UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JAN - 6 1995

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Chlorpyrifos (P.C. Code 059101) - Toxicology Data Review

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THRU: James N. Rowe, Ph.D., Section Head James N. Rowe 1/5/95
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Action requested: Review the published version (Attachment 1) of a study previously evaluated (HED Doc No. 011372, dated 12/30/94, Attachment 2): Embryotoxicity and Neurotoxicity in Rats Associated with Prenatal Exposure to Dursban; by M.A. Muto, F. Lobelle, Jr., J.H. Bidanset, and J.N.D. Wurpel; *Veterinary and Human Toxicology* 34(6):498-501; December, 1992.

Data evaluation: Additional information on the study conduct and results was included in the journal article. The purity and composition of the test substance formulation (1% chlorpyrifos, 6% xylene, 93% water) was indicated. Information pertaining to each phase of the study is detailed below.

A. Phase 1 (*in utero* exposure)

1. The days of early *in utero* dosing were expressed as GD 0-7, instead of GD 1-7. From the information provided, it cannot be determined which is correct.

2. The number of females dosed by i.p. injection was indicated (Table 1). For adequate analysis of developmental effects, it is recommended (§83-3) that at least 20 pregnant females rats be tested at each dose level.

Table 1. Number of pregnant females assigned to study

Days of treatment	Dose (mg a.i./kg)			
	Control	0.03	0.1	0.3
GD 0-7	8	10	11	7
GD 7-21	8	8	8	8

3. A summary table of physical abnormalities in offspring of dams exposed *in utero* was provided (Table 2). The fetal and litter incidences of each reported abnormality were not provided. Abnormalities were described in a rather vague manner and not characterized with the terminology standardly used in reporting developmental anomalies. Mean fetal weight data for control and treated groups were not included in the table of effects in the journal article; statistical analysis of fetal body weight data was apparently not performed. No description of the physical abnormalities produced by *in utero* exposure on GD 7-21 were included in the journal article.

Table 2. Physical abnormalities reported in pups exposed *in utero* on GD 0-7

Dose (mg a.i./kg)	Findings
0.03	Decreased body weight
0.1	Decreased body weight Small hind and fore limbs
0.3	Decreased body weight Small hind limbs Exposure of internal organs (kidney) Lack of spinal development Increased head circumference

4. The neurotoxicity data for pups exposed *in utero* were expanded slightly (Table 3), adding information on rotorod testing for pups exposed on GD 0-7 with 0.03 and 0.1 mg a.i./kg.

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Table 3. Rotorod performance in 16-day old pups following *in utero* exposure[a] - Mean(S.D.)

Days of exposure	Dose (mg a.i./kg)			
	Control	0.03	0.1	0.3
GD 0-7	39(1.5)	44(1.8)	48(1.1)*	49(2.9)*
GD 7-21	30(0.7)	N/A	N/A	46(0.9)*

a Number of falls in 2 minute trial.
N/A Report states that data were not available.
N Each data partition consisted of 20-50 rat pups, except for the 0.03 and 0.3 mg a.i./kg (GD 0-7) dose groups, where the numbers examined were 8 and 10, respectively.
* $p \leq 0.05$.

B. Phase 2 (*postnatal* exposure)

Minor corrections were made to the table of neurotoxic effects on pups exposed postnatally (Table 4), including providing a reference to the numbers of pups tested and adding statistical significance to the performance data for pups dosed on postnatal day 3 at 0.3 mg a.i./kg.

Table 4. Rotorod performance in 16-day old pups[a] - Mean(S.D.)

Postnatal day of administration	Dose (mg a.i./kg)		
	Control	0.1	0.3
3	27(1.2)	27(1.1)	38(0.9)*
10	26(2.3)	28(1.3)	33(1.4)*
12	14(1.5)	18(1.1)*	21(1.4)*

a Number of falls in 2 minute trial.
N Each data partition consisted of 12-20 rat pups.
* $p \leq 0.05$.

Conclusions/Recommendations:

The additional information as described above does not alter the conclusions of the previous data review (HED Doc. No. 011372). The study remains CORE-Supplementary (not upgradable) and is not considered to be appropriate for use in developmental toxicity risk assessment.

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Attachment 1

Embryotoxicity and Neurotoxicity in Rats Associated with Prenatal Exposure to Dursban

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ABSTRACT. DURSBAN (DB; active ingredient chlorpyrifos) is a widely-used organophosphate insecticide. The teratogenic and neurotoxic potential of DB was evaluated in rats in utero by exposing embryos on days 0-7 or days 7-21 of development. These prenatal exposures to DB (0.03, 0.1 or 0.3 mg chlorpyrifos/kg, ip) induced physical abnormalities and embryotoxicity. Rat pups which had been exposed to 0.3 mg chlorpyrifos/kg prenatally demonstrated significant behavioral neurotoxicity on postnatal day 16 in the rotorod test compared to time-matched saline-infused litters. Exposure to DB on postnatal day 3, 10 or 12 also caused neurotoxicity as evaluated by the rotorod test. Our studies suggest prenatal exposure to relatively low concentrations of DB may be associated with embryotoxicity, fetal lethality and behavioral neurotoxicity.

DURSBAN (DB) is a commercial organophosphate insecticide (Dow Chemical, Midland MI) the active ingredient of which is chlorpyrifos (CPF; 0,0-diethyl 0-(3,5,6-[tricholoropyridyl]phosphorothioate). The acute LD_{50} of CPF is approximately 200 mg/kg when administered ip to mice (1) or 118-245 mg/kg when given po to rats (2,3). The major acute toxic effects observed following CPF exposure are from inhibition of the enzyme cholinesterase and include headache, dizziness, muscle twitching, tremor, miosis, sweating, excessive urination and tightness in the chest. Acute exposure to high doses of CPF may lead to seizures, respiratory failure and death.

Clorpyrifos is reported nonteratogenic (4), yet studies on the genetic toxicity or embryotoxicity of DB or CPF remain inconclusive. Generally, there is agreement that DB (or CPF) does not cause genetic abnormalities (5, 6), but some studies suggested gene toxicity associated with CPF exposure (7). Chlorpyrifos and its metabolites caused embryotoxicity when administered to chick embryos (5).

Our studies focused on the embryotoxic and neurotoxic potential of DB and the formulation which humans are likely to be exposed to occupationally and in the home. In these preliminary studies DB was administered ip to pregnant female rats from days 0-7 or 7-21 of gestation. The litters delivered were evaluated for viability, the presence of physical abnormalities, and neurotoxicity. DB was also given ip in single exposures to immature rat pups to evaluate the neurotoxic potential following DB exposure in the early postnatal period.

MATERIAL AND METHODS

Rats were obtained from Taconic Farms (Germantown, NY) and housed in environmentally controlled rooms (temperature 20-22 C; humidity 40%-60%; 12:12 light:dark cycle, lights on 07:00). The menstral cycle of individual female rats was determined by daily vaginal smears. At ovulation 2 females were bred with a single male in the male home cage. The following day vaginal smears were performed to determine pregnancy. The presence of sperm in the in the vaginal smear denoted day zero of pregnancy. Bred females were randomly assigned to groups receiving DB daily on days 0-7 of pregnancy or days 7-21 of pregnancy. DURSBAN stock formulation consisted of: 1% CPF; 6% xylene and 93% water. Injections were made by diluting the DB stock solution with saline; saline injections were used for controls.

Prenatal Exposures

For in-utero exposure each pregnant female rat received the DB product with the dose calculated to deliver 0.03, 0.1 or 0.3 mg CPF/kg or saline ip daily. The data partitions were as follows:

0-7 days	mg CPF/kg	0.03	0.1	0.3	saline
		n=8	n=10	n=11	n=7
7-21 days	mg CPF/kg	0.03	0.1	0.3	saline
		n=8	n=8	n=8	n=8

DURSBAN was administered ip on days 0-7 or 7-21 to females confirmed pregnant (ie, sperm present in vaginal lavage). The ip injections were made in the high, midline region of the abdomen to avoid directly infusing drug or saline into the uterus or developing fetuses. Body weights were recorded daily. all females were allowed to reach term and deliver their litters. Following delivery all rat pups were evaluated for general viability, body weight and physical characteristics. Some litters remained with their dams and their postnatal development was evaluated periodically. On postnatal day 16 rats from the DB and the control infused litters were evaluated for neurotoxicity and behavioral abnormalities.

Postnatal Exposures

In another study, the effects of DB administered to neonatal rats were evaluated. DURSBAN was administered ip (0.1 or 0.3 mg CPF/kg) to groups of naive rat pups (12-2 group) on postnatal day 3, 10 or 12. Anc... group of pups received DB daily on postna... days 6-10. All pups were evaluated on po...

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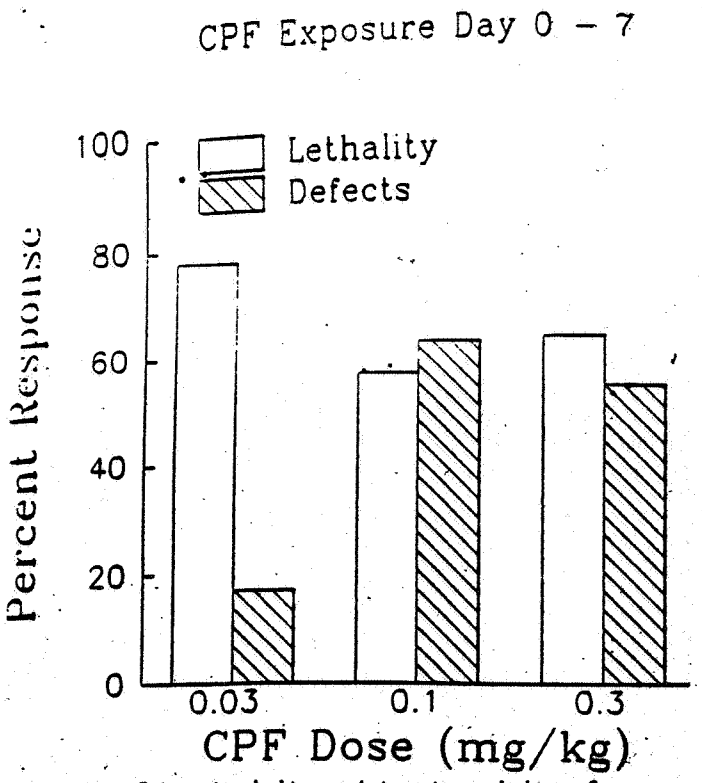

Figure 1. Embryotoxicity and teratogenicity of chlorpyrifos (CPF) administered to pregnant rats on days 0-7 of gestation. Pregnant females were injected ip with 0.03, 0.1 or 0.3 mg CPF/kg during the first trimester of pregnancy (days 0-7). Fetal lethality and physical defects were also noted at all dosages.

natal day 16 for neurotoxicity or behavioral abnormalities.

Neurotoxicity

Rat pups were evaluated on postnatal day 16 for neurotoxicity associated with pre or post-natal exposure to DB using a modification of the rotorod performance test. Sixteen-day-old rat pups were placed on a rotating rod (2 cm diameter, 8 rpm) and the number of times the pup fell from the rod was counted for a 2-min period. DURSBAN-treated pups (either pre or post-natal exposure to DB) were compared to pups from saline-infused litters. The number of falls for each pup was considered an individual data point, rather than taking an average of each litter as an individual data point.

To access behavioral development of the rats, pups were evaluated for general motor behavior and performance on the inclined screen. General motor behavior was monitored by open field observation as a subjective measure of spontaneous motor activity. The inclined screen was used to determine if failure on the rotorod or decriments in motor activity could be attributed to sedation or muscle relaxation. For the inclined screen test, rat pups were placed on an inclined screen (45° angle) and observed for 1 min. Pups which could not orient in a "head-up" position or could not maintain position on the screen were counted as failures, indicating sedation or muscle relaxation as a possible effect of DB exposure.

TABLE 1. Physical abnormalities associated with prenatal exposure to Dursban

Chlorpyrifos (mg/kg)	Abnormalities seen
0.3	decreased body weight small hind limbs exposure of internal organs-(kidneys) lack of spinal development increased head circumference
0.1	decreased body weight small hind and fore limbs
0.03	decreased body weight

Statistical Analysis

Data were analyzed statistically by ANOVA, with significance determined by post hoc comparisons of the data partitions (Neumann-Keul's test). Probabilities of 95% or greater were considered statistically significant.

RESULTS

Prenatal Exposures

The DB-injected females displayed no overt signs of toxicity and their health remained good throughout the exposure period. However, the DB caused effects on in-utero and postnatal development.

Administration of DB on days 0-7 of pregnancy increased the incidence of both lethality and physical abnormalities (Fig 1). All 3 dosages (0.03, 0.1 or 0.3 mg CPF/kg) caused a greater number of lethalities or abnormalities as compared to the saline-infused controls or to the same dose administered on days 7-21 of pregnancy. The dose-effect relation seen from the days 7-21 was absent from the days 0-7. DURSBAN (days 0-7) caused approximately 60 to 75% lethality in litters (0.03 mg/kg = 77% [26/34], 0.1 mg/kg = 57% [25/44], 0.3 mg/kg = 68% [21/31]); 15 to 65% had physical abnormalities (0.03 mg/kg = 15% [5/34], 0.1 mg/kg = 66% [29/44], 0.3 mg/kg = 55% [17/31]). The types of physical abnormalities noted are listed in Table 1.

Administration of DB on days 7-21 of pregnancy caused a dose-dependent lethality (F 2). The lowest dose caused 17% lethality 47), 0.1 mg CPF/kg caused 22% stillbirths (11/49), and 0.3 mg CPF/kg caused nearly 38% lethality (32/85). Only the highest dose (0.3 mg CPF/kg) administered days 7-21 caused any physical abnormalities (9%, 8/85; Fig . All litters delivered by the saline-infused females were grossly normal in appearance no lethalities (stillbirths) were seen.

Giving 0.3 mg CPF/kg during days 0-7 or 21 of pregnancy caused significant neurotoxicity (as measured by the rotorod test) persist into the postnatal period (Table . Both exposures caused a significant increase in the number of falls from the rotorod as compared to saline-infused controls. Prenatal exposure to 0.1 or 0.03 mg CPF/kg days 0-7 also induced neurotoxicity.

Postnatal Exposures

DURSBAN administered on postnatal day or 12 had no overt behavioral effects on

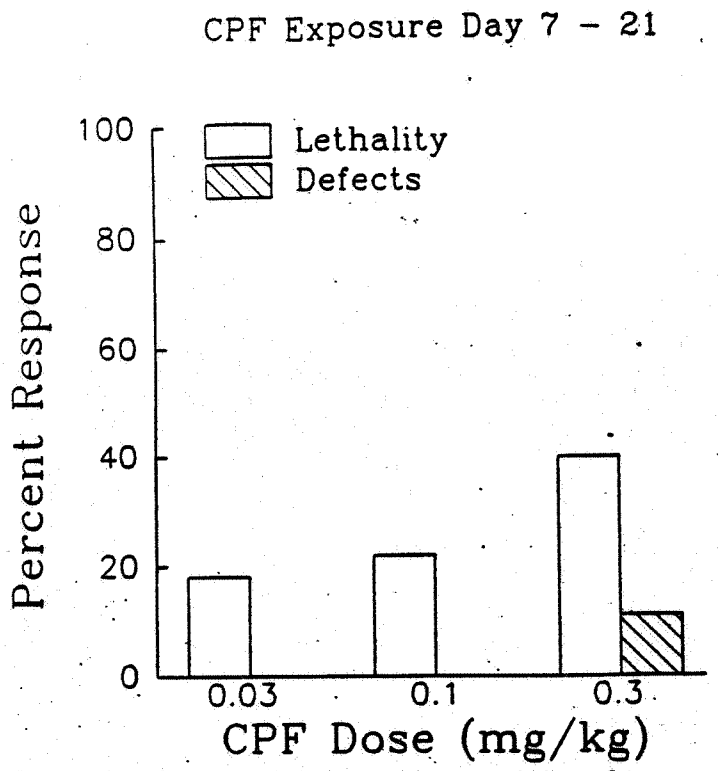

Figure 2. Embryotoxicity and teratogenicity of chlorpyrifos (CPF) administered to pregnant rats on days 7-21 of gestation. Pregnant females were injected ip with 0.03, 0.1 or 0.3 mg CPF/kg the second and third trimesters of pregnancy (days 7-21). Dose-dependent fetal lethality occurred at all dosages. Defects were only noted from 0.3 mg CPF/kg.

immature rat pups. No differences were seen in general motor behavior and in the inclined screen test between rats which received 0.1 or 0.3 mg CPF/kg or saline on postnatal day 3, 10 or 12.

However, postnatal exposure to DB caused a significant effect on performance on the rotorod in the 16-d-old rats. Rat pups receiving 0.3 mg CPF/kg on postnatal day 3, 10 or 12 had significantly increased number of falls from the rotorod (Table 3).

Administration of 0.1 mg CPF/kg did not effect rotorod performance if given on day 3 or 10 postnatally. When 0.1 mg CPF/kg was given on postnatal day 12, it significantly increased the number of falls from the rotorod only in those rat pups (Table 3).

DISCUSSION

Prenatal exposure to DB was embryotoxic and neurotoxic to the developing rat. This neurotoxicity persisted into the early postnatal period. The neurotoxicity induced appeared to be true neurotoxicity rather than failure in the rotorod test secondary to sedation or muscle weakness/relaxation. Some organophosphate insecticides cause a delayed neurotoxicity, primarily consisting of demyelinization and neural degeneration progressing from the distal extremities to finally include central structures (8,9,10); this could account for some of the subtle behavioral changes seen.

While DB is reported nonteratogenic, 2 additional factors may explain the results of our study--the solvents found in the DB formulation and the route of administration used. Xylene is a common organic solvent used in the formulation of pesticides; it can be toxic, carcinogenic and has the potential for teratogenesis (11). The embryotoxicity/teratogenicity and neurotoxicity observed in our study may have been the effects of this solvent. Alternatively, xylene may have potentiated the effects of CPF. The ip route of administration used may have exposed the developing fetus to higher concentrations than other routes of administration. It is also possible to directly inject the uterus or developing fetus. We used a high midline injection to avoid this. Some authors suggest sc injections for pregnant females to avoid these concerns (12).

Chronic exposure to organophosphate compounds may cause a delayed neuropathy consisting of demyelinization and axonal degeneration due to inhibition of a neurotoxic esterase (13). Neurotoxic esterases are inhibitors of butyrylcholinesterase found in the Schwann cells and oligodendrocytes which produce the myelin sheath (14). Although we did not observe progressive and irreversible paralysis, it is possible that the dosages of DB used altered CNS development producing a less intense neurotoxicity. When CPF was injected into adult mice, brain cholinesterase and nonspecific esterase activities were inhibited for up to 72 h (1). The much lower dosages used in our studies (ie, 0.3 mg CPF/kg versus 70 mg CPF/kg) may have only caused the behavioral neurotoxicity we observed.

CONCLUSIONS

Our results suggest that DB should be used with caution around pregnant women. Exposure due to environmental or residental DB residues may attain levels approximating those used in our studies (15). Prenatal exposure caused fetal defects and was associated w[illegible]

TABLE 2. Neurotoxicity associated with prenatal exposure to Dursban.

Chlorpyrifos (mg/kg)	Prenatal Day of Exposure 7-21	
0.3	44 ± 2.4* [illegible]	46 ± 0.4*
0.1	48 ± 1.1*	N/A
0.03	44 ± 1.8*	N/A
saline	39 ± 1.5	30 ± 0.7

Number of falls from rotorod/2 minutes mean ± S.D.). * = P<0.05. N/A data not available.
Each data partition consists of 20 - 50 rat pups, except as indicated by [illegible] and 8, respectively.

TABLE 3. NEUROTOXICITY OF DURSBAN.

Chlorpyrifos (mg/kg)	Postnatal Exposure Day 3	10	12
0.3	38± 0.9*	33 ± 1.4*	21 ± 1.4*
0.1	27 ± 1.1	28 ± 1.3	18 ± 1.1*
saline	27 ± 1.8	24 ± 2.3	14 ± 1.5

Number of falls from rotorod/2 minutes (mean ± S.D.). * = P<0.05. Each data partition consists of 18 - 20 rat pups.

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early postnatal neurotoxicity. In future studies we will evaluate the persistance of this neurotoxicity, its mechanisms and the potential effect the DB solvent and route of administration had on our results.

ACKNOWLEDGEMENTS

We thank Mr Victor Sierra for help with the vaginal smears and identification of cell types in the vaginal lavage.

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Carbaryl Distribution in Rabbit Tissues and Body Fluids

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ABSTRACT. After single po administration of ^{14}C-naphthylcarbamate, liquid scintillation assays evaluated the distribution of carbaryl in rabbit serum, liver, kidneys, small and large intestine, spleen, heart, muscles of the thigh and lungs and its excretion in urine and feces at 2,4,6 and 8 h after dosing. At 2 and 8 h radioactivity was not observed in spleen, heart, muscle and lungs, while all other tissues had increased values up to 6 h. The main excretory pathway of carbaryl was the kidneys.

Carbamate pesticides are widely used for agriculture and zootechnic purposes owing to their short environmental persistence and their low toxicity to animals due to reversible anticholinesterase activity. Their toxicological profile is well documented, while their pharmacokinetic one has been scarsely investigated. For instance, dimetilan pharmacokinetics has been studied in food-producing animals (1-3), but some pathways of its biotransformation are still unclear. Carbaryl (SEVIN, 1-naphthyl-N-methylcarbamate) is a carbamate insecticide that to this date is insufficiently characterized for its body distribution despite the large number of other pharmacokinetics studies already performed (4-7).

The literature indicates that naphthyl-1^{14}C-labelled carbaryl, when injected ip in rats, produced high radioactivity levels in plasma, urine, kidney and liver 4 h later (8). When N-methyl-^{14}C-labelled carbaryl was administered po to pregnant rats, radioactivity was detected in bone marrow, in the placental barrier, in the gut walls, and in the organs involved in metabolism and elimination (9).

The limited documentation on carbaryl body distribution and the lack of data in food-producing animals was the basis for the present study in rabbits, which was designed to obtain information on the residues produced by this compound.

MATERIALS AND METHODS

Forty healthy New Zealand female rabbits about 2.3 kg in body weight were used. The animals were housed in ventilated rooms under conditions of constant temperature and humidity, were offered drinking water at libitum, and were fed a standard balanced rabbit

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Chlorpyrifos (P.C. Code 059101) - Toxicology Data Review

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Action requested: Review the following study: *Fetotoxicity Associated with Prenatal Exposure to Dursban*; by M.A. Muto, F. Lobelle, Jr., J.H. Bidanset, and J.N.D. Wurpel; Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, NY 11439 (no date provided). A copy of the study is attached to this review.

Executive Summary

In the first segment of this study, Dursban (uncharacterized Rid-A-Bug formulation) was administered by intraperitoneal injection at dose levels of 0.03, 0.1, or 0.3 mg a.i./kg/day to pregnant female rats on days 1 through 7 or days 7 through 21 of gestation. Litters were delivered naturally. Pups were evaluated at birth for viability and external abnormalities. On day 16, the pups were evaluated for evidence of neurotoxicity (open field, inclined screen righting response, and rotorod testing). In the second segment of this study, Dursban was administered by intraperitoneal injection to rat pups (on postnatal days 3, 10, or 12; or on days 6-10) at doses of 0.1 or 0.3 mg a.i./kg to evaluate the neurotoxic potential of Dursban following exposure in the postnatal period. Neurotoxicity testing was conducted on postnatal day 16.

The study results appeared to demonstrate fetotoxic effects, observed as increased lethality, uncharacterized physical abnormalities, and evidence of neurotoxicity in 16-day old rat pups (increased falls from the rotorod apparatus), following either *in utero* or postnatal [illegible]

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administration of 0.3 mg a.i./kg Dursban (Rid-A-Bug). At 0.1 mg a.i./kg, the only effect noted was decreased rotorod performance in 16-day old pups following postnatal administration of Dursban on lactation day 12. No effects were reported at the low dose level of 0.03 mg a.i./kg. **(LOEL = 0.1 mg a.i./kg, NOEL = 0.03 mg a.i./kg)**

Core: Supplementary; not upgradable. This study is not considered to be appropriate for use in developmental toxicity risk assessment.

I. Materials and Methods

A. Phase 1 (*in utero* exposure)

Test animals: Rats (unknown strain) were obtained from Taconic Farms, Germantown, NY, and maintained in an appropriate laboratory environment. Estrus cyclicity was monitored prior to breeding (2 females:1 male); the day that sperm was confirmed in a vaginal smear was defined as Day 1 of gestation, and females were assigned to treatment groups.

Treatment: Dursban (Rid-A-Bug; no EPA Registration No. provided), containing chlorpyrifos and solvents (the report suggests either xylene or toluene) at unknown concentrations, was administered by intraperitoneal injection at dose levels of 0.03, 0.1, or 0.3 mg a.i./kg/day to pregnant female rats on days 1 through 7 or days 7 through 21 of gestation. Control animals received i.p. infusions of saline. The report states that the injections were administered midabdominally, in an effort to avoid direct infusion of test substance or vehicle into the fetuses or uteri. The number of animals assigned to each dose level was not reported.

Observations: All females were allowed to deliver their litters. Pups were evaluated for general viability, body weight, and physical characteristics. The report states that "some" litters remained with the dams and postnatal development was evaluated periodically; there was no indication of the number of litters treated in this manner or what endpoints were examined. On postnatal day 16, treated and control litters were evaluated for the presence of neurotoxicity and behavioral abnormalities. This evaluation included open field testing (no description of equipment was provided) as a subjective measure of spontaneous motor activity, a modified 2-minute rotorod test (2 cm diameter rod; 8 rpm), and a 1-minute 45° inclined screen test to determine if failure on the rotorod or decrements in motor activity could be attributed to sedation or muscle relaxation.

Analysis of neurotoxicity data: For the inclined screen test, animals which could not maintain position on the screen or orient appropriately (positive geotaxis) were counted as failures; the study authors judged this to be indicative of sedation or muscle relaxation. For the rotorod test, the number of times each pup fell from the rod was counted, and the data were analyzed for individual animals, not for average litter values. Treated and control data were compared. Data were analyzed by ANOVA, with "significance determined by *post hoc* comparisons of the data partitions" (Neumann-Keul's Test). Significance was established at $p \leq 0.05$.

B. Phase 2 (*post natal* exposure)

Test animals: There is no indication of the source or strain of naive rat pups used for this study segment.

Treatment: Dursban (diluted in saline) was administered by intraperitoneal injection, at doses of 0.1 or 0.3 mg a.i./kg, to groups of rat pups on postnatal day 3, 10, or 12; another group of pups received Dursban on postnatal days 6 through 10. Control pups received i.p. infusions of saline. The number of pups assigned to each group were not specified.

Observations: On postnatal day 16, all treated and control pups were evaluated for the presence of neurotoxicity and behavioral abnormalities, as described above.

Analysis of neurotoxicity data: As described above.

II. Results

A. Phase 1 (*in utero* exposure)

For offspring of dams dosed on gestation days 1-7, the study authors reported increases in both lethality and physical abnormalities (Table 1); control data were not provided. The article did not differentiate between stillbirths and postnatal deaths in the assessment of lethality, and the exact physical abnormalities observed were not described in any detail.

Table 1. Lethality and physical abnormalities observed in pups following *in utero* dosing of dams on GD 1-7

Finding (# dead/# total)	Dose (mg a.i./kg)			
	Control	0.03	0.1	0.3
Lethality	Not provided	26/34 77%	25/44 57%	21/31 68%
Physical Abnormalities	Not provided	5/34 15%	29/44 66%	17/31 55%

As reported by the study authors, administration of Dursban on days 7-21 of gestation caused a dose-related increase in lethality in treated litters (Table 2); control data were not provided. Physical abnormalities were reported for 8 of the 85 pups examined (9% incidence) at the 0.3 mg a.i./kg level for dams dosed on gestation days 7-21; it was stated that all control pups appeared grossly normal. The number of pups examined and the incidence of physical abnormalities for the 0.03 and 0.1 mg a.i./kg dose groups were not reported.

Table 2. Lethality in pups following *in utero* dosing of dams on GD 7-21 (no. dead/no. total)

Dose (mg a.i./kg)			
Control	0.03	0.1	0.3
Not provided	8/47 (17%)	11/49 (22%)	32/85 (38%)

It was reported that prenatal exposure to Dursban on either day 1-7 or 7-21 of gestation resulted in evidence of neurotoxicity in 16-day old pups, as measured by increased number of falls from the rotorod (Table 3).

Table 3. Rotorod performance in 16-day old pups following *in utero* exposure[a] - Mean(S.D.)

Days of exposure	Dose (mg a.i./kg)	
	Control	0.3
GD 1-7	39(1.5)	49(2.9)*
GD 7-21	30(0.7)	46(0.9)*

a Number of falls in 2 minute trial.
* $p \leq 0.05$.

B. Phase 2 (*postnatal* exposure)

According to the study authors, no differences in general motor behavior (open field testing) or in geotactic response on an inclined screen were seen between control and treated 16-day old rat pups.

A significant effect on the rotorod performance of 16-day old pups receiving 0.1 mg a.i./kg on postnatal day 12 or 0.3 mg a.i./kg on postnatal day 10 or 12 was observed; the number of falls was statistically increased (Table 4). For those pups receiving 0.3 mg a.i./kg on day 3 of lactation, the number of falls was increased by 41% from control, but statistical significance was not achieved. This increase appears to be a treatment-related effect.

No results were reported for neurotoxicity testing conducted on 16-day old pups that had been administered the test substance by i.p. injection on days 6-10 of lactation

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Table 4. Rotorod performance in 16-day old pups[a] - Mean(S.D.)

Postnatal day of administration	Dose (mg a.i./kg)		
	Control	0.1	0.3
3	27(1.2)	27(1.1)	38(0.9)
10	26(2.3)	28(1.3)	33(1.4)*
12	14(2.0)	18(1.1)*	21(1.4)*

a Number of falls in 2 minute trial.
* $p \leq 0.05$.

III. Discussion and Conclusions

Study deficiencies: This study did not meet guideline requirements for either a developmental toxicity study in rats (§83-3) or a developmental neurotoxicity study in rats (§83-6), although the protocol contains some aspects of both guidelines. The methodology and data provided did not include sufficient information to evaluate this study for quality or confirm the reported summary findings. There was no documentation provided to indicate that the study was conducted in a manner consistent with Good Laboratory Practice Standards; there was no indication that a Quality Assurance Unit audited the in-life phases of the study or the submitted report. The following information was not included in the study report:

1. The test substance used was not characterized; no EPA Registration No., solvent, or impurity information was provided.
2. The numbers of adult or juvenile animals and/or litters used to conduct this study
3. Individual or summary maternal observation data
4. Individual observation data on pups
5. Control data for the incidences of offspring lethality and physical abnormalities following *in utero* administration on GD 1-7 or 7-21
6. Low- and mid-dose data for the incidence of offspring physical abnormalities following *in utero* dosing on GD 7-21
7. Results for neurotoxicity testing conducted on 16-day old pups that had been administered the test substance by i.p. injection on days 6-10 of lactation

The study results appeared to demonstrate fetotoxic effects, observed as increased lethality, uncharacterized physical abnormalities, and evidence of neurotoxicity in 16-day old rat pups (increased falls from the rotorod apparatus), following either *in utero* or postnatal i.p. administration of 0.3 mg a.i./kg Dursban (Rid-A-Bug). At 0.1 mg a.i./kg, the only effect noted was decreased rotorod performance in 16-day old pups following postnatal administration of Dursban on lactation day 12. No effects were reported at the low dose level of 0.03 mg a.i./kg

(LOEL = 0.1 mg a.i./kg, NOEL = 0.03 mg a.i./kg)

It must be noted that the method of dosing in these studies (intraperitoneal injection) nearly ensures direct exposure of the pup or developing fetus to the test substance, as parent compound and/or metabolite, but is not comparable to normal anticipated routes of pesticide exposure (dietary, inhalation, dermal). In addition, information on the chemical composition of the Dursban formulation used in these studies is absent; i.p. administration of solvents such as xylene or toluene, which are used in pesticide formulations, might potentially elicit primary developmental or neurotoxic effects or potentiate the effects of the chlorpyrifos.

Previous developmental and reproductive toxicity studies submitted to and reviewed by the Agency were dosed by standard dietary or gavage techniques, not by intraperitoneal injection. The results of a CORE-Guideline two-generation reproductive toxicity study in rats (MRID 419303-01) demonstrated reductions in pup weights and increased pup mortality at a dietary dose of 5.0 mg/kg/day of 97.8-98.5% chlorpyrifos. In CORE-Minimum developmental toxicity studies dosed by gavage with technical grade chlorpyrifos, the following developmental effects were noted: in rats (MRID 404364-07) postimplantation loss was observed at 15 mg/kg/day; in rabbits (MRID 404364-08) fetal weights and crown-rump lengths were reduced, there was a suggestion of postimplantation loss, and there was an increased incidence of unossified 5th sternebrae and/or xiphisternum at 140 mg/kg/day; and in mice (MRID 098912) decreased fetal length and an increase in skeletal variants were observed at 25 mg/kg/day. No treatment-induced malformations were noted in any of the species tested.

IV. Conclusions

This study is determined to be CORE-Supplementary, and not upgradable. It does not meet guideline requirements for either a developmental toxicity or developmental neurotoxicity study in rats. Study and reporting deficiencies are described in detail above.

Although this study appears to demonstrate fetotoxicity (lethality, physical abnormalities, and neurotoxicity) in rat pups following *in utero* or postnatal exposure to Dursban (Rid-A-Bug), it is recommended that this study not be used as primary evidence of fetotoxicity in the regulation of the pesticide chlorpyrifos. This recommendation is based primarily on the use of an invasive method of test substance administration (intraperitoneal injection) which is not appropriate for the study of pesticide exposure, and also on the severe deficiencies in study conduct and reporting which render it impossible to adequately assess study quality and validity of reported results.

It is not possible to assess the need for a standard developmental neurotoxicity study (§83-6) with chlorpyrifos based on the results of this study. Should further data and information be provided, which would 1) indicate that the neurotoxicity observed in 16-day old pups following *in utero* exposure to chlorpyrifos was occurring at doses which were not maternally neurotoxic and 2) confirm the quality and validity of the study results represented in this report, a requirement for developmental neurotoxicity testing might be considered.