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Persistent Cognitive Alterations in Rats after Early Postnatal Exposure to Low Doses of the Organophosphate Pesticide, Diazinon

Olga A. Timofeeva^a, Cindy S. Roegge^b, Frederic J. Seidler^c, Theodore A. Slotkin^{a,c}, and Edward D. Levin^{a,c}

^aDepartment of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC, 27710, USA

^bWIL Research Laboratories, LLC, 1407 George Road, Ashland, OH, 44805, USA

^cDepartment of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710, USA

Abstract

Background—Developmental neurotoxicity of organophosphorous insecticides (OPs) involves multiple mechanisms in addition to cholinesterase inhibition. We have found persisting effects of developmental chlorpyrifos (CPF) and diazinon (DZN) on cholinergic and serotonergic neurotransmitter systems and gene expression as well as behavioral function. Both molecular/neurochemical and behavioral effects of developmental OP exposure have been seen at doses below those which cause appreciable cholinesterase inhibition.

Objectives—We sought to determine if developmental DZN exposure at doses which do not produce significant acetylcholinesterase inhibition cause cognitive deficits.

Methods—Rats were exposed to DZN on postnatal days 1–4 at doses (0.5 and 2 mg/kg/d) that span the threshold for cholinesterase inhibition. They were later examined with a cognitive battery tests similar to that used with CPF.

Results—In the T-maze DZN caused significant hyperactivity in the initial trials of the session, but not later. In a longer assessment of locomotor activity no DZN-induced changes were seen over a 1-hour session. Prepulse inhibition was reduced by DZN exposure selectively in males vs. females; DZN eliminated the sex difference present in controls. In the radial maze, the lower but not higher DZN dose significantly impaired spatial learning. This has previously been seen with CPF as well. The lower dose DZN group also showed significantly greater sensitivity to the memory-impairing effects of the anticholinergic drug scopolamine.

Conclusions—Neonatal DZN exposure below the threshold for appreciable cholinesterase inhibition caused neurocognitive deficits in adulthood. The addition of some inhibition of AChE with a higher dose reversed the cognitive impairment. This non-monotonic dose-effect function has also been seen with neurochemical effects. Some of the DZN effects on cognition resemble those seen earlier for CPF, some differ. Our data suggest that DZN and CPF affect transmitter systems

Corresponding Author: Dr. Edward D. Levin, Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Box 3412, Durham, NC 27710, USA, Phone: 1-919-681-6273; Fax: 1-919-681-3416, Email: edlevin@duke.edu.

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supporting memory function, differently, implying participation of mechanisms other than their common inhibition of cholinesterase.

Keywords

Diazinon; Organophosphate insecticides; Sex-selective effects; Non-monotonic dose-response; Cholinergic; Working Memory; Development; Radial-arm maze; Pre-pulse inhibition

Introduction

Despite recent U.S. restrictions on the use of some of the organophosphorous pesticides (OPs) in the home [32], these agents continue to be applied widely in agriculture and in residences throughout the world, representing about half the total annual amount of insecticides used [7]. One of the major concerns with these agents is their propensity to elicit developmental neurotoxicity at exposures below the threshold for any systemic symptoms, so that potentially damaging fetal or childhood exposures may go undetected until persistent functional impairments become expressed [4,18,19]. Indeed, it is now clear that, in addition to cholinergic hyperstimulation consequent to inhibition of cholinesterase activity, OPs like chlorpyrifos (CPF) elicit their adverse effects on brain development through a family of mechanisms including direct effects on neural cell replication and differentiation [20,21]. Thus, although doses of CPF or other OPs high enough to cause substantial cholinesterase inhibition disrupt the pattern of genes that are essential to neurodevelopment [5,6], widespread changes in these genes are evident at much lower exposures that lie below the threshold for cholinesterase inhibition [23,24].

If the OPs exert their adverse effects on brain development through mechanisms other than cholinesterase inhibition, then it is reasonable to expect that the various OPs might differ substantially in their effects, since aside from the shared P=S or P=O moiety, they differ widely in molecular structure. Indeed, we recently found that CPF and another OP, diazinon (DZN) show similarities but also major differences in the repertoires of gene changes elicited in the developing brain [23,24]. Furthermore, these are translated into differences in the expression of specific proteins associated with cholinergic and serotonergic synapses [10,22-25]. In the current study, we evaluated the neurobehavioral consequences of early neonatal DZN exposure using cognitive testing guidelines adapted from our earlier work on CPF [2,9,14,15]. In this case, we evaluated two doses of DZN subthreshold for producing clinical signs of acute toxicity, which were administered daily to neonatal rats on postnatal days (PND) 1-4. The lower dose did not produce detectable cholinesterase inhibition (0.5 mg/kg) and the higher dose produces barely-detectable inhibition (10-20%) in different brain areas (cerebellum, brainstem, and forebrain) [24,25] comparable to that seen with 1 mg/kg of CPF given over the same period [27]. Here we demonstrate that these otherwise subtoxic exposures to DZN nevertheless produce persistent neurobehavioral deficits present in the juvenile period through adulthood, and that the effects bear similarities to, but also significant differences from, those of CPF.

Materials and Methods

Animal Subjects

The experiments were carried out in accordance with protocols approved by the Institutional Animal Care and Use Committee and in accordance with federal and state guidelines. Thirty-six timed-pregnant Sprague-Dawley rats (Charles River, Raleigh, NC) were housed in plastic breeding cages under a 12-hr light/dark cycle (lights on at 6:00 AM) and free access to food and water. On the day of birth, the postnatal day (PND) 0, all pups were randomized and redistributed to the dams with a litter size of 12 (6 males and 6 females). Same-sexed rats from

each litter were weighed in a group and given daily subcutaneous injections of 0, 0.5, or 2 mg/kg DZN (Chem Service, West Chester, PA) on PND 1-4. Rats were randomized within treatment group on PND2-4 and in addition, dams were rotated among litters every 2-3 days to distribute any maternal caretaking differences randomly across litters and treatment groups. Each rat received an individual number on the day of weaning identified with ink markings on their tails. Before the individual markings the rats were identified by litter, which had all the pups in the same treatment condition. Because of its poor water solubility, DZN was dissolved in dimethylsulfoxide to provide consistent absorption [35] and was injected at a volume of 1 ml/kg once daily. Control rats received equivalent injections of the dimethylsulfoxide vehicle. These doses of DZN have been shown previously to span the threshold for barely-detectable cholinesterase inhibition [25] but nevertheless cause significant alterations in brain development, including the serotonin system [22,25]. Pups were weaned on PND21 and transferred to another housing room with a reverse dark-light cycle (lights off at 6:00 AM). Half of the animals from each litter was randomly selected for the battery of cognitive tests and another half was used in another study of emotional function. Weaned pups were originally housed in same-sex and same-exposure groups of 6/cage, but as they grew, they were subdivided into groups of 3 rats/cage. At the time of behavioral testing, the males were pair-housed while the females were triple-housed. All behavioral testing was carried out during the dark phase, the most active period, but in lighted environments so that the rats could access the visual cues necessary to perform the tasks. Tests were performed on 12 rats per sex per treatment group (72 animals in total), with the tests conducted during adolescence and adulthood beginning with T-maze spontaneous alternation (postnatal weeks 4-5), Figure-8 locomotor activity (week 6), prepulse inhibition (weeks 11-12), followed by radial-arm maze training (weeks 13-18) and drug challenges in the radial-arm maze (weeks 19-28). All 72 rats were examined on the mentioned above battery tests and weighted once per week to check for their growth.

T-maze spontaneous alternation

A T-maze, constructed of black painted wood, with 10-cm wide alleys and 20-cm long start area, was elevated 1 m from the floor. The rest of the stem of the maze was 45 cm long; each of the choice arms was 40 cm long and rails 1.5 cm high ran along the edges of the alleys. Rats were placed in the start area for 10 s, at which point a barrier was raised and the rat was permitted to roam about the maze, allowing 30 s to enter one of the arms. If a rat chose an arm, the barrier was then lowered to keep the rat in the arm for 30 s after which the rat was placed back in the start area for the next trial. If the rat did not choose an arm within 30 s, then it was placed back in the start area for the next trial. Five such trials were run during the one and only session and percent of alternation between left and right arms and average response latency (time between the moment of raising the barrier in the start area and the moment when rat enters one of the arms) were calculated.

Figure-8 locomotor activity

The maze consisted of a continuous enclosed alley 10×10 cm in the shape of figure-8, 70 cm long and 42 cm wide, with a 21×16 cm central arena, a 20-cm high ceiling and two blind alleys extending 20 cm from either side. Eight infrared photobeams crossing the maze alleys were used to assess locomotor activity, one located on each of the two blind alleys and three on each of two loops of the figure-8. The number of photobeam breaks was tallied during a single 1-h session. The linear and quadratic trends across twelve 5-min blocks in each session were calculated to test the hypothesis that DZN exposure affects the rate of habituation. Four of such mazes were assigned for males only, and four for females.

Prepulse inhibition (PPI)

Startle reflex and prepulse inhibition were measured using an adaptation of the classic acoustic method outlined by Swerdlow et al., [29-31] to assess sensorimotor gating. The adaptation of this test for the current study used an acoustic prepulse and a tactile startle stimulus. The test apparatus was in a sound-attenuating box within which the rats were placed inside a clear Plexiglas tube on top of a response platform. The startle reflex amplitude and PPI were measured using Med Associates Startle Reflex System (St. Albans, VT, USA). To reduce anxiety in a novel surrounding rats were introduced to the test apparatus, by placing them in the Plexiglas tube for 5 min one-two days before testing. During testing the rats were placed inside the box while a 65-dB background white noise played continuously. There was a 5-min acclimation period inside the box before testing began. The startle response was measured in three test blocks and was elicited by a 4 pounds per square inch (27.6 kilopascals) air puff to the back of the rat. The first consisted of six trials of startle alone. Block two consisted of 12 trials of startle alone, and 36 prepulse plus startle trials, with prepulse/startle delay of 100 ms. The intertrial duration was 10-20 s with a null period of 100 ms between the prepulse and startling stimulus. Trials were conducted in a random order with respect to startle and prepulse startle. There were three prepulse levels administered randomly in the prepulse startle trials (68, 71, and 77 dB pure tone). In the third block of trials, the rats were given additional trials of the startle response. The auditory stimuli had 2 ms rise/fall time. Both sexes were tested in pseudo random order. The holding tube was washed between tests.

Radial-arm maze

During the radial-arm maze training, rats had *ad libitum* access to water with daily feeding after testing, to maintain body weight at approximately 85% of free feeding level. This was determined of the weight growth in rats having free access to food and water, matching in age and treatment, but used for emotional tests. The rats were tested for working and reference memory using a 16-arm radial maze. This black-painted wood maze was at a height of 30 cm from the floor with a central platform diameter of 50 cm and 16 arms, each 10 × 60 cm, projecting radially outward from the central platform. Each arm contained a food cup 2 cm from the distal end. The test areas contained numerous visual cues. Rats were given 2 shaping sessions to familiarize them with the maze and the food reinforcements. For each shaping session, rats were placed individually in a large, opaque cylinder on the platform of the maze, given food reinforcement (halves of sugar coated cereal, Froot Loops, Kellogg's, Battle Creek, MI, USA), and allowed 10 min to eat. The rats were then trained in the radial-arm maze. The same 12 arms were baited for each rat once at the beginning of each session to assess working memory, while the other four arms were left unbaited to test reference memory in subsequent sessions. The pattern of baited and unbaited arms was consistent across testing for each rat but differed among rats. No more than 2 consecutive arms were left unbaited for any rat. Each trial began by placing the rat in an opaque cylinder on the central platform for 10 s to allow for orientation and thus avoid introducing bias as to which arm would be entered first. The rat was then allowed to enter any arm. The session lasted for up to 10 min or until all 12 baited arms were entered. Arm entries were recorded when all four paws crossed the threshold of the arm. Arms were baited only once and a repeated entry into a baited arm was counted as a working memory error. Entrance into an unbaited arm was recorded as reference memory error. Latency (seconds per arm entry) was calculated as the total session time in seconds divided by the total number of the arm entries, a parameter further characterizing motor activity. Choice accuracy was recorded as the number of errors. The animals were trained for 18 sessions on the maze, approximately 3 times per week. Males and females were tested in alternating order. The maze was cleaned between animals with a damp paper towel.

Drug challenges in the radial-arm maze

After the completion of radial-arm maze training, the rats were tested with drug challenges. Each rat received each drug dose in a repeated measures counterbalanced design (each dose was given first as often as second, as often as third, as often as fourth), with at least 2 days between challenges. The muscarinic receptor antagonist scopolamine (0, 0.4, 0.8, and 1.6 mg/kg), and 5-HT₂ receptor antagonist ketanserin (0, 0.04, 0.08, and 0.16 mg/kg), were administered sc 20 min before the beginning of the radial-arm maze test. Drugs were dissolved in isotonic saline vehicle. Scopolamine was injected in a volume of 1 ml/kg, and ketanserin was administered in a volume of 2 ml/kg due to less solubility of this compound. To exclude carryover effects of the studied drugs, the exposure to a new treatment occurred after 2-week drug-free period, during which the rats were tested with saline.

Data analysis

Data are presented as means and standard errors, with effects of DZN established by multivariate analysis of variance (ANOVA), involving factors both between subjects (dose and sex) and within subjects (test session, period within session, drug dose). A value of $p < 0.05$ was considered the threshold for significance. However, for interaction terms at $p < 0.1$, values were separated according to the interactive factors and the data reexamined for main effects [26]. When interactions of treatment with other variables were not significant, only main effects were tabulated. In situations where there was no treatment $\times$ sex interaction, values for males and females were combined for presentation but the factor of sex was retained in the statistical calculations; the standard error values as presented in the graphs thus are larger than the actual within-sex values because they include the variance between the sexes. Linear and quadratic trend analyses were used to assess progressive effects across repeated measures such as time blocks and drug doses.

Results

Neither 0.5 mg/kg/d nor 2 mg/kg/d of DZN exposure produced any signs of systemic toxicity, such as impaired gait or weight gain, weakened motor activity or loss of viability. Nevertheless, there were significant behavioral abnormalities in adolescence and adulthood disclosed on the battery tests.

In the T-maze (Fig. 1, weeks 4-5), rats exposed to either dose of DZN showed a significant reduction in response latency in early trials, just as seen previously with CPF [9,14]. In contrast, the percent of spontaneous alternation was not significantly affected by DZN treatment. The controls averaged $84.6 \pm 8.7\%$, the 0.5 mg/kg/d DZN group $86.0 \pm 8.4\%$ and the 2 mg/kg/d DZN group $86.1 \pm 7.9\%$ alternation.

For the Figure-8 locomotor activity test, (week 6) no statistically significant differences were observed (data not shown). However, sensory gating evaluated by prepulse inhibition (week 11 and 12), revealed substantial effects of DZN exposure (Fig. 2). The overall analysis showed an interaction of DZN treatment $\times$ sex ($p < 0.10$) which was followed-up by analysis of DZN treatment with each sex. Males exposed to either the 0.5 mg/kg/d ($p < 0.05$) or the 2 mg/kg/d ($p < 0.05$) dose of DZN had significant reductions in PPI vs. controls, whereas females were unaffected. PPI response in exposed males resembled values normally seen in females, thus implying that DZN treatment by affecting sex-selective response, feminised the males. Since there were no DZN-induced differences in the body weight with the rats fed regular chow the effect did not appear to be secondary to weight effects.

In the radial maze (weeks 13-18), control animals showed a sex difference in performance, with males committing fewer errors than females, primarily in the earlier session blocks (sex

× block interaction, ($p < 0.01$, not shown), in agreement with our earlier studies [2,15]. DZN exposure produced significant alterations in working memory performance in the absence of any interaction of sex × treatment, so values for males and females are shown combined (Fig. 3). The main effect of DZN was significant ($p < 0.05$) and was present in both males and females, but the effect was restricted to the group receiving 0.5 mg/kg/d ($p < 0.05$), there was no significant effect in the 2 mg/kg/d group. The non-monotonic effect of DZN was thus similar to that found previously for prenatal CPF [14], but also in contrast in that, postnatal DZN exposure did not cause sex-selective effects on working memory. The effect appeared to be selective for working memory because we did not observe any significant effects of DZN on reference memory errors or latencies (data not shown).

By the end of radial-arm maze training (week 18), error rates were indistinguishable across the treatment groups (Fig. 3). To explore the neural basis underlying performance on this task the drug challenges were begun on week 19. The muscarinic antagonist scopolamine evoked a significant ($p < 0.0001$) increase in working memory errors, with an apparent threshold at 0.08 mg/kg in the control group. Errors increased further with increased scopolamine dose. (Fig. 4). The group exposed to the low dose of DZN seemed to be more sensitive, showing a significant ($p < 0.01$) increase in errors at a normally sub-threshold dose of scopolamine (0.04 mg/kg). This effect was non-monotonic, as there was no shift in the dose-response curve in the group exposed to 2 mg/kg of DZN.

The 5HT₂ receptor antagonist ketanserin did not evoke an increase in working memory errors in the control group. This was expected given that the doses used are subthreshold for impairing memory in intact rats. However, in our previous study [2] the same doses of ketanserin affected memory function in CPF treated rats. Unlike in CPF exposed animals, ketanserin failed to increase working memory errors in either of the DZN groups indicating little or no dependence of working memory performance on this receptor input (Fig. 5).

Discussion

The current results demonstrate that early postnatal (PND1-4) exposure of rats to the OP pesticide, DZN, at doses that do not cause discernible signs of systemic toxicity, nevertheless produces long-lasting alterations in cognitive function that are present in adolescence and extending into adulthood. For a number of the observations, the behavioral impairments were similar to those identified in our previous work with CPF when exposure occurred either gestationally or postnatally [2,9,14,15]. There were also notable differences in the effect of the two agents, which might be expected.

We did not find significant changes in locomotor activity as assessed in the Figure-8 apparatus after exposure to either doses of DZN, essentially the same result that we reported for CPF exposure at a dose of 1 mg/kg/d during the same postnatal period [15]. This finding is important in two ways. First, it suggests that the differences found for other behavioral tests are rather selective, than representing global impairment of motor function. Second, this distinguishes developmental neurotoxicity from the loss of motor function that characterizes outright organophosphate-induced delayed neuropathy (OPIDN) as found in the adult [1]. Indeed, the developing organism is resistant to OPIDN because of the greater plasticity of developing peripheral projections [8]. There is a single previous report on the lasting behavioral effects of early DZN exposure [28] that indicates significant motor impairment in adult mice after exposure to 9 mg/kg/d throughout the entire gestational period. Interestingly, they did not see an increase in maze errors in the diazinon-exposed mice. In addition, gestational exposure to the low (0.18 mg/kg/d) but not the high (9 mg/kg/d) diazinon dose caused significant delay in signs of sexual maturation (vaginal opening and testes descent) [28]. The absence of motor

function impairment in our DZN treated animals was further confirmed by similar with controls response latencies in the radial arm maze.

In the T-maze alternation task, DZN-exposed rats of both sexes demonstrated significant reductions in response latency only in the early trials, likely reflecting hyperactivity in the situation that is novel and challenging. Similar results were observed in rats exposed to CPF in early or late gestational periods [9,14], but not after CPF exposure in the early postnatal period [15]. Accordingly, the actions of the two OPs may be similar, but the critical periods over which each exerts the corresponding changes in neural function can be different. In contrast to the effect on latency, we did not observe significant changes in the percent of alternation, again, a result similar to that for CPF [9,14].

In our earlier work with neonatal CPF exposure, we found that many of the behavioral effects were characterized by a distinct sex-selectivity, often resulting in a loss of normal sex differences [13,15]. Here, we used PPI as a measure of sensorimotor gating [30], and found a reduction of inhibition in DZN-exposed males but not in females; importantly, the effect of DZN was to eliminate the sex difference seen in controls, shifting the response in males to match that seen ordinarily in females. This is consistent with our earlier suggestion [3] that OP exposure can impair aspects of sexual differentiation of the brain when treatment occurs during the peak period in which these differences are first established (late gestation to early postnatal period in the rat) [16,17,33]. Although recent findings [6] indicate that prenatal cholinesterase inhibition affects expression of genes associated with sexual differentiation of the brain, our findings show that behavioral outcomes indicative of loss of sex differences can also be elicited by OP exposures below or at the threshold for anticholinesterase actions.

In contrast to the noted sex-selective effects of DZN on PPI inhibition, DZN-induced working memory deficits were common to both males and females. Viewed against our earlier work with CPF (1 mg/kg/day PND 1-4) [15], this was a surprising result. CPF, through its disruption of sexual differentiation of the brain, has opposing effects on performance in males and females: since males ordinarily commit fewer errors than females, CPF produces an increase in error rate in males but a decrease in females. Again, there may be a difference in critical period for the effects of the two OPs. Our neurochemical and gene microarray studies demonstrated that CPF and DZN have certain differences in neurotoxic effects [10,22,23,25], which may be responsible for the different behavioral outcomes. Thus, although both DZN and CPF impair memory performance, they also show disparities that reflect underlying differences in mechanism and/or dose-effect relationships. In fact, we obtained evidence that there are further fundamental differences between the two agents. CPF impairs both working and reference memory [2,15], whereas we found effects of DZN only on the former parameter. On the other hand, both agents display a non-monotonic dose-effect relationship, with deficits evident at a low dose that revert when the dose is raised just above the threshold for detectable cholinesterase inhibition [14]. This apparent anomaly is actually an expected outcome because of the positive trophic effect provided by low-level cholinergic stimulation in the developing brain. Acetylcholine serves as a trophic factor that, depending on the development stage, promotes neural cell replication and differentiation [11,34], so that if the proper balance is achieved, a small degree of cholinesterase inhibition, too low to elicit adverse systemic symptoms, might actually help to offset a few of the noncholinergic effects mediating developmental neurotoxicity. Indeed, a recent study by Laviola et al. [12], demonstrated that prenatal exposure to 5 mg/kg of CPF can produce not only negative developmental effects, but also accelerated postnatal maturation of the grasping reflex.

The drug challenge tests point to different underlying mechanisms and targets that contribute to the distinct effects of DZN and CPF exposures. The low dose of DZN rather increased while CPF, as was found earlier, decreased sensitivity to the amnesic effect of scopolamine [14,

15]. In both the current DZN study and the earlier CPF studies rats exhibiting impaired working memory function during acquisition showed an altered response to scopolamine, albeit in opposite directions. It may be the case that a modest impairment in muscarinic systems from developmental OP exposure would result in less functional reserve in muscarinic involvement in memory function thus making the critical systems vulnerable to even slight disruption by low dose scopolamine which control subjects could easily deal with. Greater disruption of muscarinic systems may make these systems unavailable for serving as a substrate for memory function, obviating the impact of muscarinic antagonist administration. This suggests that adaptive mechanisms that operate for CPF, might not come into play with comparable exposures to DZN. In our earlier work with CPF, we found that deficiencies in cholinergic inputs to the hippocampus were compensated by an abnormal dependence on serotonergic inputs, which ordinarily do not contribute significantly to these performance measures [2]. Thus, challenging the CPF-exposed animals with ketanserin, a 5HT₂ receptor antagonist, produced impaired performance, whereas it had no effect in control animals. Here, the same studies done with DZN demonstrated no compensatory increase in serotonergic contributions to working memory: ketanserin had no detected effect on performance in the DZN group.

In conclusion, our results provide evidence that otherwise subtoxic doses of DZN given to neonatal rats, even at exposures that do not cause measurable inhibition of cholinesterase, nevertheless evoke long-lasting, cognitive changes. Although administration of another OP pesticide, CPF, during the same developmental period, also produces lasting neurobehavioral damage, there are distinct differences in sensitivity and outcome that point to the importance of noncholinesterase-related mechanisms underlying the adverse effects. Our results thus reinforce the concept that the developmental neurotoxicity of OPs do not reflect a single, common mechanism and that the various agents can differ in their neurobehavioral outcomes.

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Figure 1. Early Postnatal (PND 1-4) Diazinon Exposure Effects on Spontaneous Alternation Latency in Adolescence

Effects of DZN exposure on T-maze response latency (mean±sem). The linear trend across trials was calculated and there was a significant ($p < 0.05$) treatment group effect and significantly decreases response latency in earlier trials in both the 0.5 mg/kg/d ($p < 0.05$) and 2 mg/kg/d DZN ($p < 0.03$) groups vs. control.

Figure 2. Early Postnatal (PND 1-4) Diazinon Exposure Effects on PPI Performance in Adulthood
Effects of DZN exposure on PPI inhibition (mean±sem). Males in both DZN treatment conditions had significantly ($p<0.05$) reduced PPI % relative to controls, whereas females were not affected.

Figure 3. Early Postnatal (PND 1-4) Diazinon Exposure Effects on Radial-Arm Maze Working Memory Performance in Adulthood

Effects of DZN exposure on working memory errors in the 16-arm radial maze (mean±sem). There was a significant ($p < 0.05$) main effect of DZN and as shown in the right panel there was a significant ($p < 0.05$) difference between the 0.5 mg/kg/d DZN group and controls as well as the 2 mg/kg/d DZN group averaged over the 18-session training period.

Figure 4. Early Postnatal (PND 1-4) Diazinon Exposure Effects on Radial-Arm Maze Working Memory Performance with Scopolamine Challenge

Impairment of working memory in response to scopolamine challenge in control rats and rats exposed to DZN (mean $\pm$ sem). The same DZN dose (0.5 mg/kg) group that demonstrated a significant impairment in radial-arm maze acquisition (see Fig. 3) also showed a significant ($p < 0.01$) impairment relative to controls when given a normally subthreshold (0.04 mg/kg) dose of scopolamine.

Figure 5. Early Postnatal (PND 1-4) Diazinon Exposure Effects on Radial-Arm Maze Working Memory Performance with Ketanserin Challenge

Ketanserin challenge for working memory errors in control rats and rats exposed to DZN (mean $\pm$ sem). DZN exposure did not evoke any changes in the response to ketanserin in contrast to the previous findings with CPF exposure during the same neonatal period [2,14].