Bull. Environ. Contam. Toxicol. (1987) 38:834–839

Environmental Contamination and Toxicology

Safety Related to Exposure: Dermal Dose-Red Cell Cholinesterase Response Relationships for Ethoprop and Mocap 6EC

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Ethoprop (O-ethyl S,S-dipropylphosphorodithioate), a cholinesterase (ChE) inhibiting organophosphate, is sold under the trade name of MOCAP by Rhone-Poulenc Inc. for control of soil wireworms and nematodes attacking important field and vegetable crops. MOCAP 6EC is presently being developed for use in vineyards in California. Farm Chemicals Handbook lists the acute oral LD_{50} in the rat as 61.5 mg/kg. Pesticide regulations in California presently require the development of exposure and dermal dose ChE response or dermal absorption studies to assess the hazards involved in mixing/loading and applying toxic organophosphate and carbamate pesticides.

The purpose of this study was to provide dermal dose-ChE response data on ethoprop and MOCAP 6EC. Exposure studies involving mixer/loader applicators are presently being conducted by Rhone-Poulenc Inc. The information obtained in these studies will be used to estimate the hazard to workers applying MOCAP 6EC.

MATERIALS AND METHODS

Male albino rats, SPF (Bantin and Kingman, Inc., Fremont, California) weighing 266 to 313 grams were prepared for treatment according to the method of Knaak et al. (1980). Table 1 gives the pesticides applied, the dosages, and the number of rats treated per dose level. Doses were applied to the clipped backs (25 cm^2) in 0.5 ml of acetone (ethyl parathion-positive control and technical ethoprop, 95%) or water (MOCAP 6EC, formulation containing 68.6% ethoprop) using a digital microliter pipet (Pipetman, Model P-1000 D, West Coast Scientific, Oakland, California) and spread with a glass rod to insure uniform application. After application, the animals were returned to their cages and allowed free access to food (Rat Chow 5012, Ralston Purina Company, St. Louis, Missouri) and water. During the exposure period of 72 hours the animals were observed for symptoms of poisoning, and for other reasons, such as loose collars and chewed rubber templates. Four control animals were used in each study and acted as analytical (ChE standards) as well as treatment (baseline values) controls. Blood (6.0 ml) was obtained by cardiac puncture using a disposable 6.0 ml heparinized syringe and needle and two-3.0 ml vacutainers

(green cap). In order to accomplish this, each animal was anesthetized with sodium pentothal (70 mg/kg I.P.) and a midline chest incision was made to expose the heart for proper insertion of a 22-gauge hypodermic needle. Blood was colorimetrically analyzed for cholinesterase activity and the data statistically analyzed according to the procedure of Knaak et al. (1980).

Table 1. Pesticide treatment schedule - number of animals

	Dosage ($\mu g/cm^2$)[a/]								
	0	4	20	40	80	120	160	200	240
Ethyl Parathion	4	4	4	4	4	0	0	0	0
Ethoprop/tech[b/]	8	0	4	4	4	4	8	4	4
MOCAP 6EC	4	0	0	0	0	4	4	4	4

[a/] Dose (μg of active ingredient) applied to the clipped back (25 cm^2) of 260-311g male Sprague-Dawley rats. Animals sacrificed after 72 hours. Analytical grade ethyl parathion was obtained from Chemistry Branch, California Department of Food and Agriculture, Sacramento, CA 95814. Technical grade ethoprop (95%) and MOCAP 6EC were obtained from Rhone-Poulenc Inc., Monmouth Junction, New Jersey 08852.

[b/] Two studies were run with technical ethoprop. The first study (range finding) used nominal dosages of 20, 40, 80, and 160 $\mu g/cm^2$, while the second study used nominal dosages of 120, 160, 200, and 240 $\mu g/cm^2$.

RESULTS AND DISCUSSION

Figure 1 shows the dermal dose-ChE response relationships for ethyl parathion, technical ethoprop, and MOCAP 6EC (formulated ethoprop). Tables 2, 3, and 4 provide the activity of the red blood cells in μmoles/min of -SH hydrolyzed per ml of sample, percentage of activity, and inhibition of the red cells in terms of their controls, mean weight of the animals in each group, the applied dose in $\mu g/cm^2$, the ED50 and their lower and upper limits, and the equation of the log-probit regression line. The ED50 values were determined to be 60, 162, and 147 μg of active ingredient/cm^2 of skin being treated, respectively, for ethyl parathion, technical ethoprop, and MOCAP 6EC after 72 hours of exposure. Ethyl parathion was used as a positive control in this study. In a previous study (Knaak et al., 1984) the ED50 in adult, 269 g rats was reported to be 24.3 $\mu g/cm^2$.

Technical ethoprop was approximately on-third as dermally toxic as ethyl parathion. MOCAP 6EC appeared to be slightly more toxic

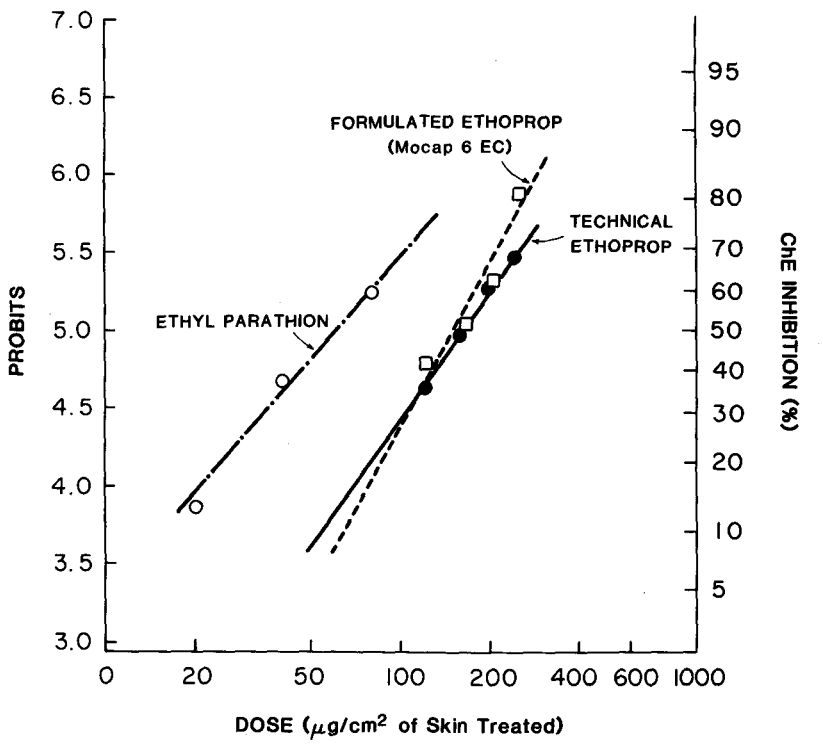

Figure 1. Dermal dose-ChE response curves for ethylparathion, MOCAP 6EC (formulated ethoprop) and technical ethoprop. Male Sprague-Dawley rats weighing 260-311 g were used. A 25 cm^2 area of skin was treated.

than technical ethoprop at the higher dosages. This difference may be due in part to the xylene base solvents present in the pesticide.

Table 5 gives the ED50 values for these pesticides in terms of $\mu g/cm^2$ of total body surface (Area = 9.64 $weight^{0.66}$) and mg/kg of body weight.

The ED10 value was included because it is considered to be the no-observed-effect level (NOEL) for acute dermal exposures to organophosphate pesticides. The ED10 values are given in terms of $\mu g/cm^2$ of total body surface and mg/kg of body weight. Under field conditions a safety factor of 10 to 100 should be applied to the NOEL. The size of the safety factor depends upon the uncertainties in scaling animal toxicity data to man and measuring exposure in the work place. Application of these safety factors (NOEL/safety factor [SF]) give dosages of 0.05 to 0.5 $\mu g/cm^2$ of

total body surface as safe levels for technical ethoprop and MOCAP 5EC.

Table 2. Dermal Dose-Red Cell Cholinesterase Response and Log-Probit Data on Ethyl Parathion

Mean[a] Rat Weight, g	Dose[b] µg/cm²	RBC[c] Activity µmoles-SH/min/ml	% Activity	% Inhibition
269	0	3.60	100.0	0.0
265	4	3.40	100.0	0.0
262	20	3.13	86.9	13.1
273	40	2.22	61.7	38.3
262	80	1.46	40.5	59.5

[a] Four rats/dose group.
[b] Twenty-five cm² of back skin was treated.
[c] Blood was analyzed 72 hours after the application of the dose. Equation for the line of best fit: Y = 2.22 (X) + 1.053; Y = probit value; X = Log10 dose. Lower limit: 56; ED50: 60; Upper limit; 64 µg/cm².

Table 3. Dermal Dose-Red Cell Cholinesterase Response and Log-Probit Data on Technical Ethoprop

Mean[a] Rat Weight, g	Dose[b] µg/cm²	RBC[c] Activity µmoles-SH/min/ml	% Activity	% Inhibition
303	0	3.24	100.0	0.0
(289)	(81)	(3.25)	(100)	(0.0)
265	121	2.06	63.5	36.5
311	161	1.68	51.9	48.2
(280)	(163)	(1.88)	(55.1)	(44.9)
311	201	1.23	38.0	61.9
313	242	1.03	31.9	68.1

[a] Four rats/dose group.
[b] Twenty-five cm² of back skin was treated.
[c] Blood was analyzed 72 hours after the application of the dose. Values in brackets were from range finding studies utilizing nominal dosages of 80, and 160 µg/cm². Equation for the line of best fit: Y = 2.793 (X) - 1.166; Y = probit value; X = Log10 dose. Lower limit: 159; ED50: 162; Upper limit: 165 ug/cm².

Table 4. Dermal Dose-Red Cell Cholinesterase Response and Log-Probit Data on (Formulated Ethoprop) Mocap 6EC

Mean[a] Rat Weight, g	Dose[b] $\mu g/cm^2$	RBC[c] Activity μmoles-SH/min/ml	% Activity	% Inhibition
271	0	4.00	100.0	6.0
275	121	2.33	58.2	41.8
276	161	1.92	48.1	52.0
280	201	1.48	37.0	63.0
289	243	0.77	19.3	80.7

[a] Four rats/dose group.
[b] Twenty-five cm^2 of back skin was treated.
[c] Blood was analyzed 72 hours after the application of the dose. Equation for the line of best fit: Y = 3.315(X)-2.189; Y = probit value; X = Log10 dose. Lower limit: 144; ED50: 147; Upper limit: 150 $\mu g/cm^2$.

Table 5. Dermal Dose-Red Cell Response Data Expressed in Terms of Total Body Surface and Body Weight.

	$ED_{50}\mu g/cm^2$	ED_{50}mg/kg	$ED_{10}\mu g/cm^2$	ED_{10}mg/kg
Ethyl[a] Parathion	3.9	5.6	1.0	1.5
Technical[b] ethoprop	9.5	13.0	3.3	4.5
MOCAP 6EC[c]	9.3	13.1	3.8	5.3

[a] mean animal weight: 266 g; skin surface area: 383 cm^2
[b] mean animal weight: 310 g; skin surface area: 425 cm^2
[c] mean animal weight: 280 g; skin surface area: 397 cm^2

Protective clothing, closed mixing/loading systems, and closed tractor cabs are effective in reducing exposure.

A recent study by Knaak et al (1986) with Nemacur 3EC showed that it was possible to reduce exposure to 1/100 of the dose producing 10% ChE inhibition (ED10 value) in laboratory rats. This level of exposure produced no blood ChE inhibitors in workers applying Nemacur 3EC.

Acknowledgments. The authors wish to thank Dale Uyeminami for technical assistance in conducting these studies.

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Received November 9, 1986; accepted December 16, 1986.