Bull. Environm. Contam. Toxicol. 22,522-529 (1979)

Teratogenicity Studies on Pesticidal Formulations of Dimethoate, Diuron and Lindane in Rats

K. S. Khera, C. Whalen, G. Trivett, and G. Angers

Bureau of Chemical Safety, National Health and Welfare, New Research Centre, Tunney's Pasture, Ottawa, Canada K1A 0L2

Dimethoate (Rogor) is a selective insecticide and is effective for the control of several phytophagous insects that infest a variety of vegetables and fruits. Residues of dimethoate have been detected at the following levels: trace amounts in potatoes and corn (DAUTERMAN et al. 1960), 0 to 0.1 ppm in wheat and sorghum grain (ROWLANDS, 1966), 0.1 ppm in olives (ALLESANDRINI, 1962), 0.7 ppm in cherries (MACNEIL et al. 1975) and as high as 0.53 ppm in corn silage and 0.125 ppm in cow's milk (BECK et al. 1968). The occurrence and rates of disappearance of dimethoate residues for fruit and vegetable crops have been reviewed (PIETRI-TONELLI et al. 1965).

UCHIDA and O'BRIEN (1967) have predicted that the acute oral LD_{50} of dimethoate for man is 30 mg/kg. Dimethoate causes hyperplasia of the haemopoietic parenchyma (STIEGLITZ et al. 1974) and chromosomal anomalies in cells of plant and animal origin (BHUNYA and BEHERA 1975). In a five-generation mouse study in which dimethoate was incorporated in the drinking water at 60 ppm (estimated intake, 9.5-10.5 mg/kg/day or 1/6 of the LD_{50} value), a reduced pregnancy rate and decreased survival and growth of pups were observed (BUDREAU and SINGH 1973).

Diuron is used as a pre- and post-emergence herbicide and to control weeds. The maximum residue concentrations permitted in Canada are 7 ppm in asparagus and 1 ppm in citrus fruits, corn, grapes, pineapple, potatoes and wheat (FOOD AND DRUG ACT 1977).

Lindane, a chlorinated hydrocarbon insecticide, is used extensively on a variety of agricultural products to control insects. It is a contact poison with long residual effects. Its residues have been detected in concentrations of 0.62 ppm in potatoes and 0.15 ppm in sugar beets (LICHTENSTEIN 1959), 1.33 ppm in harvested alfalfa and 0.46 ppm in milk (TREECE and WARE 1965), and 0.002 to 0.022 ppm in poultry, meat and fish (CORNELIUSSEN 1972, HENDERSON et al. 1971). In a total diet survey conducted in

0007-4861/79/0022-0522 $01.60

the U.S.A., residues levels of 0.003 ppm of lindane were found (CUMMINGS 1965). Data on residues of lindane in blood, milk and adipose tissue of man have been summarized (KHERA 1976). In stillborn infants, tissue levels of lindane varied from 0.06 to 0.39 ppm (CURLEY et al. 1969).

Lindane has been tested in rats for effects on reproduction. In a three-generation rat study, conducted at dietary concentrations of 25, 50 and 100 ppm of lindane, reproductive effects were noted at the two high doses but the 25 ppm dose had no effects on reproduction (HERBST and BODENSTEIN 1972).

Since the teratologic potential of these three pesticides has not been fully evaluated, a study using commercial formulations was conducted with the rat.

MATERIALS AND METHODS

The formulations tested were: (1) Cygon 4E containing 47.3% of dimethoate, O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate (Cyanamid of Canada, Niagara Falls, Ontario); (2) Karmex(R) containing 80% diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (Du Pont of Canada Toronto, Ontario); and (3) Benesan containing 50% lindane, gamma isomer of 1,2,3,4,5,6-hexachloro-cyclohexane or gamma isomer of benzene hexachloride (Chipman Chemicals Ltd., Stoney Creek, Ontario). These formulations were stored at 6°C prior to administration. The non-pesticidal ingredients constituting these formulations were unknown.

Female Wistar rats, 175-200 g in body weight, were paired overnight with proven males. The morning that a positive vaginal smear was observed was counted as day 1 of gestation. Twenty mated females were assigned to each experimental group. Dose levels, based on findings from a preliminary study were, on a per kg body weight basis: 3, 6, 12 or 24 mg of forumulated dimethoate; 125, 250 or 500 mg of formulated diuron; and 6.25, 12.5 or 25 mg of forumulated lindane. The test samples, suspended in corn oil, were administered once daily by oesophageal intubation from the sixth to the 15th day of gestation. A control group given only the vehicle, was included with each pesticide. The volume of vehicle or vehicle containing pesticide administered, was 10 ml/kg body weight. Females were weighed on the first, sixth, 15th and 22nd day (BC, before necropsy) of pregnancy. When each dam was killed on day 22 of pregnancy, the carcass, after the uterine contents were removed, was again weighed. The number of corpora lutea was determined, and the female was then necropsied. The fetuses were weighed and examined for viability and

external malformations. Early resorptions or implantation sites and fetuses dying at a late developmental stage were recorded as dead fetuses. Two-thirds of the live fetuses from each litter were studied for skeletal development following alizarin red staining. The remaining fetuses were fixed in Bouin's fluid, sectioned and examined for visceral anomalies.

A litter was considered the basic unit and the proportion of a litter having a particular attribute was calculated. The mean and the standard error of the proportions for the various test and control groups were derived. Analysis of inter-group comparisons were performed using Student's t test (DUNN 1964). Differences of $P < 0.05$ were considered siginificant. The maternal body weight data were analysed in a similar manner.

RESULTS

The pregnancy rate was variable in all groups (Table 1) and was not related to treatment. Two dams, one from the 25 mg lindane/kg group and one control from the diuron experiment, died of causes unrelated to treatment.

Average maternal body weight for the 250 mg diuron/kg and 25 mg lindane/kg groups decreased on the 15th day of pregnancy, and later at necropsy (without the uteri and their contents), but the decreases were not statistically significant. The 24 mg/kg dose of dimethoate and 500 mg/kg of diuron reduced the maternal body weight on days 15 and 22 of pregnancy and at necropsy, and these reductions were statistically significant. Eight dams administered 24 mg/kg of dimethoate manifested clonic spasms and muscular tremors during the treatment period; seven recovered, and one died on the 16th day of pregnancy. No signs of maternal toxicity were observed in other treatment groups.

The incidences of live fetuses, dead plus resorbed fetuses, and mean fetal weight in the controls and test groups treated with dimethoate, diuron or lindane are compared in Table 1. The only statistically significant effect observed was a reduction in mean fetal weight at the 500 mg/kg dose of diuron.

Dimethoate treatment at the 12 and 24 mg/kg doses was associated with a statistically significant increase in the numbers of anomalous litters (each having at least one anomalous fetus), and wavy-ribbed fetuses (Table 2). Other anomalies in the dimethoate-treated fetuses, although statistically not significant, were extra ribs, fused sternebrae, runted fetuses, hydroureter and dilated urinary bladder.

Table 1

Prenatal data from rats dosed orally with formulations of dimethoate, diuron or lindane on days 6 through 15 of gestation

Test sample	Dose (mg/kg)	No. of dams pregnant/No. initiated	No. not Pregnant at necropsy	No. of corpora lutea per pregnancy[a]	No. of live fetuses per pregnancy[a]	Dead or resorbed fetuses (%)[b]	Fetal weight (g)[a]
Dimethoate	0	17/20	3	13.9 ± 0.3	11.6 ± 0.7	6	5.2 ± 0.1
	3	17/20	3	12.7 ± 0.4	11.5 ± 0.6	5	5.1 ± 0.2
	6	15/20	5	12.7 ± 0.5	11.9 ± 0.5	5	4.8 ± 0.1
	12	16/20[c]	4	13.7 ± 0.4	12.1 ± 0.4	7	5.6 ± 0.1
	24	16/20[c]	3	13.8 ± 0.1	11.9 ± 0.5	10	5.0 ± 0.2
Diuron	0	19/20[d]	0	12.9 ± 0.3	10.5 ± 0.9	7	5.2 ± 0.1
	125	18/20	2	13.0 ± 0.4	10.5 ± 0.9	8	5.2 ± 0.1
	250	15/20	5	13.9 ± 0.6	10.9 ± 0.6	12	5.0 ± 0.1
	500	14/20	6	13.1 ± 0.3	10.5 ± 0.5	9	4.6 ± 0.1[e]
Lindane	0	18/20	2	13.2 ± 0.4	10.3 ± 0.9	7	5.1 ± 0.1
	6.25	17/20	3	12.8 ± 0.6	10.5 ± 0.8	5	5.0 ± 0.2
	12.5	19/20[d]	1	13.9 ± 0.5	12.3 ± 0.5	6	4.9 ± 0.1
	25	15/20[d]	4	13.0 ± 0.5	11.5 ± 0.7	4	5.3 ± 0.1

[a] Mean ± SE

[b] $\frac{\text{No. of resorption site + dead fetuses}}{\text{total implants}} \times 100$

[c] One dam died with dimethoate-induced signs of neurotoxicity.

[d] One dam died of causes unrelated to the treatment

[e] $P < 0.05$.

Table 2

Incidence of anomalies in fetuses from rats treated orally with formulation of dimethoate, diuron or lindane from day 6 to 15 of gestation

	Dimethoate					Diuron				Lindane			
	0	3 mg/kg	6 mg/kg	12 mg/kg	24 mg/kg	0	125 mg/kg	250 mg/kg	500 mg/kg	0	6.25 mg/kg	12.5 mg/kg	25 mg/kg
Number of anomalous fetuses/ number examined	5[a]/198	8/195	5/178	16[b]/194	33[b]/190	10/199	15/189	16[b]/164	12/147	12/185	10/179	32/234	11/172
Numbers of litters with anomalous fetus/number of litters examined	5/17	6/17	4/15	12[b]/16	10[b]/16	8/19	9/18	9[b]/15	7/14	7/18	7/17	12/19	8/15
Anomalies (number of fetuses affected)													
Wavy ribs,	1	4	2	8[b]	17[b]	3	7	7[b]	7[b]	1	1	11	2
Extra ribs	4	4	4	7	11	6	7	5	0	5	7	13	7
13 rib, rudimentary	0	0	0	0	0	0	2	1	0	0	0	5[c]	0
Sternoschisis	0	0	0	0	0	0	0	1	2	0	0	0	0
Sternebrae fused	0	0	0	0	0	0	0	0	0	1	0	0	0
Sternebrae missing	0	0	0	0	0	0	0	0	0	7	1	2	2
Calvarium delayed ossif.	0	0	0	0	0	1	4[b]	3	4	0	1	13[d]	0
Runted fetus	0	2	1	1	0	0	1	0	0	0	0	1	0
Hydroureter	0	0	0	0	0	0	0	0	1	0	0	0	0
Urinary bladder dilated	0	0	0	0	5	0	0	0	0	0	0	0	0

[a]A fetus may have more than one anomaly. [b]$p < 0.05$. [c]Occurrence limited to two litters.

[d]Only two litters effected with six anomalous fetuses in one and seven in the other.

The diuron treatment increased the frequencies (at $P < 0.05$) in the numbers of anomalous fetuses at 250 mg/kg, and wavy-ribbed fetuses at 250 and 500 mg/kg (Table 2). The incidence of delayed ossification of calvarium at 125 mg/kg dose groups was of borderline significance ($P = 0.05$). The rest of the entries in columns under the diuron dose groups of Table 2, showed no significant deviation from control values.

In the lindane-administered groups, the incidences of anomalous litters and the individual anomalies listed in Table 2 were not different from control values. Lindane treatment increased the number of rudimentary 13th rib and delayed calvarial ossification, in two litters of the 12.5 mg/kg group, but these observations were statistically not significant.

DISCUSSION

Anomalies, though of minor types and of unknown significance, were observed after the administration of dimethoate at the 24 mg/kg level - a dose clearly toxic for the dams - and 12 mg/kg, whereas no adverse effects were observed at the 6 and 3 mg/kg doses. Since the non-pesticidal ingredients were not known and constituted 52.7% of the formulation, it was not possible to pinpoint the chemical causing fetal effects. A tentative "no effect" level of 6 mg/kg therefore became apparent, pending the results of teratologic studies in cats now in progress in our laboratory. This "no effect" level would not be exceeded from exposures to the maximum residue levels in Canada: 2.0 ppm in broccoli, cabbages, cauliflowers, kale, lettuce, spinach, turnip and beet greens, pears, and apples, 1.5 ppm in citrus fruits, and 0.5 ppm on peas, peppers, and tomatoes (FOOD AND DRUG ACT 1977).

Diuron at 500 and 250 mg/kg doses manifested an increased incidence of malformed fetuses or maternal toxicity. The toxicologic effect of a 125 mg/kg dose consisted in a single anomaly of delayed ossification of the calvarium with a non-dose dependent incidence of borderline significance ($P = 0.05$). The teratologic assessment of this dose is therefore less clear, and requires that further studies be done.

The lack of teratogenicity of lindane at doses as high as 25 mg/kg tested in the present study is consistent with an earlier study conducted on four pregnant cows. These were due to calve in six to 17 weeks, and were fed 70 g of lindane each. All had convulsive seizures, but recovered and produced normal calves (MCPARLAND and MCCRACKER 1973). These studies support the level of temporary acceptable daily intake of 0.01 mg/kg and residue limits of 0.1 to 2 ppm in vegetables and fruits (FAO/WHO 1974).

REFERENCES

ALESSANDRINI, M.E.: Residue Rev. 1, 92 (1962).

BECK, E.W., JOHNSON, J.C., GETZ, M.E., SKINNER, F.B., DAWSEY, L.H., WOODHAM, D.W. and DERBYSHIRE, J.C.: J. Econ. Entomol. 61, 605 (1968).

BHUNYA, S.P. and BEHERA, J.: Current Sci. 44, 859 (1975).

BUDREAU, C.H. and SINGH, R.P.: Toxicol. Appl. Pharmacol. 26, 29 (1973).

CORNELIUSSEN, P.E.: Pestic. Monit. J. 5, 313 (1972).

CUMMINGS, J.G.: J. Assoc. Off. Agr. Chem. 48, 1177 (1965).

CURLEY, A., COPELAND, M.F. and KIMBROUGH, R.D.: Arch. Environ. Health 19, 628 (1969).

DAUTERMAN, W.C., VIADO, G.B., CASIDA, J.E. and O'BRIEN, R.D.: J. Agr. Food Chem. 8, 115 (1960).

DUNN, O.J.: Basic Statistics, 1st ed. p. 86, John Wiley and Sons, New York (1964).

FAO/WHO.: Pesticide residues in food. Report of the 1973 Joint meeting of the FAO working party of experts on pesticide residues and the WHO expert committee on pesticide residues. World Health Organization Technical Report Series No. 545. p. 32 (1974).

FOOD AND DRUG ACT, AND REGULATIONS.: Department of National Health and Welfare, Queen's Printer and Controller of Stationery, Ottawa, Canada. Table II, Agricultural Chemicals (1977).

HENDERSEN, C., INGLIS, A. and JOHNSON, W.L.: Pestic. Monit. J. 5, 1 (1971).

HERBST, M. and BODENSTEIN, G.: Toxicology of lindane. In Lindane. Edited by E. Ulman, p. 23. Verlag K. Schillinger, Freiburg, Breisgau (1972).

KHERA, K.S.: Distribution, metabolism and perinatal toxicity of pesticides with reference to food safety evaluation: A review of selected literature. In New Concepts in Safety Evaluation. Edited by M.A. Mehlman, R.E. Shapiro & H. Blumenthal. p. 369. Hemisphere Publishing Corp., Washington (1976).

LICHTENSTEIN, E.P.: J. Agr. Food Chem. 7, 430 (1959).

MACNEIL, J.D., HIKICHI, M. and BANHAM, F.L.: J. Agr. Food Chem. 23, 758 (1975).

MCPARLAND, P.J. and MCCRACKER, R.M.: Vet Rec. 93, 369 (1973).

PIETRI-TONELLI, P. DE, BAZZI, B. and SANTI, R.: Residue Rev. 11, 60 (1965).

ROWLANDS, D.G.: J. Sci. Food Agric. 17, 90 (1966).

STIEGLITZ, R., GIBEL, W., WERNER, W. and STOBBE, H.: Acta Haemat. 52, 70 (1974).

TREECE, R.E. and WARE, G.W.: J. Econ. Entomol. 58, 218 (1965).

UCHIDA, T. and O'BRIEN, R.D.: Toxicol. Appl. Pharmacol 10, 89 (1967).