BENTAZONE (172)

EXPLANATION

Bentazone was evaluated originally in 1991 (a minor correction to Annex I of the report was noted in 1992) and again in 1994. At the 27th (1995) Session of the CCPR the delegation of Germany, supported by France, drew attention to the residue definition for animal products. They preferred a definition which did not include metabolites, as in practice no residues of metabolites were found. These delegations were also of the opinion that the LOD was too low when metabolites were included in the residue definition for plant materials.

At the invitation of the Codex Secretariat comments were received from Germany, France and the USA. In addition the manufacturer submitted recent reports to clarify the residue situation. The new information is included in the present evaluation.

METABOLISM AND ENVIRONMENTAL FATE

Animal metabolism

The metabolism of bentazone, 6-hydroxybentazone and 8-hydroxybentazone was studied in lactating goats and hens.

One lactating goat (goat A) was dosed once a day with 3 mg ^{14}C-labelled active ingredient per kg body weight for 5 consecutive days, and a second goat (B) received 8 daily doses of 50 mg ai/kg bw. The dose levels correspond to 123 ppm and 1420 ppm in the feed respectively. The animals were slaughtered 24 and 4 hours after the final dose. Residue levels increased in the urine and faeces during the repeated administration of the compound. Most of the administered dose was eliminated in the urine (91.4% and 80.6%), while the faeces contained 0.6% and 5.6% respectively (BASF, 1990). The radioactivity extracted with methanol and water amounted to 98.5% from muscle, 98.1% from kidney, 95.9% from liver and 98% from fat. 90-100% of the residues in the milk were extractable with methanol. Most of the unextractable residues were released after incubation with pronase. The parent bentazone constituted about 71-96% of the total radioactive residues (TRR) in milk, 71-97% in muscle, 94-98% in fat, 91-98% in kidney, 83-84% in liver, 97-100% in urine and 71% in faeces. The bile and liver contained bentazone-*N*-glucuronide in addition to the parent compound. No 6-hydroxybentazone, 8-hydroxybentazone or AIBA (2-aminoisopropylbenzamide) could be found in the milk or tissues. Two minor metabolites at a concentration of 0.002 mg/kg bentazone equivalents were found in milk from the goat dosed at 3 mg/kg bw. The extractable residues measured in the milk and tissues are shown in Table 1 (BASF, 1991c).

Table 1. ^{14}C levels in the milk and tissues of lactating goats after dosing with 3 and 50 mg [^{14}C]bentazone/kg bw/day.

Sample	Residues, mg/kg, expressed as bentazone	
	3 mg/kg bw	50 mg/kg bw
Milk	0.029	0.29
Muscle	0.016	1.28
Fat	1.69[1]	2.85
Kidney	0.61	50.1
Liver	0.058	3.62

[1] Considered to be an outlier since it is not expected that bentazone, with a log P_{ow} of -0.45, would accumulate in fat

Two lactating goats were dosed with [^{14}C]6-hydroxybentazone at nominal dose levels of 2 mg/kg bw/day (goat A) and 40 mg/kg bw/day (goat B) corresponding to 41 ppm and 973 ppm in the feed. The single daily doses were administered for 5 and 6 consecutive days respectively. The animals were slaughtered 24 and 4 hours after the final dose. The proportions of the total administered dose excreted with the urine and faeces were 69.9% and 86.1% 4 and 24 hours after the last dose respectively (BASF, 1991a). The radioactive residues measured in various samples are shown in Table 2.

Metabolites produced by the high-dose goat were identified. In the milk, the main metabolite was identified as the sulfate of 6-hydroxybentazone (43% of the TRR), while 6-hydroxy-bentazone itself accounted for only 1%. In addition three minor metabolites were detected, each at about 5-6% of the TRR or 0.026-0.033 mg/kg bentazone equivalents. 6-hydroxybentazone was identified in the extracts of fat (94% of the TRR), kidney (73%), muscle (44%) and liver (44%). The proportion of conjugates amounted to 5% of the TRR in kidney, 7% in muscle, and 35% in liver. The liver conjugate was identified as the sulfate of 6-hydroxybentazone (BASF, 1995a). Urine and faeces contained mainly 6-hydroxybentazone.

A similar experiment was carried out with the administration of [^{14}C]8-hydroxybentazone. The doses were equivalent to 42 and 732 ppm in the feed. The proportions of the total administered dose excreted with the urine and faeces were 83.3% and 91.4% 24 hours after the final dose respectively (BASF, 1991b). The residues in the milk and tissues are shown in Table 2. The radioactivity in the urine and faeces consisted exclusively of 8-hydroxybentazone, whereas the residues in the bile included 62% of conjugates besides free 8-hydroxybentazone. Residues in the milk, muscle, fat, liver and kidneys were identified as unchanged 8-hydroxybentazone (29%, 61%, 82%, 75% and 95% of the TRR respectively) and conjugates thereof (41%, 21%, 5%, 11% and 3%; BASF, 1995b).

Table 2. Total radioactive residues in goat tissues and milk after administration of [^{14}C]6-hydroxy-bentazone and[^{14}C]8-hydroxybentazone.

Sample	TRR, mg/kg as hydroxybentazone, from			
	6-hydroxy, 41 ppm[1]	6-hydroxy, 973 ppm[1]	8-hydroxy, 42 ppm[1]	8-hydroxy, 732 ppm[1]
Milk	0.021	0.529	0.023	0.623
Muscle	0.011	0.24	0.012	0.581
Fat	0.027	0.948	0.007	0.396
Kidney	0.14	22.46	0.118	17.72
Liver	0.018	0.915	0.021	2.247

[1] Dosage expressed as equivalent level in feed

Samples from the three studies on goats described above were also analysed by a modified version of the “cold analytical procedure” (BASF, 1974). The results for milk and tissues, except liver, were in good agreement with the recoveries in fortification experiments. The low results for liver were explained by the presence of bentazone *N*-glucuronide. Methanol/HCl hydrolysis was found to be unsatisfactory for cleavage of the conjugates as it leads to the decomposition of the residues. Enzyme treatment was therefore used to release the conjugated residues.

Lactating goats were fed on a diet containing 15 ppm bentazone and 75 ppm 6-hydroxybentazone (low-level group) or 75 ppm bentazone and 150 ppm 6-hydroxybentazone (high - level group) for 21 days and were then placed on a residue-free diet. One animal from each group was slaughtered 22, 28 or 35 days after the commencement of dosing. Milk samples were collected on days 1, 7, 14, 21, 28 and 35 for analysis. Bentazone residues in all the milk samples were below the limit of determination (0.02 mg/kg). Residues of 6-hydroxybentazone from the low-dose group were <0.02 mg/kg in all samples but one (0.03 mg/kg on day 1) and the milk from the high-dose group contained residues in the range <0.02-0.07 mg/kg. No residue was detectable on the 7th day of the withdrawal period. Less than 0.1% of the applied 6-hydroxybentazone was transferred into the milk (BASF, 1981).

[^{14}C]bentazone, [^{14}C]6-hydroxybentazone and [^{14}C]8-hydroxybentazone were each administered orally to separate groups of 10 laying hens once daily for 5 days. The doses were 10 mg/hen/day, equivalent to feed containing about 100 ppm. Radioactivity was measured in the excreta and eggs from 5 hens of each group during the dosing period and up to 6 hours after the final dose, and in the tissues of all 10 hens of each group 6 hours after the final dose. The excretion of radioactivity was rapid. The mean proportions of the total cumulative dose recovered 6 hours after the final dose were 93.6% from the bentazone group, 90.2% from the 6-hydroxybentazone group and 93.1% from the 8-hydroxybentazone group. The mean concentrations of radioactivity were highest in the kidneys in all groups, followed by muscle, liver and whole blood.

After the administration of bentazone, the major radioactive component in extracts of liver, muscle, fat and eggs was the parent compound. Radioactivity in extracts of liver was associated with both bentazone (0.92 mg/kg) and its *N*-glucuronide conjugate (0.12 mg/kg). The excreta contained 45% of the total radioactive residue as bentazone, 12% as its *N*-glucuronide conjugate, and 15% as 6-hydroxybentazone.

After dosing with the 6-hydroxy- and 8-hydroxy- metabolites, the main residues in the excreta were the unchanged compounds and their glucuronide or sulfate conjugates.

The residues measured in eggs, muscle, fat and liver are shown in Table 3 (BASF, 1988).

Table 3. ^{14}C levels (expressed as mg/kg of the fed compound) in laying hens and eggs after dosing at 10 mg/bird/day with bentazone, 6-hydroxybentazone or 8-hydroxybentazone.

Sample	^{14}C, mg/kg equivalent of		
	Bentazone	6-hydroxy-B	8-hydroxy-B
Eggs (maximum)	0.15	0.023	0.029
Muscle (average)	0.39	0.032	0.023
Fat (average)	0.09	0.005	0.034
Liver	1.1	0.13	0.23

RESIDUES RESULTING FROM SUPERVISED TRIALS

The residues in crops found in supervised trials at recommended use rates are summarized in the 1991 and 1994 Evaluations.

The composition of residues in soya bean seed, forage and fodder was determined in 6 trials in two States of the USA (BASF, 1989). The summarized results are shown in Table 4.

Table 4. Residues of bentazone and its metabolites in soya bean seed, forage, hay, and fodder following two applications with 1.1 kg ai/ha.

Sample, PHI (days)	Residue range, ^{14}C as mg/kg bentazone and metabolite equivalents				Average total residue mg/kg[2]
	Bentazone	8-OH-bentazone	6-OH-bentazone	Total[1]	
Seed 78-119	<0.05	<0.05	<0.05	<0.05	<0.05 (N = 6)
Forage 24-38	<0.05-0.15	0.06-2.0	0.06-3.5	0.18-5.3	3.0-2.0 (N = 6)
Hay 36-53	<0.05-0.62	0.87-3.0	0.92-2.5	<2.2-5.9	3.3-1.5 (N = 6)
Fodder 78-119	<0.05-0.20	0.05-0.20	<0.05-0.39	<0.15-0.79	0.42-0.32 (N = 6)

[1] When none of the compounds exceeded 0.05 mg/kg of bentazone equivalents, the total is reported as <0.05 mg/kg
[2] Ranges are means at shortest and longest PHIs. When samples contained less than the LOD of one compound, its residue was assumed to equal its LOD in calculating the total residue and the mean

Residues in animal products

In the recent studies reviewed above the animals had been dosed at high levels to allow identification of metabolites. In order to estimate the expected maximum residue levels in animal products the feeding levels must be related to the maximum residues likely to occur following the use of bentazone according to GAP and to the typical composition of animal feeds.

The highest-residue diet for goats was calculated on the composition of cattle feed (EPA, 1982) assuming that goats consume the same feed as cattle in quantities proportional to their weights.

The highest national MRLs for bean forage (10 mg/kg), soya bean hay (8 mg/kg) and peppermint hay (4 mg/kg) are registered in the USA (BASF, 1995c). Taking into account the maximum percentage of these commodities in cattle feed (35%, 40% and 60% respectively)), a highest-residue diet was composited of 35% bean forage, 40% soya bean hay and 25% peppermint

hay. Since an average 550 kg cow consumes about 20 kg dry matter/day, the maximum daily intake can be calculated as follows. Dry feed contains 7 kg bean forage which is equivalent to 35 kg fresh material (20% dry matter, DM). This contains (35 kg x 10 mg/kg) = 350 mg residue. Similarly, the contributions of soya bean hay (DM = 86%) and peppermint hay (DM = 86%) to the intake of bentazone are 74.4 mg and 23.3 mg respectively. The total calculated maximum intake is about 448 mg/animal/day.

The residues in plants consist of bentazone, 6-hydroxybentazone and 8-hydroxybentazone. The hydroxy compounds need not be considered as residues in animal products because they are excreted rapidly by the animals and result in negligible residues. The proportions of the residue components, illustrated in the previous evaluations and in Table 4, vary with time and plant. For a maximum residue estimation a 1:1:1 ratio of bentazone and its two hydroxy metabolites can be assumed. Consequently the 448 mg total residue contains 149 mg bentazone which is equivalent to about 0.3 mg bentazone/kg bw/day. On the assumption of a similar intake for goats and cows, the goat A in the first metabolism study (p.??), given 3 mg/kg, was therefore overdosed by a factor of 10, and the goat B by a factor of 167 (50/0.3).

On the basis of the overdose factors, the calculated maximum residues in the edible products of goats are shown in Table 5.

The relatively high residues in the kidney and to a lesser extent in the liver of goat B can be attributed to the short interval between final dosing and slaughter, which is unlikely to occur in practice. The calculated residues in the liver and kidney of goat A therefore give a more realistic estimate of likely residues.

The factor for the overdosing of the hens in a maximum-residue situation can be calculated by assuming that the birds are eating only linseed (the highest national MRL is 1.5 mg/kg for the sum of bentazone and the two hydroxybentazones). The consumption by a 1.9 kg bird is 120 g feed. The factor for overdosing is 175. The corresponding residues are included in Table 5.

Table 5. Calculated bentazone levels in the milk and tissues of lactating goats, and the tissues and eggs of hens, from projected maximum intakes of residues in feed.

Sample	Goat A (slaughtered 24 after final dose)	Goat B (slaughtered 4 h after final dose)	Laying hens
Milk	0.003	0.002	-
Muscle	0.002	0.008	0.002 mean
Fat	0.17[1]	0.02	0.001 mean
Kidney	0.06	0.30-	-
Liver	0.006	0.02	0.006
Eggs	-	-	0.001 max.

[1] Considered to be an outlier since it is not expected that bentazone, with a log P_{ow} of -0.45, would accumulate in fat

METHODS OF RESIDUE ANALYSIS

The recent studies confirmed the applicability of the analytical procedure (BASF, 1974) described in the 1991 evaluation.

APPRAISAL

Bentazone was evaluated originally in 1991 and subsequently in 1992 and 1994. At the 27th Session of the CCPR the delegation of Germany, supported by France, suggested that residue definition for animal products should not include metabolites, as in practice no residues of metabolites were found. These delegations were also of the opinion that the LOD was too low when metabolites were included in the residue definition for plant materials.

Metabolism studies were conducted on goats and hens. In goats dosed at 3-50 mg ai/kg bw/day for 5 and 8 days, the parent bentazone constituted about 71-96% of the total radioactive residues (TRR) in the milk, 71-97% in muscle, 94-98% in fat, 91-98% in kidney, 83-84% in liver, 97-100% in the urine and 71% in faeces. The bile and liver contained bentazone *N*-glucuronide in addition to the parent compound. No 6-hydroxybentazone, 8-hydroxy-bentazone or AIBA (2-amino-*N*-isopropylbenzamide) could be found in the milk or tissues. When 6-hydroxybentazone and 8-hydroxybentazone were fed separately, residues were rapidly excreted (86.1% and 91.4% within 24 hours after the last dose respectively). The residues consisted mainly of 6-hydroxybentazone or 8-hydroxybentazone with smaller amounts of their glucuronide and sulfate conjugates.

In hens dosed at 10 mg ai/hen/day for 5 days, the major residue components were the parent bentazone and its glucuronide conjugate. No AIBA was detected.

Taking into consideration the highest residues which may occur in plant commodities and the composition of the feed of animals, it was concluded that the residues in meat, milks and eggs would not exceed the present draft MRLs of 0.05 mg/kg.

Since in the new studies no AIBA could be detected in any of the analysed tissues, milk, eggs or excreta even when extremely high doses were fed to goats and hens, the Meeting concluded that there was sufficient evidence to change the definition of the residues in animal products to bentazone alone. The change of the definition does not affect the estimated maximum residue level

(0.05* mg/kg) for meat, milks and eggs.

The Meeting also reconsidered the residue definition and the recommended limits for plant products. As the recommended maximum residue limits set at or about the limit of determination indicate undetectable residues, none of the residue components (bentazone, 6-hydroxybentazone and 8-hydroxybentazone) should be present in detectable concentration, and their LODs should not be summed. Each of the residue components can be determined individually with an LOD of <0.02 mg/kg. The LOD of 0.05* mg/kg therefore gives a 250% allowance for regulatory laboratories, where the analytical conditions might not be optimized as well as in laboratories specialized in the analysis of these compounds.

The Meeting therefore confirmed its previous recommendations for plant commodities.

RECOMMENDATIONS

In the light of the new animal metabolism studies the Meeting recommended a change in the definition of the residue in animal products.

Definition of the residue in animal products: bentazone

REFERENCES

BASF, 1974. Determination of bentazone and AIBA residues in cow and chicken tissue. BASF 74/5086. Unpublished.

BASF, 1981. Residues of bentazone and 6-hydroxybentazone in milk following dietary administration to lactating goats. BASF 81/10068. Unpublished.

BASF, 1988. The metabolism of ^{14}C-bentazone and its hydroxylated metabolites in hens. BASF 88/0432. Unpublished.

BASF, 1989. Magnitude of residues of bentazone and metabolites in soybean seed, forage, hay and fodder. BASF 89/5046. Unpublished.

BASF, 1990. Dosing of lactating goats with ^{14}C-bentazone (BAS 351 H). BASF 90/10351. Unpublished.

BASF, 1991a. Dosing of lactating goats with ^{14}C-6-OH-bentazone for the isolation and identification of metabolites. BASF 91/10702. Unpublished.

BASF, 1991b. Dosing of lactating goats with ^{14}C-8-OH-bentazone for the isolation and identification of metabolites. BASF 91/10703. Unpublished.

BASF, 1991c. The metabolism of ^{14}C-bentazone (BAS 351 H) in lactating goats. BASF 91/11137. Unpublished.

BASF, 1995a. The metabolism of ^{14}C-6-OH-bentazone in lactating goats. BASF 95/10011. Unpublished.

BASF, 1995b. The metabolism of ^{14}C-8-OH-bentazone in lactating goats. BASF 95/10062. Unpublished.

BASF, 1995c. Working paper prepared for 1995 JMPR. Unpublished.

EPA, 1982. Pesticide Assessment Guidelines, Subdivision O: Residue Chemistry. Unpublished.