Addendum 2

to the Draft Assessment Report

01 August 2001

Pyraclostrobin

Volume 3, B.6 Toxicology and metabolism

25 June 2003

Rapporteur Member State: Germany

Contents

1. Introduction

The main objective of this addendum is to adress the developmental toxicity of pyraclostrobin once more and to give recommendations for appropriate classification and labelling. Furthermore, some short-term toxicity studies in rats, mice and rabbits are reported which have been performed and submitted very recently only. Most likely, these new studies were conducted with the intention to support setting of an ARfD for this active substance. In addition, a new Ames test was submitted using a batch of with a relatively high amount (about 0.28 %) of the impurity 399379 [N-(2-((1-(4-chlorophenyl)-1H-pyrazol-3-yl)oxymethyl)phenyl)-O-methylhydroxylamine] and is also described in this addendum.

After the draft assessment report on pyraclostrobin had been completed, information regarding the occurrence of the cancerogenic and mutagenic impurity dimethylsulfate in different batches of this compound was obtained. In particular, the very low amount of this impurity in that batch which was administered in the long-term studies gives rise to serious concern. Possible consequences are discussed and opportunities for a solution proposed.

In addition, it became necessary to check once more whether the dosages in some of the toxicity studies including the long-term experiments were sufficiently high since the Canadian and US authorities doubted that the MTD had been actually reached and, therefore, expressed a possible need to repeat these studies with higher dose levels.

2. Re-evaluation of developmental toxicity with special regard to the presence or absence of maternal effects; Classification and labelling

At the meeting of the Working Group (Evaluation) in March, 2003, the Member States were informed that the Swedish authorities expressed concern about certain findings in the developmental toxicity studies in rats and rabbits when they were evaluated for national authorisation purposes. Discussions between the National Chemicals Inspectorate (KEMI), Sweden, and the applicant (BASF) on this issue had already been hold. Meanwhile, authorisation in Sweden has been granted.
The RMS was requested to comment on developmental and maternal toxicity in both species. The studies were subject to re-evaluation and the respective position papers of KEMI (KEMI, 2003) as well as of the notifier (BASF, 2003) also taken into consideration.

The outcome of this comprehensive assessment by the RMS can be summarised as follows:

Based on the findings in the developmental toxicity studies in rats and rabbits, SE notes that pyraclostrobin has the potential to induce reproductive effects and, therefore, a classification for developmental toxicity, category 3, Xn, R63 is probably warranted.

Developmental toxicity, rats
Wistar rats were orally exposed to pyraclostrobin at doses of 0, 10, 25 and 50 mg/kg bw/d from day 6 through day 19 p.c.. The NOAEL for maternal toxicity was 10 mg/kg bw/d, based on reduced food consumption and reductions in corrected body weight gain. Under the conditions of this prenatal developmental toxicity study, pyraclostrobin was not teratogenic in rats. The incidence of soft tissue and skeletal variations was significantly increased at the highest dose level of 50 mg/kg bw/d. Although all anomalies were inside the range of the historical control values, a treatment-related effect cannot be excluded. The NOAEL for developmental toxicity was 25 mg/kg bw/d. This conclusion has already been stated in the draft assessment report.

Developmental toxicity, rabbits
In the first study, Himalayan rabbits were orally exposed to at dose levels of 0, 5, 10 and 20 mg/kg bw/d from day 7 through day 28 p.i.. Maternal toxicity was substantiated by dose-related reduced food consumption with subsequent impairment in body weight gain at all dose levels only during the initial phase of the treatment. Decreased defecation occurred predominantly in the high dose group. After the initial phase food consumption and body weight gain increased and at the end of treatment the corrected body weight gain was comparable at all dose levels. A reduction in mean gravid uterus weight was observed at 20 mg/kg bw/d. There was a dose-related impact on some gestational parameters reaching statistical significance at the high dose level: Postimplantation losses were elevated mainly due to an increase in early resorptions and the mean number of live fetuses per doe was reduced.
In addition, the incidence of external or soft tissue malformations or variations was not increased but there was an apparent dose-dependent increase in the occurrence of skeletal malformations. Although the mean number of live fetuses was significantly reduced at the high dose group (due to the increased postimplantation losses) the absolute number of affected fetuses was increased at this dose level. In the intermediate and high dose groups, the percentage of fetuses with skeletal malformations was obviously higher than in the control group. Although statistical significance was not attained, the percentage of affected fetuses was outside the historical control range of the laboratory and strain. One cannot confirm that

the observed developmental effects were definitely caused by the decreased food consumption.
The NOAEL for maternal toxicity was < 5mg/kg bw/d in this study, based on the reduced food consumption on day 7 and 8 p.i.. The NOAEL for developmental toxicity was 5 mg/kg bw/d based on increased skeletal malformations at 10 mg/kg bw/d.

A second developmental study in Himalayan rabbits was requested to establish a NOAEL for maternal toxicity. The test substance was administered at doses of 0, 1, 3 and 5 mg/kg bw/d on day 7 through day 28 p.i.. There was a statistically significant reduction in food consumption and bodyweight change at day 7 and 8 at a dose level of 5 mg/kg bw/d. In addition, an effect was seen at the next lower dose of 3 mg/kg bw/d, however, since the occasionally reduced food intake at this dose level was not accompanied by an impaired food utilisation or a statistically reduced bodyweight gain, the dose of 3 mg/kg bw/d is considered to be the NOAEL for maternal toxicity. Only few fetal parameters were investigated on day 29 post insemination (early and late resorptions, live and dead fetuses, fetal body weight). Individual placental weights were also recorded. Investigations regarding external, soft tissue and skeletal findings have not been conducted.

In conclusion, the developmental toxicity study in rats revealed a NOAEL for maternal toxicity of 10 mg/kg bw/d, based on reduced food consumption and reductions in corrected body weight gain. The NOAEL for developmental toxicity was 25 mg/kg bw/d, based on increased incidences of soft tissue and skeletal variations inside the range of the historical control values.
In the developmental toxicity studies in rabbits the NOAEL for maternal toxicity was 3 mg/kg bw/d, based on the reduced food consumption (on day 7 and 8 p.i.) and body weight gain (from day 7 to 9 p.i.). The NOAEL for developmental toxicity was 5 mg/kg bw/d because of non significant increased incidences of skeletal malformations outside the historical control range of this laboratory at 10 and 20 mg/kg bw/d. It is not clear whether or to which degree any developmental effects (e.g. increased postimplantation losses) can be attributed to maternal toxicity.

Based on the findings observed in the studies in rats and rabbits, a potential of pyraclostrobin to cause developmental toxicity cannot be excluded. There is no clear relationship of the increased incidences of skeletal malformations to maternal toxicity in rabbits. Therefore, labelling for developmental effects (Cat. 3, R 63) is recommended.

3. Short term studies to support setting of an ARfD

Setting of an Acute Reference Dose (ARfD) was a crucial point in the toxicological evaluation of pyraclostrobin. In its original dossier, the notifier recommended to derive an ARfD of 0.09 mg/kg bw from the NOEL in a 4-week oral rat study using a safety factor of 100. However, since the range of parameters investigated in this experiment was rather small and since the LOAEL for maternal toxicity in both developmental studies in pregnant rabbits was lower (5 mg/kg bw/d), this suggestion could not be followed by the Rapporteur. In the draft assessment report, it was proposed to set the ARfD at 0.04 mg/kg bw on the basis of an "overall" NOAEL of 4 mg/kg bw/d for oral short-term toxicity in rodents. This value was further supported by the maternal toxicity data from the developmental toxicity studies in rabbits. Later on, the ECCO 123 peer review meeting (ECCO, 2002) agreed a slightly lower ARfD of 0.03 mg/kg bw. This value was based on the NOAEL of 3 mg/kg bw/d as established in the second developmental study in rabbits (Schilling et al., 2001, see draft

assessment report). In this trial, the focus was clearly on maternal toxicity and lower dose levels (0, 1, 3, 5 mg/kg bw/d) than in a previous full-scale rabbit teratology study were included. The ECCO meeting argued that treatment-related effects (decreased food consumption and body weight gain) became apparent from the first day of dosing already.

In the beginning of 2003, the notifier submitted a new short-term toxicity study in male and non-pregnant female rabbits and two "mechanistic" studies in rats and mice (Schneider and Hellwig, 2002; Mellert et al., 2002a and b) most probably to support setting of an ARfD which might be higher than established by ECCO. In all these trials, the duration of exposure was one week. In the following, these studies are briefly reported and assessed with special emphasis on their suitability to have an impact on setting the ARfD.

Rabbit

Report: Schneider, S. and Hellwig, J. (2002): BAS 500 F - Test study in male and non-pregnant female Himalayan rabbits. Oral administration (gavage) and administration in the diet. BASF AG, Experimental Toxicology and Ecology, Ludwigshafen/Germany; BASF RegDoc# 2002/1012052, Project no. 06R0494/96209; unpublished

Test Material: Pyraclostrobin; batch No. CP 029053; purity: 99.0 %.

Test System: Himalayan rabbits (Chbb:HM), obtained from Charles River Lab. Germany

GLP: Yes.

Test Method: Not applicable.

Acceptability: The study is considered to be supplementary because of the limited range of parameters investigated.

Material and Methods:
The test compound was administered to groups of five male and five non-pregnant female rabbits either by gavage or via the diet over a period of one week. For both routes of exposure, the dose level was 4 mg/kg bw/day. Two control groups of the same size were also included. In the control group allocated to the stomach tube application, the animals were dosed with the vehicle only (0.5 % carboxymethylcellulose CB 30.000 in doubly distilled water). The daily dosing volume was 10 ml/kg bw. In the feeding experiment, the control animals received the ground rabbit diet.
The rabbits were kept under standard conditions.
Food consumption was recorded daily and body weights were determined on days 0, 2, 4 and 6 and at sacrifice on day 7. During treatment, the rabbits were monitored daily for clinical signs of toxicity. Following sacrifice, the animals were necropsied and subjected to gross pathological examination.

Findings:
There were no clinical findings which could be indicative of a toxic effect. Body weight was not altered in any sex or group. No remarkable necropsy observations were recorded.

When the group mean values are regarded, food consumption appears lower in the treated groups. Table 1 summarises the mean food consumption for the whole treatment period and, because of the potential use of these data for setting of an ARfD, for the first day on study.

Table 1: Mean food consumption (g/animal/day) in groups of rabbits (n=5 per sex) treated with and in the respective control groups

	Gavage administration		**Feeding experiment**	
Dose level	**Control**	**4 mg/kg bw/d**	**Control**	**4 mg/kg bw/d**
Males, day 0 - 1	115.0	72.4	59.2	51.5 g
Males, days 0-7	101.2	69.1	64.1	49.1
Females, day 0 - 1	108.7	75.1	74.9	53.9
Females, days 0 - 7	101.8	81.4	61.4	47.8

It is obvious that there is a marked difference in food consumption between the two control groups in both sexes. The lower food consumption in the control groups in the feeding experiment might explain the more pronounced relative decrease, in particular in male animals, when the test substance was administered by gavage.
Individual values showed a very high variability.

Conclusion:
An effect of pyraclostrobin on food consumption in rabbits cannot be excluded because of the large difference between the control groups and because of the high interindividual variability of this parameter. Therefore, a reliable NOAEL cannot be established.
The results of this study do not contradict or invalidate the outcome of the developmental toxicity studies in pregnant rabbits. A valid comparison cannot be made since there is a huge difference between pregnant and non-pregnant does and the duration of treatment was also different. Thus, 3 mg/kg bw/d as obtained in the second rabbit developmental study is still considered as NOAEL in rabbits.

<u>Rat, mouse</u>

The principal design of the mechanistic studies in rats (Mellert et al., 2002a) and mice (Mellert et al., 2002b) was the same as in the study in rabbits described above with the exception that the animals were weighed daily. As in rabbits, 5 male and 5 female animals per dose or control group were employed. However, dose levels in the feeding experiments were different. In rats, the dietary dose levels were 0 and 34 ppm (equal to approximately 3.9 mg/kg bw/d for males, substance intake for females not given). In mice, dose levels of 0 and 18 ppm (corresponding to about 5.5 mg/kg bw/day) were used in male animals and of 0 and 15 ppm (ca 7.2 mg/kg bw/d) in females. Furthermore, there was no necropsy and no pathological examination in these studies. Since only summaries of the results have been submitted so far, these experiments are not reported in detail and the available information is considered supplementary only.

Findings:
No effect of administration neither by gavage (4 mg/kg bw/d) nor via the diet was seen in mice.

In rats, the only apparent finding which could be attributed to treatment was a lower body weight gain in the female group receiving the dietary dose than in the allocated control group which was obvious throughout the whole study period and gained statistical significance at the end of this week. No such difference was seen neither in the female group treated by gavage nor in any of the male groups (Table 2). However, food consumption in the group of female rats receiving 34 ppm also appeared somewhat lower than in the control.

Table 2: Mean food consumption (with range), body weight and body weight gain in female Wistar rats receiving

	Gavage administration		**Feeding experiment**	
Dose level	**Control**	**4 mg/kg bw/d**	**Control**	**34 ppm**
Food consumption (g/animal/day), days 0 - 7	15.9 (Range: 15.2 - 17.2)	15.7 (Range: 15.2 - 17.0)	16.3 (Range: 15.8 - 17.5)	14.9 (Range: 14.4 - 16.4)
Body weight (g), day 7	142.9	142.3	146.8	141.5
Body weight gain, days 0 - 7 (g)	19.8	19.0	23.6	19.4**

** $p < 0.01$, two-sided Welch t-test

Conclusion:
Taken together, these reductions could indicate a very weak adverse effect in female rats when pyraclostrobin is administered via the diet. It should be emphasised that the daily substance intake in this study was in the magnitude of the "overall" NOEL of 4 mg/kg bw/d established for rodents (see draft assessment report).

The recently submitted one-week studies in rabbits, rats and mice must be considered as supplemental and do not contribute much to the toxicological assessment. The occurrence of possible weak effects in rabbits and rat around a dose level of 4 mg/kg bw/day supports the ARfD of 0.03 mg/kg bw as agreed by the ECCO 123 meeting.

4. Genotoxicity testing of a batch containing a new impurity

The recent 5-batch analysis (December 2002) revealed the occurrence of the impurity N-(2-((1-(4-chlorophenyl)-1H-pyrazol-3-yl)oxymethyl)phenyl)-O-methylhydroxylamine (internal registration number of the manufacturer "399379", empirical formula $C_{16}H_{13}Cl_2N_3O$) at concentrations ranging from 0.17 to 0.28 % with a mean content in the batches investigated of 0.26 %. Thus, this is one of the very few impurities exceeding 0.1 %. In a previous 6-batch analysis (carried out in 1999), this impurity has not been identified. A search for information about this substance was performed by the Rapporteur in the EINECS database but no entry was found. The chemical structure of this impurity is similar to some of the metabolites detected in the rat metabolism study described in the draft assessment report but not completely identical with any of them.

The applicant provided an additional Ames test which had been performed with a batch of which is declared to contain 0.28 % of the impurity 399379. It must be stated that the determination of this amount was not conducted under GLP conditions and that the test material produced in 2001 was not included in the 5-batch-analysis mentioned above although the same maximum concentration was found for one of the batches there. As compared to the bacterial mutagenicity test submitted as part of the original dosssier (Engelhardt and Hoffmann, 1997, see draft assessment report), the purity of the batch used now was higher by about 1 %.
Like the previous mutagenicity studies included in the draft assessment report, this assay did not reveal any evidence of genotoxicity. Taking into account the low amount of the substance 399379 in the batches examined and its structural similarity to metabolites which have been present at least in the rat organism during the toxicological studies as well as the absence of any information suggesting adverse effects, this impurity is not considered to be of concern.
In the following, the new Ames test is reported in detail:

Report:	Engelhardt, G. and Leibold, E. (2002): Salmonella typhimurium / Escherichia coli reverse mutation assay (standard plate test and preincubation test) with BAS 500 F. BASF AG, Experimental Toxicology and Ecology, Ludwigshafen/Germany; Project no. 40M0494/964429, BASF RegDoc# 2002/1004984; unpublished.
Test Material:	Pyraclostrobin; batch No. Ch 18; purity: 99.2 %.
Test System:	Salmonella typhimurium (strains TA 100, TA 1535, TA 1537 and TA 98) and Escherichia coli (strain WP2 uvrA).
GLP:	Yes.
Test Method:	OECD 471; EEC 2000/32 (B.13/B.14).
Deviations:	None.
Acceptability:	The study is considered to be acceptable.

Material and Methods:
A new batch of pyraclostrobin was tested for its ability to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation assay. The Salmonella typhimurium strains TA 100, TA 1535, TA 1537 and TA 98 and Escherichia coli strain WP2 uvrA were exposed to the test substance dissolved in DMSO at doses ranging from 20 to 5,000 µg/plate. The dosing volume was 100 µl in all trials. The study consisted of a standard plate test and a preincubation test both with and without metabolic activation (liver S-9 mix obtained from Aroclor-1254-induced Sprague-Dawley rats). Three plates were used per dose for each strain and test condition including the controls.

For control purposes and to demonstrate the sensitivity of the test system, a sterility control, a negative control (DMSO) and appropriate positive controls were included. Positive control substances were 2-aminoanthracene in the activation experiments (different concentrations applied for testing of S. typhimurium and E. coli) and N-methyl-N-nitro-N-nitrosoguanidine, 4-nitro-o-phenylen-diamine, 9-aminoacridine or 4-nitroquinoline-N-oxide in the trials without S-9 mix.

Findings:
The stability of pyraclostrobin in the vehicle DMSO and in water has been determined analytically.
Test substance precipitation was observed at concentrations of 500 µg/plate and higher. However, no bacteriotoxic effect was observed.

The mean number of revertant colonies was not increased in any strain either with or without S-9 activation. The positive control substances gave the expected increases in revertant colonies.

Conclusion:
According to the results of the study, the tested batch of containing 0.28 % of the impurity 399379 was not mutagenic in the Ames reverse mutation assay.

5. Toxicological relevance of the impurity dimethyl sulfate

According to the substance specification for the active ingredient pyraclostrobin as included in the confidential part of the draft assessment report, the impurity dimethyl sulfate (DMS) may be contained up to a maximum amount of 0.0003 % (3 mg/kg) although analytical data suggest a measured peak concentration of only 1 ppm and this one was confined to a single batch of a total of five investigated. The recently submitted new 5-batch analysis (December 2002) revealed only traces of DMS, if any, below 0.0001 % in all batches examined.

In the chemical industry, the alkylating (methylating) and sulphating agent DMS is manufactured at high tonnages and is mainly used as an intermediate. According to the current EINECS inventory, DMS (CAS no. 77-78-1; EINECS no. 201-058-1) is classified for carcinogenicity (Category 2), mutagenicity (Cat. 3), high (oral intake) or very high (inhalation) acute toxicity, corrosivity and skin sensitisation. The risk phrases R 45, R 68, R 26 (T+), R 25 (T), R 34 (C) and R 43 have been allocated. DMS was on the 2nd priority list of the EU for dangerous chemicals and a comprehensive evaluation report from the Netherlands is available (TNO/RIVM, 1999). A review of this evaluation report by the RMS confirmed that current classification and labelling is supported by appropriate and valid experimental data. With regard to cancerogenicity, it must be stated that no long-term feeding studies are available. However, DMS caused mainly local tumours following inhalative and subcutaneous exposure and a genotoxic mechanism was assumed. Reliable assessment of reproductive toxicity is not possible because of data gaps.

The toxic properties of DMS give rise to health concern with regard to the possible occurrence even of traces of this substance in plant protection products containing pyraclostrobin as the active compound and as a potential part of the total residues on treated crops. Therefore, it was checked whether the batch containing the highest determined amount of DMS of 1 mg/kg had been sufficiently tested in the available toxicological studies to allow reliable assessment of possible effects of this impurity.

It is very important to emphasize that this batch (CP 026063) was used in all the mutagenicity tests described in the draft assessment report. These assays did not provide any evidence of genotoxic effects neither in vitro nor in vivo. Thus, it may be reasonably concluded that DMS if occcurring as an impurity of pyraclostrobin at least up to the low concentration of 0.0001 % will not induce point (gene) mutations in bacteria and mammalian cells (HGPRT gene locus), clastogenic effects or unscheduled DNA damage and repair.

Apart from the mutagenicity studies, the same batch was the test material in the acute dermal toxicity study revealing low toxicity and in the acute inhalation experiment proving a remarkably high toxicity (R 23, T). In addition, it was applied in the dermal irritation study where irritating properties have been observed but also in the eye irritation trial revealing no convincing evidence of an adverse effect. Since acute effects are concentration-dependent, it is not very likely that the critical findings in two of these studies are due to the very low amount of DMS. This assumption is also supported by the inconsistent outcome of the irritiation studies.

In all the other toxicological experiments including the long-term studies, other batches of were used. With regard to the test material in the chronic toxicity and cancerogenicity studies in rats and mice, no analytical information about the DMS content is available. It was noted that the batch used in these studies was of rather low purity (about 97 %). No evidence of cancerogenicity was obtained.

For risk assessment purposes, it must be taken into account that there are uncertainties about the presence of this potential mutagenic and cancerogenic impurity in a major part of the toxicological studies with pyraclostrobin. However, the available body of analytical information strongly suggests that the DMS concentration in nearly all batches is below or equal to but not higher than 0.0001 %. Pyraclostrobin containing this amount was devoid of a mutagenic potential. Thus, no significant health risk is to be expected when the DMS content is maintained in this very low magnitude. However, since possible effects of higher concentrations of this impurity have not been investigated or, at least, this cannot be proven in the absence of appropriate analytical data, it is proposed to change the specification for pyraclostrobin in that way that 0.0001 % DMS should be the maximum content. Contaminations of the active ingredient above this amount must not be allowed.

6. Dose selection in long-term and other toxicological studies with pyraclostrobin

In November, 2002, the Rapporteur became aware of the existence of a toxicological evaluation of pyraclostrobin prepared by Health Canada and the U.S. EPA for national registration purposes in these countries. The German health authorities were asked to participate in a phone conference dealing with critical points in the toxicological assessment. Immediately before this conference, the Canadian/U.S. evaluation was received.

The main difference between this submission and the current EU evaluation was that the North American authorities, at least at that time, did not accept a considerable part of the toxicological data package, including the long-term studies, because of their opinion that the Maximum Tolerated Dose (MTD) had not been reached. During the subsequent discussion it became obvious that different approaches were followed in the EU and North America and that studies of this type are assessed on a different legal basis. Thus, agreement could not be reached.

When the draft assessment report on pyraclostrobin was compiled and the studies from the dossier evaluated, the German authorities followed the recommendations of the current OECD guidelines, as it is common practice in the EU. For the chronic toxicity/ cancerogenicity studies, e.g., the occurrence of signs of minimal toxicity at the highest dose tested is required by the OECD Guideline 453 (adopted 12 May 1981). Such signs may be alterations in serum enzyme levels or slight depression of body weight gain (less than 10 percent). These criteria have been clearly met in the chronic toxicity study in rats as well as in the carcinogenicity study in this species at the top dose level of 200 ppm. Clear adverse effects on body weight were also noted in the long-term study in mice at 120 and 180 ppm (see draft assessment report). The same holds true for the multigeneration study in rats where clear parental and reproductive effects were seen at the highest dose level of 300 ppm.

The Canadian/U.S. requirements are based on EPA guidelines which are not mandatory to be followed in the EU evaluation process. Thus, there is no need to change our current evaluation or even to require new toxicological studies. The latter idea would clearly contravene the goal of reducing the number of laboratory animals in toxicological testing. The available database is complete and allows for comprehensive and reliable risk assessment of this active ingredient.

7. Summary

To update and complete the toxicological evaluation of pyraclostrobin, a number of new studies, chemical-analytical data and regulatory background documents were evaluated by the RMS whereas the available developmental toxicity studies as well as the analytical profile of the batches used in the toxicological studies were subject to re-evaluation.

Based on this new assessment, the following amendments to the previously drawn conclusions on should be made:

- **Developmental toxicity**

Based on the findings observed in the studies in rats and rabbits, a potential of pyraclostrobin to cause developmental toxicity cannot be excluded. There is no clear relationship of the increased incidences of skeletal malformations to maternal toxicity in rabbits. Therefore, classification and labelling for developmental effects (Cat. 3, R 63) is recommended.

- **Setting of the ARfD**

The recently submitted one-week studies in rabbits, rats and mice must be considered as supplemental and do not contribute much to the toxicological assessment. However, the occurrence of possible weak effects in rabbits and rat around a dose level of 4 mg/kg bw/day supports the ARfD of 0.03 mg/kg bw as agreed by the ECCO 123 meeting.

- **Impurity "399379" [N-(2-((1-(4-chlorophenyl)-1H-pyrazol-3-yl)oxymethyl)phenyl)-O-methyl-hydroxylamine]**

This impurity has been detected upon a recent 5-batch analysis. An Ames test was performed using pyraclostrobin containing the highest measured concentration of this substance. No evidence of genotoxicity was obtained. Based on the available data and some theoretical considerations, no risk for human health is anticipated.

- **Impurity dimethyl sulfate (DMS)**

The potential mutagenic and cancerogenic agent dimethyl sulfate may occur in traces as an impurity of pyraclostrobin technical. The content of this impurity must not exceed 0.0001 % (1 ppm) since this is the highest concentration in a batch which has been tested for mutagenicity. Thus, the proposed specification allowing for up to 3 ppm DMS should be changed since possible adverse effects of such an amount of DMS have not been sufficiently investigated.

- **Dose selection in the long-term and reproduction studies**

The dose levels employed are considered to be sufficiently high to enable comprehensive and reliable toxicological evaluation. The studies have been performed on the basis of current OECD guidelines. There is no need to change our toxicological assessment or to require new data.

8. References relied on

Only new studies and documents which have not been adressed in the original draft assessment report are mentioned here.

BASF (2003): Assessment of findings in developmental toxicity studies in rats and rabbits based on discussions with the National Chemicals Inspectorate, Sweden and BASF on pyraclostrobin (BAS 500 F) ; unpublished Working document.

ECCO (2002): Annex 8 to concise outline report of ECCO 123 peer review meeting. Pyraclostrobin. 11 March 2002. 7032/ECCO/PSD/02.

Engelhardt, G.; Leibold, E. (2002): Salmonella typhimurium / Escherichia coli reverse mutation assay (standard plate test and preincubation test) with BAS 500 F. BASF AG, Experimental Toxicology and Ecology, Ludwigshafen/Germany; BASF RegDoc# 2002/1004984, Project no. 40M0494/964429; unpublished. Owner: BASF. GLP: yes.

KEMI (2003): Memorandum pending a decision 2003-03-01,
No. F-2155-438-01 by the National Chemicals Inspectorate, Sweden; unpublished Working document.

Mellert, W. (2002a): Summary of results. BAS 500 F - Mechanistic study to determine the "Acute-Reference-Dose" in Wistar rats. Administration in the diet and by gavage. BASF AG, Experimental Toxicology and Ecology, Ludwigshafen/Germany; BASF RegDoc# 2002/1011458, Project no. 99SO494/96208; unpublished. Owner: BASF. GLP: not applicable.

Mellert, W. (2002b): Summary of results. BAS 500 F - Mechanistic study to determine the "Acute-Reference-Dose" in B6C3F1 mice. Administration in the diet and by gavage. BASF AG, Experimental Toxicology and Ecology, Ludwigshafen/Germany; BASF RegDoc# 2002/1011459, Project no. 99SO494/96210; unpublished. Owner: BASF. GLP: not applicable.

Schneider, S. and Hellwig, J. (2002): BAS 500 F - Test study in male and non-pregnant female Himalayan rabbits. Oral administration (gavage) and administration in the diet. BASF AG, Experimental Toxicology and Ecology, Ludwigshafen/Germany; BASF RegDoc# 2002/1012052, Project no. 06R0494/96209; unpublished. Owner: BASF. GLP: yes.

TNO/RIVM (1999): Dimethyl sulphate. Risk assessment. Final report on behalf of the Ministry of Housing, Spatial Planning and the Environment (VROM) in consultation with the Ministry of Social Affairs and Employment (SZW) and the Ministry of Public Health, Welfare and Sport (VWS). Contact point: Chemical Substances Bureau, P.O. Box 1, 3720 BA Bilthoven, The Netherlands.

Addendum 3

to the Draft Assessment Report

01 August 2001

Pyraclostrobin

14 October 2003

Rapporteur Member State: Germany

Contents

B.7.6 Residues resulting from supervised trials (Annex IIA 6.3; Annex IIIA 8.2)

ECCO 125 asked for additional residue data (bridging trials) with grapes to ensure that the residue trials conducted with a higher number (8) of applications and with different formulations also reflect the current GAP with only 3 applications.

Report: Study on the residue behaviour of BAS 550 F and BAS 500 F in grapes after application of BAS 536 00 F, BAS 550 09 F or BAS 500 00 F under field conditions in Germany, France (N), Italy, 2001
Jones, S.; 2002
2002/1010480, RIP2003-77

Material and methods:
In 2001, 5 trials were performed in grapes testing pyraclostrobin in the solo formulation BAS 500 00 F, but also the formulation BAS 536 00 F containing pyraclostrobin and dimethomorph was applied during this bridging program. The trials were conducted in different representative grape growing areas in Germany, France and Italy.
The plant protection products were applied three times at a constant rate of 0.16 kg pyraclostrobin/ha. In all trials, fruit samples were taken directly after the last application (0 DALA) as well as about 2, 3, 4 and 5 weeks thereafter.

The samples were analysed for pyraclostrobin with BASF method No. 445/0 which quantifies the relevant residue of pyraclostrobin and its metabolite BF 500-3 at a limit of quantitation of 0.02 mg/kg each. The results of procedural recovery experiments obtained with each analytical series were about 92% for pyraclostrobin and 90% for BF 500-3 at a fortification level of 0.02 mg/kg. Additionally, the method was validated in a separate study.

Findings:
Directly after the last application, the residue of pyraclostrobin (parent) was found between 0.27 and 2.04 mg/kg. After about 35 days which is the intended PHI of BAS 500 00 F, the total residue of pyraclostrobin in fruits ranged from 0.13 to 1.24 mg/kg. No significant difference was found between the residue levels after the treatment of the different formulations.

Residue trial details and results are summarised in Table 7.7–8.

Conclusion:
A sufficient number of residue trials in grapes have been performed with pyraclostrobin formulations in the northern and the southern part of Europe. In total, more than 40 trials over 5 years were conducted supporting different use patterns.
The results show that there is no significant difference in the residue level of trials conducted with a different number of applications and/or different formulations. In fact the new results with 3 applications and a maximum application rate show slightly higher residues than the older trials. For the calculation of a MRL it makes no difference whether 3 or 8 applications were used in the residue trials.

B.7.7 Effects of industrial processing and/or household preparation (Annex IIA 6.5; Annex IIIA 8.4)

ECCO 125 asked for a calculation of mass balances for processing from grapes to wine on basis of typical values (weights) known for this process.

Report: Study on the residue behaviour of BAS 500 F in grapes and grape process fractions after treatments with BAS 500 00 F under field conditions in Germany, 1998
Meumann H. et al. 1999
1999/10982, RIP2000-1079

Grapes: Calculation of the mass balance during wine processing
Bross, M., 2002
2002/1008777, RIP2003-81

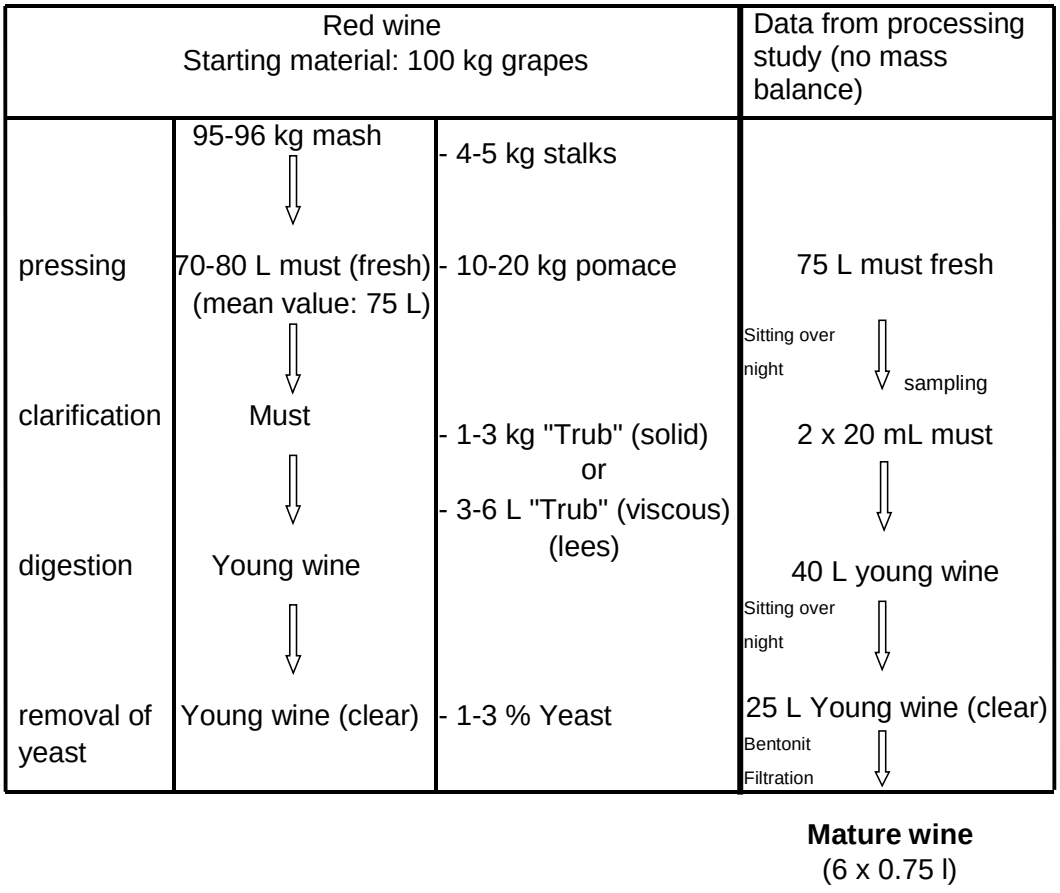
During the 1998 growing season 4 field trials, 2 each with varieties of red and white grapes were conducted in different representative wine growing areas in Germany to determine the residue levels of pyraclostrobin and its metabolite BF 500-3 in grapes and grape process fractions (juice, wine, wet pomace) (RIP2000-1079).
In order to calculate the mass balance, standard values for fraction weights were obtained from the principal investigator of this phase. The typical procedure performed at SLFA in Neustadt, consists of several aliquotation steps. The relevant procedures are summarised in Figure 7.7–1 and Figure 7.7–2.

Figure 7.7–1: Typical processing procedure for the preparation of white wine

White wine/Rose wine Starting material: 100 kg grapes			Data from processing study (no mass balance)
	100 kg mash ⇩		
pressing	70-80 L must (fresh) (mean value: 75 L) ⇩	- 15-25 kg pomace	75 L must fresh Sitting over night ⇩ sampling
clarification	Must ⇩	- 1-3 kg "Trub" (solid) or - 3-6 l "Trub" (viscous) (lees)	2 x 20 mL must ⇩
digestion	Young wine ⇩		40 L young wine Sitting over night ⇩
removal of yeast	Young wine (clear)	- 1-3 % Yeast	25 L Young wine (clear) Bentonit Filtration ⇩
			Mature wine (6 x 0.75 L)

The procedure for red wine resulted in comparable yields:

Figure 7.7–2: Typical processing procedure for the preparation of red wine



For estimating the mass balance, the fraction weights typically obtained are correlated with the residue values of pyraclostrobin obtained in the processing study.

During the calculation procedure, the weights of the individual waste products were fully considered whereas the amounts of sugar, sulfite and yeast were neglected. These amounts are negligible compared with the amount of grapes processed. For must, a density of 1.08 was assumed; the density of young wine is ~ 1. Due to missing data the last filtration step was also not taken into account.

The data for the individual trials are shown in Table 7.7–1 to Table 7.7–4.
It is obvious that most of the residue is transfered from the grapes to the wet pomace (transference: 92%, 139%, 97%, 66%). The transference for all other processing products and by products including wine is clear below 5%.

Table 7.7–1: Calculation of mass balance (white wine, trial: DU2/03/98)

White wine production				BAS 500 F Residue [mg/kg]					BAS 500 F Residue [mg/kg]		
Step	PF	Amount of PF in Liter	Amount of PF in kg	DU2/03/98	mg BAS 500 F	% of initial	Waste fraction	Amount of waste fraction in kg	DU2/03/98	mg BAS 500 F	% of initial
grapes (RAC, 35 DALT)			100	0.76	76	100	n.a.				
preparation of mash	mash		100	n.a.	n.a.	n.a.	n.a.	no losses	n.a.	n.a	n.a.
pressing	fresh must	70	75.6	n.a.	n.a.	n.a.	Wet pomace	25	2.81	70.25	92.4
Pre-clarification	must (cl.), cold	73	80	0.02	1.6	2.1	"Trub"	1-3	n.a.	n.a	n.a.
	must (cl.), heated	73	80	0.02	1.6	2.1	"Trub"	1-3	n.a.	n.a	n.a.
Fermentation	young wine	73	73	n.a.	n.a.	n.a.	n.a.	no losses	n.a.	n.a	n.a.
Removal of yeast	wine from cold must	71.5	71.5	0.02	1.43	1.9	yeast	1.5	n.a.	n.a	n.a.
Removal of yeast	wine from heated must	71.5	71.5	0.02	1.43	1.9	yeast	1.5	n.a.	n.a	n.a.

Table 7.7–2: Calculation of mass balance (white wine, trial: DU3/03/98)

White wine production				BAS 500 F Residue [mg/kg]					BAS 500 F Residue [mg/kg]		
Step	PF	Amount of PF in Liter	Amount of PF in kg	DU3/03/98	mg BAS 500 F	% of initial	Waste fraction	Amount of waste fraction in kg	DU3/03/98	mg BAS 500 F	% of initial
grapes (RAC, 35 DALT)			100	0.36	36	100	n.a.				
preparation of mash	mash		100	n.a.	n.a.	n.a.	n.a.	no losses	n.a.	n.a.	n.a.
pressing	fresh must	70	75.6	n.a.	n.a.	n.a.	Wet pomace	25	2.00	50	138.9
Pre-clarification	must (cl.), cold	73	80	0.02	1.6	4.4	"Trub"	1-3	n.a.	n.a.	n.a.
	must (cl.), heated	73	80	0.02	1.6	4.4	"Trub"	1-3	n.a.	n.a.	n.a.
Fermentation	young wine	73	73	n.a.	n.a.	n.a.	n.a.	no losses	n.a.	n.a.	n.a.
Removal of yeast	wine from cold must	71.5	71.5	0.02	1.43	4.0	yeast	1.5	n.a.	n.a.	n.a.
Removal of yeast	wine from heated must	71.5	71.5	0.02	1.43	4.0	yeast	1.5	n.a.	n.a.	n.a.

Table 7.7–3: Calculation of mass balance (rose wine, trial: DU2/02/98)

Rose wine production				BAS 500 F Residue [mg/kg]					BAS 500 F Residue [mg/kg]		
Step	PF	Amount of PF in Liter	Amount of PF in kg	DU2/02/98	mg BAS 500 F	% of initial	Waste fraction	Amount of waste fraction in kg	DU2/02/98	mg BAS 500 F	% of initial
grapes (RAC, 35 DALT)			100	0.74	74	100	n.a.				
preparation of mash	mash		100	n.a.	n.a.	n.a.	n.a.	no losses	n.a.	n.a	n.a.
pressing	fresh must	70	75.6	n.a.	n.a.	n.a.	Wet pomace	25	2.96	71.5	96.6
Pre-clarification	must (cl.), cold	73	80	0.02	1.6	2.2	"Trub"	1-3	n.a.	n.a	n.a.
Fermentation	young wine	73	73	n.a.	n.a.	n.a.	n.a.	no losses	n.a.	n.a	n.a.
Removal of yeast	wine from cold must	71.5	71.5	0.02	1.43	1.9	yeast	1.5	n.a.	n.a	n.a.

Table 7.7–4: Calculation of mass balance (rose wine, trial: DU3/02/98)

Rose wine production				BAS 500 F Residue [mg/kg]					BAS 500 F Residue [mg/kg]		
Step	PF	Amount of PF in Liter	Amount of PF in kg	DU3/02/98	mg BAS 500 F	% of initial	Waste fraction	Amount of waste fraction in kg	DU3/02/98	mg BAS 500 F	% of initial
grapes (RAC, 35 DALT)			100	0.73	73	100	n.a.				
preparation of mash	mash		100	n.a.	n.a.	n.a.	n.a.	no losses	n.a.	n.a.	n.a.
pressing	fresh must	70	75.6	n.a.	n.a.	n.a.	Wet pomace	25	1.94	48.5	66.4
Pre-clarification	must (cl.), cold	73	80	0.02	1.6	2.2	"Trub"	1-3	n.a.	n.a.	n.a.
Fermentation	young wine	73	73	n.a.	n.a.	n.a.	n.a.	no losses	n.a.	n.a.	n.a.
Removal of yeast	wine from cold must	71.5	71.5	0.02	1.43	2.0	yeast	1.5	n.a.	n.a.	n.a.

n.a.: not applicable

B.7.12 Proposed EU MRLs and justification for the acceptability of those residues (Annex IIA 6.7; Annex IIIA 8.6)

Grapes
Critical GAP North-EU: 3 * -0.16 kg as/ha, PHI 35 d
Results (results with different numbers of applications are taken for calculation):

North: 0.19, 0.23, 0.25, 0.26, 0.27, 0.36, 0.36, 0.44, 0.44, 0.47, 0.48, 0.57, 0.74, 0.76, 0.78, 0.78, 0.78, 0.82, 0.84, 0.89, 1.15, 1.27 mg/kg
R_{max} = 1.31 mg/kg, R_{ber} =1.58 mg/kg; STMR = 0.53 mg/kg
South: 0.13, 0.16, 0.18, 0.2, 0.21, 0.28, 0.34, 0.36, 0.37, 0.38, 0.39, 0.45, 0.47, 0.56, 0.59, 0.59, 0.72, 0.72, 0.83 mg/kg
R_{max} = 0.92 mg/kg, R_{ber} =1.18 mg/kg; STMR = 0.38 mg/kg

The proposed MRL is

2 mg/kg grapes

B.7.15 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8)

B.7.15.1 Chronic risk assessment

There is no necessity for a new chronic dietary risk assessment. The values reported in the list of endpoints (Version: 2. August 2002) are still valid:

ADI	0.03 mg/kg bw/d
TMDI (European Diet) (% ADI)	0.004793mg/kg bw/d (15.98 %)
NEDI (% ADI)	not calculated

On basis of the uses supported by available data the chronic risk for consumers is considered to be acceptable.

B.7.15.2 Acute risk assessment

During the evaluation process of pyraclostrobin the acute reference dose (ARfD) was set at 0.03 mg/kg bw. With this value for table grapes an acute risk was predicted when using the UK-model for toddlers and a default value for the variability factor (5).
Therefore the notifier conducted residue studies at four sites to investigate the unit-to-unit variability for pyroclostobin in table grapes.

Report: Determination of the residue variability of BAS 500 F and KIF-230 in grapes after treatment with BAS 525 00 F under field conditions in Germany and Spain, 2000
Heck, W., Benz, A., Mackenroth, C.; 2002
2002/1008790, RIP2003-80

Material and methods:
In 2000, 4 trials were performed at different sites in Spain and Germany to determine the unit-to-unit variability in grapes after application of a formulation (SE, BAS 525 00 F) containing pyraclostrobin (200 g/L) in combination with an other BASF development substance (KIF-230, 70 g/L).
The plant protection product was applied 6 times at 95, 83, 71, 59, 47 and 35 days (+/- 2 days) before commercial harvest. The application rates were increasing according to the growth stage of the plants from 0.06 to 0.16 kg pyraclostrobin/ha (formulation: 0.3 to 0.8 L/ha).
For analysis, 120 grape specimens from each trial were collected 35-36 days after the last application.

The samples were analysed for pyraclostrobin with BASF method No. 445/0 which quantifies the relevant residue of pyraclostrobin and its metabolite BF 500-3 at a limit of quantitation of 0.02 mg/kg each. The results of procedural recovery experiments obtained with each analytical series were about 88% for pyraclostrobin and 91% for BF 500-3 at a fortification levels of 0.02 mg/kg and 2 mg/kg.
Residue trial details and (mean) results are summarised in Table 7.7–9.

Findings:

Table 7.7–5: Variability of residues on bunches of grapes

	Pyraclostrobin	BF 500-3
ALO/02/00 (Spain)		
Mean [mg/kg]	0.28	0.10
Maximum [mg/kg]	0.55	0.24
Variability factor	2.0	2.5
ALO/10/00 (Spain)		
Mean [mg/kg]	0.06	0.02
Maximum [mg/kg]	0.20	0.05
Variability factor	3.1	2.3
DU2/04/00 (Germany)		
Mean [mg/kg]	0.45	0.07
Maximum [mg/kg]	1.36	0.24
Variability factor	3.0	3.7
DU4/04/00 (Germany)		
Mean [mg/kg]	0.33	0.03
Maximum [mg/kg]	1.11	0.15
Variability factor	3.4	4.5

The residue results found ranged between the LOQ and 1.36 mg/kg for pyraclostrobin and 0.24 mg/kg for BF 500-3.

Conclusion:
The results of the study indicate that it is justified to use a new variability factor in NESTI-calculations for grapes. When comparing the results for pyraclostrobin with the results obtained in studies using the same study design with other active substances (variability of residues in grapes, ECPA 2000[1]) it can be concluded that the default value of 5 is too

[1] Determination of residues variability in table and wine grapes after a tank-mix application of Anilinopyridine, Triazole, Pyrethroid, Organophosphate, and Dicarboximide crop protection products, Kaethner, M.; 2002

conservative. In case of pyraclostrobin it is proposed to use a variability factor of 3 in the deterministic risk assessment.
Since the production of table grapes is relevant especially for southern Europe and the residue level in trials from North-EU is higher than those from South-EU it is reasonable to use residue data from southern Europe only.

Table 7.7–6: NESTI-calculation (UK toddler)

Active substance:	Pyraclostrobin
ARfD (mg/kg bw):	0.03

Food portion sizes of UK toddlers aged 1.5 to 4.5 years (97.5th percentile)

Food	portion size	unit weight	variab. factor	HR (mg/kg)	Intake (mg/kg) bw	Percent of ARfD (%)
(v) Berries and small fruits						
Table grapes	158.0		3.0	0.83	0.02713241	90.44
			3.4	0.83	0.03075007	102.50

Table 7.7–7: NESTI-calculation (UK adults)

Active substance:	Pyraclostrobin
ARfD (mg/kg bw):	0.03

Food portion sizes of UK adults aged 16 to 64 years (97.5th percentile)

Food	portion size	unit weight	variab. factor	HR (mg/kg)	Intake (mg/kg) bw	Percent of ARfD (%)
(v) Berries and small fruits						
Table grapes	190.0		3.0	0.83	0.00674893	22.50
			3.4	0.83	0.00764879	25.50

Even with a variability factor of 3.4, which is the highest value obtained in the studies with pyraclostrobin, the consumption of the ARfD in case of toddlers is only slightly above 100 %.

On basis of the uses supported by available data the acute risk for consumers is considered to be acceptable.

Table 7.7–8: Residues in grapes

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Active ingredient : Pyraclostrobin (BAS 500 F)
Crop / crop group : Grapes

Federal Office for Consumer Protection and Food Safety
Division 2 - Plant Protection Products - Unit 207
D-38104 Braunschweig, Messeweg 11-12
Federal Republic of Germany

Submission date : August 2003

Content of a.s. (g/kg or g/L) : 67 g/kg
Formulation (e.g. WP) : WG
Commercial product (name) : BAS 536 00 F (004928-00)
Applicant : BASF Aktiengesellschaft

Indoors / outdoors : outdoors
Other a. s. in formulation (common name and content) : Dimethomorph 120 g/kg
Residues calculated as : Pyraclostrobin and metabolite (BF 500-3) equivalent to pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)			PHI (days)	Remarks
			kg a.s. / ha	Water L / ha	kg a.s. / hL				pyraclo-strobin	BF 500-3	Total		
	(a)	(b)				(c)		(a)				(d)	(e)
AGR/33/01 DE-53474 Ahrweiler 2003-01-07	Dornfelder	1) 01.09.01 2) 27.06.-09.07.01 3) 26.09.-29.09.01	0.161	800	0.02	01.08.01 15.08. 29.08.	stage 83	Grape	1.16 0.71 0.58 0.45 0.47	0.02 0.05 0.06 0.06 0.05	1.18 0.76 0.63 0.51 0.52	0 14 21 28 34	RIP2003-77
AGR/34/01 DE-54518 Kesten 2003-01-07	Müller-Thurgau	1) 01.08.67 2) 20.-30.06. 2001 3) 26.-28.09. 2001	0.160	800	0.02	01.08.01 15.08. 29.08.	stage 83	Grape	1.47 0.81 0.77 0.68 0.78	0.05 0.06 0.07 0.07 0.08	1.52 0.87 0.84 0.75 0.86	0 14 21 28 34	RIP2003-77
FAN/03/01 North France FR-67560 Rosheim 2003-01-07	Chardonnay	1) 01.04.95 2) 18.-30.06. 2001 3)25.09.01	0.160	800	0.02	26.07.01 08.08. 20.08.	stage 81	Grape	0.41 0.28 0.20 0.19 0.26	<0.02 0.03 <0.02 <0.02 0.03	0.43 0.31 0.22 0.21 0.29	0 15 22 29 35	RIP2003-77

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.s. / ha	Water L / ha	kg a.s. / hL	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) pyraclo- strobin	BF 500-3	Total	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
ITA/33/01 IT-27050 Mornico Losana 2003-01-07	Barbera	1) -- 2) 05.-20.06. 2001 3) 15.-28.09. 2001	0.160	800	0.02	03.08.01 14.08. 27.08.	stage 85	Grape	0.27 0.17 0.15 0.13 0.13	<0.02 <0.02 <0.02 <0.02 <0.02	0.29 0.19 0.17 0.15 0.15	0 14 22 29 35	RIP2003- 77
ITA/34/01 IT-15050 Cost Vescovato 2003-01-07	Cortese	1) -- 2) 30.05.- 12.06.01 3) 15.-25.09 2001	0.16	800	0.02	31.06.01 10.08. 23.08.	stage 85	Grape	0.81 0.80 0.61 0.66 0.59	<0.02 0.04 0.03 0.04 0.04	0.83 0.84 0.64 0.70 0.62	0 14 21 28 35	RIP2003- 77

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Active ingredient : Pyraclostrobin (BAS 500 F)
Crop / crop group : Grapes

Federal Office for Consumer Protection and Food Safety
Division 2 - Plant Protection Product - Unit 207
D-38104 Braunschweig, Messeweg 11-12
Federal Republic of Germany

Submission date : August 2003

Content of a.s (g/kg or g/L) : 250 g/L
Formulation (e.g. WP) : EC
Commercial product (name) : BAS 500 00 F (004928-00)
Applicant : BASF Aktiengsellschaft

Indoors / outdoors : outdoors
Other a. s. in formulation (common name and content) :
Residues calculated as : Pyraclostrobin and metabolite (BF 500-3) equivalent to Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)			PHI (days)	Remarks
			kg a.s. / ha	Water L / ha	kg a.s / hL				pyraclo-strobin	BF 500-3	Total		
	(a)	(b)				(c)		(a)				(d)	(e)
AGR/33/01 DE-53474 Ahrweiler 2003-01-07	Dornfelder	1) 01.09.01 2) 27.06.-09.07.01 3) 26.09.-29.09.01	0.160	800	0.02	01.08.01 15.08. 29.08.	stage 83	Grape	1.74 1.22 1.33 1.08 1.15	0.09 0.12 0.15 0.12 0.15	1.83 1.34 1.48 1.20 1.30	0 14 21 28 34	RIP2003-77
AGR/34/01 DE-54518 Kesten 2003-01-07	Müller-Thurgau	1) 01.08.67 2) 20.-30.06. 2001 3) 26.-28.09. 2001	0.160	800	0.02	01.08.01 15.08. 29.08.	stage 83	Grape	2.04 1.37 1.58 1.00 1.27	0.12 0.14 0.21 0.16 0.18	2.16 1.51 1.80 1.16 1.45	0 14 21 28 34	RIP2003-77
FAN/03/01 North France FR-67560 Rosheim 2003-01-07	Chardonnay	1) 01.04.95 2) 18.-30.06. 2001 3)25.09.01	0.160	800	0.02	26.07.01 08.08. 20.08.	stage 81	Grape	0.51 0.32 0.38 0.40 0.44	0.03 0.03 0.04 0.04 0.05	0.54 0.35 0.41 0.44 0.48	0 15 22 29 35	RIP2003-77

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.s. / ha	Water L / ha	kg a.s / hL	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) pyraclo- strobin	BF 500-3	Total	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
ITA/33/01 IT-27050 Mornico Losana 2003-01-07	Barbera	1) -- 2) 05.-20.06. 2001 3) 15.-28.09. 2001	0.160	800	0.02	03.08.01 14.08. 27.08.	stage 85	Grape	0.35 0.18 0.19 0.18 0.16	0.04 0.03 0.04 0.04 0.04	0.39 0.21 0.23 0.22 0.20	0 14 22 29 35	RIP2003- 77
ITA/34/01 IT-15050 Cost Vescovato 2003-01-07	Cortese	1) -- 2) 30.05.- 12.06.01 3) 15.-25.09. 2001	0.16	800	0.02	31.06.01 10.08. 23.08.	stage 85	Grape	1.13 1.11 0.84 1.04 0.56	0.07 0.11 0.10 0.10 0.06	1.20 1.22 0.94 1.14 0.62	0 14 21 28 35	RIP2003- 77

Table 7.7–9: Residue trials to estimate a variability factor

RESIDUES DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)
(Application on agricultural and horticultural crops)

Active ingredient : Pyraclostrobin (BAS 500 F)
Crop / crop group : Table Grapes

Federal Office for Consumer Protection and Food Safety
Division 2 - Plant Protection Product - Unit 207
D-38104 Braunschweig, Messeweg 11-12
Federal Republic of Germany

Submission date : August 2003

Content of a.s. (g/kg or g/L) : 200 g/L
Formulation (e.g. WP) : SE
Commercial product (name) : BAS 525 00 F (004928-00)
Applicant : BASF Aktiengesellschaft

Indoors / outdoors : outdoors
Other a. s. in formulation (common name and content) : KIF-230 70 g/L
Residues calculated as : Pyraclostrobin and metabolite (BF 500-3) equivalent to Pyraclostrobin

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)			PHI (days)	Remarks
			kg a.s / ha	Water L / ha	kg a.s / hL				pyraclo-strobin	BF 500-3	Total		
	(a)	(b)				(c)		(a)				(d)	(e)
ALO/02/00 ES-41700 Dos Hermanas 2003-01-07	Regina Desno	1) 2) 3) 27.07.2000	0.06 0.06 0.10 0.12 0.16 0.16	302 308 509 612 803 793	0.02	25.04.00 04.05. 15.05. 29.05. 09.06. 21.06.	stage 79	Grape bunches	0.28	0.10	0.37	36	RIP2003-80 Variability study mean of 120 single Units (bunches of grapes)
ALO/10/00 ES-41710 Utrera 2003-01-07	Cardenal	1) 2) 3) 14.07.2000	0.06 0.06 0.10 0.12 0.16 0.16	301 290 501 602 792 800	0.02	25.04.00 28.04. 04.05. 15.05. 29.05. 09.05.	stage 75	Grape bunches	0.06	0.02	0.09	35	RIP2003-80 Variability study mean of 120 single Units (bunches of grapes)

1	2	3	4			5	6	7	8			9	10
Report-No. Location incl. Postal code and date	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment kg a.s / ha	Water L / ha	kg a.s / hL	Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg) pyraclo- strobin	BF 500-3	Total	PHI (days)	Remarks
	(a)	(b)				(c)		(a)				(d)	(e)
DU2/04/00 DE-69168 Wiesloch 2003-01-07	Spät- burgunder	1) 2) 3) 04.10.2000	0.06 0.06 0.10 0.12 0.16 0.17	301 304 499 612 816 831	0.02	03.07.00 13.07. 27.07. 07.08. 17.08. 30.08.	stage 83	Grape bunches	0.45	0.07	0.52	35	RIP2003-80 Variability study mean of 120 single Units (bunches of grapes)
DU4/04/00 DE-67157 Wachenheim 2003-01-07	Riesling	1) 2) 3) 28.09.00	0.06 0.06 0.10 0.12 0.16 0.16	300 300 514 590 797 823	0.02	26.06.00 07.07. 19.07. 31.07. 11.08. 24.08.	stage 81	Grape bunches	0.33	0.03	0.36	35	RIP2003-80 Variability study mean of 120 single Units (bunches of grapes)

Remarks: (a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)
(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Note: All entries to be filled in as appropriate

B.7.17 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-6.3	Bross, M.	2002	Supplementary document to the residue section of the Annex II dossier of BAS 500 F- Summary of preliminary residue data in grapes including MRL calculation 2002/1004119 no GLP, unpublished RIP2003-76	N	BAS
AIIA-6.3	Jones, S.	2002	Study on the residue behaviour of BAS 550 F and BAS 500 F in grapes after application of BAS 536 00 F, BAS 550 09 F or BAS 500 00 F under field conditions in Germany, France (N), Italy, 2001 2002/1010480 GLP, unpublished RIP2003-77	Y	BAS
AIIA-6.3	Kaethner, M.	2002	Determination of residues variability in table and wine grapes after a tank-mix application of anilinopyridine, triazole, pyrethroid, organophosphate, and dicarboximide crop protection products no GLP, unpublished 2002/1007077 RIP2003-78	N	ECPA
AIIA-6.3	Kaethner, M.	2002	Determination of residues variability in head lettuce following a tank-mix application of anilinopyrimidine, triazole, pyrethroid, organophosphate, carbamate and dicarboximide crop protection products no GLP, unpublished 2002/1007078 RIP2003-79	N	ECPA
AIIA-6.3	Heck, W., Benz, A., Mackenroth		Determination of the residue variability of BAS 500 F and KIF-230 in grapes after treatment with BAS 525 00 F under field conditions in Germany and Spain, 2000 no GLP, unpublished 2002/1008790 RIP2003-80	Y	BAS
AIIA-6.5.2	Bross, M.	2002	Grapes: Calculation of the mass balance during wine processing no GLP, unpublished RIP2003-81	N	BAS

Addendum 4

to the Draft Assessment Report

of 13 June 2001

Pyraclostrobin

18 December 2003

Rapporteur Member State: Germany

Contents

1 Introduction

The main objective of this addendum is to replace chapter 2 of the Addendum of 19 June 2003 to the Draft Assessment Report of 13 June 2001 as well as to address the developmental toxicity of pyraclostrobin in rabbits in relation to recently submitted historical data on specific malformations and to give recommendations for appropriate classification and labelling.

At the meeting of the Working Group (Evaluation) in March 2003, the Member states were informed that the Swedish authorities expressed concern about certain findings in the developmental toxicity studies in rats and rabbits. SE notes that pyraclostrobin has the potential to induce reproductive effects and, therefore, a classification and labelling for developmental toxicity (Repr. Cat. 3; Xn; R63) is probably warranted. In the Addendum of 19 June 2003 Germany agreed that based on findings in the developmental toxicity studies in rabbits, a developmental toxic potential of pyraclostrobin could not be definitively excluded and, thus, the classification and labelling as proposed by SE was recommended.

Following later discussions of the findings in the rabbit developmental toxicity studies in Germany and at the 2003 JMPR, BASF submitted additional historical data from developmental toxicity studies in rabbits which describe the prevalence of the relevant abnormalities (vertebrae malformations) in the strain between 1991 and 2001 (Table 1). The studies are presented graphically in Figure 1. For this purpose the studies have been renumbered according to the year when they have been conducted.

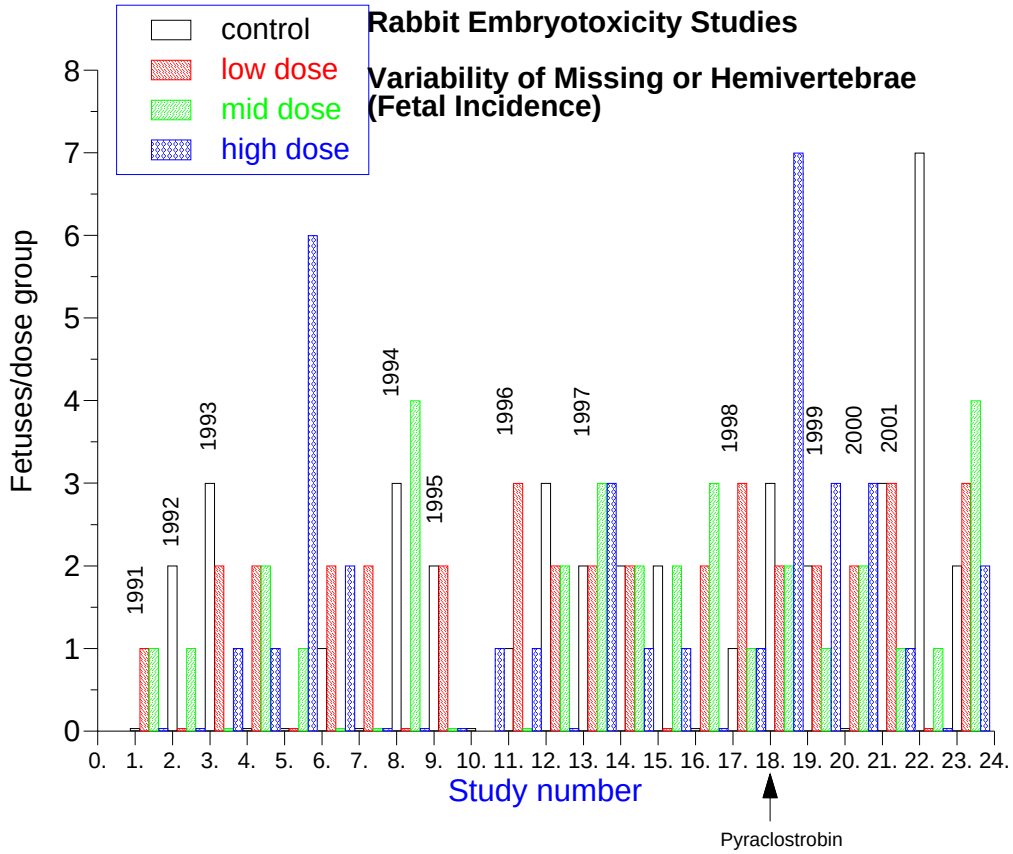
2 Re-evaluation of developmental toxicity with special regard to the presence of substance-related malformations; classification and labeling (refers to section B.4, B.6.6.2 of the draft assessement report)

The new historical control data which have been recently submitted by BASF comprises 21 full-sized studies with one control and three dose groups in addition to the study conducted with pyraclostrobin (study number 18 in Figure 1 below). Vertebral malformations occurred in two other studies with a similar prevalence as in the study with pyraclostrobin. In study number 22 (conducted in 2001) this happened in a control group. In 1993 an increased prevalence was seen in the high dose group of study number 5. BASF repeated this experiment in 1995 using this high dose plus a control group, but could not reproduce the finding (study number 10). In view of the newly submitted data it appears highly likely that the increase in vertebrae malformations observed in the high dose pyraclostrobin group is a chance finding which is unrelated to the substance tested.

In conclusion, in rabbits there is evidence of a developmental toxic effect consisting of increased post-implantation losses at and above 10 mg/kg bw/d, with a NOAEL at 5 mg/kg bw/d. The NOAEL for maternal toxicity was 3 mg/kg bw/d, based on the reduced food consumption on day 7 and 8 p.i. in the supplementary study. The historical data presented show that hemi-vertebrae or missing vertebrae are a common abnormality for this strain of rabbits, occurring in 0-3 fetuses in control and treated groups. Higher prevalences (4-7 affected fetuses per group) have been observed infrequently in controls and treated groups, including the high dose group exposed to pyraclostrobin. However, there is no convincing evidence that this finding is substance- or treatment-related.

Based on the findings observed in developmental toxicity studies in rats and rabbits, treatment with pyraclostrobin produced no evidence of teratogenic effects. Developmental toxicity occurred only at dose levels which were associated with maternal toxicity, and the embryotoxic effects (increased post-implantation losses) were likely to be secondary to the marked nutritional deficit in the dams at a critical time in gestation. Therefore, classification and labelling for developmental toxicity is no longer recommended.

Figure 1: Fetal incidence of vertebrae malformations based on historical data



3 Historical data tabulation

Table 1: Incidences of absent/misshapen vertebrae in Chbb: HM rabbits

No.	Study No.	Year	Fetal incidence				Litter incidence			
			control	low	mid	high	control	low	mid	high
1	98141	2001	3	3	1	1	3	3	1	1
2	989169	2001	7	0	1	0	5	0	1	0
3	97127	1998	1	3	1	1	1	2	1	1
4	96180	1999	2	2	1	3	2	2	1	3
5	96011	1996	1	3	0	1	1	2	0	1
6	93064	1995	2	2	0	0	1	2	0	0
7	93051	1994	3	0	4	0	3	0	4	0
8	92086	1993	3	2	0	1	3	2	0	1
9	92058	1993	0	2	2	1	0	2	2	1
10	90082	1991	0	1	1	0	0	1	1	0
11	92031	1992	2	0	1	0	2	0	1	0
12	99077	2000	0	2	2	3	0	1	2	3
13	99113	2001	2	3	4	2	2	3	4	3
14	96153	1997	2	2	3	3	2	2	3	3
15	97019	1997	2	2	2	1	1	2	1	1
16	96076	1997	2	0	2	1	2	0	2	1
17	95097	1996	3	2	2	0	2	2	2	0
18	96135	1997	0	2	3	0	0	1	3	0
19	92082	1993	0	0	1	6	0	0	1	5
20	92100	1995	0	-	-	1	0	-	-	1
21	91107	1993	1	2	0	2	1	1	0	2
22	91095	1993	0	2	0	0	0	2	0	0
		Sum	**36**	**35**	**31**	**27**	**31**	**30**	**30**	**27**
	BAS 500 F	1998	3	2	2	7	3	2	2	5