# Committee for Risk Assessment

# RAC

## Opinion

proposing harmonised classification and labelling
at EU level of

## (RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide; mandestrobin

## EC Number: -
## CAS Number: 173662-97-0

CLH-O-0000001412-86-151/F

## Adopted

## 15 March 2017

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15 March 2017

CLH-O-0000001412-86-151/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:** **(RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl] acetamide; mandestrobin**

**EC Number:** **-**

**CAS Number:** **173662-97-0**

The proposal was submitted by **Austria** and received by RAC on **28 April 2016.**

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

# PROCESS FOR ADOPTION OF THE OPINION

**Austria** 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 **31 May 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **15 July 2016**.

## ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Andrew Smith**

Co-Rapporteur, appointed by RAC: **Kostas Andreou**

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 **15 March, 2017** by **consensus**.

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

| | Index No | International Chemical Identification | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hazard Class and Category Code(s) | Hazard statement Code(s) | Pictogram, Signal Word Code(s) | Hazard state-ment Code(s) | Suppl. Hazard statement Code(s) | | |
| Current Annex VI entry | No current Annex VI entry | | | | | | | | | | |
| Dossier submitters proposal | 616-RST-VW-Y | (RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide; mandestrobin | - | 173662-97-0 | Aquatic Acute 1<br>Aquatic Chronic 1 | H400<br>H410 | GHS09<br>Wng | H410 | - | M=1<br>M=10 | - |
| RAC opinion | 616-RST-VW-Y | (RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide; mandestrobin | - | 173662-97-0 | Aquatic Acute 1<br>Aquatic Chronic 1 | H400<br>H410 | GHS09<br>Wng | H410 | - | M=1<br>M=10 | - |
| Resulting Annex VI entry if agreed by COM | 616-RST-VW-Y | (RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide; mandestrobin | - | 173662-97-0 | Aquatic Acute 1<br>Aquatic Chronic 1 | H400<br>H410 | GHS09<br>Wng | H410 | - | M=1<br>M=10 | - |

# GROUNDS FOR ADOPTION OF THE OPINION

## RAC evaluation of physical hazards

### Summary of the Dossier Submitter's proposal

Mandestrobin has been adequately tested for physical hazards. It is not explosive, oxidising, flammable or auto-flammable and does not fulfil the classification criteria for physical hazards. Therefore, classification is not required.

### Comments received during public consultation

No specific comments were received.

### Assessment and comparison with the classification criteria

Mandestrobin does not meet the classification criteria for flammable solids. Examination of the chemical structure did not indicate that mandestrobin would have any explosive or oxidising properties. RAC therefore agrees that mandestrobin **does not meet the criteria for classification as an explosive substance or an oxidising solid**.

# HUMAN HEALTH HAZARD EVALUATION

## RAC evaluation of acute toxicity

### Summary of the Dossier Submitter's proposal

Mandestrobin is of low acute oral, dermal and inhalation toxicity (oral $LD_{50}$ > 2000 mg/kg bw, dermal $LD_{50}$ > 2000 mg/kg bw and inhalation $LC_{50}$ > 4.96 mg/L air) in rats. All estimated $LD_{50}$ and $LC_{50}$ values of mandestrobin are above the criteria for classification and labelling.

### Comments received during public consultation

No specific comments were received.

### Assessment and comparison with the classification criteria

In an acute oral study carried out in female rats, a single limit dose of 2000 mg/kg bw was administered. There were no deaths, therefore the $LD_{50}$ is > 2000 mg/kg bw.

In an acute dermal study, no mortality was observed after a single dose of 2000 mg/kg bw was applied to the skin of rats. Therefore, the $LD_{50}$ is > 2000 mg/kg bw.

In an acute inhalation study in rats the $LC_{50}$ was determined to be > 4.96 mg/L in dust form (the maximum attainable concentration).

The values obtained in each study are above the guidance values for classification for dust. **No classification is proposed for acute oral, dermal or inhalation toxicity.**

## RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

### Summary of the Dossier Submitter's proposal

No human epidemiology data are available. No health effects have been recorded in workers and no poisoning incidents or clinical cases have been reported. Mandestrobin is of low toxicity after a single exposure, by all routes of administration. There were no indications of respiratory tract irritation (RTI) or narcotic effects (NE) that could conceivably be elicited by a single dose or exposure to mandestrobin. In addition, no specific non-lethal, target organ toxicity after a single exposure to mandestrobin was observed in acute toxicity studies or an acute neurotoxicity study.

### Comments received during public consultation

No specific comments were received.

### Assessment and comparison with the classification criteria

Mandestrobin has been tested in acute oral, dermal and inhalation toxicity studies in rats and also in an acute neurotoxicity study in rats. There were no effects observed that could be considered specific target organ toxicity following a single dose and therefore **classification for STOT SE is not warranted**.

## RAC evaluation of skin corrosion/irritation

### Summary of the Dossier Submitter's proposal

In a skin irritation study, mandestrobin did not irritate the intact shaved skin of rabbits. Estimated skin irritation scores are below those triggering classification and labelling.

### Comments received during public consultation

No specific comments received.

### Assessment and comparison with the classification criteria

In a single skin irritation study, mandestrobin was applied to the shaved, intact skin of rabbits. There were no skin corrosion/irritation reactions observed at any timepoint during the observation period. Therefore, **mandestrobin does not meet the classification criteria for skin corrosion/irritation.**

## RAC evaluation of serious eye damage/irritation

### Summary of the Dossier Submitter's proposal

In an eye irritation study in rabbits, mandestrobin caused mild, transient ocular irritation in rabbits. The observed average irritation scores for 24 – 72 h were: 0.22 (chemosis), 0.33 (conjunctival redness), 0 (iritis) and 0.44 (corneal opacity). These were below the criteria triggering classification and labelling.

### Comments received during public consultation

No specific comments received.

### Assessment and comparison with the classification criteria

Mandestrobin was tested for eye irritation in a study in rabbits. There were no signs of ill-health throughout the study and the Draize scores are shown below.

*Eye irritation scores following instillation of mandestrobin to the eyes of rabbits:*

| | | Individual animal scores | | | | |
|---|---|---|---|---|---|---|
| | | 1h | 24 h | 48 h | 72 h | Average (24 - 72 h) |
| Conjunctiva | Chemosis | 1, 1, 1 | 1, 1, 0 | 0, 0 , 0 | 0, 0, 0 | 0.33, 0.33, 0 |
| | Redness | 1, 1, 1, | 1, 1, 1 | 0, 0, 0 | 0, 0, 0 | 0.33, 0.33, 0.33 |
| Iris | Congestion | 0, 1, 1 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 | 0, 0, 0 |
| Cornea | Opacity | 0, 0, 0 | 1, 1, 1 | 1, 0, 0 | 0, 0, 0, | 0.67, 0.33, 0.33 |

In order to be classified as irritating to eyes (category 2), the mean scores following grading at 24, 48 and 72 h are required to be (in at least 2/3 animals) as follows:

- corneal opacity ≥ 1 and/or
- iritis ≥ 1 and/or
- conjunctival redness ≥ 2 and/or
- conjunctival chemosis ≥ 2

All ocular findings were fully reversible within 72 h of application of the test substance. **The criteria for classification for serious eye damage/eye irritation were not met.**

## RAC evaluation of skin sensitisation

### Summary of the Dossier Submitter's proposal

Following the results of a Guinea Pig Maximisation test, mandestrobin was found not to be sensitising to the skin. Therefore, classification is not warranted.

### Comments received during public consultation

No specific comments were received.

### Assessment and comparison with the classification criteria

A Guinea Pig maximisation test was performed using 20 animals in the test group and 5 animals in the positive control group. There was no evidence of any dermal reactions in any of the tested guinea pigs following the challenge dose. There were no signs of toxicity or ill-health during this test. Slight to moderate erythema and oedema were observed in the positive control animals. **In summary, mandestrobin does not meet the classification criteria for skin sensitisation.**

## RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

### Summary of the Dossier Submitter's proposal

A series of studies were carried out to investigate the effects of orally administered mandestrobin in rats (one 90-day study), mice (one 90-day study) and dogs (one 90-day study and one 1-year study) following repeated exposure over sub-chronic periods. Further data were provided over a chronic period following investigation in a 2-year study in rats and a 78-week study in mice. The effects of mandestrobin were also investigated via the dermal route in rats (one 28-day study).

The DS presented the following summary of the main findings in each of these studies:

*Summary table of repeated dose toxicity studies with mandestrobin and comparison with STOT-RE criteria.*

| Study | STOT-RE 1 | STOT-RE 2 | NOAEL and LOAEL | Adverse effects at LOAEL |
|---|---|---|---|---|
| Rat, 90 day (oral) | 10 | 100 | NOAEL<br>♂ 54 mg/kg bw/day<br>♀ 320.1 mg/kg bw/day<br><br>LOAEL<br>♂ 282.6 mg/kg bw/day<br>♀ 788.5 mg/kg bw/day | ♂ ↑ absolute and relative liver weight<br><br>♀ ↑absolute and relative liver weight; hepatocellular hypertrophy;<br>Follicular cell hypertrophy in the thyroid;<br>↑ Cholesterol levels |
| Mouse, 90-day (oral) | 10 | 100 | NOAEL<br>♂ 807.3 mg/kg bw/day<br>♀ 111.2 mg/kg bw/day | No treatment-related adverse effects observed at the highest tested dose |
| Dog, 90-day (oral) | 10 | 100 | NOAEL<br>♂ 90.9 mg/kg bw/day<br>♀ 102.7 mg/kg bw/day<br><br>LOAEL<br>♂ 267.8 mg/kg bw/day<br>♀ 304.4 mg/kg bw/day | ♂ ↑ liver weight;<br>Pigmentation of the liver<br>Centrilobular degeneration;<br>↑ alkaline phosphatase levels |
| Dog, 1-year (oral) | 2.5 | 25 | NOAEL<br>♂ 19.2 mg/kg bw/day<br>♀ 92.0 mg/kg bw/day<br><br>LOAEL<br>♂ 92.0 mg/kg bw/day<br>♀ 225.7 mg/kg bw/day | ♂ hepatocellular hypertrophy;<br>Pigmentation<br>↑ alkaline phosphatase levels<br><br>♀ ↑ relative liver weight; hepatocellular hypertrophy;<br>Pigmentation;<br>↑ alkaline phosphatase levels |
| Rat, 28 day (dermal) | 60 | 600 | NOAEL<br>1000 mg/kg bw/day | No treatment-related adverse effects observed at the highest tested dose |
| Rat, 104 weeks (oral) | 1.25 | 12.5 | NOAEL<br>♂ 105.1 mg/kg bw/day ♀ 26.7 mg/kg bw/day<br><br>LOAEL<br>♂ 375.6 mg/kg bw/day<br>♀ 135.2 mg/kg bw/day | ↓body weight and body weight gain (♂ at 991.8 mg/kg bw/day and ♀ at ≥ 135.2 mg/kg bw/day)<br>↑ liver weight (♂ at ≥ 375.6 mg/kg bw/day and ♀ at ≥ 135.2 mg/kg bw/day)<br>↑ hepatocellular hypertrophy (♂ at ≥ 375.6 mg/kg bw/day and ♀ at ≥ 135.2 mg/kg bw/day)<br>↑ hepatocellular vacuolation (♂/♀at ≥ 375.6/475 mg/kg bw/day)<br>↑ Total Cholesterol(♂ at 991.8 mg/kg bw/day and ♀ at ≥ 475 mg/kg bw/day)<br>↑ GGT (♂/♀ at 375.6/475 mg/kg bw/day) |
| Mouse, 78 weeks (oral) | 1.7 | 17 | NOAEL<br>♂ 823.9 mg/kg bw/day<br>♀ 994.0 mg/kg bw/day | No treatment-related adverse effects observed at the highest tested dose |

The main target organs in rats, mice and dogs were the liver and thyroid. All effects observed in the sub-chronic (oral and dermal) and chronic (oral) studies in rats, mice and dogs do not trigger classification and labelling of mandestrobin as STOT RE.

## Comments received during public consultation

There were no specific comments received.

## Assessment and comparison with the classification criteria

*Oral studies in rats:*

Mandestrobin was tested for repeated dose toxicity in a 90-day study and a 2-year carcinogenicity study in rats.

In the 90-day study, Wistar rats received mandestrobin in their diet in the following doses: 0, 54/61.6, 282.6/320.1, 742.7/788.5 or 1544.6/1886.5 mg/kg bw/day (males/females respectively). For a 90-day study in rats, the guidance values for classification with STOT RE 2 are 10<c≤ 100 mg/kg bw/day, therefore only effects observed at the low dose are considered relevant for classification. The main findings in this study included the increased absolute and relative liver weight (14 and 57 %, respectively), in both males and females from a dose of 282.6/788.5 mg/kg bw/day (males/females respectively). Also, hepatocellular hypertrophy, thyroid follicular cell hypertrophy and increased cholesterol (30–73%) were observed from a dose of 742.7/788.5 mg/kg bw/day (males/females, respectively). All liver and thyroid effects occurred at doses above the guidance values for classification with STOT RE.

In the 2-year carcinogenicity study, Wistar rats were examined for toxicity after 104 weeks of treatment. Rats received mandestrobin in their diet in doses of: 0, 21.0/26.7, 105.1/135.2, 375.6/475.0 or 804.3/1016.2 mg/kg bw/day (males/females respectively). Extrapolating the category 2 guidance value range for a 90-day study in rats, the equivalent guidance value range for a 104-week study would be 1.25 < c ≤ 12.5 mg/kg bw/day. At 104 weeks, there was a decrease in body weight of males and females of the top dose groups (12/20% males/females, respectively when compared to control group) and a decrease in body weight gain in males of the top dose group (18%) and in females at doses between 135.2 and 1016.2 mg/kg bw/day (18–33% decrease in bw gain compared to controls). Relative liver weight was increased at doses of 375.6 and 475.0 mg/kg bw/day in females (14–28%) and in the male top dose (14%). Microscopic evaluation of the liver and thyroid revealed increased incidences of hepatocellular hypertrophy in males from a dose of 804.3 mg/kg bw/day and in females from a dose of 475.0 mg/kg bw/day and follicular cell hypertrophy in males at the top dose only. All liver and thyroid effects occurred at doses well above the estimated guidance values for STOT RE.

Both of these oral studies in rats indicate that no classification for specific target organ toxicity is warranted.

*Oral studies in mice:*

Mandestrobin was tested for repeated dose toxicity in a 90-day study and a 78-week carcinogenicity study in mice.

In the 90-day study, CD-1 mice received 0, 204.1/251.8, 404.9/529.1 or 807.3/1111.2 mg/kg bw/day (males/females) mandestrobin in their diet, respectively. For a 90-day study in rats, the guidance value range for STOT-RE 2 classification is 10 <c≤ 100 mg/kg bw/day. All doses tested were above this range. The main findings included the decreased body weight gain in females at the top dose (26.5 % compared to controls). Both absolute and relative (15 and 22%, respectively) liver weights were increased in both males and females at the top dose, but there were no histopathological correlates.

In the 78-week carcinogenicity study in CD-1 mice, animals received mandestrobin in their diet at dose levels of 0, 82.5/99.2, 238.8/280.3 or 823.9/994.0 mg/kg bw/day. Extrapolating the category 2 guidance value range for a 90-day study in rats, the equivalent guidance value range for a 78-week study would be 1.7 < c ≤ 17 mg/kg bw/day. All doses in this study were well above these levels. Adverse effects observed were limited to an increase in liver weight (14%) in males of the top dose group at the end of the study.

The findings from both of these studies in mice indicate that no classification is warranted.

*Oral studies in dogs:*

A 90-day and a one-year repeated dose oral study exists in dogs.

In the 90-day study in Beagle dogs, animals were given mandestrobin in the diet at concentrations of 0, 90.9/102.7, 267.8/304.4 or 933.1/820.4 mg/kg bw/day (males/females, respectively). Applying the classification guidance values for a 90-day study in rats as a guide, only effects occurring at the low dose should be considered for classification purposes. There were no adverse findings at this dose. At doses above this, effects were limited to bodyweight effects and the liver effects. Males and females of the top dose had reduced terminal body weights (21 and 27% lower, males and females, respectively), when compared to the controls. Bodyweight gain was greatly reduced in males at the top dose (95%), and at the end of the study, females of the top dose group had actually lost weight. Livers in top dose animals were darkened, pigmented and enlarged. The relative liver weights were increased (46% and 48% in males and females, respectively) as compared to controls. Centrilobular degeneration was observed in all males of the top dose and all females of the mid and top dose groups.

In the one-year study, Beagle dogs received mandestrobin in their diet at doses of 0, 4.3/4.5, 19.2/20.4, 92 or 180.7/225.7 mg/kg bw/day (males/females, respectively). Only the doses of 4.3/4.5 and 19.2/20.4 mg/kg bw/day were considered relevant for classification purposes. There were no adverse effects observed at these doses. At higher doses, adverse effects in the liver were observed. These included increased relative liver weight (females only) of 27% relative to controls, hepatocyte hypertrophy and pigmentation in males and females of the top dose groups and an increase in alkaline phosphatase levels.

No classification is warranted based on these two studies in dogs.

*Dermal studies in rats:*

One 28-day study to investigate repeated dose toxicity of mandestrobin by the dermal route in Wistar rats exists. Dose levels were 0, 100, 300 and 1000 mg/kg bw/day. There were no toxicological changes observed at any dose during the course of this study and therefore the results do not indicate the need to classify for repeated dose toxicity via the dermal route.

*Conclusion:*

Mandestrobin has been tested in a number of oral studies ranging in duration of 90 days to 2 years and one dermal study of 28 days. The liver has clearly been shown to be the target organ, however in all cases, effects were only observed at high doses, significantly greater than the guidance values for classification for STOT-RE 2. There were no effects observed at doses relevant for classification, either for STOT-RE 1 or STOT-RE 2 and therefore RAC concludes that **mandestrobin does not meet the criteria for classification for STOT RE**.

## RAC evaluation of germ cell mutagenicity

### Summary of the Dossier Submitter's proposal

Mandestrobin was tested in a sufficient range of *in vitro* and *in vivo* mutagenicity assays measuring different mutagenic endpoints such as gene mutation in bacterial and mammalian cells, clastogenicity *in vitro* as well as an *in vivo* micronucleus test in mice. The results of the studies show that no mutagenic potential attributed to mandestrobin was demonstrated.

### Comments received during public consultation

Although there were no specific comments, the proposal for no classification for this endpoint was supported by the three Member State Competent Authorities that responded.

### Assessment and comparison with the classification criteria

The results of a standard bacterial reverse mutation test, the investigation of chromosome aberrations and gene mutations in cultured mammalian cells, and a mouse bone marrow micronucleus test were all negative. These four studies were all well conducted; they provide a clear profile to support **no classification for germ cell mutagenicity**.

## RAC evaluation of carcinogenicity

### Summary of the Dossier Submitter's proposal

Mandestrobin is not genotoxic. In a rat carcinogenicity study, there was no statistically significant difference between groups for hyperplasia or tumours. No increase of neoplastic findings exceeding the historical control range was observed in any organ of treated animals, with the exception of benign sex-cord stromal tumours in the ovary. 4/50 (8%) and 6/50 (12%) cases occurred in females dosed with 7000 ppm (475 mg/kg bw/d) and 15000 ppm (1016.2 mg/kg bw/d), respectively. Laboratory's historical control range for these tumours is (0-3.1%). However, the incidence in concurrent control animals (2/50 = 4%) also exceeded the historical control range. Sex-cord stromal hyperplasia occurs in aged Wistar rats, and the animals used in this study appear to have been derived from a susceptible batch. Also higher survival rates in animals at 475 mg/kg bw/d and 1016.2 mg/kg bw/d may have contributed to the higher numbers of tumours in these groups. The incidences of sex-cord stromal proliferative lesions were within historical controls for all groups. At two highest doses the body weight gain was reduced by more than 20%. This was indicative, according to the DS, that the maximum tolerated dose (MTD) was exceeded.

In *in vitro* mechanistic studies, there was no interaction of mandestrobin with the oestrogen receptor or steroidogenesis, therefore, an mechanism for ovarian tumours based on endocrine imbalance was considered to be unlikely. Furthermore, there was no accumulation or persistence of mandestrobin or its metabolites in the ovary.

In the corresponding mouse carcinogenicity bioassay, the number of tumours in any tissue was not increased by exposure to mandestrobin.

Altogether, the sex-cord stromal lesions were considered unlikely to be toxicologically relevant and no classification for carcinogenicity was proposed by the dossier submitter.

### Comments received during public consultation

Although there were no specific comments, the proposal for no classification for this endpoint was supported by all 3 of the Member State Competent Authorities that responded.

### Assessment and comparison with the classification criteria

*Study in rats*

A rat combined chronic toxicity/carcinogenicity study and a mouse carcinogenicity study are available.

Groups of 50 male and 50 female Wistar rats were administered diets containing 0 (control), 400, 2000, 7000 or 15000 ppm mandestrobin for 104 weeks (doses equivalent to (0, 21.0/26.7, 105.1/135.2, 375.6/475.0 and 804.3/1016.2 mg/kg bw/day in males/females, respectively). Satellite groups of 20 males and 20 females were used for interim sacrifice at 52 weeks.

In males, it was observed decreased body weight and body weight gain (at 15000 ppm) and toxicological alterations in the liver including increased liver weights in combination with a higher degree of hepatocellular hypertrophy and hepatocyte vacuolation (both at ≥ 7000 ppm) and

increased blood biochemistry parameters (total cholesterol and gamma-glutamyltransferase (GGT) in males at 15000 ppm).

In females it was observed that body weight and body weight gain were significantly decreased at ≥ 2000 ppm. Survival to the termination of the study was increased in the two highest dose groups. Toxicological alterations in the liver included increased liver weights (at ≥ 2000 ppm), a higher degree of hepatocellular hypertrophy and hepatocyte vacuolation (at ≥ 2000 ppm and at ≥ 7000 ppm, respectively) and increased blood biochemistry parameters (increased total cholesterol and increased GGT at ≥ 7000 ppm and at ≥ 15000 ppm, respectively).

The key findings from this study relating to potential carcinogenicity of mandestrobin are shown in the following table.

*Key findings from carcinogenicity study in rats (females)*

| **FEMALE RATS** | **0 ppm (0 mg/kg bw/day)** | **400 ppm (26.7)** | **2000 ppm (135.2)** | **7000 ppm (475)** | **15000 ppm (1016.2)** |
|---|---|---|---|---|---|
| | | | | | |
| No. of animals that died prior to termination at 104 weeks | 17/50 | 15/50 | 14/50 | 10/50 | 9/50 |
| Mean body weight | 363.6 | 353.8 | 323.2 ** | 323.2 ** | 292.5 *** |
| Mean body weight gain (week 0-104) | 220.0 | 214.5 | 180.1 ** | 179.9 ** | 147.9 *** |
| >10% reduction in body weight gain first observed | - | - | Week 68 | Week 40 | Week 11 |
| | | | | | |
| Ovary: Sex-cord stromal hyperplasia | 3/50 | 8/50 | 5/50 | 6/50 | 5/50 |
| Ovary: benign sex-cord stromal tumours | 2/50<br>4% | 0/50 | 1/50<br>2% | 4/50<br>8% | 6/50<br>12% |

** p< 0.05, *** p< 0.001

There were no malignant ovarian tumours observed in this study, but an increased frequency of **benign sex-cord stromal tumours** was seen in the ovaries of female rats at the top 2 doses.

Sex-cord stromal hyperplasia was also observed in all treatment groups, including the controls, but this was not clearly dose–related.

The tumour findings were not statistically significant when compared to the concurrent control rate by pair-wise analysis, although the increasing dose-response relationship could be considered statistically significant. The test laboratory's historical control range for benign sex-cord stromal tumours in this strain of rat (0-3.1%) was exceeded in the concurrent control group as well as at the top two doses. This indicates that the findings could be due to an inherent sensitivity of the rat ovaries in this study and not a treatment-related effect. Historical data from the test laboratory showed sex-cord stromal hyperplasia rates to vary from 2 to 48%. The increased survival rates seen in the higher dose groups may have further contributed to the findings, creating a larger pool of animals in which the tumours could develop.

Although survival of female rats was inversely related to the dose of mandestrobin, there was a clear adverse effect on body weight gain and body weight. RAC is in agreement with the DS that the effects in the top dose group were of sufficient magnitude to indicate that the

maximum tolerated dose (MTD) had been exceeded. The reduction in total body weight gain at 2000 ppm and 7000 ppm was 18 % in both cases, but the tumour incidence at 2000 ppm was only 2 % in comparison to 8 % at 7000 ppm. This indicates that the tumours may not be a direct consequence of the general toxicity observed.

In a dose-range finding fertility study and a 2-generation reproduction study in rats the group mean ovary weights were found to have decreased following treatment with mandestrobin. However, there were no associated functional changes in the reproduction parameters; mating index, fertility index, gestation index, gestation length, number of implantations and birth index all remained unaffected.

In the carcinogenicity study and in the repeated dose studies, following a full histopathological examination, ovary weight decrease that was not observed. This suggests that the findings in the reproduction studies do not provide conclusive evidence that the ovary is a target of mandestrobin toxicity and that the ovary weights decrease could be considered as incidental.

As described by the DS, there was also a minor increase in testicular interstitial cell adenomas in males at the two top dose groups: 0/50, 0/13, 0/22, 2/15 and 3/50. There were no associated increases in hyperplastic lesions in this cell type or other evidence of hormonal effects within the male reproductive tract. The observed incidences were within the historical control range provided for the test laboratory (0-6%) and therefore it seems most likely that these benign tumours were not related to mandestrobin treatment.

A dose-dependent increase in benign tumours has been found in the ovaries of female rats following treatment with mandestrobin. The tumours observed exceeded the laboratory HCD at the top two doses, but so did the concurrent control. There were no significant substance-related increases in any tumours observed in males.

*Study in mice*

Groups of 51 male and 51 female CD-1 mice were given mandestrobin in the diet at concentrations of 0 (controls), 700, 2000, and 7000 ppm for 78 weeks (doses equivalent to 0, 82.5/99.2, 238.8/280.3 and 823.9/994.0 mg/kg bw/day, males and females, respectively). Treatment with mandestrobin was well-tolerated. There were no adverse effects at any tested dose. There were no effects on survival/mortality or on the incidence or morphology of tumours to indicate any carcinogenic potential of the test substance. Altogether, mandestrobin was not found to be carcinogenic in mice.

*In vitro mechanistic studies*

Several in vitro studies provided by the Applicant were assessed by RAC to consider the potential hormonal mechanism of the observed ovarian tumours in rats. Mandestrobin had no influence on testosterone or oestradiol production in a steroidogenesis assay (OECD TG 456) conducted in the human adrenocortical NCI-H295R cell line, nor did it show agonistic or antagonistic effects on oestrogen receptor alpha or human androgen receptor in a reporter gene assay conducted in HeLa cells (OECD TG 455). The provided *in vivo* mechanistic studies investigated liver and thyroid effects, but no specific *in vivo* mechanistic study investigating potential hormonal imbalance was provided.

The absence of any mandestrobin-related hormonal effects in the *in vitro* studies, together with the negative findings in the mutagenicity/genotoxicity assays, indicate that there is no supporting mechanistic basis for the increased ovarian tumour frequency seen in rats treated with high doses of mandestrobin.

*Conclusion on classification and labelling*

Two well-conducted regulatory studies in rats and mice were provided. There was a minor increase in testicular interstitial cell adenomas in male rats s at the two top dose groups but the observed incidences were within the historical control range provided for the test laboratory (0-

6%) and therefore it seems most likely that these benign tumours were not related to mandestrobin treatment. In male and female mice there was no carcinogenic response**.** In female rats, however, there was a dose-dependent increase in benign sex-cord stromal tumours in the ovaries that exceeded both the concurrent and laboratory historical control data.

The increased frequency of tumours was found in the two highest dose groups [approx. 7000 ppm and 15000 ppm (475 and 1016 m/kg), respectively]; the highest dose exceeded the MTD clearly. Signs of toxicity (reduced body weight and body weight gain) were also evident at the second highest dose. However, there was no evidence to suggest that the general toxicity influenced the formation of tumours. Notably, the effect on body weight was similar at 2000 ppm (135 mg/kg) and 7000 ppm (475 mg/kg), but no increase in tumour frequency relative to the control group was seen at the lower of these two mid-dose groups.

The observation of an increasing trend related to treatment in a single tumour type, in female rats only, could be viewed as limited evidence of carcinogenicity in animals. However, RAC is in agreement with the DS that the available evidence is not sufficiently convincing to conclude that mandestrobin had such a carcinogenic effect in female rats. Notably:

- in a robust carcinogenicity study in mice, using similarly high doses, no tumours or effects to the ovaries were observed;
- the ovarian tumours observed in female rats were benign;
- ovarian sex-cord stromal tumours may occur in aged Wistar rats;
- compared to the concurrent control, the increase in tumours were not statistically significant when compared by pair wise analysis;
- ovarian sex-cord stromal hyperplasia was observed in all treatment groups at the end of the study, including in controls, and there was no dose-related effect;
- as the incidence and severity of the hyperplasia seen was not increased in the groups with increased benign tumours, this raised a doubt about the biological plausibility of the tumour findings;
- mandestrobin was not found to be genotoxic in a standard battery of *in vitro* and *in vivo* tests;
- *in vitro* mechanistic studies (OECD TG 456: Kubo, 2012) to investigate the influence of mandestrobin on testosterone and oestrogen yielded no positive results. However, as there are no hepatic enzymes in the adrenocortical tumour cell line used (H295R), the potential impact of liver enzyme on metabolic activation of mandestrobin is not known.
- To circumvent the limitations of the (Kubo, 2012) study, another *in vitro* mechanistic study (OECD TG 455: Suzuki, 2012) was done using mandestrobin but also its metabolites (5-COOH-S-2200, 4-OH-S-2200, 5-CH2OH-S-2200 and 5-CA-S-2200-NHM). This study also did not show agonistic or antagonistic effects on estrogen (hER$\alpha$) or androgen (hAR)receptors.

*Comparison with the criteria*

There are no data relating to the potential carcinogenicity of mandestrobin in humans, therefore there is no basis for classification in category 1A. Similarly, as there was no clear evidence of a carcinogenic response in laboratory studies with either rats or mice, a category 1B classification would not be appropriate.

As it is possible to classify a substance in category 2 for carcinogenicity on the basis of limited evidence in animal studies, this could be considered as an option for mandestrobin.

However, the weight of evidence relating to the ovarian tumour findings in female rats suggests that they were not treatment-related. There is insufficient evidence for the carcinogenicity of mandestrobin in female rats. The adverse effect seen on body weight gain was a confounding factor. Additionally, as discussed above, standard mutagenicity testing produced negative

results. A plausible mechanistic basis for the mandestrobin being a causative factor in the tumour findings is lacking.

In the absence of supporting evidence, RAC concludes there is no basis to suspect that mandestrobin may be a human carcinogen. In accordance with the criteria, **no classification for carcinogenicity is warranted**.

## RAC evaluation of reproductive toxicity

### Summary of the Dossier Submitter's proposal

Mandestrobin was tested in two two-generation reproductive toxicity study in rats (Hoshino, 2010; and Matsuura, 2012).

Teratogenicity was tested in rats (OECD TG 414 supportive study: Rhodes, 2009a; and OECD TG 414: Rhodes, J, 2012a) and rabbits (OECD TG 414 supportive study: Rhodes, 2009b; and OECD TG 414: Rhodes, 2012b)

There was no evidence of adverse effects on fertility and sexual function or on development caused by mandestrobin. No classification or labelling is proposed.

### Comments received during public consultation

Although there were no specific comments, the proposal for no classification for this endpoint was supported by all three of the Member State Competent Authorities that commented.

### Assessment and comparison with the classification criteria

***Fertility and sexual function:***

Mandestrobin has been tested in two development toxicity studies in rats, the first being a dose range-finding study (Hoshino, N.; 2010) and the second a guideline two-generation study OECD TG 416 (Matsuura; 2012).

*Dose range-finding study:*

Groups of 10 male Wistar rats were administered mandestrobin in their diet for 4 weeks before mating and throughout the mating period until the day of necropsy. Females (10/dose) were dosed daily for 4 weeks before mating, throughout the dosing period until weaning of the F1 offspring (day 21 of lactation).

Rats received 0, 244-782, 499-1429 or 1033-2441 mg/kg bw/day (dose varied depending on which period of treatment the rats were undergoing). Systemic toxicity was apparent in both males (top dose only) and female parents (from the mid dose and above). Effects on body weight in males and females were noted in the time periods before mating, during gestation and during the lactation period. However it was only females of the top dose group that had a decrease in body weight greater than 10 %. This was observed at gestation day 20 and was accompanied by a reduction in food consumption. Liver effects were observed and included a dose-dependent increase in liver weight (24 – 51 %) in males at all doses and in females from the mid dose up (37 – 65 %), brown pigmentation in the bile duct in males and females at the top dose, centrilobular hypertrophy in all males and females of the mid and top dose, brown pigmentation in hepatocytes and periductular cell infiltration in females of the top dose group only. Thyroid effects in males were apparent, with an increase in thyroid weight in all dose groups (33 – 49 %) and follicular cell hypertrophy in 5/10 males (versus 0 in controls) at the top dose group. Liver and thyroid effects observed were consistent with those observed in repeated dose toxicity studies.

Uterus weight was reduced in animals of the mid and top dose groups (40 and 54 % respectively). In females of the top dose group decreased vacuolation in the interstitial gland of the ovary was observed in 7/10 animals (versus 0 in controls) and atrophy of the uterus was also observed in 10/10 animals (compared to 0 in controls). There were no changes to reproductive parameters such as mating ability, fertility, pregnancy, parturition or nursing behaviour. The changes to the ovary and uterus were likely to be treatment-related. However, they occurred in the presence of maternal toxicity (reduced body weight of 14 % and liver toxicity) and as there were no functional changes to reproduction, they are considered not relevant for classification as reproductive toxicant.

Pup weights at birth were consistent across groups, but on day 21 (weaning day) body weight gain was reduced by 14 % in the mid dose groups and by 50 % in the top dose groups (compared to controls). Reductions in some organ weights were observed in parallel with the reduced body weights. Given the limited nature of this study (dose range finding), no clear conclusions can be made from these reductions in body weight gain in pups.

*Two generation study (reproductive Performance):*

A two-generation study, OECD TG 416 (Matsuura; 2012), was carried out in Wistar rats, administering mandestrobin in the diet daily for 10 weeks before mating for both sexes, through the mating period until the day of necropsy for males and through the mating, gestation and lactation periods until the day of weaning of F1 offspring (Day 21 of lactation) in females. There were 26 males and 26 females/dose group and the dose levels were 0, 43-163, 132-511 or 452-1688 mg/kg bw/day (depending on the period of dosing).

There were reductions in body weight gain observed in parental males of the top dose group, 11.4 % (days 0-70 prior to mating) and in parental females of the top dose group on gestation day 0-14 (11 %). In dams, this was associated with a reduction in food consumption. As observed in the dose-ranging study, the liver was the main target organ. Increased absolute and relative liver weight was observed in parental males and females from the mid dose and above and also in F1 males of the top dose and F1 females of the mid and top doses. Brown pigmentation of the bile duct occurred in parental males and females of the top dose and F1 males and females of the mid and top dose. Focal periductular cell infiltration was observed in parental and F1 males and females of the top dose groups. Proliferation of bile ducts occurred in parental and F1 females of the top dose group and hepatocyte hypertrophy occurred in parental and F1 males from the mid and high dose groups and in all dose groups of the parental and F1 females. These findings seemed to be more prevalent in the F1 generation than the parental generation. In the parental generation, 4/26 males were observed to have diffuse hypertrophy of the follicular cells of the thyroid. The liver and thyroid findings were consistent with those observed in the repeated dose toxicity studies.

As observed in the dose-ranging study, there were reductions in uterus weight in top dose parental females [16 % (abs.) and 18 % (rel.)] and ovary weights in top dose parental and F1 females [12 % (parental) and 14 % (F1)]. There were no adverse effects to reproductive performance in either the parental group or the F1 group of animals. Mating ability, fertility, pregnancy, gestation length, parturition, nursing behaviour all remained unaffected and there were no changes to oestrous cycle or sperm parameters.

Reductions in body weight were observed in top dose pups of the F1 and F2 generations, in males and females on postnatal day 14 (in males 14 % and 10 % and in females 14 % and 11 % F1 and F2 respectively) and day 21 (in males 16 % and 13 % and in females 17 % and 13 % F1 and F2 respectively). At weaning there were reductions in spleen (F1: 25 % and 20 %; F2: 23 % and 26 % males and females, respectively) and thymus weight (F1: 18 % for both males and females; F2: 14 % and 15 % males and females, respectively) in both the top dosed F1 and F2 generation males and females and a reduction in uterus weight in the top dosed F1 pups only

(14 % of controls). These reductions were all attributed to malnourishment of the dams that had reduced food consumption during the lactation period.

There was a delay in sexual maturation in both F1 males and females of the top dose group which was characterised by a delay in vaginal opening of 1.5 days and a delay of 1.6 days for preputial separation in males (compared to controls). As there were no changes to reproductive function, this delay was attributed to growth retardation. There were no external anomalies observed in F1 or F2 pups.

*Conclusion on adverse effects on sexual function and fertility:*

Decreased uterus and ovary weights (top dose females of F0 and F1) and a delay in sexual maturation (top dose males and females) were observed in F1 animals in the study by Matsuura (Matsuura, 2012). The decreased ovary weights occurred in F0 and F1 females and the decreased uterus weights occurred in F0 females only at the top dose. At this dose there was clear systemic toxicity characterised by reduced body weight and liver toxicity. In the absence of any functional reproductive changes as a consequence of these reduced organ weights, the effects are considered not relevant for classification.

With regards to the development of the offspring (F1 and F2 generation), there was evidence of reduced postnatal bodyweight in both males and females of the top-dose groups and also small reductions in spleen and thymus weight in the same groups. The study authors stated that the lower spleen weights were completely recovered to the control level by adulthood in both sexes, suggesting a transient retardation in growth. The reduced postnatal bodyweight occurred during the lactation period where dams were observed to have reduced bodyweight accompanied by reduced food consumption. Therefore, the reduced bodyweight was thought to be due to undernourishment of the dams and does not warrant classification.

The delay in sexual maturation in top dose males and females of the F1 generation was considered to be related to a retardation of growth. Therefore, **no classification is warranted for effects on fertility and sexual function**.

***Developmental toxicity:***

A dose range-finding developmental study for rats (Rhodes, 2009a) and rabbits (Rhodes, 2009b) and a guideline developmental study (OECD TG 414) are available also for both rats (Rhodes, 2012a) and rabbits (Rhodes, 2012b).

*Rats:*

*Dose range finding study:*

Pregnant female rats (7/dose group) were orally gavaged with either 0, 250, 500 or 1000 mg/kg bw/day mandestrobin from Days 6-19 of gestation. There were no unscheduled deaths during the study, mean body weight and body weight gain were unaffected.

Observations in pups were two malformed foetuses in the low dose group (250 mg/kg bw/day) and one malformed foetus in the mid dose group (500 mg/kg bw/day). There were no external foetal variations or malformations in the animals of the top dose group (1000 mg/kg bw/day). These findings showed no dose-response and were not considered treatment-related.

*Developmental effects:*

In the main study (OECD TG 414: Rhodes, 2012a), mandestrobin was administered to pregnant female Wistar rats (24/dose group) via oral gavage on Days 6-19 of gestation. The doses administered were 0, 100, 300 and 1000 mg/kg bw/day.

There were no unscheduled deaths in this study. Two dams in the top dose group were considered thin on Day 20 and this was confirmed at necropsy. There was an apparent increase in the number of dams with early intrauterine death or post-implantation loss but this lacked a

dose response and because the litter size was unchanged, it was not deemed biologically significant.

In pups, one foetus of the mid dose group (300 mg/kg bw/day) suffered a skeletal malformation and five foetuses from five litters in the top dose group (1000 mg/kg bw/day) were also found to have malformations. The malformations observed were diverse and incidence and intergroup distribution suggests that they are not indicative of an adverse effect of treatment. All findings were within the range of historical control data provided.

*Key developmental findings from developmental toxicity study in rats*

| Dose (mg/kg bw/day) | | 0 | 100 | 300 | 1000 | HCD range |
|---|---|---|---|---|---|---|
| Total external or visceral malformations: number of foetuses affected (% of foetuses)/litter incidence | | 0 | 0 | 0 | 2 (0.7)/2 | 0 - 3 (0 - 1.5 %) |
| | Kidney, severely increased pelvic cavitation | 0 | 0 | 0 | 2 | |
| Total external or visceral variations: number of foetuses affected (% of foetuses)/litter incidence | | 77 (30.9)/24 | 78 (35.7)/23 | 103 (37.4)/24 | 101 (42.4)/24 | 66 - 90 (25.5 - 52.2 %) |
| Total skeletal malformations: number of foetuses affected (% of foetuses)/litter incidence | | 0 | 0 | 1 (0.8)/1 | 3 (2.9)/3 | 0 - 6 (0 - 8.3 %) |
| | Rib cartilage shortened | 0 | 0 | 0 | 1 | |
| | Sternebrae, cleft xiphoid cartilage | 0 | 0 | 0 | 1 | |
| | Vertebral cervical arch and centrum, additional ossification site fused | 0 | 0 | 1 | 0 | |
| | Vertebral cervical arch, additional cartilaginous ventral plate fused | 0 | 0 | 0 | 1 | |
| Total skeletal variations: number of foetuses affected (% of foetuses)/litter incidence | | 122 (94.8)/24 | 108 (93.7)/23 | 128 (96.4)/24 | 120 (99.2)/24 | 76 - 113 (81.3 - 99.2 %) |
| Total number of foetuses with malformations, n (%) | | 0 | 0 | 1 (0.4) | 5 (2.1) | 0 – 7 |
| | Litter incidence | 0 | 0 | 1 | 5* | |

*Rabbits:*

Mandestrobin was tested up to a dose of 1000 mg/kg bw/day in both a dose range-finding study and a main developmental study in rabbits.

*Dose range-finding study:*

Time-mated and presumed pregnant female New Zealand White rabbits (7/dose group) each received an oral dose of mandestrobin at dose levels of 0, 250, 500 or 1000 mg/kg bw/day (by gavage) on Days 7 – 28 of gestation.

In dams, there were no treatment-related clinical findings and no treatment-related deaths. Mean body weight, body weight gain and gravid uterus weight was unaffected by treatment.

There were no treatment-related gross necropsy findings. The mean numbers of corpora lutea, implantations, mean incidences of pre- and post-implantation loss and mean litter sizes were unaffected.

In pups, there was one malformed foetus in the top dose group (1000 mg/kg bw/day). This foetus was found to have Spina bifida, severely malformed head structures and severely flexed forelimb wrist joints. There was also an increase in the number of foetuses with variations in the top dose group (8.3 % versus 0 in controls) (5 foetuses from 3 litters), this finding did not reach statistical significance. The variations included: slightly enlarged bilateral eye bulge and upper incisor not erupted.

Due to the limited nature of this study (range finding study), no conclusions can be drawn.

*Developmental effects:*

In the guideline prenatal developmental toxicity study, carried out in New Zealand White rabbits (24 time mated pregnant females/dose), animals received an orally gavaged dose of either 0, 100, 300 or 1000 mg/kg bw/day on gestation Days 7-28.

There were no unscheduled deaths of dams in this study and no significant clinical or necropsy findings.

Malformations were observed in eight foetuses from six litters in the control group, six foetuses from four litters in the low dose group (100 mg/kg bw/day), nine foetuses from seven litters in the mid-dose group (300 mg/kg bw/day) and three foetuses from three litters in the top dose group (1000 mg/kg bw/day). These findings were all diverse in nature and there did not appear to be any one finding with evidence of a dose-response that would indicate a treatment-related effect. All findings were within the historical control data ranges provided by the laboratory. Therefore, RAC agrees with the DS that the incidence and pattern of distribution is not indicative of an adverse effect of treatment and **classification for development is not warranted.**

***Conclusion on adverse effects on development***

Mandestrobin has been tested in a number of reproduction studies in rats and rabbits. There was not sufficient evidence of adverse effects on either fertility and sexual function or on development and therefore it is agreed that **no classification for this endpoint is warranted**.

# ENVIRONMENTAL HAZARD EVALUATION

## RAC evaluation of aquatic hazards (acute and chronic)

### Summary of the Dossier Submitter's proposal

Mandestrobin fulfils the criteria for the proposed classification as Aquatic Acute 1- H400, M-factor = 1 and Aquatic Chronic 1- H410, M-factor = 10 according to Regulation EC 1272/2008. Mandestrobin is toxic in acute toxicity tests to fish (*Oncorhynchus mykiss*, $LC_{50}$ = 0.94 mg/L) and aquatic invertebrates (*Americamysis bahia*, $LC_{50}$ = 0.43 mg/L). Mandestrobin is also toxic in chronic toxicity tests with aquatic invertebrates (*Americamysis bahia*, NOEC = 0.0056 mg/L). Mandestrobin is not readily biodegradable, not rapidly degradable and does not meet the CLP criteria for having a high potential for bioaccumulation based on the fish lipid corrected BCF.

***Biotic and abiotic degradation***

Two aquatic photolysis studies were available following OECD 316 (2008) and performed according to GLP principles using 14C radiolabelled mandestrobin (S-2200 R-isomer and S-2200 S-isomer). The S-2200 R-isomer and S-2200 S-isomer were tested in separate studies and were found to be stable in water. Sterile conditions were maintained throughout the study and this confirmed by the mass balances that ranged from 91 to 102% of AR for all experiments. No isomerisation between the S-2200 R- and S- isomers was observed.

There are two **hydrolysis** studies available following OECD TG 111 (April, 2004) performed according to GLP principles using $^{14}C$ radiolabelled mandestrobin (S-2200 R-isomer and S-2200 S-isomer). The S-2200 R-isomer and S-2200 S-isomer were tested in separate studies and were found to be hydrolytically stable at pH 4, 7 and 9 at 50°C. No hydrolysis of S-2167 and of S-2354 would be expected under environmental conditions.

Regarding **biodegradability**, one study on ready biodegradability and two simulation studies (one for each S-2200 isomer) on biodegradation in water/sediment systems were available. A screening test study was available following OECD 301 B Ready Biodegradability (Adopted 1981, Revised 1992) performed under GLP using an unlabeled racemic mixture of S-2200. No degradation (maximum 2 %) was determined for S-2200 during the 28 days testing period.

Two water/sediment studies were available following the OECD TG 308 (2002) performed under GLP principles using $^{14}C$ labelled S-2200 R-isomer and S-2200 S-isomer respectively. Two substance labels were used in the case of S-2200 R-isomer, benzyl-$^{14}C$ and phenoxy-$^{14}C$. For the case of the S-2200 S-isomer, only the benzyl-$^{14}C$ substance was tested. Aerobic conditions were maintained during the experiments.

With respect to the S-2200 R-isomer, the total mass balance was in the range of 97 to 101 % of AR for both systems and both labels. Formation of $^{14}CO_2$ using [benzyl-$^{14}C$] ranged from 1.4% to 3.7% of AR for the two systems used in this study while for the phenoxy-$^{14}C$ ranged from 2.3% to 2.4% of AR. The S-2200 R-isomer degraded in both water/sediment systems. The test substance transfer into sediment was relatively fast with occurrences of S-2200 R-isomer between 32 % AR and 49 % AR after 7 days in the two systems. The metabolite 2-COOH-S-2200 was detected in surface water and sediment and accounted for a maximum of 1.9 % AR, while the metabolite 5-COOH-S-2200 was detected in both water and sediment accounting for a maximum of 6.6 % AR.

The degradation rates were fitted with Single First Order (SFO) kinetics, the Order Multi-Compartment (FOMC) and the Double First Order in Parallel (DFOP) kinetics. The Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (2006) was used for the $DT_{50}$ and $DT_{90}$ estimation. The first order $DT_{50}$ values in respect to the whole system for the [benzyl-$^{14}C$] were 342 and 344 days, while

for the [phenoxy-$^{14}$C] the first order $DT_{50}$ values were 284 and 654 days. The respective $DT_{90}$ values were in the range of 942 and >1000 days.

With respect to the S-2200 S-isomer, the total mass balance was in the range of 97 to 100% of AR. The carbon dioxide generation reached a maximum of 1.3% of the AR. S-2200 S-isomer degraded in both water/sediment systems. The test substance transferred into sediment was relatively fast with occurrences of S-2200 S-isomer between 35% AR and 47 % AR after 7 days in the two systems. One metabolite, MCBX, reached up to 18% AR in the whole system (102 DAT) and up to 15.4% AR in sediment. In the surface water of both systems and in sediment of one of the systems, MCBX accounted for less than 5% AR. The metabolite 2-COOH-S-2200 was detected in one of the systems in water only and accounted for <1% AR. The metabolite 5-COOH-S-2200 was detected in both water and sediment accounting for a maximum of 1.7% AR at 60 DAT in only one of the systems.

The degradation rates were fitted with Single First Order (SFO) kinetics, the Order Multi-Compartment (FOMC) and the Double First Order in Parallel (DFOP) kinetics. The Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration (2006) was used for the $DT_{50}$ and $DT_{90}$ estimation. The first order $DT_{50}$ values in respect to the whole system for the S-2200 S-isomer were 155 and 600 days. The respective $DT_{90}$ values were in the range of 516 and >1000 days.

Based on the data presented in the two water sediment studies above, a calculation of S-2200 sediment water kinetics was performed according to FOCUS (2006) Guidance. The data on the S-isomer and on the R-isomer were combined to determine the rate of degradation of S-2200 (the racemate) in the overall system or in the water phase and determine the percentages of the formed metabolites.

Table 6 shows that for the water dissipation phase, $DT_{50}$ values of 7.8 and 19 days and $DT_{90}$ values of 64.8 and 158 days were obtained. In the overall system, SFO kinetics showed a good fit and $DT_{50}$ values of 212 and 519 days (geometric mean: 332 days) and $DT_{90}$ values of 703 and 1725 days were obtained. Due to the fact that the majority of S-2200 occurs in the sediment, the whole system $DT_{50}$ of 332 days (geometric mean) should be used for the sediment phase. No metabolite exceeds 10% in the overall system, but MCBX reaches a maximum of 9.1% after 102 days.

**Table: Dissipation and degradation rates of S-2200 in water sediment systems. (key data are highlighted in bold)**

| | **Parameter** | **Calwich Abbey [Benzyl-$^{14}$C]** | **Swiss Lake [Benzyl-$^{14}$C]** |
|---|---|---|---|
| Dissipation from water phase | Model | *FOMC* | *DFOP* |
| | Chi2 error [%] | 2.83 | 2.59 |
| | $DT_{50}$ [Day] | **7.8** | **19.0** |
| | $DT_{90}$ [Day] | 64.8 | 157.7 |
| Degradation in system | Model | SFO | SFO |
| | Chi2 error [%] | 2.02 | 1.57 |
| | $DT_{50}$ [Day] | **212 (7.2 x $10^{-8}$)*** | **519 (1.7 x $10^{-5}$)*** |
| | $DT_{90}$ [Day] | 703 | 1725 |

*P value from the t-test is given in brackets.

Based on the available information, the DS concluded that mandestrobin is considered as not rapidly biodegradable and not rapidly degradable for the purposes of classification and labelling.

***Aquatic bioaccumulation***

A Flow-Through Bioconcentration and Metabolism Study of labeled [$^{14}$C]S-2200 with Bluegill Sunfish was available following the OECD Guideline 305, US EPA FIFRA 165-4, US EPA OPPTS 850.1730 and the JMAFF 12-Nosan 8147 2-9-17 under GLP principles. Two concentrations (1.0 and 10 µg ai/L) were tested along with a solvent control (Acetone). S-2200 was stable under the test conditions and reached the steady-state plateau by day 21 of exposure. The active substance

S-2200 accumulated in whole fish with steady-state BCF values in whole fish tissues of 25 and 26 for the high and low concentration, respectively. BCF values for the total $^{14}C$ residues (TRR) were determined to be 140 and 174 for whole fish, and 253 and 331 for non-edible portions for the high and low concentration, respectively. The depuration half-life (CT50) for total $^{14}C$ residues was 2.1 d for whole fish. In the fish, the S-2200 was extensively metabolised and the major residues were the glucuronic acid conjugate and /or sulphate conjugates of hydroxylated S-2200 and the active substance itself. The potential of the metabolites S-2200-OR and S-2200-ORC to bioaccumulate in the aquatic food chain is considered to be covered by the fish bioaccumulation study conducted with the parent compound. The lipid corrected BCF was determined to be 8. Consequently, the DS considered mandestrobin to have a low potential for bioaccumulation in the aquatic environment.

***Aquatic toxicity***

There are acute toxicity data available from six fish studies, five aquatic invertebrate studies and two studies involving algae (Table 7). Chronic toxicity data are available from two early life stage toxicity fish tests studies, five studies on aquatic invertebrates and one study involving an aquatic plant (Table 7). Unless otherwise stated, all of the ecotoxicological studies on mandestrobin were performed according to GLP principles and were considered reliable and suitable for hazard classification purposes by the DS.

**Table: Summary of relevant information on aquatic toxicity (key data are highlighted in bold)**

| Test Substance | Test organism | Test condition | Exp. time | Test conc. | Results | | |
|---|---|---|---|---|---|---|---|
| | | | | | Endpoint | NOEC [mg a.s./L] | $EC_{50}/LC_{50}$ [mg a.s./L] |
| **Acute toxicity to fish** | | | | | | | |
| Mandestrobin (S-2200) | *Oncorhynchus mykiss* Rainbow trout | static | 96 hr | mm | Mortality | 0.57 | **0.94** |
| Mandestrobin (S-2200) | *Lepomis macrochirus* Bluegill sunfish | static | 96 hr | mm | Mortality | 0.56 | 2.3 |
| Mandestrobin (S-2200) | *Pimephales promelas* Fathead minnow | static | 96 h | mm | Mortality | 0.36 | 1.0 |
| Mandestrobin (S-2200) | *Cyprinodon variegatus* Sheepshead minnow | flow-through | 96 hr | mm | Mortality | 2.2 | > 2.2 |
| R-Isomer of Mandestrobin (S-2167) | *Oncorhynchus mykiss* Rainbow trout | static | 96 hr | mm | Mortality | 0.34 | 0.84 |
| S-Isomer of Mandestrobin (S-2354) | *Oncorhynchus mykiss* Rainbow trout | static | 96 hr | mm | Mortality | 1.7 | > 12 |
| **Chronic toxicity to fish- Fish early life stage toxicity tests** | | | | | | | |
| Mandestrobin (S-2200) | *Pimephales promelas* Fathead minnow | flow-through | 32 d | mm | Fry survival Growth | 0.15 | > 0.15 |
| Mandestrobin (S-2200) | *Cyprinodon variegatus* Sheepshead minnow | flow-through | 34 d | mm | Hatching success Post-hatch survival Growth | 1.3 0.30 0.64 | - |
| **Short-term toxicity to aquatic invertebrates** | | | | | | | |
| Mandestrobin (S-2200) | *Daphnia magna* Water flea | static | 48 h | mm | Immobilisation | 0.7 | 1.2 |
| S-Isomer of Mandestrobin (S-2354) | *Daphnia magna* Water flea | static | 48 h | mm | Immobilisation | 7.3 | > 14 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| R-Isomer of Mandestrobin (S-2167) | *Daphnia magna* Water flea | static | 48 h | mm | Immobilisation | 0.61 | 0.92 |
| Mandestrobin (S-2200) | *Americamysis bahia* Mysid | flow-through | 96 h | mm | Mortality | 0.22 | **0.43** |
| Mandestrobin (S-2200) | *Crassostrea virginica* Oyster | flow-through | 96 h | mm | Shell deposition | 0.29 | 2.0 |
| **Long-term toxicity to aquatic invertebrates** | | | | | | | |
| Mandestrobin (S-2200) | *Daphnia magna* Water flea | flow-through | 21 d | mm | Immobilisation Reproduction | 0.56 | 0.97 > 0.56 |
| Mandestrobin (S-2200) | *Americamysis bahia* Mysid | flow-through | 36 d | mm | Immobilisation Reproduction Growth | **0.0056** | - |
| Mandestrobin (S-2200) | *Chironomus riparius* Sediment-dwelling midge | static | 28 d | mm | Emergence Development | 8.1 | > 8.1 |
| Mandestrobin (S-2200) | *Hyalella azteca* Freshwater amphipod | flow-through | 42 d | mm | Reproduction Growth | 5.0 mg ai/kg | > 30.0 mg ai/kg |
| Mandestrobin (S-2200) | *Leptocheirus plumulosus* Marine amphipod | static-renewal | 28 d | mm | Reproduction Growth | 10.3 mg ai/kg | > 10.3 mg ai/kg |
| **Algae and aquatic plants** | | | | | | | |
| S-Isomer of Mandestrobin (S-2354) | *Pseudokirchneriella subcapitata* Freshwater green alga | static | 72 hr | mm | Biomass Growth rate | 6.0 | 12 > 12 |
| R-Isomer of Mandestrobin (S-2167) | *Pseudokirchneriella subcapitata* Freshwater green alga | static | 72 hr | mm | Biomass Growth rate | 0.13 0.26 | 0.38 **2.2** |
| Mandestrobin (S-2200) | *Lemna gibba* Duckweed | static-renewal | 7 d | mm | Growth rate Biomass | 0.32 1.2 | > 2.3 |
| ***Limited information*** | | | | | | | |
| Mandestrobin (S-2200) | | | | | Growth | | > 0.56 |

mm= mean measured

*Acute (short-term) aquatic toxicity*

Mandestrobin (S-2200, racemate) was shown to exhibit acute toxicity to fish (*Oncorhynchus mykiss*) with $LC_{50}$ = 0.94 mg/L in a static test over 96h. When the two isomers were tested individually the $LC_{50}$ was 0.84 mg/L for the R-isomer and >12 mg/L the S-isomer.

After a short-term flow-through toxicity study with the aquatic invertebrate *Americamysis bahia* the $LC_{50}$ was 0.43 mg/L. During static experiments with Daphnia magna the $LC_{50}$ for Mandestrobin S2200, the R-isomer and S-isomer gave 1.2, 0.92 and > 14 mg/L, respectively.

*Long-term aquatic toxicity*

Mandestrobin is chronically toxic to the aquatic invertebrate *Americamysis bahia* with a NOEC = 0.0056 mg/L (Table 8). The DS, while agreeing to the validity of the study, was of the opinion the NOEC (adult survival) should be 0.0056 mg ai/L, instead of a NOEC of 0.0049mg ai/L (growth, survival) proposed by the notifier of the pesticide review program.

The DS stated that the effects observed at the test concentrations 0.024 and 0.049 mg ai/L should be considered even though they were not considered statistically significant. RAC agrees with the DS and the use of a NOEC value of 0.0056mg ai/L. The NOEC 0.0056mg ai/L represents the value for an effect (cumulative number of dead mysids) that has a statistically significant difference between the treatment and the control. No assessment of the survival of second-generation mysids was conducted as the mortalities in the treatment and the control groups were too high.

*Table: Survival of saltwater mysids exposed to S-2200*

| S-2200 [µg ai/L] (mean measured) | % Juvenile Survival to Pairing (Day 15) | % Adult Survival to Test Termination (Day 36) |
|---|---|---|
| Control | 98.3 | 91.1 |
| Solvent control | 84.7 | 81.0 |
| Pooled control | - a | 86.2 |
| 5.6 | 98.3 | 81.1 |
| 11 | 91.7 | 72.9 **b |
| 24 | 88.3 *b | 77.3 |
| 49 | 100 | 74.5 |
| 84 | 100 | 66.7 ** |

* Statistically significant decrease in survival in comparison to the negative control using Fisher's Exact test ($p \leqslant 0.05$).

** Statistically significant decrease in survival in comparison to the pooled control using Fisher's Exact test ($p \leqslant 0.05$).

a There was a statistically significant difference in juvenile survival between the negative and solvent control groups ($p \leqslant 0.05$). Therefore, comparisons for juvenile survival were made to the negative control.

b While the decrease in survival was statistically significant in comparison to the negative/pooled control, it was not considered to be treatment-related since the difference was slight and was not dose-responsive.

The freshwater green algae *Pseudokirchneriella subcapitata* was tested in a static 72h experiment for both R and S isomers. For the S-isomer, $E_bC_{50}$ = 12 mg/L and $E_rC_{50}$ = > 12 mg/L, while for the R-isomer, $E_bC_{50}$ = 0.38 mg/L and $E_rC_{50}$ =2.2 mg/L.

A study on the higher aquatic plant *Lemna gibba* was performed as a static- renewal 7 day study. The final biomass EC50 was > 2.3 mg ai/L.

## Comments received during public consultation

Comments were received during the public consultation (PC) from 3 Member State Competent Authorities and they were mainly editorial comments and minor corrections to the CLH report, with no significant impact on the proposed classification. All 3 Member State Competent Authorities were in support of the proposed classification and labelling regarding aquatic hazards (acute and chronic).

An additional point was raised during the process by a Member State, where a principle agreement to the proposed aquatic classification was expressed with two additional issues raised: 1) the use of additional algal data and 2) whether additional study details on the *Americamysis bahia* test are available to the dossier submitter. The dossier submitter has provided a response by referring to the concluded prior discussions during the pesticide review program and the fact that the relevant Member States have already assessed the endpoint adequately.

## Assessment and comparison with the classification criteria

### *Degradation*

Mandestrobin is hydrolytically stable at pH 4, 7 and 9 at 50°C over 5 days. According to OECD TG 111, the expected $DT_{50}$ at 25°C would be >1 year for each isomer and hence for the racemate, S-2200. Under irradiated experimental conditions, mandestrobin degraded at a first order $DT_{50}$ value which corresponds to about 4.4 and 4.6 days respectively under environmental conditions in US/UK summer. Mandestrobin is not readily biodegradable, and it does not meet the criterion for rapid degradation in a water/sediment study with a $DT_{50}$ for the whole system of 332 days. Mandestrobin does not meet the criteria of 70 % DOC removal or 60 % depletion of the theoretical oxygen demand during a 28-day biodegradation studies.

### *Bioaccumulation*

Mandestrobin has a Log Kow =3.51 at 25 °C which is below the criterion of Kow $\geqslant$ 4. Also, the fish lipid corrected BCF was determined to be 8, namely well below the criterion BCF of $\geqslant$ 500

for bioaccumulation, and so mandestrobin is considered to have a low potential for bioaccumulation.

***Acute aquatic toxicity***

For Acute (short-term) aquatic hazard, mandestrobin fulfils the criterion of 96 hr $LC_{50}$ (for fish) $\leqslant$ 1 mg/l for fish and invertebrates. The toxicity of the active substance S-2200 to fish (*Oncorhynchus mykiss*) was $LC_{50}$ = 0.94 mg/L and for the aquatic invertebrate Americamysis bahia, $LC_{50}$ = 0.43 mg/L. These values are in the range of 0,1 < $L(E)C_{50} \leqslant$ 1 mg/L which justifies an acute M-factor of 1.

***Chronic aquatic toxicity***

For chronic aquatic toxicity, mandestrobin fulfils the criterion of NOEC $\leqslant$ 0.1 mg/L for aquatic invertebrates. The chronic toxicity of the active substance mandestrobin to the aquatic invertebrate *Americamysis bahia* is a NOEC = 0.0056 mg/L. This value is below 0.01 mg/L which is the classification threshold for category Chronic 1 for not rapidly degradable substances, and warrants a chronic M-factor of 10 (0.001 < NOEC $\leqslant$ 0.01 mg/L).

***Conclusion on Classification***

Mandestrobin is considered not rapidly degradable and has a low potential for bioaccumulation. In agreement with the DS, RAC is of the opinion that mandestrobin should be classified as:

**Aquatic Acute 1** – H400 'Very toxic to aquatic life' with an **M factor = 1**

**Aquatic Chronic 1** - H410 'Very toxic to aquatic life with long lasting effects' with an **M factor = 10.**

## 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).