12-29-92

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Tribufos (DEF®), Subchronic Inhalation Study in the Rat

TO: Bruce Sidwell PM-53
Reregistration Branch
Special Review and Reregistration Division (H7508C)

12/29/92

FROM: Robert P. Zendzian Ph.D.
Senior Pharmacologist
Toxicology Br II
Health Effects Division (H7509C)

THROUGH Karl Baetcke Ph.D.
Chief
Toxicology Br II
Health Effects Division (H7509C)

Compound; Tribufos (DEF®) Tox Chem #864

Registration #074801 Registrant; Miles

MRID #423998-01 DP barcode; D180878

Action Requested

Review the following study;

Study of the subchronic inhalation toxicity to rats in accordance with OECD Guideline No. 413; J. Pauluhn; BAYER AG, FRG; Report No: 102697; June 2, 1992; MRID 423998-01

Core Classification Guideline

Conclusion

Doses 0, 1, 2, 12 & 60 mg/m^3 nominal (0, 0.93, 2.43, 12.2 & 59.5 actual), cholinesterase inhabition RBC 12 & 60 mg/m^3 both sexes, plasma 12 & 60 mg/m^3 males, 60 mg/m^3 females, brain 60 mg/m^3 both sexes, ERG depressed a- and b- waves 60 mg/m^3 both sexes, adrenals cortical fat deposition 60 mg/m^3 both sexes.

Discussion

Tribufos (DEF®) [S,S,S-Tributylphosophorotrithioate] is an organophosphate cholinesterase inhibiting compound used as a defoliant on cotton. In a rat chronic/oncogenicity study the compound produced a retinal toxicity characterized by complete loss of the sensery layer in all rats of both sexes after 12 months of treatment at a dose level of 320 ppm (MRID 423351-01). The sensery cells (rods and cones) of the retina are the source of the a-wave of the electroretinogram (ERG). In this study depression of the a-wave was observed at the high dose (60 mg/m^3). The doses of the two studies are presented below and converted into mg/kg/day.

Inhalation study		Chronic/onco study		
mgm^3	mg/kg/day	ppm	mg/kg/day	
			males	females
0.0	0.0	0	0.0	0.0
1.0	0.3	4	0.2	0.2
2.0	0.9	40	1.8	2.3
12.0	4.5	-	-	-
60.0	22.0	320	16.8	21.1

The high dose in each study produced essentially the same mg/kg/day dose and as such the effect on the ERG in this inhalation study can be considered predictive of the retinal damage observed in the chronic/ocogenicity study.

Attachment
DER
1-liner

Data Evaluation Report

Compound Tribufos (DEF)

Citation
Study of the subchronic inhalation toxicity to rats in accordance with OECD Guideline No. 413; J. Pauluhn; BAYER AG, FRG; Report No: 102697; June 2, 1992; MRID 423998-01

11/13/91

Reviewed by Robert P. Zendzian Ph.D.
Senior Pharmacologist
Health Effects Division

Core Classification Guideline

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Materials

Tribufos (DEF)
Purity 98.8%
clear yellowish liquid
Reference No 85R-26-39
CAS No: 78-48-8
From Miles Inc Kansas City MO.

Vehicle (used as a 1/1 v/v mixture)
ethanol from BAYER AG Warehouse no 12-053
polyethylene glycol 400 (Lutrol)
from Fluka Chemikalien No. 81170

SPF-bred Wister rats, Bor: WISW (SPF-Cpb)
2-3 months of age
From Winklemann, Borchen, Paderborn, Germany

Experimental design

Ten male and ten female rats were exposed to aerosol of tribufos in polyethylene glycol 400/ethanol for 6 hours per day, five days per week for 13 weeks at nominal doses of 0 (vehicle), 1, 2, 12 and 60 mg/m^3.

Exposure

Test material was dissolved (daily) in a 1/1 mixture of polyethylene glycol 400/ethanol at concentrations calculated to produce the required concentration as an aerosol when the

aerosol was generated so as to produce the same nominal concentration of vehicle. Chamber atmosphere was sampled, from the immediate breathing zone, for particle size and concentration of test material. Operation of the aerosol generating system, stability of chamber atmosphere, chamber air flow and temperature were monitored continuously in the breathing zone of the rats.

"During exposure, the animals were exposed to DEF aerosol in Plexiglas exposure tubes. The size of the exposure tube was adjusted to the size of the rat. The exposure tubes (RHEMA LABORTECHNIK) were designed so that the tail of the rat was outside the exposure tube. In this way, hyperthermal effects can be avoided. Exposure was thus of the head nose type. By using this route of exposure, contamination of the hair coat can largely be avoided."

Range-finding tests

Doses chosen for this study were based on two range finding studies. See Appendix I for details.

Observations

Rats were weighed prior to the first exposure and weekly during the study.

"Appearance and behavior were evaluated individually on the days of exposure before and after exposure, but not during exposure (tube exposure). Rats were also evaluated on the days of no exposure."

"Rectal temperatures were determined for 5 rats per group per sex at monthly intervals immediately after exposure using a DIGIMED H 11 digital therometer with F2 sensor."

Blood samples were obtained monthly for intrim hemotological and clinical chemistry tests and at necropsy for general tests.

Intrim examination

plasma CHE
erythrocyte CHE
hematocrite
hemoglobin
leucocytes
Erythrocyted, including MCH. MCV, MCHC
thrombocytes
coagulation (Hepatoquick value)

Final examination

Hematology

RBC count
hemoglobin
WBC count
MCH
MCHC
Heinz bodies
MCV
hematocrite
platelet count
differential count
reticulocyte count

Clinical chemistry

alanine aminotransferase
albumin
alkaline phosphatase
aspartate aminotransferase
brain cholinesterase(terminal)
calcium
chloride
cholesterol
creatine kinase
creatinine
bilirubin
erythrocyte cholinesterase
gutamate dehydrogenase
magnesium
gamma-glutamyl transpeptidase
globulin
glucose
lactic dehydrogenase
phosphate
plasmacholinesterase
potassium
sodium
bilirubin
total protein
triglyceride
urea
serum protein electrophoresis

Thromboplastin time (Hepatoquick value)

Urine was collected individually during the next to last exposure week.

Urinalysis

ketone bodies
pH
bilirubin
blood
Glucose
volume
protein
urobiligen
osmolality

Opthalmological examination

"Eye examinations were performed for all rats prior to the 1st exposure and toward the end of the study period using an indirect opthalmoscope (HEINE). Five to 10 minutes prior to examination, the pupils were dilated with a mydriatic (ROCHE®). The eyes were examined for changes in the retina, vitreous humor, lens, cornea and external eye surface."

Electroretinography

"Electroretinographic (ERG) tests were performed during week 10 on 5 males and 5 females each from the control and 60 mg/m^3 groups. Since these tests revealed DEF-induced changes, ERG tests were performed prior to necropsy on animals from all groups (5 rats per group per sex) that had been acclimitized (sic) to the dark overnight." For details of the procedure see Appendix II.

Termination

At termination rats were anesthatized with sodium pentobarbital and sacrificed by esanguination. All animals were necropsied and the following tissues/organs collected for histopathological examination. Asterixed organs were weighed.

adrenals*
aorta, decending
bone
  femur
bone marrow
  femur and sternum
brain*
  cerebrum
  cerebellum
  brainstem
Coagulating gland
epididymis
  including accessory glands
  and ductuli
esophagus
exorbital lac/gland
eyes
gross lesions
harderian gland
head
  nasopharynx
  oropharynx
heart*

kidneys*
larnyx
liver*
lungs*
lymph node
  cervical
  mesenteric
  lumbar
  thoracic
mammary gland
muscle
  quadriceps femoralis
ovaries*
pancreas
parathyroid
pituitary
prostrate
rectum
salivary gland
skin
small intestine
  duodenum
  ileum
  jejunum
spinal cord
  cervical
  thoracic
  lumbar
spleen*
sternum
stomach
testes*
thymus*
thyroid*
tongue
trachea
urinary bladder
uterus
  including fallopian tubes
varina

Results

The physical parameters of the chamber atmosphere are presented in the following table from the report. Analytical air concentrations of test material were determined as 0, 0.93, 2,43, 12,2 and 59.5 mg/m^3 for the respective nominal concentrations of 0, 1, 2, 12 and 60 mg/m^3.

Table 1: Technical/Physical Inhalation Chamber Parameters

	Target Concentration (mg/m3 air)				
	vehicle	1	2	12	60
Chamber-volume (l):					
- total (l):	24.0	24.0	24.0	24.0	24.0
- inner cylinder (l):	3.8	3.8	3.8	3.8	3.8
Air flows (l/min)					
-main (via nozzle):	15.0	15.0	15.0	15.0	15.0
-total:	15.0	15.0	15.0	15.0	15.0
-exhaust:	13.0	13.0	13.0	13.0	13.0
-air changes per hour: *)	237.0	237.0	237.0	237.0	237.0
-dispersion pressure (bar):	7.1	6.5	7.5	6.5	6.4
-spray solution (g/v · %):	0.00	0.051	0.113	0.571	3.66
-vehicle (ml/m3 air):	10.0	10.0	10.0	10.0	10.0
-nominal concentration(mg/m3):	0.0	5.1	11.3	57.1	366.0
Chamber humidity/temperature:					
Relative humidity (%):	25.2	23.6	25.7	24.8	23.7
Temperature (Degree Celsius):	21.7	21.3	21.2	21.2	21.6
Particle distribution					
- MMAD (um)	0.75	0.76	0.78	0.78	0.76
- MMAD (um)	1.2	1.2	1.3	1.2	1.2
- GSD	1.5	1.4	1.4	1.4	1.4
- % < 3 um (mass related)	99.2	99.5	99.3	99.6	99.6
- number of particles (*1E6/ml)	2.9	3.2	3.3	3.5	3.0
Analytical concentrations					
- mg PE/M3 air: **)	877.5	1177.6	1197.8	1124.8	949.5
- DEF (mg/m3 air):	--	0.93	2.43	12.2	59.5
- % of nominal:	--	18.2	21.2	21.4	16.3

um = micrometer
*) = 15 * 60 / 3.8 [l * min / l]
**) = cumulative mass of PE (+ DEF) recovered by impactor (gravimetrically determined)
PE = polyethylene glycol 400
vehicle = 50:50 mixture (v/v) of PE and ethanol

Three animals were sacrificed or died on study due to nontreatment related effects. No treatment related mortality or morbidity was observed.

Signs of toxicity were observed in all animals at the highest dose but not in any lower dose. The following signs of toxicity were observed but only directly following exposure; "high-stepping gait, reduced activity, bradypnea, dyspnea, and irregular breathing, respiratory sounds, narrowed eyelids (blepharospasmus), piloerection and unpreened coat, increased menace reflex, temporarily increased aggressiveness and vocalization when touched, squatting, convulsions with spastic head movements, salivation, exophthalmus, hypothermia and miosis. Signs were considered more severe in females.

No treatment related effect was observed on body weight or weight gain.

No treatment related effect was observed on the hematological, clinical chemistry or urinanalysis parameters examined.

Effects on erythrocyte cholinesterase was observed at 12 and 60 mg/m^3 in both sexes, on plasma cholinesterase at 12 and 60 mg/m^3 in females and 60 mg/m^3 in males and in the brain at 60 mg/m^3 in both sexes. Summary data are presented in the tables below from the report.

Table 7: Cholinesterase Activity

CHOLINESTERASEN / CHOLINESTERASES							
Konz./ conc. (mg/m3)	Woche week	ERYTHROZYTEN ERYTHROCYTES kU/l	%	PLASMA PLASMA kU/l	%	GEHIRN BRAIN U/g	%
MAENNCHEN / MALES							
0	0	1.44	100	0.48	100		
0	4	0.74	100	0.56	100		
0	8	0.78	100	0.59	100		
0	12	1.18	100	0.59	100		
0	13	0.80	100	0.43	100	12.01	100
1	0	1.05+	73	0.44	92		
1	4	0.63	85	0.51	91		
1	8	0.69	88	0.54	92		
1	12	1.15	97	0.47	80		
1	13	0.76	95	0.41	95	11.78	98
2	0	1.06	74	0.43	90		
2	4	0.64	86	0.46	82		
2	8	0.62+	79	0.48	81		
2	12	1.11	94	0.46	78		
2	13	0.64	80	0.38	88	12.23	>100
12	0	0.92++	64	0.38	79		
12	4	0.37++	50	0.40	71		
12	8	0.35++	45	0.46	78		
12	12	0.45++	38	0.45	76		
12	13	0.28++	35	0.37	86	11.78	98
60	0	0.63++	44	0.22++	46		
60	4	0.09++	12	0.19++	34		
60	8	0.08++	10	0.19++	32		
60	12	0.13++	11	0.16++	27		
60	13	0.15++	19	0.22++	51	7.15++	60

Table 7: Cholinesterase Activity (cont.)

CHOLINESTERASEN / CHOLINESTERASES							
Konz./ conc. (mg/m3)	Woche week	ERYTHROZYTEN ERYTHROCYTES kU/l	%	PLASMA PLASMA kU/l	%	GEHIRN BRAIN U/g	%
WEIBCHEN / FEMALES							
0	0	1.32	100	1.36	100		
0	4	0.90	100	1.49	100		
0	8	0.62	100	1.82	100		
0	12	1.09	100	1.47	100		
0	13	0.92	100	1.28	100	11.69	100
1	0	1.20	91	1.00	74		
1	4	0.91	>100	1.15	77		
1	8	0.65	>100	1.38	76		
1	12	1.10	>100	1.51	>100		
1	13	0.93	>100	1.22	95	11.87	>100
2	0	1.35	>100	1.07	79		
2	4	0.96	>100	1.27	85		
2	8	0.69	>100	1.44	79		
2	12	1.14	>100	1.84	>100		
2	13	0.81	88	1.39	>100	11.64	100
12	0	0.99+	75	0.74+	54		
12	4	0.36++	40	0.65++	44		
12	8	0.32++	52	0.85++	47		
12	12	0.41+	38	0.87+	59		
12	13	0.33++	36	0.77++	60	11.45	98
60	0	0.67++	51	0.33++	24		
60	4	0.17++	19	0.25++	17		
60	8	0.07++	11	0.26++	14		
60	12	0.10+	9	0.25+	17		
60	13	0.12++	13	0.42++	33	6.99++	60

Comparison vs. vehicle control (U-test), + = $p < 0.05$, ++ = $p < 0.01$
Week 0-12: retro-orbital blood sampling after exposure
Week 13: blood sampling by heart puncture one day after the last exposure
Brain: CHE-activity after Triton pre-treatment of tissue

No treatment related effects were observed in the eyes by direct opthalmoscopic examination.

Results of the electroretinographic examinations showed depression of amplitude of the a-wave and the b-wave at 60 mg/m^3. Results are summarized in the tables below from the report.

Table 1: ERG Test: Means

ERG Mittelwerte/means							
conc. (mg/m3)	Woche/ week	I	II	III	IV	V	VI
männlich/male							
cont.	10	240.6	685.8	35.3	4.1	8.9	45.2
60	10	189.1*	654.8	29.0*	3.8	8.5	40.8*
weiblich/female							
cont.	10	237.5	786.0	30.6	3.9	9.8	47.1
60	10	190.6**	621.9**	30.8	3.8	8.4**	43.7
männlich/male							
cont.	13	249.6	715.8	34.3	3.5	8.9	42.2
1	13	251.1	698.1	36.0	4.1	9.1	43.8
2	13	256.4	671.4	38.1	4.1	8.9	43.7
12	13	260.2	671.4	39.1	4.1	9.2	41.7
60	13	130.4**	569.8	24.3*	3.8	8.4	45.9
weiblich/female							
cont.	13	238.7	735.5	32.6	3.9	8.9	44.3
1	13	240.3	677.7	35.7	4.1	9.8	43.1
2	13	211.6	656.0	32.4	4.1	9.8	43.3
12	13	233.9	652.7	36.6	4.1	8.9	42.0
60	13	129.8**	600.2**	21.8**	4.2	8.6	43.3

* = $p < 0.05$; ** = $p < 0.01$ vs. control (ANOVA)

I : a-Amplitude (uV)
II : b-Amplitude (uV)
III : Verhältnis/ratio a-Amplitude/b-Amplitude (%)
IV : Beginn/onset time a-Amplitude (ms)
V : Maximum/peak time a-Amplitude (ms)
VI : Maximum/peak time b-Amplitude (ms)
cont. : Vehikel-Kontrolle/vehicle control
conc. : Target-Konzentration/target concentration

Table 2: ERG Test: Percent Difference from Control

ERG Prozentuale Abweichung von der Kontrolle/ difference in percent of control							
conc. (mg/m3)	Woche/ week	I	II	III	IV	V	VI
männlich/male							
60	10	-21*	-5	-18*	-7	-5	-10*
weiblich/female							
60	10	-20**	-21**	1	-3	-7**	-7
männlich/male							
1	13	1	-2	5	17	2	2
2	13	3	-6	11	17	0	4
12	13	4	-6	14	17	3	-1
60	13	-48**	-20	-29*	9	-6	9
weiblich/female							
1	13	1	-8	10	5	1	-3
2	13	-11	-11	-1	5	1	-2
12	13	-2	-11	12	5	0	-5
60	13	-46**	-18**	-33**	8	-3	-2

* = $p < 0.05$; ** = $p < 0.01$ vs. control (ANOVA, see Table 1)

I : a-Amplitude
II : b-Amplitude
III : Verhältnis/ratio a-Amplitude/b-Amplitude
IV : Beginn/onset time a-Amplitude
V : Maximum/peak time a-Amplitude
VI : Maximum/peak time b-Amplitude
conc. : Target-Konzentration/target concentration

No apparent treatment related effects were observed at necropsy. Organ weight data showed an increase in absolute and relative adrenal weights in both sexes at 60 mg/m^3 which was statistically significant in the males.

Histology showed minor changes in the nasal and paranasal cavities an lungs across all groups which may be considered as secondary to inhalation of the vehicle. The adrenals showed a treatment related increase in cortical fat desposition which was statistically significant in the 60 mg/m^3 males.

APPENDIX I

Range Finding Studies

DEF

Page_____ is not included in this copy.

Pages 14 through 33 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Tox Chem No. tribufos (DEF)

File Last Updated _____

Current Date _____

Study/Lab/Study #/Date	Material	EPA MRID No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
13-week inhalation, rat; Bayer; Report No 102697; Jun 2, 1992	Tech 98.7%	423998-01	Doses 0, 1, 2, 12 & 60 mg/m^3 nominal (0, 0.93, 2.43, 12.2 & 59.5 actual), cholinesterase inhabition RBC 12 & 60 mg/m^3 both sexes, plasma 12 & 60 mg/m^3 males, 60 mg/m^3 females, brain 60 mg/m^3 both sexes, ERG depressed a- and b-waves 60 mg/m^3 both sexes, adrenals cortical fat deposition 60 mg/m^3 both sexes.	N/A	Guideline