Image 1

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

014448

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

December 21, 2000

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

Subject: 032501: 21-day dermal toxicity study in rabbits with disulfoton (MRID# 45239601).

DP Barcode: D270001
Submission: S587194

From: David G Anderson David G Anderson 12/4/2000
RRB-2 HED (7509C)

To: Christina Scheltema
SRRD (7508)

Thru: Alan Nielsen, BSS Alan Nielsen 1/18/2001
RRB-2, HED (7509C)

The registrant submitted data on a 21-day dermal toxicity study in rabbits. The DER for the study is attached. The reference and Executive Summary is below.

CITATION: Flucke, W (1988) S 276 Technical grade Disulfoton: Study of the Subacute Dermal Toxicity to Rabbits. Bayer AG., Germany. Study Number 98347. Report No. 116342, January 5, 1988. MRID 45239601. Unpublished.

SPONSOR: Bayer Corporation, Stillwell, KS.

EXECUTIVE SUMMARY: In a 21-day dermal toxicity study in rabbits (MRID 45239601), disulfoton (97% a.i.%) was administered dermally to New Zealand White (HC:NZW) rabbits (5/sex/dose) at dose levels of 0, 0.8, 1.0 or 3.0 mg/kg/day for 21-days. Plasma, erythrocyte cholinesterase was determined day -2, 8, 15 and 21. Brain cholinesterase was determined at termination on day 21. Plasma and erythrocyte cholinesterase were compared with day -2 values while brain cholinesterase was compared with concurrent control values. Clinical observations, chemistry and histological examination of tissues were conducted.

Body weight was slightly decreased and statistically significant (-3% compared with controls) during the last 2 weeks of the study at 3.0 mg/kg/day in females. Clinical signs consistent with cholinergic signs occurred in males at the end of the study. Muscle spasm, tremors, diarreha, and/or difficulty in breathing in 4 aminals and one male death occurred at 3.0 mg/kg/day toward the end of the study. One female was lathargic and had difficulty breathing on the last day of the study at 3.0 mg/kg/day. No differences attributed to treatment were noted in organ weights or clinical chemistries other than cholinesterase activity.

Plasma cholinesterase was statistically significantly inhibited in males at 1.0 and 3.0 mg/kg/day at day 15 (22%) and 21 (24%) and at day 8 (63%), 15 (70%) and 21 (65%),

respectively. In females, plasma cholinesterase was statistically significantly inhibited only at 3.0 mg/kg/day and only on day 15 (61%) and 21 (61%), but it was 44% inhibited on day 8 (not statistically significant). Erythrocyte cholinesterase was statistically significantly inhibited in males days 8 (53%), 15 (56%) and 21 (62%) at 3.0 mg/kg/day and day 21 (17%) at 1.0 mg/kg/day. In females, erythrocyte cholinesterase was statistically significantly inhibited on days 8 (42%), 15 (55%) and 21 (51%) at 3.0 mg/kg/day, but at 1.0 mg/kg/day it was statistically significantly inhibited on days 15 (28%) and 21 (25%) only. Although, erythrocyte cholinesterase was inhibited in females 30% at 1.0 mg/kg/day on day 8, it was not statistically significant, possibly due to the high standard deviation in day -2 values used for comparision. However, concurrent control females and the 0.8 mg/kg/day dose group showed 21% and 24% erythrocyte cholinesterase inhibition on day 8, respectively, compared with the -2 day values. Thus the 30% erythrocyte cholinesterase inhibition in females on day 8 at 1.0 mg/kg/day was not considered biologically significant. At termination, brain cholinesterase was 55% inhibited in males and 27% inhibited in females only at 3.0 mg/kg/day (neither were marked as being statistically significant, but they were depressed according to the report author). Due to the timing of sample collection in females, depression in brain cholinesterase values seen for females, probably had time to partly reverse before collection.

There was no definitive indication from these data that there was or was not accumulation of the test material, which caused increased cholinesterase depression with days on study, however, frequently the day 15 and/or day 21 values were nominally lower than the day 8 cholinesterase activity values, and cholinergic clinical signs occurred in animals after day 15.

The overall NOAEL was 0.8 mg/kg/day for any day of dosing. The overall LOAEL is 1.0 mg/kg/day based on statistically significant inhibition of plasma cholinesterase in males by day 15 and statistically significant inhibition of erythrocyte cholinesterase inhibition in females by day 15. Significant plasma and erythrocyte cholinesterase inhibition occurred by day 8 only at 3.0 mg/kg/day in males and females.

This study is classified **acceptable** and satisfies the Subdivision F guideline requirement for a 21-day dermal study in rabbits (82-2).

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

Disulfoton **21-Day Dermal Rabbits (§82-2)**

EPA Reviewer: David Anderson, Ph.D. 12/4/2000
Reregistration Branch 2 (7509C)

EPA Secondary Reviewer: Robert Fricke, PhD 1/12/01
Registration Branch 2 (7509C)

DATA EVALUATION

RECORD

STUDY TYPE: 21-Day Dermal Study - rabbits
OPPTS Number: 870.3200 OPP Guideline Number: §82-2

DP BARCODE: D270001 SUBMISSION CODE: S587194
P.C. CODE: 0325501 TOX. CHEM. NO.: 341
REREG. CASE NO.: 0102 CAS REG. NO.: 298-04-4

TEST MATERIAL (PURITY): Disulfoton Technical (≥97% a.i.)

SYNONYMS: O,O-Diethyl S-[2-(ethylthio)ethyl]phosphorodithioate

CITATION: Flucke, W (1988) S 276 Technical grade Disulfoton: Study of the Subacute Dermal Toxicity to Rabbits. Bayer AG., Germany. Study Number 98347. Report No. 116342, January 5, 1988. MRID 45239601. Unpublished.

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Body weight was slightly decreased and statistically significant (-3% compared with controls) during the last 2 weeks of the study at 3.0 mg/kg/day in females. Clinical signs consistent with cholinergic signs occurred in males at the end of the study. Muscle spasm, tremors, diarreha, and/or difficulty in breathing in 4 aminals and one male death occurred at 3.0 mg/kg/day toward the end of the study. One female was lathargic and had difficulty breathing on the last day of the study at 3.0 mg/kg/day. No differences attributed to treatment were noted in organ weights or clinical chemistries other than cholinesterase activity.

Plasma cholinesterase was statistically significantly inhibited in males at 1.0 and 3.0 mg/kg/day at day 15 (22%) and 21 (24%) and at day 8 (63%), 15 (70%) and 21 (65%), respectively. In females, plasma cholinesterase was statistically significantly inhibited only at 3.0 mg/kg/day and only on day 15 (61%) and 21 (61%), but it was 44% inhibited on day 8 (not statistically significant). Erythrocyte cholinesterase was statistically significantly inhibited in males days 8 (53%), 15 (56%) and 21 (62%) at 3.0 mg/kg/day and day 21 (17%) at 1.0 mg/kg/day. In females, erythrocyte cholinesterase was statistically significantly inhibited on days 8 (42%), 15 (55%) and 21 (51%) at 3.0 mg/kg/day, but at 1.0 mg/kg/day it was statistically significantly inhibited on days 15 (28%) and 21 (25%) only. Although, erythrocyte cholinesterase was inhibited in females 30% at 1.0 mg/kg/day on day 8, it was not statistically significant, possibly due to the high standard deviation in day -2 values used for comparision.

However, concurrent control females and the 0.8 mg/kg/day dose group showed 21% and 24% erythrocyte cholinesterase inhibition on day 8, respectively, compared with the -2 day values. Thus the 30% erythrocyte cholinesterase inhibition in females on day 8 at 1.0 mg/kg/day was not considered biologically significant. At termination, brain cholinesterase was 55% inhibited in males and 27% inhibited in females only at 3.0 mg/kg/day (neither were marked as being statistically significant, but they were depressed according to the report author). Due to the timing of sample collection in females, depression in brain cholinesterase values seen for females, probably had time to partly reverse before collection.

There was no definitive indication from these data that there was or was not accumulation of the test material, which caused increased cholinesterase depression with days on study, however, frequently the day 15 and/or day 21 values were nominally lower than the day 8 cholinesterase activity values, and cholinergic clinical signs occurred in animals after day 15.

The overall NOAEL was 0.8 mg/kg/day for any day of dosing. The overall LOAEL is 1.0 mg/kg/day based on statistically significant inhibition of plasma cholinesterase in males by day 15 and statistically significant inhibition of erythrocyte cholinesterase inhibition in females by day 15. Significant plasma and erythrocyte cholinesterase inhibition occurred by day 8 only at 3.0 mg/kg/day in males and females.

This study is classified **acceptable** and satisfies the Subdivision F guideline requirement for a 21-day dermal study in rabbits (82-2).

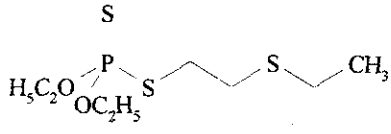
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: S 276 disulfoton technical = Di-Syston®
   Description: Colorless oily liquid
   Lot/Batch #: Reference# 79-R-225-40 (Mobay Chemical Corp.)
   Purity: 96.7% to 97.8% a.i.

   CAS #: 298-04-4
   Structure:



2. Vehicle and/or positive control: Cremaphor/None

3. Test animals: Species: New Zealand White rabbits
   Strain:(HC:NZW)
   Age and weight at study initiation: 11-16 weeks of age; body weight range - males, 2-3 kg
   Source: Intrerfauna, UK

Diet: Feed for Rabbits, 100 g/day
Water: Municipal tap water, ad libitum
Environmental conditions:
Temperature: 22°±2° C
Humidity: 50%
Air Changes: 10 times/hr
Photoperiod: 12-Hour light/dark cycle
Acclimation period: 13 Days

B. STUDY DESIGN:

1. In life dates - Start: Not given End: not given
2. Animal assignment

Five rabbits each sex were randomly assigned to the test groups as indicated in the study design table below. Assignments were made based on body weight.

Study design:

Test Group	Nominal Dose to Animal (mg/kg/day)[b]	Animals Assigned	
		Male	Female
1	0	5	5
2	0.8	5	5
3	1.0	5	5
4	3.0	5	5

[a] = Dose levels were based on the results of another 21-day rabbit dermal study at 0, 0.4, 1.6, 6.5 mg/kg/day. In that study, because 0.4 was the NOAEL and mortalities occurred at 6.5 mg/kg/day, an intermediate dose level of 0.8 mg/kg/day was chosen between the NOAEL and LOAEL.

3. Treatment preparation

Twenty-four hours before treatment and two times per week during treatment, hair was shorn from the backs to the flanks of the rabbits. The treatment area was about 6x8 cm. The treatment site was left uncovered. After treatment the animals were restrained to prevent oral intake. The site was treated for 6 hours per day, 5 day/week for 21 days. The treatment site was washed after each 6 hour treatment.

Disulfoton was diluted with physiological saline and applied in 2% Cremaphor. Stability and concentration of the test materials were analyzed and were within acceptable limits.

4. Statistics

The values for the test populations (dose groups) were compared with those for the control population using the significance test (U-test of Mann-Whitney and Wilcoxan) at the significance level of alpha = 5% and alpha = 1%.

C. METHODS:

1. Observations

All animals were observed for morbidity and mortality at least once daily.

2. Body weight

Animals were weighed pretest, once weekly throughout the study, and at necropsy.

3. Food consumption and compound intake: Food consumption for each animal was measured weekly.

4. Sampling for Blood:

Blood was collected from the ear vein of each animal at -1 or -2 day prior to administration of the test substance, and at day 8, day 15 and day 21 or 22 (termination for females), approximately 16 hours after the end of the treatment. The animals were fasted 16 hours prior to blood collection. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocytes
	Blood clotting measurements*	X	Heinz bodies
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		
	(Activated partial thromboplastin time)		

* Required for chronic toxicity studies.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*		
X	Chloride*		Urea
X	Potassium*		Creatinine*
X	Phosphorus		Glucose*
X	Sodium*		Direct bilirubin
	ENZYMES		Bilirubin[b]
X	Alkaline phosphatase (APh)		Total serum protein (TP)*
X	Cholinesterase (ChE)[a]		
X	Aspartate aminotrnasferase (ASAT/GOT)		
X	Alanine aminotransferase (also ALT, SGPT)*		

[a] Plasma, brain, erythrocyte cholinesterase

7. Cholinesterase Measurements

Plasma and erythrocyte cholinesterase were measured in all test animals from blood drawn on day-1 or-2 and day 8, 15 and 21 or 22, approximately 16 hours after the start or end of the treatment day. The study was started on a Thursday and blood drawn 16 hours after dosing ended and at the beginning of dosing on day 8, Friday. Thus blood was sampled from animals pre-dosing, then dosed for 2 days, followed by 2 days of rest and 4 days of dosing for the day 8 sampling, then 1 day of dosing, followed by 2 days of rest and 4 days of dosing for day 15 sampling and so on for day 21 or day 22.

Terminal measurements were taken on whole brain homogenate for cholinesterase activity in all test animals. Males were terminated on day 21, but females were held (presumably not dosed) an extra 24 hours prior to sacrifice due to logistical problems (page 42). Thus as stated

in the report that brain cholinesterase in females may have partly reversed (page 42) at all dose levels during this time. The values for female brain cholinesterase inhibition may be in doubt for this reason.

The brain cholinesterase values were compared with concurrent controls. All plasma and erythrocyte cholinesterase depressions were compared with pretreatment values.

8. Urinalysis

Urine was collected from the test animals prior to study termination. The test animals were fasted overnight prior to urine collection. The CHECKED (X) parameters were examined.

X	Appearance	X	Glucose
	Volume	X	Ketones
X	Specific Gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)	X	Nitrite
X	Protein	X	Urobilirubin
		X	Leukocytes

10. Sacrifice and Pathology

All test animals were sacrificed on schedule at study termination and were subject to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	Organ systems		Sex organs		NEUROLOGIC
XX	Thyroids	XX	Testes	XX	Brain*
XX	Heart	XX	Epididymides		
XX	Lungs	XX	Overies		
XX	Liver	XX	Uterus		
XX	Kidney*				
XX	Adrenals				
XX	Spleen*				

II. RESULTS

A. Observations

1. Mortality - One male rabbit died on day 17 with clinical signs of cholinesterase depression.

2. Clinical Signs - Slight clinical signs were seen at 3.0 mg/kg/day only. One rabbit showed slight muscle spasms on day 21, another showed diarrhea (day 16 and 17), and still another showed tremors, slight muscle spasms, difficulty breathing and diarrhea, and died on day 17. One female showed difficulty breathing and lethargy on day 21.

B. Body weight and weight gain

Slight differences in mean body weights and body weight gains between the 3.0 mg/kg/day treatment male and female groups (body weight was significantly decreased in females, but not in males) and control groups were observed during the last 2 weeks of the

study (about -3% when compared with controls).

C. Food consumption

1. Food consumption - No definitive differences in food consumption (g/animal/day) were observed between the treatment and control groups.

D. Blood work

1. Hematology - No differences in hematological parameters between the treatment and control groups. Differences in some groups from control were not considered to be treatment-related.

2. Clinical Chemistry - No differences in clinical blood chemistry between the treatment and control groups were considered to be treatment-related.

3. Cholinesterase Levels - The levels of cholinesterase found along with standard deviations are reported in Table 1, 2 and 3. Statistical analyses were conducted on these data and the results reported in these Tables 1, 2, and 3. The information on % inhibition are reported in Tables 4, 5 and 6 along the results of the statistical analyses for convienence in identifying significant results conducted on the data from Table 1, 2, and 3. The data and information in Tables 4, 5, and 6 on percentage inhibition were calculated by the reviewer. The relevance of the statistical analyses and the "p" values were checked by Hans Allender.

As can be seen in Table 1 and 4, plasma cholinesterase activity was depressed or inhibited statistically significantly in males at 3.0 mg/kg/day on day 8, 15 and 21, and at ≥1.0 mg/kg/day on day 15 and 21, but females showed significance changes only at 3.0 mg/kg/day on day 15 and 21.

Table 2 and 5, shows that erythrocyte cholinesterase activity was depressed or inhibited statistically significantly in males and females on days 8, 15 and 21 at 3.0 mg/kg/day, but at 1.0 mg/kg/day in males only on day 21 and in females on day 15 and 22.

Table 3 and 5 shows the results from brain cholinesterase activity or inhibition. Although none of the results were statistically significant, the report author indicates that the brain cholinesterase depression or inhibition in males and females at 3.0 mg/kg/day on day 21 or 22 was significantly depressed. No effects were seen in males or femnales at 0.8 and 1.0 mg/kg/day on brain cholinesterase activity.

Table 1: Plasma cholinesterase levels in male and female rabbits treated with disulfoton [a]

Dose level/Study days	-2	8	15	21
Plasma cholinesterase in males±standard deviation (n=5)				
0 mg/kg/day	0.53±0.098	0.48±0.113	0.50±0.092	0.47±0.086
0.8 mg/kg/day	0.58±0.140	0.49±0.120	0.45±0.095	0.42±0.119
1.0 mg/kg/day	0.51±0.047	0.44±0.043	0.40±0.022**	0.39±0.013**
3.0 mg/kg/day	0.57±0.113	0.21±0.099**	0.17±0.054**	0.20±0.057** [b]
Plasma Chloinesterase in females±standard deviation (n=5)				
0 mg/kg/day	0.56±0.055	0.52±0.054	0.52±0.041	0.48±0.054
0.8 mg/kg/day	0.55±0.105	0.46±0.084	0.43±0.071*	0.43±0.059
1.0 mg/kg/day	0.60±0.097	0.47±0.090	0.44±0.079	0.45±0.090
3.0 mg/kg/day	0.62±0.161	0.35±0.112	0.24±0.105**	0.24±0.096**

[a] = Data taken from page 48 of MRID# 45239601. [b] = One male died at day 17, thus only 4 males were available for this determination. *, ** = $p \leq 0.05$ & ≤ 0.01, respectively.

Table 2: Erythrocyte cholinesterase levels in male and female rabbits treated with disulfoton [a]

Dose level/Study days	-2	8	15	21
Erythrocyte cholinesterase in males±standard deviation (n=5)				
0 mg/kg/day	1.78±0.120	1.70±0.158	1.82±0.682	1.62±0.210
0.8 mg/kg/day	1.74±0.256	1.67±0.221	1.84±0.515	1.56±0.246
1.0 mg/kg/day	1.60±0.479	1.65±0.210	1.64±0.318	1.33±.0.097*
3.0 mg/kg/day	1.96±0.250	0.93±0.147**	0.86±0.108**	0.75±0.022 [b]
Erythrocyte chloinesterase in females±standard deviation (n=5)				
0 mg/kg/day	2.05±0.356	1.62±0.357	1.93±0.444	1.82±0.276
0.8 mg/kg/day	1.92±0.140	1.45±0.172	1.76±0.539	1.53±0.138
1.0 mg/kg/day	1.91±0.318	1.34±0.172	1.37±0.210*	1.44±0.155*
3.0 mg/kg/day	1.91±0.226	1.10±0.176*	0.85±0.211**	0.94±0.179**

[a] = Data taken from page 49 of MRID# 45239601. [b] = One male died at day 17, thus only 4 males were available for this determination. *, ** = $p \leq 0.05$ & ≤ 0.01, respectively.

Table 3: Brain cholinesterase in males and female rabbits at termination and dosed with disulfoton. [a]

Dose/Days on study	Day 21	Day 22
	Males	Females
0 mg/kg/day	3.41±0.607	2.89±0.375
0.8 mg/kg/day	3.15±0.422	3.02±0.370
1.0 mg/kg/day	3.43±0.543	2.69±0.489
3.0 mg/kg/day	1.52±0.179 [b]	2.10±0.750

[a] = Data taken from page 50 of MRID# 45239601. [b] = One male died at day 17, thus only 4 males were available for this determination. *, ** = p≤0.05 & ≤0.01, respectively.

Table 4: Percentage inhibition of plasma cholinesterase levels in male and female rabbits treated with disulfoton

Dose level/Study days	-2	8	15	21
Percentage inhibition of plasma cholinesterase in males (n=5)				
0 mg/kg/day	0	9.4%	5.7%	11%
0.8 mg/kg/day	0	16%	22%	28%
1.0 mg/kg/day	0	14%	22%**	24%**
3.0 mg/kg/day	0	63%**	70%**	65%** [b]
Percentage inhibition of plasma cholinesterase in females (n=5)				
0 mg/kg/day	0	7.1%	7.1%	14%
0.8 mg/kg/day	0	16%	22%*	22%
1.0 mg/kg/day	0	22%	27%	25%
3.0 mg/kg/day	0	44%	61%**	61%**

[a] = Data calculated by the reviewer from Table 1. [a] = Data taken from page 48 of MRID# 45239601. [b] = One male died at day 17, thus only 4 males were available for this determination. *, ** = p≤0.05 & ≤0.01, respectively.

Table 5: Percentage inhibition of erythrocyte cholinesterase levels in male and female rabbits treated with disulfoton [a]

Dose level/Study days	-2	8	15	21
Percentage inhibition of erythrocyte cholinesterase in males (n=5)				
0 mg/kg/day	0	4.5%	-2%	9%
0.8 mg/kg/day	0	4%	-6%	10%
1.0 mg/kg/day	0	-3%	-2%	17%*
3.0 mg/kg/day		53%**	56%**	62% [b]
Percentage inhibition of erythrocyte cholinesterase in females (n=5)				
0 mg/kg/day	0	21%	5.9%	11%
0.8 mg/kg/day	0	24%	8.3%	20%
1.0 mg/kg/day	0	30%	28%*	25%*
3.0 mg/kg/day	0	42%*	55%**	51%**

[a] = Data calculated by the reviewer form Table 2. [b] = One male died at day 17, thus only 4 males were available for this determination. *, ** = $p \leq 0.05$ & ≤ 0.01, respectively.

Table 6: Percentage inhibition of brain cholinesterase in males and female rabbits at termination and dosed with disulfoton. [a]

Dose/Days on study	Day 21	Day 22
	Males	Females
0 mg/kg/day	0	0
0.8 mg/kg/day	7.6%	-4%
1.0 mg/kg/day	-1%	6.9%
3.0 mg/kg/day	55% [b]	27% [c]

[a] = Data calculated by the reviewer from Table 3. [b] = One male died at day 17, thus only 4 males were available for this determination. [c] = Since females were sacrificed 24 hours later than males, some reversibility within this 24 hour period may have occurred. *, ** = $p \leq 0.05$ & ≤ 0.01, respectively.

E. Urinalysis

No treatment-related differences in urine parameters were observed in any of the treatment groups.

F. Sacrifice and Pathology

1. Organ weight - No treatment-related differences in absolute or relative organ weights were observed in dogs in the treatment groups compared to the control weights.

2. Gross pathology - No treatment-related differences in post mortem gross pathology were

observed between treated and control dogs. All findings occurred randomly and sporadically in all study groups.

3. Microscopic pathology

a) Non-neoplastic - No treatment-related microscopic post mortem differences were observed between the treated and control groups. Background changes occurred randomly and sporadically in all study groups.

Spleen weights were increased in females at 3.0 mg/kg/day, but it apparent increase was attributed to 2 rabbits in the controls that low.

No skin changes from treatment were noted.

III. DISCUSSION

A. Investigator's Conclusions: The only definitive changes with treatment were decreases in male plasma at 1.0 and 3.0 mg/kg/day, starting at day 15 and day 8, respectively.

Erythrocyte cholinesterase was decreased in both sexes at 1.0 and 3.0 mg/kg/day. Marked changes were seen only at 3.0 mg/kg/day starting a day 8.

Marked decreases in brain cholinesterase occurred in males at 3.0 mg/kg/day (Inhibition was 55.5%. In females 27% brain cholinesterase inhibition occurred at 3.0 mg/kg/day.

None of the data on cholinesterase inhibition show an accumulation effects (time dependent increase in cholinesterase inhibition) during the 3-week dosing period. However, as noted in the clinical observations, signs showed only after study day 16 at 3.0 mg/kg/day dose level.

B. Reviewer's Discussion

Initial ambiguities in the proximity or timing of blood collections for cholinesterase analyses and dosing were clarified by the registrant in an E-Mail message. Clarification of the timing of blood collection is satisfactory. The following was extracted from the E-Mail message. "The animals were treated on working days but not during week-ends. An overview on the timeline of blood and brain collections is given in the following table.

day of week	Th	Fr	Sa	So	Mo	Tu	We	Th	Fr	Sa	So	Mo	Tu	We	Th	Fr	Sa	So	Mo	Tu	We	Th	Fr
study day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
treatment day	1	2			3	4	5	6	7			8	9	10	11	12			13	14	15		
activities performed	⊗	⊗			⊗	⊗	⊗	⊗	⇧ ⊗			⊗	⊗	⊗	⊗	⇧ ⊗			⊗	⊗	⊗	⇧ ♂ ?	♀?

⇧ blood collection for CHE determination; ⊗ dermal exposure

♂? / ♀? brain collection in male / female animals for CHE determination"

IV. Study Deficiencies

No significant deficiencies or deviations from Subdivision F were noted in this study.

13544

012674

Chemical:	**Disulfoton**
PC Code:	**032501**
HED File Code	**13000 Tox Reviews**
Memo Date:	**12/21/2000**
File ID:	**TX014448**
Accession Number:	**412-02-0006**

HED Records Reference Center
12/04/2001