



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

004517

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MEMORANDUMOFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: ASPON® (O,O,O,O-Tetrapropyl dithiopyrophosphate)  
Reregistration: Toxicology Data  
EPA Reg. No. 476-2109

CASWELL #845A

FROM: George W. Robinson, D.V.M. *George W. Robinson*  
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Registrant: Stauffer Chemical Co.  
Richmond, CA 94804

The registrant, in response to requirements as set forth in the USEPA Aspon Registration Standard of September 30, 1980, submitted the following toxicity studies: acute delayed neurotoxicity (hen), subchronic 21-day dermal (rabbit), teratology (rabbit), and acute oral (dog). A second teratology study in the rat has been promised.

The registrant states that all of the required toxicology studies have been completed. According to TOX files the following additional data gaps will continue to exist for: (1) acute dermal toxicity; (2) subchronic 90-day feeding in rodent and non-rodent; (3) reproduction, 2-generation; (4) chronic toxicity in rodent and non-rodent; and, (5) oncogenicity in mice and rats.

Review of currently submitted toxicity studies follows.

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I. Acute Delayed Neurotoxicity Study with Aspon® Technical in Adult Hens by G.L. Sprague and T.R. Castles, Richmond Toxicology Laboratory, Stauffer Chemical Co., #T-10589, 12/9/82; ID No. 476-2109

Test Material: Aspon® Technical (Composite Lot #4921-24-22), purity 92.0%

Positive Control Material: Tri-o-tolylphosphate (TOCP), purity > 97%.

Vehicle Negative Control: Corn Oil (Mazola, Lot #48001-05820)

Test Animals: Adult White Leghorn Hens, 14 months old, weighing 1.3-2.6 kg, in egg production. Hens were caged individually in a controlled laboratory environment with feed and water ad libitum.

Procedure:

Aspon Technical (undiluted), TOCP in corn oil (positive control), and corn oil (negative control) were administered by oral intubation on study day 1 to adult hens as outlined below:

<u>Material</u>	<u>Dose</u>	<u>No. of Hens</u>
Aspon Technical	1.0 g/kg bw	10
Aspon Technical	10.0 g/kg bw	14
TOCP	0.50 g/kg bw	15
Corn Oil	10.0 g/kg bw	15

All hens were observed daily for signs of toxicity and walking behavior scores were recorded weekly. Repeat identical doses of test and control materials were administered orally to respective groups of hens on study day 22.

Individual body weights and food consumption were recorded every 4 days and weekly egg production was recorded for each hen. Observations continued through study day 43 (21 days after 2nd dose), the day of termination.

Surviving hens were anesthetized, exsanguinated and perfused via the heart with a cold 4% formaldehyde-1% glutaraldehyde (pH 7.2). Intact brain, spinal cord and sciatic nerves were removed, fixed, sectioned and stained. Histopathological examination was performed on sections of (1) the cerebellum with attached medulla, (2) the brain stem, (3) mid-cervical, mid-thoracic and lumbo-sacral spinal cord, and (4) sciatic nerves from both legs.

Results:

Plasma cholinesterase and brain acetylcholinesterase inhibition was determined following single, oral doses of 32, 100 and 320 mg/kg bw and 1 and 10 g/kg bw of Aspon. Significant dose-dependent inhibition of both enzymes was detected at all dose levels at 24 hours post-treatment (Brain AChE-25% at 32; Plasma ChE-50% at 32; Brain AChE-70% at 320, Plasma ChE-85% at 320).

Four of 14 hens dosed with 10 g/kg bw Aspon died: 2 within 5 days after the first dosing and 2 within 6 days of the second. There were no deaths in 10 hens dosed with 1 g/kg bw Aspon. Three of 15 hens treated with TOCP died late in the study with advanced paralysis. One of 15 hens treated with corn oil died with a perforated intestine on study day 16.

Within 1 week after treatment with Aspon, hens at both dose levels exhibited motor incoordination, non-vocal behavior, moderate to severe diarrhea, soft-shelled egg, postural and coordination difficulties. Motor incoordination in Aspon-treated hens was detected within 2-3 days after each treatment and lasted less than 1 week and was therefore transient. This transient motor incoordination was also manifest by the elevated mean walking behavior score in the high dose hens after the first treatment with Aspon and in both low and high dose hens after the second treatment. Motor incoordination in TOCP-treated hens appeared on study days 9-13 and the severity increased with time. The constant increase in walking behavior scores and leg weakness in TOCP-treated hens were also indicative of progressive motor impairment.

Body weight loss, reduced food consumption and decreased egg production were other transient effects of Aspon treatment. In the TOCP-treated hens, weight loss was progressive, food consumption remained significantly lower after the second treatment, and egg production almost ceased during the last 2 weeks of the study.

Neurohistologic changes in Aspon-treated hens at both dose levels were similar to those observed in corn oil treated hens. Proliferative changes in the brain and spinal cord (lymphocytic perivascular cuffing and focal gliosis) and peripheral nerves (lymphocytic foci and Schwann cell hyperplasia) were of similar severity (mild) and frequency in Aspon and corn oil treated hens. Axonal degeneration in the spinal cord in Aspon-treated hens was mild and randomly located in similar tracts as observed in corn oil treated hens. Peripheral nerve axonal degeneration usually occurred unilaterally and was mild in Aspon and corn oil treated hens.

In addition to the random, solitary neurohistologic changes observed in the corn oil treated hens, moderate to severe axonal degeneration was observed in the brain and all spinal cord regions in 100% of the TOCP treated hens. This axonal degeneration was accompanied by focal reactive gliosis and was limited to specific tracts or funiculi of the brain and spinal cord. Moderate to severe bilateral axonal degeneration was also present in peripheral nerves of all TOCP treated hens.

Conclusion:

There were neither clinical signs nor neurohistopathologic changes indicative of delayed neurotoxicity in adult White Leghorn hens treated twice (21 days apart) with Aspon Technical by oral intubation at dosages of 1.0 and 10.0 g/kg bw. Plasma cholinesterase and brain acetylcholinesterase were significantly inhibited by all doses greater than 32 mg/kg bw Aspon.

TOCP treated hens (positive control) exhibited leg weakness and motor incoordination with delayed onset that increased in severity with time. Also, neurohistopathologic changes characteristic of delayed neurotoxicity were localized in specific tracts and funiculi of the brain and spinal cord of hens treated with TOCP.

Negative for acute delayed neurotoxicity (10 g/kg/bw)  
LEL < 32 mg/kg bw (reduced plasma ChE and brain AChE)

Classification: Core-Minimum

II. Subchronic 21-Day Dermal Toxicity Study in Rabbits by T.J. Davidson and P.H. Becci, Food and Drug Research Laboratories, Inc., No. 7489, 3/14/83; ID No. 476-2109.

Test Material: Aspon Technical, Lot #145; Ref. #4921-24-22

Vehicle: Mineral Oil (Aspon was diluted in mineral oil daily)

Test Animals: Male and Female New Zealand White rabbits, weighing 2-4 kg, from the New York State Rabbit Development, Hartwick, N.Y. Rabbits were caged individually in a controlled laboratory environment with feed and water ad libitum.

Procedure:

Rabbits were randomly assigned to one of 4 groups of 5/sex which received the test material at 0.1, 0.5 and 2.0 mg/kg bw and the vehicle control (mineral oil) at 2 mg/kg bw by dermal application 5 days per week for 3 consecutive weeks. Hair was clipped from a 130 cm<sup>2</sup> area on the back of each rabbit prior to initial dosing and once per week thereafter. Individual doses were spread evenly on the exposed skin with a glass rod. The test site was covered with gauze and an occlusive binder. Six hours later coverings were removed and any excess material on the test site was wiped off with gauze.

The rabbits were observed twice daily for signs of toxicity and mortality. Draize dermal irritation scores were recorded daily before applying test or control material to the test site. Each rabbit was weighed prior to initial dosing, the 7th day of each week, and at termination. Food was measured for a 48-hour period on each weekend.

Blood samples were collected from all rabbits prior to initial dosing and at termination for hematological and clinical chemistry determinations.

Surviving rabbits were sacrificed on study days 22 or 23. All rabbits, including those sacrificed moribund, underwent complete gross necropsies. Adrenal glands, kidney, liver and testes (males) of each rabbit were weighed at necropsy. Skin (treated and untreated), kidneys, liver, testes/ovaries and tissue samples of all gross lesions were fixed in 10% neutral buffered formalin, trimmed, dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Results:

All rabbits survived to study termination except one mid-dose female which was found dead on day 3 of the study; this death was not related to the test material.

Very slight to mild erythema occurred sporadically during the first week in males of treated and vehicle control groups, but was absent in all females at the end of the first week. Low grade mean erythema scores appeared in females, and persisted in males during the second week; scores were similar in both sexes in high dose and vehicle control groups. During the third week, mean erythema scores gradually decreased in both sexes in the vehicle control groups while remaining unchanged in the high dose groups. However, no significant differences were detected in the incidence or severity of erythema in high dose and vehicle control groups. Very slight edema occurred sporadically and infrequently in treated and control groups during the 3-week study period and was unrelated to the test material.

Mean body weight, mean food consumption, and absolute and relative weights of liver, kidneys, adrenals or testes (males) were comparable in treated and control groups. No significant differences were noted among groups in any of the hematological determinations. Mean serum cholinesterase levels at termination in the high dose female group were significantly less than levels in the vehicle control. Mean terminal serum cholinesterase values were 16 to 18% less than pre-test values in high dose males and females and appeared to be related to the test material. Red blood cell cholinesterase levels were comparable in treated and control groups. Terminal mean potassium levels in mid and high dose males were significantly less than the pre-test means for these groups. Mean potassium levels were significantly reduced in low, mid and high dose males compared to vehicle control males. The greatest reduction occurred in mid dose males. Mean potassium levels at termination in females were similar to pre-test means and were comparable among the groups of females. There were no gross lesions at necropsy or histopathological findings which were related to the test material.

Conclusion:

Aspon Technical, at 0.1, 0.5 and 2.0 mg/kg bw by dermal application 5 days/week for 3 consecutive weeks, was not lethal and produced no overt clinical signs of toxicity in rabbits. Serum cholinesterase levels were significantly reduced in females in the high dose group; high dose males also exhibited reduced serum cholinesterase values.

NOEL = 0.5 mg/kg/day  
LEL = 2.0 mg/kg/day (reduced serum ChE in both sexes)

Classification: Core-Minimum

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III. A Teratology Study in Rabbits with Aspon by M.D. Nemec,  
WIL Research Laboratories, Inc., Proj. No. WIL-27005,  
4/14/83; ID No. 476-2109.

Test Material: Aspon Technical, WRC#4921-24-22,  
Lot #MP-145; light, yellow liquid from  
Stauffer Chemical Co. (considered 100% pure)

Vehicle: Mazola®; 100% Pure Corn Oil (diluent for Aspon)

Test Animals: Female New Zealand White rabbits, weighing  
3.0 - 4.6 kg, from Langshaw Farms, Augusta,  
Michigan. Rabbits were caged individually  
in a controlled laboratory environment with  
food and water ad libitum.

Procedure:

Female rabbits were randomly assigned by computer to one of 4 groups of 18 which received test material at 3, 10 and 30 mg/kg bw/day and the vehicle control (Mazola corn oil) at 0.5 ml/kg bw/day by gavage for 13 consecutive days on gestation days 6 through 18. All dose levels were adjusted to a dosage volume of 0.5 ml/kg. Individual doses at initiation of treatment were based on gestation day 6 body weights.

Semen was collected with an artificial vagina from 6 resident males of the same strain and obtained from the same source as females. Fresh semen, diluted in physiological saline to a final concentration of more than 3 million motile sperm/ml, was pipetted into the vagina of each female. Each inseminated female received an immediate i.v. injection of 100 U.S.P. units of human chorionic gonadotropin to stimulate ovulation. The day of insemination was gestation day 0.

All females were observed daily for appearance and behavior, clinical signs of toxicity and mortality from gestation days 0 through 29. Individual body weights were recorded on days 0, 6, 12, 18, 24 and 29 of gestation for all females. Daily food intake was recorded for the following intervals of gestation: days 0-6, 6-12, 12-18, 18-24 and 24-29. Food consumption was calculated and reported as g/animal/day and g/kg/day.

All surviving females were sacrificed, pups delivered by cesarean section on gestation day 29 and dams examined for gross internal morphological changes. Intact uteri were extirpated, weighed and incised for examination of contents. Numbers and position of all live and resorbed fetuses, implantation sites and corpora lutea were recorded. Live fetuses were weighed, sexed and examined for external, visceral and skeletal variations and malformations.

Results:

One female in the high dose group was sacrificed on gestation day 22 because of a broken leg. Three females, one each in low, mid and high dose groups, aborted on gestation day 29 and were not included in analyses.

Mean maternal body weight gains were substantially less in the high dose group than in the vehicle control group during the treatment period (gestation days 6-8) and 6 days post-treatment (gestation days 18-24). In the low and mid dose groups, mean maternal body weight gains were greater than in the vehicle control group during the same gestational intervals. There were no significant differences in food consumption for treated and vehicle control groups. No gross lesions were observed in dams at sacrifice which were related to Aspon treatment.

Fertility rates were comparable for dams of all groups. There were no significant differences in the mean number of implantation sites, resorptions and corpora lutea for treated and control groups. No dead fetuses were observed in any on the dams. Litter size, fetal sex distribution and mean fetal body weights were comparable in treated and control groups. The incidence of malformations and developmental variations in fetuses and litters of treated groups was not significantly different from that of the control group, with gallbladder absent or small and major blood vessel variation (left carotid arising from the brachiocephalic trunk) the most frequently reported variations in all groups.

Conclusion:

Aspon Technical, at doses of 3, 10 and 30 mg/kg bw by gavage on days 6 through 18 of gestation in New Zealand White rabbits, was not teratogenic or fetotoxic. Reduced maternal body weight gain was evident in the high dose group.

Teratogenic NOEL > 30 mg/kg bw/day  
Fetotoxic NOEL > 30 mg/kg bw/day  
Maternal NOEL = 10 mg/kg bw/day  
Maternal LEL = 30 mg/kg bw/day (reduced bw gain)

Classification: Core-Minimum



IV. Acute Oral Toxicity Study in Dogs - Aspon Technical by  
J.A. Trutter, Hazleton, Laboratories America, Inc.,  
Proj. No. 132-159, 1/21/83; ID #476-2109

Test Material: Aspon® Technical, Ref. No. 4921-24-22,  
light yellow liquid (considered 100% a.i.)

Test Animals: Male and Female Beagle dogs weighing 13.4 -  
17.1 kg (males) and 11.2-17.2 kg (females)  
from Erick Stock, Gelnhausen, W. Germany.  
Dogs were caged individually in a controlled  
laboratory environment with feed and water  
ad libitum.

Procedure:

Dogs were randomly assigned by body weight and sex to  
the following groups:

1. Each of 3 groups of 4 males received test material  
at dosages of 200, 600 and 2000 mg/kg bw.

2. Each of 3 groups of 4 females received test material  
at dosages of 60, 180 and 600 mg/kg bw.

Each dog received a single oral dose of test material in  
one or more gelatin capsules on study day 1. All dogs were  
observed for signs of toxicity at 1 and 4 hours post-treatment  
and twice daily thereafter. Individual body weights were  
recorded on study days 1, 7, 14 and 15 and weekly food consump-  
tion was measured on days 7 and 14. Blood samples were collected  
from each dog prior to treatment, 4 hours post-treatment and on  
study day 7 for plasma and erythrocyte cholinesterase analyses.  
All dogs were sacrificed on study day 15 and underwent complete  
gross necropsies.

Results:

All dogs survived to termination of the 14-day study. Female  
dogs in all dose groups (60, 180 and 600 mg/kg bw) and male dogs  
in the low dose group (200 mg/kg bw) appeared normal throughout  
the study. Tremors were observed in one mid-dose (600 mg/kg bw)  
male at 4-hours post-treatment and in one high dose (2000 mg/kg  
bw) male on each of days 2-5. Emesis was observed in one high  
dose male at 2-hours post-treatment and in another high dose  
male on study day 2. All dogs appeared normal during the second  
week of the study.

Mean plasma ChE levels decreased 43-68% in all dose groups at 4-hours post-treatment and remained depressed, to a lesser degree, on study day 7 with a dose-related trend. Erythrocyte ChE levels were decreased in all dose groups at both post-treatment intervals; however, an inverse related trend occurred in female dogs.

Conclusion:

No acute oral LD<sub>50</sub> was determined since no mortality resulted from the dosages administered to dogs in this study. Plasma and erythrocyte cholinesterase inhibition was the principal indicator of Aspon toxicity.

LD<sub>50</sub> > 2,000 mg/kg bw (males) (HDT)  
LD<sub>50</sub> > 600 mg/kg bw (females) (HDT)  
ChE NOEL < 200 mg/kg bw (males) (LDT)  
ChE NOEL < 60 mg/kg bw (females) (LDT)

Classification: Toxicity Category III; Core-Minimum

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