7-31-81 OPP-1166 TXR-903

000903

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: An Experimental Use Permit No. 7969-EUP-EU for the Herbicide 2-[1-(ethoxyimino)-butyl]-5 [2-(ethylthio) propyl]-3-hydroxy-2-cyclohexen-1-one (BAS: 9052 H; Trademarked Poast®, and Temporary Tolerances for Residues of this Chemical (PP 0G2396). Accession Nos. 099536, 243319 and 099537; 099535, 099538, 099802.
Caswell No. 72A

Proposed Tolerances:
0.25 ppm in or on Soybeans
0.05 ppm in or on eggs, milk, and the meat, fat,and byproducts of cattle goats, hogs, horses, poultry and sheep.

FROM: Minnie R. Sochard, Ph.D.
Toxicology Branch/HED (TS-769) Minnie R. Sochard 7/31/81

TO: Robert Taylor, PM #25
Registration Division (TS-767)

THRU: Krystyna K. Locke, Acting Section Head
Section II, Toxicology Branch/HED (TS-769) Krystyna K. Locke 7/31/81

THRU: Christine Chaisson, Acting Branch Chief
Toxicology Branch/HED (TS-769)

Petitioner: BASF Wyandotte Corporation
Parsippany, New Jersey

Action Requested:

BASF Wyandotte Corporation requests an Experimental Use Permit, for the herbicide BAS9052 H (Poast®). They also propose that temporary tolerances for residues of this chemical be established as Follows:

0.25 ppm in or on Soybeans
0.05 ppm in or on eggs, milk, and the meat, fat,and byproducts of

Recommendations:

1. Toxicology Branch has no objections to the issuance of an EUP for the herbicide BAS 9052H and temporary tolerances for residues of this chemical established as proposed.

2. Toxicological data support the issuance of the EUP and requested tolerances (see Eight Point Free Standing Summary).

3. Label modifications should be implemented to reflect Category I toxicity of the formulated product as evidenced by primary eye irritation and primary dermal irritation studies.

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4. Toxicology Branch concurs with RCB's recommendation that the petitioner modify the product label so that only non-phytotoxic oil concentration additives approved for this pesticide use are mentioned on the label. A similar change should be made in the description of the EUP program which deals with the use of a non-phytotoxic oil concentration to be added to a spray solution of this pesticide.

5. Presently available data do not indicate the presence of nitrosamines as metabolites of BAS9052 H.

Description of EUP Program:

The Experimental Use Permit Program proposes that BAS9052 H (trade name Poast®) is to be applied as a post emergence application to soybeans and grass weeds at an early stage of their growth at the maximum recommended application rate of 0.5 lb. a.i. per acre (broadcast basis). In some cases, a second application may be needed 2-4 weeks after the first application for complete control of less sensitive grasses. Applications are timed to the growth stage of the grasses. Soybeans are tolerant to this chemical at all stages of growth. The addition of a non-phytotoxic oil concentrate to the spray solution is always recommended. The EUP proposes the use of 2400 pounds of active ingredient on a total of 8,000 acres of soybeans, with a 70 day last application-to-harvest interval. The grazing of treated soybean fields or the feeding of treated soybean forage or hay to livestock is prohibited by label restrictions. The finished product, at 1600 gallons, will be imported. (The total quantity proposed for shipment is 1568.83 gallons, with active equivalent of 2400 pounds.)

Description of Chemical and Formulations:

A. Substance Identificat on

1. Chemical Name

2-[-1-(ethoxyimino)-butyl]-5-[2-(ethylthio) propyl]-3- hydroxy -2-cyclohexen-1-one.

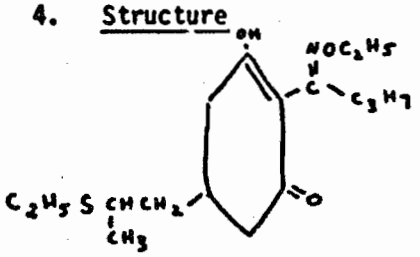
2. Synonyms

Poast® Herbicide
NP-55
BAS 9052 H
BAS 9052 OH (formulated product)

3. Purity of Technical Material

As currently produced BAS 9052 H is > 94.9% pure.

4. Structure



5. Other Physical/Chemical Data:

Empirical Formula $C_{17}H_{29}NOS$
Molecular Wgt. - 327.5
Color: reddish brown
Odor: characteristic of sulfur - containing compounds
Physical State: oily liquid
Specific Gravity: 1.043 (25° C)
Boiling Point > 90° C (at $4X10^{-5}$ mmHg)
Vapor pressure < $1X10^{-6}$ mmHg (20° C)
pH 4.2 (48 ppm aqueous solution)
Dissociation constant pKa 4.61
Octanol/Water partition coefficient 21/27

Solubility

In water: 24.5 ppm (25° C)
In Organic Solvents (g/100 gms solvent): Methanol > 100
Acetone > 100
Methylene dichloride > 100
Hexane > 100
Ethyl acetate > 100
Benzene > 100

Stability:

Stability in Water
Estimated half-life of 5.0 ppm at 25° C:
at pH 3.0 = 43.3 hours
at pH 6.0 = 962.7 hours
at pH 9.0 = 4077 hours

Stability in Acidic or Basic Solution
Estimated half-life of 5.0 ppm at 25° C:
in 0.1 NHCl = ca 8 hours
in 0.1 Na OH = 792 hours

Referenced Petitions - None.

Formulations:

Poast® Herbicide:

Active ingredients - percent by weight

The product for which registration is being sought is Poast® Herbicide, an emulsifiable concentrate containing 20% BAS 9052H (1.35 lb. act/gal).

2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio propyl]-3-hydroxy -2-cyclohexen-1-one.....20%

Inert ingredients.....80%

[redacted]

OPP:HED:TOX: M.SOCHARD:sb 3/4/81 TS-769 Rm. 816 CM 2 #1

Sethoxydim scientific review

Page _____ is not included in this copy.

Pages 5 through 10 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

_x_ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

___ FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Summary of Toxicity Data

Eight Point Free Standing Summary

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Summary of selected toxicology data considered in setting the tolerances:

a) Data on Technical NP-55

STUDY	RESULTS	TOX CATEGORY	CORE CLASSIFICATION
Acute Oral LD50, Rat	3.125 gms/kg - males 2.676 gms/kg - females	III	Guideline
Acute Dermal LD50, Rat	> 5.0 gms/kg, males & females	III	Guideline
Acute Inhalation LC50, Rat (4 hours)	6.03 mg/L, males 6.28 mg/L, females	III	Guideline
Primary Eye Irritation, Rabbit	No irritation	IV	Guideline
Primary Dermal Irritation, Rabbit	No irritation	IV	Guideline
Dermal Sensitization, Guinea Pig	Negative	-	Minimum
14-Week Mouse Feeding Study	NOEL = 300 ppm	-	Minimum
14-Week Rat Feeding Study	NOEL = 300 ppm	-	Guideline
26-Week Dog Feeding Study	NOEL = 120 ppm	-	Minimum
2-Year Mouse Feeding Study (1-Year interim report)	NOEL - Cannot be determined from the interim report; also, female 12 mos. blood chem. data absent.	-	Supplementary
2-Year Rat Feeding Study (1-Year interim report)	NOEL - Cannot be determined from the interim report; also, urinalysis absent from protocol.	-	Supplementary
Teratology Study, Rats	Teratogenicity NOEL: 250 mg/kg /day (highest dose tested) (Maternal NOEL = 40 mg/kg/day maternal LEL = 100 mg/kg/day [significantly decreased adrenal weight]).	-	Guideline
Teratology Study, Rabbits	Teratogenicity NOEL 160 mg/kg/day Teratogenicity LEL = 480 mg/kg/day (Increased number of a variety of random effects including skeletal, visceral abnormalities, reduced fetal weight, changes in male/female ratios) (Maternal NOEL = 160 mg/kg/day Maternal LEL= 480 mg/kg/day [severe weight loss, 5/16 deaths, 6/16 abortions, reduction in number of litters and viable fetuses]).	-	Minimum

STUDY	RESULTS	TOX CATEGORY	CORE CLASSIFICATION
Mutagenicity Studies:			
i. Rec-assays and forward mutations, B. subtilis, E. coli, S. typhimurium	Negative at concentrations of chemical to 100%	-	Minimum
ii. Mouse host-mediated assay - S. typhimurium	Negative at up to 2.5 gms/kg/bw/day of chemical	-	Minimum
Two Generation Reproductive Study in Rats. (23-week interim report with addendum)	No conclusions can be drawn from this interim report with addendum. No reports on necropsies or tissues saved.	-	Supplementary
Metabolism Study - Rats	Tissue accumulation of chemcial negligible and excretion extremely rapid, assuming DMSO vehicle doesn't affect storage or excretion of the chemical.	-	Guideline
b) Data on Formulated Product - BAS 9052 OH			
Acute Oral LD50, Rat	4918.7 mg/kg	III	Guideline
Acute Dermal LD50, Rat	> 4000 mg/kg	III	Guideline
Acute Inhalation LC50, Rat	> 7.6 mg/L	III	Guideline
Primary Eye Irritation, Rabbit	P.I. = 32 (24-hrs.); 35 (48-hrs.); 29 (72 hrs.) Tox. Category I, (scarring in 5/6 animals at 8 days, corneal opacity, in one animal at 8 days).	I	Minimum
Primary Dermal Irritation, Rabbit	P.I. = 4.0 (numerical score indicates moderately irritating but 1 animal with necrosis & 5/6 with severe scaling at 8 days upgrades category to I).	I	Minimum

2. Summary of Data Considered Desirable But Lacking for This Action:

a. The registrant indicated a six month feeding study in dogs is in progress but data has not yet been received.

3. Action Being Taken to Obtain the Lacking Information

The petitioner described a six month feeding study in dogs, undertaken by Nisso Institute, would be completed in January of 1981.

4. Summary of Other Tolerance Data for This Pesticide

Since this is a new chemical, no other tolerances have been published to date. See attached printout, dated February 2, 1981.

5. The total of the tolerances under consideration can be found on the attached printout. The total tolerances under consideration to be granted utilize 19.61 percent of the Provisional Maximum Permissible Intake. The total Maximum Residue Concentration contribution to the average daily diet would be 0.0353 mg/day/1.5 kg. The individual TMRC contribution to the average daily diet is noted on the printout.

6. Acceptable Daily Intake Data

Dog - Beagle mg/kg	ppm	S. F.	PADI mg/kg/day	MPI mg/kg/day
3.0	120	1000	0.0015	0.0900

7. There are at this writing no pending regulatory actions against the registration of this pesticide.

8. There are no other relevant considerations in setting these tolerances.

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No CFR Number BAS 9052 H 6/26/81

File last updated 6/26/81

ACCEPTABLE DAILY INTAKE DATA

Dog	NOEL	S.F.	ADI	MPI
mg/kg	ppm		mg/kg/day	mg/day(60kg)
3.000	120.00	1000	0.0030	0.1800

Current Action PP# 0G2396

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Soybeans(148)	0.250	0.92	0.00344
Meat,inc poultry(89)	0.050	13.65	0.01039
Milk&Dairy Products(93)	0.050	28.62	0.02146

MPI	THRC	% ADI
0.1800 mg/day(60kg)	0.0353 mg/day(1.5kg)	19.61

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Detailed Review of Studies

Acute Studies, Technical NP-55 (BAS 9052 H)

1. Acute Oral Toxicity of NP-55 in Rats; Report C1; Nisso Institute; October 18, 1979. Tab. C1; pp 1-43; Accession No. 099536, Chemical No. 0G2396

   Protocol - Six groups of male SD-SLC rats (6 weeks old), each group consisting of 10 rats, were fasted overnight and then given by intragastric intubation the following doses of undiluted technical NP-55: 2083, 2500, 2739, 3000, 3286 and 3600 mg/kg. Similar groups of female rats were given doses of 2200, 2569, 3000, 3503, 4091 and 4777 mg/kg. All animals were observed for mortality and signs of toxicity for 14 days. Body weights were recorded at 0, 1, 2, 3, 7 and 14 days. Gross necropsies were performed on all animals that died during the study and on all survivors at the end of the 14 day observation period.

   Results - Most mortalities occurred within the first 2 or 3 days following dosing. Signs of toxicity observed in these animals were ataxia, ventral position, sedation, hypotonia, lacrymation, salivation, tremor, paralysis, fall of body temperature, urine incontinence diarrhea (at 10 minutes to several days). Gross necropsies in these animals revealed dark reddish lungs and hemorrhagic stomachs. Surviving animals showed similar signs of toxicity but to a lesser degree. Decreases in body weights were observed for 3 days with recoveries by 7 days. Gross necropsies on these animals at 14 days were negative.

   Acute Oral LD50, males = 3125 (2957-3341) mg/kg
   Acute Oral LD50, females = 2676 (2391-2919) mg/kg

   Toxicity Category III, Core Study - Guidelines.

2. Acute Dermal Toxicity of NP-55 in Rats; Report C2; Nisso Institute, October 18, 1979; Tab. C2; pp 1-77; Accession No. 099536.

   Protocol - Three groups of male Wistar-SLC rats (6 weeks old), each group consisting of 10 rats were depilated at application sites and the sites abraded to penetrate the stratum corneum but not the dermis. The following doses of undiluted technical NP-55 were applied to the abraded skin sites and kept there in contact for 24 hours: 1000, 2000 and 5000 mg/kg. Similar groups of female rats were given the same doses. All animals were observed for mortality and signs of toxicity for 14 days. Body weights were recorded at 0, 1, 2, 3, 7 and 14 days. Gross necropsies were performed on all survivors at the end of the 14 day observation period.

   Results - There were no mortalities in any group. No toxic signs were observed in any animals in all groups. Body weights decreased one day following dosage, but recovered thereafter. Gross necropsies of all survivors showed no pathological changes.

   Acute Dermal LD50, males = > 5000 mg/kg
   females = > 5000 mg/kg

   Toxicity Category III, Core Study - Guidelines

3. Acute Inhalation Toxicity Study of NP-55 in Rats; Report C3; Nisso Institute; October 18, 1979, Tab. C3; pp 1-58, Accession No. 099536

Protocol - Five week old, SD-SLC male rats were divided into three groups of 10 animals each, designated experimental, vehicle control and negative control groups. Animals were restrained with heads inserted into a nebulizing chamber fitted with an ultrasonic nebulizer (NE-U-10). Experimentals were exposed for 4 hours to a nebulized mixture of technical NP-55 (25%) and dimethylsulfoxide (DMSO, 75%). Vehicle control animals were exposed for 4 hours to a nebulized mixture of 25% water and 75% DMSO. Negative control animals were exposed for 4 hours to air. Three similar groups of female rats were similarly treated. Air flow rates, actual chamber concentration of materials near the breathing zones of the animals, temperature and humidity in the chambers were measured at 1, 2, 3 and 4 hours. Air flow rates ranged from 162.5 ml/sec to 191.0 ml/sec. Particle size measurements were taken twice hourly during exposure. Particle size averaged 0.614 u $\pm$ 0.060 in chambers of males and 0.615 u $\pm$ 0.049 for females. Nominal average concentration for males was 39.7 mg/L and 41.1 mg/L for females for 4 hours exposure. Actual chamber concentrations of chemical were 6.03 mg/L for males and 6.28 mg/L for females. Observations for toxic signs were made hourly on the day of dosing and once thereafter for 14 days. Body weights of animals were measured at the beginning of dosing, after 4 hours and at 1, 2, 3, 4 and 14 days. Surviving animals were necropsied at 14 days.

Results - No mortalities occurred in any groups. Lacrymation and salivation were observed at 2 hours, lasting to 4 hours after inhalation exposure in both experimental and vehicle control groups, but were absent thereafter. Body weights in all groups decreased at 4 hours but recovered thereafter, with retarded recovery in experimental animals. No other toxic effects were observed. No significant changes were seen in organs at necropsy of survivors.

Acute Inhalation LC50 (4 hours) in males = 6.03 mg/L
in female = 6.28 mg/L

Toxicity Category III, Core Study - Guidelines

4. Primary Eye Irritation Study of NP-55 in Rabbits; Report C4, Nisso Institute; October 18, 1979, Tab. C4; pp. 1-10; Accession No 099536

Protocol - 0.1 ml of undiluted NP-55 was instilled into the left eyes of 9 male Japanese White rabbits (age about 3 months). The right eyes served as controls. The treated eyes of 3 rabbits were flushed for one minute with lukewarm water, 20-30 seconds after instillation. The treated eyes of the remaining 6 rabbits remained unwashed. Observations were made examining the eyes for corneal opacity, iritis, conjunctival redness, chemosis or discharge at 1, 2, 3, 4 and 7 days after treatment. Grading and scoring of irritation were performed by the method of Draize.

Results - No eye irritation was observed in any of the treated animals. PI = 0 (no irritation).

Toxicity Category IV, Core Study - Guidelines.

5. Primary Dermal Irritation Study of NP-55 in Rabbits. Report C5; Nisso Institute; October 18, 1979, TAb. C5; pp 1-10 ; Accession No. 099536.

Protocol - Six male Japanese White rabbits about 3 months old were clipped free of hair at application sites one day prior to treatment. 0.5 ml of technical NP-55 was introduced under 3 cm^2 gauze patches at two intact and two abraded skin application sites on each animal. The abrasion penetrated the stratum corneum but not the dermis. Plastic material was used to cover the gauze patches in order to retard evaporation and keep the test substance in contact with the skin for 24 hours on the restrained animal. After 24 hours the wrapping was removed, the skin sites wiped (not washed) to remove remaining test substance and readings of dermal lesions made at 24 and 72 hours after treatment. Primary skin irritation was scored according to the method of Draize.

Results - No significant changes were seen in both intact and abraded skin sites at 24 and 72 hours after application. Results indicate NP-55 is non-irritating.

Primary skin irritation index = 0
Toxicity Category IV, Core Study - Guidelines

6. Dermal Sensitization Study of NP-55 in Guinea Pigs; Report C6; Nisso Institute, October 18, 1979, Tab. C6; pp. 1-25; Accession No. 099536.

Protocol - Three groups of young adult male Hartley guinea pigs weighing 273-358 g were prepared for intradermal injection by shaving off a strip of hair on each side of the animals from flank to trunk. Groups of ten animals were injected intradermally starting at one end of one strip and choosing a new site for each succeeding injection as follows: Group one was injected on day one with 0.05 ml 10% NP-55 in corn oil; subsequently, animals were injected with 0.1 ml three times weekly on alternate days for a total of 10 injections. Animals were rested 2 weeks and challenged intradermally with 0.1 ml test material. Group two was injected with 20% NP-55 in corn oil using an injection dosage and schedule similar to that of Group One. Group Three, the positive control group was injected using the same dosage and schedule as for groups one and two, except that the injected chemical consisted of 0.1% p-phenylene diamine (PPDA) in corn oil.

Skin reactions including erythema, edema and other lesions were scored at 24 and 48 hours after each application by Draize's method. Individual body weight of all animals were measured up to the 35th day.

Results - Experimental animals in groups one and two showed little change in response after the challenge injections in comparison with averages of previous injections. Controls, however, showed a similar non-sensitizing response. Therefore, both positive controls and experimental animals showed no evidence of dermal sensitization. Because positive controls showed no reaction, and the experimental material was diluted but showed no toxic effects in dermal irritation tests, the test is down graded to core-minimum data.

Dermal Sensitization - Negative.

Core - Minimum Data.

Acute Studies, Formulation (BAS 9052 OH)

7. Report on the Acute Oral Toxicity of "BAS 9052 OH" in the Rat. Report C7; BASF Gewerbehygiene und Toxikologie; July 18, 1980, Tab. C7; 7 pages (in German); Accession No. 099536, English Translation, 7 pages; Accession No. 243319.

Protocol - Ten groups of Sprague-Dawley rats, each group consisting of 10 animals (5 males and 5 females, mean weights 180 g) were fasted 15-20 hours, and then administered by gavage the following single dosages of BAS 9052 OH (0.5% aqueous carboxymethylcellulose form formulated product): 21.5, 46.4, 100.0, 215.0, 1000.0, 3160.0, 3830.0, 4640.0 5,000.0 and 6810.0 mg/kg. Animals were observed for 14 days for mortalities and pharmacotoxic effects. Body weights were measured prior to dosage, and at 2-4 days, 7 days and 14 days after treatment. Dead and surviving animals were necropsied. LD50 and 95% confidence intervals were calculated for males, females and males plus females by the probit method.

Results - Dyspnea, apathy, spastic gait and poor general state were symptoms in all dosage groups. Abnormal position, staggering, piloerection and salivation were observed in the 4 highest dosage groups. Atonia, pain reflex absent, narcotic like state and exciccosis were present in the 3 highest dosage groups. Body weight were depressed at the beginning of the test regimen and remained lower over time at the highest dosage levels to 7 days with recovery at 14 days. The acute LD50 for males was approximately 5000 mg/kg with no 95% confidence limits reported. The acute LD50for females was 4385.8 mg/kg with 95% confidence limits between 3549.5 and 5376.3. For males and females, the combined LD50 was 4918.7; 95% confidence limits were between 4538.6 - 5508.6. At necropsy, dead animals showed heart dilatation, acute congestive hyperemia with atonic, diarrheaic intestine. Sacrificed animals showed several cases of adhesions of forestomach to liver, spleen, peritoneum with thickened forestomach and focal thickenings.

Acute LD50 males = 5000 mg/kg
Acute LD50 females = 4385.8 mg/kg
Acute LD50 males plus females = 4918.7 mg/kg

Toxicity Category III, Core Study - Guidelines

8. Report on the Study of the Acute Dermal Toxicity of "BAS 9052 OH" in the Rat. Report C8; BASF Gewerbehygiene und Toxikologie; July 18, 1980; Tab. C8, 3 pp. (in German); Accession No. 099536; In English, 3 pp.; Accession No. 243319.

Protocol - Four Groups of 10 Sprague-Dawley, SPF breed rats (5 males and 5 females per group, mean weight 218 g for males and 182 g for females) were clipped free of hair on the lateral and dorsal part of the trunk 15-24 hours prior to application of the test material. The following four dosages of BASF 9052 OH were applied uniformly to test sites on the skin of 50 cm^2: a 50% aqueous preparation in a dose of 400 mg/kg and 1000, 2000, and 4000 mg/kg undiluted. Treated skin areas were covered with inert foil and secured with adhesive tape. After 24 hours, the chemicals were washed off with warm water or a mixture of water and Lutrol and dried with cellulose. Mortalities within 14 days were noted. Observations were made for pharmacotoxic signs and local irritation and surviving animals necropsied after 14 days. (Departure from guidelines protocol-material washed rather than wiped off skin.)

Results - No mortalities occurred in any groups up to 14 days. Pharmacotoxic signs were noted but not reported as correlated with any dosages. Signs of skin irritation (erythema, edema) occurred in the 4000 mg/kg group returning to normal by 7 days. Similar signs to a lesser degree, dimishing in amount with dosage were found in remaining groups; these also returned to normal by 7 days. Necropsy of sacrificed animals showed no abnormalities.

Acute Dermal LD50, male and female rats = > 4000 mg/kg

Toxicity Category III, Core Study - Guidelines

9. Report on the Determination of the Acute Inhalation Toxicity LC50 of BAS 9052 OH, as a Liquid Aerosol After a 4-Hour Exposure in Sprague-Dawley Rats. Report C9; BASF Gewerbehygiene und Toxikologie; Tab. C9; pp. 1-13 (in German); July 7, 1980; Accession No. 099536; In English; 14 pp., Accession No. 243319.

Protocol - Two groups of Sprague-Dawley rats (10 males, mean body weight 193 g and 10 females, mean body weight 177 g) were exposed by the inhalation route to a nominal concentration of 28.05 mg/L formulated BAS 9052 OH aerosolized in a dynamic inhalation system (model 940 Schlick two-component atomizer) with an air flow of 1.25 ml/sec. Animals were restrained so that snouts projected into the inhalation chamber. The mean analytical concentration of chemical near the noses of the animals was 7.64 mg/L, a dose which permitted determination of a no effect level. Particle size was not reported. Exposure of the animals was for 4 hours. Body weights were measured at the beginning of th experiment, after 7 days and 14 days. Animals were observed for mortalities and pharmacotoxic effect. Necropsies were performed on surviving animals at the end of 14 days. Changes in body weight over the experimental period were determined by comparison with a group of untreated controls (10 males, mean weight 193 g and 10 females, mean weight 183 g). Calculation of the LC50 (4 hours) was by the binomial test.

Results - There were no mortalities. Symptoms among experimentals included discharge from eyes and nose; lid closure; dyspnea; staggering gait; crouching posture; apathy; ruffled and slightly sticky fur. No symptoms were seen after 6 days. Body weight gain of males was retarded in comparison with controls; this was not seen with females. No abnormalities were seen at necropsy. The LC50 (4 hours) was calculated to be greater than 7.64 mg/L at the 1% level of significance.

LC50 (4 hours), male and female rats = > 7.6 mg/L

Toxicity Category III, Core Study - Guidelines

10. Report on the Study of the Primary Irritation of "BAS 9052 OH" on the Eye of White Rabbits. Report C10, BASF Gewerbehygiene und Toxikologie; Tab. C10 ; pp. 1-3 (in German); August 15, 1980, Accession No. 099536; In English 6 pp. Accession No. 243319.

Protocol - 0.1 ml of undiluted "BAS 9052 OH" was applied to the conjunctival sac of the lower right eyelid of three male and three female (3.55 and 3.39 kg mean weights) White Vienna rabbits. Eyes of the animals were not washed after treatment. Animals were observed for eye irritation and scored according to the Draize method at 24, 48 and 72 hours and 8 days following treatment. Observation were not made beyond 8 days.

Results - The primary eye irritation average indexes were 32, 35 and 29 at 24, 48 and 72 hours respectively. At 8 days redness was observed in the conjunctivae of all animals, discharge in one animal and very slight corneal opacity in one animal. In addition, scarring was seen in 5 of 6 animals at 8 days.

P.I. Index = 32 (24 hours); 35 (48 hours) and 29 (72 hours).

Toxicity Category I (scarring in 5/6 animals at 8 days; persistence of very slight corneal opacity in one animal at 8 days), Core study - Minimum data.

11. Report on the Study of the Primary Skin Irritation of "BAS 9052 OH" on the Dorsal Skin of White Rabbits, Report C11; BASF Gewerbehygiene und Toxikologie; Tab. C11; pp. 1-3 (in German) August 15, 1980, Accession No. 099536; In English, 5 pp. Accession No. 243319.

Protocol - 0.5 ml of BAS 9052 OH was placed on intact and abraded dorsal skin of 4 female and 2 male White Vienna rabbits (mean weight 3.53 and 3.58 kg, respectively). Application sites were 2.5 X 2.5 cm; exposure was for 24 hours. Observations for erythema and edema were made at 24 and 72 hours and at 8 days. Reactions were scored according to Draize. Necrotic changes were confirmed by gross pathology.

Results - Erythema and edema were observed in all abraded animals at 24 and 72 hours on all intact animals at 24 hours and on 5 of 6 intact animals at 72 hours. Severe scaling was observed at 8 days in 4 of 6 intact and 3 of 6 abraded animals. Confirmed necrosis (millet-seed size) was seen at 8 days in one animal. It was concluded however, that "...8 days after application... erythema and severe scaling indicated severe skin irritation". The primary irritation index was 4.0.

P.I. Index = 4.0 (moderately irritating. However, despite the numerical score which indicates "moderately irritating" the finding of one animal with necrosis and 5/6 with severe scaling at 8 days necessitates upgrading of the toxicity category to Toxicity Category I).

Toxicity Category I, Core Study - Minimum.

Subchronic and Chronic Studies, Technical NP-55 (BAS 9052 H)

12. Subacute Feeding Study of NP-55 in the Mouse, Report C12, Nisso Institute; Tab C12, pp. 1-140; December 18, 1978, Accession No. 099536.

Protocol - Five groups of six week old ICR mice, each group consisting of 20 males and 20 females were fed a diet containing technical NP-55 dissolved in acetone to make the following doses administered for 14 weeks: 2700 ppm, 900 ppm, 300 ppm, 100 ppm and 0 ppm. Mean NP-55 intakes over the study period in mg/kg/day were as follows for males and females, respectively: 373.6 and 486.3 (2700 ppm group), 137.1 and 164.4 (900 ppm group), 45.6 and 52.7 (300 ppm group), 15.4 and 17.2 (100 ppm group). The 0 ppm group was the vehicle control (acetone added to diet). Animals were observed daily for pharmacotoxic symptoms, mortalities, physiological changes, central nervous system effects. Body weights were measured weekly. Estimates of food consumption were made weekly for 4 weeks and biweekly thereafter. Estimation of water intake were made at the termination of feeding. At the termination of the experiment, hematology, urinanalysis, blood chemistry, gross necropsies, organ weight measurements and histopathology were performed on each animal.

Results - No mortalities occurred in any groups and no pharmacotoxic signs were observed over the 14 week observation period. Body weight gains for males of the 2700 ppm dose group were significantly retarded ($P > 0.05$) as compared with controls. Food consumption for that group was also decreased. Water intake for all groups was similar. Hematological testing showed increases in mean corpuscular volume and mean corpuuscular hemoglobin in males of the 300 ppm and 900 ppm groups and a decrease in erythrocytes in males of the 2700 ppm group compared with controls. No significant differences between group were seen in urinalysis tests. Blood urea nitrogen (BUN) was increased in males of the 2700 ppm group. Total protien and albumin were increased in males of the 900 ppm group. (Differences cited above were significant to at least $P > 0.05$.) No significant differences between experimental and control groups in other blood chemistries (sodium, potassium, alkaline phosphatase, lactic dehydrogenase, glutamic oxaloacetic transaminase, glucose, total bibirubin or albumin/globulin ratio). Gross necropsies revealed a few scattered changes in stomach, kidneys and bladder found in controls as well as experimentals. Livers of males in the 900 ppm and 2700 ppm groups were heavier than controls ($P < 0.001$). Organ/body weight ratios were significantly larger for both males and females of the 900 ppm group ($P < 0.01$) and 2700 ppm group ($P < 0.001$). Hearts of females in the 27000 ppm group were significantly larger ($P < 0.05$).

On histopathological examination, swollen liver cells were found in 100% of 900 ppm and 2700 ppm males and in 50% of 2700 ppm females. Other microscopical changes were found in thymus, lung (1 adenoma, 5 subacute pneumonias), submaxillary gland, liver, kidney, bladder and small intestine (amyloid deposition in females of 300 and 900 ppm groups) which were, in general, random. The target organ for the effect of NP-55 was the liver; the no effect level was 300 ppm.

Adverse Effects - Liver showed increase in weight at highest dosages in males and increased organ/body weight ratios for males and females at highest dosages. Swollen liver cells were found in all males at highest dosages and in half of females at the highest dose.

NOEL = 300 ppm

Core Study - Minimum data.

13. Subacute Feeding Study of NP-55 in Rats, Report C13, Nisso Institute, Tab. C13, pp. 1-207; October 18, 1978; Accession No. 099536.

Protocol - Six groups of six week old Wistar-SLC rats, each group consisting of 20 males and 20 females were fed a diet containing technical NP-55 dissolved in acetone to make the following doses administered for 14 weeks: 2700 ppm, 900 ppm, 300 ppm, 100 ppm, 33 ppm and 0 ppm. Mean NP-55 intakes over the study period in mg/kg/day were as follows for males and females respectively: 196.34 and 200.45 (2700 ppm group), 60.43 and 66.8 (900 ppm group), 20.12 and 21.43 (300 ppm group), 6.75 and 7.08 (100 ppm group), 2.25 and 2.42 (33 ppm group). The 0 ppm group was the vehicle control group (acetone added to diet). Animals were observed daily for mortalities pharmacotoxic symptoms, physiological changes and central nervous system effects. Body weights were measured weekly. Food consumption was estimated weekly for 4 weeks and biweekly thereafter. Water consumption estimates, urinalysis, hematology tests were done at 4 and 14 weeks. For urinalysis, 10 males and 10 females from each group were tested. For hematology 8 males and 8 females from each group were tested. Blood chemistry, gross necropsies, organ weights and histopathologies were performed on all surviving animals at 14 weeks.

Results - No mortalities occurred in any groups and no pharmacotoxic signs were observed over the 14 week test period. Total weight gains for the 2700 ppm group were significantly retarded ($P < 0.001$) but other dosage groups were similar to controls. Food consumption in the 2700 ppm group was depressed but of other groups was similar to controls. Control and experimental groups water consumption were similar. On hematological examination, mean corpuscular hemoglobin decreased for 100 ppm males and increased for females of 100 ppm and 2700 ppm groups. Platelet increases occurred for 100 ppm females, 900 ppm males and females, and 2700 ppm males. Total leucocytes increased in 2700 ppm males and females. Urinalysis showed no significant differences between controls and experimentals. The following differences in blood chemistries between experimentals and controls were noted: protein increased in 33 ppm females, 300 ppm males and females and 2700 ppm males. Albumin increased in 33 ppm and 300 ppm males, but decreased in 300 ppm, 900 ppm and 2700 ppm females. Glutamic pyruvic transaminase decreased in the 2700 ppm group. Alkaline phosphatase decreased in 900 ppm and 2700 ppm females. Glucose decreased in 2700 ppm females. Bibirubin increased in 900 and 2700 ppm groups. Total cholesterol increased in 100 ppm females, 900 ppm males and all of 2700 ppm group. Total calcium increased in 33 ppm males and decreased in 300, 900, and 2700 ppm females.

At gross necropsy, random changes seen in organs of experimentals and controls included thymus petichia, lung, stomach, small intestine and testes changes. Organ weights of liver were increased at high doses, but to a significant level in 900 ppm males and 2700 ppm females. In 2700 ppm males, heart, lung, spleen, kidney and adrenals were significantly decreased in weight. In 2700 ppm females, lung, spleen and adrenals were significantly decreased in weights. Organ/body weight ratios differed from controls as follows: livers of 900 ppm groups and 2700 ppm groups were larger. Brain was larger in the 2700 ppm group,

but spleen was smaller. Thymus of females in the 2700 ppm group was smaller. On histopathological examinations, pathological changes were observed in heart, lungs, kidneys, testes and thymus, but were not NP-55 related. However, swollen liver cells at incidences of 100% and 25% in 2700 ppm males and females respectively and at 60% incidence in 900 ppm males were attributable to NP-55 treatment. Such hepatic abnormalities were considered responsible for growth retardation, decreased food efficiency and increased serum cholesterol and bilirubin. The target organ of NP-55 is considered the liver with a no effect level of NP-55 at 300 ppm.

Adverse Effects - The liver at greater than 300 ppm is the target organ for NP-55 in the rat. Associated effects are increases in cholesterol and bilirubin and increase in size and organ/body weight ratio of the liver, with swollen cells as histopathological findings.

NOEL = 300 ppm

Core Study - Guidelines.

14. Twenty-Six Week Feeding Study in Dogs (of NP-55), Report C14; Hazelton Laboratories America, Inc., Project No. 886-103; Tab. C14; pp. 1-147. March 25, 1980, Accession No. 099536.

Protocol - Four groups of male beagle dogs (Hazelton Research Animals Inc., Cumberland, Va.) aged 30-34 weeks, weighing between 6.7-13.7 kg, each group consisting of 6 animals, were fed Wayne® dog meal containing the following amounts of technical NP-55 for a period of 26 weeks: 0 ppm (controls); 120 ppm (3.0 mg/kg/day); 600 ppm (15.0 mg/kg/day) and 3000 ppm (75.0 mg/kg/day). Four similar groups of female dogs weighing between 5.4-11.8 kg were similarly fed for 26 weeks. Appropriate diet was fed once weekly for the first six weeks and twice weekly thereafter, and was available ad libitum. All dogs were observed daily for mortality and signs of pharmacotoxic effects for the first 2 weeks and weekly thereafter. If clinical signs were seen, observations were made daily thereafter. Body weights and food consumption were recorded weekly to week 6 and twice weekly thereafter. Clinical chemistry, hematology and urinalysis were performed on all dogs prior to treatment and at weeks 4, 8, 13 and 26. Blood was collected by jugular puncture. Urine was collected from cage floor runoff except for phenolsulphonthalen determination for which urine was collected by catheterization. Hematology studies included hematocrit, hemoglobin, erthyrocyte and platelet counts, total and differential leucocyte counts, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Clinical chemistry tests included total protein, albumin, alkaline phosphatase, total and direct bilirubin, blood urea nitrogen, calcium, lactic acid

dehydrogenase, bromosulphalen dye retention, phenolsulfonphthalen dye excretion, fasting glucose, serum glutamic pyruvic transaminase, serum glutamate - oxaloacetic transaminase, total globulin, albumin/globulin ratio, potassium and total cholesterol. Urinalysis included appearance, pH, specific gravity, glucose, ketones, total protein, bilirubin, urobilinogen, reducing substances and microscopic examination of sediment. Opthalmoscopic examinations were performed initially and terminally on all dogs with an indirect opthalmoscope and a hand held slit limp, using a mydriatic. Surviving dogs were sacrificed after 26 weeks by exsanguination while under the effect of Surital® anesthesia. Complete necropsies were done on all dogs and included total body weight measurement and organ-body weight ratio of brain, pituitary, thyoid, heart, liver, lung, kidney, adrenal, testes with epididymus (males) and ovaries (females). Preserved tissues from all dogs which died or were sacrificed were sectioned, stained with hematoxylin and eosin and examined microscopically. These included brain, pituitary, thoracic and lumbar spinal cord, eyes, salivary glands, thyroid with parathyroid, thymus, trachea, esophagus, lungs with bronchi, heart, aorta, liver, gallbladder, spleen, intestine (duodenum, jejunum and ileum), large intestine, mesenteric lymph node, urinary bladder, prostate (males), testes with epididymus (males), ovaries and uterus (females), mammary skin, costachondral junction, bone marrow (femur) and sciatic nerve with adjacent muscle. Statistical comparisons were made on the following: body weight changes (weeks 0-4, 0-8, 0-13, 0-26); food consumption (weeks 4, 8, 13, 26); hematology and clinical chemistry and absolute and relative organ weight data. Data of control groups were compared to treated groups of the same sex by Barlett's test first and the 1-way classification of variance (ANOVA). If Barlett's and ANOVA yielded significant results, a multiple pairwise comparison procedure (Games and Howell) was used to compare group mean values. If only ANOVA gave significant results, Scheffe's multiple pairwise comparison procedure was used to compare group mean values. All analyses were evaluated at the 5% probability level.

Results - Non-treatment related effects included non-specific dermatitis, injected scleras, lacrimation, soft stools or diarrhea, sores on body. One high-dose male (75 mg/kg/day) was sacrificed moribund as a result of complications due to urinary calculi-obstructed urethra. No other mortalities occurred. No treatment-related behavioral symptoms were noted. The most significant treatment related clinical symptoms included cystitis and/or urinary calculi among low, mid and high dose males observed during catheterization and in the high dose moribund male. One low dose, 2 mid dose and 2 high dose males had such symptoms. Occasional instances of bloody urine were found among low, mid and high dose males. No significant changes in body weights attributable to NP-55 were observed over the 26 week test period. Treatment related changes, including those statistically significant that were found in clinical chemistry, hematology, urinalysis, gross necropsy and histopathology are listed in Table I.

TABLE I

Summary of Statistically Significant Changes in Various Characteristics of Dogs Treated with NP-55

Effects at Various Weeks of Treatment, Low, Mid and High Doses of NP-55

CHARACTERISTIC		LOW - 120 ppm	MID - 600 ppm	HIGH - 3000 ppm
RBC	MALE	8 weeks ↓	-	-
	FEMALE	-	-	8 weeks ↓
WBC	MALE	-	-	26 weeks ↑
	FEMALE	-	4,8,26 weeks ↑	4,26 weeks ↑
MCHC	MALE	-	-	-
	FEMALE	13 wk ↓	-	-
SGOT	MALE	-	-	-
	FEMALE	26 weeks ↓	-	-
LDH	MALE	-	4,8,13 ↓	4,8,13 ↓
	FEMALE	-	-	-
ALKPHOS.	MALE	-	26 ↑	4,8,13,26 ↑
	FEMALE	-	-	-
BSP	MALE	-	-	-
	FEMALE	-	-	13 weeks ↑
BIT	MALE	-	-	-
	FEMALE	8 weeks ↑	4 weeks ↑	-
FASTING GLUCOSE	MALE	-	-	-
	FEMALE	-	-	26 weeks ↓
TOTAL CHOLEST.	MALE	-	-	-
	FEMALE	-	4, 13 ↑	8 weeks ↑
POTASSIUM	MALE	-	-	-
	FEMALE	-	26 weeks ↑	-
CALCIUM	MALE	-	13 weeks ↓	-
	FEMALE	-	-	-
TOTAL ALBUMIN	MALE	-	-	4,8,26 ↓
	FEMALE	-	-	4,8,13,26 ↓
A/G RATIO	MALE	-	-	-
	FEMALE	13 wk ↓	13 weeks ↓	8,13 ↓
PSP	MALE	-	4,8,13,26 ↓	4,8,13,26 ↓
	FEMALE	8, 26 ↓	4,8,13,26 ↓	4,8,13,26 ↓

NECROPSY

CHARACTERISTIC		LOW - 120 ppm	MID - 600 ppm	HIGH - 3000 ppm
URINARY CALCULI PRESENT	MALE	+ (1/6)	+ (2/6)	+ (3/6)
	FEMALE			
LIVER WGT. INCREASE	MALE	-	+ *	+
	FEMALE	-	-	+*
THYROID WGT. INCREASE	MALE	-	+ *	+ *
	FEMLAE	+	-	+
LUNG WGT. INCREASE	MALE	-	-	-
	FEMALE	+	-	-

HISTOPATHOLOGY

CHARACTERISTIC		LOW - 120 ppm	MID - 600 ppm	HIGH - 3000 ppm
RENAL PELVIS PATHOLOGY	MALE	-	+	+
	FEMALE	-	-	-
CYSTITIS URINARY BLADDER	MALE	+	+	+
	FEMALE	+	+	+

* Relative increase, not statistically significant

Adverse Effects - Random effects on RBC, MCH, SGOT, BIT, and lung weight were seen in 120 ppm (7.5 mg/kg/day) animals. Effects of NP-55 showing a dose-response relationship were seen in 600 ppm and 3000 ppm animals in LDH, A/G, PSP excretion, urinary calculi present, liver and thyroid weight, and pathology in renal pelvis and urinary bladder. A/G ratio of 120 ppm animals showed a dose-response relationship, but this observation was not considered significant. Cystitis was observed in 2/6 males and 3/6 females at the lowest dose, 2/6 males and 2/6 females at the mid dose and 2/6 males and 3/6 females at the highest dose. The above effects were not observed in control animals.

NOEL = 120 ppm

Core Study - Minimum data.

Commentary:

1. NP-55 is reported as slightly unstable at room temperature, but stable at -22°C (Report C1-C13). In the present study, NP-55 was stored at room temperture from November 30, 1978 to January 4, 1979; then refrigerated from January 4, to March 6, 1979 (study week 6) and frozen from March 6 to termination of the experiment. (In reports C1-C13, it was noted that careful procedures were used [i.e. freezing] to prevent NP-55 treatment materials from deterioration.)

2. Dosage of NP-55 were prepared according to the 96.1% purity stated by the sponsor, but no testing was done to confirm purity or to determine if deterioration had occurred during storage.

3. No report has been received to date concerning homogeneity or accuracy of NP-55 feeding mixture (although sponsor, as per telephone conversation of November, 1980, said this information would be sent).

4. At the start of the feeding experiment, NP-55 food mixture was offered once weekly for 6 weeks to the young dogs and was available ad libitum, suggesting the possibility that non-uniformity of dosage could easily occur.

5. The results of this study are at variance with those obtained and reported by Nisso Institute (Reports C12 and C13). This is discussed in the accompanying commentary (Report C14 Comment):

   Statement for Toxicological Study No. 001. Reason to Make Additional Test of Six Month Dog Feeding Study of NP-55. Nisso Institute for Life Science Tab. Report C14 Comment. December 27, 1979; Scientific Comment on Six Month Dog Feeding Study of NP-55.

   This commentary deals with the findings reported by Hazelton Laboratories America Inc., in their six month feeding study, employing a diet of 120 ppm; 600 ppm and 3000 ppm in beagle dogs. In all treated animals a significant

decrease in PSP excretion was noted and acute cystitis of urinary bladder and pyelitis of the renal pelvis were observed microscopically. Because Nisso Institute had previously performed two subacute toxicity studies (rat and mouse, see reports C12 and C13), Nisso Soda Co. and found the target organ for compound NP-55 was the liver, another subchronic dog feeding study of NP-55 was begun in order to confirm the differences in target between dogs and rodents. The second report is in progress and the final report is expected in January 1981.

14. Addendum to Twenty-Six Week Toxicity Study in Dogs of NP-55. Report C14 Hazelton Laboratories America, Inc. Tab. C14, pp. 1-147. Accession No. 099536. Analysis of Diet, Accession No. 099802, December 8, 1980, pp. 1-3 and Tables 1-5.

Protocol - Two-gram samples of dietary feed in duplicate, were analyzed to determine the concentration of NP-55 in canine food for Project No. 886-103. Three-hour extracts were made with methylene chloride, the extract evaporated and analysis made of aliquots for the 120, 600 and 3000 ppm dietary levels using high pressure liquid chromatography.

Results - The analytical method was successful in demonstrating near quantitative recovery techniques, but revealed that a homogeneity problem was present in bulk preparation of the animal diet. The homogeneity problem was overcome by switching from the Hobart blender to pre-mixing diet in a Waring blender, thereby achieving target concentration of NP-55 in the feed mix. However, analysis of the diet mixes used throughout the canine feeding studies revealed failure to achieve target doses. (The Hobart mixer was evidently used to prepare the diet for the feeding studies.) (See Table 2 of this report.) Average percent of target dose for the 120 ppm diet varied from 16.0 to 76.5% of target; for the 600 ppm dose diet, average percent ranged from 77.32 to 87.8% of target andd for the 3000 ppm dose diet, the average percent of target dose ranged from 82.7 to 110.2%.

Comments - The non-uniformity of diet dosage, inadequate storage procedures for the administered chemical as well as the method of diet administration (for the latter two see detailed toxicological review - Protocol and comment) have a deleterious effect on the quality of the feeding test protocol and probably affect the results.

15. Chronic Feeding Study of NP-55 in Mice Report C15 and RD 8067. Nisso Institute for Life Sciences. Interim Report, (Fifty-Two Week Study). Includes analytical results of NP-55 in animal diet for the long term oral dosing study in mouse - first 52 weeks data in 104 weeks feeding test. February 25, 1980. Accession 099357, Tab. C15 (no page numbers) and RD 8067, pp. 1-8.

Protocol - Five groups of male BD, F_1 mice (C57Bl/6 X DBA), aged 6 weeks were respectively fed diet (CE 2®, CLEA, Japan) containing the following doses of technical NP-55: Control group, 0 ppm (100 animals); 120 ppm (70 animals); 360 ppm (70 animals) and 1080 ppm (70 animals). In mg/kg/day, these doses were respectively, 0 mg/kg/day, 6, 18, 54 and 162 mg/kg/day. Similar groups of female mice were similarly treated. For the diet, NP-55 was dissolved in acetone and mixed with 5 kg of diet, filtered (1.5 mm mesh) and allowed to stand at room temperature overnight. The mixed diet was mixed into 11 kg of untreated diet and kept at - 20°C until fed to animals or analyzed, for a mixmum of two weeks. Analysis of diet samples showed recovery rates of 95% for 40 ppm and 120 ppm samples and 96% for 360 ppm and 1080 ppm samples.

Animals were examined daily for clinical signs and mortality. Body weight and food consumption were measured weekly for 14 weeks and biweekly thereafter. Hematology and urinalysis tests were done at 0, 6 and 12 months of feeding. Blood chemistry tests were done at 0 and 12 months. At 12 months, 10 males and 10 females from each group, randomly selected, were sacrificed and necropsied. Organ/body weight ratio were determined and histopathology examination performed.

Results - No clinical symptoms attributable to feeding of NP-55 were noted. Mortalities were noted in the following groups which were not attributable to feeding of NP-55: 1/100 in males of the 0 ppm group, 1/70 each in males of the 40 ppm and females of the 40 ppm group, 2/70 in 120 ppm females and 1/70 in both 360 ppm males and 360 ppm females. Decreased food consumption and increased food efficiency were found in males of the 360 ppm and 1080 ppm groups. No significant differences in water consumption were seen between treated and control groups. Urinalysis values for all animals indicated no significant differences between control and treated groups. A few random elevated ketone values were found among female animals of the treated groups. Although statistically significant differences from control values were found among treated mice for hematology and clinical chemistries (see Table I), no dose-response relationship was shown. At necropsy, weight of liver, heart and spleen were significantly elevated in 1080 ppm dose males. Organ/body weight ratios of liver and spleen were also significantly elevated in 1080 ppm dose males. For females at the 1080 ppm level doses, organ weights were not elevated, but organ/body weight ratios were significantly elevated for liver at 360 ppm and 1080 ppm dose levels. The thymus/body weight ratio for 1080 popm females was

also significantly elevated. The body weight average for 120 ppm females was significantly lower than the control value, while brain and thymus organ/body weight ratios were significantly higher than control values. While statistically significant, the findings in the 120 ppm group of females appear unrelated to NP-55 administration. At necropsy, gross pathological lesions were found in males and females (lungs) and female ovaries (cysts) which appeared to be random and unrelated to NP-55 administration, as they were found similarly in untreated controls. Liver from 360 ppm dosed and 1080 ppm dosed males showed swollen cells and fatty degeneration. This was not seen in any treated female groups. Two cystadenomas (benign) were found in females; one in the 40 ppm group and one in the 360 ppm group. One pulmonary adenoma (benign) was found in females of the 0 ppm group. During the one year feeding period, five males and five female werre necropsied as a consequence of death or moribund sacrifice. One female (120 ppm group) had liver and spleen hemongioma (benign). Three malignant tumors were found as follows: Reticular cell sarcoma, liver (0 ppm male); abdominal wall fibrosarcoma (360 ppm male) and thymus malignant lymphoma (1080 ppm male).

Adverse Effects - No adverse effects can be definitively determined from this interim report, although interim results suggest NP-55 affects the liver.

NOEL - No "no observable effect level" can be determined from this interim report.

LEL - No "lowest effect level" can be determined from this interim report.

Core Study - Supplementary

Comments - One page of blood chemistry values for females at 12 months appears to be missing from this study. (Data missing includes Glucose, BUN, Cholesterol, Bilirubin, Protein, Albumin and A/G ratios.)

TABLE I

Statistically Significant Changes From Control Values In NP-55 Treated Mice

CHARACTERISTIC	MALE/FEMALE	Dosages ppm 40	120	360	1080
Hematology (0,6,12 mos.)					
RBC	M	-	6 mo ↓	6 mo ↓	6 mo ↓
	F	-	-	-	6 mo ↓
PVC	M	-	6 mo ↓	6 mo ↓	6 mo ↓
	F	-	-	-	6 mo
Hb	M	-	6,12 mo ↓	6 mo ↓	-
	F	-	-	-	-
MCV	M	-	-	-	6 mo ↓
	F	-	-	-	-
MCH	M	-	-	-	6 mo ↑
	F	-	-	-	-
PLATELETS	M	6,12 mo ↓	6,12 mo ↓	6,12 mo ↓	6,12 mo ↓
	F	-	-	-	-
TOTAL LEUCOCYTES	M	6 mo ↓	6 mo ↓	6 mo ↓	-
	F	-	-	-	-
Blood Chemistry (0, 12 mos.)					
GLUCOSE	M	-	-	-	12 mo ↑
	F	*Data missing			
A/G RATIOS	M	-	-	12 mo ↓	12 mo ↓
	F	*Data missing			
ALP	M	-	-	-	-
	F	12 mo ↑	12 mo ↑	-	-

* 12 mos. data on females missing: Glucose, BUN, Chol., Bil., T.Prot., Alb, A/G Ratios.

16. 104-Week Chronic Dietary Study in Rats (of NP-55). 52-Week Interim Report. Hazelton Laboratories America, Inc., July 29, 1980. Accession No. 099537; Tab. C16, pp. 1-168.

Protocol - Four groups of male weanling albino (Fischer 344, CDF) rats, obtained from Charles River Breeding Labs. Inc., each group consisting of 55 animals were fed a basal diet of Purina Rodent Laboratory Chow® containing the following concentrations and designations of NP- 55 respectively: 0 ppm (control), 40 ppm (low), 120 ppm (mid) and 360 ppm (high) for 52 weeks. Four similar groups of female rats were similarly treated. Procedures for the preparation of the diet mixture were changed on week 26 of the study, due to a homogeneity problem discovered during analysis of the feed for concentrations of NP-55. For the first 26 weeks of the feeding studies, diets were prepared by blending measured NP-55 in a Hobart blender with 5 kg dose diet and adding the mixture to the total amount of diet feed required and blending again. The assayed values of this diet were consistently lower than the target values. The problem was corrected by the use of a Waring blender, which provided an NP-55/diet blend within the acceptable 10% limit of error for the target values. The corrected procedure was used to prepare diets for the remainder of the feeding study. Two lots of NP-55 were used for the feeding study; lot No. PN 1-2, purity 96.1% was used for weeks 1-30 and lot No. PN-3 was used for weeks 31-52. Animals were fed twice weekly and were individually housed. Animals were observed daily for deaths or moribund condition. Weekly examinations were made for pharmacotoxic effect; body weight and food consumption measurements were made for the first 13 weeks and biweekly thereafter. Eight rats of each sex from each group were selected for hematology studies at weeks 0, 18, 35, 51 and for clinical chemistries on weeks 0, 26 and 51. Urinalysis tests were not reported. Hematology tests included hematocrit (HCT), hemoglobin (HGB), erythrocyte counts (RBC), platelet counts (Plate), total leucocytes (WBC), differential leucocytes, mean corpuscular volume (MCV) and mean corpuscular hemoglobin content (MCHC). Clinical chemistry tests included total protein (T. Prot), albumin (ALB), alkaline phosphatase (ALK. PHOS.), bilirubin (BIT), blood urea nitrogen (BUN), serum glutamic oxaloacetic transaminase (SGOT), fasting glucose, serum glutamic pyruvic transaminase (SGPT), total globulin, albumin/globulin ratio (ALB/GLOB), calcium, potassium (POTAS), lactate dehydrogenase (LDH) and total cholesterol (T. CHOL). Ophthalmoscopic examinations were performed on all rats at weeks 0, 26 and 52 employing 1% Mydriacyl® for pupil dilation and a binocular indirect opthalmoscope for examination. Gross necropsies were performed on dead or moribund sacrificed animals during the study and at 52 weeks, when 5 rats of each sex from each group were exsanguinated under sodium pentabarbitol. Organ weights and organ/body weight ratios were determined for brain, heart, liver, kidneys, testes with epididymis and lungs before fixation. Following fixation, thyroid, adrenals and ovaries were similarly measured. In addition to those of the above organs, tissues from pituitary, thoracic spinal cord, eyes, salivary glands, parathyroids, thymus, oesophagus, trachea, aorta, stomach, pancreas, duodenum, jejunum, ileum, colon, mesenteric lymph nodes, urinary bladder, prostate, uterus, skin, rib junction, bone marrow, nerve with muscles, mammary gland and any unusual lesions were fixed, sectioned, stained with hematoxylin and eosin and

microscopically examined. Multiple statistical methodology including procedures to assure homogeneity, measure variances, permit comparisons between groups and determine significance of differences from control values at the 5% probability level, were employed.

Results - Two control females, one mid-dose male and one high dose female died or were sacrificed moribund during the first 52 weeks of the study. There were no treatment-related clinical symptoms in these animals. No significant body weight differences were found in all groups at week 52, although statistically significant differences (higher/lower values) in body weight were occasionally encountered during the course of the study in males and females. For hematology and clinical chemistries, Table I shows those tests in which statistically significant differences from untreated control values were found. Although the values were significant statistically, no dose-response relationship was shown by the results of any tests listed (Table I). No treatment-related opthalmoscopic changes were noted at weeks 26 or 52. Pathologic changes were observed in 3 control females, 3 low-dose males, one low-dose female, one mid-dose female and one mid-dose male which were attributable to damage from blood sampling from the orbital sinus of the animals for hematology and clinical chemistry tests. At necropsy, no significant gross findings attributable to NP-55 administration were found in animals which died or were sacrificed moribund during the study, or in those animals sacrificed after 52 weeks. On examination of organ weight data, and organ/body weight ratios, no treatment related effects were noted. An incidental unexplained finding in low dose female was a sigificantly decreased terminal body weight for the group necropsied. The terminal average weight of this sub group of 5 low dose females was 30 grams lower than the average weight of the 55 number original group measured one week prior to sacrifice. The brain/body weight ratio of the 5 number low dose sub group was significantly elevated. Histopathology studies revealed spontaneous lesions and incidental findings primarily in liver and heart, which were scattered throughout all groups relatively uniformly. The heart lesions observed included degenerative cardiomyopathy and the liver lesions included some focal hepatitis and infiltration by mononuclear cells. Early changes of nephropathy observed among all males was attributed to aging, characteristic of Fischer rats. One female (high dose group) had a cortical adenoma of the adrenal gland and an alveolar/bronchiolar carcinoma was found in a male rat of the 120 ppm group. The adenoma was considered not uncommon in aging rats. The latter carcinoma, rarely found in rats, was considered spurious.

Adverse Effects - No adverse effects can be determined from this interim report.

NOEL - No "no observable effect level" can be determined from this interim report.

LEL - No "lowest effect level" can be determined from this interim report.

Core Study - Supplementary data.

Comments - A serious defect in this study is the absence of urinalysis data, particularly since the study submitted includes observations of pathology in the urinary excretory system ("progressive chronic nephropathy commonly observed in old male F344 rats....."). It is recommended that urinalysis reports accompany the final reports and that any data which could fill the urinalysis data gaps in the present reports be filled if resulting from an oversight.

TABLE I

Statistically Significant Changes In Clinical Chemistry and Hematology Values

		Dose Level of NP-55			
CHARACTERISTIC	MALE/FEMALE	0 PPM	40 PPM	120 PPM	360 ppm
Hematology					
HGB	M	-	-	34 wk↓	-
	F	-	-	-	-
PLATE	M	-	-	18 wk↑	34 wk↑
	F	0 wk↓	-	-	-
MVC	M	-	-	-	18 wk↓
	F	-	0 wk↓	-	-
MCH	M	-	18 wk↓	18 wk↓	18 wk↓
	F	-	-	-	-
MCHC	M	-	-	34 wk↓	34 wk↓
	F	-	-	-	-
Clin. Chem.					
ALB/GLOB	M	-	-	-	-
	F	-	-	51 wk↓	-
CALCIUM	M	-	-	-	26 wk↑
	F	-	26 wk↑	26 wk↑	26 wk↑
T. CHOL.	M	-	-	51 wk↓	-
	F	-	-	-	-
ALK PHOS.	M	-	-	-	26 wk↓
	F	-	-	-	-
BIT	M	-	-	51 wk↑	-
	F	-	-	-	-
SGOT	M	-	-	-	26 wk↓
	F	-	-	-	-
SGPT	M	-	-	-	26 wk↓
	F	-	-	-	-
LDH	M	-	26 wk	-	26 wk↓, 51 wk↑
	F	-	-	-	51 wk↑

17. Teratogenicity Study of NP-55 in Rats. Nisso Institute for Life Science, Kanagawa, Japan; April 17, 1980. Accession No. 099537, Tab. C17, pp. 1- 76.

Protocol - Five groups of female SPF Sprague-Dawley rats, 14 weeks old (SLC Japan), consisting of 24 rats per group, were individually mated overnight to similar male rats , examined for presence of vaginal plugs or presence of spermatozoa in vaginal smears, housed 3 animals per cage and fed a basal diet of CA-1 (CLEA, Japan). On days 7-17 of pregnancy the five groups of rats were fed the following doses of NP-55 or aspirin (in 1% aqueous sodium carboxymethyl cellulose plus 1% Tween 80) by gavage in a volume of 2 ml/kg; 0 mg/kg (vehicle control), 40 mg/kg, 100 mg/kg, 250 mg/kg or 0 mg/kg plus 200 mg/kg aspirin (positive control). The high dose (250 mg/kg) of NP-55 was equal to 1/10 of an LD50 value and was expected to produce some fetal or maternal toxicity without maternal death. The low dose (40 mg/kg) was expected to produce no observable adverse effects attributable to NP-55. Aspirin, as a well characterized teratogen, was expected to produce teratogenic effects. Body weight of dams was measured daily and animals were observed for pharmacotoxic signs throughout the test period. Animals were sacrificed after chloroform anesthesia and necropsied on day 21. Gross pathological changes were noted and organ weights of liver, spleen, kidney, ovaries and gravid uterus were measured. Organ/body weight ratios were determined. Examination of fetuses included: number of implantation sites, dead or resorbed fetuses and live fetuses. Dead or resorbed fetuses were classified in two stages: early deaths (only placental remains) or late deaths (both placental and fetal remains). Living fetuses were fixed, alizarin red stained and examined for skeletal abnormalities. Two groups of skeletal variations (Kimmel's method) were characterized, based on 14th or 15th rib changes; rudimentary if less than 1/2 the length of the 13th rib and extra if half or longer than half of the preceding rib. The remaining one third of each litter was fixed and examined for internal abnormalities. Sectioning (method of Wilson) was used to measure head and cervical regions; thoracic, abdominal and pelvic viscera were examined by microdissection (method of Nishimura). Skeletal and internal examinations were aided by dissection microscopy. Statistical analysis was employed to evaluate the incidence of abnormalities using the litter as the experimental unit, with differences detected at the $P < 0.05$ level of significance.

Results - No deaths occurred in any group of rats and pregnancy rates were closely similar. Although significant body weight decreases were observed in 100 mg/kg and 250 mg/kg NP-55 treated animals, recovery of weight gains occurred by day 21 and no significant changes in weight were observed in those groups as compared with other groups except for the aspirin treated group. In the aspirin treated group, depression of weight gain occurred on days 16-19 of pregnancy with the net gain slightly larger than the control group. On examination of organ weights, mean absolute weight of adrenals was significantly decreased in the 100 mg/kg NP-55 group compared with the control group. In the 250 mg/kg NP-55 group, mean absolute and relative weights of liver increased and adrenals decreased. No significant changes in organ weights of spleen, kidney or ovaries was observed in any NP-55 treatment group. In the aspirin treated group, mean absolute and relative weights of liver increased and adrenals

decreased in comparison with controls. Two dams of the 40 mg/kg NP-55 group and one of the 250 mg/kg groups had reddish adrenals; another of the 250 mg/kg group showed dilation of the renal pelvis. No significant changes due to NP-55 administration were observed in any dams.

On observation of fetuses, mean implantation sites, corpora lutea and litter size were unaffected in all groups, but fetal weights in the aspirin group were significantly less than the control group. Numbers of dead or resorbed fetuses were slightly larger in the 250 mg/kg NP-55 group and the aspirin group than in controls, but values were not statistically significant. No dose-related response to NP-55 was seen. Sex ratios were similar in all groups. The number of dams having fetuses with some abnormalities was significantly increased in the aspirin treated group ($P < 0.001$). The few observed extenal abnormalities in fetuses of the NP-55 treated groups were as follows: club foot (1 fetus = 0.3%), waved tail (1 fetus = 0.3%) in the 40 mg/kg NP-55 group; exencephalia (1 fetus = 0.3%) and club foot (1 fetus = 0.3%) in the 250 mg/kg NP-55 group. External variations included small fetuses (2 fetuses = 0.7%) in the 250 mg/kg group. Ecchymosis was observed in all groups except the 100 mg/kg NP-55 group, but was highest in the 250 mg/kg NP-55 group. In the control group, microcephalia (1 fetus = 0.3%) was seen. In the aspirin treated group, a high incidence of external abnormalities occurred, including craniorachischisis (16 fetuses = 5.1%), cerebral hernia (2 fetuses = 0.6%), subcutaneous edema (1 fetus = 0.3%), short tail (1 fetus = 0.3%), waved tail (1 fetus = 0.3%) and gastroschisis (1 fetus = 0.3%). In fetuses of the NP-55 groups, a slightly increased incidence of 14th rib, bilobed thoracic vertebrae and total variations were not significant compared with controls. Waved ribs (1 fetus = 0.5%) was seen in the 40 mg/kg NP-55 group. Ossification of sternebrae was significantly accelerated in the 100 mg/kg NP-55 group and slightly accelerated in the 250 mg/kg NP-55 group. Ossification of proximal hind foot phalanges was significantly retarded in fetuses of the 250 mg/kg NP-55 group. All of the aspirin group fetuses had skeletal variations including statistically significant increases of 14th and 15th ribs, splitting and bilobed thoracic vertebrae, supernumerary vertebrae, waved ribs, depressed ossification of cervical cetra, forefoot and hindfoot proximal phalanges and hindfoot metatarsus and decreased number of sacral and caudal vertebrae. Abnormalities of soft tissue in NP-55 fetuses, though not statistically significant, included hypoplasia of kidney (1 fetus = 1.0%) and right aortic arch abnormality (1 fetus = 1.0%) observed in 2 different litters of 250 mg/kg NP-55 treated groups. In the aspirin group retroesophageal right subclavian artery (1 fetus = 0.9%), unbilateral absence of kidney (1 fetus = 0.9%) and dilated renal pelvis (3 fetuses = 2.8%) were found in different litters. Control fetus observations included dilated renal pelvis (2 fetuses = 1.8%) and anophthalmia (1 fetus = 0.9%) in different litters. The study conclusion was that NP-55 is not teratogenic in rats. Retardation of ossification of hind foot proximal phalanges at 250 mg/kg NP-55, accelerated ossification of sternebrae at 100 mg/kg NP-55, and increased ecchymosis in fetuses at 250 mg/kg NP-55 were not considered to be evidence of teratogenesis but were considered within the range of normal variations in the fetuses.

Teratogenicity - No terata were found in any groups dosed with NP-55. The aspirin treated, positive control group did show a spectrum of terata characteristic of aspirin treatment.

NOEL = 250 mg/kg NP-55

Maternal Adverse Effects - significant increased liver/body weight ratio and decreased adrenal/body weight ratio at 250 mg/kg NP-55. Significantly decreased adrenal weights at 100 mg/kg NP-55.

NOEL = 40 mg/kg/day NP-55

LEL = 100 mg/kg NP-55 (significantly decreased adrenal weights).

Core Study - Guidelines

18. Teratology Study in Rabbits (of NP-55) International Research and Development Corporation. May 23, 1980, Accession No. 099537, Tab. C18, pp. 1-33; Appendix I (no pagination); Appendix II (6 pp.) Plus Protocols; Appendix III (no pagination).

Protocol - Four groups of sexually mature virgin female New Zealand White rabbits (Langshaw Farms, Augusta, Mi.) aged 7 months, acclimated and free of parasitic coccidiae, each group consisting of 16 animals, were artificially inseminated and induced to ovulate. On days 6-28 of gestation, the four groups of rabbits were fed the following dosages of NP-55 by gavage, respectively, suspended in 0.5% carboxymethylcellulose as a vehicle in a constant volume of one ml per kg: 0 mg/kg (vehicle control), 40, 160 and 480 mg/kg. Animals were observed daily prior to and following treatment for mortality and pharmacotoxic signs to day 29 of gestation. Animals showing signs of abortion or premature delivery were sacrificed and examined for gross evidence of morphological changes on the day signs appeared. Intact fetuses were examined and preserved as well as those tissues deemed necessary to confirm other findings. Gross necropsy was performed on all animals not surviving to scheduled sacrifice to determine cause of death, with tissues preserved and fetuses examined and preserved as noted above. Maternal body weights were measured on days 0, 6, 12, 18, 24 and 29 of gestation. On day 29, surviving females were sacrificed by an overdose of sodium pentobarbitol in the marginal ear vein, the uterus excised and weighed and the fetuses removed. The number and location of viable fetuses, early and late resorptions and the number of total implantations and corpora lutea recorded. Thoracic and abdominal cavities were examined for gross morphological changes. Uteri of non-gravid appearing females were opened and preserved for examination of pregnancy status. Fetuses were weighed and examined for external malformations and variations, including palate and eye. Visceral malformations and variations and sexes were determined by dissection, including brain (mid cornal slice). The heart was dissected by the method of Staples. Eviscerated, skinned fetuses were fixed, cleared and stained with Alizarin Red S (method similar to that of Dawson) for skeletal examination. Statistical procedures compared treatment to control groups at a significance level of $P < 0.05$. Statistical analysis was used to compare male/female sex distribution, number of litters with malformations, number of early and late resorptions and post implantation loss. The mean number of viable fetuses, total implantations, corpora lutea and

mean fetal body weights were compared by analysis of variance with appropriate supplementary tests to judge significance of differences.

Results - For maternal observations, there were no differences in appearance or behavior during the gestation period between treatment and control groups. A summary of deaths and abortions is abstracted in Table I. Cause of deaths prior to sacrifice of dams could not be determined. In addition to data in Table I, red fluid in the bladder was found in one of each of the 160 mg/kg and 480 mg/kg rabbits. One control and two 480 mg/kg rabbits had pale livers. Mean body weights of 40 mg/kg/day rabbits were similar to controls; weights of 160 mg/kg/day rabbits were slightly less than controls during test week one and days 18-24. The 480 mg/kg rabbits showed a severe loss in body weight during NP-55 administration and over the entire gestation period, with a concomitant decrease in mean uterine weight due to increased early resorptions. There were no statistically significant differences in mean numbers of viable fetuses, late or early resorptions, total implantations, corpora lutea, fetal sex distribution or mean body weight in the 40 or 160 mg/kg/day NP-55 groups. In the 480 mg/kg/day group, however, statistically significant decreases in mean viable fetuses and increases in numbers of early resorptions and post implantation losses were observed. The number of gravid females in this group was less than half of the other groups. Total corpora lutea numbers and implantations in the 480 mg/kg/day group were similar to controls, although mean fetal weights were slightly lower. Male/female ratios were virtually reversed in the high dose group (ca 33/67) compared with the controls (ca 60/40).

On examination of the fetuses, no significant differences were found between those of 40 mg/kg or 160 mg/kg group and controls in the number of litters with malformations. However, the 2 litters from the 480 mg/kg group did exhibit a varity of malformations. 100% (8 fetuses) had full 13th ribs and 27 presacral vertebrae were found in 5 fetuses (63%). The sample size of fetuses from the 480 mg/kg/day group was considered too small for appropriate comparison and evaluation for teratogenic effects. No teratogenic effects were seen in fetuses at dose levels of NP-55 of 160 or 40 mg/kg/day administered to dams during days 6-28 of gestation. However, evidence for a variety of teratogenic effects was seen in two litters of the 480 mg/kg/day group. The sample size of that group was considered too small to permit appropriate comparisons with the control group.

Teratogenicity NOEL = 160 mg/kg/day

LEL = 480 mg/kg/day (increased number of a variety of random effects, including skeletal and visceral abnormalities, reduced fetal weight, changes in male/female ratios).

Maternal Toxicity NOEL = 160 mg/kg/day

LEL = 480 mg/kg/day (severe weight loss, 5/16 deaths, 6/16 abortions, reduction in number of viable litters and fetuses).

Core Study - Minimum data.

Comments - This study does not permit adequate evaluation of teratogenic effect of NP-55 in rabbits because of severe maternal and fetotoxic effects at the high dose level of 480 mg/kg/day. A better dose regimen would have been to substitute or include one dose between 160 mg/kg/day and 480 mg/kg/day.

TABLE I

Maternal Observations For Animals Treated On Gestation Days 6-28

Dose Group - mg/kg	No. Animals and Day of Death	No. Animals and Day of Abortion	Comment
0 Group	1 (day 25) [a]	1 (day 25) 1 (day 27)	Day 25 Animal died and aborted
40 Group	0	1 (day 27)	
160 Group	1 (day 2)[ab]	0	
480 Group	1 (day 24), 1 (day 25) 3 (day 26)[ac]	1 each (day 19, 21,22,24,26,29)[d]	Day 26 animal died & aborted

a No cause of death determined
b Treatment not yet initiated
c Two of 5 animals showed black or brown foci on stomach mucosa
d Fetuses aborted day 29, treated & examined with those at study termination.

19. Mutagenicity Testing of NP-55 in Microbial Systems. The Institute of Environmental Toxicology, Toxicology Division, BASF Wyandotte Corporation, Agricultural Chemicals Division, Parsippany, New Jersey; November 21, 1979. Accession No. 099537, Tab. C19, pp. 1-7.

Protocol - The rec-assay employing wild (H17) and deficient (M45) strain of Bacillus subtilis was used to determine the DNA damaging capability of NP-55. For the ability of NP-55 to induce reverse mutations, Escherichia coli strains WP2 hcr (TRP-) and Salmonella typhimurium strains TA1535 and TA100 (His-) were used to detect base change mutations; frame shift mutations were detected by using S. typhimurium strains TA1537, TA1538 and TA98 (all His-). For the rec-assay, frozen cultures (-80°C) of B. subtitis H17 and M45 were thawed and streaked on agar plates. NP-55 at concentrations of 1, 5, 10, 25, 50 and 100% (v/v) in DMSO was applied to filter paper discs (10 mm diameter) in volumes of 0.02 ml placed so as to cover the starting parts of the bacterial streaks. Kanamycin at 10 ug/disc and Mitomycin C at 0.1 ug/disc were used as negative and positive controls, respectively with DMSO alone used as a vehicle control; all in similar volumes. The tests were read by measuring the length of the inhibition zone of each streak following overnight incubation of all agar plates at 37°C. For reverse mutation assays, with and without metabolic activation, frozen cultures (-80°C) of the E. coli and S. typhimurium strains were thawed and suspended in phosphate buffer and mixed in molten top agar (0.6% agar and 0.5% Na Cl) at a rate of 1/10 (v/v). The molten agar contained 0.5 mM L-typtophan for the E. coli and 0.5 mM l-histidine and 0.5 mM D-biotin for the S. typhimurium strains. The S-9 metabolic activation component was isolated from liver homogenate from an Aroclor 1254-induced Sprague-Dawley male rat (age 7 weeks) using standard techniques. For the reverse mutation tests without metabolic activation (MA), 0.1 ml of NP-55 and 0.5 ml S-9 mix were added to 2.0 ml top agar and mixed. Molten top agars were layered over minimal medium agar plates containing Vogel Bonner E medium and all plates incubated at 37°C for 2 days. DMSO controls were included. For positive controls, AF-2 (2-(2 furyl)-3-(5-nitro-2-furanyl) acrylamide, 0.1 ug/plate, 0.25 ug/plate and 0.05 ug/plate; B -propiolactone 50 ug/plate, 9-aminoacridine 200 ug/plate, 2-nitrofluorene, 50 ug/plate and 2-aminoanthracene, 10 ug/plate were used. (The 2-aminoanthracene requires addition of S-9 MA to demonstrate its reverse mutation capability.) All tests were run with and without MA.

Results - In the rec-assay, NP-55 produced inhibition zones of 1 mm (highest concentration) or less, (at lower concentration) to no inhibition at the lowest concentration. The DMSO control was negative. Kanamycin, the negative control, showed similar negative inhibition (difference in inhibition between Wild type H17 and M45 deficient strain was 1, considered negative). Mitomycin C, the positive control, yielded inhibition zones for M45 and H17 of 8 and 0.5 mm respectively, a marked difference in inhibition between the 2 strains of B. subtilis, indicating the (expected) positive result. In the reverse mutation assay, NP-55 produced no increase in the number of revertant bacterial colonies in any strain with or without S-9 MA. Positive controls induced revertant colonies in all strains with or without MA, except for 2-aminoanthracene, which induced revertant colonies only with MA.

Conclusion - NP-55 is not mutagenic as measured in bacterial systems such as the rec-assay employing B. subtilis strain M45 and H17, or in reverse mutation assays employing E. coli WP2 hcr or S. typhimurium strains TA1535, TA100, TA1537, TA1538 and TA98.

Core Classification - This study is valid according to methodology in the current literature.

20. Mutagenicity Study of NP-55. Host Mediated Assay, Nisso Institute for Life Sciences, Kanagawa, Japan; May 8, 1980. Accession No. 099537 Tab. C20, pp. 1-14.

Protocol - Two trial groups of male CD-1 mice (Charles River, Japan, Inc.) aged 5 weeks, each trial group consisting of 4 sub groups of 5 mice per group, were all treated with Salmonella typhimurium strain G46 (His-) at a constant volume, different dosage per trial group, to test for revertant bacteria following pre treatment with NP-55 (experimentals), vehicle (negative control) or dimethylnitrosamine (DMN: positive control). Experimental material NP-55 was prepared as follows: NP-55 was suspended in 1% aqueous carboxymethylcellulose containing Tween 80 (CMC - Tween 80) to provide dosages of 310, 496, 1080 and 2500 mg/kg/body weight. Negative control material consisted of CMC-Tween 80 alone. Positive control material was DMN prepared to provide 100 mg/kg/body weight. The pretreatment regimen for mice was as follows, administered by gavage: for Trial group one, each sub group of 5 mice received respectively, 310 mg/kg/body weight NP-55 for 7 days, 496 mg/kg/body weight NP-55 for 7 days, vehicle control (CMC-Tween 80) for 7 days and one dose on day 7 of DMN. For Trial group two, each sub group of 5 mice was administered, respectively, 1080 mg/kg/body weight for 2 days, CMC-Tween 80 for 2 days and one dose on day 2 of DMN. Immediately following final administration of the pre treatment test material, animals were challenged intraperitoneally (I.P.) with 2.0 ml of (unspecified) medium containing S. typhimurium at concentrations of 1.4×10^8 bacteria/ml (trial one animals) and $3.2 \times k\ 10^8$ bacteria/ml (trial two animals). After 3 hours, animals were sacrificed by cervical dislocation, 2.0 ml physiological saline injected I.P. and peritoneal fluid (P.F.) withdrawn. To tally surviving bacteria from negative controls, minimal agar plates (0.1 uM D-biotin and 1.5 uM L-histidine) were spread with 0.1 ml of dilutions of P.F. to 10^6 made in saline. To count revertant colonies from experimental and DMN animals, 0.1 ml of undiluted P.F. was spread on minimal agar plates. All plating was done in triplicate, plates were incubated for 48 hours at 37°C and revertants and survivors counted. Revertant frequency per 10^8 surviving bacteria was calculated.

Results - Significantly (not statistically computed) increased numbers of revertants were found for positive controls, but NP-55 treatment showed no differences from negative control values at all treatment levels.

Conclusion - NP-55 at 2500 mg/kg/body weight for 7 day (highest dose tested) is not mutagenic in the host mediated assay employing S. typhimurium strain G46.

Core Classification - Valid study according to methodology in the current literature.

21. Two Generation Reproduction Study in Rats (of NP-55) (23 Week Interim). International Research and Development Corporation, May 23, 1980. Tab. C21; (no pagination - ca 14 pp.). Also: Addendum to the 23 Week Interim (May 23, 1980), July 11, 1980, No Tab, no pagination (ca 7 pp.) Accession No. 099537.

Protocol - Male and female Charles River COBS CD® weanling rats, consisting of three treatment groups and one control group, each group containing 12 males and 24 females, were acclimated for 10 days. Animals were individually housed except during mating, when one male and two females were housed together, and during lactation. Two additional groups, consisting of one treatment and one control group were initiated 4 months later. The interim report contains 23 weeks data on the first 4 groups and 20 weeks data on the additional 2 groups. Rats were fed a basal diet (Certified Rodent Chow® #5002) to which NP-55 was added by blending to provide the following feeding regimen: 0 ppm, 40 ppm, 120 ppm and 360 ppm respectively, for the first 4 groups and 1080 ppm and 0 ppm for the two groups added later. After 4 weeks, the dosage level for the 1080 ppm group was increased to 2160 ppm; after 9 weeks, this dosage level was increased to 3240 ppm because of a lack of toxicological effect at the two previous levels. After 14 weeks of treatment, the F_0 parental rats were housed in units of one male and two females to initiate the F_1 generation, allowing a maximum period of 15 days for mating. Females were examined daily for presence of a vaginal plug or sperm seen on vaginal smears, which time was considered gestation day 0 and females were then housed separately. Females were examined for parturition three times daily at the end of gestation. Lactation day 0 was the day all pups in a litter were found. F_1 pups were weighed as litters on days 0, 4, 7, 14 and individually weighed on day 21. After weaning, 12 male and 24 female pups were selected to become F_1 parents. Five males and five females of the F_1 pups were necropsied and tissues saved. Ten males and ten females of the F_0 parental rats were similarly treated. At this stage, the high dose, 3240 ppm and 0 ppm groups added later had not yet produced offspring.

Results - No changes attributable NP-55 administration were seen in parents and offspring of the F_0 and F_1 generations examined to the date of the interim report. Characteristics compared included behavior, appearance, survival, food consumption, weight gain of parents and pups. No differences in litters, male and female fertility indices, pup survival or pup body weights were observed on comparison of the 40, 120 and 360 ppm dosage levels against controls.

Addendum Results - (Data for the conclusion of the first generation study of the 3240 ppm group and the concurrent control group.) Mean parental body weight of the 3240 ppm group and the mean pup body weights at birth through weaning were slightly less than the control group. There were no changes in appearance, behavior, survival, fertility, length of gestation or survival of pups through weaning seen in these groups of rats. In neither the interim nor the addendum to the interim are there reports on necropsies or tissues saved for further studies.

Reproductive Effects - No conclusion can be drawn from this interim report with addendum.

Core Category - Supplementary data.

22. The Metabolism of NP-55 in Rats. Fine Chemicals Research Laboratory. July 1980, Tab. C22; Accession No. 099537.

Protocol - Four groups of male and female rats (Fischer strain, Charles River, Japan) aged 9 weeks (groups A, B and D) or 8 weeks (group C) were treated as outlined in Table I to determine the metabolic fate of radioactively labeled technical NP-55. The radioactive sample of NP-55 [(*4-^{14}C) - NP-55] was synthesized (Japan Atomic Engery Research Institute) with a specific activity of 10.3 m Ci/mM and a radiochemical purity of 98%. Five males and five females from each group selected as representative of the group by uniformity of plasma levels of ^{14}C-NP-55 were then individually housed and tested until 95% of the administered radioactive dose was eliminated (48 hours), and then sacrificed. During the 48 hours period, blood, urine and feces were collected for analysis of metabolites and measurement of radioactivity. Blood samples were collected as 0.25, 0.5, 1.0, 2, 3, 4, 6, 12, 24 and 48 hours following treatment. Urine and feces were separately collected on day 1 and 2 from cage apparatus arranged to receive them and during sampling procedures for blood. At 48 hours sacrifice, rats were exsanguinated from the carotid artery, with the plasma separated from blood by centrifugation. Residual bladder urine was pooled with the previously collected 2 day urine sample. Tissues and structures removed weighed and analyzed for metabolites and radioactivity included fat, gonads (testes plus epididymes of males or ovaries of females), spleen, kidney, liver, heart, lung, urinary bladder, femoral muscle and femoral bone. Remaining structures reserved as the carcass were weighed. Radioactivity in samples was measured by liquid scintillation spectrophotometry with external standard, using appropriate methodology for liquid or solid samples. Statistical methods were used to determine means and standard errors for all analytical procedures with the Student's t test applied to determine differences between 2 groups and a 5% level of significance was adopted.

Results - During the 48 hour test period, all groups showed an average percent of 78.5% administered radioactivity excreted into urine and 20.1% in feces. Less than 2% of the administered radioactive dose was tissue-associated. In the tissues, residual levels of ^{14}C-NP-55 were highest in liver, with values of 0.6 ppm in groups A, B and C and 13 ppm in group D (the high dose group). The extent of absorption was almost 100% in groups B, C and D, based on radioactivity excreted in urine by group A (intravenous administration considered highest absorption rate). Of remaining tissue-bound ^{14}C residues, one half remained bound in groups A, B and C and one third in group D (high dose group).

Conclusion - Excretion of NP-55 in rats in extremely rapid and tissue accumulation is negligible, assuming the DMSO vehicle does not affect excretion or storage of the chemical.

Core Study Category - Guidelines

TABLE I

RAT METABOLISM STUDY
(4- ^{14}C)-NP-55 DOSING

PROCEDURES	RAT GROUPS A	B	C	D
Dosage with unlabeled NP-55 prior to challenge with labeled NP-55	-	-	14 daily oral doses 10 mg/kg, 100 ul/ animal DMSO vehicle	-
Number of animals per group	11 males 11 females	8 males 8 females	8 males 8 females	8 males 8 females
Challenge Total conc. NP-55	10 mg/kg	10 mg/kg	10 mg/kg	325 mg/kg
conc. radioactivity (4-^{14}C) NP-55	10 uCi/25 ul	10 uCi/300 ul	10 uCi/300 ul	10 uCi/300 ul
Vehicle	DMSO	DMSO	DMSO	DMSO
Final number of animals selected & tested in metabolism study*	5 males 5 females	5 males 5 females	5 males 5 females	5 males 5 females

*Animals selected to be representative of the group by uniformity of plasma levels of ^{14}C-NP-55.