

4-25-96

Reviewed by: William F. Sette, Ph.D. *William F. Sette*
Peer-Review Section
Science Analysis Branch
Health Effects Division (7509C)

Secondary reviewer: Robert F. Fricke, Ph.D. *Robert F. Fricke 25 Apr 96*
Toxicology Branch II
Health Effects Division (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Neurotoxicity Study in Rats 81-8

TOX. CHEM NO: 011A

PC Code 098301

DP Barcode D209417

Submission No. S477129

MRID NO. 43442301

TEST MATERIAL: Aldicarb

SYNONYMS: Temik, 2-methyl-2-(methylthio) propionaldehyde O-(methylcarbamoyl) oxime.

STUDY NUMBER: 97235

SPONSOR: Rhone-Poulenc

TESTING FACILITY: Bio-Research Labs, Montreal, Canada

TITLE OF REPORT: An Acute Study of the Potential Effects of orally administered Aldicarb, technical grade, on behavior and neuro-morphology in rats.

AUTHOR(S): Robinson K, Brooks W, Broxup B

REPORT ISSUED: 9/28/94

CONCLUSIONS: In an acute neurotoxicity study (MRID 43442301), groups of 22 Sprague-Dawley rats were given acute oral doses by gavage of 0, 0.05, 0.1, or 0.5 mg/kg of Aldicarb. 12 rats/sex/dose were examined by a Functional Observational Battery (FOB) and a Motor Activity test (MA) prior to the study; at the time of peak effect, 0.5 hours after dosing for the FOB and 1 hour after dosing for MA; and on post-dosing days 7 and 14. Blood (plasma, red blood cell, whole blood) and whole brain cholinesterase (ChE) determinations were made on 5 rats/sex/dose at the estimated time of peak effect, 0.75 hours, and 8 hours after dosing. On day 15, 6 rats/sex/dose were perfused and the high dose and control groups subjected to neuropathological examination.

No deaths or significant effects on body weight or food consumption were noted.

0.5 hours after 0.5 mg/kg, a variety of clinical signs of ChEI were seen in the FOB, including: tremors, lacrimation, salivation, decreased body temperature, increased respiration, decreased arousal, activity, and reactivity, and decreased fore and hind limb grip strength. Automated motor activity was also significantly decreased 1 hour after dosing in this group (74-82%). At 0.1 mg/kg, only fore limb grip strength in females was significantly

decreased (17%, $p < 0.05$). No significant behavioral effects were seen on days 7 and 14. The NOEL for behavior was 0.05 mg/kg.

Toxicologically significant effects on blood ChEs were seen in all dose groups 0.75 hours after dosing, but there was recovery by 8 hours after dosing.

0.75 hours after 0.5 mg/kg, whole brain ChE (45% M, 50% F) and all 3 blood measures of ChE were significantly decreased (whole blood, 65-76%; plasma, 92-94%; RBCs, 51-54%; M, Fs respectively).

0.75 hours after 0.1 mg/kg, whole blood (61% M, 54% F), plasma ChEs (86% M, 73% F) and RBCs (47% M, 31% F) were decreased in both sexes, though statistical significance was inconsistent. No significant effects on brain ChE were seen after 0.1 mg/kg (10% M, 16% F), or at 0.05 mg/kg.

In the 0.05 mg/kg group after 0.75 hours, there were smaller changes in RBCs (5% M, 8% F), whole blood (15% M, 29% F), and plasma (33% M, 47% F). While none of these were statistically significant by the limited method used, the relatively large differences in plasma ChEI were even more convincing in comparison to their own pre-exposure measures and concluded to be toxicologically significant.

No neuropathological changes were seen microscopically or grossly. Ophthalmoscopic examinations were also negative. While, in females given 0.5 mg/kg whose nervous systems were perfused, brain weights were statistically significantly decreased, brain weights in other females were unaffected, and this was not considered toxicologically significant.

For this study, then, the NOEL is < 0.05 mg/kg for ChEI measures, with toxicologically significant plasma ChE inhibition at 0.05 mg/kg; with significant ChE inhibition in blood and some behavioral changes at 0.1 mg/kg, and marked changes in behavior and decreases in ChEs in blood and brain, at 0.5 mg/kg.

LOEL = 0.05 mg/kg for plasma ChEI

NOEL < 0.05 mg/kg

The study is classified as acceptable and satisfies the requirement for an acute neurotoxicity study according to Guideline 81-8.

A. MATERIALS:

1. Test compound: Aldicarb technical, Lot No. 25DEQ89, 99% purity was stored at 4 degrees C in the dark. Dosing solutions were prepared by serial dilutions in double distilled and deionized water prior to dosing and refrigerated at 4 degrees C until use. Dosage volume was 2 ml/kg. Stability of solutions were analyzed and found stable, i.e., at $> 90\%$, for all dose solutions.

2. Test Subjects 88 male and 88 female Sprague-Dawley, Cr1:CD(SD)BR rats from 48-53 days of age on day of treatment, with males weighing 235-294 g and females, 172-202g, served as subjects.

Subjects were individually housed in rooms with 12 hour light/dark cycles, temperatures of 22 +/-3 degrees C, and 30-70% humidity. They received food and water ad libitum.

B. STUDY DESIGN: Subjects were randomly assigned to groups, balancing assignment with respect to body weights between groups. There were 4 groups, who received 0, 0.05, 0.1 or 0.5 mg/kg. Groups consisted of 3 replicates of 4 rats/sex/dose for the main study.

Ten rats/sex/group underwent ChE determinations. For the ChE rats, 5 rats/sex/dose had samples taken at pre-study and approximately 0.75 hours after dosing, while 5 rats/sex/dose had samples taken pre-study and 8 hours after dosing.

For the behavior/pathology groups, there were 12 rats/sex/dose subjected to behavioral assessment, and 6 /sex/dose perfused and prepared histologically for neuropathological examination.

C. METHODS AND RESULTS:

There were daily clinical exams, body weights taken prior to dosing, weekly along with observations, and prior to sacrifice. Food consumption was measured weekly.

A Functional Observational Battery was administered prior to treatment, 0.5 hours after dosing, and on days 7 and 14 after dosing, by technicians unaware of the treatment group of each subject. This battery, which conformed to EPA's guideline, consisted of observations of body position and general state in the home cage, ease of removal and reaction to removal, observations in a standard arena of clinical signs, motor behavior, and autonomic signs; handling observations including extensor thrust, body tone, corneal, visual placing, pinna, and toe and tail pinch reflexes; auditory startle and air righting reflex; body temperature; hind limb splay by an inked foot method (2x), and fore and hind limb grip strength (mean of 2 measures).

Following the FOB, each animal was placed in a San Diego Instruments motor activity chamber (figure 8) for one hour, commencing roughly 1 hour after exposure.

For ChE measures, pre-exposure blood measurements were made via a tail vein. On the day of dosing, measures made either at 0.75 hours or 8 hours after dosing were made from the abdominal aorta under ether anesthesia. Subjects were then exsanguinated and brains removed, weighed, and assayed for ChE.

All analyses were made by the Ellman method modified for a Hitachi 717 analyzer. Measures of whole blood and plasma were made. Red blood cell measures were calculated. Whole brain ChE was also measured.

Six rats/sex/dose were anesthetized and perfused with Ringer's solution, followed by 3% glutaraldehyde/paraformaldehyde solution, and placed in 10% buffered formalin (carcass, brain, spinal cord, limbs, minus skin, thoracic and abdominal organs). Tissues from high dose and control animals were processed for neuropathological examination. Tissues from other groups were kept in formalin. Gross

examinations of neural tissue from all groups were made.

CNS sections were embedded in paraffin, cut into 6 micron sections, and adjacent sections stained with H&E, Kluver-Barrera, Holmes and PTAH stains and examined by light microscopy. Brain weight, length, and width were recorded. CNS sections included: 6 brain sections, cervical, thoracic, and lumbar spinal cord cross sections; cross and longitudinal section of skeletal muscle (H&E stain); a cross section of basal tail; and any grossly abnormal tissues.

Peripheral nerve sections (sciatic, 3 cross, 1 longitudinal; sural, 1 cross; and tibial, 1 longitudinal and cross sections) and CNS ganglia from ventral and dorsal cervical and lumbar roots (and Gasserian ganglion) were embedded in plastic, sectioned at 0.5 microns, and stained with toluidine blue.

Ophthalmoscopic examinations were performed on all animals prior to exposure and between days 12-14 after exposure by indirect ophthalmoscopy (fundoscopic) and, after mydriatic, by slit lamp (biomicroscopic).

If homogeneity of variances were found, most data were analyzed by one way analyses of variance, with Dunnett's test for significant comparisons. Otherwise, a Kruskal-Wallis test followed by Dunn's or Wilcoxin's was used. For qualitative FOB data, Fisher's exact test was used. A repeated measures ANOVA was used for motor activity data for total counts, and for a linear constructed variable, which evaluated the rate of linear change, i.e., pattern of activity within a session. An analysis of covariance using the pre-exposure data as the covariant was performed with t tests for post-hoc comparisons.

A quality assurance statement, documenting the QA program used for the study, was provided.

RESULTS

There were no deaths or sacrifice of morbid animals, nor any significant incidence of signs seen during clinical evaluations (given twice daily and from "a more detailed examination" on days 1, 8, and 15 after dosing. There were no meaningful effects of treatment on body weights or food consumption.

Functional Observational Battery (FOB)

Significant effects seen in rats receiving 0.5 mg/kg at 0.5 hours after the dose and their incidence are summarized in Table 1.

Table 1. Functional Observational Battery Data: Significant Effects for 0.5 mg/kg rats (#affected/#tested)

HOME CAGE	MALES	FEMALES
lying down	4/12	7/12
coarse tremors (slight)	6/12	1/12
coarse tremors (moderate)	6/12	11/12
slight ataxia	6/12	7/12
ARENA		
gait changes (slight)	5/12	8/12
coarse tremors (slight)	5/12*	1/12*
coarse tremors (moderate)	6/12	10/12
increased respiration	9/12	6/12
reduced activity	11/12	9/12
decreased arousal	10/12	9/12
increased grooming	8/12	6/12
increased lacrimation	7/12	5/12
eye deposits; chromo-dachrorhea	6/12	
salivation (moderate)	8/12	10/12
decreased tailpinch	7/12	6/12
decreased rearing (% diff from controls)	- 80%	-90%**
decreased body temperature degrees C vs. controls	36.3 vs 38.1	35.4 vs 38.3

* the other rat in each of these groups had slight tremors described as fine.

** In females, while statistically significantly decreased rearing was seen in all dose groups, the study authors discounted this effect based on a high control group mean in comparison to pre-exposure values, which do not reveal big differences. The data in ascending dose group order are:

Rearing after dosing: 17.83, 13.17, 12.92, 1.75 (SDs 2-5);

Pre-exposure: 12.92, 11.42, 10.42, 9.83 (SDs 3-5).

The day of dosing control group mean is much higher than any other data point and the pre-exposure comparison demonstrate an effect only at the highest dose on this measure.

Significantly decreased forelimb and hind limb grip strength

were seen in both sexes after 0.5 mg/kg. For females, at 0.1 mg/kg, forelimb grip strength was statistically significantly reduced (17%); a 9% decrease in hind limb strength was not statistically significant, though it suggests that this was a general rather than a specific effect.

MEAN GRIP STRENGTH in g (S.D.)

DOSE (mg/kg)	MALES		FEMALES	
	FORELIMB	HINDLIMB	FORELIMB	HINDLIMB
0	1293	705	1187	670
s.d.	136	149	129	109
0.05	1250	702	1080	648
s.d.	136	109	180	98
0.1	1135	675	987* (-17%)	608 (-9%)
s.d.	200	171	184	84
0.5	806* (-38%)	368* (-48%)	841** (-29%)	522** (-22%)
s.d.	198	80	138	75

* p<0.05, ** p<0.01; % difference from controls

Tables 31-2 show the group mean motor activity counts for each interval for the session on the day of dosing. There were significant overall decreases in motor activity for both sexes, 74% for males and 82% for females, at 0.5 mg/kg, with no significant differences at lower doses, or on days 7 and 14.

Cholinesterase Measurements

Tables 37-40 from the study report show the blood ChE data for males and females prior to dosing and 0.75 and 8 hours after dosing.

In high dose males (Table 37) prior to exposure there was a statistically significant difference (22%) in plasma ChE levels in comparison to controls.

At 0.75 hours after dosing, in males, all 3 blood measures showed dose-related decreases. By the study statistical analyses, all 3 measures at 0.5 mg/kg were significantly decreased, but at 0.1 mg/kg, only the RBCs and the whole blood measures were significantly decreased, despite an 86% mean decrease in plasma ChE!

In females, at 0.75 hours after dosing, all 3 blood measures were also significantly decreased at 0.5 mg/kg, while by the study

statistics, at 0.1 mg/kg only the whole blood measures were significantly affected, despite mean decreases of 30.6% in RBCs and 73% in plasma!

No measures were reported as statistically significant at 0.05 mg/kg for either sex. At 0.05 mg/kg, the mean decreases relative to vehicle controls were:

Males: 5%, RBCs; 15%, whole blood; 33%, plasma.

Females: 8.5%, RBCs, 29%, whole blood; 47%, plasma.

For individual rats in the 0.05 mg/kg dose group, % differences from their pre-exposure values for plasma were:

Males: 14%, 59%, 63%, 79%, 15% (46%+/-30%);

Females: 65%, +8%, 52%, 76%, 28% (43%+/-33%).

Eight hours after exposure, in males, no significant differences in blood ChEs were seen, with the largest mean difference seen in plasma, which was decreased 20%, in the 0.5 mg/kg group. Similarly, in females 8 hours after exposure, no significant differences for females were reported, and the largest mean decrease noted among the 3 blood measures was a 17% decrease in plasma in the 0.5 mg/kg group.

Brain ChEs were decreased 0.75 hours after exposure to 0.5 mg/kg, by 45% in males and 50% in females. After 0.1 mg/kg, mean decreases were 15.6% for females and 9.6% for males. Only at 0.5 mg/kg were these effects reported as statistically significant. 8 hours after dosing, no significant effects were seen and the biggest mean difference from controls was a roughly 10% decrease in 0.5 mg/kg males.

Ophthalmoscopic Examinations

No treatment related effects were seen.

Histopathology

No gross pathology was found in any group that was related to treatment. No histopathological effects were found in high dose animals, so lower dose animals were not examined at the light microscope level.

A statistically significant decrease in the brain weights of female rats at 0.5 mg/kg was noted among the groups perfused (1.70g vs 1.52g, control vs high dose). However, no differences were seen in brain weights of female rats on whom brain ChE measurements were made, (1.707g vs 1.754g; 1.717g vs 1.708 g, controls vs high dose, 0.75 and 8 hours, respectively), so this effect does not seem to be reliable or consistent.

D. DISCUSSION

This was a generally well conducted study. A number of effects seen merit some further comment. The only significant behavioral effect seen at 0.1 mg/kg was a 17% decrease in forelimb grip

strength in females. Hind limb grip strength was decreased 9% and was not statistically significant. In males, smaller changes were seen at this dose level in these measures. At 0.5 mg/kg, larger decreases in grip strength were seen, along with a host of changes consistent with ChE inhibition. While this is an isolated effect, it is statistically significant, dose dependent, consistent with ChE inhibition, and so there is no real reason not to consider it toxicologically significant.

The interpretation of cholinesterase data is complicated by a disparity between the amount of inhibition seen and the lack of their apparent statistical significance. This could be due to limitations in the statistical methods, the variability in the method of analysis, or the number of subjects used. For a study such as this, with pre-exposure measurement and repeated measures in 2 sexes, the most powerful analysis would have been one which incorporated all of those variables, i.e., some kind of repeated measures analysis of covariance.

At 0.1 mg/kg, an 86% decrease in mean plasma ChE was not statistically significant, and in females, mean decreases of 30.6% in RBCs and 73% in plasma were similarly not found to be statistically significant. At 0.05 mg/kg, no measures were reported as statistically significant for either sex, where the mean decreases relative to vehicle controls were:

Males: 5%, RBCs; 15%, whole blood; 33%, plasma.

Females: 8.5%, RBCs, 29%, whole blood; 47%, plasma.

For individual rats in the 0.05 mg/kg dose group, % differences from their pre-exposure values for plasma were:

Males: 14%, 59%, 63%, 79%, 15% (46%+/-30%);

Females: 65%, +8%, 52%, 76%, 28% (43%+/-33%).

Given: 1) this general within subject set of results, i.e., greater than 50% decreases in 3/5 rats of both sexes relative to their own pre-exposure levels;

2) the fact that all the measures generally recovered after 8 hours;

3) that, (absent a plasma difference of 22% in pre-exposed males), no pre-exposure differences were noted to suggest serious or systematic methodological problems, see e.g., variability in pre-exposure or 8 hour data;

It is hard to see how such large differences should not be regarded as toxicologically significant.