ENVIRONMENTAL IMPACT ANALYSIS REPORT

A. Date : October 20, 1982

B. Name of applicant: Pitman-Moore, Inc.

C. Address : Washington Crossing, New Jersey 08560

D. Environmental Information

1. Describe the proposed action

Pitman-Moore, Inc. has submitted a New Animal Drug Application for STRESNIL* (azaperone) Injection to the Food and Drug Administration pursuant to section 512(b) of the Federal Food, Drug and Cosmetic Act. The subject New Animal Drug Application (NADA 115-732) provides for the use of STRESNIL Injection in swine. The drug is indicated for the control of aggressiveness when mixing or regrouping pigs weighing up to 80 lbs. of body weight. The recommended dose of azaperone for the pig is 2.2 mg/kg (1 mg/lb.) of body weight given by deep intramuscular injection either behind the ear and perpendicularly to the skin or in the back of the ham. One ml of STRESNIL will treat 40 lbs. of body weight. Each ml of STRESNIL Injection, available in 20 ml vials, contains: azaperone, 40 mg; tartaric acid, 14 mg; sodium bisulfite, 2 mg; methylparaben, 0.5 mg; propylparaben, 0.05 mg; sodium hydroxide (to adjust pH).

a. The purpose of the action

STRESNIL Injection is recommended for use when pigs from different litters or pens are brought together, since they often fight in an attempt to establish a social order. Such fights can last a few hours, a few days or even a whole week. The intensity and duration of the fights depends on the inborn aggressiveness of the animal. The weakest animals are forced into the least favorable parts of the pen where they are the last to reach the feeder trough. Besides cuts, scratches and even more serious wounds, these fights may cause mortality and reduce growth. Following a single dose of STRESNIL, pigs may be mixed; fighting is eliminated or greatly reduced.

b. The environment to be affected

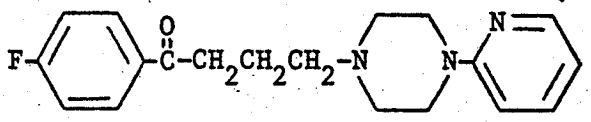
STRESNIL will primarily be used in pigs under confinement in hog complexes. In these areas, there is usually an intense production of growing pigs. The pigs are mixed, approximately 100 pigs, into pens having concrete or slat floors. Waste is washed off into waste pools or holding tanks where it may be further disseminated. The primary mode of introduction of the product into the environment is excretion by the target animal.

*Trademark

D. Environmental Information (Continued)

1. Describe the proposed action (Continued)

c. Chemical and physical properties of the product

Generic name	:	azaperone
Chemical name	:	4'-fluoro-4-[4-(2-pyridyl)-1-piperazinyl] butyrophenone
Structural formula	:	
Molecular formula	:	$C_{19}H_{22}FN_3O$
Molecular weight	:	327.40
Chemical Abstract No.	:	CAS 1649-18-9
Appearance	:	Almost white to slightly yellowish powder
Melting range	:	Between 92 and 95°C
Solubility	:	The solubility of azaperone in various solvents is given in Table 1.
Stability of the chemical substance	:	Azaperone is affected by UV light, but the molecule remains intact after acid and base hydrolysis (see summary in Exhibit 1).
Octanol/water partition coefficient	:	A determination of the octanol/water partition coefficient for azaperone is described in Exhibit 2.

d. Pharmacological properties

Azaperone is a neuroleptic of the butyrophenone series of which haloperidol (HALDOL*, McNeil Pharmaceutical, Spring House, Pennsylvania) is an exemplary representative and widely used in human medicine.

The pharmacological profile of azaperone is that of a typical neuroleptic drug. The properties have been extensively studied in mice, rats, dogs and on isolated tissues. The results of these tests are summarized below; reports of the studies are included in NADA 115-732 for STRESNIL* Injection, dated May 24, 1978, Volume 14, Exhibits 77-83, Pages 4462-4560.

*Trademark

D. Environmental Information (Continued)

1. Describe the proposed action (Continued)

d. Pharmacological properties (Continued)

- Azaperone is a typical neuroleptic drug; it induces catalepsy and palpebral ptosis in handled rats; it antagonizes the amphetamine- and apomorphine-induced agitation and stereotyped movements in rats, and it has pronounced antiemetic properties in dogs.

- In addition, azaperone has sedative properties. Like all known sedative neuroleptics, azaperone is more active against amphetamine-induced agitation than against amphetamine-induced stereotyped movements in rats. Azaperone potentiates the hypnotic effects of pentobarbital in mice. These effects were more pronounced with azaperone than with the other reference drugs.

- Azaperone has potent alpha adrenergic blocking properties in rats, as illustrated by its high anti-shock activity; and at very low dose levels, the drug also protects rats against lethal doses of epinephrine or norepinephrine.

- Azaperone has weak antihistaminic and anti-adrenergic activity in vitro; the drug is also devoid of anti-cholinergic and anti-serotonergic activity in vitro. Azaperone is devoid of a specific constipating effect.

e. Toxicological properties

In toxicity studies conducted in laboratory animals, azaperone was characterized as having a wide margin of safety. A summary of these studies is given in Exhibit 3.

f. Metabolism data

The metabolism of tritium-labeled azaperone in the pig was determined cooperatively by Pitman-Moore, Inc. and Janssen Pharmaceutica, Beerse, Belgium. In this study, tritium-labeled azaperone was injected intramuscularly to pigs at 4 mg/kg -- approximately twice the proposed use level of 2.2 mg/kg (1 mg/lb.). The drug was rapidly absorbed from the site of injection, metabolized and approximately 69% of the administered dose (average of six pigs) was excreted in the urine and feces. Of the total amount excreted, an average of 56% of the dose administered was excreted with the urine, while 13% was in the feces (Table 2); thus, the main excretion route for azaperone and its metabolites in the pig is in the urine.

D. Environmental Information (Continued)

1. Describe the proposed action (Continued)

f. Metabolism data (Continued)

Three metabolites and the parent compound azaperone accounted for approximately 80% of the total radioactivity in the urine. The metabolites were azaperol, 5-hydroxy azaperone and 5-hydroxy azaperol. Further analysis showed that the two hydroxy metabolites were labile, excreted as glucuronides and unstable *in vitro*.

The metabolic pathway of azaperone can be summarized as follows:

Azaperone → 5-hydroxy azaperone†
↓ ↓
Azaperol → 5-hydroxy azaperol†

†excreted as glucuronides and broken down *in vitro* to depyridinated products

Details of metabolism of azaperone in the pig are included in the study report located in the STRESNIL* Injection NADA 115-732, dated May 24, 1978, Volume 2, Exhibit 12, Page 206.

2. Describe the probable impact of the proposed action on the environment, including primary and secondary consequences

a. Describe probable adverse and beneficial environmental effects of use

An assessment of the environmental concentration in localities where STRESNIL Injection is intended to be used is given in Exhibit 4. Based on this assessment, it is concluded that insignificant levels of the drug are excreted into the environment.

Pursuant to 21 CFR 25.1(g)(1), Pitman-Moore, Inc. hereby requests that the approval by the Food and Drug Administration of the New Animal Drug Application for STRESNIL Injection warrants exemption from the requirements of an Environmental Impact Statement in accordance with the following 21 CFR 25.1 provisions:

21 CFR 25.1(f)(1)(ii)(e)(1): "Under prescription on a limited number of animals"

*Trademark

D. Environmental Information (Continued)

2. Describe the probable impact of the proposed action... (Continued)

a. Describe probable adverse and beneficial... (Continued)

21 CFR 25.1 (f)(1)(ii)(e)(2): "In the treatment of a disease or condition which requires individual dose administration"

21 CFR 25.1 (f)(1)(ii)(e)(3): "In animals which metabolize the drug so that no significant quantities of the drug are excreted into the environment"

b. Describe measures taken to avoid or mitigate potential adverse environmental effects

No adverse environmental effects are anticipated.

c. Analyze the environmental impact of the manufacturing process(es) of the article that is the subject of the requested action

No pollutants are emitted from the manufacturing process of STRESNIL* Injection. Please refer to Exhibit 5 for an assessment of the environmental impact of the manufacturing process.

3. Describe the probable adverse environmental effects that cannot be avoided

There are no adverse environmental effects expected with the use of STRESNIL Injection.

4. Evaluate alternatives to the proposed action

Since there are no potential hazards, this does not apply to the approval of a New Animal Drug Application for STRESNIL Injection.

5. Describe the relationship between local short-term use of the environment with respect to the proposed action and the maintenance and enhancement of long-term productivity

STRESNIL Injection should not present any problems with regard to any deleterious effects on local short-term use of the environment or the maintenance and enhancement of long-term productivity.

6. Describe any irreversible and irretrievable commitment of resources that would be involved if the proposed action should be implemented

There are no foreseeable instances involving irreversible negative impacts upon the environment.

*Trademark

D. Environmental Information (Continued)

7. Discuss the objections raised by other agencies, organizations or individuals that are known to the applicant

There are no known objections.

8. If the proposed action should be taken prior to 90 days from the circulation of a draft environmental impact statement or 30 days from the filing of a final environmental impact statement, explain why

Since there are no anticipated short-term or long-term effects on the environment with the use of STRESNIL* Injection, the New Animal Drug Application should preclude the need for an environmental impact statement.

9. Risk-benefit analysis

There is no anticipated environmental impact with the use of STRESNIL Injection; therefore; only the positive benefits derived from its use as a neuroleptic to control aggressiveness in pigs need be considered.

E. Certification

This certifies that the information furnished in this Environmental Impact Analysis Report is true, accurate, and complete.

October 20, 1982
Date

Orville W. Ostmann /RJS
Orville W. Ostmann, Ph.D.
Director, Regulatory Affairs

*Trademark

Table 1: Solubility of Azaperone In Various Solvents at Room Temperature

Solvents	g azaperone per 100 ml solution
water	0.001
octanol	0.05
0.01 N acetic acid	0.20
hexane	0.22
0.01 N hydrochloric acid	0.30
0.01 M tartaric acid	0.54
propylene glycol	1.27
2-propanol	1.58
0.1 N acetic acid	1.93
0.1 N hydrochloric acid	2.41
diethylether	3.18
polyethylene glycol 200	3.35
ethanol	3.41
polyethylene glycol 400	4.08
0.1 M tartaric acid	4.24
polyethylene glycol 600	4.61
1 N hydrochloric acid	5.90
methanol	6.24
acetone	11.66
ethyl acetate	12.83
1 N acetic acid	17.04
chloroform	>25
benzene	26.70
1 M tartaric acid	35.80

Table 2: Excretion of Total Radioactive Material, as a Percentage of the Administered Dose, After Intramuscular Administration of Azaperone-^{3}H at a Dose Level of 4 mg/kg†

	Time interval	% of administered dose						
		48 hours		24 hours		72 hours		
		No. I	No. II	No. III	No. IV	No. V	No. VI	→ Pig No.
URINE	0-24h	66.87	62.34	59.35	31.74	53.45	29.15	
	24-48h	7.51	3.67	—	—	7.60	2.67	
	48-62h	—	—	—	—	2.32	2.21	
	Bladder	0.07	2.99	0.83	4.74	0.01	0.00	
	Total	74.45	69.00	60.18	36.48	63.38	34.03	→ Average 56.25%
FAECES	0-24h	3.34	2.03	15.75	13.88	7.62	17.56	
	24-48h	4.22	1.85	—	—	3.42	4.98	
	48-72h	—	—	—	—	1.85	1.49	
	Total	7.56	3.88			12.89	24.03	→ Average 12.99%
Total in excreta		82.01	72.88	75.93	50.36	76.27	58.06	→ Average 69.25%

†Corresponds to Table 32 (page 345) of the report "Metabolism of Azaperone in Swine" located in STRESNIL* (azaperone) Injection NADA 115-732 dated May 24, 1978, Volume 2, Exhibit 12, page 206.

Stability of the Chemical Substance

Data were generated to define and identify the decomposition products of azaperone. Details of these studies were previously included in the STRESNIL* (azaperone) Injection Amendment to NADA 115-732 dated May 25, 1979, Volume 2, Exhibit 6, Page 158. In these studies, azaperone was subjected to ultraviolet light, acid hydrolysis and base hydrolysis in order to demonstrate the chemical degradation of the drug. As a result, azaperone was degraded by ultraviolet light but the molecule remained intact after acid and base hydrolysis. A decomposition product after ultraviolet light exposure was identified as p-fluorobenzoic acid. Other decomposition products were speculated but not conclusively identified. A summary of these studies is presented.

A. Exposure to Ultraviolet Light

In order to degradate azaperone, 20.00867 g of reference standard azaperone was dissolved in 500.0 ml of methanol and reacted in an ultraviolet photochemical reactor for 41 hours. After 41 hours, the solution was transferred to a 500 ml volumetric flask and diluted to volume with methanol. The solution had the characteristic odor of benzoic acid. The sample was diluted and assayed for azaperone using a gas liquid chromatographic method[1] (Method No. D77111 reported in NADA 115-732 Amendment dated May 25, 1979, Volume 2, Exhibit 7, Page 169). The color of the solution before the reaction was pale yellow and after the reaction, was dark amber. 81.4% azaperone was recovered.

To determine what the remaining 18.6% was, the following was done:

1. A 50.0 ml portion was titrated for p-fluorobenzoic acid using 0.1 N sodium hydroxide and phenolphthalein T.S. as the indicator. The 50.0 ml contained 59.88 mg of p-fluorobenzoic acid, which is equivalent to the breakdown of 1.399 g of azaperone, or 7.0% of the original 20.00867 g (1 g of azaperone will yield 428 mg of p-fluorobenzoic acid.)

2. The remaining 11.6% of decomposition could not be conclusively identified. Since p-fluorobenzoic acid was identified, the pathway of decomposition was either A or C, as shown in Figure 1.

[1]Method was used without internal standard.
*Trademark

Stability of the Chemical Substance (Continued)

B. Acid Hydrolysis

400.83 mg of reference standard azaperone was hydrolyzed in 75 ml of 0.1 N hydrochloric acid for one hour. The solution was transferred to a 100 ml volumetric flask, and diluted to volume with 0.1 N hydrochloric acid. A 20.0 ml aliquot was assayed according to Method No. D77111.

98.2% azaperone was recovered, equivalent to 393.6 mg.

C. Base Hydrolysis

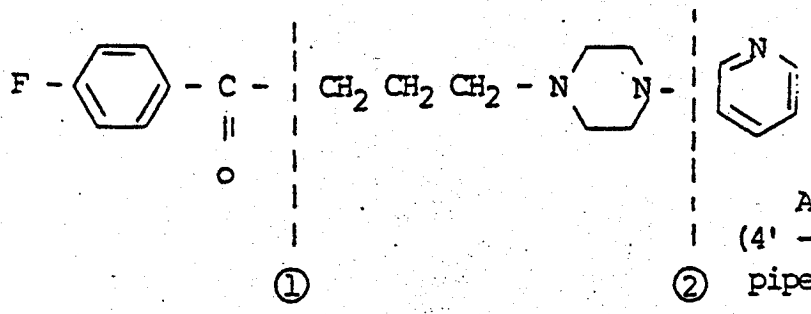
399.94 mg of reference standard azaperone were hydrolyzed in 75 ml of 0.1 N sodium hydroxide for one hour. The sample had an oil layer in it, and upon cooling, a white flocculent precipitate formed. The sample was transferred to a 200 ml volumetric flask, using alcohol to aid in the transfer. The remaining solids were dissolved in alcohol in a sonic bath, and added to the first portion. The sample was diluted to volume with alcohol, and a 40.0 ml aliquot was assayed according to Method No. D77111.

102.3% azaperone was recovered, equivalent to 409.1 mg.

FIGURE 1

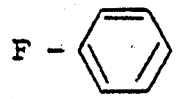
Decomposition Products of Azaperone

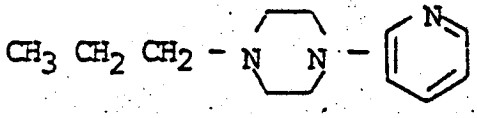
1. The following schematic indicates the points at which degradation is likely to occur:



Azaperone
(4' - fluoro - 4 - [4-2-pyridyl)- 1 - piperazinyl] butyrophenone)

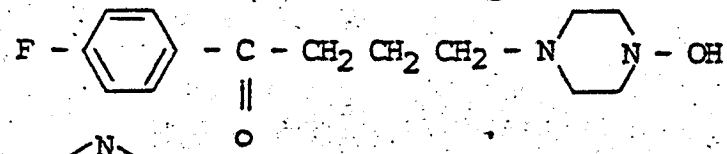
A) if degradation occurs at point ① , it would yield

1)  p-fluorobenzoic acid

2) 

1 - n - propylpiperazine pyridine

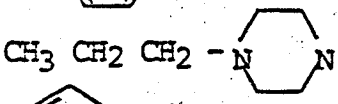
B) if degradation occurs at point ② , it would yield

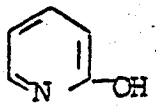
1) 

2)  pyridine

C) if degradation occurs at points ① and ② together, it would yield

1)  p - fluorobenzoic acid

2)  1 - n - propylpiperazine

3)  2 - hydroxypyridine

n-Octanol/Water Partition Coefficient

A determination of the n-octanol/water partition coefficient for azaperone was attempted, but the results were inconclusive; however, the solubility of azaperone in each phase alone was determined. A summary of the test results is presented.

Summary

The solubility of azaperone was found to be approximately 0.5 mg/ml in octanol and 0.01 mg/ml in water. Since partition coefficients should be determined at low concentrations well below the solubility limit in either phase and since a second determination should be performed at one-tenth the concentration of the first, a preliminary test was conducted to first determine if azaperone in octanol, at a concentration close to the solubility limit, would distribute into the aqueous phase. In this attempt, 12.45 mg of azaperone (99.1% purity) was dissolved in 100 ml of octanol (highest purity available) and further diluted with octanol to provide a standard octanol concentration of 1.245 mg azaperone per 100 ml. Ten milliliters of this solution (total 0.1245 mg azaperone) were added to 25, 50 and 75 ml of distilled water and mixed at half speed on a mechanical shaker for 60 minutes. The mixtures were centrifuged at 3,000 rpm for 15 minutes. After centrifugation, both the octanol and water layers were analyzed using gas chromatography.

The result of the preliminary test was that azaperone could not be detected in any of the aqueous layers tested even though increasing volumes of water (i.e., 25, 50 and 75 ml) were used for the extractions. A slight turbidity was noticed in each aqueous layer. Since azaperone at the relatively high concentration used could not be quantitated in the aqueous phase, it was uncertain whether partitioning had occurred or whether other interfering factors were involved, e.g., azaperone as a hydrophobic molecule was adhering to the glassware.

It was concluded that additional specific analytical recovery techniques would be needed to determine whether acceptable data have been generated and that this could not be accomplished at Pitman-Moore, Inc. because of the lack of additional instrumentation that would be required. Such modifications to the methodology might include:

- ultracentrifuge the octanol/water mixture after shaking
- perform a turbidimetric analysis to ensure absence of an emulsion
- increase the volume of the aqueous phase to 100-200 ml
- use a more sensitive analytical procedure (mass spectrometry)
- extract azaperone from the aqueous phase using chloroform

Based on this experimentation, the solubilities of azaperone in octanol and in water were found, but the results of the determination of the octanol/water partition coefficient were inconclusive.

Summary of Toxicity Studies of Azaperone

The toxicological profile of azaperone was determined in laboratory animals in studies consisting of acute, subacute, chronic and reproductive trials. Highlights of these studies are presented. Full reports are contained in the STRESNIL* (azaperone) Injection New Animal Drug Application (NADA 115-732), dated May 24, 1978, Volumes 6 through 14, consecutively.

A. Acute Toxicity

1. LD_{50} - Mice, Rats, Guinea Pigs

The acute toxicity of azaperone was evaluated by Janssen Pharmaceutica, Beerse, Belgium. In these tests, azaperone was administered to male mice and male rats by three different routes of administration, i.e., intravenously (IV), subcutaneously (SC) and orally, and in male guinea pigs orally. Ten animals were used per dose level. The individually caged animals were observed for up to three days after IV and SC injection and for up to seven days after oral administration by gavage. In mice, the LD_{50} values (mg/kg body weight) were 42 (IV), 179 (SC), 385 (oral); in rats, 28.1 (IV), 450 (SC), 245 (oral); and in guinea pigs, 202 (oral). Gross behavioral phenomena observed in mice and rats were palpebral ptosis and sedation at all dose levels tested. At lethal doses, tremors and occasionally clonic seizures occurred in all three species.

In another study, the acute intravenous toxicity of azaperone and two metabolites, azaperol (R 2138) and 5-hydroxy azaperol (R 34189), were evaluated in male adult albino mice. The drugs were given as aqeous solutions on an mg/kg weight basis. The LD_{50} values (mg/kg body weight) were calculated to be 38.1 (24.7-58.8) for azaperone; 56.3 (41.6-76.3) for azaperol; and 150 (111-202) for 5-hydroxy azaperol.

2. Single Dose, Oral and Subcutaneous - Dogs

The acute toxicity of azaperone in dogs was evaluated by Janssen Pharmaceutica, Beerse, Belgium. Azaperone was injected subcutaneously at 2.5, 5, 10, 20 and 40 mg/kg body weight and given orally by gavage up to 20 mg/kg body weight. At least 10 animals per dose level were used and azaperone was given in an amount of 0.5 ml/kg body weight. No mortality occurred. Gross behavioral phenomena were ptosis, sedation and occasionally vomiting after oral administration.

*Trademark

A. Acute Toxicity (Continued)

3. Single Dose, Intravenous and Intramuscular - Dogs

The acute toxicity of azaperone in purebred beagle dogs was evaluated by Food and Drug Research Laboratories, Inc., New York, under the supervision of M. Shelanski, M.D., C.M. Groups of two adult male dogs per group received a single injection of azaperone at 10 and 20 mg/kg intravenously or 20 and 40 mg/kg intramuscularly. Heart rate, respiration and gross behavioral effects were monitored for up to eight days post-injection. No mortality occurred. At lower dosage levels, both intravenously and intramuscularly, muscular tremors and tranquilization were seen. All dogs exhibited extreme muscle incoordination, decreased reaction to painful stimuli and increased sweating. The immediate response of the animals to the higher dosages were tachycardia, increased respiratory rate and opisthotonus. Within 10 minutes, tonic and clonic convulsions ensued. This was followed by a Parkinsonian-like tremor and subsequent tranquilization. These findings subsided in all dogs irrespective of dosage or route of injection 2 1/2 to 3 hours after drug administration.

4. Single Dose, Intravenous and Intramuscular - Cats

The acute toxicity of azaperone in cats was evaluated by Food and Drug Research Laboratories, Inc., New York, under the supervision of M. Shelanski, M.D., C.M. Groups of two female cats per group received single injections of azaperone at doses of 30 and 60 mg/kg body weight intravenously or 60 mg/kg body weight intramuscularly. The animals were observed daily for appearance, behavior and survival for seven days. No mortality occurred. A variety of pharmacologic responses including temporary bradycardia, increased respiratory rate, tremors and/or convulsions, impaired locomotion and ability to stand were noted at both 30 and 60 mg/kg intravenous dose levels. The effects were temporary, approximately 5-10 minutes in duration, at the lower dose. The intramuscular dosage produced considerably less effects at the equivalent higher dose with no convulsions being observed.

B. Subacute Toxicity

1. Fourteen-day Repeated Feeding - Rats

A 14-day repeated feeding study in Wistar rats was conducted by Janssen Pharmaceutica, Beerse, Belgium, under the supervision of R. Marsboom, D.V.M. Groups of five male and five female rats per group received azaperone daily in the diet for 14 days at concentrations of 0, 0.8, 3.10, 12.5 and 50 mg/kg food. This provided doses of azaperone from 0.069

B. Subacute Toxicity (Continued)

to 6.97 mg/kg/day (males) and from 0.065 to 6.94 mg/kg/day (females). Initial and final body weight of each individual rat and food consumption over the total experimental period were recorded. Behavioral observations of the rats included scoring for palpebral ptosis and catelepsy and by conducting an "open field" test (Niemegeers, et al, Arzneim. Forsch. 24:1798-1806, 1974). In the open field test, rats were placed in an arena in which the frequency of diameter crossings (ambulation), rearing movements and defecation were recorded over a three minute period. No statistically significant difference in body weight, food intake or behavior could be detected between the treated and control male or female rats.

2. Fifteen-week Repeated Feeding - Rats

A 15-week oral safety evaluation of azaperone in 80 Wistar rats was conducted by Janssen Pharmaceutica, Beerse, Belgium, under the supervision of R. Marsboom, D.V.M. Three groups of rats with 10 males and 10 females per group received azaperone daily in the diet for 15 weeks at concentrations of 10, 40 and 160 mg/100 g food. This provided approximately a daily oral dose of 10, 40 and 160 mg/kg body weight. A fourth group of rats served as controls and received the basic laboratory diet only for the same period of time. All animals survived the experiment except for one 160 mg/100 g food dosed rat which was sacrificed in a moribund condition on the 66th day of the experiment. Autopsy revealed obstruction of the intestines and abscessus of the colon. Health, behavior and appearance was normal in controls and dosed groups. Food consumption decreased in males at 40 and 160 mg/100 g food and in females at 160 mg/100 g food. Body weight decreased in males at 160 mg/100 g food. Terminal hematological studies were normal. Terminal serum analyses gave normal results except for a slight decrease of cholesterol in the 160 mg/100 g food dosed males and females. At the 160 mg/100 g food dose level, urobilinogen increased in males. Gross pathological changes were not observed. The histological examination of organs and tissues evidenced some changes in the female genital organs and hypophysis of the 160 mg/100 g food dosed females. These features are commonly encountered after the administration of neuroleptic drugs.

3. Thirteen-week Repeated Subcutaneous Injection - Rats

The safety evaluation of azaperone, injected subcutaneously in Wistar rats for 13 weeks, was conducted by Janssen Pharmaceutica, Beerse, Belgium, under the supervision of R. Marsboom, D.V.M. Groups of 10 male and 10 female rats per group were injected subcutaneously with azaperone, six days per week, at doses of 2.5, 10 and 40 mg/kg body weight. A fourth similar group served as controls and was injected subcutaneously with a sodium chloride solution (0.9%). One high dosed female (40 mg/kg) died accidentally on the fifth day of the

B. Subacute Toxicity (Continued)

experiment. All other males and females survived. Rats receiving azaperone were sedated for a period of about two hours after drug administration. This effect was due to the central nervous system depressant properties of the compound. Dosed rats were indistinguishable from the controls regarding health. Dosages at 2.5 and 10 mg/kg had no adverse effect on average body weight. At 40 mg/kg, there was a significant ($P<0.001$) decrease in average body weight of the males. Female body weight at this dose level was unaffected. Hematocrit, hemoglobin, red and white blood cell counts in all dosage groups closely approximated the historical normal values for rats in this laboratory. Differential count indicated a slight increase of the granulocytic series in 10 and 40 mg/kg dosed males and in 40 mg/kg dosed females. Terminal biochemical determinations and terminal qualitative urinalyses gave normal results. Necropsy of animals belonging to the 2.5 and 10 mg/kg dosage groups failed to reveal any dose or drug-related changes. Some animals dosed at 40 mg/kg showed a discolor of the liver. Weight of the thymus in these high dosed animals significantly decreased. Histological studies failed to reveal any pathological variations.

4. Three-month Oral Toxicity - Dogs

A three-month oral toxicity study of azaperone in 24 purebred beagle dogs was conducted by Janssen Pharmaceutica, Beerse, Belgium, under the supervision of R. Marsboom, D.V.M. Groups of three male and three female beagles per group were administered azaperone orally in gelatin capsules once daily, six days a week for three months at doses of 1.25, 5 and 20 mg/kg body weight. A similar group served as controls and received capsules containing 250 mg lactose. Behavior and appearance were normal at 1.25 and 5 mg/kg, but at 20 mg/kg, sedation was observed for some hours after dosing. Body weight increase was normal for all groups and no deaths occurred. Blood pressure was not affected and heart rate and electrocardiogram were satisfactory. Within groups, variations of hematology, blood chemistry and urinalysis existed; however, no clinical importance was attached to these findings. Gross pathology failed to reveal any dose or drug-related changes. The relative weight of the liver slightly increased at the 5 and 20 mg/kg doses. Histological control of the organs and tissues also failed to reveal any dose or drug-related changes.

C. Chronic Toxicity

1. Six, Twelve and Eighteen-Month Chronic Feeding - Rats

A six, twelve and eighteen-month chronic feeding study of azaperone in rats was conducted by Janssen Pharmaceutica, Beerse, Belgium, under the supervision of R. Marsboom, D.V.M. Groups of 10 male and 10 female Wistar rats per group received diets containing 0, 10, 40 or 160 mg of azaperone per 100 grams of food, which provided approximately 0, 10, 40 or 160 mg/kg body weight. Fresh diets were prepared weekly. A total of 18 animals out of 240 died during the experiment and nine other rats had to be sacrificed in a moribund state before the end of the study. Gross pathology observations in these animals failed to reveal any drug or dose-related effects. With exception of a dose-related sedative effect in all dosage groups, no other drug-related effects were seen on health, behavior and physical appearance. No adverse effect on food consumption and body weight gain was noted in rats in the 10 and 40 mg/100 g food dosage groups; whereas, in the 160 mg/100 g food groups, food consumption and body weight gain significantly decreased in both males and females. Azaperone did not produce any adverse effects as determined by hematology, serum analysis, urinalysis,, gross pathology and organ weights. As could be expected after the administration of a neuroleptic at the high dose of 160 mg/100 g food, a tendency to a prolonged anoestral aspect of the female genital tract was seen and within the pituitary, more extended chromophobe tissue in both sexes and more conspicuous erythosinophilic tissue in the females.

2. Twenty-four Month Chronic Study - Dogs

A twenty-four month chronic toxicity study of azaperone in purebred beagle dogs was conducted by Janssen Pharmaceutica, Beerse, Belgium, under the supervision of R. Marsboom, D.V.M. Groups of three males and three females per group received azaperone orally in gelatin capsules at doses of 1.25, 5 and 20 mg/kg body weight, once daily, six days a week, for 24 months. A control group (three males and three females) was similarly treated with capsules containing 250 mg lactose. All animals survived the 24-month experiment except for one high dose male (20 mg/kg) which died during the 64th week of the study from uremia, which was not drug-related. Azaperone did not produce any drug or dose-related effects on heart rate, electrocardiogram, blood pressure, hematological or the biochemical parameters measured.

D. Reproductive Studies

1. Teratology Study - Mice

A test was conducted in mice by McNeil Laboratories, Inc., Fort Washington, Pennsylvania, under the supervision of M. Danilovitz, in which pregnant females were dosed with azaperone daily by gavage from day six through day 15 of pregnancy. The dose levels were 0 (saline), 0 (vehicle), 2.5, 10 and 40 mg/kg/day. Twenty-nine pregnant females were used at each dose level. Female mice were sacrificed on day 18 of presumed pregnancy. Number of fetuses, uterine placement, live and dead fetuses, early and late resorptions and number of corpora lutea were recorded. All fetuses were examined for external anomalies. One third of the fetuses were examined for visceral anomalies. The other two thirds were cleared and bone stained with alizarin. Azaperone was not teratogenic in mice in this study.

2. Teratology Study - Hamsters

A test was conducted in hamsters by McNeil Laboratories, Inc., Fort Washington, Pennsylvania, under the supervision of M. Danilovitz, in which pregnant hamsters were dosed with azaperone daily by gavage on days six through 10 of pregnancy. The dose levels were 0 (saline), 0 (vehicle), 2.5, 10 and 40 mg/kg body weight. Twenty-six pregnant females were used at each dose level. Female hamsters were sacrificed on day 15 of presumed pregnancy. Number of fetuses, uterine placement, live and dead fetuses, early and late resorptions and number of corpora lutea were recorded. All fetuses were examined for external anomalies. One third of the fetuses were examined for visceral anomalies. The other two thirds were cleared and bone stained with alizarin. Azaperone was not teratogenic in hamsters in this study.

3. Emrbyotoxicity and Teratogenicity - Rats

A test was conducted in rats by Janssen Pharmaceutica, Beerse, Belgium, under the supervision of R. Marsboom, D.V.M., in which pregnant female rats were dosed with azaperone daily by gavage on days six through 15 of pregnancy. The dose levels were 0 (saline), 2.5, 10 and 40 mg/kg body weight. Twenty-five pregnant females were

D. Reproductive Studies (Continued)

used at each dose level. The females were sacrificed the morning of the 22nd day after insemination. The fetuses were delivered by Caesarean section. The dams were examined for number and distribution of dead and live embryos in each uterine horn, presence of empty implantation sites, and embryos undergoing resorption. All fetuses were carefully examined for any external anomalies. Radiographic examinations were carried out for all fetuses of the control group and the 40 mg/kg group, but not for the lower dosed groups. Rat fetuses of each litter were randomized for dissection (one third), clearing and bone staining with alizarin (two thirds). Azaperone was not embryotoxic or teratogenic in rats in this study.

4. Embryotoxicity and Teratogenicity - Rats

A test was conducted in rats by Janssen Pharmaceutica, Beerse, Belgium, under the supervision of R. Marsboom, D.V.M., in which pregnant female rats were injected subcutaneously with azaperone from day six through day 15 of pregnancy. The dose levels were 0 (saline), 2.5, 10 and 40 mg/kg body weight. Twenty pregnant females were used at each dose level. The females were sacrificed the morning of the 22nd day after insemination. The fetuses were delivered by Caesarean section. The dams were examined for number and distribution of dead and live embryos in each uterine horn, presence of empty implantation sites, and embryos undergoing resorption. All fetuses were carefully examined for any external anomalies. Radiographic examinations were carried out for all fetuses of the control group and the 40 mg/kg group, but not for the lower dosed groups. Rat fetuses of each litter were randomized for dissection (one third), clearing and bone staining with alizarin (two thirds). Azaperone was not teratogenic in this study.

5. Embryotoxicity and Teratogenicity - Rats

A test was conducted by Janssen Pharmaceutica, Beerse, Belgium, under the supervision of R. Marsboom, D.V.M., in which pregnant female rats were injected subcutaneously with azaperone daily during the first 21 days of pregnancy. Dose levels were 2.5, 10 and 40 mg/kg body weight. Twenty pregnant females were used at each dose level. A total of

D. Reproductive Studies (Continued)

350 control rats were given subcutaneous injections of isotonic sodium chloride solution (0.9%) in 1 ml volumes. The females were sacrificed the morning of the 22nd day after insemination. The fetuses were delivered by Caesarean section. After opening the uterus, the distribution of placental sites, the number of dead and live fetuses, the correlation with the number of implantation sites, early and late resorptions were noted. All fetuses were carefully examined for any external anomalies. Radiographic examinations were also carried out for all fetuses. Rat fetuses of each litter were randomized for dissection and for clearing and bone staining with alizarin. Azaperone was not teratogenic in this study.

6. Embryotoxicity and Teratogenicity - Rabbits

A test was conducted by Janssen Pharmaceutica, Beerse, Belgium, under the supervision of R. Marsboom, D.V.M., in which pregnant female rabbits were given azaperone daily on day six through 18 of pregnancy. The dose levels were 0 (saline), 2.5, 10 and 40 mg/kg body weight. Fifteen pregnant females were used at each dose level. The females were sacrificed the morning of the 22nd day after insemination. The does were then necropsied and checked for gross abnormalities and for pregnancy. The fetuses were individually weighed and immediately examined for any external anomalies. They were put on a radiator-plate at a temperature of 30°C for 24 hours and survival rate was calculated. Radiographic examinations were carried out for all fetuses. One third of the fetuses were also examined for visceral anomalies. The other two thirds were used for clearing and bone staining with alizarin. Azaperone was not teratogenic in this study.

7. Effects During Peri- and Post-natal Period - Rats

A test was conducted in female Wistar rats by Janssen Pharmaceutica, Beerse, Belgium, under the supervision of R. Marsboom, D.V.M., to evaluate the safety of oral administration of azaperone during late pregnancy and lactation. Groups with 25 females per group were administered azaperone orally by gavage in doses of 2.5, 10 and 40 mg/kg body weight, once daily from day 16 of pregnancy throughout a three-week lactation period. A similar group served as controls and received saline according to the same schedule. There was little or no difference between the controls and treated dams in regard to litter size. No abnormalities were noted. Average body weights of the pups were comparable for all groups. Survival rate of the pups slightly decreased in the 40 mg/kg treated group.

D. Reproductive Studies (Continued)

8. Effects on Female Reproductive Performance During Three Generations - Rats

A test was conducted in young adult female Wistar rats by Janssen Pharmaceutica, Beerse, Belgium, under the supervision of R. Marsboom, D.V.M., to evaluate the safety of oral administration of azaperone on dams and their offspring for three generations. Eighty virgin female rats, three to four months of age and weighing 200-220 g, were used in the initial phase (20 females per treatment group). From day six through day 15 of pregnancy, the females were fed diets containing 0, 10, 40 or 160 mg of azaperone per 100 grams of food. Prior to and after the period of organogenesis and throughout lactation, the female rats received the basal nonmedicated rat diet. In the second generation study, 125 virgin females randomly chosen from the young born from the first generation were mated with 63 males, also born from mothers in the first generation. The young females were treated in the same manner at the same dose levels as their mothers. In the third generation study, a total of 173 females and 87 males were randomly chosen from the young born in the second generation study. The females were treated like their mothers and grandmothers but they were sacrificed on the morning of their 22nd day after insemination.

No mortalities occurred among the females of the control or treated groups in all three generations. No adverse effect on body weight, food consumption or pregnancy rate of the dams were seen. Litter size, birth weight and survival rate of pups and percentage of live fetuses were similar for control and treated groups; thus, no embryotoxic effect occurred in the three generations. There were no fetal abnormalities observed which were attributable to the treatments.

9. Dominant Lethal Mutation Test - Mice

A dominant lethal mutation test of azaperone in mice was conducted by Janssen Pharmaceutica, Beerse, Belgium, under the supervision of R. Marsboom, D.V.M. Groups with six fertile males per group were administered azaperone once orally by intubation at doses of 10, 40 and 160 mg/kg body weight. A similar group of six males received saline only (negative control) while another six males were treated with cyclophosphamide at 210 mg/kg, orally, as a positive control. Following treatment, the males were mated with untreated virgin females and further transferred at weekly intervals to successive groups of untreated virgin females for eight consecutive weeks. All females were autopsied 15 days

D. Reproductive Studies (Continued)

after exposure to the males and the number of corpora lutea, early and late embryo deaths, live implants and pseudo-pregnancies were counted. Dominant lethal mutations were evaluated directly by enumeration of embryonic deaths and indirectly on the basis of pre-implantation losses calculated from the reduction in the number of implants per impregnated female in experiment as compared to the implant per impregnated female in control. This experiment did not demonstrate any mutations induced by azaperone in any stages of the mice male germ cells.

ENVIRONMENTAL ASSESSMENT

An assessment of the introduction of substances into the environment as a result of the use of STRESNIL* (azaperone) Injection in swine and the fate of emitted substances in the environment is presented. This analysis and calculations were patterned after an internal working guideline of the Food and Drug Administration, i.e., "Appendix A: Environmental Impact Data Profile".

I. Introduction of Substances Into the Environment

A. Number of Animals Potentially Treated

Of a total of 87.8 million pigs farrowed (1983 estimate), it is projected that 4.4 million pigs (5%) may potentially be treated with a single dose (1 ml) of STRESNIL Injection. This is based on a Pitman-Moore market survey.

B. Estimated Concentrations of Substances in Excreta of Target Animals

As determined in the metabolism study of azaperone in swine (STRESNIL Injection, NADA 115-732, dated May 24, 1978, Volume 2, Exhibit 12, Page 206), azaperone was metabolized to three metabolites: azaperol, 5-hydroxy azaperone and 5-hydroxy azaperol. These three metabolites, plus the parent compound azaperone, accounted for approximately 80% of the total radioactivity excreted in the urine. Using the data derived in the metabolism study, the estimated concentration of metabolized azaperone in the urine, the predominant excretion route, after treatment at the recommended dose (2.2 mg/kg or 1 mg/lb.) was calculated to be 27.7 ppm (see Table 3 for calculation).

C. Estimated Concentration of Substances Expected to Run Off from an Open Air Animal Growing Facility

The concentration of metabolized STRESNIL Injection expected in run off from an open air facility was estimated to be 0.14 ppm. This was determined using the calculation shown below. The following assumptions were used:

1. 69% of the administered dose is excreted into the environment,
2. an animal growing facility contains 1,000 pigs,
3. all pigs weigh 40 lbs. at the time of treatment, and
4. each pig receives a single injection at the recommended rate (1 mg/lb).

*Trademark

C. (Continued)

Example

Quantity of "Y" in Excreta (mg)		Number of Animals in Facility	÷	Weight of 2 inches[1] Run Off from the Facility (kg)	=	mg "Y"[2] / kg Run Off (ppm)

[1]One acre-inch of water = 102,750 kg (=liters)
[2]Calculation assumes all of "Y" to be contained in run off

Calculation

69% of 40 mg Per Pig (28 mg)	X	1000 Pigs	÷	205,500 (kg)	=	0.1362 ppm in Run Off

D. Estimated Concentration of Substances Expected When Excreta of Target Animals are Incorporated into Agricultural Soil

Example

Concentration of "Y" in Excreta[1] (mg/kg)		Application[2] Rate of Excreta Into Soil (kg/acre)	÷	Weight of Soil[3] Per Unit Area at 6 Inch Depth of Incorporation (kg soil/acre)	=	mg "Y" / kg soil

[1]Dry weight basis
[2]Typical application rate = 4.5×10^3 kg/acre (dry weight)
[3]909,000 kg soil per acre

Calculation

$27.7 \text{ mg/kg}^{1} \times 4.5 \times 10^3 \text{ kg/acre} \div 909{,}000 \text{ kg soil/acre} = \frac{0.1371 \text{ mg}}{\text{kg soil}}$

[1]Figure derived from calculation shown in Table 3.

II. Fate of Emitted Substances in the Environment

It is concluded that the use of STRESNIL* (azaperone) Injection in the pig does not result in a significant accumulation of metabolites in the environment because:

- The 5-hydroxy metabolites of azaperone were found to be labile and unstable in vitro (NADA 115-732, dated May 24, 1978, Volume 2, Page 206).
- Azaperone is subject to photodegradation

*Trademark

- The dilution factor provided by the soil and rainfall further reduces the environmental concentrations of any emitted substances.

- Based on the metabolism study and the photodegradation characteristics of azaperone, there is a low probability that the chemical will significantly bioconcentrate.

Because STRESNIL* Injection will be restricted to use by or on the order of a licensed veterinarian (prescription drug) and its low order of toxicity, it can reasonably be expected that the use of the drug will not pose any hazards to the environment.

*Trademark

Table 3: Calculation of the Concentration of Metabolized Azaperone In the Urine of the Pig[1] After a Single Intramuscular Dose of 4 mg/kg

Pig No.	Slaughtered After (hours)	Weight[2] kg	Dose mg/kg	Total mg	% Dose in Urine[3]	Total mg in Urine	Total ml of Urine	Concentration in Urine mg/ml
I	48	15	4	60	74.45	44.67	793	0.0563
II	48	18.7	4	74.8	69.00	51.61	705	0.0732
III	24	21.3	4	85.2	60.18	51.27	756	0.0678
IV	24	12.9	4	51.6	36.48	18.82	468	0.0402
V	72	22.5	4	90	63.38	57.04	1,360	0.0419
VI	72	17.7	4	70.8	34.03	24.09	1,040	0.0231

Average concentration in urine = 0.0504 mg/ml (50.4 ppm).

Conclusion

If a dose of 4 mg/kg administered intramuscularly in the pig results in 50.4 ppm of metabolized azaperone in the urine, then a dose of 2.2 mg/kg should result in 27.7 ppm in the urine.

Calculation

$$\frac{4 \text{ mg/kg}}{50.4 \text{ ppm}} = \frac{2.2 \text{ mg/kg}}{X \text{ ppm}}$$

$X = 27.7$ ppm

[1]Calculation derived using the study "Metabolism of Azaperone in Swine" dated May 12, 1976, located in STRESNIL* Injection NADA 115-732, dated May 24, 1978, Volume 2, Exhibit 12, Page 206.
[2]Taken from Table 29 (Page 342) of the above cited report.
[3]Taken from Table 31 (Page 344) of the above cited report.

*Trademark

Analysis of the Environmental Effects of the Manufacturing Process

STRESNIL* (azaperone) Injection may be manufactured by Pitman-Moore, Inc., Washington Crossing, New Jersey, 08560, or by an alternate manufacturer - Taylor Pharmacal Company, Decatur, Illinois, 62525. Analysis of the environmental effects at each manufacturing location is presented on the following pages.

*Trademark

An Analysis of the Environmental Effects of the Manufacturing Process at Pitman-Moore, Inc. in Accordance with 21 CFR 25.1(g)(2)

1. An identification of the pollutants expected to be emitted

   No pollutants are emitted from the manufacturing and packaging process at Pitman-Moore, Inc. Any discardable materials (refuse) will be disposed of via normal refuse disposal methods.

2. A citation of applicable Federal, state and local emission requirements

   a. Air Pollution Control Programs

      Federal requirements: 40 CFR, Chapter 1, Subchapter C, Part 52, Subpart FF - New Jersey

      State requirements : New Jersey Air Pollution Control Code

      (1) Chapter 7: Control and Prohibition of Pesticides from Manufacturing Processes. Effective Date: March 27, 1972

      (2) Chapter 13: Ambient Air Quality Standards. Effective Date: March 5, 1973

   b. Water Pollution Control Programs

      Rules and Regulations for the Preparation and Submission of Plans for Sewer Systems and Wastewater Treatment Plants. New Jersey State Department of Environmental Protection. Dated: July, 1970

   c. Hazardous Waste Management System

      Federal requirements: 40 CFR, Parts 260 to 265

      State requirements : Title 7, Chapter 26 of the New Jersey Administrative Code

3. A certification that such emission complies with these requirements.

   There are no polluting emissions associated with the manufacturing and packaging of STRESNIL* Injection at Pitman-Moore, Inc.

   Pitman-Moore, Inc. certifies compliance with all emission requirements.

AUG - 5 1982
Date

Orville W. Ostmann
Orville W. Ostmann, Ph.D.
Director, Regulatory Affairs

*Trademark

xiii. An analysis of the environmental impact of the manufacturing process(es) of the article that is the subject of the requested action as specified under 21 CFR 25.1(g).

1. An identification of pollutants expected to be emitted:

   None.

2. A citation of applicable Federal, State and local emission requirements.

   There are no polluting emissions involved from the processing at Taylor Pharmacal Company.

3. A certification that such emission complies with said requirements.

   Applicable Federal, State or local emission requirements are being complied with by Taylor Pharmacal Company.

   Solid waste is disposed of at a landfill dump, E.P.A. Permit No. 11580402. Liquid aqueous processing water is discharged to the sanitary sewer monitored by the Decatur Sanitary District Permit No. 11580402, and is in compliance with all State and Federal Standards.

F. Stanley Taylor

F. Stanley Taylor, President

Date June 24, 1982