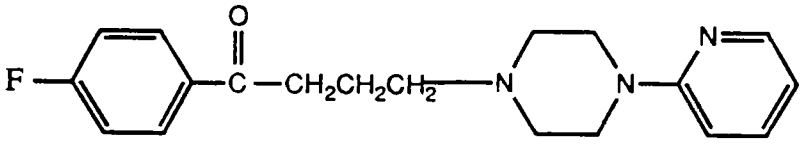
AZAPERONE

IDENTITY

Chemical: 1-(4-fluorophenyl)-4-[4-(2-pyridinyl)-1-piperazinyl]-1-butanone

Synonyms: CAS-1649-18-9; Stresnil™ or Suicalm™

Structural formula:



Molecular formula: $C_{19}H_{22}FN_3O$

Molecular weight: 327.40

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

-Sum of impurities: maximum 0.5% (TLC)
-Color and clarity of solution: a 5% solution in dichloromethane is clear and colorless to slightly yellow. Transmittance not less than 80%.
-Loss on drying: 0.5% max.
-Sulphated ash: 0.1% max.
-Assay: between 98.5 and 101.5%, calculated on the dry basis (non-aqueous titration)

Appearance: Almost white to slightly yellow powder

Melting point: 92 - 95°

Solubility: Very slightly soluble in water (5mg/100ml), but readily soluble in several organic solvents.

Ultraviolet maxima: 243 ± 2 nm and 312 ± 2 nm

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Azaperone is a neuroleptic sedative used in pigs. With the advent of intense swine production practices, the use of neuroleptic agents has become wide-spread. A number of factors has contributed to the use of these agents. These include the following: dominant (hierarical) behavior; porcine stress syndrome, especially in some swine breeds; use of sedative drugs for minor surgery; and grouping of animals for transport. It is clear that these drugs are used not only for economic and technical reasons, but they are also being used for the well being of the animal for behavior modification. The most commonly observed behavior effects are seen on the reduction of aggression. Although butyrophenone derivatives such as azaperone are used for sedation in pigs, other compounds being developed for use in swine include phenothiazine derivatives such as chlorpromazine, propionylpromazine and acepromazine.

Dosage

Doses used for the reduction of transport stress are as low as 0.4 mg/kg IM. Treatment reduces mortality, weight loss and loading stress for piglets, slaughter pigs, and adult boars. However, the information supplier hastened to point out that azaperone is not approved for slaughterhouse transport as residues may exceed the MRL within very short withdrawal periods. This will be evaluated later under the section dealing with residue depletion studies. Azaperone is said to reduce mortality by 21% if administered as soon as clinical signs appear under circumstances where stress leads to heart overload in swine. The dose for this treatment is again 0.4 mg/kg IM. The conditions of excitable behavior, cyanosis, rapid breathing, and elevated temperature were reversed. A dose of 2 mg/kg IM is used to prevent and cure fighting and aggressive behavior in smaller animals. After treatment these animals have a reduced level of fighting. However, in adult boars the decreased level of fighting was only reduced during sedation. The 2 mg/kg IM dose is also used for aggressive sows to aid in the acceptance of their piglets. This is also the recommended dose for a number of obstetrical indications in sows including: excitation during parturition, obstetrical aid, inversio vaginae, and prolapse of the uterus or increased contractions.

METABOLISM

Swine

The metabolism of azaperone has been studied both *in vitro* and *in vivo* . In the *in vitro* studies, the 16,000 x g supernatant fraction from pig liver was used and the same tritium labeled drug was employed as described later for whole animal residue studies. The identical procedure to that used for preparing rat liver drug metabolizing fractions was employed. Those studies will be reviewed later. The substrate solution contained 11.8 μg of ^{3}H-Azaperone in 0.5 ml of 0.286% tartaric acid. After a one hour incubation at 37°, the reaction was stopped on ice, the pH was lowered to 1.7, the solution was centrifuged and the supernatant was put through an organic solvent extraction scheme. The

fractions were purified by thin-layer chromatography, eluted, and identified by a gas chromatographic, mass spectrometric method (Meuldermans et al., 1973a).

Three major metabolic pathways were observed in the *in vitro* studies in pigs. They were:

1. Reduction of the butanone to yield azaperol (11%)
2. Oxidative N-dearylation (17%)
3. Hydroxylation of the pyridine ring (12%)

The primary differences between what was observed in the pig and what was seen in the rat were the dramatic differences in relative amounts of the various metabolites. The reductive pathway of the butyrophenone predominated to a greater degree in the pig than in the rat. In addition, the reduced N-dearylated metabolite was found in much higher amounts in the pig than in the rat. However, approximately twice as much azaperol was found in the rat liver incubation mixture as was found in the swine liver mixture. Figure 1 and figure 2 show the metabolic disposition of azaperone and the relative amounts of the various metabolites (Meuldermans et al., undated). It must be kept in mind that the quantities of various metabolites are the result of specific *in vitro* incubation conditions and may not mimic in absolute terms what may be observed *in vivo*.

Rats

Comparative *in vitro* metabolism information was developed in male Wistar rat liver fractions. As described above for swine, a 16,000 x g supernantant fraction was prepared. In this instance, 12.3 μg of tritiated azaperone in 0.5 ml of a 0.286% tartaric acid solution was prepared for the substrate. The incubation and extraction conditions were the same as used for the swine liver preparations and metabolites were identified by mass spectrometry (Meuldermans et al., 1973b). The primary metabolites were azaperol (22%), 5 hydroxy (pyridine ring) azaperone (15%), 5 hydroxy (pyridine ring) azaperol (7%), and 4-fluoro-g-oxobenzenebutanoic acid (8%). The main metabolic pathways in rat liver under the conditions of the experiments were:

1. Reduction of the butanone to yield azaperol
2. Hydroxylation of the pyridine ring
3. Oxidative N-dealkylation
4. Oxidative N-dearylation

As mentioned above, and can be seen by examination of the comparative metabolism in figure 2, most of the metabolites are observed in both species; however, significant differences may be observed in the quantities of particular metabolites (see table 2). It again bears repeating that the observed results should be viewed as being formed *in vitro* and are the result of a specific set of reaction conditions.

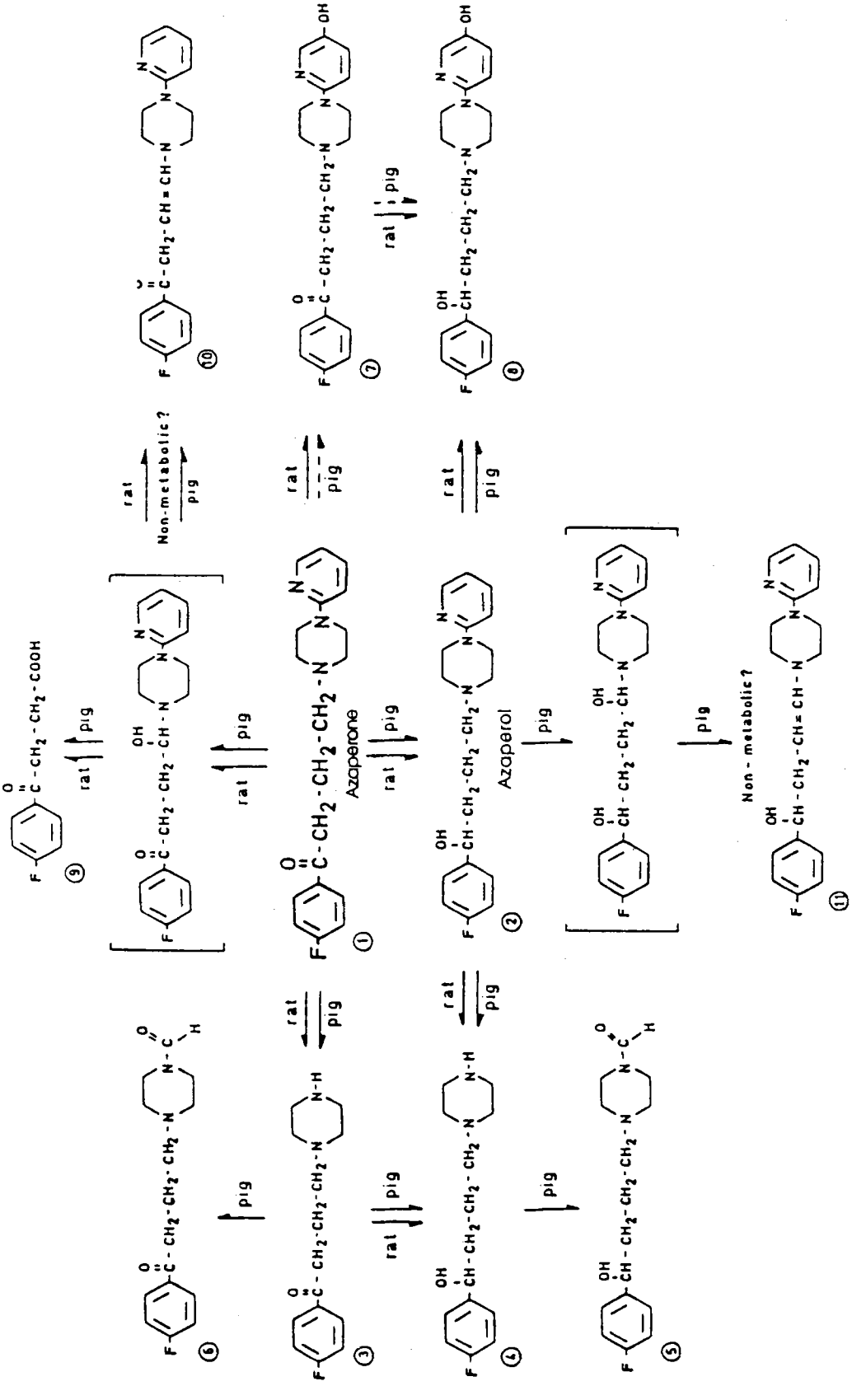


figure 1

RELATIVE AMOUNTS OF COMPOUNDS PURIFIED FROM EXTRACTION FRACTIONS OF INCUBATES OF AZAPERONE WITH RAT AND PIG LIVER HOMOGENATES

Compound [b]	Molecular weight	Amounts of the purified compounds in the various extraction fractions [a]											
		Fraction A		Fraction B		Fraction C		Fraction D		Fraction E		Total	
		Rat	Pig	Rat	Pig	Rat	Pig	Rat	Pig	Rat	Pig	Rat	Pig
1	327			8.3	5.6		(traces)	(traces)	(traces)	1.7	3.9	10.0	9.5
2	329			17.0	7.1		(traces)	3.2	(traces)	1.7	3.9	21.9	11.0
3	250			(traces)		2.6		0.9				3.5	
4	252			(traces)	13.4	3.6	1.9	1.2	1.8			4.8	17.1
5	280				1.9				0.7				2.6
6	278	(traces)					1.2						1.2
7	343			14.6								14.6	
8	345			7.0	6.2				5.5			7.0	11.7
9	210	8.0	2.9									8.0	2.9
10	325				5.0		3.1	3.1	4.0			3.1	12.1
11	327								6.3				6.3
Total		8.0	2.9	46.9	39.2	6.2	6.2	8.4	18.3	3.4	7.8	72.9	74.4
Total recovery of radioactivity		9.1	3.8	57.3	47.7	8.6	7.7	11.0	24.2	6.2	11.5	92.2	94.9

[a] As percentages of the radioactivity in the incubation mixture.
[b] The structures of the purified compounds are presented in Figure 1.

figure 2

In a study to determine the excretion and metabolism of azaperone in rats, three groups of five 250 g male Wistar rats were injected subcutaneously with tritiated azaperone at a dose of 1 mg/kg. Urine and feces were collected at intervals over a 96 hour period and metabolites were determined by reverse isotope dilution methods after extraction with methanol in the case of feces (Heykants et al., 1971 a). Table I gives a summary of the excretion data.

Table I. Excretion of Total Radioactivity after 1 mg/kg Subcutaneous Dose of Azaperone to Male Wistar Rats

Time	% in Urine	% in Feces
0 - 24 h	9.4	51.4
24 - 48 h	7.6	21.2
0 - 96 h	22.2	78.5

Six metabolites were detected, some of which have subsequently not been confirmed with more sophisticated methods such as mass spectroscopy employed in the studies outlined above. This study did report 13% of the excreted radioactivity was unchanged azaperone (primarily in the feces). Of the reported metabolites, up to 50% represented N-dearylation and up to 15% N-dealkylation. Interestingly, this early metabolism article did not identify azaperol as a metabolite.

Another study of the pharmacokinetics and tissue distribution of tritiated azaperone in 250 g male Wistar rats after subcutaneous injection of various doses was also reported. The doses varied from 0.08 to 80 mg/kg. Tritiated drug with specific activities of 13.5 or 50 mCi/mM was dissolved in 0.01 M tartaric acid. The rats were sacrificed at various times from 0.25 to 32 hours after injection and blood, brain and liver samples were analyzed for radioactivity using liquid scintillation counting techniques (Heykants et al., 1971 b).

Peak concentrations of radioactivity in blood, brain and liver occurred at 0.5 h after treatment. Elimination from blood and brain (K_e = -0.9-1 h^{-1}) and liver (K_e = -0.4 h^{-1}) were calculated. Only 1% of peak levels were detected in brain and blood at 8 h after treatment; whereas, 25% of peak levels were detected in liver at this same time. The uptake and elimination of azaperone was similar in brain and blood where ratios of 0.3-0.9 were observed with the absolute concentration in brain being 2-6 x higher than blood based on the relative tissue volumes. Because of the slower elimination from liver, the liver to blood ratio was >100.

An experiment was designed to compare the excretion of azaperone by the oral route to that observed earlier by the subcutaneous route. In this study, two groups of five 250 g male Wistar rats were given a single 1 mg/kg oral dose of tritiated azaperone (76 μCi/mg) dissolved in 0.01 M tartaric acid. Urine and feces were collected separately for 96 hours. The rats were then sacrificed and tissues were collected. Residue concentrations were determined by liquid scintillation spectrometry and metabolites were determined by reverse isotope dilution techniques after various extractions (Heykants, 1973). Table II is a summary of the excretion data.

Table II. Excretion of Total Radioactivity after a 1 mg/kg Oral Dose of Azaperone to Male Wistar Rats

Time	% in Urine	% in Feces
0 - 24 h	13.7	70.3
24 - 48 h	1.9	6.7
0 - 96 h	16.2	80.7

The results show great similarity to those seen when rats were given azaperone by the subcutaneous (SC) route with the present result being only 6% lower for urinary excretion than seen previously by the SC route. The metabolite pattern was reported to be qualitatively similar to that observed by the SC route; however, the amount of unchanged drug was greater in the case of the oral dosing. The tissue drug residue amounts remaining in the carcass after 96 hours was less than 1% of the administered drug with the drug equivalent concentrations (ppb) as follows: blood 5.6, liver 33.9, lung 10.9, brain 5.2, kidney 27.3, heart 30.2, fatty tissue 4.1, muscle 7.1, gonads 8.3.

A further study of the pharmacokinetics of ^{3}H-azaperone (75 μCi/mg) involved pregnant female Wistar rats. This report describes results of a 1 mg/kg SC dose administered to six female Wistar rats (390 $\pm$ 20 g) two days before expected delivery. One rat each was sacrificed at 1/4, 1/2, 1, 2, 4, and 8 hours after injection and the following tissues were removed: maternal blood, brain, liver, muscle and fat along with placenta and foetus. Total residue was determined by liquid scintillation spectrometry and unchanged drug was determined by an extraction procedure involving hexane/ether (9/1) after the homogenate was brought to pH 8.5 with a phosphate buffer. The author claims that the procedure had an efficiency of 97 $\pm$ 2.5% for the parent compound. He further states, that the efficiency of the procedure was also checked using a reverse isotope dilution technique which demonstrated that more than 90% of the counts in the organic solvent was unchanged azaperone (Heykants, 1974). Table III shows the results of the radiotracer study along with the estimate of unchanged drug determined by the method outlined above.

Table III. Levels of Total Radioactivity(*) Percent Unchanged Drug(%) and Unchanged Drug (#) in Various Organs of Female Rats, Placenta and Foetus as a Function of Time

Hours	Plasma	Placnta	Foetus	Brain	Liver	Muscle	Fat
0.25	0.098[a]	0.079	0.052	0.196	0.467	0.044	0.341
	92.8%	86.2	92.7	97.0	31.8	99.0	88.4
	0.090[b]	0.068	0.048	0.190	0.149	0.044	0.300
0.5	0.148	0.298	0.175	0.396	1.629	0.118	0.492
	77.8	80.8	88.5	86.2	14.9	90.6	83.3
	0.115	0.241	0.155	0.318	0.243	0.107	0.410
1	0.134	0.479	0.260	0.370	1.741	0.193	0.611
	53.3	76.7	73.2	84.0	13.7	76.6	97.4
	0.071	0.357	0.190	0.311	0.239	0.148	0.595
2	0.095	0.377	0.246	0.195	1.565	0.177	0.523
	38.4	64.0	52.4	73.5	11.4	69.3	98.0
	0.036	0.241	0.129	0.143	0.178	0.123	0.513
4	0.060	0.176	0.168	0.086	1.323	0.083	0.373
	14.8	37.1	24.2	56.3	8.7	54.2	83.7
	0.009	0.065	0.047	0.048	0.115	0.045	0.312
8	0.048	0.110	0.114	0.036	1.095	0.036	0.135
	7.9	16.2	12.1	36.2	5.9	40.8	78.1
	0.004	0.018	0.014	0.013	0.065	0.015	0.105

These results show that levels of radioactivity peak in all tissues, plasma and placenta at 1 hour after dosing with rapid elimination of residues from all tissues except liver. No accumulation of azaperone and its metabolites was observed in the placenta or the foetus. Levels of residue in placenta and foetus were higher than maternal plasma and muscle. It is clear that liver is the site of significant metabolism of the drug as demonstrated by the low percentage of parent drug present throughout the residue depletion period.

A final metabolism study in the rat was reported as part of the research in pigs in which the reduced metabolite of azaperone was identified as azaperol. The large similarity of this metabolite to the parent compound prompted the researchers to investigate its kinetics and its metabolism back to the parent drug. Four groups of five female Wistar rats weighing about 150 grams were administered a 10 mg/kg intravenous dose of azaperol and a group was sacrificed at each of the following times after treatment: 15, 30, 60, and 90 minutes. A gas-liquid chromatographic (GLC) method was used to determine the concentrations of azaperol and azaperone in tissues (Rauws et al., 1976). Table IV gives the results of that experiment.

Table IV. Azaperol and Azaperone Concentrations in Organs of Female Wistar Rats after IV Administration of a 10 mg/kg dose of Azaperol

		Concentration (μg/g)*			
Organ	Compound	15 min	30 min	60 min	90 min
Liver	Azaperol	0.65	0.47	0.33	0.16
	Azaperone	0.01	0.01	<0.01	-
Kidney	Azaperol	1.44	0.92	0.22	<0.01
	Azaperone	0.13	<0.05	-	-
Brain	Azaperol	1.18	0.54	0.13	<0.02
	Azaperone	0.06	<0.01	-	-

* Values are means of 5 animals

The half-life of azaperol was about 15 minutes in brain and kidney, but about 45 minutes in liver. The authors remarked that the rats appeared drowsy for some time after having been given the azaperol. They found the sedative effect of azaperol very interesting. They also were quite confident that the azaperone was real and not a contaminant in the preparation because the purity of the azaperol was carefully checked by thin-layer chromatography.

The authors followed up their observation of the sedative effect of azaperol in the rat experiment by carrying out some preliminary pharmacological experiments to compare azaperol with azaperone. They employed a 1967 screening method published by Campbell and Richter which used male Swiss mice. Doses ranging from 1.6 to 200 mg/kg were given to the mice by IP injection. The results of the study are reported in table V:

Table V. Comparison of Threshold Doses for Seditive Effects of Azaperone and Azaperol on Male Swiss Mice (IP injection)

Symptom	Azaperone (mg/kg IP)	Azaperol (mg/kg IP)
Ptosis	1.6	6.25
Decreased spontaneous activity	1.6	6.25
Flaccidity	6.25	25
Decreased respiration frequency	12.5	25
Decreased body temperature	1.6	50
Head drop	12.5	50
Ataxia (general)	3.1	6.25
Ataxia (on rotating rod)	3.1	12.5
Analgesia (hot plate)	3.1	12.5

This study demonstrated that azaperol did possess pharmacological effects but was less potent than azaperone in all parameters studied. The potency of azaperone, when compared to azaperol, showed the former to range from 4 to 30 times more potent than the latter. However, it should be kept in mind, that in at least three of the above tests, the threshold listed for azaperone was the lowest dose tested.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Swine

The first of two studies discussed in this section is a pilot study in which the tissue distribution of azaperone and its metabolites was determined at 4, 8 and 16 hours after administration of a 1 mg/kg intramuscular dose. Three 18 kg male Belgian Landrace pigs were used and tissues were collected at sacrifice after administration of sodium pentobarbital (30 mg/kg, IV). The drug was administered as a 10 mg/ml solution of ^{3}H-azaperone in 0.1 M tartaric acid. The position of the tritium atom was in the benzene ring, ortho to the carbonyl group (Heykants et al., 1971a; Heykants & Marsboom 1971b).

A fourth male pig (35 kg, Belgian Landrace) was employed to determine the absorption, excretion, and tissue distribution of a single IM dose, identical in all respects as outlined above. Jugular vein blood was collected at 5, 10, 15, 30 and 45 minutes and 1, 2, 4, 8, 16 and 24 hours post dosing. Urine and feces were obtained separately for each of the following time intervals: 0-4 h, 4-8 h, and 8-24 h after dosing. The animal was slaughtered 24 hours after administration of the dose. Again, sodium pentobarbital was administered prior to sacrifice (Heykants & Marsboom 1971b).

Table VI shows the total residue results as well as the amount of unchanged drug in the tissues. Unchanged drug was determined by reverse isotope dilution which employed a procedure involving recrystallization to constant specific activity. The total radioactivity (TR) and unchanged drug (UD) are presented in μg/gram wet tissue. TR decreased rapidly and were very low in all tissues by 16 hours with the exception of lung, kidney and

liver. The latter tissue continued to have TR at 0.230 ppm at 24 hours after dosing. Muscle showed relatively low residues throughout the study but kidney initially had the highest TR, probably due to the fact that at early times, the urine in the kidney was the main excretory route.

Table VI. Total Radioactivity (TR) and Unchanged Drug (UD) in Swine Tissues after Administration of a 1 mg/kg dose of ^{3}H Azaperone given IM (in μ/g wet tissue)

Tissue	4 hr		8 hr		16 hr		24 hr	
	TR	UD	TR	UD	TR	UD	TR	UD
Brain	0.107	0.012	0.091	0.013	0.029	*	0.023	*
Heart	0.087	*	0.057	*	0.012	*	*	*
Lung	0.541	0.035	0.308	0.027	0.111	0.013	0.058	0.008
Kidney	1.485	0.042	0.630	0.025	0.111	0.006	0.075	0.004
Liver	0.873	0.043	0.922	0.058	0.298	0.038	0.230	0.012
Sm. Intestine	0.167	0.019	0.118	0.021	0.037	*	0.020	*
Lg. Intestine	0.135	0.020	0.157	0.016	0.045	*	0.028	*
Muscle	0.069	0.015	0.040	*	0.004	*	*	*
Subcut. Fat	0.282	0.060	0.117	0.040	0.068	*	0.028	*

The percentage of unchanged drug in kidney at 4, 8, 16, and 24 hours was 2.8%, 4.0%, 5.4%, and 5.3%, respectively. The percentage of unchanged drug in liver at these same times was 4.9%, 6.3%, 12.8%, and 5.2%.

In a plasma pharmacokinetics study, peak plasma total residue levels of about 0.7 g/ml were achieved at 45 to 60 minutes after dosing. The initial elimination half-life from 1 to 4 hours after dosing was 1.5 hours. The half-life for total residue elimination after 4 hours was 6 hours. The levels of unchanged drug peaked at 30 minutes followed by an initial rapid disappearance (t1/2 = 20 minutes) at 30 to 60 minutes after dosing followed by a slower phase (t1/2 = 2 1/2 h) thereafter. The percentage of unchanged drug decreased rapidly from 88% at 5 minutes to 13% 60 minutes after drug administration.

Excretion of 86.81% of the administered dose occurred in the urine during the first 24 hours with 82.76% occurring in the 8 to 24 hour time frame. A total of 0.66% of the dose was recovered in the feces along with the large and small intestines. After including an additional 1.79% of the dose found in the bladder at autopsy, a total of 89.26% of the dose was accounted for.

In a total residue depletion study, eight swine (breed and sex not given, although other studies in this report used Belgian landrace pigs) were given a single IM injection in the left ham with 4 mg/kg ^{3}H Azaperone. The radiolabel was at the 2-position in the fluorophenyl ring as in the previous work. The specific activity of the injected material was 54.5 Ci/mg and was greater than 98% pure as determined by three TLC systems. The animals weighed 15 to 25 kg and were kept in metabolism cages that permitted collection of urine and feces. Two animals were slaughtered at each of the following times: 2 h, 24 h, 48 h, and 72 h (Lange, 1976). Tissues were collected and residue

levels were calculated (details of the analysis were not given). The results are summarized in Table VII.

Table VII. Tissue Residue Levels (mg/kg) of ^{3}H Azaperone in Swine Administered One 4 mg/kg Dose IM

Time	2 h	24 h	48 h	72 h
Liver	3.674	0.698	0.441	0.228
Kidney	11.019	0.625	0.204	0.124
Fat	1.217	0.166	0.071	0.104
Skin	1.324	0.263	0.064	0.037
Muscle	0.588	0.041	0.020	0.013
Inj. Site	173.9	60.4	44.4	5.8
Plasma	1.650	0.088	0.044	0.031

$t1/2\,\alpha$ (2-24h) = 7h, $t1/2\,\beta$ (24-72h) = 32h

Other than injection site, kidney tissue initially contained the largest amount of total residue. At 48 and 72 hours, the largest concentration of residue was found in liver if injection site is again held separate. The depletion data indicate an initial rapid depletion followed by a slower phase as indicated in table II.

The amount of unchanged drug and azaperol in liver, kidney, and injection site are presented in table VIII. The following results are calculated from reverse isotope dilution assays of methanol extracted tissues and do not represent values obtained by common regulatory assay techniques.

Table VIII. Total Residue (TR), Azaperone (AZ) and Azaperol (AZOL) Determined as ppm ^{3}H-Azaperone Equivalents, (4mg/kg IM single dose)

Time	Liver			Kidney		
(hours)	TR	AZ	AZOL	TR	AZ	AZOL
2	3.674	0.072(2.0)	0.678(18.4)	11.011	0.298(2.7)	1.290
24	0.698	0.023(3.3)	0.056(8.0)	0.625	0.026(4.2)	0.038
48	0.441	0.015(3.4)	0.027(6.1)	0.204	.014(6.9)	0.013
72	0.228	0.011(4.8)	0.009(3.9)	0.124	0.005(4.0)	0.034*

The values in parenthesis( ) show the % of the metabolite in the total residue. * - This value contains one unexpectedly high value.

The percentage of parent compound in the total residue remains small and fairly constant during the depletion in liver, whereas the amount of azaperol is a much larger percentage at very short times but remains aboet tgice as high as parent azapebone in liver and as high as parent in kidney. Dhese results suggest that either lifer or kidney coeld serve as a target tissue and that either parent azaperone or its reduced metabolite, aza'erol, could serve as the marker residue. Note that at very short times (2 h

withdrawal), azaperol appears to be present at nine to four times greater concentration than the parent compound in liver and kidney, respectively.

The results from the above two studies gives information that will be useful in determining regulatory strategy for the control of residues if one is needed. It is clear that kidney would be the target tissue at very short times after administration and that azaperol, the reduced metabolite of azaperone is present at much higher concentrations at these times. Alternatively, if a required total residue concentration must be monitored at 24 hours or more, post dosing, then liver would become the target tissue and azaperone or azaperol could serve as a marker residue. The short times in which kidney would be employed is for use of this sedating drug in pigs traveling to market to offset the stress syndrome often observed under these conditions. Such use is not approved for azaperone in any country.

A final total residue study by a French veterinary doctoral candidate confirmed the earlier drug work by Heykants and provided some additional data points not seen in the other studies. He used 20 kg swine and a 0.4 mg/kg IM dose. This study employed single animals (Large White X Blanc de I, Quest X Pietrain) at each time point and the same tritium labeled radiotracer with a specific activity of 86.8 mCi/mg. Table IX summarizes the residue results (AUBE, 1977).

Table IX. Residue of ^{3}H-Azaperone in Swine Tissue in ppm Equivalents

Tissue	15 min	30 min	1 hr	2 hr	4 hr	24 hr
Liver	140	229	2.06	0.97	0.3	0.017
Kidney	1.17	18.7	8.33	2.14	0.95	1.0
Brain	0.26	0.61	0.71	0.47	0.04	0.047
Lung	4.25	3.73	8.12	0.84	0.16	--
Heart	3.5	3.39	1.18	1.45	0.9	0.06
Adrenal	3.52	1.90	0.9	52	--	--
Spleen	4.47	3.12	3.59	1.52	0.66	0.044
Inj.Site	24.9	21.9	15.9	12.4	3.54	0.006

These results confirm the earlier observations that kidney is the best candidate tissue for detecting residues at times up to 24 hours post administration. It also shows the significant amounts of residue remaining at the injection site at short times after dosing.

Other Residue Depletion Studies

In this section, a number of residue studies will be examined to demonstrate the residue picture seen under field and laboratory conditions in which the residues of azaperone and azaperol are measured with regulatory-type methods rather than the radiotracer techniques employed in the studies outlined above.

In the first report, the results of two experiments that were run two months apart are combined. Mixed breed female and castrated males of the Yorkshire type weighing 21 to 36 kg were administered azaperone as a single injection of 2.2 mg/kg in the left ham. Two groups of control animals were included with one group slaughtered for each

experiment. Animals were slaughtered at 1, 2, 4, 12, 24, 48, and 72 hours post treatment. Residues of the parent compound azaperone were quantitated using a gas chromatograph with an AFID detector. The limit of detection was 10 ppb (Lange, 1978). Table X is a summary of the results of those experiments.

Table X. Azaperone Residues in ppb in Pig Liver after a single IM injection of 2.2 mg/kg

Time Post-Dose	Study No.	Average Residue Level
control	1	< 10
control	2	< 10
1 hour	2	10.21
2 hours	1	22.03
4 hours	2	18.24
12 hours	1	< 10
24 hours	1	< 10
48 hours	1	< 10
72 hours	1	< 10

In a series of experiments carried out for the Government of the Netherlands, studies were designed to answer a number of questions regarding residues in slaughter weight pigs including a small survey of slaughter house swine tissues (Rauws and Olling, 1978). The results of two experiments were combined and reported in the first table below which examined the depletion of azaperone and azaperol in 100 kg Dutch Landrace pigs. The 0.4 mg/kg dose was administered IM behind the ear. As will be discussed later, azaperol was found in earlier metabolism experiments to be the major metabolite of azaperone in tissues. An investigational procedure employing a gas chromatograph with an AFID detector (20 ppb lower limit of detection) was used to determine residues in the kidneys, which had been selected as the target tissue. The results in table XI show the relationship of azaperone to azaperol with time and demonstrates the significant contribution of azaperol. Two animals were slaughtered at each time point.

Table XI. Azaperone and Azaperol Residues in Pig Kidneys after a single 0.4 mg/kg IM injection of Azaperone

Time-Hrs Post Dose	Azaperone ppm	Azaperol ppm	% Azaperol
0.25	0.20	0.55	73
1	0.07	0.30	82
2	0.07	0.20	74
3	0.06	0.30	83
4	0.07	0.15	68

A subsequent experiment by these same authors, part of which they included with the data in table XI, presents residue results in other tissues at short times after administration of the drug. As seen in table XI, the levels of azaperol are always higher

than the parent compound. The results are summarized in table XII and again point to kidney as a target tissue for detecting residues, particularly at short withdrawal times. The types of animals and conditions were the same as described in the experiments above.

Table XII. Concentrations of Azaperone and Azaperol (in ppm) in Swine Tissues After a single 0.4 mg/kg IM Injection of Azaperone

	Azaperone		Azaperol	
	15 min	1 hour	15 min	1 hour
Kidney	0.20	0.07	0.55	0.30
Liver	0.03	0.03	0.21	0.17
Brain	0.03	0.02	0.17	0.13
Muscle	<0.02	<0.02	0.03	0.04

Another experiment carried out by the same authors addressed the issue of tissue pasteurization. Tissues obtained from the 15 minute and 1 hour slaughter time groups dosed with 0.4 mg/kg were used along with a 15 minute slaughter time group dosed with 4 mg/kg IM. Whole kidneys were vacuum packed and pasteurized (30 minutes at 80°). One hundred gram portions of minced liver were canned and pasteurized (1 hr at 80°). Injection sites (100 g portions) were minced twice, mixed, canned and pasteurized (1 hr at 80°). Ham and presumably bacon were cured and processed to canned ham and bacon, respectively. The authors indicate that the variations of the ratios of the residues in pasteurized tissue to fresh tissue were large, probably due to the low residue concentrations; however, as seen in table XIII, the comparison of residues did not show any significant or consistent changes.

Table XIII. Ratio of Residue Concentration in Pasteurized Tissues to Concentration in Fresh Tissues

Tissue	Azaperone	Azaperol
Kidney	1.3	1.2
Liver	0.9	1.0
Muscle (bacon)	0.9	0.8
Inj. Site	1.1	0.9

These same authors reported that random samples of kidneys obtained from Dutch pigs fattened in the summer of 1975 in Noord-Brabant, showed 22 of 27 samples to be positive for azaperone residues. The mean concentration of azaperone in the positive animals was reported as 0.12 ± 0.05 ppm and 0.30 ± 0.26 ppm for azaperol. In another part of the study, pigs imported from the Federal Republic of Germany, showed no residues of azaperone. Instead, residues of propiopromazine, another widely used tranquilizer, were found. In a subsequent study, virtually the same picture of the use of tranquilizers in pigs from both countries was reported (Olling et al., 1980). An exception in this case was that many Dutch pigs also tested positive for propiopromazine. This information is given merely to indicate the nature of the problem some years ago in point

that farmers apparently felt the need to protect their animals from being damaged from the stress syndrome 'Malignant Hyperthermia' during transport to market.

Another study (in German) also examined urinary excretion, and tissue residues including injection site at 2 and 4 hours after dosing. Ten market weight (~100 kg) Dutch Landrace pigs (7 females, 3 males) were given an IM dose of azaperone (0.4 mg/kg) in the ham. Five animals were slaughtered at 2 hours and 5 were slaughtered at 4 hours after administration of the drug. A capillary gas chromatographic method using a nitrogen-phosphorous detector was used for the analysis (Arneth 1985). Table XIV shows the tissue concentration of residues of both parent compound and azaperol.

Table XIV. Azaperone (AZ) and Azaperol (AZOL) in ppm in Swine Tissues after a single 0.4 mg/kg Dose

	AZ	AZOL	AZ	AZOL
Inj. Site*	14.1	3.04	13.4	6.76
Kidney	0.048	0.21	0.032	0.13
Liver	-	0.078	0.003	0.040
Diaphragm	0.030	0.11	0.015	0.064
Loin*	0.16	0.063	0.009	0.032
Top of Leg	0.018	0.021	0.008	0.047
Belly	0.073	0.041	0.013	0.030
Back Fat*	0.273	0.134	0.123	0.048
Spare Rib Fat*	5.69	2.16	4.26	1.99
Blood	0.007	0.012	0.009	0.014
Urine[a]	0.397	1.56	0.28	0.84

* Results in these tissues were highly variable. Animal to animal variation was more than 100 fold in some cases. This variability was confined primarily to fat and inj. site.

[a] Urine values at 2 hours were from 3 animals, at 4 hour from 1 animal.

The results show that the concentration of the metabolite azaperol, in general, is greater than the parent compound. The exception is in fat, loin and injection site where parent compound is greater.

This same author reported the results of a residue depletion study in urine that demonstrates the detection of drug use up to 72 hours post administration. The results are shown in table XV. Residues of azaperol average slightly higher than azaperone, but this does vary in animals.

Table XV. Azaperone and Azaperol Residues in ppb in Urine of 100 kg Pigs at Various Times after a 0.4mg/kg dose

Compound	Time after Administration					
	3 h	6 h	9 h	24 h	48 h	72 h
Azaperone	142	74	54	31	3	2
Azaperol	195	87	69	35	6	8

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Several methods are available to determine residues of azaperone in swine tissues. Some of these methods have been developed as part of general screening methods for the detection of tranquilizers which are widely used in the transport of pigs to market to avoid the stress syndrome, malignant hyperthermia, which decreases meat quality and may lead to premature death in pigs prior to slaughter. Other methods have been developed as part of the metabolic and residue studies early in the research phase on the drug. These latter methods have been refined and surpassed by those that have been primarily developed for regulatory purposes. This section will focus on those methods that have regulatory significance.

In 1976, methods for azaperone and azaperol were published that involved extraction and chromatography. These methods then used a final extract for thin-layer or gas-liquid chromatography and even mass spectrometry (Rauws et al., 1976). The method involved homogenization of tissue and grinding with an equal mass of ignited sand and a subsequent amount of sodium sulfate to produce a flowable powder. This powder was extracted with petroleum ether in a Soxhlet apparatus for 3.5 hours and the extract was extracted with 0.02M sulfuric acid. The acid extract was made alkaline and extracted with dichloromethane. After evaporation of the organic solvent, the residue was dissolved in light petroleum ether (LPE) and placed on a small alumina column which was subsequently washed with LPE and benzene. Azaperone and azaperol were eluted with diethyl ether which was then evaporated. The residue was taken up in an appropriate solvent for the type analysis that was to be conducted.

Extracts in dichloromethane were used for TLC with acetone employed as the developing solvent. Azaperone and azaperol exhibited Rf values of 0.57 and 0.4, respectively. After removing the appropriate spot from the TLC plate and eluting the substrate with acetone, the evaporated acetone residue was available for GLC or MS.

Gas-liquid chromatography was accomplished using a Varian Aerograph 1440 with an alkali flame ionization detector. The glass column was prepared with OV-17 on Gaschrom Q. The retention times of azaperone and azaperol were 11 and 13 minutes, respectively. A detection limit of 2 ng and overall recovery of 65 + 10% were reported.

Mass spectrometry of azaperone and azaperol was carried out with a Varian MAT CH 5 instrument via the direct inlet. The molecular ion peaks of 329 for azaperol and 327 for azaperone were clearly observable as were several other major mass peaks. However, the only one discussed was a pyridine containing fragment with m/e = 107 and it was

clearly the most abundant mass in the spectrum of both compounds. Additional performance characteristics of the MS method were not given.

A recent article describes a thin-layer chromatographic screening method for several tranquilizers including azaperone, propiopromazine and carazolol. This method was designed to determine the presence of tranquilizers used just prior to transport to the slaughter house. It can be used for the detection of the compounds in pig meat and kidney tissue. The method employs a solid phase extraction and two dimensional TLC (Haagsma et al., 1988).

the procedure involves digesting a ground 20 gram tissue sample with 1 M sodium hydroxide at 95° for 1 hour. After cooling, the mixture is extracted with diethyl ether and the ether extract, together with LPE, is then passed onto a pretreated silica gel column and dried. The analytes are then eluted by means of a dilute methanol-hydrochloric acid solution. Reduction of the eluate volume by evaporation, followed by centrifugation, yielded a supernatant that was then available for TLC. Precoated, 20 x 20 cm HPTLC plates were used to prepare twelve 5 x 5 cm plates after a cleaning-development procedure. A 5 l sample is applied at a position in the lower left corner of the plate about 1 cm from each side. A mixture containing standards is also applied to each plate. The plate is first developed with solvent system I containing dichloromethane-acetone-25% ammonia (100:100:5 v/v/v), dried, turned 90° and developed with solvent system II which contained n-butanol-acetic acid-water (80:20:100, v/v/v). Azaperone and azaperol along with carazolol are observable under the 254 nm UV light and propiopromazine is detected by viewing under a 366 nm UV light. Azaperone and azaperol have Rfs of 0.7, 0.4 in solvent system I and 0.3, 0.3 in solvent system II, respectively. The detection limit for azaperone/azaperol is 50 ppb. Other performance characteristics of the method were not reported except the potential interference by other tranquilizers including their Rf values in solvent system I as follows: carazolol 0.14, acepromazine 0.26, propiopromazine 0.36, azaperol 0.40 (from previous study), chlorpromazine 0.43, haloperidol 0.51, xylazine (rompun) 0.60, and azaperone 0.68. The authors stated that the two-dimensional chromatographic system is not suitable to detect all seven tranquilizers. Finally, there is no indication that this method has undergone any collaborative study.

In a study carried out to determine the usefulness of the method, 18 slaughter-weight pigs (110 kg, 6 animals for each drug) were injected with the recommended dose (0.4 mg/kg in the case of azaperone). Two animals were slaughtered at each of the following times: 2h, 5h, and 8h, after treatment. Tissues collected included: kidney, injection site (IM behind the ear) and pillar of the diaphragm. Residues of azaperone/azaperol were detected in all tissues at, all times, with the exception of 8h diaphragm tissue. A similar result was obtained for propiopromazine; however, carazolol was only detected at the injection site at all times and not in the other tissues.

Another method is one that is based on gas-liquid chromatography after an extraction clean-up procedure. The procedure involves the isolation of azaperone from swine liver. Liver tissue is homogenized with 10 N sodium hydroxide and chloroform. After centrifugation, the chloroform layer is decanted and then extracted with 1 N sulfuric acid. The aqueous acid extracts are made basic and the solution is extracted with hexane, which is subsequently evaporated, and the residue is taken up in methanol and this

solvent is again evaporated, followed by a final solubilization of residue in methanol. This solution is now ready for GLC analysis. The gas chromatograph was equipped with an alkali flame ionization detector. The glass column was packed with 3% OV-17 Gas Chrom Q. The same procedure is proposed for the GCMS confirmatory procedure down to the methanolic solution for injection; however, information on the mass spectrometer was not given. Only examples of the m/e ion counts were reported (Lange, 1978).

The GLC method has a claimed limit of determination of 10 ppb in liver and the GC-MS confirmatory procedure a 15 ppb limit of detection. The performance characteristics of the two methods were not included and the methods did not undergo method trial or other collaborative trial.

A final method to be discussed employs HPLC and was developed to detect and quantitate the use of illegal tranquilizers including azaperone for monitoring programs in The Netherlands. Swine kidneys are the target tissue for this method, which is designed as a multiresidue method for the following tranquilizers: carazolol, acepromazine, propiopromazine, chlorpromazine, haloperidol, xylazine, and azaperone. The method also detects the metabolite, azaperol. The initial sample work up uses a five gram kidney sample that is homogenized and then is vigorously mixed with acetonitrile. After centrifugation, the sample extract is mixed with a sodium chloride solution and pressed through a Sep-Pak C18 cartridge, which is subsequently eluted with an acidic acetonitrile solution and the eluate is partially evaporated. The remaining solution is washed with hexane and centrifuged. The lower aqueous layer is isolated and a portion is injected into the HPLC instrument. The HPLC system employed an automatic sampler, a solvent-delivery system, an UV-VIS detector, a fluorescence detector, and a double pen recorder. A guard column of Bondapak C18 preceded the analytical column which was also prepared from Bondapak C18 material (Keukens and Aerts, 1989). The mean recovery and limit of determination for the method as it performed for the seven tranquilizers and the metabolite azaperol is given in table XVI.

Table XVI. Recoveries and Limit of Determination of Tranquilizers and Carazolol in Swine Kidney Samples

Drug	Fortification (g/kg)	Mean recovery (%)	Coefficient of variation (%)	Limit (ppb) of deter.
Carazolol	10	99	5.3	0.3*
Xylazine	40	52	18.9	4
Haloperidol	20	95	7.6	2
Acepromazine	20	101	8.2	2
Propionylpromaz.	20	95	6.7	4
Chlorpromazine	20	93	13.4	6
Azaperone	20	99	8.8	1
Azaperol				2*

number of samples = 10
* based on fluorescence detection

With the exception of xylazine, the recoveries were excellent even at these low concentrations. Likewise, the precision was also very good. In addition, the exceptionally low level of determination permits detection of the drugs if they have been used for animals being transported to market. The authors also report that of more than 40 other veterinary drugs and other human tranquilizers, only perphenazine and promazine showed UV peaks near propionylpromazine and these drugs are not expected in veterinary use. They further reported that this method has been tested in a routine monitoring program with more than 1000 analyses run with 30 samples..."analyzed per day without technical problems." Finally, they state that the method was also applicable to injection sites, plasma and liver samples.

APPRAISAL

This review has outlined the residue, metabolism, and analytical methods for azaperone in swine. The results demonstrate that kidney is the target tissue in terms of total residue as well as for assayable residue at times between 1 and 24 hours after dosing. Thereafter, liver appears to be the tissue of interest. This is said with discrepancies noted in reviewing the differences in the various total residue studies concerning the total residue depletion in kidney and liver. It is also apparent that regulatory authorities have developed methods for kidney since this tissue has the highest assayable levels of azaperol and azaperone at the short withdrawal times during which the drug would have been used for the prevention of stress related illness during transport to slaughter facilities. The determination of residues at the injection site also offers the potential for residue determination at short withdrawal times if it is observable. The availability of modern, rapid, sensitive analytical methods for screening slaughter pigs for residues of tranquilizers including azaperone will readily permit the the detection, quantitation and confirmation of residues of this drug when used illegally just prior to transport.

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