Bull. Environ. Contam. Toxicol. (1989) 43:95–100

Bulletin of Environmental Contamination and Toxicology

# Effects of a Low OP Dose on Seed/Bead Discrimination in the Kangaroo Rat, *Dipodomys*

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The assessment of toxic substance effects on wildlife at the population level must not only include such important parameters as direct lethal effects and reproductive impairment but subtle physiological and behavioral changes that are important to life processes. One such process is the procurement of food in as efficient a manner as possible to maximize net energy gain, avoid predation, and minimize exposure to severe detrimental climatic factors. There are many excellent examples of how toxic substances influence reproductive success in mammals as widely diverse as the California sea lion (Zalophus californianus) (DeLong et al. 1973) and primates (Allen and Barsotti 1976). Important physiological changes have also been demonstrated, primarily related to the induction of the mixed function oxidase system in the liver and these changes become evident in increased breakdown of endocrine steroids and alteration in metabolic rate (Warren et al. 1978, Zepp et al. 1974, Braham and Neal 1974, Ege and Peterle 1985). Behavioral changes reported as a result of toxic substance exposure to mammals include domestic sheep (Van Gelder et al. 1970), and laboratory mice (Spyker et al. 1972, Peterle and Peterle 1971). Chronic exposure to toxic substances may alter the animals susceptibility to diseases and parasites by alteration of the immune system responses as in recent seal (Phoca vitulina) mortality in the Baltic Sea (Reijnders 1986). How physiological and behavioral effects that have been observed in the laboratory influence the animal in the field during normal life processes is unknown. Not only is the impact on the individual important but we do not know how to extrapolate these potential effects to the population level. Over 20 years ago, Warner et al.(1966:245) pointed out "the folly of assuming that lack of evidence is the same as negative evidence". There is a continued challenge to study the subtle physiological and behavioral effects of toxic substances on wildlife because the information contributes to general knowledge and potentially leads to the prediction of effects of toxins as additive stressors to the individual animal and subsequently to possible detrimental population effects (Anderson et al. 1986).

This study, involving the effects of an organophosphate on the discriminatory ability of the kangaroo rat (D. merriami) relates a subtle chronic effect to an important life process; that of gathering food in a hostile environment.

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Kangaroo rats are widely distributed in arid and semi-arid regions of North America where they live in burrows and feed, mainly at night, on scattered seeds found in the soil surfaces (Burns 1958). Soil surfaces are sifted for seeds with the small sensitive forepaws, the manus, and potentially palatable foods are stuffed into fur-lined cheek pouches for later, more detailed examination in the safety of the burrow. Because the animal is exposed to predation and arid conditions while searching on the soil surface for food, it is advantageous to stuff the cheek pouches with highly acceptable high energy food as rapidly as possible. Burns (1958) found that kangaroo rats could select a seed and insert it into their cheek pouch at a modal rate of one seed per 0.2 sec. The ability of the kangaroo rat to discriminate between seeds and beads was tested by Burns (1958) and later by T. F. Isaacs (unpub. rpt. Dept. Biol. Kenyon College, Gambier, Ohio nd). Kangaroo rats picked up and stuffed both seeds and beads into their pouches, but if allowed to examine the contents of their pouches, they rapidly learned and subsequently no longer picked up beads. Isaacs (nd) reported that after three trials all experimental kangaroo rats learned to pick up only seeds.

These behavioral patterns were adapted to test the effect of a single low-dose oral exposure of an organophosphate, methyl parathion, on the ability of wild kangaroo rats to discriminate seeds from beads in laboratory tests. Because the rapid selection and stuffing of suitable seeds is so important a life process and directly related to nutritional status, water balance and exposure to predation, its impairment by a toxic substance could influence survival of the individual organism and ultimately could result in population declines.

## MATERIALS AND METHODS

CERL personnel obtained approximately 30 wild-trapped Merriam's kangaroo rats from a commercial trapper in southern California in mid-March 1987. The rats were held individually in 38 l aquaria with a layer of sand, a container to serve as a burrow and a screen top. Animals were fed ad libitum an ordinary bird seed mixture with sunflower and oat and rye grain added. They were supplied with ad-libitum sources of water. Only red-light was used constantly; temperatures averaged 22 C. At the end of the experimental trials, the rats averaged 32.9g (SD 3.4) with males being slightly heavier. After 2 weeks of acclimation to the laboratory facility, the rats were starved for 12 to 48 hrs and placed in a neutral cage for 5 min with exposure to equal numbers of seeds, hulled rye seeds, and beads, oat pearls (3 x 6mm, D. J. Darice Inc., Strongsville, Ohio). The oat pearls were approximately the same size as the rye seed, but had a shiny coating of imitation nacre. The mixed pile of seeds and beads were placed on a sand substrate in equal numbers of 75 each. After the rat had examined the pile of seeds/beads, stuffed its cheek pouches or after a period of 5 min had elapsed, we placed it in a closed container with the inhalation anesthetic Metofane (R) (Pitman-Moore, Inc., Washington Crossing, N.J.) for 2-2.5 min. Seeds and beads were expelled from the cheek pouches of anesthetized rats, and counted. Rats were returned to their home cages and either immediately fed, or again tested in the neutral cage approximately 2-3 hrs later in each trial. If the rats were fed, they began to eat almost immediately after recovering from the anesthetic, in about 3-4 min.

Following 4 pre-treatment trials, the rats were ranked according to their ability to discriminate seeds from beads and the closest two rats were paired for subsequent tests. We selected a dose of one-half the $LD_{50}$ of methyl parathion for laboratory rats. The range of $LD_{50}$ levels for the laboratory rat is 9-25mg/kg (Meister 1987). LD-50 levels were not obtained prior to dosing because of insufficient numbers of test animals. We administered 5mg/kg in corn oil, given in a single dose by gavage. Control rats received only corn oil. One of each pair was randomly selected to be the treated animal. The final experiment included 22 kangaroo rats, equally divided between treated and control. Because we did not expect any different responses between the sexes, we did not sort or pair the animals according to sex. The experimental group contained 13 males and 9 females. Five of the females and 6 of the males were treated. Two rats were dosed pre-treatment to be certain the level was not lethal and these rats were used to determine ChE brain levels subsequent to the experiment. ChE levels were determined in 2 treated and 2 control animals (A. Fairbrother, methods developed by CERL). Twelve hours after being orally dosed and after 24 hrs of starvation, the rats were again placed in neutral tanks and exposed to the seed/bead mixture. Each rat was given the same mixture used in previous trials and each rat was exposed only to the same individual mixture assigned only to that rat. If seeds were eaten, they were replaced for the next trial so the mix would again contain equal numbers of seeds and beads. The dosed rats were again tested at 24 hrs post-treatment, which was 36 hrs of starvation. Following the final trial, rats were fed and had access to water. Throughout the trials the animals all had access to water when in their home cages. Approximately one year later (March 1988), the same rats were used in an additional test of seed/bead discrimination. Methods were similar to the original trials except the rats were dosed with 7.5mg/kg methyl parathion and the behavioral tests were conducted 2 hours post-treatment rather than 12 and 24 hours post-treatment as in the initial test.

## RESULTS AND DISCUSSION

Pre-treatment- Eight (36%) of the rats never picked up a bead during the 4 tests with the seed/bead mixture; 6 picked up 1-3 beads and 9 others stuffed 9-131 beads into their pouches during the 4 trials. All rats that initially picked up beads apparently learned to avoid the beads and picked up fewer beads in subsequent trials. During the 4th trial, only 4 animals picked up any beads at all. All animals picked up the rye seeds and numbers selected ranged from 7 to 224 for the total of 4 trials. Some of the rats were extremely consistent in the number of seeds per trial. In 4 consecutive tests we observed the following total numbers of seeds per animal; 24, 20, 27, and 31; 55, 55, 38, and 56, for 2 different rats.

The animals were treated following 12 hrs of starvation at approximately 0800-1000. None of the treated animals died as a direct result of the oral dose of methyl parathion, 5mg/kg in corn oil. None of the control animals died immediately post-treatment following an oral gavage of corn oil.
Post-treatment- Twelve and 24 hrs after treatment the animals were again tested and only 1 of 11 treated

animals picked up 4 beads in the test immediately following treatment and no beads in the next 3 tests. Two control animals picked up 14 and 25 beads during the 4 tests, none after the first 3 trials. The number of seeds taken during the post-treatment trials ranged from 0 to 266 per animal for the 4 tests. The data were analyzed in two ways, by total Chi-square and by paired t-tests. The Chi-square data set included all of the sums of seeds and beads selected during the pre and post-treatment tests by the 22 test rats (Table 1).

Table 1. Seeds and beads selected by control and treated (5mg/kg, methyl parathion) kangaroo rats, April, 1987 CERL.

| | Pre treatment | | BEADS | Post treatment |
|---|---|---|---|---|
| | Obs. | Expt. | Obs. | Expt. |
| Treated rats | 219 | 206 | 4 | 17 |
| Control rats | 286 | 299 | 39 | 26 |

Chi-square= 17.76, df 1; P 0.01 = 6.64

| | Pre treatment | | SEEDS | Post treatment |
|---|---|---|---|---|
| | Obs. | Expt. | Obs. | Expt. |
| Treated rats | 1241 | 1228 | 1194 | 1207 |
| Control rats | 1168 | 1181 | 1175 | 1162 |

Chi-square =0.56, df 1; P 0.10=2.76

The Chi-square tests show that the treated rats picked up significantly (P 0.01) fewer beads following treatment. Interpretation must be somewhat cautious, because only one treated animal selected beads post-treatment. Because of the wide variance in the numbers of seeds and beads selected among the treatment and paired groups, none of the t-tests was significant. When treatment and control groups were compared in total and when the paired groups were tested, the t values were not significant.

Two animals were dosed with 5mg/kg of methyl parathion prior to treatment and examined for ChE inhibition. Two control animal brain ChE values were 98.1 and 92.3 percent. ChE levels in the brains of the two treated rats were 75.5 and 77.2 percent indicating a depression of about 18-19 percent. Lethal effects are generally assumed to begin at approximately 50 percent ChE inhibition.

No differences in seed/bead discrimination were observed in the 1988 trials. Only 2 of 24 rats picked up any beads. ChE analyses of two treated rats immediately following the behavior trials showed no reduction in activity compared to 2 control rats. Following the behavior trials in 1988, an LD-50 test was conducted on 20 remaining rats. The calculated LD-50 was 97.1mg/kg with a 95% CI range of 42.6 to 178.6mg/kg(SAS probit analysis). AChE values (Table 2) include those of surviving rats in the 90 and 143mg/kg treatment groups.

Table 2. Summary of mean AChE values(mu/min/ml)

| Treatment mg/kg | N | Deaths | Mean | Range |
|---|---|---|---|---|
| Control | 3 | 0 | 141 | 138-146 |
| 35 | 4 | 0 | 138 | 138-139 |
| 56 | 4 | 1 | 100 | 63-138 |
| 90* | 4 | 3 | 89 | 70-130 |
| 143* | 4 | 3 | 82 | 64-121 |

*Includes surviving individuals tested 72 hours post-treatment

LD-50 81.7mg/kg+ 95%CI 42.6-178.6mgkg.

Because of the low number of animals tested, 11 pairs, at a single dose level, it might be premature to suggest that the methyl parathion treatment actually enhanced the ability of the rats to learn to discriminate seeds from beads. In fact, the hypothesis was just the opposite, we anticipated that the treatment would interfere with the rats' ability to sort seeds from beads. Richardson and Glow (1967) tested laboratory rats and suggested that low ChE influenced function not learning. If our results are true, this might be an instance of a hormesis effect where a slight toxic insult, an 18 to 19 percent inhibition of ChE activity, might cause the animal to overcompensate and make them more sensitive to their surroundings. These results are presented more to describe a potential model for testing low-dose effects on an important behavioral activity that is easily quantified and one that is crucial to ultimate survival of the animal in the wild. If we can begin to understand how low doses of toxic substances and combinations of toxins can influence important life processes we may begin to better evaluate the population impacts of the ubiquitous xenobiotics distributed throughout our global environment.

Acknowledgments. Performed while TJP was visiting the CERL of USEPA. I thank the staff and administration for their kind hospitality ; B. Williams, who acted as my host, R. Bennett and A. Fairbrother for discussions and the ChE analyses, R. Bentley and C. Stock who assisted with the behavioral trials, and T. Murphy, Director, and H. Kibby who agreed to my visit. R. Bentley conducted the 1988 trials.

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Received October 31,1988; accepted January 12, 1989.