AZAPERONE

First draft prepared by
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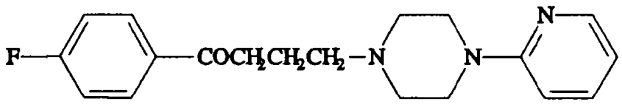
IDENTITY

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

Synonyms: CAS-1649-18-9, R-1929 (sponsor code)
Stresnil or Suicalm (Trademarks, Janssen)

Structural formula:



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

Molecular weight: 327.40

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Azaperone (sum of impurities maximum 0.5%)

Appearance: Almost white to slightly yellow powder

Melting Point: 92 - 95°C.

Solubility: Very slightly soluble in water (50 mg/l) and readily soluble in organic solvents especially, dichloromethane, N,N-dimethylformamide, tetrahydrofuran, toluene, 2-butanone, acetone and ethyl acetate.

Optical rotation: None

Ionization constant: pKa2 = 4.3, pKa1 = 7.5

UV_{max}: 243 nm and 312 nm.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Azaperone is a butyrophenone derivative used as a neuroleptic sedative in pigs. The most commonly observed behavioral effect is the reduction of aggression.

Dosages

The dose to reduce aggressive behaviour is 2 mg/kg body weight (BW) intramuscularly (i.m.). The dose for the non-approved use to reduce transport stress prior to slaughter is as low as 0.4 mg/kg BW.

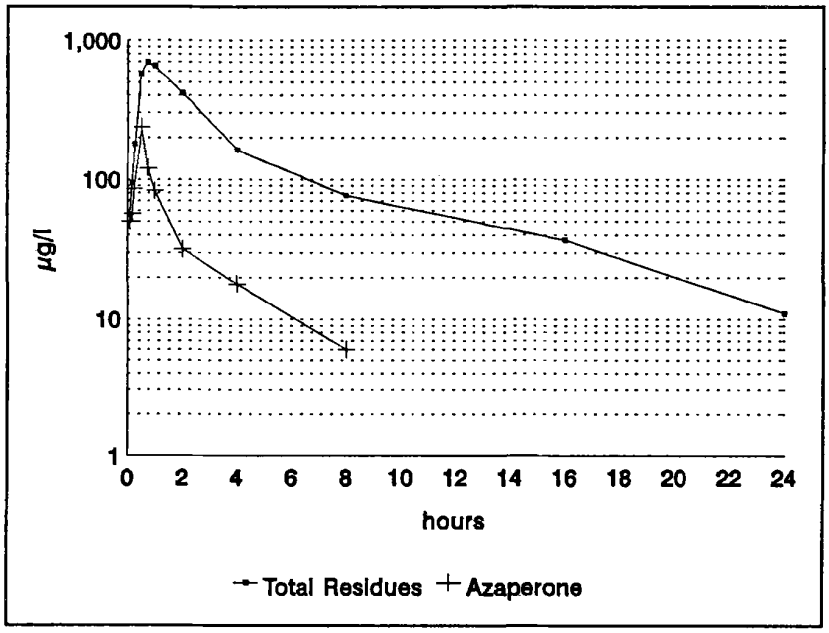
METABOLISM

Pharmacokinetics

Pig

One pig weighing 35 kg was injected i.m. with a single dose of 1 mg/kg BW of tritiated (^{3}H) azaperone (specific activity (SA) 13 mCi/mM). Blood, urine and faeces were collected over a 24 hour period after dosing and analyzed for total radioactive residues and additionally in the case of blood (plasma) the amount of unchanged drug was measured (method unclear)(K10). The results for blood are shown in Figure 1 and indicate a rapid absorption of the drug into the blood and reaching peak concentrations in about one hour. Thereafter there was a steady depletion with an elimination half-life after 4 hours of approximately 6 hours for the total residues and 2.5 hours for azaperone. At four hours more than 90% of the residue was metabolites of azaperone indicating rapid metabolism and degradation of the parent drug.

Figure 1. Plasma levels of total radioactivity and unchanged azaperone in one pig after i.m.i. of 1 mg/kg BW of tritiated azaperone.



About 89% of the total radioactivity was excreted in the urine during the first 24 hour after dosing, while less than 1% was found in the faeces.

In a second study (K19) pigs were dosed intramuscularly with ^{3}H-azaperone at 4 mg/kg BW. 60% of the radioactivity was excreted in the urine and 15% in the faeces in a 62 hour collection period.

In a new study (K22) using the recommended dose of 2 mg/kg BW, plasma levels of azaperone and azaperol were measured and shown to decline very rapidly (see below).

Rat

After subcutaneous injection of azaperone at 1 mg/kg BW 78.5% of the dose was excreted in the faeces and 22.3% in the urine during a four day period (K1). This in marked contrast to the target species where the major route of excretion is the urine.

Metabolism in Food and Laboratory Animals

No new data was requested or submitted. The metabolism in the pig and the rat were fully reviewed at the 38th JECFA (FAO 41/4). In summary the data showed extensive metabolism in both species, using both *in vivo* and *in vitro* studies. The main metabolic pathways were:

i) reduction of the butanone
ii) hydroxylation of the pyridine group
iii) oxidative N-dealkylation
iv) oxidative dearylation.

The primary differences between what was observed in the pig compared to the rat were the large differences in the relative amounts of the various metabolites. The reductive pathway of the butyrophenone predominated to a greater degree in the pig than in the rat. Also the reduced N-dearylated metabolite was found in much higher amounts in the pig than in the rat. Rat liver incubates had twice the amount of azaperol than pig liver homogenates.

Azaperone had 4-30 times the biological potency of azaperol in mice (Rauws et al., 1978). The activity of azaperol may be associated with its ready interconversion with azaperone. None of the other metabolites were shown to have significant neuroleptic activity (see V8672).

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

The sponsors presented data at the 38th JECFA and no further data was requested. Two studies were performed in pigs and used doses of azaperone of either 1 mg/kg or 4 mg/kg BW. After administration of 1 mg/kg BW of ^{3}H-azaperone intramuscularly to pigs the results are given in Table 1.

Table. 1. Total radioactivity (TRA) and unchanged drug (UD in pig tissues after administration of a 1 mg/kg BW dose of ^{3}H-Azaperone given i.m.

Tissue	µg/kg wet tissue							
	4 hour		8 hour		16 hour		24 hour	
	TRA	UD	TRA	UD	TRA	UD	TRA	UD
Brain	107	12	91	13	29	nd	23	nd
Heart	87	nd	57	nd	12	nd	nd	nd
Lung	541	35	308	27	111	13	58	8
Kidney	**1485**	**42**	**630**	**25**	**111**	**6**	**75**	**4**
Liver	**873**	**43**	**922**	**58**	**298**	**38**	**230**	**12**
S-Intestine	168	19	118	21	37	nd	28	nd
L-Intestine	135	20	157	16	45	nd	20	nd
Muscle	**69**	**15**	**40**	**nd**	**4**	**nd**	**nd**	**nd**
Subcut Fat	**282**	**60**	**117**	**40**	**68**	**nd**	**28**	**nd**

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

The second study used eight pigs weighing 15-25 kg and were dosed intramuscularly with 4 mg/kg BW ^{3}H-azaperone. The results are summarised in Table 2.

Table 2. Total radioactivity (TRA), unchanged drug (UD) and azaperol (*) in pig tissues after administration of a 4 mg/kg BW dose of ^{3}H-Azaperone given i.m.

	µg/kg wet tissue							
Tissue	**2 hour**		**24 hour**		**48 hour**		**72 hour**	
	TRA	UD	TRA	UD	TRA	UD	TRA	UD
Kidney	**11019**	**298** **1290***	**625**	**26** **38***	**204**	**14** **13***	**124**	**5** **34*,a**
Liver	**3674**	**72** **678***	**698**	**23** **56***	**441**	**15** **27***	**228**	**11** **9***
Muscle	**588**	**44** **258***	**41**	**4** **2***	**20**	**2** **0.8***	**13**	**1.0** **0.4***
Fat	**1217[b]**	**444** **954***	**166**	**70** **50***	**71**	**15** **10***	**104**	**13** **6***
Skin	1324	151 523*	263[b]	244 188*	64	15 12*	37	3 4*
Inj. Site	173900	131120 35300*	60420	53470 3080*	44380	35100 3500*	5805	4050 450*

* is azaperol; [a] one value unexpectedly high.
[b] note this value is lower than sum of identified residues.

Other Residue Depletion Studies (with Unlabeled Drug)

Pigs

The sponsors have submitted a new study (K22) determining the magnitude and decline of residues of azaperone in the edible tissues (including the injection site) and plasma of pigs after dosing with 2 mg/kg BW intramuscularly. Twenty pigs weighing 79-96 kg were dosed and they were slaughtered in groups of four (2 males and 2 females) at 1, 2, 3, 5 and 7 days after injection and tissue and plasma samples collected and analyzed by HPLC/UV for residues of azaperone and azaperol. Plasma was also collected at 3 hours and 24 hours from all the pigs. Plasma levels of both azaperone and azaperol declined rapidly. Three hours after dosing the average level was 23 µg/l for azaperone and 44 µg/l for azaperol. From 24 hours onwards the plasma levels of both compounds were not detectable (LOD 5 µg/l) except for 2 animals in which the values at 24 hours were 13 and 21 µg/l for azaperone and 9 µg/l for azaperol. One animal out of four tested had a level of 7 µg/l for azaperone at 48 hours.

The results for the residues in the edible tissues are summarised in Table 3. No residues were detected (LOD 25 µg/kg) at 5 days or 7 days after dosing. No residues of azaperone were detected in liver or kidney and no residues of azaperol were detected in muscle. In one pig muscle sample at day 2 residues of 76 µg/kg for azaperone were detected. By day 3 the only detectable residues were azaperone in one fat sample and azaperol in one kidney sample.

Table 3. **Residues (μg/kg) of azaperone (AZAP) and azaperol (AZOL) in tissues of pigs after intramuscular injection of azaperone at 2 mg/kg body weight.**

	Day 1		Day 2		Day 3	
	AZAP	AZOL	AZAP	AZOL	AZAP	AZOL
Muscle	nd	nd	76	nd	nd	nd
Liver	nd	64, 71, 80	nd	36, 56	nd	nd
Kidney	nd	57, 64, 74, 63	nd	28, 28	nd	28
Fat	31, 41, 44	79, 75, 60, 29	26	34, 26	44	nd
Skin	60, 92	29, 36, 63, 66	nd	nd	nd	nd
Lard	26	26, 42	nd	nd	nd	nd

No residues were detected at day 5 or on day 7. nd means no residues were detected in all four pigs per collection time (LOD 25 μg/kg).

The injection site 317-510 g was excised and the residues of azaperone and azaperol measured. The results are shown in Table 4. There were significant residues remaining at the injection site for at least five days. By day 7 residues were detectable in only one of the four pigs. Azaperone appears to be partially metabolised to azaperol at the site of injection.

Bound Residues/Bioavailability

No data was submitted and no new data was requested.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The analytical methods available were fully reviewed at the 38th meeting of JECFA. They included methods for measuring both azaperone and azaperol by gas chromatography, thin layer chromatography and HPLC. It was concluded that the methods were sensitive enough for monitoring residues of interest and particulary for the determination of residues if the drug had been used immediately prior to the transport of pigs to slaughter.

The sponsors submitted new data (K34) showing that their HPLC method had been further developed and optimised to give better sensitivity and precision. Tissues (amount not given) were ground in a Waring blender and then homogenised in water. The chlorine analogue of azaperone was added as an internal standard before extraction with heptane-isoamyl alcohol (98.5:1.5, v/v). The extract was extracted into 0.1*N* sulphuric acid and back extracted at a high pH into the organic mixture. The extract was dried and dissolved in the HPLC mobile phase. HPLC was performed in the reverse phase with UV detection. Calibration curves were obtained for fortified tissues using azaperone and azaperol and a constant amount of internal standard. The concentrations in actual samples were obtained by interpolation on daily repeated calibration curves.

The recoveries of azaperone, azaperol and the internal standard were >90% for all tissues and plasma. The limit of quantification was 25 μg/kg for tissues and 5 μg/l for plasma. The accuracy in tissues ranged from 92 to 102% for azaperone and 92 to 109% for azaperol. The precision ranged from 1.7 to 6.5% for azaperone and 0.2 to 8.3% for azaperol. The residues were stable in deep frozen (-20°C) tissues and plasma for at least two months.
[Note: There is no repeatability study for incurred material and the internal standard and spikes are added to the extract after homogenisation.]

Table 4. Residues of azaperone and azaperol at the injection sites of pigs dosed intramuscularly with azaperone at 2 mg/kg body weight.

	Day 1	Day 2	Day 3	Day 5	Day 7
Azaperone					
Conc., range (mg/kg)	6.96 - 51.9	2.29 - 71.8	0.16 - 11.1	4.23 - 29.6	<0.025 - 0.16
Mean (mg)	31.6	24.9	4.11	11.9	0.045
Total, range (mg/kg)	2.99 - 19.1	0.99 - 36.6	0.07 - 4.16	1.93 - 9.68	0.066 (1)
Mean (mg)	13.4	12.1	1.60	4.22	0.066
Azaperol					
Conc., range (mg/kg)	1.29 - 8.25	0.37 - 3.84	0.03 - 1.68	0.54 - 1.44	<0.025 - 0.045
Mean (mg)	5.17	1.84	0.62	1.00	0.03
Total, range (mg/kg)	0.56 - 3.86	0.16 - 1.96	0.01 -0.63	0.17 - 0.48	0.019 (1)
Mean (mg)	2.21	0.86	0.24	0.38	0.019

The number in parenthesis indicates only one sample out of the four was positive.
Where no residue was detected the nominal value of the LOD was used for the calculation of the mean value.

APPRAISAL

Azaperone, a neuroleptic agent for use in pigs, was evaluated at the 38th JECFA. No ADI or MRL were recommended. JECFA requested "Studies on the concentrations of residues of azaperone and azaperol in both muscle and fat of pigs treated with azaperone over a three day period.".

The sponsors have answered the request in full and also provided further information on residues in other edible tissues (see Table 3).

Pigs were injected intramuscularly with azaperone at the recommended dose of 2 mg/kg body weight (BW). They were slaughtered in groups of four at 1, 2, 3, 5 and 7 days after the injection. The residues in edible tissues of both azaperone and azaperol were measured by a new and improved method using HPLC with an LOD of 25 μg/kg. The results are shown in Table 3. No residues were detected in any tissue at 5 and 7 days. The only residues detected at day 3 were residues of azaperone in one pig fat sample and of azaperol in one kidney sample. In the muscle samples, 19 out of 20 pigs had no detectable residues and one sample showed a residue of azaperone (but not azaperol) on day 2. The results for fat indicated that residues were found in all four pigs on day 1, two pigs on day 2 and one pig on day 3. The concentration of the residues in all tissues did not exceed 100 μg/kg at any sampling time.

Maximum Residue Limits

As suggested at the 38th meeting both kidney and liver were possible marker tissues. There was great variation in the ratios of azaperone to azaperol in different tissues and at different sampling time points and this made it almost impossible to use one of the compounds as the sole marker of the total residues. A more reliable marker of total residues was obtained if the sum of both compounds were used. The ratio of the concentration of azaperone plus azaperol as a percentage of total residues was not known for the recommended dose (2 mg/kg BW), however, for the 4 mg/kg BW dose it was possible to calculate the ratio over a three day withdrawal time. The mean value for the percentage at all sampling times was 27% (range 6 - 115%) and at 72h after injection the mean average percentage was 11%.

The residues as azaperone equivalents which have neuroleptic activity have to be considered in the setting of MRLs. However only azaperone and azaperol are thought to possess significant neuroleptic potency and are approximately 10% of the total residues in tissues; the remaining 90% of residues can be discounted in calculating the MRLs.

The Committee recommends temporary MRLs of 60 μg/kg for muscle and fat and 100 μg/kg for liver and kidney

of pigs expressed as the sum of azaperone and azaperol. Using these values for the MRLs and taking into account the factors above, the ingested residues of parent drug and azaperol are muscle (300 g) 18 μg, liver (100 g) 10 μg, kidney (50 g) 5 μg and fat (50 g) 3 μg. The total is 36 μg/day.

The Committee noted that there were residues of azaperone and azaperol at the injection site and that their concentrations can exceed the MRL set for muscle tissue during a 7 day withdrawal period.

REFERENCES

K1. V579. Heykants, J., Pardoel, L. and Janssen, P.A.J. (1971). On the distribution and metabolism of azaperone (R1929) in the rat and the pig. Arzneim.-Forsch. (Drug Res.) 21, 982-984.

K10. V1282. Sponsor submission of Janssen. Heykants, J. and Marsboom, R. The absorption, excretion and tissue distribution of azaperone (R1929) in the pig.

K19, V4874. Sponsor submission, originally of Pitman-Moore. Armand, F., Lange, Ph. (1976), Metabolism of azaperone.

K22. V8142. Sponsor submission of Janssen. Tissue residues in pigs after a single intramuscular dose of azaperone at 2 mg/kg.

K34. V8143. Sponsor submission of Janssen. (1992). HPLC-UV method for the determination of azaperone and azaperol in animal tissues and plasma: validation and application to the azaperone residue study in pigs after intramuscular administration.

V8672. Sponsor submission of Janssen. (1994). Executive review report on the toxicological, pharmacokinetic and pharmacodynamic documentation related to azaperone.

Rauws, A.G., Olling,J., Freudenthal, J. and Ten Ham, M. (1976). Azaperol, a new metabolite of the veterinary butyrophenone tranquillizer Azaperone. Toxicol. Appl. Pharmacol. 35, 333-339. [Also submitted by sponsor as Report K11, V1782]