Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

bentazone (ISO); 3-isopropyl-2,1,3-benzothiadiazine-4-one-2,2-dioxide

EC Number: 246-585-8
CAS Number: 25057-89-0

CLH-O-0000006912-71-01/F

Adopted

10 December 2020

10 December 2020

CLH-O-0000006912-71-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **bentazone (ISO);**
3-isopropyl-2,1,3-benzothiadiazine-4-one-2,2-dioxide

EC Number: **246-585-8**

CAS Number: **25057-89-0**

The proposal was submitted by **The Netherlands** and received by RAC on **2 October 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **28 October 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **10 January 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Nathalie Printemps**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **10 December 2020** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	613-012-00-1	bentazone (ISO); 3-isopropyl-2,1,3-benzothiadiazine-4-one-2,2-dioxide	246-585-8	25057-89-0	Acute Tox. 4* Eye Irrit. 2 Skin Sens. 1 Aquatic Chronic 3	H302 H319 H317 H412	GHS07 Wng	H302 H319 H317 H412			
Dossier submitters proposal	613-012-00-1	bentazone (ISO); 3-isopropyl-2,1,3-benzothiadiazine-4-one-2,2-dioxide	246-585-8	25057-89-0	**Retain** Skin Sens. 1 **Add** Repr. 2 **Modify** Acute Tox. 4 **Remove** Aquatic Chronic 3	**Retain** H317 H302 **Add** H361d **Remove** H412	**Retain** GHS07 Wng **Add** GHS08	**Retain** H317 H302 **Add** H361d **Remove** H412		**Add** oral: ATE = 1640 mg/kg bw	
RAC opinion	613-012-00-1	bentazone (ISO); 3-isopropyl-2,1,3-benzothiadiazine-4-one-2,2-dioxide	246-585-8	25057-89-0	Repr. 2 Acute Tox. 4 Skin Sens. 1	H361d H302 H317	GHS08 GHS07 Wng	H361d H302 H317		oral: ATE = 1600 mg/kg bw	
Resulting Annex VI entry if agreed by COM	613-012-00-1	bentazone (ISO); 3-isopropyl-2,1,3-benzothiadiazine-4-one-2,2-dioxide	246-585-8	25057-89-0	Repr. 2 Acute Tox. 4 Skin Sens. 1	H361d H302 H317	GHS08 GHS07 Wng	H361d H302 H317		oral: ATE = 1600 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Bentazone (ISO) is a herbicide approved as an active substance in plant protection products. It has an existing entry in Annex VI of the CLP Regulation. The dossier submitter (DS) addressed the following hazards: acute oral toxicity, skin sensitisation, reproductive toxicity and environmental hazards.

Bentazone technical dry active ingredient is a free acid. In the dossier, studies were also provided on bentazone sodium, which is a derivative of bentazone. RAC considers the studies performed on this sodium salt also relevant to address bentazone human health hazards.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute oral toxicity

Summary of the Dossier Submitter's proposal

Seven acute oral toxicity studies were available in rats, two in rabbits, two in Guinea pigs, one in dogs and one in cats. The calculated oral LD_{50} in rats varied from 850 to 2470 mg/kg bw. The lowest $LD_{50,}$ derived from the most reliable studies, was found to be 1640 mg/kg bw in rats (Doc. No. 83/114). Based on this study, supported by other reported LD_{50} values in rats, rabbits, cats and dogs, the DS proposed to classify bentazone as Acute Tox. 4 (H302). The DS also suggested an acute toxicity estimate (ATE) value of 1640 mg/kg bw.

Comments received during consultation

One Member States Competent Authority (MSCA) agreed with the proposed classification Acute Tox. 4 (H302) for acute oral toxicity.

Assessment and comparison with the classification criteria

Rats

Six acute oral gavage toxicity studies in rats were performed with bentazone as free acid. In addition, one study was available with bentazone sodium salts. Bentazone was administered as aqueous carboxymethyl cellulose (CMC) solution except in one of the studies. In this study (Doc. No. 69/0013), tragacanth suspension was used.

Only two studies were considered acceptable by the DS and rated Klimisch score 2 (Doc. No. 83/114 and 83/113). Other studies were only considered as supportive because test methods and results were not described in detail. Nevertheless, in these studies, RAC notes that details on the number of animals used and results on mortality were available and allow their use for classification purposes. The main limitation for most of the available studies was a lack of information on the purity of the test material.

Consistent results were obtained in rats between the studies. The LD_{50} values were all within the range of Acute Tox. 4 criteria (300-2000 mg/kg bw) except in one study were the LD_{50} was above 2000 mg/kg bw.

There is no clear indication that sex or strain greatly influence the toxicity of bentazone in rats. With regards to the vehicle, the lowest LD_{50} value was obtained in the oldest study using tragacanth. Nevertheless, similar results were obtained with CMC 8% suggesting that the vehicle may not be the main factor in the observed increased toxicity. Differences in purity of the tested technical material may explain the variations in the observed LD_{50} values. Nevertheless, data on purity are lacking to make a firm conclusion.

***Table**: Summary of LD_{50} values obtained in rats with bentazone*

Test material	**Strain**	**Mortality per dose groups (mg/kg bw, males/females)**	**LD_{50} results (mg/kg bw)**	**Ref. (Doc. No.)**
Bentazone in aqueous CMC 0.5% solution Purity: 93.9% n= 5/sex/group	Wistar	2610: 5/5 1780: 2/4 1210: 0/1	LD_{50}, female: 1470 (1080-1990 CI[1]) LD_{50}, male: approximately 1780 LD_{50}, combined: 1640 (1400-1920 CI)	83/114
Bentazone in aqueous CMC 0.5% solution n= 5/sex/group	Wistar	2610: 5/3 1780: 2/3 1210: 1/1 825: 0/2	LD_{50}, males: 1780 LD_{50}, females: 1790 LD_{50}, combined: 1710	83/113
Bentazone in aqueous CMC 0.5% solution Purity: 94.6% n= 10/sex/group	Sprague-Dawley	3732: 10/9 3110: 8/7 2592: 6/5 2160: 6/3 1800: 2/0	LD_{50}, males: 2340 (2208-2480 CI) LD_{50}, females: 2470 (2058-2964 CI)	78/053
Bentazone in aqueous CMC 0.8% n= 5/sex/group	Sprague-Dawley	2000: 5/5 1600: 4/4 1250: 2/4 1000: 0/2	LD_{50}, combined: 1220 (1056-1409 CI)	73/022
Bentazone sodium salts in aqueous CMC 0.8% n= 5/sex/group	Sprague-Dawley	2000: 5/4 1600: 3/2 1250: 1/2 800: 0/0	LD_{50}, combined: 1356 (1148-1601 CI) as free acid	73/023
Bentazone in aqueous CMC 8% n= 5/sex/group	Sprague-Dawley	2000: 5/4 1600: 5/5 1250: 3/3 1000: 4/1	LD_{50}, combined: 1050 (847-1302 CI)	72/051
Bentazone in 2-16% tragacanth suspension n= 5/sex/group	Sprague-Dawley	1600: 5/5 1250: 5/5 1000: 5/3 800: 2/2 400: 0/0 200: 0/0	Approximately 850	69/0013

[1]CI: 95% Confidence Interval

Rabbits

There are two studies available in rabbits. The first study is not considered acceptable as only 2 animals were used (Doc. No. 69/005). In the second study, insufficient details on study methods and study results (Neuschl *et al.*, 1993) did not allow RAC to assess the reliability of the study. Nevertheless, RAC agrees with the DS that both studies support the proposed classification as Acute Tox. 4 (H302) as the LD_{50} values were in the range of 300-2000 mg/kg bw.

Guinea pigs

Two studies were available in Guinea pigs (Doc. No. 74/035 and 91/10147). In these studies, bentazone was administered as 4-16% CMC solution. The combined LD_{50} was found to be about 1100 mg/kg bw in both studies. Detailed results on male and female were not available. The results of these studies also support classification as Acute Tox. 4 (H302).

Dogs and cats

Both studies in cats and dogs were considered unacceptable due to insufficient number of animals per groups. Moreover, according to the authors, it was not possible to calculate an LD_{50} in the dog study. In cats, an LD_{50} was found to be 500 mg/kg bw. Nevertheless, due to the low number of animals per groups, mortality rate dose-response was unclear: 1/2 at 2000 mg/kg bw, 1/2 at 1000 mg/kg bw and 3/6 at 500 mg/kg bw.

Overall conclusion

RAC agrees with the DS's proposal to **classify bentazone as Acute Tox. 4 via the oral route** based on the rat studies and supported by the other available studies.

Based on the most recent study in rats using bentazone with known purity (Doc. No. 83/114), RAC agrees with the DS to set an **ATE at 1600 mg/kg bw** (rounded value).

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS summarised in the CLH report two *in vivo* studies in Guinea pigs on skin sensitisation properties of bentazone, a Guinea pig maximisation test (GPMT) performed with bentazone and an open epicutaneous test (OET) performed with bentazone sodium formulation (600 g/L bentazone sodium).

In the GPMT assay, similar to OECD TG 406, bentazone was found to be a skin sensitiser. Positive reactions in 12/20 animals after the first challenge, 6/20 after the second challenge and 16/20 after the third challenge, were observed after intradermal induction with 5% bentazone (Doc. No. 86/195). Challenge was performed with bentazone as 50% test substance in aqueous solution.

No CLP criteria are available for classification based on an OET test. At a 50% concentration of sodium salts for induction and challenge, a positive response was observed in 25% of the animals after the first challenge and 38% after the second challenge (Doc. No. 86/221). No clear dose-response was observed in the assay as no reaction was observed when the undiluted test material was used.

Based on the positive response in the GPMT assay, supported by the positive results in the OET test, the DS concluded that bentazone should be classified as Skin Sens. 1 (H317).

According to the CLP criteria, a classification in category 1A is required, based on a GPMT study, when a positive reaction is observed in 60% or more of the animals after intradermal induction with 0.1-1%. In the GPMT, the positive response was observed at 5% but no lower intradermal concentrations were tested to fully exclude a classification in category 1A. Therefore, no sub-categorisation was proposed by the DS.

Comments received during consultation

One MSCA agreed with the DS' proposal.

One industry representative provided a negative Buehler assay on bentazone sodium formulation (700 g/L bentazone sodium) to support a sub-categorisation of bentazone in Skin Sens. 1B. The DS did not find a clear explanation for the inconsistency in the results between the negative Buehler and the positive maximisation study. Nevertheless, the DS responded that, based on the available data, classification for bentazone is warranted.

The results of a Buehler assay performed with a formulation containing 700 g/L bentazone sodium salts was provided by an industry representative during the generic consultation. The study was performed in male Hartley Guinea pigs according to OECD TG 406 (GLP compliant) and was negative. The undiluted test substance was topically applied using an occlusive 25 mm Hill top chamber. No sensitisation response was observed at challenge in both controls (n=10) or test animals (n=20) after 24 and 48 hours.

Assessment and comparison with the classification criteria

RAC agrees that based on the positive GPMT, a classification of bentazone as Skin Sens. 1 is warranted. The OET study is considered supportive of a classification but of lower weight than the GPMT study. The negative Buehler assay obtained with a formulation containing bentazone sodium is not sufficient to dismiss the positive results obtained with bentazone. The Buehler assay is of lower sensitivity than the GPMT. Overall, RAC agrees to **classify bentazone as Skin Sens. 1 (H317) without sub-categorisation** as sub-category 1A cannot be excluded based on the results of the GPMT.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

The DS based its evaluation on two multigeneration reproductive toxicity studies in rats. In these studies, no effects on parameters on sexual function and fertility were observed. Moreover, no effects on reproductive organs were observed in repeated-dose toxicity studies.

In addition, published studies on spermatogenesis and hormonal activity were reported in the dossier. Although a positive antiandrogenic effect was noted at high concentrations *in vitro* (Recombinant yeast assay), the antiandrogenic activity was not observed in higher tier studies. No other findings were seen.

Overall, the DS proposed no classification.

Development

In total, in rats, five oral prenatal developmental toxicity studies were available on bentazone technical. In addition, one study (including a preliminary study) was available with a formulation containing bentazone sodium (Agrichem file No. R22) and another was available with an unknown formulation containing bentazone (Al-Mahdi and Lofti, 1988). Only two studies in rats were considered reliable by the DS (Doc. No. 86/421 and Agrichem file No. R22). Other studies were only considered as supportive data.

Two prenatal oral developmental toxicity studies were available in rabbits and did not show developmental toxicity.

Based on post-implantation losses, not secondary to maternal toxicity, observed in some but not in all developmental toxicity studies in rats, the DS proposed to classify bentazone as Repr. 2, H361d.

In the repeated dose toxicity studies, bentazone induced blood coagulation impairments and haemorrhaging. A structural resemblance of bentazone with vitamin K and anticoagulants such as warfarine was suggested during the EFSA expert meeting on bentazone. The similarity was not supported by the DS. Moreover, as post-implantation losses were not observed with another anticoagulant, flocoumafen, the DS concluded that the increase in post-implantation loss observed with bentazone was not secondary to the potential decrease in coagulation impairments and haemorrhaging.

Effects on or *via* lactation were not evaluated in the CLH dossier.

Comments received during consultation

Sexual function and fertility

No specific comments were received.

Development

One MSCA considered the case borderline between Repr. Cat. 2 and Cat. 1B. The MSCA highlighted that the studies did not fully comply with the most recent version of the OECD TG 414 as a shorter duration of treatment was performed (gestation day (GD) 6-15 instead of GD6 to the day before sacrifice).

One industry representative considered that bentazone should not be classified. Two main reasons were highlighted.

- Resorptions observed in rats may have been secondary to maternal toxicity. Indeed, in the available studies, only food consumption and body weight effects were investigated while the most sensitive parameters identified in the repeated-dose dietary toxicity studies were water consumption, haematology, clinical chemistry and kidney weight. The bolus administration (gavage) may have enhanced these maternal effects.
- Resorptions observed following gavage administration may not be relevant to human under realistic exposure scenarios. Resorptions were not induced by bentazone following dietary administration. There is marked differences in toxicokinetic between bolus and dietary administration. Indeed, a possible saturation of excretion was identified after bolus administration starting between 80 and 160 mg/kg bw (mechanistic toxicokinetic study, Doc ID 2011/1262233).

The DS agreed that saturation of excretion was observed at lower dose of bentazone compared to higher dose in this toxicokinetic study. Nevertheless, they considered that it is unclear how this would be related to oral administration via the diet. Maternal toxicity, induced by gavage administration, may have been observed at dose where no effects were seen in the repeated-dose dietary toxicity studies. However, the DS pointed out that no relation between maternal toxicity and resorptions were seen in the developmental toxicity studies. Indeed, resorptions were not seen in the dietary teratogenicity study although maternal toxicity was observed. In addition, the DS also pointed out that the use of gavage resulted in a more constant and more precise exposure of rats during gestation as it is not affected by the fluctuation in food consumption. The DS also remarked that classification is based on intrinsic hazards of a substance and does not take into account exposure consideration.
The industry representative also provided several comments on the reliability of the available studies:

- The dietary study (Agrichem Doc. No. 22) should be scored Klimisch score 1 (reliable without limitations) instead of Klimisch score 3 (unreliable) due to dietary route of administration. Indeed, other routes of administration are allowed in the OECD TG 414 if justified. In this case, the study was intended to clarify if the observed effects by gavage would also be observed by dietary exposure. As resorptions were not observed via dietary exposure, resorptions may be the consequence of a peak plasma effect that would not occur under realistic condition of exposure. The DS agreed that the study was well performed and could be used for classification purposes.
- Developmental toxicity studies from 1971, (Doc. No. 71/0041) and the El-Mahdi and Lofti (1988), were of poor quality and should be excluded from the overall weight-of-evidence. The DS acknowledged the limitations and responded that these two studies were only used as supplementary data.

Finally, regarding mechanism of action, the industry representative disagreed that bentazone is structurally similar to warfarin. They pointed out that blood coagulation impairments were different. In addition, the blood effects induced by bentazone occurred at much higher concentration than the concentration where resorptions were seen. Thus, they considered that inhibition of blood coagulation is not a relevant mode of action for the increased post-implantation losses observed in the developmental rat toxicity studies.

Assessment and comparison with the classification criteria

Sexual function and fertility

No effects were seen in the multigenerational studies, available in rats. RAC notes that the top dose used in the 3-generation study (Doc. No. 73/010), around 20 mg/kg bw/d, may have been insufficient to fulfil the requirements of OECD TG 416. Nevertheless, in the 2-generation study (Doc. No. 89/0068), the top dose of bentazone (around 253 mg/kg bw/d) induced some toxicity. Therefore, the top dose in this study was sufficient to cover the reproductive potential of the test substance. Nevertheless, RAC notes that several endpoints (e.g. sperm parameters, sexual maturation) were not investigated as this was not recommended in OECD TG at the time of the study.

RAC agrees with the DS that the positive findings in the YAS-assay were insufficient for classification.

No relevant effects were noted in the repeated dose toxicity studies.

In conclusion, RAC agrees with the DS's proposal that **no classification for sexual function and fertility is warranted for bentazone**.

Development

In the developmental prenatal toxicity studies performed in rabbits, no effects relevant for classification were observed.

In rats, the main findings highlighted by the DS for classification were the increased incidence of resorptions in some studies. In addition, the DS pointed out that foetal delayed development was induced by bentazone at a non-maternally toxic dose.

<u>Resorptions</u>

The table below summarises the incidences of resorptions observed in the prenatal developmental toxicity studies performed by gavage (as described in the CLH dossier or study reports).

Study	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Maternal toxicity	Reliability (RAC assessment)
Bentazone				
Doc. No. 86/421 Wistar rats Oral, gavage GD6-15 N=25 Purity: 97.8% Vehicle: CMC Doses: 0, 40, 100, 250 mg/kg bw/d	100	250 (late resorptions: 0, 0, 0.3, 14.4% at 0, 40, 100 and 250, respectively)	250: Slight decrease in food consumption	According to OECD TG 414 Acceptable with limitations: shorter exposure duration, insufficient top dose level.
Doc. No. 78/039 SD rats Oral, gavage Purity: 92.5% GD6-15 N=27-29 Vehicle: CMC Doses: 0, 22.2, 66.7, 200 mg/kg bw/d	200	/	None (up to 200 mg/kg)	Similar to OECD 414 (Re-test of Doc. No; 71/0041) Acceptable with limitations: - non-GLP; - insufficient top dose level; - shorter exposure duration.
Doc. No. 71/0041 SD rats Oral, gavage GD6-15 N=20-32 Two control groups Purity: not reported Vehicle: 1% aqueous tylose suspension Doses: 0, 22.2, 66.7, 200 mg/kg bw/d	66.7	200 (total resorptions: 66.3 % vs 7.6% in control, due to late resorptions)	None reported in the CLH dossier	Similar to OECD TG 414 Reliability not assignable: - study report in German; - no GLP status; - stability of test material not checked (analysis not performed); - no statistical analysis reported; - purity not provided.
Formulation containing bentazone				
Agrichem file No. R22 Formulation containing bentazone sodium salts (purity: 600 g/L bentazone Na) Wistar rats Oral, gavage GD6-15 N=25 Vehicle: water Doses: 0, 5, 30, 180 mg/kg bw/d	360 (eq. to about 216 mg/kg bw /d bentazone sodium and 198 mg/kg bw/d bentazone as free acid*)	/	360 mg/kg bw/d (eq. to 198 mg/kg bw/d bentazone free acid*): slight reduction in bw and food consumption	According to OECD TG 414 Acceptable with limitations: - insufficient top dose level: top dose not chosen based on maternal toxicity in the dose-range finding study (none observed) - short exposure duration
Dose range-finding study, Agrichem file No. R463 Formulation containing bentazone sodium salts	50 (eq. to 30 mg/kg bw/d bentazone Na or 27.5 mg/kg bw/d	150 (total resorptions) 450 (eq. to about 247 mg/kg bw/d pure bentazone*) (late resorptions 29% vs 0% in	None (up to 450 mg/kg bw/d), no effects seen at necropsy	Non-guideline range-finding study

(purity: 600 g/L bentazone Na) Wistar rats N=5 GD6-15 Vehicle: 4% CMC aqueous solution Doses: 0, 50, 150, 450 mg/kg bw/d	bentazone as free acid*)	controls)		
El-Mahdi and Lofti, 1988 Basagran formulation containing bentazone Single administration (GD8, 11,14 or 16) Sacrifice at GD 20 Doses: 0, 12, 43.2, 96 mg/kg bw/d	/	25 (eq. to 12 mg/kg bw/d bentazone) (increased resorption rate)	Not reported	Unreliable - non-GLP - not similar to OECD TG 414 - purity and origin of test material not provided - no information on the coformulants - low number of animals (reported 3/groups) - no foetal visceral examination - no detailed results - vehicle not reported - maternal toxicity not reported

*considering a purity of 60% and that 1 mole of bentazone sodium will give 0.916 moles of bentazone

The table below summarised the incidences of resorptions observed in the prenatal developmental toxicity studies performed by dietary administration (as described in the CLH dossier or study reports).

Study	**NOAEL (mg/kg bw/d)**	**LOAEL (mg/kg bw/d)**	**Maternal toxicity (mg/kg bw/d)**	**Reliability (RAC reliability assessment)**
Bentazone				
Doc. No. 84/066 SD rats Oral, diet N=23 GD0-21 Purity: 93.9% Doses: 0, 162, 324, 631 mg/kg bw/d	631	/ (Early resorption observed in 11/13 implants in the dams with intrauterine haemorrhage (402)[1]. No increased resorption vs control observed in other dams)	631: emaciation and intrauterine haemorrhage in one animal, some clinical signs, decrease bw gain and food consumption, and increased water consumption	Similar to OECD TG 414 Acceptable
Doc. No. 89/0068 2-generation study Wistar rats Oral, diet Purity: 97.8% Doses: 0, 200, 800, 3200 ppm	238	/	238: reduced food consumption and body weight 56: reduced food consumption	According to OECD TG 416 Acceptable
Doc. No. 73/010 3-generation study SD rat Oral, diet Doses: 0, 20, 60, 180 ppm	15	/	/	Acceptable with limitations - not similar to OECD TG 416 - prior to GLP - no information on origin and purity of test material - insufficient dose levels

[1] Information retrieved by RAC in the study report

Oral gavage studies

Post-implantation losses were increased in two developmental toxicity studies (Doc. No. 86/421 and Agrichem file No. R463). In both studies, the increase was observed at the top dose only (≥ 250 mg/kg bw/d) and was due to late resorptions.

The first study (Doc. No. 86/421) was performed according to OECD TG 414 with some deviations. The main limitation was the insufficient top dose level as no maternal toxicity was observed. Post-implantation losses were statistically significantly increased at the top dose level of 250 mg/kg bw/d. At this dose, a decrease in the number of live foetuses was noted. A statistically significant reduction in foetal weight and an increase of foetuses with delayed ossifications were also noted at 250 mg/kg bw/d. At this dose, female rats did not show any signs or symptoms. No differences in mean body weight gain were noted in dams. Only a slight statistically significant decrease of mean food consumption was noted from GD6-11.
The table below summarises the observed resorptions in this study:

Dose (mg/kg bw/d)	**0**	**40**	**100**	**250**
Post-implantation losses (%)[1]	7.4	8.3	8.7	22*
Embryonic resorption (%)[1]	7.4	8.3	8.4	7.5
Foetal resorption (incidence) (%)[1]	0 0	0 0	1 0.3	44 14*
Foetal weight (%)	4.8	4.9	4.9	4.2*

*p<0.05; [1] Information retrieved by RAC in the study report

The second positive study is a range-finding prenatal developmental toxicity study (Agrichem file No. R463). In this study, doses up to 450 mg/kg bw/d of a formulation containing bentazone sodium have been tested (eq. to around 270 mg/kg bw/d bentazone sodium or 247 mg/kg bw/d bentazone as free acid). At the top dose, an increase in late resorption was noted. The increase in late resorptions was well above the historical control values provided in the study report of the main study (maximum 1.3% or 4 incidences in 9 studies). An increase in early resorption was also noted in the study but without dose-response. At 450 mg/kg bw/d, female rats did not show any toxicity (body weight gain, food consumption, clinical signs). No differences in mean body weight gain were noted. Only a slight statistically significant decrease of mean food consumption was seen from GD6-11. The table below summarises the observed resorptions in this study:

Dose (mg/kg bw of the formulation)	**0**	**50**	**150**	**450**
Post-implantation losses (%)	9.7	13.6	29.3**	39**
Embryonic resorption (%)[1]	9.7	13.6	29.3**	10.2
Foetal resorption (%)[1] Incidence	0	0	0	28.8 ** 17

** p<0.01; [1] Information retrieved by RAC in the study report

In the main study (Agrichem file No. R22), the top dose level was reduced to 360 mg/kg bw/d instead of 450 mg/kg bw/d used in the range-finding study. As no maternal toxicity was noted in the range-finding study at this dose, the use of a lower top dose level is questionable. No resorptions were seen up to 360 mg/kg bw/d of the test article (around 216 mg/kg bw/d bentazone sodium or 198 mg/kg bw/d bentazone free acid). The use of a lower top dose level in this study does not allow assessors to confirm or dismiss the resorptions induced at higher dose levels in the range-finding study.

In the study Doc. No. 71/0041, an increase in the incidence of resorptions (also mainly due to late resorption according to the German study report) was observed at 200 mg/kg bw/d in the absence of maternal toxicity. In this study, no analytical check of the substance was performed. Anasarca was also noted in the study. Nevertheless, these effects were not observed in a retest

study (Doc. No. 78/039). Thus, RAC considered the results observed in Doc. No. 71/0041 questionable at 200 mg/kg bw/d. This is supported by the absence of resorptions seen at around 200 mg/kg bw/d in the Agrichem file No. R22 study.
The top dose used in the retest study Doc. No. 78/039 was below the dose at which resorptions occurred in the two positive studies. The top dose of 200 mg/kg bw/d did not induce any maternal toxicity.

Overall, late resorptions were induced by bentazone at ≥ 250 mg/kg bw/d in two studies (Agrichem file No. R463 range-finding study and Doc. No. 86/421). Uncertain results were observed at 200 mg/kg bw/d in Doc. No. 71/0041 as the effects were not observed at this dose in a retest study and in the main Agrichem file No. R22 study. Only slight maternal toxicity was noted at ≥ 250 mg/kg bw/d.

Dietary studies

One dietary study (Doc. No. 84/066), performed with bentazone, was performed with a method similar to OECD TG 414. No increase resorptions were seen up to 631 mg/kg bw/d. At this dose, maternal toxicity was noted as shown by some clinical signs, decreased body weight gain, food consumption, and increased water consumption. Resorptions (early embryonic resorption) were only seen in one dam in the study at this dose. This dam showed emaciation and intrauterine haemorrhage. No late resorptions were seen in this dam.

No post-implantation losses were noted in the multigenerational studies (Doc. No. 73/010 and 89/0068) performed in rats up to 238 mg/kg bw/d.

Overall, no post-implantation losses were noted following dietary administration up to 631 mg/kg bw/d bentazone.

Delayed development

Decreased foetal weight was also noted in most of the gavage studies. This effect was not associated with maternal toxicity. Delayed development was also noted in the study Doc. No. 86/421.

In the two-generation toxicity study (Doc. No. 89/0068), the highest dose levels caused a decrease in body weights of pups during lactation. Reduction in food consumption and weight gain, as well as kidney and liver effects in parents were observed.

Gavage vs dietary exposure

The mechanistic toxicokinetic study (Doc. No. 2011/1262233) investigated the area under the curve (AUC) vs. dose ratio relationships following gavage administration (40 to 500 mg/kg bw) of bentazone.

The AUC versus dose ratio relationships indicated that the internal dose is over proportional to the oral gavage dose. According to the summary of the study, "*this effect may be based on active excretion of the test substance or its metabolites with saturation at higher doses, yielding to over proportional internal doses with increasing dose when a threshold dose (saturation of excretion) is reached. Within the current study, this effect starts between actual dose levels of 84.7 and 165.0 mg/kg bw (calculated as bentazone-sodium corresponding to 1.09 x dose of bentazone acid).*"

Saturation at higher doses is acknowledged and may lead to a non-linear dose-response curve at these higher dose levels by gavage. There is no relationship with dietary exposure provided in the dossier to compare to those results obtained by gavage.

RAC agrees with the DS that the classification is based on the intrinsic hazard properties of the substance. There is no evidence in the dossier suggesting that gavage oral studies would not be relevant for hazard assessment.

Maternal toxicity

Only slight maternal toxicity was noted in the gavage studies at around 250-270 mg/kg bw/d. No repeated dose toxicity studies were available by gavage. It is unknown if other maternal effects (blood effects, kidney effects, water consumption) were present in these studies.

In the dietary prenatal developmental toxicity study (Doc. No. 84/066), maternal effects were noticeable at ≥ 631 mg/kg bw/d.

In the rat dietary sub-acute toxicity study (Doc. No. 81/10240), performed with bentazone, haemorrhages were seen in kidneys and ovaries at ≥ 555 mg/kg bw/d. At ≥ 1000 mg/kg bw/d, in addition to mortality and body weight changes, changes in haematological parameters were noted (haematocrit, haemoglobin, and prolonged blood coagulation time). In the 90-day rat study (Doc. No; 87/0173), changes in haematological parameters, and prolonged blood coagulation time was seen at ≥ 250 mg/kg bw/d in the presence of mortality in males (4/10 males) and body weight changes in females. In the older 90-day study (Doc. No. 70/008), only changes in kidney weight were noted at about 80 mg/kg bw/d in the presence of retarded body weight gain.

Specific organ toxicity was generally reported in the presence of general toxicity in rats (retarded body weight gain or food consumption effects). Although potential blood findings could be expected in the dietary study at 631 mg/kg bw/d (Doc. No. 84/066), no resorptions were seen. There was therefore no clear relationship between maternal toxicity and the induction of resorptions.

It may also be noted that in the dietary prenatal developmental toxicity study, intrauterine haemorrhage and a body weight decrease were seen in one dam at the top dose (according to the study report). In this dam, only early resorptions were observed and no late resorptions were seen. This finding supports the view that the increase in haemorrhages may not always result in an increase in post-implantation losses due to late resorptions observed with bentazone.

In addition, RAC agrees that there is no structural similarity with the anticoagulants such as warfarin and that known anticoagulants did not consistently induce these types of effect.

Thus, overall RAC agreed with the DS that the increase in late resorption observed in the gavage studies could not be attributed to maternal toxicity.

Comparison with criteria

Evidence for developmental effects associated with bentazone was observed in rats.

Reduced foetal weight observed at non-maternal toxic dose in most of the gavage studies may be treatment-related but are insufficient for classification.

Effects on post-implantation losses (due to foetal resorptions) are relevant for classification. The effects were seen in two prenatal developmental gavage studies testing doses ≥ 250 mg/kg bw/d. RAC considers the effects not secondary to maternal toxicity. At lower dose, resorptions were only induced in one out of 3 studies, but an analytical check of the test substance was not performed. In the three studies lacking effects, top dose levels were only up to 200 mg/kg bw/d.

No effects were seen in rabbits or in rat studies with dietary administration.

As the classification is based on the intrinsic properties of the substance, studies performed by gavage in rats were considered relevant for classification.

RAC notes that in all the gavage studies, insufficient dose levels were used, and the study duration was shorter than recommended in the current OECD TG 414. Developmental toxicity was

probably not sufficiently investigated in these studies and more particularly in studies lacking effects.

Overall, based on post-implantation losses and delayed development, both considered not to be secondary to maternal toxicity, a classification of bentazone is warranted. RAC agrees with the DS proposal to **classify bentazone as Repr. 2 (H361d).** Category 1B is not considered appropriate in view on the inconsistencies observed.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Bentazone acts as a selective post-emergent herbicide against broadleaved weeds and is currently classified as Aquatic Chronic 3 – H412 in Annex 3.1 of the CLP Regulation. The DS proposed to remove the classification based on:

- Aquatic plants being the most sensitive species under acute testing with a 7d-E_rC_{50} of 12 mg/L, which was above the classification cut-off 1 mg/L. Thus, bentazone is not to be classified for acute aquatic toxicity.
- Aquatic plants were the most sensitive species under chronic testing with a 7d-E_rC_{10} of 3.2 mg/L. The substance was not rapidly degradable and had low potential for bioaccumulation. Considering that the lowest effect concentration was above cut-off of 1 mg/l for not rapidly degradable substances, bentazone is not to be classified for chronic aquatic toxicity.

Degradation

Abiotic degradation

Bentazone contains no functional groups that can hydrolyse such as esters, amides or epoxides. No hydrolysis was observed in aqueous photolysis studies in dark conditions. The DS concluded that bentazone was hydrolytically stable at 25°C at pH 5, 7 and 9.

There were three studies available on phototransformation in water which showed that direct photolytic degradation in water occurred. The DT_{50}s ranged from 47.8 hours to 5.12 days in sunlight. Several photolytic degradation products were formed. Degradation products exceeding 10 % were identified in a separate study: 3-isopropyl-2,3-dioxo-5-oxocyclopenteno [d]-1 H-2,1,3-thiadiazine-4(3H)-one-6-carbonic acid, 2-[(isopropyl-amino)-carbonyl] phenylsulfamic acid) and 8-hydroxy bentazone. According to the DS, this occurred under laboratory conditions and it seemed unlikely that under environmentally more relevant conditions, e.g. deeper and more turbid waters with lower light intensities, the transformation products would be formed in significant amounts. The EFSA conclusion document on bentazone noted a low risk to aquatic organisms from these three transformation products (EFSA Journal 2015;13(4):4077).

The estimated half-life in air (hydroxyl radicals) was 2.06 hours (QSAR, AOPWIN v.1.92a).

Biotic degradation

There was no experimental ready biodegradability study available. QSAR estimations with BIOWIN v.4.10 models 5 and 6 predicted bentazone to be not readily degradable.

In a non-guideline, non-GLP biodegradation screening study, degradation after 117 days at 20°C based on residual measurements was 0% at 10 and 20 mg/L.

In a non-GLP BBA[1] guideline IV 5-1 water/sediment study using ^{14}C-bentazone under aerobic conditions two water/sediment systems were tested for 100 days at 20° at dark at test concentration of 0.34 mg/L. The degradation DT_{50}s for the total system were 688 and 940 days at 20°C. Dissipation DT_{50}s were 701 and 678 days in water and 595 and 568 days in sediment. Mineralisation was 2.6% after 100 days. One transformation product was detected at >10% i.e. N-methylbentazone, reaching maximum of 13% AR in one of the systems with the formation being reversible. Non-extractable residues amounted to approximately 15% AR in both systems at test end. The DS also presented one water/sediment study and one water/soil study to be used as supportive for the low biodegradation potential of bentazone. No DT_{50}s could be determined in these studies. Mineralisation was low in both studies.

Regarding the transformation product N-methylbentazone, the EFSA conclusion document noted that it was more toxic to fish and aquatic invertebrates than bentazone (see Aquatic toxicity).

There were four aerobic soil degradation studies available:

	SETAC Lysimeter Guideline, non-GLP	**OECD TG 307, GLP**	**OECD TG 307, GLP**	**OECD TG 307, GLP**
DT_{50}, days	15.9 at 20 °C (dissipation) 610 (degradation; NER attributed to parent)	45.1 at 20°C (dissipation) 352 (degradation; NER attributed to parent)	18.5 at 20°C (dissipation)	30.9, 33.0, 43.4, and 49.1 days at 20 °C (dissipation)

The DS concluded that bentazone was slowly degraded in soil and that determination of degradation DT_{50} values was hampered by adsorption of bentazone to soil.

The DS also presented one draft OECD TG 307 study where mineralisation of 14.9% AR after 117 days showed that bentazone is not quickly mineralised, which was used as supportive data.

The DS concluded that bentazone is not rapidly degradable for classification purposes.

Bioaccumulation

Bioaccumulation of ^{14}C-labelled bentazone was studied in *Lepomis macrochirus* under flow-through conditions according to US EPA 165-4 and OECD TG 305. Fish were exposed to 5 mg/L bentazone for 28 days. Steady state condition was reached after day 7. The reported BCF values were 0.4, 2.2 and 1.4 L/kg for edible, non-edible and whole body, respectively. Lipid content was not measured, and the fish length and weight at the end of the test were not reported. Thus, correction for growth dilution and normalisation to 5% lipid content could not be done. Metabolites were measured but no data were presented thus the reported BCF values were based on total radioactivity. The BCF values were well below the classification threshold of 500 L/kg, which indicated a low potential for bioaccumulation.

There were two studies that determined the n-octanol/water partitioning coefficient of bentazone under acidic, neutral and alkaline conditions. Both studies were conducted according to EEC

[1] Biologische Bundesanstalt für Land- und Forstwirtschaft - Federal Biological Research Center for Agriculture and Forestry

method A8, i.e. shake flask methodology. For non-charged substances the ratio of the concentration in n-octanol and water is referred to as the log K_{ow}. The charge of ionisable organic compounds, like bentazone, is depended on pH, and thus the distribution at a certain pH is referred to as log D_{ow}. The log D_{ow} determined at a pH where the substance is present in the neutral form corresponds to the log K_{ow}. For bentazone log D_{ow} values of 1.54, -0.94 and -1.32 had been determined at pH 4, 7, and 9 in one test and 0.77, -0.46 and -0.55 at pH 5, 7 and 9 in the other test, respectively. The pKa of bentazone had been reported to 2.50 and 3.51, and the molecule was increasingly present in the neutral form at pH values below the pKa. The highest log D_{ow} of 1.54 determined at pH 4, which could be considered representing a worst-case for environmental conditions, approached the log Kow. As the highest log D_{ow} of 1.54 was well below the classification threshold of log K_{ow} 4, bentazone was considered to have a low potential for bioaccumulation.

Aquatic toxicity

Bentazone can degrade to N-methylbentazone (max 13%) in water-sediment systems, with the formation being reversible. The EFSA conclusion document noted that N-methylbentazone was more toxic to fish and aquatic invertebrates than bentazone[1]. Considering that degradation of bentazone in water was a slow process, that the formation of N-methylbentazone was reversible, and that the presence of N-methylbentazone would result in a more conservative assessment of bentazone toxicity, the classification was conducted by the DS based on studies conducted with bentazone.

Some of the available studies were performed with the sodium salt of bentazone. The ecotoxicological results with sodium bentazone were considered relevant for bentazone because in solution and, therefore, also in biological systems both substances will dissociate and form the same anion. In case of quantitative results, the extrapolation was corrected for differences in molecular weight.

Acute Aquatic Toxicity

Table: *Summary of reliable information on acute aquatic toxicity*

Method	**Test material**	**Species**	**Results (***	**Remarks**	**Reference, reliability**
Fish					
OECD TG 203, GLP, static	Bentazone 94%	*Lepomis macrochirus*	96h-LC_{50}: >100 mg/L, no effects	nominal (actual conc. ~100% of nominal)	Anonymous 1986a Klimisch 1
OECD TG 203, GLP, static	Bentazone-Na 100%	*Cyprinus carpio*	96h-LC_{50}: >916 mg/L, no effects (corrected for sodium)	nominal (actual conc. ~100% of nominal)	Anonymous 1983 Klimisch 2

[1] In the RAR the following aquatic toxicity data were reported for N- methylbentazone: fish 96h-LC50 of 8.56 mg/L (mean measured); fish 28d-NOEC of 0.23 mg/L (nominal with actual ~100% of nominal); aquatic invertebrates 48h-LC50 of 26.5 mg/L (mean measured); daphnia 21d-NOEC of 2.0 mg/L (nominal with actual within 20% of nominal); algae 72h-ErC50 of 37.7 mg/L (nominal with actual within 20% of nominal); and lemna 7d-ErC50 of 35.8 mg/L (mean measured).

OECD TG 203, GLP, static	Bentazone-Na 91.9%	*Pimephales promelas*	96h-LC_{50}: >104 mg/L, no effects (corrected for sodium)	limit test, mean measured	Anonymous 2011a Klimisch 2
ASTM E 729-88; EPA FIFRA-E 540/9-82-024, GLP, flow-through, sea water	Bentazone (BAS 351 H-tech a.i.) Purity: 53.0%; no information on impurities	*Cyprinodon variegatus*	96h-LC_{50}: >136 mg/L, no effects	mean measured	Anonymous, 1991 Klimisch 2
Aquatic invertebrates					
OECD TG 202, GLP, static	Bentazone 98.4%	*Daphnia magna*	48h-EC_{50}: >100 mg/L no sublethal effects	nominal (actual conc. ~100 % of nominal)	Jatzek, 2003b Klimisch 2
EPA-E 540/9-82-024, ASTM E 729-88, GLP, sea water	Bentazone (BAS 351 H-Tech a.i.) Purity: 53.0%	*Mysidopsis bahia*	96h-LC_{50}: >132.5 mg/L bentazone no sublethal effects	mean measured	Graves and Smith, 1991a Klimisch 1
EPA-E 540/9-82-024, ASTM E 729-88, GLP, sea water	Bentazone (BAS 351 H) Purity: 53.0%	*Crassostrea virginica*	96h-LC_{50}: >109 mg/L bentazone 38.3% shell inhibition at 109 mg/L	mean measured	Graves and Smith, 1992a Klimisch 2
Algae and other aquatic plants					
OECD TG 201, GLP, static	Bentazone (BAS 351 H) Purity: 98.4%	*Pseudokirchneriella subcapitata* (current name *Raphidocelis subcapitata*)	72h-E_rC_{50}: 33.3 mg/L	nominal (actual conc. ~100% of nominal, but determined in replicate without algae)(**	Jatzek, 2003b Klimisch 2
OECD TG 221, GLP, static	Bentazone Purity: 100%	*Lemna gibba*	7d-E_rC_{50}: 12.0 mg/L (dry weight) 25.3 mg/L (frond numbers)	mean measured (78.4-104.8 % of nominal)	Hoffmann, 2011b Klimisch 1
OECD TG 221; OPTS 850.4400 (draft), GLP, static	Bentazone-Na Purity: 91.9%	*Lemna gibba*	7d-E_rC_{50}: 17.0 mg/L (dry weight) 21.0 mg/L (front numbers) (corrected for sodium)	mean measured (74.8-110.3 % of nominal)	Hoffmann 2011a Klimisch 1

(* The Dossier Submitter had corrected the results for purity. RAC does not see this as a common practice and hence no correction for purity is done in this table.

(** considering that the log K_{ow} is 2.34 and the low adsorption potential of bentazone, (median Kf,oc value of 25.2 L/kg) this is not seen to invalidate the study (dissipation of the substance due to binding to algae is not foreseen)

There were four reliable studies available with 96-h LC_{50} values for fish ranging from >100 to >916 mg/L. No effects were observed in the studies.

There were data available for three aquatic invertebrate species, i.e. a 48h-EC_{50} of >100 mg/L for *Daphnia magna*, a 96h-LC_{50} of >132.5 mg/L for *Mysidopsis bahia* and a 96h-EC_{50} of >109 mg/L for *Crassostrea virginica.*

In the only available reliable algae test, the 48-hour ErC_{50} was 33.3 mg/L for *Pseudokirchneriella subcapitata*. In the two *Lemna* tests the mean measured 7-day E_rC_{50} values were 12.0 mg/L (dry weight) and E_rC_{50} of 17.0 mg/L (dry weight). The respective E_rC_{50} values for frond number were 25.3 mg/L and 21.0 mg/L.

The DS concluded that aquatic plants were the most sensitive species, with a 7d-E_rC_{50} of 12 mg/L. The value is above the classification cut-off value of 1 mg/L and the DS concluded that bentazone does not warrant classification for acute aquatic toxicity.

Chronic Aquatic Toxicity

Table: *Summary of reliable and relevant information on chronic aquatic toxicity*

Method	Test material	Species	Results	Remarks	Reference, reliability
Fish					
OECD TG 210; US EPA-FIFRA 72-4; EPA-OPPTS 850.1400, GLP Fish early life stage toxicity test Flow-Through	Bentazone-Na (BAS 351 H-Na) Purity: 91.9%	*Pimephales promelas*	35d-NOEC (survival, body weight and length): 9.0 mg/L (corrected for sodium) limit test	mean measured	Anonymous, 2011b Klimisch 1
Aquatic invertebrates					
OECD TG 211, GLP, semistatic	Bentazone 480 g/L SL (formulated product) Purity: 480 g/L (~40% bentazone)	*Daphnia magna*	21d-NOEC: 32 mg/L (expressed as bentazone)	nominal (actual conc. ~100% of nominal)	Migchielsen, 2001 (** Klimisch 2
OECD TG 211, GLP, semistatic	Basagran (BAS 351 32 H; formulated product) Purity: 40.4% bentazone	*Daphnia magna*	21d-NOEC (reproduction): 101 mg/L (expressed as bentazone)	nominal (actual conc. ~100% of nominal)	Jatzek, 1989a (** Klimisch 2

Algae and aquatic plants					
OECD TG 201, GLP, static	Bentazone (BAS 351 H) Purity: 98.4%	*Pseudokirchneriella subcapitata* (current name *Raphidocelis subcapitata)*	72h-E_bC_{10}: 9.9 mg/L	nominal (actual conc. ~100% of nominal, but determined in replicate without algae) (***	Jatzek, 2003b Klimisch 2
OECD TG 221, GLP, static	Bentazone Purity: 100%	*Lemna minor*	7d-E_rC_{10}: 3.2 mg/L (frond numbers) 3.3 mg/L (dry weight)	mean measured (78.4-104.8 % of nominal)	Hoffmann, 2011b Klimisch 1
OECD TG 221, GLP, static	Bentazone-Na Purity: 91.9%	*Lemna minor*	7d-E_rC_{10}: 3.2 mg/L (dry weight) 3.5 mg/L (frond number) corrected for sodium	mean measured (74.8-110.3 % of nominal)	Hoffmann, 2011a Klimisch 1

(* The Dossier Submitter had corrected the results for purity. RAC does not see this a common practice and hence no correction for purity is done in this table.

(** These two references had changed place in the CLH Report – Now corrected according to RAR

(*** considering that the log Kow is 2.34 and the low adsorption potential of bentazone, (median Kf,oc value of 25.2 L/kg) maintenance of the test concentrations is expected (dissipation of the substance due to binding to algae is not foreseen)

The DS also presented in the CLH Report studies that were rated Klimisch 3 and 4. These included acute and chronic fish tests, acute *Daphnia* tests, six algae tests, and two *Lemna* tests. The DS did not use this data for classification. One *Lemna* test was considered reliable but the result was given only as an E_yC_{50} value and, thus, was not used for classification.

The only reliable chronic fish test was a 35-day Fish early life stage toxicity test with *Pimephales promelas*. The test was performed as a limit test. The mean measured 35d-NOEC was 9.0 mg/L for survival, body weight and length.

There were no chronic invertebrate studies available with bentazone. There were two reliable studies with formulated products, and as such it could not be excluded that effects could at least partially be attributed to other constituents of the formulations. However, since there are no long-term toxicity studies with aquatic invertebrates exposed to just bentazone, the available data was used by the DS, representing a worst-case approach. The studies report 21-day NOECs of 101 and 32 mg/L when expressed as bentazone. Both values are based on nominal test concentrations that have been analytically verified.

There was one reliable algal inhibition study yielding an E_rC_{10} of 9.9 mg/L. For the duckweed *Lemna* there were two reliable studies with the growth rate endpoint. The lowest toxicity values were a mean measured E_rC_{10} of 3.2 mg/L (frond numbers) from one study and an E_rC_{10} of 3.2 mg/L (dry weight) from the other.

The DS concluded that aquatic plants were the most sensitive species, with a 7d-E_rC_{10} of 3.2 mg/L. The value is above the classification cut-off of 1 mg/L for not rapidly degradable substances and the DS concluded that bentazone does not warrant classification for chronic aquatic toxicity.

Comments received during consultation

Five MSCAs agreed that no acute classification for the aquatic environment was necessary for bentazone. Four agreed also that no chronic classification was needed.

One MSCA brought up a surface tension of 45.6 mN/m suggesting that bentazone was a surface-active substance. The DS answered that in the CLH Report all reported surface tension values were above 60 mN/M which would mean that bentazone is not a surface-active substance. No reference to the lower value was given by the MSCA and, thus, the lower value could not be verified by the DS. In addition, the MSCA was of the opinion that there was information, especially for algae and aquatic plants, to classify bentazone with Aquatic Chronic 2, H411. They also referred to a *Xenopus* study with a NOEC below 1 mg/L. According to the DS the information presented by the MSCA was assessed in the pesticide assessment process and the algae studies were considered unacceptable because of no analytically verified test concentrations and outdated test guidelines. No effects were reported in the *Xenopus* study (Orton *et al*. 2009).

One MSCA specifically agreed with the DS not to use aquatic toxicity values of the metabolite N-methylbentazone for classifying bentazone. Another MSCA also supported not using the metabolite toxicity values for acute classification but felt that the relevance of metabolite data and explanation of as how the toxicity of N-methyl-bentazone is considered in the classification of bentazone, should be discussed. The DS reminded that the more toxic effects of the metabolite are already seen in the study results. They thought that no further discussion was needed but acknowledged the calculation made by the MSCA being supportive of not classifying bentazone for environment.

One MSCA requested more information on a chronic fish test (OECD TG 210) giving a 35-day NOEC of 9 mg/L based on survival and body weight. According to the MSCA there was a significant increase in body weight in treatment fish which was considered not relevant, as an endpoint, in the CLH Report. The DS agreed that a significant increase in body weight should not have been discarded *a priory* as not being adverse. The DS did not have access to the study reports used in the pesticide assessment and therefore they could give no more information on the test details. However, the original assessment in the RAR had been discussed with other Member States and EFSA during the pesticide renewal procedure. The DS pointed out that in case this study was discarded, there would be a data gap for chronic fish toxicity. Using the surrogate approach with the lowest acute effect study results 96-h LC_{50} of >94 mg/L for *Lepomis macrochirus* would, in DS's opinion, also result in no chronic classification.

One MSCA thought that surrogate approach should be considered for invertebrate chronic toxicity as for invertebrates only formulation studies were available. They noted that also by using the surrogate approach bentazone would not be classified for environmental hazards. The DS explained that by expressing the effect concentrations based on the active substance, all toxicity was attributed to the active substance. Therefore, they did not see the surrogate approach necessary but acknowledged that it is supportive to their proposal.

One MSCA also noted that although bentazone was a herbicide, the tested algal and *Lemna* species did not appear to be sensitive. In contrast, non-target terrestrial plants were highly sensitive (RAR, 2014). This difference in sensitivity might be due to the selective mode of action of bentazone which is targeted towards broadleaved weeds. It may therefore be necessary to revise the classification if data on other plant species become available in the future. The DS agreed to the revision of classification in case new data for more sensitive primary producers come available.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal that bentazone is not rapidly degradable:

- bentazone was hydrolytically stable
- there was no ready biodegradability study available; QSAR estimation (BIOWIN v.4.10) predicted bentazone not to be readily degradable
- there was no surface water simulation test available
- the dissipation half-lives in a water/sediment test were >16 days in water (701 and 678 days); moreover, the detected transformation product N-methylbentazone (reaching maximum 13%) fulfils the criteria for classification as hazardous to the aquatic environment

As supportive information, it was acknowledged that it was also not demonstrated that bentazone is ultimately degraded in a soil simulation tests with a half-life of <16 days (degradation DT_{50}: 352 and 610 days).

Bioaccumulation

RAC agrees with the DS that bentazone has a low potential for bioaccumulation. In the fish 28-day bioaccumulation test (OECD TG 305) with *Lepomis macrochirus*, the whole body BCF was 1.4 L/kg. Data was lacking for correction of growth dilution and normalisation to 5% lipid content. The BCF value is below the classification threshold of 500 L/kg.

The n-octanol water partitioning coefficient was determined in two studies using shake flask methodology where the log D_{ow} determined at a pH where the substance was present in the neutral form corresponds to the log K_{ow}. The pK_a of bentazone had been reported as between 2.50 and 3.51, and the molecule was increasingly present in the neutral form at pH values below the pK_a. The highest log D_{ow} of 1.54 determined at pH 4, which could be considered representing worst-case for environmental conditions, approached the log K_{ow}. The Log D_{ow} of 1.54 is below the classification threshold of 4.

Aquatic toxicity

RAC agrees with the DS not to use the ecotoxicity data on the degradation product N-methylbentazone for classification of bentazone. N-methylbentazone was detected in water sediment systems at maximum 13% after 30 days in one system and 7% in the other. N-methylbentazone was nearly exclusively found in the water phase. Considering that degradation of bentazone is slow, that the formation of N-methylbentazone was reversible, and that the presence of N-methylbentazone especially in chronic aquatic tests would result in a more conservative assessment of bentazone toxicity, RAC agrees to base the classification on studies conducted with bentazone.

<u>Acute Aquatic Toxicity</u>

There were reliable data available on acute toxicity of bentazone for fish, invertebrates, algae and the aquatic plant *Lemna (gibba* and *minor)*. Aquatic plants were the most sensitive species, with a 7-day E_rC_{50} of 12 mg/L for *Lemna*.

The lowest acute toxicity value being a 7-day E_rC_{50} of 12 mg/L for algae iss above the classification limit of 1 mg/L. Based on Table 4.1.0 (a) RAC agrees that bentazone does not warrant classification for <u>acute</u> aquatic hazards.

Chronic Aquatic Toxicity

There are reliable data available on chronic toxicity of bentazone for fish, algae and aquatic plant *Lemna*.

For invertebrates, there were two studies conducted with formulated products. The studies reported 21-day NOECs of 101 and 32 mg/L for *Daphnia magna*, when expressed as bentazone. Both values were based on nominal test concentrations that had been analytically verified. Although the DS admitted that it cannot be excluded that effects at least partially were attributed to other constituents of the formulation, they concluded to use the study results for classification.

As regards data on formulations, RAC does not agree with the DS. RAC is of the opinion that formulation studies can be used for classification only when there is detailed information on properties and effects of the substances in the formulation. No data on the formulations are given in the CLH Report. Co-formulants serve different purposes in the products and might have an effect to the overall toxicity of a product. Therefore, RAC considers that the classification should be based on data on bentazone itself.

The lowest chronic toxicity value, 7-day E_rC_{10} of 3.2 mg/L, is above the classification limit of 1 mg/L. Bentazone was not rapidly degradable and had a low potential for bioaccumulation. Based on Table 4.1.0 (b)(i), bentazone is not to be classified for long-term hazard.

In the absence of chronic invertebrate data available with bentazone, the lowest acute toxicity value for invertebrates is a 48-h EC_{50} of > 100 mg/L for *Daphnia* and the substance is not rapidly degradable. In the acute *Daphnia* test, no sublethal effects were observed in the study. Based on Table 4.1.0 (b)(iii), the surrogate approach would lead to no classification for environmental hazards.

Overall, RAC agrees that **bentazone does not fulfil the criteria for short-term or long-term aquatic hazards and therefore does not warrant classification.**

The substance is a herbicide although the tested algal and *Lemna* species do not seem to be sensitive. This might be due to the selective mode of action of bentazone. Other aquatic plant species might be more sensitive. Therefore, in case new data comes available on more sensitive species the classification might have to be revisited.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).