1030

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The Effects of the Subchronic Feeding of the Organophosphate Famphur to Rats

The Effects of the Subchronic Feeding of the Organophosphate Famphur to Rats. BLACK, W. D., VALLI, V. E., CLAXTON, M. J., AND MARCEAU-DAY, M. L. (1979). *Toxicol. Appl. Pharmacol.* 50, 167–170. Famphur was mixed in the feed and fed to 190 rats at three dosage levels: 1, 3, and 25 ppm. The rats were killed after 45 or 90 days exposure and one group receiving 25 ppm Famphur for 90 days was maintained on a Famphur-free diet and killed after 42 days. Famphur did not cause a significant change in food intake or weight gain of the rats. It did cause a marked reduction ($p < 0.05$) in plasma cholinesterase activity at 25 ppm in all groups except male rats at 45 days, in whole blood cholinesterase at 3 and 25 ppm, and in brain cholinesterase at 25 ppm. Plasma glutamic oxalacetic transaminase, glutamic pyruvic transaminase (GPT), and alkaline phosphatase activities were normal in all animals except for a slight but statistically significant ($p < 0.05$) increase in GPT activity of female rats in the 3- and 25-ppm 90-day treatment group. Hematological and postmortem examinations revealed no abnormal findings except for a significant ($p < 0.05$) increase in the packed cell volume of female rats exposed to 3 ppm for 45 days.

Famphur (Famphos, Warbex) is the topical systemic phosphorothioate insecticide, *O,O*-dimethyl *O*-[*p*-(dimethylsulfamoyl)-phenyl] phosphorothioate, used for control of lice and warbles (*Hypoderma* sp.) in cattle (Drumond *et al.*, 1970) and reindeer (Kurzaw, 1970). It caused marked depression of cholinesterase activity in blood of cattle (Palmer, 1971) and reindeer (Erne and Nordkvist, 1970). In addition, Brahman cattle are more susceptible than Hereford cattle to the toxic effects of this agent (Palmer, 1971).

In the last 15 years the published literature reveals little about the toxic effects of this chemical in laboratory animals despite the fact that it is widely used for insect control in livestock destined for human consumption. Gatterdam *et al.* (1967) described the absorption, metabolism, and excretion of Famphur in sheep and calves. In addition these authors reported an oral LD50 of 27 mg/kg in mice. Smith and Goulding (1970) observed cholinesterase inhibition in rabbits exposed to Famphur and in ectoparasites (ticks and mosquitoes) feeding on these animals. The cholinesterase inhibiting properties of the organophosphates have been recognized as their major acute toxic effects for many years (Adrian *et al.*, 1947). The long-term effects of these compounds are less clearly understood.

The purpose of this study is to examine the effects of feeding this compound to rats for up to 90 days, at three dosage levels, to follow cholinesterase activity in plasma, whole blood, and brain, and to compare these findings with plasma enzymes, body weight gain, postmortem findings, and food consumption.

METHODS

Animals and animal care. One hundred and ninety male and female Wistar rats, weighing approximately 100 g, were used in this study and housed individually at the University of Guelph Central Animal Facility in hanging type rat cages with free access to food and water. The rats were randomly divided into four groups of equal sexes according to drug treatments, control (50 animals), 1 ppm (40

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9276

animals), 3 ppm (40 animals), and 25 ppm (60 animals). Five male and five female rats were selected from the control group and used as precontrols for the examination of hematological parameters. A further five male and five female rats in each treatment group were sacrificed after 45 days of exposure to the chemical and 15 male and 15 female rats in each group were sacrificed after 90 days of exposure. From the 25-ppm group an additional 10 male and 10 female rats were maintained on a diet free of chemical for an additional 42 days before sacrifice.

Feed preparation. The animals were fed a mash prepared by grinding commercial rodent laboratory chow cubes with a hammer mill. Feed was prepared, fresh weekly, by dissolving technical grade Famphur (98.7%) obtained from Cyanamid of Canada, Scarborough, Ontario in acetone to make a 1 mg/ml stock solution which was mixed with an aliquot of rat chow. This concentrated mixture was mixed with larger quantities of reground material to give the following concentrations of Famphur: 25, 3, 1 and 0 ppm. The rats were fed the control and experimental diets *ad libitum* and the amount consumed was determined during Weeks 1, 6 and 13.

Sample collection and analysis. Blood was obtained from the orbital sinus of rats, under light ether anesthetic, at 1 to 2 days prior to the sacrifice. About 1.5 ml of blood was collected in heparinized tubes and stored in ice. A 0.4-ml aliquot of the blood was frozen (−20°C) for whole blood cholinesterase determinations; plasma was removed from the remaining heparinized blood and stored frozen (−20°C) for enzyme determinations.

At the termination of drug exposure the rats were killed with ether. The brain samples were removed, placed in ice-cold saline, and transported to the laboratory for immediate analysis. Brain tissue was minced finely with scissors and an aliquot transferred to a chilled homogenizer tube where it was diluted 1:10 with ice-cold saline, homogenized briefly, and returned to the ice bath.

Cholinesterase determinations were carried out on the fresh brain tissues, on frozen whole blood and frozen plasma using the technique of McOsker and Daniel (1959).

Commercial kits obtained from General Diagnostics, Toronto, Ontario were used for determination of the following plasma enzymes: GOT (glutamic oxalacetic trasaminase); GPT (glutamic pyruvic transaminase); AP (alkaline phosphatase). The enzyme determinations were carried out on plasma maintained frozen until assayed.

Hematology. Blood samples, collected in EDTA at the time of bleeding were used for determination of packed cell volume, total white blood cell, and differential counts. Standard manual methods were used for all hematological determinations.

Postmortem examination. Gross postmortem examination was carried out on all rats killed in the experiment. In addition the brain, spinal cord, thyroid, heart, liver, kidney, adrenals, and gonads were removed and placed in buffered formalin fixation solution. These tissues were sectioned, mounted on glass slides, stained with hematoxylin and eosin, and examined for histopathological lesions.

Statistics. Statistical analysis using analysis of variance and calculated least significant difference was carried out as described by Steel and Torrie (1960). Student's t determinations were calculated using the methods of Alder and Roessler (1977).

RESULTS AND DISCUSSION

Rats exposed to Famphur at the levels used in the experiment exhibited no reduction in the rate of body weight gain ($p > 0.05$) or food intake. The rats that were receiving 25 ppm for 90 days were reluctant to eat the food once the chemical was removed at the start of the 42-day period in which they were maintained on Famphur-free diet.

Plasma cholinesterase activities (Table 1) were significantly depressed ($p < 0.05$) by the diet containing 25 ppm Famphur. The diets containing 1 and 3 ppm also depressed plasma cholinesterase in female rats after 90 days exposure. Control male cholinesterase activities were very low in the 45-day experiment, lower in fact than the 1-ppm group. This may reflect a chance observation with a small number of animals, since it is inconsistent with the observation of the other control groups.

Whole blood cholinesterase activities were significantly depressed ($p < 0.05$) by 3 and 25 ppm. Significant depression of brain cholinesterase was observed at 25 ppm, but not at 3 ppm.

The increase in whole blood cholinesterase activities noted after the 42-day recovery period in the male and female rats may indicate a rebound phenomenon following the extended period of cholinesterase depression. It may also reflect an increase in enzyme activity due to age since the control whole blood cholinesterase activities increased con-

TABLE 1

WHOLE BLOOD, PLASMA, AND BRAIN CHOLINESTERASE ACTIVITIES OF RATS FOLLOWING EXPOSURE TO FAMPHUR IN THE DIET[a]

Day	Sex		Exposure (ppm)			
			Control	1	3	25
45	M (5)	WB[b]	33.0 ± 5.9	31.9 ± 6.6	21.4 ± 3.4[c]	19.9 ± 5.7[c]
		P	12.5 ± 2.9	19.7 ± 6.7[c]	16.9 ± 2.6	8.3 ± 3.4
		B	544 ± 142	582 ± 88	509 ± 52	313 ± 38[c]
45	F (5)	WB	42.4 ± 9.8	39.6 ± 5.6	30.1 ± 4.4[c]	21.2 ± 1.6[c]
		P	53.8 ± 13.4	41.0 ± 9.3	47.8 ± 13.1	20.2 ± 5.6[c]
		B	488 ± 96	532 ± 123	492 ± 132	248 ± 35[c]
90	M (15)	WB	37.6 ± 5.3	33.2 ± 6.3	31.9 ± 8.5[c]	20.5 ± 7.8[c]
		P	20.2 ± 3.9	17.9 ± 2.6	17.9 ± 4.8	10.2 ± 4.7[c]
		B	403 ± 59	410 ± 44	407 ± 36	264 ± 36[c]
90	F (15)	WB	49.5 ± 7.1	44.4 ± 11.1	42.3 ± 8.9[c]	24.2 ± 7.1[c]
		P	66.9 ± 17.2	51.7 ± 16.9[c]	54.7 ± 11.6[c]	31.9 ± 11.4[c]
		B	407 ± 44	405 ± 62	393 ± 50	204 ± 31[c]
132[d]	M (10)	WB				42.7 ± 4.6[e]
		P				17.9 ± 3.2
		B				396 ± 4.2
132[d]	F (10)	WB				59.4 ± 5.5[e]
		P				63.9 ± 13.8
		B				382 ± 6.3

[a] Values are means ± SD (μmol SH liberated/g wet tissue/hr at 37°C). Number of animals is given in the parentheses.
[b] WB, whole blood; P, plasma; B, brain.
[c] Significantly different from control ($p < 0.05$).
[d] 90-day chemical exposure followed by 42-day recovery period.
[e] Significantly different from the 90-day control ($p < 0.05$).

sistently throughout the period of the study (Table 1). Brain and plasma cholinesterase were not significantly greater than these enzyme activities in the control 90-day group.

Studies of the plasma GOT and AP activities in the rats treated with Famphur revealed no significant difference from the controls. A slight increase in plasma GPT (μmol pyruvate/min/liter at 37°C) was observed at 90 days in the female rats, 7.29 ± 2.16 (3 ppm) and 8.03 ± 2.68 (25 ppm), which was significantly different than the controls 5.80 ± 1.07 ($p < 0.05$). The changes, while not great, appeared to be dose dependent.

Hematological studies did not reflect any changes in either the total white blood cell count or differential count. An altered packed cell volume was observed in the 3-ppm female animals at 45 days; however this was not observed in any other group and could not be correlated to any other observable difference. Neither the physical appearance of this group of animals nor the other parameters studied suggest that this was a toxic reaction.

Postmortem examinations and histopathological examination of tissues did not reveal any lesions attributable to Famphur at the dosages administered.

In summary, the administration of Famphur caused a marked and consistent depression of cholinesterase enzymes. It did not produce detectable changes in plasma GOT or alkaline phosphatase enzymes, but

a slight increase in GPT activity. No other significant changes could be attributed to Famphur in this study.

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