EXPERIMENTS ON THE TOXICOLOGY OF 2 : 3 : 5 : 6-TETRACHLORONITROBENZENE

By G. A. H. Buttle and F. J. Dyer

From the Department of Pharmacology, School of Pharmacy, University of London

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The experiments described were carried out to determine the toxic effects of 2:3:5:6-tetrachloronitrobenzene (T.C.N.B.) when given in varying levels in the diet of mice and rats. This material is being used for the treatment of potatoes to prevent sprouting and rotting, and it is important to determine whether any small quantity which may remain on the potatoes after they have been stored could be harmful.

A. Chronic Toxicity in Mice

Experiment No. 1. Two groups of 12 mice each received a diet containing the substance over a period of 31 days. After a preliminary trial, dose-levels of 672 mg. and 67·2 mg./50 g. of diet were chosen and a third control group of 12 mice received 50 g. of unmedicated food daily. The food consumed by each group was determined by difference between the amount of food offered and the amount left over. Table I shows the total weight of food consumed by each group during the 31-day period, the average weight of food consumed daily per mouse and the average weight of drug consumed daily per mouse for each group.

TABLE I

Summary of food and drug consumption during 31-day mouse feeding tests

Dosage mg. per 50 g. of Food	Total Food Consumed per Group (g.) in 31 Days	Average Daily Food Consumed per Mouse (g.)	Average Daily Drug Consumed per Mouse (mg.)
672 mg.	898	2·4	32·4
67·2 mg.	1083	3·0	4·1
Control—Nil	1251	3·4	—

The average weights of the medicated and control mice were determined at the start of the experiment and at the end of the 31-day feeding period. Table II gives the values found and the calculated average gains in weight.

TABLE II

Average body-weight changes

Dosage mg. per 50 g. of Food	Average Body Weight in Mice		Average Gain in Weight (g.)
	At Start (g.)	At Finish (g.)	
672 mg.	18·1	18·1	0
67·2 mg.	19·1	30·0	10·9
Control—Nil	21·6	32·3	10·7

The mice receiving the dosage of 672 mg. per 50 g. of food showed no gain in body-weight during the 31-day period; whereas the animals receiving 67·2 mg. appeared to gain weight at a rate approximately equal to that of the control group.

No unfavourable symptoms of toxicity were observed in either of the experimental groups. The animals were killed at the end of the 31-day feeding period and post-mortem examination revealed no detectable changes in the organs of either of the groups.

It, therefore, appears that 4·1 mg. of tetrachloronitrobenzene administered daily for 31 days to mice produces no detectable changes and has no adverse effect on the rate of growth as compared with that of the controls. A daily dose of 32·4 mg. definitely retards growth, but produces no other symptoms of toxicity and no detectable tissue damage.

Experiment No. 2. A group of 24 mice, weighing about 25 g. each received a diet containing 13·68 g. of the drug per 100 g. of food in an attempt to give each mouse 570 mg. per day. Due to reduced food intake in comparison with the controls, the treated mice took only 250 mg. of drug per day. A second group of 12 mice weighing approximately 19 g. each served as non-medicated controls. 5 of the 24 experimental mice died on the third and fourth days, and the experiment was therefore discontinued on the fourth day.

Post-mortem examination of the 5 dead animals showed fatty degeneration of the liver, and some fatty changes were also observed in the histological sections of the spleen and kidney.

The results of these two experiments are summarised in Table III. For simplicity the daily dose of drug has been converted to terms of a 20 g. mouse.

TABLE III

TOXICITY TO MICE

Daily Dose mg./20 g. Mouse	Average Weight of Food Consumed per Mouse per Day (g.)	Number of Days on Diet	Results
200	1·8	4	5/24 died of fatty degeneration of liver.
35	2·4	31	Growth completely inhibited. No other changes observed.
4·3	3·0	31	Normal growth. Average weight increase of 10·9 g. No other changes observed.
Controls	3·4	31	Average weight increase of 10·7 g.

In each of the last three groups 12 mice were used and in the first group there were 24.

It appears from these results, that the daily consumption by mice of 4·3 mg. per 20 g. of body-weight (215 mg./kg.), has no harmful effects when given over a period of 31 days. A daily dose of 35 mg./20 g. of body-weight, or 1750 mg./kg., inhibited growth but caused no other

toxic effects. A daily dose of 200 mg./20 g. of body-weight or 10,000mg./kg., caused the death of 5 out of 24 mice in 4 days.

B. Chronic Toxicity in Rats

The following experiment was carried out to determine the possible chronic effect in rats. For this purpose weanling albino rats received various amounts of drug in their diet for a period of 10 weeks. Observations were made on growth rate, and symptoms of toxicity such as weakness and diarrhœa were looked for. 30 animals were uniformly distributed in regard to litter mates, sex and initial body-weight into 3 groups of 10 rats each, with a further 10 for the control group. The animals were fed *ad lib.* and weighed every 2 days. The dosages for the medicated groups were 4 mg. in Group A, 20 mg. in Group B and 100 mg. in Group C per 5 g. of diet. Group D was the control.

The amount of food consumed by each group was determined, and the average dose consumed by each rat was calculated. The changes in body-weight over the 10-week period for the 4 groups and the corresponding amounts of food and drug consumed by the rats are summarised in Table IV.

TABLE IV

	GROUP			
	A	B	C	D
Average daily food consumption, g.	8·5	8·5	2·0	8·5
Drug administered, mg. :—				
Per 5 g. diet	4·0	20·0	100·0	0
Mean daily intake	6·8	34·0	40·0	0
Per kg. body-weight per day*	57	400	1111	0
Mean body-weight of Rats, g. :—				
At end of week No.—				
0	41	39	41	39
1	36	34	31	34
2	46	44	28	53
3	74	47	(28)	76
4	87	60	(28)	90
5	108	69	†	110
6	126	80	†	124
7	143	95	†	140
8	161	103	†	154
9	176	118	†	172
10	197	131	†	196
Average change in weight	+ 156	+92	− 13	+ 157

* Calculated from the mean of initial and final body-weights. † All dead.

No deaths occurred among the rats in the Groups receiving 57 mg. and 400 mg. of drug per kg. of body-weight per day. 9 of the 10 rats in Group C receiving 1111 mg. per kg. per day died by the end of 3 weeks and the remaining rat died in the fifth week.

It will be noted that the rats in Group A, taking 57 mg./kg. of body-weight per day, gained weight at the same rate as the control Group D. Group B, which consumed about 400 mg./kg. per day, gained at a slower rate than the control animals and their total gain in weight was

only 67 per cent. of that of the control group. The animals of Group C receiving 1111 mg./kg. quickly lost weight and all were dead by the fifth week.

No toxic symptoms were shown by the animals of Groups A and D. At the dosage of 400 mg./kg. per day the rats showed no changes other than a staining of the fur on the belly indicating excretion of a pigmented substance in the urine. Those in Group C receiving 1111 mg./kg. of body-weight showed very obvious toxic symptoms. At the end of one week the animals had stained fur, some had diarrhœa and all appeared weak and sickly. All died by the end of the fifth week.

In summary, these experiments indicate that the oral ingestion of 6·8 mg. of tetrachloronitrobenzene daily by young rats, or an approximate dose of 57 mg./kg./day for 10 weeks, produces no reduction of growth or toxic symptoms. A dose-level of 34 mg./day or 400 mg./kg./day caused a diminished rate of growth, but no other toxic symptoms and no deaths, but a dose-level of 1111 mg./kg./day produced a rapid loss of weight and the death of all animals within five weeks.

C. Inhalation Tests

It was considered desirable to test the possible hazards arising from the inhalation of the powder, in order to assess the risks to which workers might be exposed while engaged in dusting potatoes and inhaling air heavily laden with the dust.

Six young rats weighing from 40 to 50 g. were divided into 2 groups so that litter mates were present in each group. All animals were subjected to a stream of air blown from an air pump for a period of half-an hour, 3 times daily for 10 days. The rats in the first group served as controls and were exposed to pure air only. The animals in the second group received air which was blown over finely powdered tetrachloronitrobenzene. The increase in weight of the treated rats was similar to that of the controls and the treatment with tetrachloronitrobenzene dust appeared to be without effect.

D. Tests in Rabbits

Experiments were also carried out to test the possible effect of irritation of the eye and skin of rabbits.

No untoward reactions or symptoms of irritation were observed following instillation of the material into the eyes of rabbits each day for one week.

The pure compound was rubbed on the shaved skin of rabbits on 2 successive days. Blackening and induration of the skin were observed on the sixth day, but there were no general toxic effects. In further experiments with the material diluted in china clay to a concentration of 20 per cent. and 2 per cent. there was slight discolouration but no

induration. It appears that high concentrations of the substance caused some superficial change in the rabbits' skin, but no general effects.

Conclusions

The experiments show that a daily dose of 215 mg./kg. of body-weight for mice and 57 mg./kg. for rats produces no ill-effect. On the other hand, dosages of 1750 mg./kg. of body-weight in mice, and 400 mg./kg. in rats cause an inhibition of growth rate.

Experiments on pigs Scorgie[1] indicate that the toxicity of the substance in these animals is of the same order as that in rats. So far as the work goes, therefore, there is no evidence of an increase in toxicity in the larger animals. How far the findings can be applied to effects of tetrachloronitrobenzene on man is at present a matter for speculation.

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Reference

1. Abrams, Scorgie and Willis, *Vet. J.* (in the Press).