Low doses of 2,4,5-trichlorophenoxyacetic acid are behaviorally teratogenic to rats

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Summary. Pre-natal exposure of rats to 2,4,5-T has long-term effects on behavior. A test for novelty responding detects abnormalities after exposure to a single dose as low as 6 mg/kg on day 8 of gestation. This is well below doses reported to be morphologically teratogenic, and raises concern for human exposure.

Sanderson and Rogers[2] have shown that doses as low as 7 and 13 mg/kg of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) administered to incubating chicken eggs can produce behavioral abnormalities of increased fear-related activity and learning deficits in the hatched chickens. To assess the possible relevance of these findings to humans it is necessary to extend these studies to a mammalian species, and we now report that 2,4,5-T can cause behavioral abnormalities in the pups of mother rats treated with single doses as low as 6 mg/kg of 2,4,5-T which is only some 2% of the LD_{50} dose for adult rats. It is also considerably less than the lowest doses which have been reported to cause morphological teratogenicity in other strains of rats using 2,4,5-T with similar or higher levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD, contamination; e.g. Khera and McKinley[3] reported increased skeletal anomalies in Wistar rats exposed to daily doses of 2,4,5-T, contaminated with less than 0.5 ppm TCDD, given at doses of 100–150 mg/kg from days 6–15 of gestation but no significant morphological effects were found at lower doses.

A single oral dose of a 0.25 ml maize oil suspension of 2,4,5-T (Sigma), containing 0.03 ppm of TCDD (Australian Government Analytical Laboratories), was administered to pregnant outbred Long-Evans rats on day 8 of gestation. (Day 0 = presence of vaginal plug.) Control animals received 0.25 ml of the vehicle alone. Two experiments were conducted. Experiment A involved control subjects and groups treated with either 25 or 100 mg/kg of 2,4,5-T on day 8 of pregnancy. When significant results were obtained at these doses, a 2nd experiment, B, was conducted using controls and treatment groups given either 6 or 12 mg/kg of 2,4,5-T administered on day 8 of pregnancy. All litters were culled to 8 pups on day 2 after birth, and weaned at 23 days. No significant effects on litter sizes, sex ratios, gestation time, pup weights or gross morphology were found. Although the treatment had no effect on the body weight of the pups, brain weight after perfusion on day 2 of life was found to be significantly elevated in a small, mixed-sex sample of the culled pups which had been exposed to 100 mg/kg of 2,4,5-T (mean brain wt $\pm$ SE was 0.55 ± 0.01 g for 9 treated pups, and 0.49 ± 0.01 g for 7 controls, $0.002 < p < 0.02$, 2-tailed U-test). This could result from 2,4,5-T's ability to cause transient hyperthyroidism[4]. However, no qualitative or quantitative structural differences were detected in the sizes of the cerebral cortex, hippocampus, cerebellum or corpus callosum (measured by S. Singh, Monash University). Also, this affect on brain weight may be transient since no significant differences in perfused brain weight were detected in a sample of adult brains measured after completion of the behavioral testing (6 controls and 6 rats exposed to 100 mg/kg on day 8 of gestation).

Open field testing of the pups was conducted between 65 and 75 days after birth, according to the procedure described by Sjödén and Söderberg[5]. Latency, ambulation, rearing, grooming and defaecation were scored in a 4-min period. Analysis of variance (sex $\times$ dose) of data in experiment A (sample sizes 8–17) revealed significant main effects of sex and dose and interaction between sex and dose for ambulation ($p < 0.05$ for each). T-tests between the control group and treatment groups showed that the significant dose effects were due to an elevation of ambulation in both sexes of offspring of mothers which had been treated with 25 mg/kg 2,4,5-T ($0.01 < p < 0.025$), and in the male offspring of mothers treated with 100 mg/kg 2,4,5-T ($0.025 < p < 0.05$) (see fig. 1). The main effects of sex were in the expected direction (higher ambulation and rearing and lower latency and grooming scores in females). This confirms previous reports by Sjödén and Söderberg[5], but they administered only the 100 mg/kg dose of 2,4,5-T. Indeed, in females the 25 mg/kg dose produced a marked elevation in ambulation; whereas the 100 mg/kg dose had no effect. It is possible that non-specific effects of 2,4,5-T on open field behavior may partially or completely mask effects of higher doses.

In experiment B (sample sizes 20–25 for each treatment group) an interaction between sex and dose was found for ambulation. As no main effects of sex or dose were found for ambulation in this experiment, the effects of the lower doses of 2,4,5-T are not clearly demonstrated in a broad screening test like the open field.

Sjödén and Söderberg[4] reported that the elevated ambulation observed in male pups of females exposed to 100 mg/kg 2,4,5-T was confined to novel environments, and interpreted it as increased exploration. However, they ignored the fact that fear may be the most important factor contributing to this response on the first time that rats are tested in the open field[6-8]. We therefore devised a 'Novelty Test' to score exploration. It has proved to be much more sensitive measure for the behavioral teratogenicity produced by 2,4,5-T. The male rats in experiment A were tested at 5 months of age, and those in experiments B at

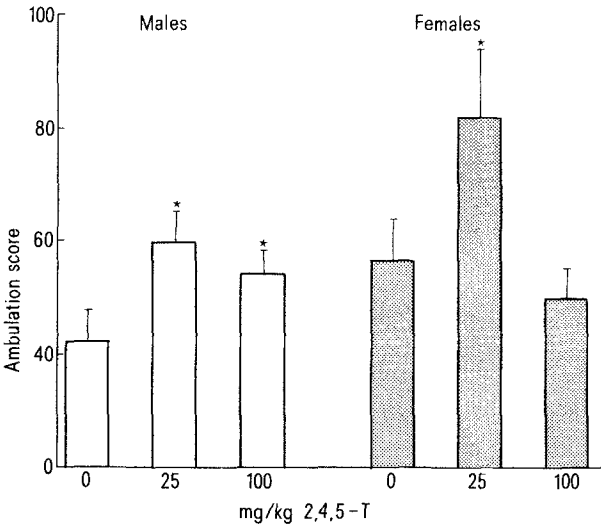

Figure 1. Ambulation scores for rats in experiment A tested in the open field (4-min duration), scored as the number of times the rat crossed with both fore-paws a line of the grid pattern marked on the floor. The doses of 2,4,5-T are those administered to the pregnant mother on day 8. Male and female pups were tested when 65–75 days old. Means + SE are plotted. Asterisk indicate significance of t-test comparisons with controls. * $0.01 < p < 0.025$.

approximately 65 days old. A grey plastic cylinder (6 cm diameter, and 12 cm length) with black, circular, 1-cm stripes was secured in the center of an arena 76 cm × 76 cm with 10 cm high, grey walls, the floor being demarkated with 12.5-cm squares. Over a 2-min test period we scored latency to move from a starting square in a corner of the arena, number of entries with 2 front paws into the zone comprising the 4 central squares containing the novel stimulus, number of times an animal's head (up to its eyes) was inserted into the cylinder, amount of defaecation and the number of times the novel object was sniffed. The latter score served only as an estimate largely correlating with entry into the central zone, since it was not always possible to be certain whether an animal had sniffed the object more than once in rapid succession. Before testing the rats were given at least 3 weeks' experience in their home cages with cardboard cylinders similar to the novel object.

Non-parametric analysis was performed on the data, experiments A and B being analyzed separately (fig. 2). Significant heterogeneity occurred between doses in both experiments A and B for entry into the central zone and sniffing the novel stimulus (Kruskal-Wallis one-way analysis of variance; $0.001 < p < 0.01$ for both experiments A and B). Two-tailed Mann Whitney U-tests were performed between the control and treatment groups. There was a significant reduction of entry into the central zone for pups exposed to all doses of 2,4,5-T, even 6 mg/kg ($p < 0.002$). The differences between experiments A and B in the control scores for this measure are likely to be due to age differences between the pups in each experiment. Although it was a less accurate measure, the number of times the animals sniffed the novel stimulus was also found to be decreased by 2,4,5-T exposure (Kruskall-Wallis analysis of variance; $0.01 < p < 0.02$ for experiment A and $p < 0.001$ for experiment B). Two-tailed U-tests showed that there was a significant decrease in sniffing for pups exposed to 12 and 100 mg/kg 2,4,5-T (see fig. 2 for p-values). Pups exposed to 2,4,5-T prenatally were also less likely to explore the novel object by placing their heads inside it; in experiment A 43% of the controls inserted their heads in the pipe compared to only 13% of the 25 mg/kg and 11% of the 100 mg/kg treatment groups ($p = 0.019$, χ^2 test). There was no significant effect on latency to move or defaecation in this test.

Increased fear rather than exploration is likely to be the explanation for increased ambulation in 2,4,5-T exposed rats tested in the open field. If so, this is consistent with Sjödén and Söderberg's report of increased defaecation in 2,4,5-T treated rats in the open-field situation[5]. The open field is useful in initial screening for behavioral effects, but its lack of specificity makes it difficult to interpret and less sensitive than tests like the Novelty Test.

We cannot say to what extent our findings are due to the action of 2,4,5-T itself or to its highly toxic contaminant TCDD, or possibly to an interaction betwen the 2 chemicals[9]. However, the purity of 2,4,5-T used in this study with respect to TCDD was better than or equal to that of 2,4,5-T commercially available in most countries. Lavy et al.[10] have calculated that a knapsack sprayer can receive up to 1.85 mg/kg 2,4,5-T on the skin surface in a spraying operation lasting 180 min. Although we realize that not all of this amount would be absorbed, it is essential to mention that this is a conservative estimate not taking into account the fact that many individuals are repeatedly exposed, and not including intake via inhalation. Inhalation is an important route of intake when 2,4,5-T is sprayed aerially; this mode of application has been estimated to produce inhaled doses of 2–10 mg/kg/h for adult humans[11]. Given these considerations, we feel that our results of behavioral teratogenicity produced by a single exposure of pregnant rats to as little as 6 mg/kg of the acid form of 2,4,5-T raise concerns about safety factors in 2,4,5-T use by humans, particularly when the ester form is used[12].

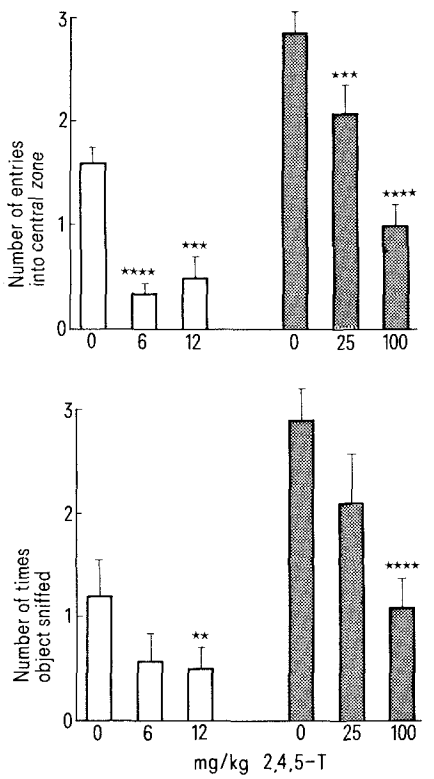

Figure 2. Males tested on a 'Novelty Test'. Means and SE are plotted for number of times the rat entered the central zone containing the novel object and number of times it sniffed the novel object in a testing period of 2 min. Data for experiments A (right side) and B (left side) are plotted separately since the rats in each were tested at different ages. The asterisks indicate significant differences from the appropriate control group, 2-tailed U-tests, ** $0.02 < p < 0.05$, *** $0.002 < p < 0.02$, **** $p < 0.002$.

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