RB-1166
TAR-2575

Coberly

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

002575

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MAR 9 1983

MEMORANDUM

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

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769)

Budd 3/9/83

SUBJECT: PP #2F2670 and EPA #7969-LI.
Poast™ in or on Soybeans.
Toxicology Branch Response to 6
Submissions from Registration Division.

W A Burnam 3.9.83

FROM: Minnie Sochard, Ph.D. and Edwin R. Budd
Toxicology Branch, HED (TS-769)

Minnie Sochard 3/4/83
Edwin R. Budd 3/9/83

TOX Chem. No. 72A

Petitioner: BASF Wyandotte Corporation
100 Cherry Hill Road
P. O. Box 181
Parsippany, N.J. 07054

This memorandum deals with the following submissions from RD for PP 2F2670 and EPA #7969-LI , Poast™ in/on soybeans:

1. Registration package, received, EPA, April 15, 1982. Due July 10, 1982. Accession Nos. 070817, 070818, 070819, 070815, 070816, 070814, and 070820. Record No. 66214 and 66215.

2. Revised Section F. Date received, EPA, September 3, 1982. Due November 8, 1982. No data. Record No. 77026.

3. Re-revised Section F, Date received, EPA, September 7, 1982. Due November 12, 1982. No data. Record No. 77184.

4. Label change. No data. Date received, EPA, January 26, 1983. Due April 4, 1983. Record No. 88613.

10/62

5. Supplement I. Accession No. 249451. Date received, EPA, February 3, 1983. Due May 26, 1983. Record No. 89594.

6. Additional Toxicology Data. Accession No. 249241. Date received, EPA, January 12, 1983. Due May 10, 1983. Record No. 87565.

Action Requested:

BASF Wyandotte has requested (1) registration of the formulated product, POAST® Herbicide (EPA# 7969-LI) and (2) permanent tolerances for the herbicide Poast™ in or on raw agricultural commodities as follows*:

Soybeans	3.0 ppm
Meat, fat and meat byproducts of cattle, goats, hogs, horses, poultry and sheep	0.2 ppm
Milk	0.05(N) ppm
Eggs	0.5 ppm

* Revised Section F received 2/3/83 (accession # 249451).

Recommendations:

1. TB has no objections to conditional registration of POAST® Herbicide or to the above proposed tolerances subject to the conditions specified below in 3.

2. TB has been advised by RCB (on 3/2/83) that the proposed tolerance of 3.0 ppm on soybeans may be too low and that a higher tolerance on soybeans may be necessary. Also under review at this time in TB and RCB is another petition (PP 2F2748) for which a tolerance of 5.0 ppm for POAST on cottonseed is expected to be proposed. TB would have no objection to either of the following tolerance combinations for soybeans and cottonseed:

	Maximum Tolerance
soybeans (alone)	12.5 ppm
no cottonseed	N/A
soybeans	10.0 ppm
cottonseed	5.0 ppm

The reason for presentation of these maximum tolerances is explained more fully below in 8.

3. As discussed more fully below in 8, TB recommends that conditional registration of POAST® Herbicide be approved subject to the following conditions:

a. That BASF Wyandotte agrees to perform and submit to EPA additional toxicity studies using the POAST hydroxy metabolite(s) as the test material. The number and type of these studies will be agreed upon by BASF Wyandotte and TB in the near future.

b. That BASF Wyandotte agrees to perform and submit to EPA additional studies requested by RCB.

4. The human hazard signal word on the most recent proposed label for POAST® Herbicide (dated 2/1/83) is correct and is DANGER, due to the toxicity category I classification for skin and eye irritation. Although BASF requested that the signal word be changed to CAUTION based on the results of a second eye irritation study, TB does not concur with this request. For more information on this matter, see the TB review dated 8/31/81 by M. Sochard and the review of the second eye study (in this review).

5. The proposed label (dated 2/1/83) does not appear to conform to type size and standard formating requirements - which require appropriate warnings and a precautionary paragraph under the general heading of "Precautionary Statement" and a subheading of "Hazard to Humans and Domestic Animals" and repetition of the signal word (DANGER). TB defers to RD the proper type size and label formating for this product. The warnings and precautionary paragraph should be appropriate for a toxicity category I skin and eye irritant. A copy of the 2/1/83 label is attached.

6. TB concurs with BASF's proposal that the second 6-month dog study should replace the first 6-month dog study.

7. RCB has deferred to TB as to whether or not the hydroxylated metabolites MU-2, MU-1 and MU need to be included in the tolerance regulation (see RCB review of 5/21/81 by E. Zager).

Studies with radioactive labeled BAS9052 H (active ingredient in POAST) show that in soybeans the hydroxymetabolites MU-1, MU-2 and MU account respectively for 23%, 8% and 12% of total recovered radioactive metabolites. Thus, individuals eating treated soybean products may be exposed to a total of 43% hydroxymetabolites.

TB believes that these hydroxylated metabolites, which obviously comprise a very large percentage of the residue on soybeans, should be included in the tolerance regulation. TB also believes this issue to be resolved in that the most recent tolerance statement, dated 2/1/83, adequately covers the parent compound and its metabolites.

8. TB is concerned about the potential toxicity of the hydroxylated metabolites MU, MU-1 and MU-2 since they comprise a very large percentage of the residue on soybeans and toxicity studies using these metabolites as test materials are not available at the present time - except for acute oral LD_{50} studies recently submitted in accession #249241. Additional studies with these metabolites have been made a conditional requirement for registration of POAST® Herbicide for use on soybeans (see #3 above). TB has recently met with BASF several times regarding this problem. As a result of these meetings and in consideration of additional information and rationale submitted by BASF in Supplement I (accession #249451), TB has concurred with BASF's suggestion that a "provisional Maximum Permissable Intake (MPI)" can be established for these hydroxylated metabolites, based on the occurrence of hydroxylated metabolites in a rat metabolism study at a level of 0.8% of the administered dose. See the addendum at the end of this review for a review of this rat metabolism study. BASF's suggestion, with which TB concurs, is basically the following (with modifications and additional statements by TB):

> The study on which the ADI and MPI will be set for this chemical is the 2-year chronic feeding/oncogenicity study on rats. This study has a NOEL of 360 ppm or 18 mg/kg/day. Using a safety factor of 100, the ADI is 0.18 mg/kg/day and the MPI (for a 60 kg person) is 10.8 mg/day. Since the rat metabolism study indicated that 0.8% of "parent" POAST may be converted to hydroxylated metabolite(s), it is reasonable to assume that 0.8% of the MPI (10.8 mg/day) is due to the hydroxylated metabolite(s). Therefore a "provisional MPI" for hydroxylated metabolites may be established at 0.8% of 10.8 mg/kg or 0.0864 mg/day.
>
> Utilizing the concept that the portion of the TMRC due to hydroxylated metabolites should not exceed the "provisional MPI" for hydroxylated metabolites (0.0864 mg/day), the maximum possible TMRC for these hydroxylated metabolites was set at 0.0864 mg/day. Until additional toxicity studies are received on these metabolites, TB

will not permit this value to be exceeded. Any combination of crops may be used, however, in calculating the contributions to this TMRC. Back calculations for soybeans alone (with no other crops considered), utilizing a food factor of 0.92%, would permit a maximum residue of 12.5 ppm for residues of parent and metabolites on soybeans (assuming 50% of the residue is hydroxylated metabolites). Since BASF is also expected to propose establishement of a 5.0 ppm tolerance on cottonseed, a TMRC contribution for hydroxylated metabolites on this crop, again assuming 50% of the residue is hydroxylated metabolites, has been calculated, with a food factor of 0.15%, to be 0.0056 mg/day. Subtracting this from 0.0864 mg/day yields 0.0808 mg/day available for contributions from other crops. A tolerance of 11.7 ppm for parent and metabolites on soybeans would approximate this figure. Thus, a tolerance of 10.0 ppm on soybeans and 5.0 ppm on cottonseed could be supported.

Summary of Toxicity Data and Eight Point Free Standing Summary

1. Summary of selected toxicology data considered for these actions:

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	Guidelines
Acute Dermal LD50, Rat	> 5.0 gms/kg, males & females	III	Guidelines
Acute Inhalation LC50, Rat (4 hours)	6.03 mg/L, males 6.28 mg/L, females	III	Guidelines
Primary Eye Irritation, Rabbit	No irritation	IV	Guidelines
Primary Dermal Irritation, Rabbit	No irritation	IV	Guidelines
Dermal Sensitization, Guinea Pig	Negative	-	Minimum
14-Week Mouse Feeding Study	NOEL = 300 ppm	-	Minimum
14-Week Rat Feeding Study	NOEL = 300 ppm	-	Guidelines
Six Month Dog Feeding Study	NOEL = 20 mg/kg/day (males and females) LEL = 177 mg/kg/day - males 223 mg/kg/day - females (values based on analysis of diet and food) Non-specific anamia, liver effects, possible kidney effects.	-	Guidelines

002575

STUDY	RESULTS	TOX CATEGORY	CORE CLASSIFICATION
2-Year Mouse Chronic Feeding/Oncogenicity Study	NOEL = 120 ppm (=18 mg/kg/day) LEL = 360 ppm (non-neoplastic liver lesions) Not oncogenic in BDF1 mice.	-	
	As a chronic feeding study	-	Guidelines
	As an oncogenic study	-	Guidelines
2-Year Rat Chronic Feeding/Oncogenicity Study	NOEL $\geq$ 360 ppm (highest dose tested, (= 18 mg/kg/day)	-	
	As a chronic feeding study	-	Guidelines
	As an oncogenic study	-	Guidelines
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]),	-	Guidelines
Teratology Study, Rabbits	No terata up to and including 160 mg/kg/day. Effects observed at 480 mg/kg/day (increased number of a variety of random effects including skeletal, visceral abnormalities, reduced fetal weight, changes in male/ female ratios) considered due to extreme toxicity in dams and not to test material. 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

002575

STUDY	RESULTS	TOX CATEGORY	CORE CLASSIFICATION
Two Generation Reproductive Study, Rats	No reproductive effects. NOEL = 360 ppm	-	Guidelines
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
Metabolism Study - Rats	Tissue accumulation of chemical negligible and excretion extremely rapid, assuming DMSO vehicle doesn't affect storage or excretion of the chemical.	-	Guidelines
b) Data on Formulated Product - BAS 9052 OH (EPA #7969 - LT)			
Acute Oral LD50, Rat	4918.7 mg/kg	III	Guidelines
Acute Dermal LD50, Rat	>4000 mg/kg	III	Guidelines
Acute Inhalation LC50, Rat	>7.6 mg/L	III	Guidelines
Primary Eye Irritation, Rabbit Study No. 1	P.I. = 32 (24-hrs.); 35 (48-hrs.); 29 (72 hrs.) (scarring in 5/6 animals at 8 days, corneal opacity, in one animal at 8 days).	I	Minimum
		TOX	CORE
Primary Eye Irritation, Rabbit Study No. 2 (present review)	P.I. indexes - Washed eyes = 6.0, 6.0, 1.3, 0.7 and 0.0 at respectively 24, 48, 72 hrs. and 4 and 7 days.	I	Minimum

002575

STUDY	RESULTS	TOX CATEGORY	CORE CLASSIFICATION
	Unwashed eyes = 32.0, 31.0, 28, 17.6, 7.7 at respectively, 24, 48, 72 hrs and 4 and 7 days.		
Primary Dermal Irritation, Rabbit	P.I. = 4.0 Tox. Category I (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:

Additional toxicity studies using hydroxylated metabolite(s) as test material. See # 3 and 8 under recommendations.

3. Action being taken to obtain the lacking information or other additionally needed information.

See # 3 under recommendations.

002575

STUDY	RESULTS	TOX CATEGORY	CORE CLASSIFICATION

4. A summary of other permanent tolerances granted for this herbicide.
None.

5. See attached computer printouts (an following pages) for:

   a. soybeans (oil) at 3.0 ppm (plus secondary residues in meat, milk, poultry and eggs), and

   b. soybeans (oil) at 10.0 ppm (plus secondary residues in meat, milk, poultry and eggs)

Note: - RCB has informed TB (on 3/2/83) that increasing the soybean tolerance from 3.0 to 10.0 ppm will not alter the secondary residue levels in meat, milk, poultry and eggs.

6. The 2-year rat chronic feeding/oncogenicity study with a NOEL of 360 ppm (or 18.0 mg/kg/day) was used to set the ADI. The safety factor employed was 100. The ADI is 0.18 mg/kg/day. The MPI is 10.8 mg/day (for a 60 kg person).

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

8. Other relevant considerations in setting these tolerances.

See recommendations #1 through #8.

002575

No CFR Number BAS 9052 H 3/2/83

a.

File last updated 2/17/83

NOEL change not recorded per

ACCEPTABLE DAILY INTAKE DATA

002575

RAT,Older	NOEL	S.F.	ADI	MPI
mg/kg	ppm		mg/kg/day	mg/day(60kg)
18.000	360.00	100	0.1800	10.8000

Current Action 0G2396

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Soybeans (oil)(148)	3.000	0.92	0.04132
Meat,inc poultry(89)	0.200	13.85	0.04154
Milk&Dairy Products(93)	0.050	28.62	0.02146
Eggs(54)	0.500	2.77	0.02078

NPI	TMRC	% ADI
10.8000 mg/day(60kg)	0.1251 mg/day(1.5kg)	1.16

****

No CFR Number BAS 9052 H 3/2/83 b.

NOEL change not recorded per

File last updated 2/17/83

ACCEPTABLE DAILY INTAKE DATA 002575

RAT,Older NOEL mg/kg	ppm	S.F.	ADI mg/kg/day	MPI mg/day(60kg)
18.000	360.00	100	0.1800	10.8000

Current Action 0G2396

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Soybeans (oil)(148)	10.000	0.92	0.13772
Meat,inc poultry(89)	0.200	13.85	0.04154
Milk&Dairy Products(93)	0.050	28.62	0.02146
Eggs(54)	0.500	2.77	0.02078

MPI	TMRC	% ADI
10.8000 mg/day(60kg)	0.2215 mg/day(1.5kg)	2.05

****

00257!

Description of Chemical and Formulations:

A. Chemical Identification

1. Chemical Name (active ingredient)

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

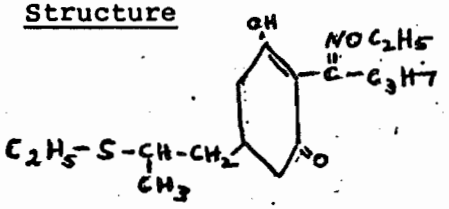
2. Synonyms

Poast®
NP-55
BAS 9052 H (technical product)
BAS 9052 OH (formulated product)
Sethoxydim (proposed common name for the a.i.)

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)

INFORMATION WHICH MAY REVEAL THE IDENTITY OF AN INERT INGREDIENT IS NOT INCLUDED

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

B. Formulations:

1. Poast® Herbicide (EPA #7969-LI)

Active ingredient - percent by weight

The product for which registration is being sought is Poast® Herbicide, an emulsifiable concentrate containing 18% a.i. (1.5 lb. a.i/gal).

2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio propyl] -3-hydroxy-2-cyclohexen-1-one.....18.0%

Inert ingredients.....82.0%

[redacted]

CONFIDENTIAL

CONFIDENTIAL

CONFIDENTIAL

002575

Detailed Review of Studies

Acute Study Formulation

1. Primary Eye Irritation Study of NP-55, 20% EC in Rabbits. Report No. 0056, November 27, 1981. Nisso Institute for Life Science pp. 1-11. Accession No. 070820. (Second Eye Study).

Protocol:

0.1 ml of undiluted NP-55 (BAS 9052) formulated to contain 20% a.i. was placed on the everted right lids of 9 Japanese white male rabbits aged 3-4 months old (averaging 3-4 kg). Eyelids of treated eyes were held together for one second and released. Untreated eyes served as controls. Eyes of 3 treated animals were flushed with lukewarm water for one minute; the remaining 6 animals were not further treated. Observations and scoring according to the Draize method were done at 1, 2, 3, 4, and 7 days after treatment. Readings were discontinued after 7 days.

Results:

Mean Primary Irritation (PI) scores were 6.0, 6.0, 1.3, 0.7 and 0.0 at respectively, 1, 2, 3, 4 and 7 days for washed eyes. Values for unwashed eyes for the same reading days were 32, 31, 28, 17.6 and 7.7. These latter values were similar to those obtained in an earlier study with the same formulation, (memo of August 31, 1981 M. Sochard, Ph.D.) which were 32, 35 and 29 at respectively 23, 48 and 72 hours, with corneal and conjunctival damage persisting through day 8 (observations not made beyond 8 days). In that study, there was no group of "washed eyes" for comparison. In the present study washed eyes exhibited no eye injuries persisting beyond day 4. Unwashed eyes showed corneal and conjunctival injury through day 7.

PI Index: Washed eyes: 6.0 (24 hrs); 6.0 (48 hrs); 1.3 (72 hrs); 0.7 (4 days); 0 (7 days).

Toxicity Category = I (Corneal and conjunctival injury present in 3/6 unwashed eyes at 7 days.)

Core Category = Minimum data

Reviewer's Comments:

Observations should have been carried out to 21 days or until all signs of injury were absent, whichever came first.

2. Two Generation Reproduction Study in Rats with NP-55. (Final Report). International Research and Development Corporation, November, 1981. 80 pages, 8 appendices, Accession No. 070814. Study No. 449-001.

Protocol:

A. Breeding and Dosage Schedules:

Male and female Sprague-Dawley 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. 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 (concurrent control) 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. Diets were analyzed periodically to assure desired concentration of test article. Test article (NP-55) was the technical grade, 95% purity. 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. Similar procedures were used to obtain the F_{2a} litters, test for fertility, etc.

002575

Because insufficient litters were obtained in the F_{2a} generation for the 3240 ppm treatment group and its concurrent control group, the F_1 parents of these two groups were remated to produce the F_{2b} litters. (The breeding and dosing schedules and breeding schematics are presented in Exhibits 1 and 2 attached.)

The 120 ppm group was sacrificed on week 28 of the study because it was considered non-essential. Without this group, three treatment groups remained which were adequately spaced dosage groups, i.e., 40, 360, and 3240 ppm.

B. General Observations:

Animals were observed twice daily for changes in appearance and/or behavior. Body weights were measured weekly on adult rats; pregnant females were weighed on gestation days 0, 6, 15, 20 and on lactation days 0, 4, 7, 14 and 21. Food consumption was recorded weekly for adults except during the 15 day mating period, when animals were left undisturbed. Spermatogenesis evaluations were made on selected males from each treatment and control group by microscopic examination of the epididymus at sacrifice for the presence of mature sperm of normal morphology.

C. Statistics:

Statistical analyses compared treatment with control groups and significant differences at $p < 0.05$ or $p < 0.01$ levels were sought. Statistical analyses were made at the following times during the study: F_0 generation - week 14 and 23, F_1 generation - week 24, 40, (0 ppm, 40 ppm, 360 ppm groups), week 36 (3240 and 0 ppm concurrent controls groups), week 54 (0 ppm, 40 ppm, 360 ppm) and week 56 (3240 ppm and 0 ppm concurrent control groups). Statistical analyses were also used for comparisons of: fertility indices for males and females, live-born pup numbers and pup survival indices at 0, 4, and 21 days.

Analytical procedures included one way ANOVA, Barlett's test for homogeneity of variances, t-test (Steel and Torrie) for equal equal or unequal variances. Multiple comparison tables (Dunnetts) were used to determine significant differences.

D. Pathology:

Animals were selected for necropsy at intervals during the study as shown in Exhibit 2. Examination

002575

included external as well as abdominal, thoracic and cranial cavities in situ and following dissection. Microscopically detected abnormalities were recorded. Tissues from F_1 parents, F_1 and F_{2a} pups, weighed fresh included heart, liver, testes, kidneys, spleen; tissues weighed post-fixation included adrenals, and thyroids/parathyroids. Sections of tissues from F_0, F_1 parental and F_1, F_{2a} pups placed in phosphate buffered neutral formalin included aorta, colon, esophagus, eyes, ileum, jejunum, lungs, mesenteric lymph nodes, ovaries, pancreas, pituitary, prostate, salivary gland, seminal vesicles, sciatic nerve, skeletal muscle, skin, stomach, testes, epididymus (all adult F_1 male animals), thymus, trachea, urinary bladder, uterus, cervix. Animals which died during the study or were moribund sacrificed were similarly examined but no body or organ weights were measured. Hematoxylin/eosin stained sections examined microscopically from F_1 parents and F_1 and F_{2a} pups included: adrenals, colon, heart, ileum, jejunum, kidneys, liver, lungs, ovaries, spleen, stomach, testes with epididymus, thyroid, urinary bladder, prostate, uterus and cervix.

Results:

Few statistically significant differences were seen between dosage groups or between parental groups and subsequent generations. Differences which might be biologically significant and the few significant findings are described below:

1. Fertility Indices:

Since the parental F_1 produced fewer than 20 litters (F_{2a} litters) from the 3240 ppm and concurrent controls (0 ppm group), the F_1 parents were remated to produce the F_{2b} group. A comparison of fertility indices (see Table I) revealed no statistical differences between groups. The fertility indexes of 3240 ppm F_1 females and males appear slightly depressed in comparison with controls, which probably account for the smaller number of litters. This effect, not statistically significant, may be biologically meaningful.

002575

TABLE I

Comparison of Fertility Indexes

Dose	F_0 Parents Females %Pregnant	F_0 Parents Males %Fertile	F_1 Parents* Females %Pregnant	F_1 Parents* Males %Fertile	F_1 Parents† Females %Pregnant	F_1 Parents† Males %Fertile
0	70.8	83.3	79.2	91.7	-	-
40	70.8	83.3	82.3	100.0	-	-
120**	83.3	91.6	-	-	-	-
360	91.7	100.0	75.0	83.0	-	-
3240***	70.8	75.0	66.7	75.0	70.8	91.7
0***	79.2	83.3	70.8	91.7	75.0	91.7

* Parents of F_{2a} pups
† Parents of F_{2b} pups
** 120 ppm group discontinued after 28 weeks as "non essential" (see protocol).
*** 2 groups added later in study (3240 ppm and 0 ppm concurrent control).

2. Parental Body Weights:

Body weights, determined at the termination of the study, are given in Table II.

TABLE II

Parental Body Weights
Comparisons in Dosage Groups

Parents		Average Weights Within Dosage Groups (ppm) 0	40	120	360	3240	0*
F_0	Males	574g	586g	563g	593g	540g	552g
	Females	308	307	314	310	293**	321
F_1	Males	649	649	-/	629	587***	627
	Females	346	344	-	343	271	273

* 0 ppm concurrent control with 3240 ppm group.
** Significantly different from control, $p < 0.01$.
*** Significantly different from control, $p < 0.05$.
/ 120 ppm group discontinued (see protocol).

As can be seen, F_0 females at 3240 ppm and F_0 males at 3240 ppm weighed significantly less than controls. A dose-response relationship is not shown. No significant differences were seen on comparisons of adult body weights. No differences were seen on comparison of pup weights from all litters. Pups weights of F_{2b} litters are not given.

3. Macroscopic Findings:

Effects which were not statistically significant but might be of biological significance are shown in Table III. Included are macroscopic observations on lung and kidneys. For ease of comparison, the term "lung effects" includes congestion, foci, consolidation, firm area, edematous, collapsed. The term "kidney effects" includes observations of mottled red, foci, cyst and pale. (Effects of NP55 technical were observed in liver of animals [Rev. of Aug., 1981] but such were not found here.) Although some kidney and lung effects were seen (Table III), a dose relationship was not indicated.

TABLE III

Macroscopic Findings Among Animals

		Dosage Groups (ppm)					
Lung Effects*	Sex	0	40	120	360	3240	0†
F_0 Adult	M	3/10	1/10	5/10	4/10	0/12	0/12
	F	5/10	2/10	4/10	3/10	I/10	0/10
F_1 Adult	M	0/10	2/10	-	3/10	1/12	0/11
	F	1/10	3/10	-	3/10	4/10	0/10
Pups F_1	M	1/5	4/5	0/5	1/5	0/5	0/5
	F	0/5	3/5	3/5	1/5	0/5	1/5
Pups F_{2a}	M	0/5	0/5	-	0/5	0/5	0/5
	F	0/5	0/5	-	0/5	0/5	0/5
Pups F_{2b}	M					-**	-**
	F					-**	-**

* Congestion, foci, consolidation, firm area, edematous, collapsed
** No data
† Concurrent control with 3240 ppm group

002575

Kidney Effects*	Sex	Dosage Groups (ppm) 0	40	120	360	3240	0†
F_0 Adults	M	4/10	4/10	3/10	5/10	2/12	5/12
	F	2/10	1/10	2/10	0/10	0/10	1/10
F_1 Adults	M	4/10	4/10	0/12	8/10	0/12	0/11
	F	2/10	3/10	1/24	2/10	1/10	0/10
Pups F_1	M	0/5	0/5	1/5	0/5	0/5	0/5
	F	0/5	0/5	-	0/5	0/5	0/5
Pups F_{2a}	M	2/5	0/5	-	1/5	0/5	1/5
	F	0/5	0/5	-	0/5	0/5	0/5
Pups F_{2b}	M					--**	--**
	F					--**	--**

* Mottled red, foci, cyst, pale.
** No data.
† Concurrent control with 3240 ppm group.

4. Organ Weights:

Data was not submitted for F_0 generation adult organ weights for the following dosage groups: 0 ppm, 40 ppm, 120 ppm, and 360 ppm. Individual animal data was available for the 3240 ppm group and its concurrent control group from which Table IV below was prepared. Statistically significant differences between treated and control group organ weights for F_1 audults are shown in Table V below.

TABLE IV

F_0 Generation Organ Weights and Body Weights

Dose	Sex	Weights in Grams Body Weights	Spleen	Liver	Kidney	Testes	Heart
3240	M	540.0	.76	19.7	3.9	3.7	1.7
	F	287.0	.62	14.6	2.6	-	1.4
0 ppm	M	541.5	.77	20.5	4.0	3.7	1.8
	F	320.0	.66	16.6	2.8	-	1.7

No significant differences are seen between treatment and control groups.

002575

TABLE V

F_1 Generation Adults
Statistically Significant ($p < 0.01$ or $p < 0.05$)
Increases in Organ Weights Compared with Controls

Organ	Sex	Dosage Groups		
		40	360	3240*
Adrenals	M	+	-	+
	F	+	+	+
Thyroid	M	+	+	+
	F	+	+	-
Liver	M	-	-	+
	F	-	-	-
Spleen	M	-	-	-
	F	-	-	+
Testes	M	-	-	+

* 3240 ppm dosage groups compared with concurrent, 0 ppm controls.

5. Fertility/Spermatogenic Effects:

No evidence for decreased fertility or pathologic changes in spermatogonia were noted in any groups throughout the study.

6. Pup Macroscopic Observations:

The 3240 ppm groups had fewer pups than the concurrent controls. The F_1 pups were 13% fewer and the F_{2b} pups were 11% fewer then the concurrent controls. No other differences of statistical or biological significance were found among such characteristics as mean number of live pups, failure to deliver, number of dead pups at birth or following lactation, etc.

7. Histological (Microscopic) Observations:

Effects from individual animal data indicating differences from normal are given in Table VI. No data are given for F_0 generation animals. Data are for F_1 adults (20 animals per group), F_1 pups (10 animals per group) and F_{2a} pups (10 animals per group). There are no individual animal data for

F_{2b} pups at other than high dose and concurrent controls. The report states that data from F_{2a} pups are sufficient for pathological evaluation. No pathological changes were seen in tissues from F_{2a} pups, although "juvenile testes" was reported in all male pups among dosage groups of that generation.

TABLE VI

Abnormalities Noted in Histological Preparations
(From Individual Animal Data)

		Dosage Groups					
		0 ppm	40 ppm	120 ppm	360 ppm	3240 ppm	0 ppm
A.	F_1 Parents						
	Kidney	6/20	9/20	-*	6/20	0/20	2/20
	Liver	7/20	8/20	-*	4/20	3/20	1/20
	Lung	4/20	6/20	-*	5/20	0/20	0/20
	Thyroid	1/20	2/20	-*	1/20	0/20	0/20
B.	F_1 Pups						
	Kidney	1/10	2/10	1/10	1/10	0/10	0/10
	Liver	2/10	1/10	0/10	4/10	4/10	0/10
	Lung	5/10	10/10	9/10	9/10	1/10	1/10

* Not reported.

The kidney, liver, lung and thyroid effects observed do not demonstrate a dose-response relationship but rather appear to be random effects.

Conclusions:

No statistically significant effects attributable to the test article were observed among treatment groups at 40 ppm and 360 ppm when compared with controls. Groups treated with 3240 ppm showed statistically significant decreases in weight as adults (Table II) for F_0 females and F_1 males. F_1 adults showed possible treatment related effects in that adrenal weights of females and thyroid weights of males were increased over the treatment range of doses (Table V). A reduction in the number of surviving pups was observed at 3240 ppm for F_1, F_{2a} and F_{2b} progeny. "Juvenile Testes" were reported without qualification for all male pups of the F_{2a} generation. Since some biologically significant toxicological effects were noted among high dose animals, the systemic NOEL = 360 ppm. No reproductive effects were seen.

Systemic NOEL = 360 ppm.
Core category - Guideline.

002575

Study Title and Decription

104-Week Chronic Dietary Study of NP-55 in Rats. Final Report. Report #C25, Parts I and II, pp. 1-1186. December, 1981. Hazelton Laboratories, America, Inc. Accession No. 070815 and 070816.

Submitted By:

BASF Wyandotte Corporation.
Parsippany, New Jersey.

Background:

BASF Wyandotte Corporation has submitted a rat 2 year chronic feeding/oncogenesis study as part of the requirement to support registration and tolerances for POAST™ (technical chemical NP-55).

Test Material

Technical NP-55	Technical NP-55
Lot No. PN 1-2	Lot No. PN-3
Purity 96.1% (weeks 1-30)	Purity 94.8% (weeks 31-104)

Summary of Study

Fischer 344, CDF rats (55 males, 55 females) per dose group were administered technical NP-55 in the diet at dose levels of 0 (controls), 40, 120 or 360 ppm. Clinical chemistry was performed on 8 animals from each group at week 0, 26 and 51, and hematology evaluations on 8 animals per group on weeks 0, 18, 35 and 51. At one year, 5 rats/sex per group were exsanguinated and completely necropsied. At termination, all animals were necropsied and a virtually complete set of standard tissues was examined microscopically for nearly all rats. Animals which were moribund sacrificed or died during the study were similarly examined.

No treatment-related effects were noted with regard to mortality, clinical observations, hematology, clinical chemistry, body weights (though mid dose male weights were significantly below control values at 18, 26 & 34 weeks) and food consumption. A significantly higher growth weight was seen for high dose females at 26, 34 and 52 weeks. Organ weights and organ/body weight ratios revealed no differences from control values at the end of the study. However, mid dose males and low dose females showed respectively lower kidney weight values and lower body weight values at 53 weeks.

002575

Low dose females also had lower brain/body weight values at this time. Non-neoplastic lesions observed microscopically were not considered due to the test material. These included in descending frequency lesions in heart, liver, kidney, male testes and female uterus. Neoplastic lesions included benign tumors and malignancies, none of which appeared to show a dose response relationship. Many of these occurred with extremely low frequency or were present with roughly similar incidence in control animals, or were common in historical control animals of similar strains and ages. Under the conditions of this study, NP-55 is not oncogenic in ~~mice.~~ rats.

NOEL $\geq$ 360 ppm (Highest Dose Tested, = 18 mg/kg/day)

Core Classification

As a chronic feeding study - Guidelines
As an oncogenicity study - Guidelines

Detailed Review of Study

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 concentration and designations of NP-55 respectively: 0 ppm (control), 40 ppm (low), 120 ppm (mid) and 360 ppm (high dose) 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 concentration 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 purity 94.8% was used for weeks 31-52. Animals were fed twice weekly and were individually housed. Animals were observed daily for deaths or moribund conditions. Weekly examination were made for pharamacotoxic effect; body weight and food consumption measurements were made for the first 13 weeks and biweekly thereafter. Eight rats of each set from each group were selected for hematology studies at week 0, 18, 35, 51 and for clinical

chemistries on weeks 0, 26 and 51. Hematology tests included hematocrit (HCT), hemoglobin (HBG), erythrocyte counts (RCB), 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 area 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 ophthalmoscope 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 for each group were exsanguinated under sodium pentabarbitol. Organ weights and organ/body weight ratios were determined for brain, heart, liver, kidneys, testes with epididymus 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 muscle, 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 comparison between groups and determine significance of differences from control values at the 5% probability level, were employed.

Results

I. Diet.

Diet analysis showed values close (within 10 percent) of target doses. Oxidation was found to occur with the product M-SO produced in the low dosage group diet. Analysis for the test article plus amounts of M-SO produced accounted for the correct amount of test chemical added. (The problem of adequate blending of test article with diet to produce a uniform dosage was encountered in other reviews concerning this chemical.)

II. Animal Survival.

Animals surviving were tallied at 104 and 106 weeks. Terminal sacrifices were performed at 106 weeks. At 104

weeks, survival ranged from 64% (low dose females) to 86% (high dose males). Percentage survivals are given in Table 1. No unusual signs or symnptoms were observed among surviving animals.

III. Body Weights and Food Consumption.

Although a significantly higher growth rate is reported for high dose female rats at 26, 34 and 52 weeks, a graph of subsequent weights at 10 week intervals (refer to Fig. 2) reveals no significant differences between treated and controls from weeks 52-104. A reference is made to mid-dose male rat weights as being significantly lower than controls at 18, 26 and 34 weeks. This difference is seen at weeks 52-104, (refer to Fig. 1) but is not statistically significant, since the deviations around the means encompass all four dosage group means. The mean total food consumption values for the mid-dose males was found to be significantly decreased on comparison with controls. The biological significance of this observation is not clear. Food consumption values for all other groups showed no significant differences from controls.

IV. Ophthalmoscopic Findings

Unilateral cataracts were present in 6/43 high dose males at 104 weeks, but the low number and absence of these findings in other groups indicate this pathology to be spurious. No other ophthalmic pathology or findings related to treatment were found.

Table 1

Survival of Male and Female Rats

		Dose Groups			
		0 ppm	40 ppm	120 ppm	360 p
Week	Sex	% Survivors			
104	M	82 (41/50)**	84 (42/50)	80 (40/50)	86 (4
	F	70 (35/50)	64 (32/50)	88 (43/50)	76 (3
106*	M	78 (39/50)	82 (41/50)	80 (40/50)	82 (4
	F	68 (34/50)	62 (31/50)	84 (41/50)	76 (3

* Animals terminated at 106 weeks rather than at 104 weeks.
**Actual numbers in parentheses.

002575

V. Pathology

A. Clinical Chemistry and Hematology

Table 2 shows statistically significant changes in clinical chemistry and hematology measurements compared against control values. At 104 weeks, the 120 ppm males had a decrease in globulin. At 87 weeks 360 ppm males had increases in hematocrit, hemoglobin and red blood cell counts. At 79 weeks, 360 ppm males had a decrease in albumin. At the termination of the study, no significant changes were found in leucocyte counts, platelets, mean corpuscular volume, mean corpuscular hemoglobin concentration, albumin/globulin ratio, calcium, potassium, total cholesterol, alkaline phosphatase, total bilirubin, blood urea nitrogen, glucose, serum glutamicoxaloacetic transaminase, serum glutomic-pyruvic transaminase, and lactic acid dehydrogenase. In summary, no treatment-related results were seen in clinical chemistry or hematology values among groups administered the test chemical.

B. Gross Pathology

No treatment-related pathology was found. Age-related lesions were found which are common in aging Fischer rats.

C. Organ Weights and Organ/Body Weight Ratios.

At 53 weeks, male rat kidney weights of the 120 ppm group were statistically significantly lower than control rat kidney weights. At 53 weeks, female body weights of the 40 ppm group as well as brain/body weights ratios were significantly lower than those of contols. At 107 weeks, no statistically significant difference in organ weights and organ/body weight ratios were found on comparison with controls.

Table 2

002575

Statistically Significant Changes in Clinical Chemistry and Hematology Values During Weeks 0-104 of Study.

Characterists	Male/Female	0 ppm	40 ppm	120 ppm	360 ppm
Hematology					
Hematocrit	M	-	-	-	87 wk↑
	F	-	-	-	-
Hemoglobin	M	-	-	34 wk↓	87 wk↑
	F	-	-	-	-
RBC count	M	-	-	-	87 wk↑
	F	-	-	-	-
Clincial Chemistry					
Albumin	M	-	-	-	79 wk↓
	F	-	-	-	-
Globulin	M	-	-	104 wk↓	-
	F	-	-	-	-
Albumin/Globulin	M	-	-	51 wk↓	-
	F	-	-	-	-
Calcium	M	-	-	-	26 wk↑
	F	-	26 wk↑	26 wk↑	26 wk↑
Total Cholesterol	M	-	-	51 wk↓	-
	F	-	-	-	-
Alkaline Phosphatas	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
	F	-	-	-	51 wk↑

D. Histology

No treatment-related significant findings were observed among animals from all dosage groups at termination, or among animals found dead or moribund-sacrificed. Changes which were observed were those commonly seen in 0 ppm (control) animals and which were commonly seen in aging rats of the Fischer strain.

002575

1. Lesions observed.

At termination, the heart was most commonly observed to have lesions. In descending frequency, the liver, kidney, male testes and female uterus were found to have lesions as well. Lesions of the heart included degenerative cardiomyopathy and focal non-suppurative myocarditis. Lungs frequently had lymphoid hyperplasia and pulmonary artery mineralization, (the latter also found in other areas of the cardiovascular system). Livers showed pericholangitis, hepatitis and bile ducts frequently showed hyperplasia. Kidneys of male rats showed progressive nephropathy. The testes of most males had hyperplasia of interstitial cells. Female rats had frequent uterine endometrial stromyl polyps. The spleen was frequently found to have extramedullary hematopoeisis and increased pigment (hemosiderin), the latter mostly in male animals. Cortical vacuolization, hyperplasia, hypertrophy and angiectasis was found in the adrenal glands. The thyroid lesions were "C" cell hyperplasia and/or follicular cell hyperplasia. Hyperplasia also was found in the pancreas among islet cells. Endometrial stromyl polyps were frequently found in the uterus, characterized by stromal cell proliferation. Among most male animals including controls, the testes showed evidence of commonly occurring benign tumors.

Table 3 summarizes common histopathology findings among male and female rats. The histopathology findings for thyroid in males and females compares reasonably with tumor findings. The occurrence of mononuclear infiltration in males and females, and the lymphocytic hyperplasia probably reflects the monocytic leukemias.

002575

Table 3. Common Histopathology Findings Among Male and Female Rats on Study

Organ	MALES Dose Group 0 ppm	40 ppm	120 ppm	360 ppm
1. Thyroids				
"C" Cell Hyperplasia	4/47(9)*	3/49(6)	3/46(7)	746(15)
Cystic Follicles	1/47(2)	6/49(12)	3/46(7)	3/46(7)
2. Ileum				
Lymphocytic Hyperplasia	1/48(2)	2/46(4)	1/44(2)	5/48(10)
3. Prostate				
Epithelial Hyperplasia	7/48(15)	20/50(40)	7/49(14)	23/48(48)
Granulomatous prostatitis	0/48(0)	8/50(16)	1/49(2)	11/48(22)

Organ	FEMALES Dose Group 0 ppm	40 ppm	120 ppm	360 ppm
1. Thyroids				
"C" Cell Hyperplasia	7/48(15)	6/45(13)	8/49(16)	10/46(2
2. Liver				
Areas of cellular in-tration	7/49(14)	14/50(28)	12/50(24)	15/50(3
3. Kidneys				
Focal mononuclear infiltration	1/49(2)	2/48(4)	6/50(12)	5/48(10
4. Stomach-dilated mucosal gland	16/48(33)	16/50(32)	34/50(68)	37/50(7
mononuclear infiltration	0	1/50(2)	1/50(2)	5/50(10
5. Mesenteric Lymph Nodes				
Reticular cell hyperplasia	14/49(28)	20/48(42)	19/50(38)	7/43(16
Lymphangiectasis	4/49(8)	24/50(58)	29/50(58)	11/43(2
Lymphocytic hyperplasia	4/49(8)	10/48(20)	14/50(28)	3/43(7)

* % in ( ).

2. Tumors found.

Although a large number of benign tumors and malignancies were observed, none showed a dose response relationship, many appeared with an extremely low frequency, and are considered spurious.

The incidences of selected benign and malignant tumors are shown in Tables 4 (male rats) and 5 (female rats). In the pituitary, the most common tumors were adenomas, with a high incidence in both male and female groups. Spontaneous tumors of the pituitary are common in older rats of the Fischer strain (J.D. Burek, Pathology of Aging Rats. CRC Press, Inc., West Palm Beach, FL 33409, 1978.) Pituitary carcinomas were found in female rats with a low incidence in control and treatment groups alike. Lung tumors were adenocarcinomas, found in 1 female control, 1 mid dose and 1 high dose female. Lung cancers are rare in rats; the ones described here are considered spurious on the basis of their low incidence and lack of dose response relationship. Liver neoplastic nodules were found in males and females at a low incidence and showed no dose-response relationship and are not considered significant.

Thyroid tumors of the "c" cell type are not uncommon in aging Fischer rats nor are pheochromocytomas of the adrenal glands, islet cell adenomas, interstitial cell tumors of the testes of aging male rats, or endometrial stromyl polyps in the uterus of aging female rats. Monocytic leukemia is commonly found in aging Fischer rats. The above mentioned tumors are seen with similar frequency in controls and treated rats in the present study. Of the remaining tumors, the pituitary carcinomas (females), adrenal cortical carcinomas, pancreatic islet carcinomas, etc., are either found with very low frequency (e.g., leiomyosancoma or scirrhous carcinoma of the uterus) or present at low frequency in controls and treatment groups (e.g., islet cell adenoma in males, pituitary carcinoma in females, liver neoplastic nodules in females and males, and hepatocellular carcinoma in males).

Conclusion and Discussion

This study shows that dosing Fischer rats with NP-55 results in a no observed effect level (NOEL) of 360 ppm (highest dose tested).

Although a variety of tumors and lesions were observed, none showed a dose response relationship, others were of such infrequency as to be considered spurious, and some were infrequent and similar in incidence in control and treated animals.

Table 4. Tumors of Interest in Male Rats in Percents

Organ	Dose Group 0 ppm	40 ppm	120 ppm	360 ppm
1. Pituitary Adenomas	26(13/49)*	26(13/50)	8(4/47)	34(17/50)
2. Adrenals Pheochromocytomas	6(3/50)	4(2/50)	2(1/50)	6(3/50)
Malignant pheochromocytomas	0	0	4(2/50)	2(1/50)
Bilateral pheochromocytomas	0	2(1/50)	0	0
(pheochromocytomas, TOTAL)	[6(3/50)	6(3/50)	6(3/50)	8(4/50)]
Cortical adenomas	0	6(3/50)	2(1/50)	4(2/50)
3. Thyroid				
"C" cell adenomas	4(2/47)	10(5/49)	6(3/46)	2(1/46)
"C" cell carcinomas	0	4(2/49)	2(1/46)	10(5/46)
Follicular cell carcinoma	0	0	4(2/46)	4(2/46)
4. Hematopoeitic System monocytic leukemia	22(11/50)	16(8/50)	14(7/50)	18(9/50)
5. Liver				
neoplastic nodules	6(3/50)	8(4/50)	8(4/50)	2(1/50)
hepatocellular carcinoma	0	6(3/50)	4(2/50)	2(1/50)
(Liver tumors, TOTALS)	[6	14	12	4]
6. Pancreas				
Islet cell adenoma	6(3/50)	10(5/50)	4(2/49)	4(2/50)
Islet cell carcinoma	0	4(2/50)	14(7/49)	4(2/50)
7. Tissue masses mesothelioma	4(2/50)	4(2/50)	6(3/49)	6(3/50)
8. Skin keratoacanthoma	2(1/49)	0	4(2/50)	6(3/50)
9. Testes interstitial cell tumors				
Bilateral	88(44/50)	88(44/50)	84(42/50)	90(45/50)
Unilateral	8(4/50)	6(3/50)	8(4/50)	8(4/50)
Malignant	0	0	2(1/50)	0

* Actual numbers in parentheses.

002575

Table 5. Tumors of Interest in Female Rats in Percents

Organ	Dose Group 0ppm	40ppm	120ppm	360 ppm
1. Pituitary				
Carcinoma	4(2/48)*	8(4/50)	6(3/50)	8(4/50)
Adenoma	55(27/49)	58(26/45)	61(30/49)	56(27/48)
2. Adrenals				
Cortical adenoma	4(2/49)	2(1/49)	2(1/50)	0
Cortical Carcinoma	0	0	2(1/50)	2(1/48)
Pheochromocytoma	0	2(1/49)	2(1/50)	2(1/48)
3. Thyroid				
"C" cell adenoma	2(1/48)	4(2/45)	4(2/49)	2(1/46)
"C" cell carcinoma	4(2/48)	4(2/45)	2(1/49)	2(1/46)
4. Lung				
alveolar/bronch-iolar adeno carcinoma	2(1/49)	0	2(1/50)	2(1/50)
5. Liver				
Neoplastic nodules	6(3/49)	6(3/50)	4(2/50)	0
6. Pancreas				
Islet cell carcinoma	2(1/48)	4(2/48)	2(1/50)	2(1/47)
Islet cell adenoma	0	2(1/48)	2(1/50)	0
7. Uterus				
endometrial stromal polyp	32(16/50)	26(13/50)	36(18/50)	32 (16/50
endometrial stromyl carcinoma	8(4/50)	6(3/50)	8(4/50)	4(2/50)
scirrhous carcinoma	4(2/50)	8(4/50)	0	0
Adenocarcinoma	0	0	0	10(5/50)
Leiomyosarcoma	0	0	2(1/50)	0
8. Mammary gland				
Adenocarcinoma	12(6/50)	4(2/49)	8(4/49)	2(1/49)
Adenocarcinoma	0	0	2(1/49)	4(2/49)
Fibro adenoma	2(1/50)	16(8/49)	10(5/49)	10(5/49)
9. Hematopoeitic system				
monocytic leukemia	24(12/50)	36(18/50)	14(7/49)	16(8/50)

* Actual numbers in parentheses.

The conclusion is that no oncogenic effect for NP-55 was evident in this study.

This study is assigned a Core Guidelines Classification.

NOEL = $\geq$ 360 ppm (Highest Dose Tested)
= 18 mg/kg/day

Study Title and Description:

Chronic Feeding Study Combined With Oncogenicity Study of NP-55 in Mice; Nisso Institute for Life Science, Kanagawa, Japan; Study report dated August 31, 1981; Laboratory No. 0049. Three volumes (EPA Accession numbers 070817-070819).

Submitted by:

BASF Wyandotte Corporation
Parsippany, New Jersey

Test Materials:

Technical NP-55
Lot No. PN-1-2
Purity: 95.4%

Summary of Study

Technical NP-55 was administered in the diet to male and female BDF1 mice for 24 months at dosage levels of 0 (control), 40, 120, 360 and 1080 ppm. One hundred mice of each sex were used as controls and 70 mice of each sex for each dosage level of NP-55. At 12 months, 10 mice of each sex from the control and each dosage level group were sacrificed and examined. Clinical observations, hematological, blood chemistry and urinalysis tests were performed. All animals were necropsied and a virtually complete set of standard tissues was examined microscopically for nearly all mice.

There were no apparent effects of the test material on mortality, clinical observations, hematological, blood chemistry (except for increased GPT and GOT values at 24 months in the 1080 ppm male group), or urinalysis determinations. Food consumption for the 1080 ppm male mice tended to be higher than male controls for most of the study. Mean body weight for the 1080 ppm male and female mice were significantly lower than their respective controls for most of the study and were considered to be related to the test material.

Organ weights and organ/body weight ratios showed no apparent changes due to the test material except for increased liver weights and liver/body weight ratios for male and female mice at 1080 ppm. Gross necropsies showed no effects due to the test material. Non-neoplastic lesions observed microscopically were not considered to be due to the test material except for liver effects in male mice at 360 ppm (minimal effects) and at 1080 ppm and in female mice at 1080 ppm. Those liver effects included local granulomatous inflammation, fatty degeneration, swelling of hepatocytes and hemosiderin deposition.

Neoplastic lesions observed during the study were predominantly malignant lymphomas and malignant neurilemomas. These lesions were not considered to be related to the test material since they were observed at roughly equal incidences in both control and NP-55 treated mice. In addition, these types of tumors are known to occur in historical control mice at similar incidences. Liver and lung tumors and all other observed tumors were not considered to be related to the test material. Under the conditions of this test, NP-55 was not oncogenic in mice.

NOEL = 120 ppm
LEL = 360 ppm (non-neoplastic liver lesions)
Not oncogenic in BDF1 mice.

Core Classification.
As a chronic feeding study - Guidelines
As an oncogenicity study - Guidelines

Detailed Review of Study

Test Animals:

Specific pathogen-free BDF1 (C57BL/6 x DBA/2) male and female mice, from Shizuoka Laboratory Animal Agricultural Cooperative Association. Aged 5 weeks when purchased and 6 weeks at beginning of test diet administrations.

Study Design:

Male and female mice, following an acclimatization period of 1 week, were divided into ten groups and fed technical NP-55 continuously in the diet for 24 months at the following nominal concentrations for the entire duration of the study.

002575

NP-55 Dosage	Sex	Number of Animals
Untreated Diet (Control)	M	100
Untreated Diet (Control)	F	100
40 ppm	M	70
40 ppm	F	70
120 ppm	M	70
120 ppm	F	70
360 ppm	M	70
360 ppm	F	70
1080 ppm	M	70
1080 ppm	F	70

At 12 months, 10 males and 10 females from each group were sacrificed, necropsied and examined histopathologically.

An additional 10 males and 10 females were used at zero months for hematological and urinalysis tests and an additional 21 males and 21 females for blood chemistry tests.

Animal Maintenance:

All mice were group housed with 2 or 3 mice of the same sex in the same group per cage. Standard animal husbandry techniques were utilized. Temperature and humidity were maintained at 22.7 ± 0.8°C and 54 ± 0.8%, respectively. Illumination was a daily cycle of 12 hours of light and 12 hours of darkness. Food and tap water (in bottles) were available to all animals ad libitum. Mean volumes of water intake were estimated at 6, 12, 18 and 24 months. Each mouse was identified individually.

Preparation of Test Diet and Administration:

A powdered mouse diet (CE-2) was the basal diet to which appropriate amounts of technical NP-55 were added (after dissolving in acetone). Acetone alone was added to the control diet. All 5 diets were supplied biweekly and stored in a deep freezer till used. Samples of diet from each lot were analyzed for NP-55.

002575

Food consumption was determined weekly for the first 14 weeks and biweekly thereafter. Based on food consumption and body weight data, the mean dosage of NP-55 was calculated weekly for the first 14 weeks and biweekly thereafter, (in terms of mg/kg/day).

Duration of Study:

Test diets were continuously available to test animals for 106 weeks - from October 24, 1978 to November 11, 1980.

Clinical Observations:

Observations for clinical signs of pharmacological and toxicological effects were made daily on all animals. Palpations for tissue masses were also made daily. Individual body weights were determined weekly for the first 14 weeks and biweekly thereafter.

Hematological Tests:

Erythocyte count, hematocrit, hemoglobin, total leukocyte count, differential leukocyte count and platelet count were performed on 10 males and 10 females at 0, 6, 12 and 18 months and on nearly all survivors at 24 months. MCV, MCH and MCHE parameters were also calculated.

Blood Chemistry Tests:

Sodium potassium, glucose, blood urea nitrogen, total cholesterol, total protein, albumin, total bilirubin, alkaline phosphatase, lactic dehydrogenase, glutamic pyruvic transaminase and glutamic oxaloacetic transaminase were performed on 7 males and 7 females at 0 months, on 10 males and 10 females at 12 months and on nearly all survivors at 24 months. All analyses were determined by a Technicon Auto Analyzer® (SMA 12/micro system).

Urinalysis Tests:

Urinalysis determinations (volume, color, specific gravity, pH, protein, glucose, occult blood, ketone body, urobilinogen and bilirubin) were performed on 10 males and 10 females at 0, 6, 12 and 18 months and an all survivors at 24 months. Test mice were housed individually in metabolism cages for 24 hours to collect the urine samples.

002575

Gross Necropsy:

Gross necropsies were performed on all animals dying or sacrificed in extremis during the study, on all animals sacrificed at 12 months (planned interim sacrifice) and on all suviving animals at termination of the study. Organ weights and organ/body weight ratios for the following organs were determined for 10 males and 10 females at 12 months and for all survivors at termination: brain, thymus, heart, lungs, liver, spleen, kidneys and testes.

Histopathological Examination:

The following organs and tissues from nearly all mice from all groups were examined microscopically:

Brain (3 lobes)	Adrenals
Pituitary	Stomach
Eyes	Pancreas
Harderian glands	Deuodenum, Jejunum, Ileum
Salivary gland	Colon
Thyroids (parathyroid)	Mesenteric lymph node
Thymus	Urinary bladder
Esophagus	Testes, epididymis
Trachea	Ovaries
Lung	Uterus
Heart	Skin
Aorta	Bone marrow
Liver (2 lobes)	Nerve with muscle
Spleen	Mammary gland
Kidneys	Unusual lesions

In addition, the spinal cord (2 levels), nasal cavity, nasopharynx and middle ear were examined in a minimum of 10 animals of each sex at each dosage level.

Tissues and organs were stained with hematoxylin and eosin. Perodic acid -SCHIFF (PAS) and Sudan III staining was performed on the liver, and kidney. All microscopic abnormalities were graded for severity on an 0 scale from 1 (slight) to 4 (extreme).

Study Results

Feed Analysis: (see Tab. AC-14, Accession #070820).

Fresh diets, prepared at 1 week intervals throughout the entire duration of the study, yielded the following ppm on analytical analyses.

002575

Nominal ppm	Analytical ppm (range)
0	0
40	36-43
120	108-131
360	344-386
1080	1000-1233

Food and Water Consumption:

Food consumption for 1080 ppm males tended to be higher (than male controls) from week 5-54 and very slightly higher from week 56-104. For 360 ppm males, food consumption tended to be very slightly higher from week 5-54. Food consumption for 1080 pm females tended to be very slightly higher from week 3-70. Statistical analyses were not performed but the above differences were small and were not likely to be statistically significant except perhaps for the 1080 ppm males. Food efficiency determinations were highly variable both within groups and from group to group. No consistent patterns were discernible upon a visual inspection of the data.

No meaningful differences in water consumption were observed between control and dosed groups during the study.

Mean Dosages of NP-55 (in mg/kg/day):

Mean dosages of NP-55 (in mg/kg/day) for the entire 106 week feeding period, based on food consumption and body weights, were calculated and are presented below.

Nominal Dosage (ppm)	Sex	Mean Dosage ± S.D. (mg/kg/day)
0	M	0.00 ± 0.00
0	F	0.00 ± 0.00
40	M	4.48 ± 0.98
40	F	4.85 ± 1.28
120	M	13.77 ± 3.18
120	F	14.86 ± 4.05
360	M	41.16 ± 9.80
360	F	44.33 ± 12.11
1080	M	134.46 ± 30.15
1080	F	142.85 ± 34.85

Mortality:

For male mice, the percentages surviving the 106 week study period for 0, 40, 120, 360 and 1080 ppm groups were

88.9%, 66.7%, 81.7%, 90.0% and 86.7% respectively. The actual numbers of surviving animals were 80, 40, 49, 54, 52, respectively (recall that at 52 weeks, 10 males were sacrificed from each group). For the first 78 weeks, mortality rates were comparable for all male groups. After 78 weeks, survival tended to be slightly lower in the 40 ppm group. No toxicological significance was attached to this finding.

For female mice, the percentages surviving the 106 week study period for 0, 40 120, 360 and 1080 ppm groups were 63.3%, 66.7%, 56.7%, 60.0% and 66.7% respectively. The actual numbers of surviving animals were 57, 40, 34, 36, and 40 respectively (recall that at 52 weeks, 10 females were sacrificed from each group). For the first 90 weeks, survival tended to be slightly higher in the 2 highest dosage groups. After 90 weeks, mortality rates were comparable for all female groups.

There was no apparent relationship between mortality rates and the test material in any of the treated groups.

Body Weights:

Mean body weights for 1080 ppm male mice were significantly lower from week 11 to termination of the study. Mean body weights for 40 ppm male mice were signficantly lower from week 76 to termination of the study and for 360 ppm male mice from week 32 to week 46. Mean body weights for 1080 ppm female mice were significantly lower from week 4 to week 72.

The decrased body weights for 1080 ppm male and female mice are considered to be related to the test material. Body weight changes in other groups were generally inconsistent and/or not dose related and are not considered to be related to the test material.

Nominal Dosage (ppm)	Mean Body Weights at 104 Weeks (gms) Males	Females
Control	43.7	38.4
40	41.4*	39.9
120	42.2	37.5
360	42.9	38.4
1080	39.3***	37.6

* Significantly different from control at $p<0.05$
***Significantly different from control at $p<0.001$

Clinical Signs:

Clinical signs observed during the study were infrequent, sporadic, not dose related and clearly not related to the test material.

Hematological Observations:

RBC Parameters:

Male mice - At 6 months, erythrocyte count, hematocrit and hemoglobin tended to be significantly decreased for 1080, 360 and 120 ppm animals. Also, MCV and MCH were significantly increased for 1080 ppm animals. At 12, 18, and 24 months, no consistent effects were observed.

Female mice - At 6 months, erythrocyte count, hematocrit were significantly increased for 1080 ppm animals. At 18 months, erythrocyte count, hematocrit and hemoglobin tended to be significantly increased for 1080, 360, and 120 ppm animals. At 12 and 24 months, no consistent effects were observed.

Platelet Count:

Male mice - At 6 months, animals treated with test material tended to have significantly decreased platelet counts. No effects were observed at 12, 18, or 24 months.

Female mice - At 6, 12, 18 and 24 months, no effects were observed.

Leukocyte Parameters:

Male mice - At 6 and 12 months, total leukocyte counts tended to be significantly decreased in animals treated with the test material. Similar effects were not observed, however, at 18 and 24 months. Differential leukocyte counts were essentially negative for effects.

Female mice - Total and differential leukocyte counts were essentially negative for effects.

The hematological observations described above were generally inconsistent with time and/or not dose related. None of these observations are considered to be related to the test chemical.

002575

Blood Chemistry Observations:

The following statistically significant differences were observed during the study:

Glucose: decreased at 12 months in 1080 ppm males; increased at 24 months in all treated females (probably due to low control value).

A/G ratio: decreased at 12 months in 40, 360, and 1080 ppm males; increased at 24 months in 40, 120 and 1080 females.

LDH: decreased at 24 months in 40 and 360 ppm males; decreased at 24 months in 360 ppm females.

GPT: increased at 24 months in 1080 ppm males (approximately 300% of control value).

GOT: increased at 24 months in 1080 ppm males (approximately 150% of control value).

ALP: increased at 12 months in 40 and 120 ppm females.

Total Cholesterol: decreased at 24 months in 1080 ppm females.

Total Bilirubin: decreased at 24 months in 1080 ppm females.

Albumin: increased at 24 months in 120 ppm females.

Sodium: increased at 24 months in 40 and 120 ppm females.

The only differences likely to have toxicological significance are the increases in GPT and GOT at 24 months in the 1080 ppm males which suggests possible liver damage in these animals.

Urinalysis Observations:

In male and female mice at 6, 12, 18 and 24 months, differences from control gorups were sporadic, inconsistent, not dose related and apparently not related to the test material.

002575

Organ Weight and Organ/Body Weight Ratios:

In the 1080 ppm male mice at the 12 month interim sacrifice and at the 24 month final sacrifice, statistically significant increases ($p<0.001$) were observed for absolute liver weights and for relative liver/body weight ratios. In the 1080 ppm female mice at the same sacrifices, statistically significant increases were again observed for absolute liver weights and liver/body weight ratios, but to a lesser magnitude than in the males. The increased liver weights and liver/body weight ratios for male and female mice at 1080 ppm are considered to be related to the test material.

Dosage Group	12-Month Sacrifice		24-Month Sacrifice	
	Mean Liver Wt.(gm)	Mean Liver BW ratio	Mean Liver Wt. (gm)	Mean Liver BW ratio
Males				
0	1.34	3.37	1.69	4.01
40	1.41	3.49	1.63	4.03
120	1.44	3.33	1.76	4.33
360	1.42	3.56	1.84	4.42
1080	1.80***	4.30***	2.11***	5.53***
Females				
0	1.15	3.13	1.53	4.18
40	1.08	3.18	1.71	4.47
120	1.07*	3.27	1.56	4.30
360	1.16	3.44*	1.52	4.05
1080	1.22	3.62**	1.76*	4.80*

*Significantly different from control at $p<0.05$
**Significantly different from control at $p<0.01$
***Significantly different from control at $p<0.001$

002575

Other differences from controls in organ weights and/or organ/body weights ratios were occasionally observed in males for heart, spleen, gonads, brain and lung; and in females for brain, thymus, heart, and kidney. These differences were sporadic, inconsistent and/or not dose-related and are not considered to be related to the test material.

Gross Necropsy Observations:

The most frequently observed gross lesions in animals sacrificed at the end of the study were:

lymph nodes, enlarged or masses (M and F)
abdominal fat, polypoid (M)
lungs, masses and nodules (M)
lungs, colored zones (M and F)
liver, masses and nodules (M)
spleen, masses or enlarged (M and F)
seminal vesicles, enlarged or colored (M)
ovary, cyst (F)
uterus, mass (F)

For none of these grossly observed lesions was there a consistent dose-related increase that appeared to be attributable to ingestion of the test material. Incidences were reported with about equal frequency at all dosage levels and controls.

Histopathological Examination, Non-neoplastic Lesions:

For mice which died during the study, were sacrificed in moribund condition or were sacrificed at termination of the study, the following non-neoplastic lesions were frequently observed in control and test groups with about equal frequencies and therefore are not considered to be attributable to the test material but rather to the general health status of the animals.

organ, lesion	% incidence (range)* males	females
lung, chronic inflamation**	62-80	37-65
thymus, atrophy**	95-100	91-100
kidney, cytoplasmic vacuolization**	82-90	low
kidney, nephrosis	15-40	low
jejunum & ileum, amyloid	19-49	low
spleen, extramed. hematopoiesis	low	17-22
ovary, epithelial cyst	-	17-27

* There were 90 mice examined in the control groups and 60 mice examined in each test group.

** Also observed in mice sacrificed at 12 months (interim sacrifice - 10 mice/sex/group).

002575

The predominant non-neoplastic lesions observed in this study which were considered attributable to the test material occurred in the liver. For mice which died during the study, were sacrificed in moribund condition or were sacrificed at termination of the study, the following liver lesions were considered to be related to the test material.

Incidence and Percent of Non-neoplastic Liver Lesions in Male Mice

Lesion	Control	40 ppm	120 ppm	360 ppm	1080 ppm
Inflammation, focal granulomatous*	18/90 20%	12/60 20%	15/60 25%	13/60 22%	48/60 80%
Fatty degeneration**	1/90 1%	2/60 3%	0/60 0%	5/60 8%	52/60 87%
Swelling**	0/90 0%	0/60 0%	0/60 0%	1/60 2%	56/60 93%
Hemosiderin deposition	0/90 0%	1/60 3%	1/60 2%	4/60 7%	38/60 63%

* Also increased in 1080 ppm male mice at 12-month interim sacrifice.

** Also increased in 360 and 1080 ppm male mice at 12-month interim sacrifice.

Incidence and Percent of Non-neoplastic Liver Lesions in Female Mice

Lesion	Control	40 ppm	120 ppm	360 ppm	1080 ppm
Inflammation, focal granulomatous*	7/90 8%	16/60 27%	5/60 8%	22/60 37%	6/60 10%
Fatty degeneration	6/90 7%	0/60 0%	1/60 2%	1/60 2%	15/60 25%
Swelling	None Reported				
Hemosiderin deposition	3/90 3%	2/60 3%	5/60 8%	1/60 2%	2/60 3%

* Also observed in female mice at 12-month interim sacrifice, but not in a dose-related manner.

002575

In male mice at 1080 ppm, there is clearly a statisically significant increase in liver lesions. In male mice at 360 ppm, there is minimal evidence of increased incidence of liver lesions. In female mice at 1080 ppm, there appears to be a possibly statistically significant increased incidence of fatty degeneration in the liver. The increaseed incidence of focal granulomatous inflammation in female mice at 40 and 360 ppm is not dose related. The NOEL for liver lesions in this study is set at 120 ppm.

Another non-neoplastic lesion possibly related to the test material in male mice is hypertrophy of the islets of Langerhans in the pancreas. The evidence, however, is only suggestive at best.

Incidence and Percent of Hypertrophy of Islets of Langerhans

Group	Control	40 ppm	120 ppm	360 ppm	1080 ppm
Males					
12-mo. sac.	1/10 10%	2/10 20%	5/10 50%	0/10 0%	3/10 30%
all others	19/90 21%	27/60 45%	33/60 55%	25/60 42%	22/60 37%
Females					
12-mo. sac.	2/10 20%	2/10 20%	1/10 10%	1/10 10%	2/10 20%
all others	1/89 1%	1/60 2%	0/60 0%	3/60 5%	1/60 2%

Other apparently sporadic non-neoplastic changes were observed but were of low frequency, not dose-related and not considered to be related to treatement.

Histopathological Examination, Neoplastic Lesions:

The overall incidence of animals with one or more tumors of any kind is presented below. It is apparent that the test material did not increase the overall incidence of tumors in the treated groups.

002575

Number of Animals with One or More Tumors of any Kind

Dosage Group	No. Exam.	Males		Females	
		No.	%	No.	%
Control	90	65	72.2	63	70.0
40 ppm	60	42	70.0	44	73.3
120 ppm	60	48	80.0	37	61.7
360 ppm	60	39	65.0	44	73.3
1080 ppm	60	30	50.0	39	65.0

The frequent occurrence of malignant lymphoma and of malignant neurilemoma in many animals in all groups dominated the neoplastic findings in this study. In both males and females, the primary lesions were generally found in numerous tissue and organs and, in addition, frequently metastasized to other tissues and organs. A few reticulum cell sarcomas were also diagnosed. Primary malignant lymphomas were particularly prominant in the lymph nodes of males and females. Primary malignant neurilemomas were particularly prominent in the uteri of females. The frequency of occurrence of these tumor types is presented below. Since nearly all animals in all groups were examined histopathologically (with only a very few exceptions), this data is presented for all the animals in the study. Note, however, that the table entries are numbers of tumors (not numbers of animals with tumors).

Malignant Lymphoma/Reticulum Cell Sarcoma - Number of Tumors

Dosage Group	No. Exam.	Males				Females			
		Primary Tumors*	Metastatic Tumors	Total Tumors		Primary Tumors*	Metastatic Tumors	Total Tumors	
				No.	Mean/Animal			No.	Mean/Animal
Control	60	15	27	42	.47	23	86	109	1.21
40 ppm	60	13	25	38	.63	20	52	71	1.20
120 ppm	60	19	34	53	.88	10	25	35	.58
360 ppm	60	16	26	42	.70	21	83	104	1.73
1080 ppm	60	6	26	32	.53	19	83	102	1.70

* Number of primary tumors = number of animals with tumor type.

In spite of the observed slight increases for malignant lymphoma/reticulum cell sarcoma in males at the lowest dosage levels, and in females at the 2 highest dosage levels, there does not appear to be any consistent pattern or dose-response relationship that would suggest these responses are due to the test material. This tumor type is often observed in historical control mice at similar frequencies.

002575

Malignant Neurilemoma - Number of Tumors

Dosage Group	No. Exam.	Males				Females			
		Primary Tumors*	Metastatic Tumors	Total Tumors		Primary Tumors*	Metastatic Tumors	Total Tumors	
				No.	Mean/Animal			No.	Mean/Animal
Control	90	6	9	15	.17	14	22	36	.40
40 ppm	60	5	10	15	.25	14	43	57	.95
120 ppm	60	2	0	2	.03	16	38	54	.90
360 ppm	60	4	6	10	.17	10	35	45	.75
1080 ppm	60	6	22	27	.45	10	21	31	.52

* Number of primary tumors = number of animals with tumor type.

In spite of the observed slight incrases for malignant neurilemomas in males at the highest dosage level and in females at the lowest 3 dosage levels, there does not appear to be any consistent pattern or dose-response relationship that would suggest these responses are due to the test material. This tumor type is often observed in historical control mice at similar frequencies. Note: According to L. Karza, TB pathologist, other pathologists frequently classify this tumor type differently as types of sarcomas, Schwanomas, fibromas, etc.

002575

Other frequently observed tumors were benign and malignant hepatomas in the liver and adenomas and adenocarcinomas in the lungs. The incidences for these types of tumors are presented below. It is apparent that the test material did not increase the incidences for these tumor types in the treated animals.

Liver Hepatomas - Number of Animals

Dosage Group	No. Exam.	Males				Females			
		Hepatoma, benign		Hepatoma, malignant		Hepatoma, benign		Hepatoma, malignant	
		No.	%	No.	%	No.	%	No.	%
Control	90	11	12.2	8	8.9	3	3.3	0	0.0
40 ppm	60	9	15.0	3	5.0	0	0.0	0	0.0
120 ppm	60	6	10.0	5	8.3	1	1.7	1	1.7
360 ppm	60	5	8.3	3	5.0	5	8.3	0	0.0
1080 ppm	60	6	10.3	10	16.7	3	5.0	2	3.3

002575

002575

Lung Adenomas and Adenocarcinomas - Number of Animals

Dosage Group	No. Exam.	Males				Females			
		Adenomas		Adenocarcinomas		Adenomas		Adenocarcinomas	
		No.	%	No.	%	No.	%	No.	%
Control	90	31	34.4	3	3.3	7*	7.8	0	0.0
40 ppm	60	6	10.0	6	10.0	5	8.3	2	3.3
120 ppm	60	17	28.3	4	6.7	3	5.0	1	1.7
360 ppm	60	8	13.3	4	6.7	1	1.7	2	3.3
1080 ppm	60	5	8.3	0	0.0	3	5.0	2**	3.3

* Additional adenoma observed in 12-month sacrifice animal.
** 1 Adenocarcinoma described as being a metastatic tumor.

Tumor types other than those described above did sporadically occur but were distributed more or less evenly across all groups (including controls), were of low frequency and did not appear to be related to ingestion of the test material. These tumor types included hemangiomas/hemangioscarcomas (particularly in livers of males), cystadenomas in ovaries of females, adenomas in pituitaries of females, and others.

The Metabolism of NP-55 in Rats. Supplement to Report RD 8025. Tab C-22, Accession No. 099537, Fine Chemicals Research Laboratory, Dated July, 1980. Also: Fine Chemicals Research Laboratory, Report RD 8148, Dated August 17, 1981, 25 Pages. Accession No. 249451.

TOX Chem No. 72A

Protocol:

Four groups of male and female rats (Fischer strain, Charles River Labs., Japan) aged 9 weeks (Groups A, B and D) or 8 weeks (Group C) were treated as indicated in Table I, to determine the metabolic fate of radioactively labeled technical NP-55. The radioactive sample of NP-55 [t4-^{14}C-NP-55] was synthesized (Japan Atomic Energy Research Institute) with a specific activity of 10.3 mCi/mM and a radiochemical purity of 98%. Unlabeled NP-55, 99.8% pure, lot No. TK 9112 was also used in this study.

Five males and 5 females from each group selected as representative of the group by uniformity of plasma levels of NP-55 were then individually housed and tested until 95% of the administered radioactive dose was eliminated (48 hours). Animals were then sacrificed. During the 48 hour test period, blood, urine and feces were collected for analysis of metabolite and measurement of radioactivity. Blood samples were collected at 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, as well as during sampling procedures for blood. At sacrifice, rats were exsanguinated from the carotid artery and the plasma was separated from the blood by centrifugation. Residual bladder urine was pooled with the previously collected urine samples. Tissues and structures were removed and weighed and analyzed for metabolites and radioactivity. Included were fat, testes plus epididymus of males and 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.

Mass spectrometry was used to identify main metabolites. Minor metabolites were identified and confirmed by mass spectrometry following purification by thin layer chromatography and column chromatography. Final extracts of the minor metabolites were analyzed and quantified by high performance liquid chromatography (HPLC).

TABLE I

($4-^{14}C$) NP-55 Dosing Protocol
Rat Metabolism Study

	Rat Groups			
Study	A	B	C	D
Final number of animals tested	5 males 5 females	5 males 5 females	5 males 5 females	5 males 5 females
Dosages with unlabeled NP-55 prior to challenge with labeled NP-55	- -	- -	14 oral daily doses 10/ mg/kg 100 ul each	- -
Challenge- Total conc. NP-55	10 mg/kg i.v.	10 mg/kg [illegible]	10 mg/kg [illegible]	325 mg/kg [illegible]
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

Results:

During the 48 hours test period, all groups showed an average 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 the liver with values of 0.6 ppm in Groups A, B, C and 13 ppm in Group D (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 1/3 in D (high dose group).

The group of primary interest in the elucidation of the metabolites of NP-55 was Group A, because of the amount of administered test chemical and the mode of administration which provided the best model for exposure to the animal's metabolic functions.

During the course of the study, mass spectrometry revealed the 3 main metabolites, which were: M-SO, M1SO and M2SO. The four minor metabolites were identified as M-SO_2, M1SO_2, M2SO_2 and 5-OH-M-SO_2. The latter is identical to the metabolite MU-1 found in NP-55 - treated soybeans.

In Group A animals, 0.8% of the administered radioactivity was identified in the urine as hydroxymetabolite(s), represented by 6-OH-MsSO_2. Rather than 0.7% as indicated by the authors, 0.8 is being used, in accordance with suggestions made by Maxi Jo Nelson (RCB review, in preparation, 3/2/83) in which the validity of this result (0.8%) was verified by RCB.

00257

Acute Oral Toxicities of Main Metabolites of NP-55 in Rats. Nisso Institute for Life Science. Dated October 30, 1980. Accession No. 249241. Part I, 48 Pages, Part II, 60 Pages and Part III 8, Pages.

TOX Chem No. 72A

Protocol: Parts I, II, III

Acute oral toxicity tests were done in Sprague-Dawley - SLC rats, 10/sex/group with purified metabolites of NP-55 (purity between 98-99%). Oral dosing was by intubation in the 6 week old animals. Dose concentration of each metabolite was 5000 mg/kg in 1% carboxymethylcellulose with Tween 80.

Animals were observed at one hour and observations continued for 14 days, twice daily. Observatons included physical signs and symptoms of pharmacological and toxic effects including deaths, central nervous system effects, pupillary reactions. At 14 days, remaining animals were sacrificed and necropsied, as were all dead animals prior to sacrifice time. Behavioral effect as well as body weights were noted.

Results:

Part I

Metabolites Tested	LD50 Males	LD50 Females
M-SO	> 5000 mg/kg	> 5000 mg/kg
M-SO_2	> 5000 mg/kg	> 5000 mg/kg
M1-S	> 5000 mg/kg	> 5000 mg/kg
M1-SO_2	> 5000 mg/kg	> 5000 mg/kg
M2-S	> 5000 mg/kg	> 5000 mg/kg
M2-SO_2	> 5000 mg/kg	> 5000 mg/kg
M1-SO +	≤ 5000 mg/kg	≤ 5000 mg/kg
M2-SO +	≤ 5000 mg/kg	≤ 5000 mg/kg

Part II

M1-SO +	3080 (2953-3175) mg/kg	-
M2-SO +	5573 (4942-7435) mg/kg	-

Part III

MU-1 (5-OH-MSO_2)	> 5000 mg/kg	-

#m22

Sethoxydim scientific review

Page _____ is not included in this copy.

Pages 58 through 63 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.