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Toxicological Effects in Rabbits Induced by Endosulfan, Lindane, and Methylparathion Representing Agricultural Byproducts Contamination

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Southern Spain is a dry area where water is conservatively used in agriculture to produce crops for human consumption and profit. The major crops are vegetables (eg. tomatoes, peppers), fruits (eg. lemons, oranges, apricots) and ornamental plants (eg. carnation flowers). These cultivated crops result in large amounts of agricultural byproducts. These byproducts have economic importance for use as animal feeds in rabbits and ruminants, to compensate for lack of forages due to the water shortage (Moreno and Ocio, 1988).

Pesticide contamination resulting from agricultural practices for pest control limit the safe use of these products. The main problems that result are clinical and subclinical effects leading to losses in animal performance or in residue contamination of animal products which may later be consumed by humans (Cerón, 1993).

The purpose of this work is to study the effects that can be produced by endosulfan, lindane and methylparathion, pesticides heavily used in this area, that frequently contaminate agricultural byproducts (Cerón, 1993). The rabbit, which is used in Spain as a meat source, was employed as an experimental animal to study clinical, hematological, and biochemical effects and pesticide residues resulting in tissues following endosulfan exposure. These results provide experimental evidence of the potential problems associated with feeding agricultural byproducts previously sprayed with these pesticides.

MATERIALS AND METHODS

Twenty-eight healthy male New Zealand rabbits weighing 2.2-2.6 kg were obtained from "Murcia University Animal Resources Center". The animals were housed in individual cages and fed a commercial diet ad-libitum with the following composition: 87% dry matter, 7.5% ash, 17% crude protein, 16% crude fibre, 2.5% ether extract and 57% nitrogen free extract. Fresh water was always available.

Rabbits were divided in four groups (C, L, E and M) of seven animals. Group C were the control rabbits not treated with any pesticide. In Groups L and M, rabbits

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received a daily oral dose of commercial lindane (90% a.i.) at 4.21 mg/kg body weight, or commercial methylparathion (35% a.i.) at 1.73 mg/kg body weight for 28 days. Rabbits in Group E received a single oral dose of endosulfan (35% a.i.) at 15.13 mg/kg body weight. These doses were calculated based on the amount of these pesticides that would be ingested by a rabbit fed carnation byproduct freshly sprayed with each compound (Cerón, 1993). The requisite quantity of each of these pesticides was suspended in 5 ml of tap water and given by gavage.

Throughout the study, animals were observed daily for toxic signs. Feed intake and body weight were measured every seven days. Blood samples were collected by venipuncture of the marginal ear vein prior to dosing, 30 minutes after the first dose, on days 7,14,21,28, and 7 days after the last pesticide administration. Four animals of each group were killed at 28 days and liver, brain and peri-renal fat samples were taken. These samples were also obtained from the other three animals 7 days after the last administration (surviving animals in group E were killed at 35 days). All tissue samples were stored at -20^{0}C until analysis.

Hematological parameters were estimated by standard methods used in the Veterinary Clinical Pathology Laboratory of Murcia University (Cerón, 1993). Red Blood Cells (RBC) were counted in an automatic cell counter (Coulter Counter, Coulter Electronics LTD, England), and White Blood Cells by a Neubauer haemocytometer. Packed Cell Volume (PCV) was determined using the microhematocrit method. Hemoglobin concentration (Hb) was measured by the cyanomethemoglobin technique. Total plasma protein concentration was measured using an Atago SPR-T2 refractometer (Atago instruments, Japan).

Plasma urea and activities of plasma alkaline phosphatase (ALP, EC. 3.1.3.1.) were measured by Merck diagnostic kits (Darmstadt, Germany). Aspartate aminotransferase (AST, EC. 2.6.1.1.) and alanine aminotransferase (ALT, EC. 2.6.1.2.) were similarly measured by commercial ITC diagnostic kits (Barcelona, Spain). Acetylcholinesterase (AChE, EC. 3.1.1.7.) activity was measured according to the Ellman method (1961) with the modifications made by Cerón (1993).

Pesticides were extracted from tissues using the method of Mes et al. (1980) as modified by Luna et al. (1993). Residues of lindane and endosulfan were determined by gas chromatography in an Autosystem Perkin Elmer chromatograph under the following conditions:
- Column: 30 m x 0.25 mm i.d. silica column packed with 0.25 m of SPB-5.
- Detector: electron capture, ^{63}Ni source.
- Carrier gas: Nitrogen at a flow rate of 60 ml/min.
- Operating temperatures: injector, 250°C; detector, 300°C and column 250°C.

A Sigma 4B Perkin Elmer chromatograph was employed to determine methylparathion residues under these conditions:
- Column: 30 m x 0.75 mm i.d. glass column packed with 1 m of SPB-5.
- Detector: Termionic nitrogen phosphorus-specific detector (NPD).
- Carrier gas: Nitrogen at a flow rate of 15 ml/min.
- Operating temperatures: injector, 250^{0}C; detector, 250^{0}C and column 200^{0}C.

Recovery experiments with lindane and methylparathion added to different tissues and following the procedure described above indicated a recovery of 97% and 96.8% respectively. Recoveries with endosulfan I, II and endosulfan sulfate were of 86.7%, 88.4% and 85.5% respectively.

Analysis of data was performed using a Statgraphics program. Multiple variant analysis was utilized to compare changes of parameters over time within groups (feed intake and body weight were compared between groups). A level of $p<0.05$ was considered significant (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Acute poisoning occurred in three rabbits 10-15 minutes following administration of endosulfan and progressed to death within 15-20 minutes. Clinical signs observed during this time were clonic-tonic convulsions, fine generalized tremors and charging motions against the cage walls which progressed to lateral recumbency accompanied by tremors and running movements of the forelimbs. Two other animals also developed signs within 15-20 minutes characterized by generalized tremors, depression leading to sternal recumbancy and forelimb extention. These animals died two to three hours following the clinical onset. The remaining rabbits in the group ($n=2$) developed prostation, watery diarrhea and anorexia which lingered 3-4 days after exposure. These rabbits progressively recovered with the exception of a complication involving left forelimb rigid extension that was persitent in one rabbit during the experimental course. No change in body temperature appeared in the animals of this group. The clinical signs observed in the rabbits were similar to descriptions previously reported for acute cases of endosulfan toxicoses (Gupta and Chandra, 1975; Nicholson and Cooper, 1977). Due to the acute poisoning, no more endosulfan doses were given to this group. Neither clinical signs of poisoning nor deaths were found in any animals of other groups.

Animals that survived after ingestion of endosulfan had lower values of feed intake and weight gain than the control rabbits (Table 1). The rabbits gained no weight and actually lost weight during the first week following ingestion. Decreased weight gain in rabbits has been described with other organochlorine pesticides (Steinek et al., 1982). Rabbits treated with lindane and methylparathion gained less weight than control rabbits comparing day 0 with day 28. Feed intake was also less than in the control group. Decreased feed intake could explain the significant decreased weight gains found in treated animals. Prolonged exposure to low levels of pesticides can interfere with metabolic processes and not allow the normal utilization of nutrients, by motility alterations or pathological lesions in the gastrointestinal tract (Shull and Cheeke, 1983).

Red Blood Cells (RBC), Packed Cell Volume (PCV) and Hemoglobin (Hb) were decreased (PCV and Hb with $p<0.01$) in animals that survived one week after the administation of endosulfan, followed by a recovery to normal values during the remaining course of observation (Table 2). Hematological changes in the rabbits treated with lindane and methylparathion were minimal.

Table 1. Feed intake and body weight

Feed Intake (g)

	Days	0-7	7-14	14-21	21-28	28-35	Mean (0-28)
Group	C	907±25	1078±30	1221±24	1090±31	1200±27	1074.0±64.4
Group	E	133±15	200±21	225±18	240±23	290±25	199.5±23.6*
Group	L	778±20	764±12	771±21	780±27	950±30	733.2±36.3*
Group	M	806±23	785±15	985±21	964±34	1185±24	885.0±52.0*

Body Weight (kg)

Days		0	7	14	21	28	35	Mean
G.	C	2,3±0.4	2,6±0.6	2,7±0.6	3,0±0.3	3,2±0.3	3,3±0.1	2,7±0.5
G.	E	2,5±0.5	2,0±0.3	2,2±0.6	2,2±0.4	2,3±0.3	2,4±0.3	2,2±0.5*
G.	L	2,4±0.8	2,4±0.9	2,5±0.8	2,6±0.9	2,7±0.1	2,8±0.2	2,5±0.4*
G.	M	2,2±0.4	2,3±0.3	2,5±0.5	2,7±0.5	2,7±0.3	2,9±0.3	2,5±0.3*

* $p<0.001$

The significant biochemical test results are shown in Table 3. Plasma urea concentrations did not change in animals treated with any pesticide. Apparently the doses employed in this study were not high enough to produce renal damage, which would have resulted in high urea concentrations. Methylparathion did not decrease acetylcholinesterase activity in plasma until the twenty-first day of exposure. The decreases in AchE determined in our study did not reach levels lower than 25% of normal activity, which is the degree of depression thought necessary before clinical signs became apparent (Meerdink, 1989). Some recovery of cholinesterase activity occurred by 7 days after the last methylparathion administration.

In animals treated with lindane and endosulfan, a significant increase of plasma ALP and ALT activities were found immediately following initiation of dosing. The early increase of ALT and ALP activities suggests a toxic effect on the liver with hepatocyte damage (elevated ALT) and bile duct alterations (elevated ALP) (Raina et al., 1990; Bush, 1991). A significant increase of AST was observed in all samples taken in these groups. The increase of AST activity could be a result of general tissue damage, particularly liver, muscle and heart (Boyd, 1983). In the rabbits treated with methylparathion, there was an increase in ALP activities throughout the study, but no significant changes were found in AST or ALT activities. Raina et al. (1990) explained that organophosphorus insecticides have been shown to cause a pronounced increase in the permeability of artificially prepared lipid membranes and that this cellular damage may release phosphatases into the systemic circulation. Variations in ALP, AST and ALT could be influenced by decreased feed intake, since this fact can produce a liver damage (Blood and Radostits, 1992).

No endosulfan residues were detected in animals of the control group or the endosulfan treated rabbits that survived for 35 days. Compared with other organochlorines, endosulfan is of short persistence in warm blooded animals having little accumulation in tissues because most (80%) of its metabolites are hydrophilic (Smith, 1991). In the five rabbits that died after endosulfan

Table 2. Hematological parameters in control (Group C) and treated rabbits (Group E=endosulfan, Group L=lindane, Group M=methylparathion).

		Time						
		0	30 min	7 d	14 d	21 d	28 d	35d
Red blood cells (mll/mm3)	Group C	5.8±0.0	5.9±0.1	5.8±0.0	6.2±0.0	5.8±0.1	6.0±0.1	6.0±0.1
	Group E	6.0±0.0	6.0±0.2	5.1±0.3	5.4±0.1	5.5±0.1	5.5±0.1	5.7±0.1
	Group L	5.8±0.2	5.7±0.1	6.2±0.3	6.1±0.2	6.2±0.2	6.3±0.1	6.3±0.1
	Group M	5.9±0.1	5.5±0.2*	6.1±0.1	6.3±0.2	5.5±0.0	5.7±0.1	5.9±0.1
Packed cell volume (%)	Group C	37.1±0.3	37.6±0.4	36.97±0.3	37.2±0.7	38.1±0.7	38.7±0.4	39.3±0.6
	Group E	37.4±0.4	38.5±1.6	25.50±0.5***	29.0±1.0***	32.0±1.0***	34.0±2.0	36.0±1.0
	Group L	38.0±0.5	38.8±1.5	35.00±0.7*	36.1±1.1*	34.8±0.6*	36.0±0.8*	38.0±0.6
	Group M	37.1±0.3	34.4±0.6*	35.57±0.5*	36.0±0.4*	37.2±0.6	37.1±0.6	37.0±1.1
Hemoglobin (g/dL)	Group C	13.1±0.2	12.8±0.0	14.2±0.5	13.8±0.3	13.5±0.3	13.3±0.3	12.8±0.8
	Group E	13.9±0.2	14.2±0.8	9.9±0.7**	10.6±0.4**	11.0±0.5**	11.5±0.5**	12.0±0.8
	Group L	12.8±0.4	13.1±0.6	13.7±0.2	11.9±0.3	13.5±0.5	13.1±0.7	13.2±0.7
	Group M	13.1±0.2	14.9±0.4	14.2±0.8	13.6±0.6	13.1±0.2	13.0±0.4	13.5±0.2

Values after 30 min in endosulfan group are mean of two animals.
* $p<0.05$
** $p<0.01$
*** $p<0.001$

Table 3. Biochemical parameters in control (Group C) and treated rabbits (Group E=endosulfan, Group L=lindane, Group M=methylparathion).

		Time						
		0	30 min	7 d	14 d	21 d	28 d	35 d
Ache (μmol/min/mL)	Group C	0.42±0.01	0.42±0.01	0.40±0.01	0.40±0.01	0.39±0.01	0.41±0.01	0.41±0.01
	Group E	0.42±0.01	0.40±0.01	0.40±0.02	0.42±0.02	0.40±0.01	0.41±0.01	0.40±0.01
	Group L	0.40±0.01	0.42±0.01	0.41±0.01	0.40±0.01	0.40±0.01	0.40±0.01	0.41±0.01
	Group M	0.40±0.01	0.39±0.01	0.38±0.02	0.38±0.02	0.33±0.01*	0.32±0.01*	0.36±0.01*
ALP (U/L)	Group C	139.7±7.3	145.0±8.9	132.8±11.0	155.4±6.6	139.1±6.2	160.0±4.0	159.0±6.6
	Group E	150.7±6.9	245.4±25.3**	156.9±8.2	165.2±22.3	134.7±5.3	140.0±5.0	130.0±10.1
	Group L	160.0±7.7	242.3±14.6**	201.0±16.8**	154.6±12.0	166.8±11.7	170.0±6.5	165.0±7.6
	Group M	130.0±12.1	194.6±31.1*	156.6±16.2	157.3±8.7	214.8±24.6*	223.8±24.3*	200.0±20.8*
AST (U/L)	Group C	21.0±1.1	16.5±1.3	21.6±2.3	22.9±2.8	21.0±2.2	22.0±1.6	24.0±3.7
	Group E	23.1±1.6	100.1±13.3***	101.0±4.0***	102.8±21.5***	109.2±10.9***	97.0±12.0***	90.0±4.0***
	Group L	22.5±2.3	76.0±10.1***	70.2±4.5***	70.3±6.5***	62.0±5.2***	69.8±6.1***	60.0±3.2***
	Group M	21.0±1.4	22.8±6.0	25.7±7.8	26.4±7.4	25.7±6.7	26.0±0.8	23.0±2.0
ALT (U/L)	Group C	52.0±4.7	60.5±7.7	55.8±4.5	59.1±4.6	51.0±3.8	49.2±1.7	50.0±7.6
	Group E	59.0±2.8	92.5±10.5*	73.2±3.0	57.1±13.9	66.0±1.0	70.0±10.2	60.0±7.0
	Group L	56.1±2.1	110.6±14.6***	74.1±2.4***	48.9±9.7	44.2±7.2	50.0±5.8	52.0±1.5
	Group M	56.7±1.6	57.7±10.0	64.9±14.4	55.8±11.6	51.1±6.8	39.9±7.9	44.0±5.5

Values after 30 min are mean of two animals.
* $p<0.05$
** $p<0.01$
*** $p<0.001$

administration, endosulfan I residues were found in liver (1.53-2.79 ppm) and brain (1.59-2.26 ppm), but no detectable amounts appeared in peri-renal fat. Only endosulfan I was found in liver and brain, and no endosulfan II or sulfate were detected. Gupta (1978) reported that endosulfan II disappeared rapidly from brain as well as from plasma and that endosulfan I was assumed to be more stable.

Animals of the control group had lindane residue levels in fat between 1.90 and 3.57 ppm. The existence of residues in control animals has also been seen by Davey and Johnson (1974). Rabbits treated with lindane and sacrified at 28 days had levels between 38.51 and 61.85 ppm of this compound. Lindane residues in fat of Group L animals killed 7 days after the last dose of lindane showed lower levels (12.31-21.52) when compared with animals sacrified on day 28. Decreases in lindane residues (approximately 90%) of fat from sheep and rabbits that were removed from lindane exposure for one month has been decribed by other authors (Collet and Harrison, 1968; Mosha and Gyrd-Hansen, 1986). No residues of methylparathion were found in fat, liver and brain of animals that ingested this pesticide nor in the control group. The lack of residues in general of organophosphorous compounds in animal tissues is probably due to their fast metabolism and hydrolysis (Biehl and Buck, 1987).

In conclusion, some potential harmful effects of pesticide exposure representing agricultural byproducts contamination have been identified. Continual monitoring for these insecticides in agricultural byproducts is desirable to avoid deleterius effects or contamination of the food chain.

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