blood cell (RBC) cholinesterases were significantly decreased on day 1 both at the end of the 6 hour exposure period and one hour after the exposure period. At 2 and 4 hours after exposure, changes were minimal. At both 500 mg/kg and 1000 mg/kg on day 3 one hour after dosing, both plasma and red blood cell (RBC) cholinesterases were also significantly decreased. Day 3 changes were somewhat larger than day 1 effects at 1000 mg/kg. Brain inhibition at 1000 mg/kg was 9.9%, but this failed to achieve statistical significance. While at 250 mg/kg, plasma inhibition was roughly 20% and RBC inhibition was about 10%, neither was statistically signficant. Overall, for this study, there was reasonable dose dependence in all 3 measures.

Positive control rats orally dosed at 0.1 mg/kg, and sampled 45 minutes after dosing, showed plasma inhibition of 86%, RBC inhibition of 38%, and brain inhibition of 16%, all considered significant.

The ChE results for the males and female rats in the definitive study are shown in Tables 4 and 5, respectively. In males, statistically significant effects were seen only in plasma in the mid-dose 250 mg/kg rats on days 1, 5, 8, 12, and 19. At 500 mg/kg, plasma inhibition ranged from 0.3%-30.5%, but in no case was this statistically significant. Except for day 15,(ChEI 30.5%), the level of inhibition never exceeded 15%. RBC inhibition ranged from +4% to -5% at 250 mg/kg, and from -3% to -11% at 500 mg/kg and never attained statistical significance. There were also no significant effects on brain ChEI at any dose (2.8%, 3.2%, and 3.4% at 100, 250 and 500 mg/kg).

In females (Table 5), there were no consistent effects on blood or brain ChE measures. At 500 mg/kg, plasma inhibition ranged from 11-33%, but was never statistically significant. RBC ChEI ranged from 3-15.5%, and only the 15.5% measure, from day 8, was statistically significant. Anomalously, on day 12, RBCs from the 250 mg/kg dose, were significantly *increased (21%)*. Other ChE changes seen at 250 mg/kg were less than the changes at 500 mg/kg.

TABLE 3. Preliminary Study II: Cholinesterase Measurements (mU/mL) Mean ± S.E. *p<0.05

Dose (mg/kg)	0	250	500	1000
Day 1				
Plasma 0 hour (end of 6 hr exposure)	332 ± 30	267 -19.5% ± 40	178* -46.4% ± 31	217* -34.7% ± 28
+1 hour	323 ± 32	249 -23% ± 44	175* -46% ± 31	152* -53% ± 42
RBCs 0	3890 ± 181	3154 -19% ± 311	2964* -23.8% ± 257	2656* -31.7% ± 116
+1 hour	3510 ± 103	3083 -12% ± 202	3012 -14% ± 272	2419* -31% ± 261
Day 3 (+1 hour)				
Plasma	300 ± 27	252 -16% ± 45	151* -49.7% ± 28	83* -72.3% ± 23
RBC	3890 ± 87	3439 -11.6% ± 240	2893* -25.6% ± 186	2348* -39.6% ± 235
Brain	6594 ± 152	6575 -0.29% ± 223	6525 -1% ± 284	5941 - 9.9% ±321

TABLE 4. Definitive Study Cholinesterase Data for Male Rats (+ 1 hour after exposure) (mU/mL) Mean ± S.E. * p< 0.05; **p< 0.01

Dose (mg/kg)	0	100	250	500
Plasma				
Day 1	332 ±14	321 ±14	272** -18.1% ±11	292 -12% ±28
Day 5	333 ±15	300 ±12	252** -24.3% ±8	332 - 0.3% ±25
Day 8	350 ±21	354 ±16	293* -16.3% ±12	354 + 1.1% ±22
Day 12	337 ±16	341 ±13	269* -20 % ±14	287 -14.8% ±28
Day 15	371 ±13	347 - 6.5% ±13	302 -18.6% ±13	258 -30.5% ±27
Day 19	363 ±17	332 - 8.5% ±13	274** -24.5% ±11	317 -12.7% ±21
RBCs				
Day 1	3252 ±126	2979 - 8.4% ±119	3246 -0.2% ±191	2947 -9.4% ±145
Day 5	3246 ±110	3068 - 5.5% ±69	3388 +4.4% ±167	2965 -8.7% ±125
Day 8	3188 ±226	3157 -1.0% ±67	3276 +2.8% ±107	3083 -3.3% ±63
Day 12	3269 ±167	3365 +2.9% ±103	3113 -4.8% ±83	2905 -11.1% ±107
Day 15	3608 ±96	3498 -3% ±95	3558 -1.4% ±78	3231 -10.45% ±169
Day 19	3142 ±154	3157 +0.5% ±95	3068 -2.4% ±72	2935 -6.6% ±126
Brain				
Day 19	7126 ± 122	6929 -2.8% ± 67	6899 -3.2% ± 134	6883 -3.4% ± 140

TABLE 5. Definitive Study Cholinesterase Data for Female Rats (+1 hour after exposure) (mU/mL) Mean ± S.E. *p< 0.05.

Dose (mg/kg)	0	100	250	500
Plasma				
Day 1	962 ±87	1057 ±128	901 -6% ±68	850 -12% ±104
Day 5	921 ±73	978 ±117	882 -4% ±116	817 -11 % ±98
Day 8	1167 ±73	1234 ±144	991 -15% ±100	780 -33 % ±139
Day 12	1064 ±63	1188 ±158	1034 ±100	902 -15% ±164
Day 15	1234 ±113	1376 ±140	1075 -13% ±110	1095 -11% ±103
Day 19	1140 ±72	1238 ±139	1008 -12% ±111	897 -21% ±182
RBCs				
Day 1	3231 ±152	3015 ±155	3409 + 5% ±83	3053 - 5% ±86
Day 5	3172 ±176	2935 ±126	3009 ±74	2861 -10% ±108
Day 8	3246 ±59	3098 ±233	3291 ±89	2742* -15.5% ±132
Day 12	2979 ±202	3276 +10% ±105	3617* +21% ±136	2890 -3% ±186
Day 15	3646 ±161	3691 ±106	3572 -2% ±117	3483 -4% ±67
Day 19	3276 ±116	3261 ±107	3127 -5% ±103	2816 -14% ±176
Brain				
Day 19	7047 ± 70	7085 ±134	7134 ± 77	6679 -5% ± 136

III. DISCUSSION

In the 21 day study, both sexes of rats showed 20-30% reductions in body weight gain at the 100 mg/kg and 250 mg/kg that were attenuated at 500 mg/kg (11-16%). This lack of dose dependent body weight changes at the high dose, and its consistency between males and females is puzzling. Overall food consumption showed a similar pattern, with the high dose group similar to controls. This suggests that in the 21 day study, exposure was not increased at 500 mg/kg.

The overall NOAEL for ChEI in the definitive study is 100 mg/kg, based on significant plasma inhibition in male rats at 250 mg/kg. But ChEI effects in the 21 day study at the 500 mg/kg dose were also less than in the lower dose groups. Table 6 shows a comparison between the PS II and the definitive study. The level of effects seen were generally comparable in males and females. RBC effects at both doses were much less in the definitive study. At 500 mg/kg, plasma effects were much less in the main study, while at 250 mg/kg, plasma effects were very similar between studies. Since the body weight and ChE effects are similar in pattern, this suggests that exposure at 500 mg/kg was not greater than at 250 mg/kg in this later study. The lack of clear cut effects on RBCs at either dose in the definitive study suggests difficulty with the assay.

TABLE 6. Comparisons of preliminary study to definitive study ChE results

		PS II (males)	Definitive (males)	Definitive (Females)
Plasma	250 mg/kg	16-23%	18-25%	3-15%
	500 mg/kg	46-50%	0.3-30.5%	11-33%
RBC	250 mg/kg	12-19%	4+4.8%	5 +21%
	500 mg/kg	14-26%	3-11%	3-16%

Table 7 shows a comparison of the oral positive controls in PS II in comparison to data from other acute rat studies, one from the acute neurotoxicity study (81-8), and the other from an EPA study. The oral positive control data on ChEI looks generally comparable to the data from an earlier acute rat neurotoxicity study by the registrant and with data by an EPA investigator. This would support the performance of the assay in the laboratory. The oral control group, however, was part of the PS II study, and not the subsequent definitive study. So assay problems as the source of the inconsistencies in the definitive study cannot be ruled out.

TABLE 7. Comparison of results in male rats in 3 acute oral exposure studies.

	plasma ChEI	RBC	Brain	clinical signs
0.1 mg/kg (pos cons)	86%	38%	17%	None
0.1 mg/kg (81-8)	86%	47%	10%	none in males[1]
0.1 mg/kg (Moser et al.)	85% (whole blood)		12%	males; 30% dec in MA;

One difference between the preliminary and main studies was the use of the Elizabethan collar in the definitive study for the time between the end of the exposure period and the blood sampling, though it seems unlikely to this reviewer that the exposure regimen described here would leave much material (given granular pellets and washing at the end of exposure) or opportunity (a small area on the back) for such exposure. Also, the plasma 250 mg/kg effects were similar between the studies.

Another potential set of difficulties in this new dermal procedure is limited moisture, lack of effective skin contact for the full dose, or other reasons that may have contributed to the disparity in findings in the definitive study. Additional commments concerning the application device that may have contributed to the inconsistency in the results were provided by Dr. Zendzian, and are appended to this DER. On the other hand, in the preliminary study, there was reasonable dose response progression between 250 and 1000 mg/kg.

So, while neither potential difficulty seems to be completely account for the problems, the inconsistency in the results is a problem that undercuts the validity of the study and the findings at all of the doses. Because this is a novel procedure, it seems reasonable to ask for further investigation to ascertain what the source of the inconsistent results may be.

A number of possible modifications could help to address these issues. One would be to employ the radiometric assay, for which EPA in North Carolina can provide guidance.

---

[1] Moser found a 30% decrease in motor activity, but no other clinical signs. The acute neurotoxicity study did not find changes in MA at 0.1 mg/kg.)

Second, consider greater moistening of the granules. In older acute dermal toxicity studies, dry granules of aldicarb(10GV) were reported as having an LD50 of 4.58 g/kg (458 mg/kg of active ingredient), when applied to the clipped skins of rats (MRID 00060195). Wetted granules gave a 4 hour LD50 of 44 mg/kg of active ingredient (MRID 00101942). Temik 10G was noted as giving similar findings. In either case, here up to 1000 mg/kg yielded no lethality with a slightly wet preparation and what is a newer formulation. This is much less effect than would be predicted. A better accounting of the mass balance of the granules might also provide useful information

Use of a dermal positive control group or groups could also be useful by reflecting the kinetics and dynamics of this exposure route. In a preliminary dermal toxicity study reported by the registrant, as little as 2.5 mg/kg of aldicarb technical applied dermally in water caused considerable cholinesterase inhibition. Only the following details were reported: "The dose levels in the study were 0, 2.5, 5.0, and 10.0 mg/kg, applied as a single dermal dose in water. The mean RBC inhibition was 42%, 46%, and 41% respectively for each dose compared to control and the mean plasma inhibition was 91%, 94%, and 94% respectively." Our current data requirements call for 21 day dermal toxicity studies using both the TGAI and the EP, which the granular represents. While less than a full study of the active ingredient may be needed, a dermal control group could help focus on the source of differences seen here.

Because this is a preparation that may prove useful for a variety of pesticides, we hope that these procedures can be worked out. But at present, the data do not provide a consistent coherent data set and consequently must be classified as unacceptable.

Attachment 1. Comments from R.P. Zendzian on the dermal application procedure.
21-Day Dermal toxicity Study with Temik 15G Grit (14.75% aldicarb) 013268
MRID446361-01

Comments on the application device

Robert P. Zendzian PhD

Examination of the results of the study and the characteristics of the 'application device' utilized leads to the conclusion that the device can result in uneven and unpredictable distribution of the dosing material over the exposed area of the skin. This distribution can result in unpredictable and irreproducable absorption of the test chemical and subsequent toxic effects. It is suggested that further testing of the device is necessary before it can be accepted for dosing.

For this study an 'application device' was utilized. The device was described as "The weighed Temik grit dose, applied dry in the granulated formulation, was poured into a self-adhesive, foam rubber dam (7/16" deep), measuring 2" x 2" in size with a 1" x 1" opening (which allowed the test compound to lie as a single layer), covered by filter paper (figure 2). the rat was rolled onto the self-adhesive foam rubber dam (Figure 3). the foam rubber dam was secured to the animal with adhesive wrap, Vetwrap and adhesive tape over the Vetwrap (Figures 4 and 5). When the Vetwrap was applied over the foam rubber dam, the latter was compress such that the test material was confined in an area in close approximation to the skin (figures 4 and 5)."

This device is a takeoff from a device used to protect the application site in dermal absorption studies performed by the same laboratory. In a dermal absorption study the rubber dam, with a cutout opening, is applied directly to the shaven and washed back of the rat. The dose of pesticide, dissolved or suspended in water, is applied to the area of the back within the cutout and spread evenly. The dose is dried and the test material remains spread evenly over the exposure area. Gauze is then glued on top of the rubber dam to protect the application site and avoid loss of test material by falling or being scraped off. Applied in this manner the dose remains evenly spread and in close contact with the skin.

In contrast, figure 2 shows the Temik test material to be loosely and unevenly spread in the application device so that one may expect it to be more or less scattered over the application site. Dermal absorption of the test material is a product of dose, skin contact area and duration of exposure. With the device utilized skin contact area can vary in an unpredictable manner producing significant variation in the dose absorbed and subsequent toxic effects. Also, the device exposes one square inch of skin while dermal toxicity studies, acute or repeated dose, expose an area equal to 10% of the skin surface.

I suggest a screening experiment of the application device using Temik . Five rats of one sex to be dosed at 1000 mg/kg for six hours with the device and five rats of the same sex to be dosed at 1000 mg/kg for six hours with the test material held directly in contact with the skin by a bandage. The surface area exposed must be the same for each group. Animals must be observed for toxic effects and blood for cholinesterase determination taken before dosing and one hour after dosing.