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Concentration and Toxicity of Trifluralin in CD-1 Mice, Presented Intragastrically or Intraperitoneally

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In considering factors which can influence toxicity in experimental administration of compounds, differences associated with routes of administration receive frequent attention; those which might be attributable to differences in the concentration at which any given dose might be presented tend to be considered less often. Further, such reports have generally dealt with aqueous solutions. The *parenteral* route is usually found to produce toxic effects at a lower dose and in a shorter time than *per ora* presentation. (See BOYD 1972, and CASARETT and DOULL 1975, for example, for detailed discussions).

The report which follows presents the results of experiments involving administration of the herbicide Trifluralin, dissolved in corn oil, delivered at different concentrations for each dose, either by intragastric intubation or intraperitoneal injection. The investigations produced several surprising results. These included the fact that, in *per ora* administration there were clear cut differences in toxicity such that for each dose which did cause death, there was an inverse relationship between toxicity and concentration. This relationship was not seen in the intraperitoneal treatment groups. Further, there was no apparent difference in LD-50 in the two routes, and the time to death was greater in the intraperitoneally treated animals.

MATERIALS AND METHODS

Random-bred female CD-1 mice were purchased from Charles River Breeding Laboratories (Wilmington, Ma.) at 25gm size. They were housed five animals per cage, provided with water and Wayne Blox Chow *ad libitum*. They were weighed at the start of the experiment. Body weight averaged 36.6gms (N=235) at the time of treatment. Each animal received a single dose of the test compound on a body weight basis, either through intragastric intubation or intraperitoneal injection. Each experimental group consisted of five animals which continued to be housed together. Toxicity was defined as the proportion of treated animals in each group which died within 14 days of treatment. Mortality was recorded daily and representative animals were autopsied. Surviving animals were weighed again at 24 hours, 7 days, and 14 days after treatment.

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Trifluralin is the Eli Lilly brand of α,α,α-Trifluoro-2,6-dinitro-N,N-di-N propyl-p-toluidine. Samples were weighed, melted by gentle heating and dissolved in reagent grade corn oil (Fisher Scientific Co.). For some experiments each dose was weighed and dissolved separately; for others only the highest concentration was weighed, lower concentrations being made up by dilution with the corn oil vehicle. The compound is bright orange in color, has a melting point of 48°C, is insoluble in water, highly soluble in acetone and in the corn oil vehicle used.

Four discrete intubation experiments were peformed and several series were repeated on three occasions. In all, there were 23 concentration-dose combinations, and the modal number of animals per combination was 15. The first two series presented constant volumes of intubate with varying amounts of Trifluralin providing doses of Trifluralin ranging from .375 - 3.0 gm/kg in one series (total volume 3.3 ml/kg) and 2.5 - 10.0 gm/kg in the second (total volume 11.7 ml/kg). Volumes of corn oil (as distinct from solute) were calculated for each different dose. Corn oil controls (3.3 and 11.7 ml/kg respectively) completed the design.

The third and fourth series presented constant volumes of corn oil with varying amounts of dissolved Trifluralin, resulting in varying total volumes of intubate. In one series, doses between 2.5 and 7.5 gm/kg were presented in 3.3 ml of corn oil/kg body weight. The range of intubate volumes was thus 4.5 - 9.1 ml/kg. The fourth series presented doses of .375 - 3.0 gm Trifluralin/kg in 8.33 ml corn oil/kg, resulting in intubate volumes which varied from 8.6 - 10.7 ml/kg.

The replications were of the latter type of experiment (i.e. constant volume of corn oil).

The intraperitoneal experiments consisted of two replications in which constant volumes of corn oil were injected, each containing different doses of Trifluralin. Seven volumes were used, between 3.3 and 23.3 ml/kg. The larger volumes were easily tolerated in the intraperitoneal route. The doses of Trifluralin varied between .375 and 10.0 gm/kg, with corn oil controls in all but two groups. Not all doses were presented in all volumes, but each dose was presented in at least four different concentrations.

RESULTS

Per Ora Route - No differences attributable to the different series could be detected, and the pooled data for mortality in response to treatment are presented in Table 1. At each dose-level which was presented in several different concentrations, mortality was higher in the groups receiving a larger volume of corn oil in the intubate. That is to say, the same dose of Trifluralin was more toxic in more dilute solutions. No mortality occurred at

TABLE 1.

Percent mortality in female CD-1 mice following a single gastric intubation of Trifluralin in corn oil

Volume of Corn Oil Intubated (ml/kg body wt.)	Dose of Trifluralin (gm/kg Body Weight)								
	10.0	7.5	5.0	3.0	2.5	1.5	0.75	0.375	0
< 3.0	-	-	-	(0,0) 0	-	(0) 0	(0) 0	(0) 0	-
3.3	(2) 40	(0,0,0) 0	(1,0,0) 6.7	(1,1,0) 13.3	(0,0,0) 0	(0,0,0) 0	-	-	(0) 0
6.7	-	(2) 40	-	-	-	-	-	-	-
8.3	-	(3,2,1) 40	(1,4,1) 40	(1,2,1) 26.7	(2,2,2) 40	(0,0,0) 0	(0,0,0) 0	(0) 0	-
10.0	-	-	-	-	(3,3,0) 40	(1,1,0) 13.3	-	-	(0,0) 0
11.7	-	-	-	-	-	-	-	-	(0) 0

Legend: Each number in parenthesis is the number killed per group of five animals which constitutes a single replicate.

doses below 1.5 gm/kg. Nearly all mortality occurred within 24 hours of treatment. The few animals which died one or two days later were noticeably unwell at 24 hours following treatment. Orange discoloration of urine and of the fur in the perineal area of treated animals suggested excretion of water soluble metabolites. EMMERSON and ANDERSON (1966) report at least ten colored metabolites of Trifluralin present in the urine of rats treated with the compound. Autopsy revealed orange discoloration of fat and intestine but not caecum. Slight orange-yellow discoloration of the inner surface of the skin and of the stomach was also seen. The discolored tissues suggest the deposition of the compound or lipid soluble metabolites. None of the other organs or tissues showed any grossly pathological signs or any discoloration.

There were no major differences in weight changes during the course of treatment, between different treatment groups or between replicates within each group. Indeed combined weights of all survivors (N=198) at 24 hours (36.9 gm), seven days (36.5 gm) and fourteen days (36.8 gm) were remarkably constant.

Intraperitoneal Route - The data for frequency of mortality are presented in Table 2. In all but one of the 34 dose-volume combinations (3 gm Trifluralin/kg in 23.3 ml/kg corn oil) there was good agreement between replicates. For this route of administration there was no relationship suggested between concentration and toxicity. Interpolation suggests an LD 50 of about 4.6 gm Trifluralin/kg body weight. Of the 124 animals which died in treatment, although the majority died within one day (N=54), or two days (N=43), there was considerable variation, some surviving to ten (N=1), eleven (N=4) or even twelve days (N=2) following treatment. The average survival times ($\bar{X} \pm SD$) of those which died was 2.47 ± 2.54 days. Death was generally more rapid at higher doses, (7.5 and 10.0 gm/kg) and within each of the higher doses, death was more rapid at lower concentrations.

In all 34 Trifluralin-treated and all seven corn-oil control groups, there was a weight loss among survivors at 24 hours after treatment. Three control and seven treated groups showed some recovery of body weight by one week after treatment; the remaining groups showed a further weight reduction. At termination of the experiment, 14 days after treatment, 17 groups showed some recovery of body weight; the others continued to lose weight. Continued weight loss was more pronounced at the higher doses at which there were a significant number of survivors (3.0 and 5.0 gm Trifluralin/kg body weight), regardless of the volume of corn oil in which the dose was presented.

DISCUSSION

Our data for toxicity of Trifluralin in the mouse, obtained from injection, are in agreement with published values for oral presentation (WORTH 1970); STECHER *et.al.* 1968, p. 1074). There

TABLE 2.

Percent mortality in female CD-1 mice following a single intraperitoneal injection of Trifluralin in corn oil

Volume of Corn Oil Injected (ml/kg body wt.)	Dose of Trifluralin (gm/kg Body Weight)							
	10.0	7.5	5.0	3.0	1.5	.75	.375	0
3.33	-	-	-	(0,0) 0	(0,0) 0	(0) 0	(0) 0	(0) 0
5.0	-	-	-	(1,0) 10	(0,0) 0	(0) 0	(0) 0	(0) 0
8.33	-	(3,4) 70	(1,0) 10	(0,0) 0	(0,0) 0	(0) 0	(0) 0	(0) 0
11.67	(5,5) 100	(5,5) 100	(3,5) 80	(1,0) 10	(0,2) 20	(0) 0	(0) 0	(0) 0
16.67	(5,5) 100	(4,4) 80	(3,2) 50	(0,0) 0	(0,0) 0	-	-	(0) 0
20.0	(5,5) 100	(5,5) 100	(5,3) 80	(1,0) 10	-	-	-	(0) 0
23.3	(4,5) 90	(5,5) 100	(3,5) 80	(5,0) 50	-	-	-	(0) 0
Average % Killed	97.5	90.0	60.0	11.4	4.0	0	0	0

Legend: Each number in parenthesis represents the number killed per group of five animals.

was no suggestion of enhanced toxicity via the *i.p.* route. It is difficult to estimate an LD 50 for *per ora* administration in our study since toxicity varied dramatically with concentration for any given dose. The phenomenon of increased toxicity with increased dilution has been reported for *per ora* administration of aqueous solutions by several authors for a variety of compounds (FERGUSEN 1962; MOORE *et.al.* 1960; BOYD *et.al.* 1969; BALAZS 1970). Explanations for this phenomenon include solvent toxicity (BOYD 1972), especially when very large volumes are presented (CONSTANTOPOULOS and BOYD 1968), and increase in rate of emptying of the stomach with enhanced intestinal absorption (MOORE *et.al.* 1968). Solvent effects of vegetable oils may include a laxative effect (CASARETT and DOULL 1975, p. 136), but observation of the fecal pellets of treated mice did not suggest any such solvent effect in the present study.

The major finding, of increased toxicity with increasing dilution in the *per ora* administration, could be attributed to enhanced absorption of the compound in larger vehicle volumes while it is transiently present in the gut. This interpretation is consistent with the failure to demonstrate this phenomenon in *i.p.* presentation, since in the latter case the compound continues to be present in the closed abdominal cavity, and rapid absorption is not as crucial for toxicity to become manifest. Other observations also support a somewhat slower, more prolonged absorption from the *i.p.* presentation. These include the greater time to death, and pronounced and prolonged weight loss following *i.p.* presentation but not *per ora* presentation. One might expect a slower uptake of material from an oil solvent in the peritoneal cavity relative to its rate in the gut. Thus all the data are consistent.

In addition to being an interesting demonstration of an inverse relationship between toxicity and concentration of a compound in an oil solvent, these data suggest that not only dose but also concentration should be reported for a meaningful assessment of toxicity of various compounds. Differences in concentration of intubate might well contribute to the wide variations in reported LD 50 for Trifluralin in rats, for example. These values vary from 500 mg/kg (CHRISTENSEN and LUGINBYHL 1974, p. 773), to 3,700 mg/kg (THOMPSON 1973, p. 48), to more than 10,000 mg/kg (WORTH 1970).

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