# European Commission
## Peer Review Programme

# Paraquat
**Volume 3**

**Annex B**

# ADDENDUM

**to the Draft Assessment Report and Proposed Decision of the United Kingdom prepared in the context of the possible inclusion of the above active substance in Annex I of Council Directive 91/414/EEC**

**Summary, Scientific Evaluation and Assessment**

**MAY 2000**

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**CONTENTS - continued**

## B.3 DATA ON APPLICATION AND FURTHER INFORMATION

B.3.5.1.3 Procedures for cleaning application equipment (IIIA 4.2)

The notifier has submitted the following calculations to support the effectiveness of cleaning procedures for application equipment.

The cleaning procedures recommended that the spray tank should be washed out twice with a quarter filled tank of clean water. Using the following calculation the notifier is able to determine that the likely residues of 'Gramoxone' left after the decontamination procedure are minimal.

Initial use of sprayer with 'Gramoxone' - assumptions:

- 47 litres of spray solution left in tank before washing and after first and second washings.
- spray tank size is 1500 litres
- 2 washings of 150 litres ($1/10^{th}$ volume - worst case as recommendation states quarter tank) each are used.

The minimum recommended spray dilution for paraquat-containing formulations is 11 g paraquat ion/l (tractor).

Amount left in the tank before washing is 47 x 11 = 517 g ai

First washing with 150 litres of water gives a dilution of 517 g ai in 197 litres of water = 2.62 g ai/l
Amount left in tank before second washing is 47 x 2.62 = 123.1 g ai

Second washing with 150 litres of water gives a dilution of 123.1 g ai in 197 litres of water = 0.62 g ai/l.
After emptying a total of 29.1 g ai remains.

Next use of sprayer - assumptions:

- 47 litres of previous washings left in tank = 29.1 g ai
- Next spray of 22 litres/ha with a full 1500 litre tank

Sprayed at 22 litres/ha this gives a contamination level of 1.3 g ai/ha.

This contamination level is 846 times less than the maximum recommended application rate of 1100 g ai/ha and is therefore not considered to be significant.

On the basis of calculation it can be concluded that the washing procedure will result in decontamination of spraying equipment. If further information is required this could be addressed at Member State level.

## B.4 METHODS OF ANALYSIS

### B.4.1 Technical active substance and plant protection product (IIA 4)

#### B.4.1.1 Technical active substance (IIA 4.1)

Paraquat cation and the impurities 1-methyl-4-pyridin-4-ylpyridinium ion (R009170), 1-methyl-4-pyridin-2-ylpyridinium ion (R011698), and 1-methyl-4-(1-methyl-2-oxopyridin-4-yl)pyridinium ion (R030499) were determined in technical material concentrate by capillary electrophoresis. The following conditions were employed.

| | |
|---|---|
| column: | 65cm (57cm effective length) |
| temperature: | 20°C |
| electrolyte solution: | 50mM sodium acetate trihydrate adjusted to pH 4.6 with glacial acetic acid |
| detection: | 257 nm |
| polarity: | minvoltage (kV)<br>00<br>0.225<br>1225 |
| internal standard: | ethyl viologen dibromide |

The report authors emphasised the need for pre-conditioning and equilibration of the system.

The method of analysis was validated as shown below:

| Paraquat cation | |
|---|---|
| precision | Seven sample weights at 0.6 - 1.25X where X is the recommended compound weight |
| linearity | Determined over 0.6 - 1.25X where X is the recommended compound weight |
| accuracy | Analysis of four synthetic samples |
| specificity and interference | A sample of pure paraquat dichloride and a sample of paraquat technical; were examined by capillary electrophoresis and UV spectra taken at the peak apex. the spectra were compared.<br>Injections of solvent (blank), typical technical paraquat and internal standard were made under the same conditions. Electropherograms were examined for co-migrating impurities. |
| stability of paraquat cation solution | Determined over 27 days |
| R009170, R030499 | |
| precision | Samples of paraquat standard were fortified at 5 concentrations and six determinations made of each sample. |
| linearity | Validated over the impurity concentration range 0.05 - 1.95% (R009170) and 1% R030499 |
| accuracy | Samples of paraquat standard were fortified at 5 concentrations and six determinations made of each sample. |
| specificity and interference | Samples of impurity reference materials and a sample of paraquat technical; were examined by capillary electrophoresis and UV spectra taken at each peak apex were compared with those of the reference material. |
| stability of impurity solution | Determined over 118 hours |
| limit of quantification | Samples analysed at a fortification level of 0.04% w/w |

Acceptable electropherograms were submitted.

### B.4.1.2 Impurities (IIA 4.1)

**a** **R009170, R030499**
See 4.1.1

**b** **Volatile impurities**
The volatile impurities were determined by capillary GC-FID with octadecane as an internal standard. The method was used to determine [redacted]

The method was validated for linearity and recovery using the impurities [redacted]

Acceptable chromatograms were submitted.

**c** **Volatile (solvent type) impurities**
Solvents were determined by capillary gas chromatography-FID with chlorobenzene as an internal standard. [redacted]

Acceptable chromatograms were submitted.

Table 4.1 Summary of method validation (active substance and plant protection product)

| | linearity (linear between) (mg/l) | precision - repeatability | accuracy (%) | interference |
|---|---|---|---|---|
| **Technical active substance** | [redacted] | [redacted] | [redacted] | none |
| [redacted] | [redacted] | [redacted] | [redacted] | none |
| [redacted] | [redacted] | [redacted] | [redacted] | none |
| [redacted] | [redacted] | [redacted] | [redacted] | none |

## B.4.2 Residues in treated plants, plant products, foodstuffs and feedingstuffs (IIA 4.2.1)

### B.4.2.1 Plants

### a Hops

Hop samples were refluxed in sulphuric acid/water for 5 hours. The samples were cooled and filtered. After dilution with water the solutions were percolated through cation exchange resin and the columns washed with deionised water, 2M HCl and 2.5% ammonium chloride. Paraquat and diquat were eluted with saturated ammonium chloride and an aliquot of the eluate was passed through a C18 cartridge prior to analysis. Analysis was by HPLC using a phenyl column with UV detection at 258nm paraquat and 310 for diquat. The mobile phase was water:methanol with sodium-1-octanesulfonate (0.1%), diethylamine (1%), orthophosphoric acid (1.0%). The validation are summarised in table 4.2. The recovery data were corrected for the mean apparent residue in control samples.

Residues in control samples were 0.0085 - 0.0137 mg/kg for paraquat and 0.005 - 0.0032 for diquat.

Table 4.2 Summary of method validation data on hops

| Fortification level | paraquat recovery (corrected) | paraquat recovery uncorrected | diquat recovery | diquat recovery uncorrected |
|---|---|---|---|---|
| 0.05 | 88, 83, 85, 96 | 110, 105, 105, 116 | 84, 83, 83, 88 | 91, 90, 87, 92 |
| 0.1 | 78, 80 | 91, 89 | 88, 88 | 90, 90 |
| 0.5 | 84, 84 | 86, 86 | 96, 96 | 97, 96 |
| 1.0 | 82, 86 | 83, 87 | 90, 92 | 90, 92 |

## B.4.5 Summary of methods of analysis

Acceptable methods of analysis based on capillary electrophoresis were submitted for paraquat and three impurities. Remaining volatile impurities were analysed by capillary GC. Validation data were not submitted for all possible impurities but it is considered that the information submitted is sufficient to allow the determination of paraquat and the organic impurities present at greater than 0.1% w/w in the technical material. However validation data are still required for the determination of sodium ions, ammonium ions and total ionisable chlorine.

An acceptable method of analysis for paraquat and diquat residues in hops was submitted. The method is based on acid extraction, followed by clean-up on a cation exchange column and analysis by HPLC-UV. The limit of quantification was 0.05 mg/kg.

## B.5 MAMMALIAN TOXICOLOGY

At ECCO 32, the mammalian toxicology meeting, the need for further data was identified in several areas. These requirements for further data were confirmed at ECCO 36, the Regulatory Decisions ('Overview' meeting). The requirements identified were as follows:

i) it was noted that short term inhalation studies on rats were submitted by the main notifier to the Netherlands as part of national requirements but these were not present in the dossier. The main notifier was asked to submit these subacute studies and it was stated that they should be taken into account in relation to operator exposure by inhalation (data requirement no. 4.2).

ii) a reassessment of the long term study on rats was needed to determine a NOAEL value with regard to eye effects and lung tumours (data requirement no. 4.3).

iii) it was considered that the figure of 0.3 % for dermal penetration from a compromised human study seems to be too low and clarification with regard to the *in vivo* study was requested (data requirement no. 4.4).

iv) in relation to operator exposure, a field study under European conditions was proposed for estimation of operator exposure (data requirement no. 4.5).

v) in relation to worker and bystander exposure the need for further information was identified (data requirement no. 4.6).

All of the above requirements have been fulfilled by the main notifier. The Rapporteur's detailed evaluation of these data is given below.

In addition the main notifier's summary is given in the updated Evaluation Table together with the Rapporteur Member State's comments and conclusions.

## B.5.3 Short term toxicity

### B.5.3.3 Other routes (IIA 5.3.3) - Inhalation toxicity studies on paraquat

The main notifier has submitted 4 reports (DP 58865; 58867; 58869; 58879) on the effects of repeated inhalational exposure to paraquat on respiratory tract pathology and levels of paraquat in the lung. The 4 reports represent an original investigation and a repeat following a very similar protocol. None of the work is GLP compliant (performed in 1978 pre-GLP) but all the reports have QA statements. Investigations of recovery/clearance were included. All concentrations are given as paraquat ion. The results from the 2 studies show good reproducibility.

a. First 3 week inhalation study

Groups of Sprague Dawley rats were exposed (whole body) to a fine paraquat aerosol (MMAD <2μm) for 6h per day, 5 days/week for 3 weeks (15 exposures). Histopathological examination of the respiratory tract was performed on animals (8/sex/test group, 16 controls) sacrificed after the 15th exposure and 2 weeks after the 15th exposure. Exposure concentrations were confirmed by analysis as 0.012, 0.11, 1.28 and 0.49μg paraquat/litre. Due to deaths at 1.28μg/litre no histopathology was performed on these animals and the 0.49μg/litre concentration was added.

Body weight gain and food consumption were reduced initially in all male treatment groups; females were unaffected. Clinical signs, predominantly brown nasal staining were seen at 0.11μg/litre and above. Histological examination detected lesions of the larynx at 0.49 and 0.11μg/litre (ulceration, necrosis, inflammation, keratinisation and metaplasia) and lesions of the lung at 0.49μ g/litre (macrophage aggregation, damage to bronchiolar epithelium, loss of cilia and clara cell characteristics and some thickening of the alveolar walls). The larynx lesions showed evidence of recovery after 2 weeks, but the lung lesions did not. No effects were noted at 0.012μg/litre.

DP58865

b. Paraquat levels in lungs from study a.

The findings (Table 5.1 overleaf) showed evidence of a plateau after about 5 exposures and a clearance on cessation of exposure with a $t_{1/2}$ of approximately 1 day.

Table 5.1 Paraquat concentrations in lungs of rats (means of 4/sex) exposed by inhalation (μg/g wet weight)

| | no. of exposures 5 | no. of exposures 15 | no. of exposures 15 + 1 day recovery | no. of exposures 15 + 2days recovery | no. of exposures 15 + 3 days recovery |
|---|---|---|---|---|---|
| 0.012μg/litre | 0.1* | 0.1* | 0.1* | nd | nd |
| 0.11μg/litre | 2.1 | 1.7 | 1.3 | 0.7 | 0.4 |

* some values below detection limit of *ca* 0.1 μg/g.

DP58867

c. Second 3 week inhalation study

One aim of this study was to investigate the reduced body weight gain seen in all treated male groups in the first study - it was proposed that this may have been due to the need to expose controls under slightly different conditions to test animals. This study on Sprague Dawley rats involved 2 control groups (saline aerosol and no aerosol) and exposure groups receiving 0.012 or 0.11μg/litre for a range of 6 hour exposure periods, from 1 exposure to 15 exposures over 3 weeks, together with recovery groups. The aerosol was again very fine with MMAD of <0.7μm. Histology was restricted to the respiratory tract of animals sacrificed after 1 or 3 exposures with macroscopic examinations of other animals.

Body weight gain and food and water consumption were similar in all groups. The only clinical sign of note was brown nasal staining which was seen at similar frequency in some controls and test animals. Histological examinations showed effects consistent with those reported previously on the larynx of all animals 3 days after one exposure to 0.11μg/litre. The findings were more severe 1 day after 3 exposures. No effects were seen at 0.012μg/litre. No adverse findings were reported in animals examined macroscopically after 15 exposures.

DP58869

d. Paraquat levels in lungs of animals exposed by inhalation.

Male Sprague Dawley rats were exposed to paraquat aerosols (MMAD<0.7μm) at 0.014, 0.106 or 0.532μg/litre for 6h/day for up to 15 exposures over 3 weeks. Some animals were allowed to recover for up to 6 days. Lungs were removed and analysed for paraquat. The results (Table 5.2 overleaf) are consistent with the previous study, showing a plateau after 4 exposures and a clearance with a $t_{1/2}$ of 1 to 2 days. A small number of female rats were exposed on 5 or 15 occasions and had lung paraquat concentrations similar to equivalent male animals.

Table 5.2 Paraquat concentrations in lungs of rats (means of 5 males) exposed by inhalation (µg/g wet weight)

| Exposure | No. of exposures 1 | No. of exposures 1 + 1 day recovery | No. of exposures 1 + 3 days recovery | No. of exposures 4 | No. of exposures 10 | No. of exposures 15 | No. of exposures 15 + 1 day recovery | No. of exposures 15 + 3 days recovery |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| 0.014µg/l | nd | nd | nd | 0.2 | 0.04 | 0.16 | nd | nd |
| 0.106µg/l | 0.56 | nd | 0.02 | 1.3 | 1.3 | 1.4 | 0.82 | 0.53 |
| 0.532µg/l | 1.7 | 1.0 | 0.6 | 4.7 | 1.5 | 2.4 | 1.7 | 0.5 |

DP58879

**Conclusions**

The studies show consistent effects on the respiratory tract. Paraquat is toxic by inhalation, and is classified as such, however a clear NOAEC of 0.1µg/litre can be determined for lung lesions and 0.01µg/litre for overall respiratory tract pathology. Tissue levels reached a plateau after approximately 4 days and cessation of exposure resulted in a reasonable rapid reduction in lung paraquat levels (t1/2 of 1-2 days).

These studies were carried out using atmospheres generated from the concentrated product not the diluted spray.

Very fine particles were used in the study (< 2µm MMAD) and are not considered relevant to those generated during application of paraquat by hydraulic sprayers under the existing and proposed conditions of use.

## B.5.5 Oral long term toxicity and carcinogenicity

### B.5.5.1 Long term dietary study in rats (IIA 5.5) - reassessment of the long term study on rats with regard to eye effects and lung tumours

#### B.5.5.1.1 Eye effects

The main notifier has provided a review of the lenticular findings in the chronic rat study assessing the no effect levels at 90 days and 103 weeks and at study termination.

The examination was confined to ophthalmoscopy observations which are considered to be more sensitive than histopathological assessment for changes in the lens as they can be observed clinically and are shown to be both time and dose dependant.

The relevant lenticular changes of toxicological importance (in order of severity) and induced by paraquat were as follows:

a) suture line opacity
b) posterior polar opacity/cataract
c) posterior capsular opacity/cataract
d) radial cataract
e) cataract
f) resorption cataract

This judgement was made on the basis that these were the only lenticular changes which were increased in top dose rats (irrespective of the time point of the observation).

Based on increased incidences of any of the above changes in any dose group the following no effect levels were determined:

Table 5.3 No effect levels determined between 14 and 118/119 weeks on basis of lenticular changes

| | No effect level determined |
|---|---|
| 14 weeks | in excess of 150ppm (the highest dose tested) for both sexes |
| 52 weeks | in excess of 150ppm (the highest dose tested) for both sexes |
| 79 weeks* | 150ppm for females and 75ppm for males |
| 103 weeks | 25 ppm for both sexes |
| 112/113 weeks* | less than 25 ppm for males |
| 118/119 weeks* | 25 ppm for females (males terminated at 112/113 weeks) |

*detailed incidence of eye effects at these times is given below.

The no effect levels summarised in Table 5.3 are based on the following incidences of lenticular effects:

79 weeks
The notifier states that the judgement for males at 79 weeks was based on the incidence of cataract as follows:

| 0ppm | 0ppm | 25ppm | 75ppm | 150ppm |
|---|---|---|---|---|
| 0/20 | 0/20 | 1/20 | 0/20 | 3/20 |

On this basis the evidence for an effect in males at 150ppm is considered weak and questionable by the notifier.

102/103 weeks

This conclusion is based on suture line opacity and cataracts.

MALES

| | 0ppm | 0ppm | 25ppm | 75ppm | 150ppr |
|---|---|---|---|---|---|
| Suture line opacity | 0 | 0 | 1 | 14 | 1 |
| Posterior polar opacity/cataract | 3 | 0 | 1 | 8 | 19 |
| Posterior capsular opacity/cataract | 0 | 0 | 0 | 3 | 24 |
| Radial cataract | 0 | 0 | 1 | 2 | 8 |
| Cataract | 1 | 1 | 2 | 3 | 5 |
| | | | | | |
| TOTAL Cataracts | 4 | 1 | 4 | 16 | 56 |

FEMALES

| | 0ppm | 0ppm | 25ppm | 75ppm | 150ppr |
|---|---|---|---|---|---|
| Suture line opacity | 1 | 0 | 0 | 9 | 1 |
| Posterior polar opacity/cataract | 0 | 0 | 0 | 5 | 30 |
| Posterior capsular opacity/cataract | 2 | 5 | 4 | 6 | 12 |
| Radial cataract | 0 | 0 | 0 | 2 | 5 |
| Cataract | 1 | 1 | 1 | 1 | 4 |
| | | | | | |
| TOTAL Cataracts | 3 | 6 | 5 | 14 | 51 |

These data strongly support the position that at 102/103 weeks the clear NOEL for lenticular effects in males and females is 25ppm paraquat ion.

112/113 weeks
This conclusion was based on treatment-related increases in suture line opacity and posterior polar opacity:

i) Incidence of suture line opacity:

| 0ppm | 0ppm | 25ppm | 75ppm | 150ppm |
|---|---|---|---|---|
| 8/33 | 6/34 | 17/33 | 0/28 | 0/37 |

ii) Incidence of posterior polar opacity:

| 0ppm | 0ppm | 25ppm | 75ppm | 150ppm |
|---|---|---|---|---|
| 2/33 | 2/34 | 5/33 | 12/28 | 1/37 |

118/119 weeks
Based on an intergroup comparison of the numbers of rats with any significant (i.e. paraquat-induced) lenticular lesion at any stage of the study the following conclusions were made:

Over the entire study, up to 112/113 weeks for males and 118/119 weeks for females, the no-effect level for males was less than 25ppm, the no-effect level for females was in excess of 25ppm.

| | 0ppm | 0ppm | 25ppm | 75ppm | 150ppm |
|---|---|---|---|---|---|
| males | 19 | 14 | 31 | 39 | 47 |
| females | 32 | 42 | 38 | 43 | 48 |

**B.5.5.1.2 Re-reading of lung slides from chronic rat study.**

The main notifier has submitted 2 documents relating to re-reading of the slides from the chronic rat study on paraquat (DP 58881 & 58901).

a. 1986 report by W.M. Busey

This report gives lower incidences of lung tumours (Table 5.4) than those given in the original study report and reproduced at table B5.17 of the EC Monograph. However, it does identify a dose-related hyperplastic and fibrotic response at 150 and 75 ppm not evident in the original study report. In the opinion of Dr Busey, the findings at 25ppm were similar to those in controls. The evaluation was performed 'blind' of treatment group and sex.

Table 5.4 Incidences of lung lesions in the chronic rat study with paraquat as reviewed by Busey in 1986 (/70 animals)

| | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0 | 25 | 70 | 150 | 0 | 0 | 25 | 75 | 150 |
| Bronchioalveolar adenoma | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Bronchioalveolar carcinoma | 1 | 1 | 2 | 2 | 2 | 0 | 0 | 1 | 1 | 1 |
| Squamous cell carcinoma | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 |
| Adenomatous hyperplasia | 2 | 3 | 7 | 9 | 16* | 5 | 5 | 5 | 7 | 7 |
| Alveolar wall fibrosis | 1 | 4 | 6 | 9 | 11* | 11 | 10 | 11 | 17 | 15 |

* p<0.05 by Fisher exact test 1 way

DP58901

b. 1983 report by Ishmael & Godley

The evaluation of slides was performed blind. Statistical tests included a Peto correction for longevity. As with the previous re-evaluation, a hyperplastic response to paraquat was evident, but not a neoplastic one (Table 5.5), but unlike the review by Busey, fibrosis was not reported. The report clearly defines the type of lesions considered to be hyperplastic and those which are neoplastic [even though the terminology of 'adenomatosis' to describe non-neoplastic effects is not straight forward]. The reviewers concluded that neither adenomas nor carcinomas showed an association with paraquat administration. The NOEL for non-neoplastic lesions was considered to be 25ppm.

Table 5.5 Incidences of lung lesions in the chronic rat study with paraquat as reviewed by Ishmael & Godley (/70 animals)

| | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0 | 25 | 70 | 150 | 0 | 0 | 25 | 75 | 150 |
| Adenoma (NOS) | 0 | 0 | 2 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| Carcinoma (NOS) | 1 | 1 | 2 | 1 | 3 | 0 | 0 | 1 | 1 | 0 |
| Adenomatosis | 2 | 4 | 5 | 8 | 11* | 4 | 4 | 5 | 4 | 13* |
| Alveolitis | 12 | 15 | 8 | 15 | 11 | 9 | 8 | 11 | 14 | 18* |
| Pneumonitis | 0 | 0 | 0 | 2 | 7* | 0 | 5 | 1 | 3 | 1 |

* p<0.05 by Fisher exact test 1 way

DP58881

### B.5.5.1.3 Conclusions

i) Eye effects

The main notifiers reassessment of the eye effects is consistent with the conclusions reached in the monograph. They agree that there is no true NOAEL in males at the latest time points (post week 104 when such studies are normally terminated). The proposal in the monograph, to use the lowest dose (25ppm) as a minimal effect level, is still considered applicable as ocular lesion severity and incidence were only increased marginally at this dose and were only evident at a time when most chronic toxicity studies have been terminated.

It is concluded that had the study been terminated at 103/104 weeks (guideline requirement) a clear NOAEL of 25ppm would have been available. However, the rapporteur considers that the lenticular changes seen late in the study cannot be ignored, but as the effects are only seen in rats, not mice or dogs, the relevance to man must be questioned. Taken together these support the use of 25ppm as a minimal effect level and it is considered that there is no basis for the use of an extra uncertainty factor on these effects.

ii) Lung effects

The additional reports of lung pathology findings are consistent with the re-evaluation presented in the monograph. It is noted that the reviewing pathologists, reading the slides 'blind', differed in their descriptions of the lesions. However, the consistent interpretation is that whilst there are hyperplastic lesions produced by paraquat, there is no clear evidence of a neoplastic effect. A NOAEL for lung lesions was 25 ppm.

### B.5.10.2 Acceptable Daily Intake

The relevance of the new information provided on the eye and lung effects seen in the long term dietary study in the rat is considered below.

ADI proposed in Monograph

In the monograph the rapporteur concluded that the paraquat was not carcinogenic nor directly toxic to reproduction. The dog was found to be more sensitive to the repeat dose effects of paraquat than either mice or rats.

The NOAEL of 0.45mg/kg bw/d from the 1 year dog study was considered the appropriate value to use in deriving the ADI with a safety factor of 100 giving an ADI of 0.0045mg paraquat ion/kg bw. This NOAEL value gives a margin of 2 with respect to the LOEL seen in the study (lung lesions) and a margin of 2.5 with respect to the minimal effect level (eye effects) seen in the chronic rat study at 25ppm (≡1.0-1.3mg/kg bw/d).

ADI proposed by ECCO

At ECCO 32 a new ADI value of 0.002 mg/kg bw/day was proposed based on the LOAEL value of 25 ppm (≡1.0-1.3mg/kg bw/d) for eye lesions seen in the 119 week rat study. A safety factor of 500 was used given that no NOAEL value available from this study. A requirement was identified for a reassessment of the long term study on rats to determine a NOAEL value with regard to eye effects and lung tumours.

Rapporteur conclusion

The main notifier has provided a re-assessment of the eye effects and lung effects seen in the 2 year rat study, the rapporteur's conclusions on these studies have been given above.

On the basis of the information submitted, and the evaluation contained in the monograph, it is considered that the appropriate ADI for paraquat should be set at 0.004mg paraquat ion/kg bw using the NOAEL of 0.45mg/kg bw/d from the 1 year dog study with a safety factor of 100. This is considered a more robust basis on which to set the ADI than the rat study for the following reasons:

- the dog is more sensitive to the repeat dose effects of paraquat than either the rat or mouse;
- the eye effects seen in the 2 year rat study at the lowest dose level (25ppm, = 1.0 -1.3mg/kg bw/d) can be considered as a minimal effect level. Ocular lesion severity and incidence were only increased marginally at this dose and

were only evident at a time (110 weeks and beyond) when most chronic toxicity studies have been terminated;

- the eye effects are only seen in rats not mice or dogs, their relevance to humans is questionable.

The rapporteur notes that the use of the dog study to set the ADI is consistent with the position adopted by the JMPR in 1986 and with that reached and confirmed by the US EPA in their recent (1995) review.

### B.5.10.3 Acceptable Operator Exposure Level

The rapporteur proposed both a short and long term AOEL for paraquat, details are given on page 97 of the Annex B (Volume 3) to the UK report on paraquat dated September 1996.

The exposure pattern of paraquat is considered to be short-term at specific periods during a year and not continuous exposure throughout the year. As data indicate that paraquat is rapidly cleared from the body in general and specifically from the lung, it is appropriate to use a short/medium term systemic AOEL for the assessment of paraquat. The appropriate value is 0.0005mg/kg bw/d based on the 90 day dog study with a 100 fold uncertainty factor and corrected for 10% oral absorption.

## B.5.12 Dermal penetration (IIA 5.12)

The concerns at the ECCO meeting were that under occluded conditions (such as those which may be experienced by operators wearing PPE) with formulated product, dermal penetration may be closer to the value of 1.4% seen with diquat under similar conditions. There are no disagreement that paraquat only penetrated skin to a limited extent but the concerns of a number of attendees were that the case was not convincing enough to show that under typical European operating conditions it would be less than 1%.

Rapporteur conclusion

The published report by Feldman and Maibach (DP58885) investigated the dermal penetration in humans of a number of pesticides including diquat. The main notifier contends that diquat can be used as a surrogate for paraquat. A case is made to support this contention and it is noted that the two compounds have similar chemical properties (e.g. water solubility ~650g/l, log Kow ~4.5). See B5.14.1 para 1. The paper reports that the urinary excretion of i.v. diquat is ~60% over 5 days and that the dermal penetration was 0.3% under unoccluded conditions. Under occluded conditions a value of 1.4% is presented (a 5 fold increase), increasing to 3.8% if the skin is damaged. It should be noted that gloves are only required to be worn for mixing and loading. Occlusion is considered to increase absorption only over long periods and assuming occulsion for the entire working period is probably a conservative assumption.

The key reference on dermal penetration of paraquat is Wester *et al* (DP17081). This uses human volunteers. They were exposed to $^{14}$C-methyl labelled paraquat dichloride for 24 hours under 'normal' conditions (not specifically occluded) before washing. Urinary excretion was measured for up to 120 hours and corrected for urinary excretion of an i.v dose given to monkeys (59%). Total urinary excretion was independent of application site (hand, leg, forearm) and in the range of 0.2 - 0.4% under unoccluded conditions using unformulated material - approximately 0.1% of this was in the first 24 hours. This result is consistent with the data on diquat, indicating a value of 0.5% would be expected under occluded conditions over the 24 period used for the AOEL. The value of <0.5% is based on a 5 fold increase under occluded conditions (based on diquat data) and a 24 hour penetration of 0.1% under unoccluded conditions for paraquat.

## B.5.14 Exposure data

The exposure pattern of paraquat is short-term at specific periods during a year and not continuous throughout the year, therefore the short/medium term systemic AOEL of 0.0005mg/kg bw/d is used as the basis for the assessment of exposure (see section B5.10.3 of this addendum).

## B 5.14.1 Operator exposure (IIIA 7.2.1.2)

Plant protection products containing paraquat are used for weed control. The range of products and application rates authorised across the European Union are broadly similar. 'Gramoxone' (a soluble concentrate containing 200 g paraquat/litre) is a representative product for agricultural use.

In response to the requirement identified during the ECCO process for a field study under European conditions an exposure study for operators applying paraquat through knapsack sprayers has been submitted by the main notifier. A surrogate exposure study, where operators applied diquat via knapsack sprayers has also been submitted as additional supporting information.

A rationale for the use of diquat data to support uses of paraquat has been provided by the main notifier (Appendix 1 to ECCO reference no 1929/ECCO/BBA/97).

Human *in vivo* and *in vitro* dermal penetration data, as reported in Annex B, Section B 5.1.3 of the diquat and paraquat monographs, have shown diquat and paraquat are almost identical in respect to their diffusion potentials through skin. Biological monitoring data for diquat are therefore also applicable to paraquat.

### B 5.14.1.1 Measurement of Operator exposure

a) In 1997, operator exposure of 20 mixer-loader applicators using knapsack sprayers to apply 'Gramoxone' (an SL formulation containing 200 g paraquat/litre) in a citrus orchard near Riba Roja, Spain was monitored. The study was conducted according to GLP Regulations, 1997, however the field facility was not, at the time, part of the UK Compliance monitoring programme. There were no management approved procedures for routine facility operations but the procedures used in this phase of the study were documented in the study plan.

Application Procedure

Product was supplied in 1 litre polyethylene terephtalate (PET) containers. Each spray applicator used a 'Pulmic PM 118 knapsack sprayer of 18 litres capacity fitted with flat fan nozzles. Sprayers were fitted with pressure regulating valves to maintain application at 1 bar.

Each worker mixed, loaded and applied 12 tank fills (15 litres spray solution per fill) of 'Gramoxone' over an approximate 6 hour period. Target concentration

was 1 part product per 100 parts water. Target application rate was 3 to 4 litres product per hectare (600 – 800 g a.s/ha). Dilution rate was 300 – 400 litres spray solution per hectare. On this basis each operator handled approximately 18 litres 'Gramoxone' over a six hour day (360 g a.s per day).

Areas treated were similar, consisting of an orchard of well established trees. Weed cover was fairly light and patchy.

Workers wore standardised clothing of long sleeved cotton shirt, long cotton trousers and rubber boots. The clothing was unused and issued to the workers prior to application. In accordance with the label recommendation, protective (nitrile) gloves and a face shield were worn during mixing and loading.

Observations on work and hygiene practices are provided. These indicate some workers smoked during the exposure period. There were also incidents of operator contamination from equipment maintenance, tank overflow and handling contaminated equipment without gloves. See Table 5.1

Climatic data were recorded hourly at each site. These data indicated temperatures of 11°C to 17.5°C, relative humidity of 72% to 95% and wind speeds of 0 to 3.2 km/h
(2 mph).

Table 5.1: Summary of worker practice observed during the worker exposure study.

| Worker Number | Notifier's Observations |
|---|---|
| 1 | There was some spillage of solution on the outside of the spray tank during one mixing. Tended to be a little sloppy with filling the tank with water. Sprayed well. Smoked a cigarette on completion of spraying. No major incidents. |
| 2 | On one occasion cleaned the nozzle and washed filter with bare hands. No major incidents. |
| 3 | After the 3rd tank some leakage was noted on the left buttock and splashes on the back which were observed to increase. No major incidents. |
| 4 | No major incidents. |
| 5 | Worker had a problem with a blocked filter which needed cleaning. No major incidents. |
| 6 | On one occasion some dilute product was spilt on the back while bending down. No major incidents. |
| 7 | Slightly overfilled sprayer on first load. Some problems with leaking sprayer on back and buttocks, resulting in the need to change sprayers. Forgot to wear gloves on two occasions when mixing. No major incidents. |
| 8 | On one occasion slightly overfilled sprayer. No major incidents. |
| 9 | Workers clothing was observed to be wet, but mainly from the dew and sloppy filling by pouring water over the outside of the tank. No major incidents. |
| 10 | Slightly overfilled sprayer on one occasion. Wet clothes from the dew and sloppy filling of spray tank. No major incidents. |
| 11 | Forgot to wear face shield on one occasion when mixing. One load was made up by No. 12 while No.11 was returning from breakfast. On another occasion 12 assisted and 11 forgot to wear his face shield again. Observed to have large wet patch on back. No major incidents. |
| 12 | Product spillage onto tank lid. Occasionally sprays his feet. Small amount of spray mix noted on shirt. Makes up one load for No. 11 and assists with 2nd load. No major incidents. |
| 13 | Some water spilled on outside of knapsack. No major incidents. |
| 14 | Shirt wet due to leakage from the top of the sprayer. Lid not tight enough. No major incidents. |
| 15 | Shirt noted to be very damp. Smoked on one occasion between loads. Did not wear face shield on one occasion when mixing. Spilt dilute solution over hands while washing sprayer. No major incidents |

Table 5.1 continued.

| Worker Number | Notifier's Observations |
|---|---|
| 16 | On one occasion only wore one glove during mixing. No major incidents. |
| 17 | Back of shirt and shoulder area wet due to leaking from seal around hydraulic pump. No major incidents. |
| 18 | Small area of spillage on back and shoulder. No major incidents. |
| 19 | Wet patch observed on back and top of trousers. Removed top off bottle without gloves and put finger in the bottle top. No major incidents. |
| 20 | Sprayed through area of thick weeds and walked through sprayed area. Encountered problem with pressure in sprayer. Cleaned nozzle with bare hands. Shirt was observed hanging out of his trousers on one occasion. No major incidents |

Analysis

Absorption of paraquat was measured by collecting complete 24 hour urine samples for a 7 day period. This comprised one day prior to exposure as a baseline day (workers had no contact with paraquat for 5 days prior to exposure), the exposure day and 5 days afterwards. As a check on whether there had been any extraneous contamination of the urine sample with paraquat, two separate collections were taken on the day of exposure (day 2). The first during application, the second immediately after.

Analysis was by radioimmunoassay method [Levitt, T (1979)]. Determination of paraquat in clinical practice using radioimmunoassay. Proc. Anal. Div. Chem. Soc. Vol. 16 : p72-76. The method of analysis was submitted.

Creatinine excretion was measured to assess collection of total urine output. Analysis of creatinine was using the Jaffe reaction [Jaffe, MZ (1986). Physical Chem 10: 391. Reflab test kit for creatinine, Medical Analysis Systems Inc., Camarillo, CA 93012]. A published reference was detailed but not submitted.

Control and fortified field recoveries were prepared for each day of exposure and for 2 days post exposure. Samples were prepared at the study location. Control urine was supplied by persons having no contact with paraquat. Urine samples were fortified at 1, 10 and 20 ng/ml using a stock solution of 100 ng/ml paraquat in saturated ammonium chloride. Samples were frozen under the same conditions as workers urine.

Results

Field recovery for urine samples fortified at the 10 and 20 ng/ml were acceptable and ranged from 80 – 142 %. At the 1 ng/ml fortification rate 6 of the 12 samples were below the limit of determination. Recoveries from the remaining samples were satisfactory, ranging from 113 to 128%. The main notifier is unable to provide an explanation for the low recoveries seen at the 1 ng/ml fortification rate. Supported by previous work on the stability of paraquat in urine samples (as part of the pecan sprayer exposure study, Meier and Findley 1995) no adjustment for field recovery has been made to the worker urine samples. Paraquat was not detected in any of the control samples.

Reanalysis of the 0,1,10 and 20 ng/ml fortification samples after completion of the study showed the concentrations to be 0, 0.92, 10.6 and 20.7 ng/ml respectively. Field recoveries were adjusted to reflect the post-study analysis of the stock solutions. The 24 hour urine volumes and creatinine concentrations demonstrated completeness of collection by the workers. The amounts of paraquat absorbed (summarised in Table 5.2) were calculated from the amount excreted in workers urine corrected for the percentage of a parenteral dose excreted in the urine of monkeys (59%, Wester, *et al*, 1984).

Table 5.2 Paraquat absorption by mixer-loader-applicators during use in citrus orchards in Spain

| Subject i.d | Body weight | Amount absorbed (mg) | Amount absorbed /kg body weight (ng/kg bw/day) |
|---|---|---|---|
| 1 | 74 | 12.3 | 166 |
| 2 | 80 | 0.77 | 9.6 |
| 3 | 96 | 8.95 | 93.2 |
| 4 | 77 | 18.56 | 241 |
| 5 | 80 | 5.83 | 72.9 |
| 6 | 76 | 19.38 | 255 |
| 7 | 73 | < L.O.D | < L.O.D |
| 8 | 67 | 4.6 | 68.7 |
| 9 | 65 | 4.01 | 77.6 |
| 10 | 111 | 13.76 | 124 |
| 11 | 70 | 25.69 | 367 |
| 12 | 75 | 18.15 | 242 |
| 13 | 90 | < L.O.D | < L.O.D |
| 14 | 75 | 30.6 | 408 |
| 15 | 58 | 9.16 | 158 |
| 16 | 61 | 2.2 | 36.1 |
| 17 | 69 | 6.18 | 89.6 |
| 18 | 84 | 10.83 | 129 |
| 19 | 71 | 12.28 | 173 |
| 20 | 70 | 19.11 | 273 |
| | | Geometric mean | **77** |
| | | 75$^{th}$ percentile | **241** |

L.O.D for assay used = 0.75 ng/ml

Paraquat was detected in urine samples of eighteen of the twenty workers. No paraquat was detected in any of the urine samples collected prior to exposure. On the day of application (day 2) paraquat was found in urine samples of eleven workers. In seventeen of the twenty workers paraquat was totally eliminated within 72 hours of applying the product (day 4). Paraquat was only detected in the urine of one worker after day 5.

Workers 6, 11, 12, 14 and 20 produced the highest absorbed doses of paraquat, with worker 14 having the highest (408 ng/kg bw/day). Whilst all workers appear to have demonstrated reasonable compliance with hygiene standards during mixing/loading and product application, some contamination of these operators clothing was observed (Table 5.1). Workers 2 and 16 had the lowest absorbed dose above the limit of determination, which was approximately 10% of those having the highest. Although no major incidents were reported for either of these two workers, worker 16 was observed failing to use appropriate PPE when mixing/loading on one occasion and worker 2 handled contaminated equipment on one occasion (Table 5.1).

The geometric mean of absorbed dose of paraquat for all workers involved in the study was 77 ng/kg bw/day, equating to 15 % of the short term AOEL. The 75th percentile value is 241 ng/kg bw/day which is 48 % of the AOEL. Worker 14 had the highest absorbed dose of paraquat (408 ng/kg bw/day), this equates to 81% of the AOEL.

(Findley M, Chester G and Wiseman J. 1998)

b) In 1996, exposure of 20 mixer-loader-applicators using knapsack sprayers to apply 'Reglone' (an SC formulation containing 200 g diquat per litre) to a banana plantation in Guatemala was monitored. The study was designed to measure the potential dermal and inhalation exposure to and absorption of diquat by workers. Field and analytical phases of this study were compliant with US EPA GLP Regulations. This study was submitted as additional supporting information.

Application Procedure

All workers participating in the study mixed, loaded and applied the product. Knapsack sprayers used were 'Guarany Plus 16' (16 litres capacity) fitted with floodjet nozzles. Target spray concentration was 2 g diquat per litre and the target application rate was 3 litres 'Reglone' / ha (600 g diquat). The adjuvant 'Agral 25' was added to at a concentration of 0.4% of the spray solution. Exposure time varied between 275 minutes and 323 minutes. Total diquat applied per worker ranged from 2.88 kg a.s to 3.84 kg a.s.

Workers were provided with 100% cotton clothing comprising ; long sleeved shirt, long trousers, rubber boots and long socks. The clothing served as the dermal exposure dosimeters. Protective gloves and faceshield were supplied and worn during mixing and loading.

Spraying was a mixture of spot and broadcast application and took place over flat areas of fairly dense plantation, with gullies every 100 metres. Workers sprayed both types of terrain. Whilst spraying the gullies it was often necessary for the lance to be held at chest and/or face height, resulting in many of the workers clothing becoming contaminated. High temperatures and humidity had a significant affect on the comfort of the operator, with arm to face contact being observed for most workers . One worker (No. 3) experienced contamination due to defective equipment. (See Table 5.3.)
Application times ranged from 275 minutes (2.88 kg diquat mixed/loaded/applied) to 323 minutes (3.84 kg diquat mixed/loaded/ applied).

Climatic data were recorded hourly at each site. These data indicated temperatures of 23.7°C to 36.44°C, relative humidity of 57% to 107% and wind speeds of 0 to 9.65 km/h (6mph).

Table 5.3: Summary of worker practice observed during the worker exposure study.

| Worker Number | Notifier's Observations |
|---|---|
| 1 | Many banana leaves were observed on the ground making access sometimes difficult. No major incidences. |
| 2 | Worker initially held the lance around chest & face height but then generally kept it low. Some glove to hand contamination observed. |
| 3 | Knapsack observed to leak slightly from pressure cylinder, leaked on top of trousers and back of left leg. Land very wet and muddy and some tall weeds. |
| 4 | Some spraying was done up the sides of banks and spraying tall grass in the canals. |
| 5 | Worker was generally fairly careful, on occasions spraying high sided gullies and banks with lance at chest and face height. Occasionally wiped brow with sleeve. |
| 6 | Worker often sprayed in deep gullies resulting in nozzle being held at chest and face height. Some arm to face contact. Drank occasionally. |
| 7 | Worker sprayed deep steep sided canals. Faceshield and bare hands in contact with gloves. Occasionally wiped forehead with hand. Nozzle often above head height. Tended to work quickly. |
| 8 | Sprayed deep sided ditches with nozzle at head height. Difficult access area necessitated nozzle being held at waist height. Hand to glove contact observed. Wiped brow with sleeve. |
| 9 | Worker sprayed in canals a lot, at one stage the nozzle blocked. |
| 10 | Workers trousers observed to be very muddy below the knees. Waved lance around frequently and tried to cover the area quickly. |
| 11 | Worker sprayed a lot of canal area with the lance held at head height. |

Table 5.3 : Continued

| Worker Number | Notifier's Observations |
|---|---|
| 12 | Worker sprayed a lot of canal area at head height. Shirt and front of trousers observed to be very wet. |
| 13 | Worker observed to operate very quickly, on occasions a little careless. |
| 14 | Area very muddy. Often sprayed in water filled canals with difficult access. Angle of the nozzle badly directed on one occasion, which caused excessive wetting of trousers. |
| 15 | Many tall weeds reaching knee height. Contamination noted on shirt where straps rub. Sprayed in gully at shoulder height. |
| 16 | Many tall weeds reaching knee height. Contamination noted on shirt where straps rub. Sprayed in gully at shoulder height. |
| 17 | Worker held lance in his left hand and was fairly cautious during the application |
| 18 | On the flat areas worker kept the nozzle low. In deep gullies the lance was often held at chest and face height. On occasions worker was observed wiping forehead with sleeve. |
| 19 | Worker was thorough, kept nozzle low but often sprayed his trousers. |
| 20 | Lance often around face height in deep gullies. Wiped face and brow. |

Analysis

Dermal exposure

Potential dermal exposure was measured by analysis of the cotton clothing provided to and worn by workers during the application day. A handwash procedure was used to assess hand contamination.

Inhalation Exposure

A glass fibre filter housed in an IOM sampler attached to the collar of the workers clothing was used to determine the inhaleable fraction of the spray. The filter was connected to a personal air sampling pump which were run for the duration of the exposure period.

Analytical method references are provided in page 133-147 of the report.

Systemic absorption

The absorption of diquat was measured by collection of the workers urine over a 7 day period. Sampling commenced on the day prior to exposure and ceased on the first void of the sixth day following exposure. As a check on whether there had been any direct contamination of the urine during the application period, two separate collections were made on the exposure day (day 2). Subjects were instructed to exercise caution so contamination of urine with extraneous diquat did not occur and to keep samples out of direct sunlight.

Diquat was extracted from urine using solid phase extraction and converted to the dipyridone derivative which was then determined by liquid chromotography with fluorescence detection. The method of analysis was submitted.

Creatinine excretion was measured to provide an assessment of compliance in respect to collection of total urine output. The concentration of creatinine was measured using a test kit [Reflab test kit for creatinine, Medical Analysis Systems Inc., Camarillo, CA 93012] based on the Jaffe reaction [Jaffe, MZ (1986)].

Field recovery

Dermal exposure

A spray strength solution of diquat (2 g a.s/l) was made up on each day of application and applied to 100 $cm^2$ of clothing at two volumes, 0.1 ml/sample and 0.01 ml/sample. Untreated control clothing, placed away from the treatment area, was used to measure background exposure. Samples were run for the duration of the exposure period and processed in the same manner as the test samples.

Inhalation exposure

Spray strength solution of diquat, made up on the day application, was applied to glass fibre filters at 0.1 and 0.01 ml/sample. Untreated control filters were attached to personal air sampling pumps in the same way as those used by the workers. These were placed away from any likely source of diquat contamination. Samples were run for the duration of the exposure period and processed in the same manner as the test samples.

Urine

Control and fortified field recoveries were prepared for each day of exposure. Samples were prepared at the site field laboratory. Control urine was supplied by persons having no previous exposure to diquat. Samples were taken to the site for the exposure day and kept at ambient temperature. Recovery urine samples were fortified at 2, 10 and 50 ng/ml. These samples were stored under the same ambient and frozen conditions as the test samples. Recovery and test samples were analysed concurrently.

Results

Field recovery for urine samples at the 2, 10 and 50 ng/ml fortification rates were 87% (range 69%-114%), 85.1% (range 65.9% - 111%) and 98.1% (range 67.8% – 130%) respectively. Recoveries below 5% were recorded for six samples (two from each fortification rate). The notifier considers this was due to error in the fortification of the samples and excluded theses results when calculating mean recovery. Diquat was not detected in any of the control samples. No adjustment for field recovery has been made to the worker urine samples.

There was significant variation seen in the field recoveries for both clothing and handwash. See Table 5.4. Recoveries for clothing and handwash samples were acceptable on days 1 and 3, however, on days 2, 4 and 5 these fell to below 60%. The notifier has stated that day 2 recoveries may have been affected by exposure to direct sunlight, as it was necessary to relocate the field laboratory on this day. Field recoveries for the glass fibre filters ranged from 70% to 81%.

Table 5.4 Total Potential Dermal Exposure

| Worker no. | Clothes - % field recovery (mean) | Clothes (mg/sample) | Hands - % field recovery (mean) | Hands (mg/sample) | Total potential dermal exposure (mg/kg bw/day) |
|---|---|---|---|---|---|
| 1 | 90 | 31.8 | 89 | 1.12 | 0.62 |
| 2 | 90 | 42.8 | 89 | 1.26 | 0.75 |
| 3 | 90 | 34.9 | 89 | 1.46 | 0.62 |
| 4 | 90 | 69.2 | 89 | 0.87 | 1.06 |
| 5 | 59 | 148* | 54 | 6.48* | 1.91 |
| 6 | 59 | 135* | 54 | 6.48* | 2.17 |
| 7 | 59 | 196* | 54 | 9.83* | 2.52 |
| 8 | 59 | 101* | 54 | 5.63* | 1.90 |
| 9 | 80 | 51.1 | 125 | 1.29 | 1.01 |
| 10 | 80 | 90.6 | 125 | 4.48 | 1.51 |
| 11 | 80 | 46.1 | 125 | 2.57 | 0.82 |
| 12 | 80 | 60.5 | 125 | 1.34 | 1.29 |
| 13 | 56 | 115* | 45 | 3.98* | 1.87 |
| 14 | 56 | 64.6* | 45 | 7.31* | 1.23 |
| 15 | 56 | 52.8* | 45 | 0.98* | 1.00 |
| 16 | 56 | 87.3* | 45 | 6.49* | 1.75 |
| 17 | 61 | 133* | 29 | 3.69* | 2.62 |
| 18 | 61 | 29.6* | 29 | 3.66* | 0.56 |
| 19 | 61 | 67.7* | 29 | 6.14* | 1.12 |
| 20 | 61 | 63.7* | 29 | 8.79* | 1.12 |

| | Geometric mean | 75th percentile | Min | Max |
|---|---|---|---|---|
| | mg/kg bw/day | | | |
| Total Potential Dermal Exposure | 1.24 | 1.88 | 0.56 | 2.62 |

* Denotes value adjusted for stated field recovery

Table 5.5 Total Potential Inhalation Exposure

| Worker no. | Air filter (mg/sample) | Inhalation exposure (mg/m3) | *Total potential inhalation exposure (ng/kg bw/day) |
|---|---|---|---|
| 1 | 0.29 | 0.0005 | 75 |
| 2 | 0.66 | 0.0011 | 169 |
| 3 | 0.21 | 0.0003 | 50 |
| 4 | 0.28 | 0.0005 | 60 |
| 5 | 0.22 | 0.0004 | 37 |
| 6 | 0.21 | 0.0003 | 47 |
| 7 | 0.71 | 0.0012 | 123 |
| 8 | 0.68 | 0.0011 | 177 |
| 9 | 0.45 | 0.0007 | 135 |
| 10 | 0.27 | 0.0004 | 63 |
| 11 | 0.42 | 0.0007 | 101 |
| 12 | 0.24 | 0.0004 | 62 |
| 13 | 0.26 | 0.0005 | 62 |
| 14 | 0.36 | 0.0007 | 86 |
| 15 | 0.49 | 0.0009 | 130 |
| 16 | 0.42 | 0.0008 | 111 |
| 17 | 0.21 | 0.0004 | 57 |
| 18 | 0.16 | 0.0003 | 33 |
| 19 | 0.18 | 0.0003 | 45 |
| 20 | 0.16 | 0.0003 | 31 |

* Total potential inhalation exposure is calculated using a breathing rate of 29 l / minute

| | Geometric mean | 75th percentile | Min | Max |
|---|---|---|---|---|
| | ng/kg bw/day | | | |
| Total Potential Inhalation Exposure | 73 | 115 | 31 | 169 |

A reference range for creatinine excretion for male adults is 1.43 g to 2.20 g over a 24 hour period (Dorland's Medical Dictionary, 28th Edition). Measured creatinine excretion in the study showed that although no worker's urine samples were within these limits on all collection days, on the days where diquat was detected in samples (days 2 and 3) daily excretion of creatinine was acceptable for most workers, suggesting full collections were made on these days. For four workers (Nos 4, 5, 6, and 12) there was considerable variability in the daily excretion of creatinine, suggesting that these workers urine collections were incomplete. However, in the majority of cases where low creatinine excretion was observed, theses samples corresponded to collections made near the end of the study, where any concentrations of diquat present were below the limit of quantification. The non-compliance in collection of total urinary output for certain workers is therefore not considered to have affected the overall estimation of systemic exposure.

The absorbed dose (summarised in Table 5.6) is calculated by adjusting the diquat excreted in workers urine for the percentage of an intravenous dose (61%) excreted in urine (Feldman and Maibach, 1974).

Table 5.6 Diquat absorption by mixer-loader-applicators during use in banana plantations in Guatemala

| Subject i.d | Body weight (kg) | Amount a.s handled (kg) | Amount diquat absorbed – corrected for 61% excretion (ng/kg bw/day) |
|---|---|---|---|
| 1 | 53.1 | 3.2 | 92 |
| 2 | 59 | 3.2 | 64 |
| 3 | 59 | 2.88 | 26 |
| 4 | 65.8 | 2.88 | 54 |
| 5 | 80.8 | 3.84 | 77 |
| 6 | 63.6 | 3.52 | 111 |
| 7 | 81.3 | 3.84 | 126 |
| 8 | 56.3 | 3.84 | 195 |
| 9 | 51.8 | 3.52 | 15 |
| 10 | 62.7 | 3.52 | 52 |
| 11 | 59 | 3.52 | 589 |
| 12 | 47.7 | 3.52 | 228 |
| 13 | 63.6 | 2.88 | 33 |
| 14 | 58.1 | 2.88 | 31 |
| 15 | 53.6 | 2.88 | 30 |
| 16 | 53.6 | 2.88 | 189 |
| 17 | 52.2 | 3.52 | 464 |
| 18 | 59 | 3.52 | 48 |
| 19 | 65.8 | 3.84 | 23 |
| 20 | 64.5 | 3.84 | 59 |

| | |
|---|---|
| Geometric mean | **75** |
| 75$^{th}$ percentile | **142** |

Limit of quantitation for assay used = 0.5 – 3.77 ng/ml

Diquat was detected in urine samples of all twenty workers. On the pre-exposure day diquat was detected in the sample of Worker 12. This worker's sample contained diquat at just above the limit of quantification (0.71 ng.ml). On the day of application (day 2) diquat was found in urine samples of all workers. In fifteen of the twenty workers diquat was eliminated within 24 hours of applying the product (day 3). No diquat was detected in the urine of any worker after day 3.

Workers 8, 11, 12, 16 and 17 produced the highest absorbed doses of diquat, with worker 11 having the highest (589 ng/kg bw/day). Worker 11 was the only worker who exceeded the short term AOEL for paraquat (118%). It is noted that this worker was involved in treatment of canals with the spray lance being held at head height. This is considered to be unrepresentative of applications practised under European conditions.

In the study potential inhalation exposure (geometric mean 73 ng/kg bw/day) was significantly lower than potential dermal exposure (geometric mean 1.24 mg/kg bw/day). The geometric mean of absorbed dose of diquat for all workers was 75 ng/kg bw/day. The 75th percentile value is 142 ng/kg bw/day.

From the notifiers observations (Table 5.3) workers having the highest potential and systemic exposures do not appear to have mixed, loaded or applied the product differently from others participating in the study. All workers appear to have demonstrated generally good compliance with hygiene standards during mixing/loading and product application. However, the spraying at chest and/or head height in the gullies caused significant contamination of their clothing.

(Findley M and Hall M 1997)

Conclusion

Summary of studies

| | | Geometric mean | 75th percentile |
|---|---|---|---|
| **European study** (Spain) | amount paraquat absorbed (ng/kg bw/day) | **77** | **241** |
| | as percentage of the short term AOEL | **15 %** | **48 %** |
| **Guatemalan study** (supporting study using diquat) | amount diquat absorbed (ng/kg bw/day) | **75** | **142** |
| | expressed as percentage of the short term AOEL for paraquat | **15 %** | **28.4 %** |

The main notifier has conducted a field study under European conditions. The study, conducted in Spain in November 1997, involved the application of paraquat through knapsack sprayers. An absorbed dose was determined for eighteen of the twenty participants. As summarised above, the mean absorbed dose of paraquat (75th percentile value) is 241 ng/kg bw/day which is 48% of the short-term AOEL (AOEL of 0.0005 mg/kg bw/day equivalent to 500 ng/kg bw/day). The analytical method used for this study (LOD = 0.75 ng/ml) is more sensitive than that used in the previously reported Sri Lankan operator exposure study (30 ng/ml, Chester *et al*, 1993), which failed to determine paraquat in the urine of Sri Lankan workers over 5 consecutive days following mixing and loading and application of paraquat.

The workers generally followed the label recommendation for mixing and loading and used the recommended PPE. The workers also appear to have demonstrated reasonable hygiene standards during mixing/loading and application. Whilst the highest absorbed dose values may be attributed to contamination of the operators clothing with spray solution during application, most operators in the study were observed either mixing and loading or using the spray equipment incorrectly on at least one occasion. The study is therefore considered be a realistic assessment of the use of paraquat applied via a knapsack sprayer under representative EU conditions. The study demonstrates that under representative conditions of use exposures are within the AOEL.

In addition a supporting study involving the application of diquat by knapsack sprayers has been submitted. The study was conducted in Guatemala. In this study contamination of workers clothing appears to have been much greater than that reported in the Spanish study. The high temperatures and humidity also caused more frequent hand to face contact with spraying often reported to have been conducted at head and chest height. As summarised in the table above, the mean absorbed dose of diquat (75th percentile value) was 142 ng/kg bw/day which is 28.4 % of the short-term AOEL for paraquat.

Diquat and paraquat are almost identical in their physical and chemical behaviour with respect to dermal penetration. The amounts of diquat absorbed by workers in comparison to their potential dermal exposure supports the conclusion that paraquat and diquat are poorly absorbed through skin.

As the quantity of product mixed, loaded and applied by operators are similar in both studies, exposure values for absorbed dose determined from the Guatemala study support the findings of the Spanish study, that operator exposure from knapsack use of paraquat would be within the AOEL where protective gloves and a face-shield are worn during mixing and loading.

### B.5.14.2 Bystander exposure (IIIA 7.2.2)

Operator and worker biomonitoring studies show exposure to be within the (short-term) AOEL. Bystander exposure would be expected to be lower than worker exposure and is therefore considered to be acceptable.

### B.5.14.3 Measurement of worker exposure (IIIA 7.2.3.2)

An estimate of exposure to workers re-entering treated crops was provided by the RMS. Assuming a theoretical foliar residue from use of Gramoxone of 110 mg as/m² (based on an application rate of 1100 g paraquat/ha) a 60 kg worker would have to systemically absorb all the residue from about 0.1 m² of foliage to achieve a dose equal to the AOEL. However, it was unknown what level of dermal contact is likely to occur. In view of this further information on bystander and worker exposure was requested to support the main notifiers proposal for a re-entry period of 24 hours after treatment.

In 1995, exposure of 25 workers entering a cotton crop treated with 'Starfire' (an SL formulation containing 23.2 % w/w paraquat) in Georgia, USA was monitored. With the exception of the climatological data the study is in accordance with the requirements of GLP.

Experimental Procedure

In the study paraquat was used as a desiccant rather than as a herbicide. Although desiccant use of paraquat is not supported by the main notifier in the EU, this use pattern is considered to represent a worst case for worker re-entry due to the potential for significant and prolonged contact with treated foliage.

Three sites were used for the study. Cotton height was estimated to be 88 – 163 cm, 29-197 cm and 89-192 cm at sites 1, 2 and 3 respectively. 'Starfire' was applied via ground based hydraulic sprayer. Target application rate was 2.92 litres product per hectare (677 g a.s/ha). Actual application rate ranged from 2.8 litres/ha (662 g a.s. /ha) to 3.4 litres per ha (792 g a.s/ha). Dilution rate was 90.7 litres spray solution per ha.

Exposure from two re-entry periods was assessed, these being a) from spray dry on the foliage to four hours post application and b) approximately 24 hours post application. The study was conducted at three sites (fields) and took place over six consecutive days. Different areas of the same field were used for the 4 hour and 24 hour re-entry.

Typical activities for a crop consultant inspecting a cotton crop following desiccation involve handling bolls to determine if they are about to open and handling weeds or crop stems to assess suitability (moisture content) of the crop for harvest. Participating workers spent a minimum of 15 minutes in the field conducting the above tasks whilst walking 100 feet into the field. Workers then crossed one row over, exited the field and remained outside the crop for 10 to 15 minutes before re-entering. This procedure was repeated a total of ten times to simulate travelling to and inspecting 10 different sites over a working day. Each subject spent approximately 2.5 hours inside the field and 2.5 hours outside the field. Twenty five workers participated in the study. There were to be thirteen workers for each re-entry period, however Worker 08 withdrew from the study prior to field exposure. Each of the participants was familiar with the tasks they were asked to perform.

Workers clothing was short or long sleeved shirt, long trousers, socks and work shoes or boots. A hat was worn by some workers. Observations on work and hygiene practices in and out of the crop are provided. These indicate some workers ate, drank and smoked during the exposure period. Hand/forearm contact with workers face/neck was also observed as workers wiped away perspiration (Table 5.7).

Table 5.7 Summary of worker practice observed during the worker exposure study

| Site Number 1 | Notifiers observations |
|---|---|
| 02 | Cotton was shoulder to head high. Subject removed shirt after 4 entries, drank soft drink outside field, and carried drink into field. |
| 03 | Cotton was shoulder to head high. Frequent hand to face contact in the field. Drank water and soft drink outside field and carried drink into field |
| 04 | Cotton was shoulder to over head high. Subject drank outside field and carried drink into field. Ate peanuts in and out of field. Frequent sleeve to face contact. |
| 05 | Subject frequently adjusted glasses. Always carried a face towel with which he wiped his hands, face, and mouth. Frequently uses lip ointment. Smoked a cigarette outside the field and carried a plastic cup into the field with a drink. |
| 10 | Subject frequently adjusted glasses Wiped face with forearm, and scratched neck. Frequently smoked, ate and drank outside the field. |
| 11 | Subject often wiped his face and forehead with his hands and forearm. Put his pen in his mouth. |
| 16 | Constant hand contact with mouth, hair, neck and face. Rubbed eyes frequently. Drank soft drink and had lunch out of field. |
| 17 | Subject often wiped mouth, brow, and forehead with hands. Often drank and ate outside the field. |

| Site Number 2 | Notifiers observations |
|---|---|
| 00 | Cotton was shoulder high. Wiped ears and face with hands and adjusted sunglasses. Ate peanuts outside the field and placed ice in mouth. Took a drink into the field during last entry. |
| 01 | Cotton was about shoulder height. Subject ate and drank outside the field and occasionally wiped face with hands. Obtained ice with hands and placed in drink. Touched lips with fingers. |
| 06 | Cotton was waist to head high and leaves touched face and neck area. Subject infrequently scratched nose and neck and foreheads. Subject ate and drank outside field. |
| 07 | Cotton was over the subject's head and was very dense and difficult to walk through. Subject had lots of foliar contact with bare arms. Frequently drank outside the field. |
| 14 | Cotton was chest to over head height. Subject removed long-sleeved shirt, but not undershirt, each time upon exiting field. Subject constantly touched face and neck and drank outside the field. |
| 15 | Cotton was chest to over head height. Subject frequently touched face and neck. Ate and drank outside the field drinks. Took off shirt on one occasion. |
| 19 | Subject always removed shirt, but not tee-shirt, outside the field. Frequently adjusted sunglasses and wiped face with hands or shirt sleeve. Outside field subject chews, ate and drank. |
| 24 | Subject constantly touched face with hands or shirt sleeve. Drank outside field. |

| Site Number 3 | Notifiers observations |
|---|---|
| 20 | Crop is waist to chest high. Subject constantly touched face with hands or wiped with handkerchief. Drank water outside of field. |
| 21 | Crop is waist to chest high. Subject infrequently touched his face. Drank water or refreshment outside of field. |
| 22 | Crop is waist to over head high. Subject frequently drank outside the field. |
| 23 | Crop is waist to chest high. Subject smoked a pipe both in and out of the field. |
| 09 | Subject frequently wiped his face with his tee-shirt and hands, frequently chewed tobacco both in and out of the field. Had lunch outside the field. |
| 12 | Crop is waist to head high. Subject scratched his head often. Ate and occasionally drank and chewed tobacco. |
| 13 | Subjects smoked cigarette and took refreshments both in and out of the field. |
| 18 | Subject had frequent hand-to-mouth contact by chewing tobacco throughout the day. |
| 25 | Subject drank and smoked a cigarette outside the field. Had lunch after washing hands. |

Climatic data were recorded hourly at each site during the exposure period. These data indicated temperatures of 21°C to 33°C, relative humidity of 32% to 76% and wind speeds of 0 to 12.8 km/h (8 mph).

Analysis

Complete 24 hour urine samples were collected for a 7 day period to measure absorption of paraquat. This comprised one day prior to exposure as a baseline day (workers had no contact with paraquat for 6 days prior to exposure), the exposure day and 5 days afterwards. To detect extraneous contamination, urine from the day of field exposure was collected in two parts. The first upto the subject changing clothing and washing hands, the second upto the first collection of the following day (day 3).

Analysis for paraquat was by radioimmunoassay [Levitt, T (1979)] and for creatinine excretion using the Jaffe reaction [Jaffe, MZ (1986)].

Control and fortified field recoveries were prepared for each of the six exposure days. Samples were prepared at the study location. Control urine was supplied by persons having no contact with paraquat. Urine samples were fortified at 0,10, 20 and 50 ng/ml using a stock solution of paraquat prepared in saturated aqueous ammonium chloride. Field recovery samples were stored under ambient conditions in the observers vehicle on the day of exposure, then frozen under the same conditions as worker urine samples. Recovery and worker samples were analysed concurrently.

Results

Paraquat was not detected in any of the unfortified samples. Recovery for urine samples fortified at the 10 ng/ml and 20 ng/ml were acceptable and ranged from 90 – 124 %. On this basis no adjustment for field recovery has been made to the worker urine samples. The 50 ng/ml samples were not analysed as the main notifier considered this concentration was not relevant to the concentrations observed in the test samples.

The 24 hour urine volumes and creatinine concentrations demonstrated completeness of collection by the workers. As no paraquat was detected in day 2 or day 3 samples for any subjects the main notifier concluded analysis of further samples (days 4-6) would be unlikely to contain detectable concentrations of paraquat and no further analysis of samples was made.

Paraquat was not detected in any samples for workers re-entering 24 hours post application and was only detected in a single sample from those entering the crop 4 hours after application (Worker 19). The concentration of paraquat in this sample was below the limit of determination (10 ng/ml) and was estimated to be 6 ng/ml. Total amount of paraquat excreted was calculated to be 0.0024 mg/day based on the urine volume of 400 ml. The amount absorbed was 0.0041 mg/day (based on 59% of a parenteral dose excreted in the urine of monkeys (Wester, *et al*, 1984)) giving an absorbed dose of 0.00004 mg/kg bw/day.

(Findlay M and Iwota T 1995)

Conclusion

The main notifier has submitted a worker re-entry biological monitoring study. The study, conducted in the USA, involves re-entry into cotton crops following the use of paraquat as a desiccant. It is noted that desiccant uses are not supported in the EU review programme and that uses supported in the EU involve application for weed control under circumstances were there is little need for re-entry or inspection of treated crops. It is therefore accepted that the study can be considered to represent a worse case assessment of dermal exposure for workers inspecting a paraquat treated crop.

Paraquat was not detected in any samples for workers re-entering 24 hours post application and was only detected in a single sample from those entering the crop 4 hours after application. The absorbed dose of paraquat for this worker was 0.00004 mg/kg bw/day which is 8% of the (short-term) AOEL.

Although all workers wore long trousers and some wore long sleeved shirts, the potential for dermal contact with treated foliage was reasonably high owing to the height of the crop, which often exceeded the height of the workers. Worker behaviour also indicates frequent hand to face contact as workers wiped away perspiration.

These data show worker exposure to paraquat would be within the AOEL and support the main notifiers proposal for a 24 hour re-entry period. A shorter re-entry period could also be supported by the submitted data.

### B.5.14.4 Conclusions

It is concluded that the exposure studies demonstrate that operators handling and using paraquat under the proposed conditions of use will not exceed the AOEL and that, on the same basis, it can concluded that bystander exposure will not exceed the AOEL.

The worker biomonitoring exposure study is considered to demonstrate that workers re-entering treated crops after the application of paraquat will not exceed the AOEL.

### B.5.15 References relied on

| Annex point | Author | Date | Title and Company reference | GLP | Pub. | PPDB Ref. |
|---|---|---|---|---|---|---|
| IIIA 7.2.1.2 | Findlay M, Chester G and Wiseman J. | 9/4/99 | Paraquat: Worker exposure during mixing and loading and application of 'Gramoxone' with knapsack sprayers. **Report No. WER004** | Yes | No | 59276 |
| IIIA 7.2.1.2 | Findlay M and Hall M | 1997 | Diquat : Worker exposure during mixing and loading and application of 'Reglone' with knapsack sprayers. **Report No. CTL/P/5379** | Yes | No | 71074 |
| IIIA 7.2.1.2 | Chester G Jones N Woollen BH | 1989 | Paraquat: dermal exposure of, and absorption by Sri Lankan tea plantation workers. **TMF3189 3G/3** | No | No | |
| IIIA 7.2.1.2 | Meier, D.J. and Findlay M | 1995 | Paraquat: Worker exposure during mixing, loading and application of 'Gramoxone Extra' to pecans using vehicle-mounted, ground boom equipment. Zeneca Ag products. **RR 95019B** | | No | |
| III A 7.2.3.2 | Iwata T and Findlay M | 1995 | Worker exposure during re-entry into paraquat treated cotton fields : Biological monitoring in Georgia in 1994. Zeneca Ag products. **RR 95010B** | Yes | N0 | 58887 |

Additional references

(i) Wester, R.C., Maibach, H.I., Bucks, D.A.W. and AuFrere, M.M. (1983). *In Vivo* Percutaneous Absorption of Paraquat from Hand, Leg and Forearm of Humans. J. Tox. Environ. Health. 14: pp 759-762.

(ii) Levitt, T (1979)). Determination of paraquat in clinical practice using radioimmunoassay. Proc. Anal. Div. Chem. Soc. Vol. 16 : p72-76.

(iii) Feldman, R.J. and Maibach, H.I. (1974). Toxicol. and Applied Pharmacol. 28, pp 126-132

(iv) Dorland's Medical Dictionary. 28th Edition. Published by WB Sanders.

## B.8 ECOTOXICOLOGY

At ECCO 31, the ecotoxicology meeting, the need for further data was identified in several areas. These requirements were confirmed at ECCO 36, the Regulatory Decisions ('Overview' meeting). The requirements to be addressed by the main notifier were identified as follows:

i) data on the effects of the active substance on bird reproduction - if no data are available the risk to birds can be mitigated by restriction to certain uses where birds are not affected;

ii) further information on the toxicity of the active substance to hares required (these data should address the risk to hares from the use of paraquat and include data on, for example, the residues of paraquat on treated vegetation, the risk from grooming as well as data on the toxicity of paraquat to hares) - if no data are available, the risk for hares can be mitigated by restriction to certain uses where hares are not affected;

iii) a laboratory study using appropriate sediment concentrations (i.e. to simulate the range of application rates) investigating the chronic risk to sediment dwelling invertebrates (e.g. *Chironomid sp.*);

iv) data on the toxicity of the active substance to *Lemma* spp;

v) data on the toxicity of the active substance to *Typhlodromus pyri*;

vi) the risk of uses on canal and ditch banks {this was agreed as a requirement to be addressed at Member State level}.

The data requirements identified as necessary for Annex I inclusion, i) to v) above, have been addressed by the main notifier. The Rapporteur's detailed evaluation of these data is given below.

In addition the main notifier's summary is given in the updated Evaluation Table together with the Rapporteur Member State's comments and conclusions.

Details of the proposed uses of paraquat are as previously discussed in the Monograph. In most cases the risks for all organisms and from all proposed uses can be adequately covered by the worst case use of paraquat as a 200 g/l SL formulation applied at 1100 g a.s./ha in arable and 'non-crop land' situations or alternatively in orchard and forestry situations at 1000 g a.s./ha. (Note, all doses, toxicity end points and Predicted Environmental Concentrations (PECs) used refer to paraquat in its ionic form).

## B.8.1 Effects on birds (IIA 8.1, IIIA 10.1)

### B.8.1.1 Acute risk to birds

When originally assessed and discussed at ECCO 31, the acute and short term dietary risks to birds were considered acceptable following the refinement of certain exposure assumptions. The acute risk assessment was however based on acute oral toxicity studies which were not conducted to modern standards (nature of the test substance unclear, not to recognised guidelines and not to GLP). These reported avian LD50's for paraquat ranging from 35 to 144 mg a.s./kg bw. The worst case LD50 of 35 mg a.s./kg for mallard duck was however considered appropriate for use in the subsequent acute risk assessment.

For clarification purposes the Notifier has now submitted an additional acute oral toxicity study on mallard which has been conducted to modern standards. This has been evaluated and is summarised below:

Table 8.1 The acute toxicity of paraquat to mallard duck

| Species | Test substance | Acute oral LD50* (95% CL) | Acute oral NOEL* | Test guideline# | Reference |
|---|---|---|---|---|---|
| mallard duck *Anas platyrhynchos* | paraquat dichloride tech. concentrate (32.3% w/w paraquat ion) | 54 mg/kg bw (41.7-70.7) | 18.1 mg/kg bw based on mortality | US EPA 71-1 | Johnson, 1998 |

* End points based on paraquat ion.
# Study conducted in accordance with test guideline and to GLP.

The above LD50 of 54 mg/kg is within the range of values previously determined for paraquat and is close to the LD50 of 35 mg as/kg for mallard duck previously used in the acute risk assessment. Since the new value is greater than the earlier LD50 it will not significantly alter the original risk assessment and the acute risk to birds remains acceptable.

### B.8.1.2 Risk to breeding birds

Based on the available reproductive toxicity data and the patterns of use and exposure, the ECCO group realised a potential risk to reproducing birds feeding on contaminated insects. The $TER_{lt}$ was calculated as 3.9 for insectivorous birds (the assumed realistic worst case route of exposure), this was following refinement using data from a residue study on contaminated insects treated at 1000 g a.s./ha (see B.8.11 of Monograph). A mean residue level of 7.6 mg a.s./kg insect was used from this study based on the mean concentrations over 0-56 DAT for a mixture of small and large insects. This was compared with the NOEL of 30 mg a.s./kg diet from a reproductive toxicity study on mallard evaluated at B.8.1.2 (Beavers and Fink, 1982b) which was considered acceptable for use in risk assessment. The resulting $TER_{lt}$ of 3.9 was below the Annex VI trigger of 5 and

further information on the effects of paraquat on birds was requested to complete the assessment.

### B.8.1.3 New information submitted on the long term risk to birds

The main notifier has submitted a scientifically reasoned case to address the risk to breeding birds from use of paraquat. This paper [Zeneca document: Risk assessment for the effect of long term exposure of birds to paraquat residues in their diet. Author: P. J. Edwards, dated 7 May 1999 (accompanied by notifiers letter dated 15 May 1999)] is available as an accompanying document. The supporting references cited in the notifier's paper have all been made available to the Rapporteur.

### B.8.1.4 Critical assessment of Notifier's scientifically reasoned case

The majority of the assumptions used in the notifier's reasoned case above are considered valid. The data sets used are also considered reliable and appropriate for use in a European wide risk assessment.

The refinements used in the Monograph for the original reproductive risk assessment for insectivorous birds took account of the mean residue level over time instead of the initial exposure concentration and also an average of residues on small and large insects (insects being considered the worst case route of exposure). This new case also includes a factor to account for the proportion of contaminated food which would be in the diet of various bird species (72 species considered). This was based on the time spent in the field or 'crop usage' but it is conservative in assuming that birds forage evenly throughout the day and it equates all time in the crop with time feeding. The data on orchard usage includes time spent in the trees as well as time on the ground where paraquat would actually be applied.

The diet of the chosen birds is also refined using factors of either 0, 0.25, 0.5, 0.75 or 1 (where 1 = 100% in diet) applied to either vegetation, earthworms, other vertebrates, seeds and insects. This was based on dietary information on a large number of species. The seeds were further subdivided into husked or de-husked (this reduced the residues to approx 13%). The risks from contaminated seed were probably lower than anticipated due to the fact that many plants would be treated at early growth stages and would therefore not survive to produce seed. Any seed still produced (even part-ripe) would also be more prevalent later in the year when birds are less likely to be breeding. The insect food category was further subdivided into large, small and all insects. Vegetation and fruit accounted for little in this assessment since fruit would not be available until autumn (and probably would not be produced from treated vegetation), green herbage would also die after 2-3 days and would be unlikely to contribute significantly to chronic exposure. The assessment is also refined by focussing on those bird species which frequent the areas where paraquat is most commonly used, i.e. for seed bed preparation in maize and sugar beet fields, in potato crops and in orchards.

In line with the original Monograph, the notifier's assessment indicates that birds with a high proportion of insects in their diet are most at risk, particularly as the proportion of insects consumed during the breeding season increases and insects form a large part of the diet of nestlings. Seed eaters which do not dehusk are also considered a high risk group, although as discussed, this may result from an overestimation of exposure.

The notifier has taken the approach of determining Time Weighted Average (TWA) concentrations, this was based on actual concentrations and dissipation rates for various food items treated at 1000 g a.s./ha. The TWA dietary concentrations were calculated for 18 weeks (the duration of the reproductive study) and 6 weeks (stated to be the length of a typical brood cycle), the TWA residues over 6 weeks were the highest and gave the most conservative TERs. The reproductive toxicity figure used was the same 18 week NOEL for mallard of 30 ppm in diet as used previously. Based on these assumptions (using the 6 week TWA residues), examples of the lowest TERs for key bird species with a high proportion of insects in their diet are given in Table 8.2 (note, some of these species (asterisked*) also include a large amount of seed as well as insects in their diet). The TERs for the predominantly seed-eating pigeons and doves were all fairly similar for each scenario (range 7-20) and only examples are given in the tables (marked #). Although these TERs are low, this is probably due to an overestimated risk from the consumption of husked seeds.

**Table 8.2 a, b and c: Lowest long term TERs for key bird species with a high proportion of insects in their diet in different situations (based on 6 week TWA residue levels)**

a) Seed bed preparation:

| Species | TERlt from use at 1.1 kg |
|---|---|
| meadow pipit (*Anthus pratensis*) | 20 |
| grey partridge (*Perdix perdix*)* | 20 |
| pheasant (*Phasianus colchicus*)* | 18 |
| red legged partridge (*Alectoris rufa*)* | 16 |
| stock dove (*Columba oenas*)# | 12 |
| skylark (*Alauda arvensis*)* | 11 |

* The TERs for these spp. were based on a large proportion of husked seed as well as insects in the diet

b) Potato crops:

| Species | TERlt from use at 1.1 kg |
|---|---|
| meadow pipit (*Anthus pratensis*) | 14 |
| pheasant (*Phasianus colchicus*)* | 19 |
| skylark (*Alauda arvensis*)* | 12 |
| tree sparrow (*Passer montanus*)* | 15 |
| yellow wagtail (*Montacilla flava*) | 16 |
| stock dove (*Columba oenas*)# | 12 |

* The TERs for these spp. were based on a large proportion of husked seed as well as insects in the diet

c) Orchards:

| Species | TERlt from use at 1.1 kg |
|---|---|
| chaffinch (*Fringilla coelebs*) | 23 |
| willow warbler (*Phylloscopus trochilus*) | 13 |
| collard dove (*Streptopelia decaocto*)# | 7 |

**Conclusions**

The notifier has addressed the risk to breeding birds using a reasoned case. The approach taken has involved consideration of the species potentially exposed, information of diets up to and during the breeding season including consideration of the proportion of diet taken from treated fields/crops. This has involved determination of Time Weighted Average (TWA) concentrations based actual concentrations and dissipation rates for various food items treated at 1000 g a.s./ha.

The Annex VI TERlt trigger for increased concern regarding reproductive risk to birds is 5. Table 8.2 on page 40 presents the lowest long term TERs for key bird species with a high proportion of insects in their diet in different situations. The long term TERs were all in excess of the Annex VI trigger, ranging from 11 to 23 for these species considered to be most at risk. The majority of the TERs calculated for other crop relevant birds were greater than those presented in Table 8.2 and were well in excess of the trigger (see Tables 2-4 in Zeneca document: Risk assessment for the effect of long term exposure of birds to paraquat residues in their diet - author: P. J. Edwards dated 7 May 1999). Overall, on the basis of this assessment, the reproductive risk identified for birds is considered to be low.

These TER calculations are however, based on time weighted average (TWA) concentrations of paraquat in the diet. The use of this practice could be questioned due to uncertainty over 'time to effect' considerations. It is not known, or clear from reproductive toxicity studies whether any reproductive effects are due more to initial toxicity early (or at a key time point) in the reproductive period or to constant low level exposure and toxicity throughout the period. This has a greater bearing in the field since initial concentrations may be significantly higher than a TWA due to the dissipation of residues over time.

However, few applications of paraquat are likely to coincide precisely with the key period in the breeding cycle of exposed birds. Therefore, using the TWA exposure level might well be appropriate but it could be considered less than worst case in some circumstances and using initial residue levels would be more conservative. A worse case assessment of exposure for two species considered to be at high risk is conducted below:

> From the TERs above in Table 8.2, an example of one of the predominantly insectivorous species most at risk in arable situations is the meadow pipit with long term TERs of 14-20, this has taken account of all the refinements mentioned above, including the 6 week TWA calculations. The diet of the meadow pipit was considered to be mainly small insects, the TWA residues on small insects over 6 weeks exposure were calculated to be approximately 37% of the initial residues (7.4 ppm as against 20 ppm), therefore correcting the lowest TER (14) accordingly for initial residue levels gives a TER of 5.18. Similarly correcting the orchard TER for the willow warbler of 13 to account for the initial residues on small insects gives a TER of 4.81.

The rapporteur considers that, although using the TWA residue may underestimate the risk, using the initial residues (as given above) will overestimate the risk. Therefore, considering the various other worst case assumptions used, these lower TERs of 5.18 and 4.81 for insectivorous birds using initial residue data are concluded to be conservative and not considered indicative of a long term risk to birds from paraquat use.

Overall, it is concluded that the majority of the assumptions used in the reasoned case presented by the notifier are valid. The data sets used are considered reliable and appropriate to a European wide risk assessment. It is concluded that these data support the refined assessment of risk and provide a basis for a more robust consideration of the actual risk posed to reproducing birds from the use of paraquat. It is considered that the available data supports the conclusion that under field conditions the long term TERs will be greater than the Annex VI trigger of 5 and that there will be no unacceptable impact on reproducing birds following use of paraquat according to the proposed conditions of use.

## B.8.2 Effects on terrestrial vertebrates other than birds (IIIA 10.3)

### B.8.2.1 Acute risk to terrestrial vertebrates other than birds - risk to hares

When originally assessed and discussed at ECCO 31, the acute and short term risks to mammals from exposure through consumption of contaminated seeds and invertebrates were considered acceptable. The worst case route of exposure, presenting the highest risk, was however, considered to be through consumption of recently treated vegetation. One of the animals thought to be particularly at risk from such exposure was the hare (*Lepus* spp.) as these animals live and feed predominantly within the crop, cereal stubbles being a favoured habitat. There were also a few confirmed, and a larger number of unconfirmed incidents, linking the use of paraquat with the deaths of hares.

In the Monograph, the acute TER for the hare was calculated to be 5, this was less than the Annex VI trigger of 10 and indicated a potential risk. There was also thought to be a potential risk from ingestion of paraquat residue when grooming wet fur. The ECCO group therefore recognised a potential risk and further information was requested.

### B.8.2.2 New information submitted on the acute risk to hares

In response to this request for further information, the main notifier has responded with a scientifically reasoned case supported by numerous referenced papers. This paper 'Review of the factors affecting the decline of the European Brown Hare, *Lepus europaeus* (Pallas, 1778) and the use of Wildlife Incident data to evaluate the significance of paraquat' by P.J. Edwards, M R Fletcher and P Berny, (due for publication in June 2000) is available as an accompanying document. The supporting references cited in the notifier's paper have all been made available to the Rapporteur.

### B.8.2.3 Critical assessment of Notifier's scientifically reasoned case

The data requirement proposed as a result of ECCO 31 was worded as follows: 'Further information on the toxicity of the active substance to hares (these data should address the risk to hares from the use of paraquat and include data on, for example, the residues of paraquat on treated vegetation, the risk from grooming as well as on the toxicity of paraquat to hares)'.

The notifier's reasoned case above does not address this requirement by submitting further toxicity or exposure data, instead the factors affecting the decline in European Brown Hare populations are discussed with specific reference to wildlife incident data and any poisonings which implicate paraquat. The data sets used are considered reliable and appropriate for use in a European wide risk assessment.

It is apparent that hare populations were in decline throughout much of Europe before the widespread use of paraquat and this also occurred in areas where paraquat was not widely used. There is no evidence of any causal link being

established between the long term decline in hare populations and the use of paraquat, the most probable explanation for the decline is due to overall changes in farm management and cropping practices. On a smaller scale it is apparent from the toxicity information and a no-choice enclosure study (de Lavaur, Grolleau *et al*, 1973) that paraquat can cause lethal effects in hares where exposure is inevitable. However, in a choice study (Grolleau, 1981) paraquat reduced feeding on tillers and no mortality was seen. In a real field environment, this repellency of paraquat (due to local irritation) and the availability in most situations of alternative forage, will probably provide an opportunity for hares to avoid an acute lethal dose.

The conclusion that such mitigating factors are operating in the field is supported by the relative lack of confirmed paraquat poisoning incidents from the UK Wildlife Incident Investigation Scheme (WIIS) and the French Sanitary Surveillance Scheme for Wildlife (SAGIR). The results from such wildlife incident recording schemes need to be treated with caution, since a lack of reported incidents does not necessarily mean that none have occurred. This is particularly the case with smaller birds and mammals which are less readily observed and which either die under cover or are quickly scavenged. Hares however, are conspicuous animals living in open fields rather than burrows, they are a sought after game species and also one of conservation concern. This means that unusual hare incidents are likely to be noticed and reported. Both of the reporting schemes mentioned have accumulated a significant number of hare incidents since their inception. In the WIIS scheme, 2 out of 104 (2%) hare incidents were associated with paraquat use but they were not diagnosed as the cause of death (NOTE: a WIIS incident may include more than one animal) and in the SAGIR scheme (where one incident = one animal) 8 out of 13588 (0.06%) incidents were confirmed as due to paraquat use. Given the reasonable sample sizes, there is no reason to suppose that the overall proportions of actual incidents involving paraquat use are significantly greater than the levels reported. Indeed, due the possible confusion of symptoms with the effects of EBHS, the older WIIS incidents in particular, may be an overestimate of actual paraquat poisonings.

On an overall population scale it is considered therefore, that paraquat is unlikely to have contributed significantly to the decline of hares or to a large proportion of the reported incidents. However, particular areas that have not been completely addressed are the relative numbers of incidents that may occur on a local (field or farm) scale due to paraquat use and whether these and the effects on individual animals are acceptable. The role of grooming in determining exposure has also not been discussed explicitly, however the overall effect from all routes of exposure, including grooming has been considered. It is likely that hares foraging in a field recently treated with paraquat will experience irritation and ulceration of the nose, lips and tongue through feeding on treated vegetation and/or through grooming wet fur. This appears to be sufficient to make them stop feeding for a while and/or seek untreated food and habitat, in most environments this will be available as alternative forage or neighbouring untreated fields. The reference papers submitted indicate that a hares' foraging range normally encompasses a number of fields and boundary habitats and it is unlikely that all fields in an area would be treated with paraquat at the same time. Where incidents have occurred

this may be due to large fields being treated where little alternative habitat was available. Sublethal effects and reduced feeding may indirectly contribute to mortality in such circumstances where insufficient untreated forage is available.

Using the assumption of typical current European population densities of 1-10 hares per 100 ha (taken from various of the reference papers submitted) and using the confirmed incidents from the SAGIR scheme as a basis that 0.06 % of hare deaths could be attributed to paraquat, then mortalities attributable to paraquat on local scale is considered to be minimal. Young hares (leveretts) may be more at risk and poisoning incidents are less likely to have been reported but viewed against the many natural dangers (predation, disease) and other threats (e.g. being crushed by farm machinery) faced by leveretts, the numbers likely to be harmed by paraquat should also be relatively very low.

Overall it is concluded that the information submitted supports the contention that there is not a causal link between the decline in European Brown Hare populations and use of paraquat. The data supports the conclusion that under field conditions there will be no unacceptable impact on hare populations following application of paraquat according to the proposed conditions of use. In this context, it is considered that in field situations, the reported repellency of paraquat (due to local irritation) coupled with the availability of alternative forage will not result in significant hare mortalities arising from the use of the plant protection product.

Whilst it is concluded that under field conditions there is no unacceptable impact on hares, Member States may wish to consider labelling in order minimise the risk of exposure. Hares usually rest during daylight hours and are considered to be most at risk from dusk to dawn when they are actively foraging. As a result of earlier concerns regarding the effects of paraquat on hares, the UK implemented labelling on paraquat products indicating that, for uses with a high probability of exposure, fields should be sprayed 'early in the day'. This was intended to give still active hares time to avoid being oversprayed and allow residues to dry and desiccation/wilting of treated foliage to occur before dusk when hares would start feeding again.

## B.8.3 Effects on aquatic organisms (IIA 8.2, IIIA 10.2)

When previously assessed via ECCO 31, the acute and chronic risks to fish, *Daphnia* and algae were considered to be low. Based on a standard 1 m spray drift scenario, the calculated TER values for each group indicated a low risk to aquatic life. According to the available data, algae were the most sensitive species to the active substance (and the tested formulations) with a 96h $E_bC50$ of 0.075 mg a.s./l for *Pseudokirchneriella subcapitata* (syn. *Raphidocellis subcapitata* and *Selenastrum capricornutum*). It was noted however that a study was also available on higher aquatic plants (*Lemna gibba*) but that it was missing from the Dossier. This study has now been submitted along with some additional algal toxicity studies (on different taxonomic groups). These new studies are evaluated below.

A data gap was previously identified in the Monograph regarding the risk posed to sediment dwelling organisms and a confirmatory data requirement was proposed to address this risk. Tests on *Chironomus riparius* using two different methods have now been performed and are also evaluated below.

### B.8.3.1 Toxicity of the active substance

#### B.8.3.1.1 Toxicity to algae and higher aquatic plants

The newly submitted studies on algae and *Lemna* are summarised in the following Table 8.3:

Table 8.3: The toxicity of paraquat ion to algae and *Lemna*.

| Species | Test type and duration | Actual conc. (as % of nominal) | EC50, µg/l paraquat ion (95% CL) | NOEC, µg/l paraquat ion | Test guideline* | Reference |
|---|---|---|---|---|---|---|
| **Algae (IIA 8.2.6)** | | | | | | |
| *Anabaena flos-aquae* | 120h static | mean measured range 72-86, overall mean 79 | measured $E_bC50$: 4.9 (1.9-9.2) $E_rC50$: 7.8 (5.2-12.1) | measured 1.0 (based on biomass) 4.3 (based on growth rate) | EPA FIFRA 123-2 | DVSmyth *et al*, 1992 ref: BL4579/B |
| *Navicula pelliculosa* | 96h static | not determined as below LOD, (nominals used) | nominal $E_bC50$: 0.23 (0.17-0.31) $E_rC50$: 0.34 (0.24-0.48) | nominal 0.21 (based on both biomass and growth rate) | EPA FIFRA 123-2 | DVSmyth *et al*, 1992 ref: BL4464/B |
| *Navicula pelliculosa* | 96h static in the presence of sediment[#] | range 45-97 at day 0, (nominals used)[@] | nominal $E_bC50$ and $E_rC50$: >290 | nominal 290 (based on both biomass and growth rate) | modified OECD 201 and EU, L383A C3 | DVSmyth and N Shillabeer, 2000, ref: BL6800/B |
| **Higher aquatic plants (IIA 8.2.8)** | | | | | | |
| *Lemna gibba* | 14d semi-static | mean measured range 75-94 (nominals used) | nominal $E_bC50$: 45 (42-50) $E_rC50$: 37 (33-42) | nominal 21 (based on both growth rate and biomass) 5.2 (based on pale fronds and reduced root growth) | EPA FIFRA 123-2 | DP 48926 D V Smyth *et al*, 1992 ref: BL4493/B |

***** Test conducted without deviation and in accordance with GLP unless otherwise stated.

[#] The study was run for 120 hours but the algae had stopped growing (in control and treatment groups) by 96h due to nutrient exhaustion, end points are for 96h. Sediment was sandy loam field soil 2.2% OM.

[@] Although the range of measured concentrations fell below usual standards, the measure concentration at the top two doses was 86% and 97% of nominal and given that no effects were seen at these concentrations, the use of nominals is considered appropriate.

The Notifier has also submitted a number of studies in the public domain which address the toxicity of paraquat to a wide range of algal species (in particular the published papers ref.: Ibrahim, 1990 and Cullimore, 1975). None of these studies were performed according to internationally recognised guidelines or to GLP but the paper by Ibrahim gives comparable 96h EC50s of 29, 54 and 61 µg paraquat ion/l for *Scenedesmus dimorphus*, *S. quadricauda* and *Ankistrodesmus facatus* respectively. The EC50s from the Cullimore study were over 30 days and were far in excess (100-10000 µg a.s./l) of those reported in Table 8.3, the methodology was not comparable.

### B.8.3.1.2 Effects on sediment dwelling organisms (IIA 8.2.7)

Data have been submitted on the effects of paraquat on larvae of the midge *Chironomus riparius*. Two different methods were used, the first used the spiked sediment ASTM method, the second used the spiked water BBA method. These studies are summarised below:

a) In a 21-day toxicity study on *Chironomus riparius* using technical paraquat dichloride (34.1% paraquat ion) mixed with radiolabelled $^{14}$C-paraquat, the sediment phase of a sediment/water system was dosed with a nominal 100 mg paraquat ion/kg dry weight sediment. The sediment used was based on the OECD 207 standard earthworm soil and contained 70% silica sand, 20% kaolinite clay, 10% peat and 5 g/kg $CaCO_3$, (OC content was 4.2%). Treated sediment (200 g dry weight) was mixed with 2 litres of overlying water in 3 litre glass vessels. The water had a total hardness of 172 mg/l $CaCO_3$ and a pH of 8.4-8.7, DO was 9.0-9.1 mg/l. There were five test and four control replicates, these were maintained at 19.5-20.2°C under a 16:8 light:dark photoperiod. The first instar chironomid larvae (25 per replicate) were added after a 3 day settling period (day 0) and were fed ground fish food and *Chlorella* algae. From day 10 the test systems were observed daily for emergence of adults and the numbers of males and females was recorded. Samples of water and sediment were taken for analysis of paraquat concentrations.

The concentration of $^{14}$C-paraquat in the sediment phase was determined to be 94 mg/kg at day 0. In the water phase the concentrations (and %AR) at day 0, 7, 14 and 21 were 0.02 mg/l (0.2), 0.064 mg/l (0.6), 0.121 mg/l (1.2) and 0.026 mg/l (0.3) respectively. It is clear that very little partitioning of paraquat back into the water phase occurred. Adults emerged from day 12 or 13 up until day 18 and there were no differences between patterns, numbers or sexes of emerging adults between the control or treated replicates. The cumulative total emergence was 97% and 99% in the control and treated groups with respective mean emergence times of 14.1 and 14.0 days.

It was concluded that paraquat applied to sediment at a concentration of 100 mg /kg had no effect on the survival or development of *Chironomus riparius*. The EC50 would have been >100 mg a.s./kg sediment with a NOEC of 100 mg a.s./kg.

The test was conducted to GLP and according to ASTM and SETAC guidelines.

(ref: Hamer, 1998, report No.RJ2649B)

b) In a 21-day toxicity study on *Chironomus riparius* using technical paraquat dichloride (34.1% paraquat ion) mixed with radiolabelled $^{14}$C-paraquat, the water phase of a sediment/water system was dosed at a nominal 0.367 mg paraquat ion/l water. The sediment used was based on the OECD 207 standard earthworm soil and contained 70% silica sand, 20% kaolinite clay, 10% peat and 5 g/kg $CaCO_3$, (OC content was 4.2%). The sediment (267 g dry weight) was mixed with 2.5 litres of water in 3 litre glass vessels, this gave a sediment depth of 2cm with 18 cm of overlying water. The water had a total hardness of 173 mg/l $CaCO_3$

(approx. 136 at end of study) and a pH of 7.7-8.18, DO was 6.7-8.8 mg/l. There were four test and control replicates, these were maintained at 19.2-20.6°C under a 16:8 light:dark photoperiod. The first instar chironomid larvae (25 per replicate) were added at day 0 and were fed ground fish food. From day 10 the test systems were observed daily for emergence of adults and the numbers of males and females was recorded. Samples of water and sediment were taken for analysis of paraquat concentrations.

The concentration of $^{14}$C-paraquat in the water phase was determined to be 0.295 mg/l at day 0 (80.6% of nominal), this had declines to 3.5% (0.013 mg/l) at day 7 and 1.0% of nominal (0.0036 mg/l) by day 21. In the sediment phase the concentrations at day 21 was 3.2 mg/kg (92.5% nominal AR). It is apparent that there was significant partitioning of paraquat to the sediment. Adults emerged from day 13 up until day 17 or 19 and there were no differences between patterns, numbers or sexes of emerging adults between the control or treated replicates. The cumulative total emergence was 96% in both control and treated groups with the same mean emergence time of 14.4 days.

It was concluded that paraquat applied to the water at a concentration of 0.367 mg/l had no effect on the survival or development of *Chironomus riparius*. The EC50 would have been >0.367 mg/l with a NOEC of 0.367 mg/l.

The test was conducted to GLP and according to BBA guidelines.

(Hamer and Ashwell, 1997, report No.RJ2392B)

### B.8.3.2 Risk to aquatic organisms

All areas of risk to aquatic organisms from spray drift of paraquat have been addressed via the Monograph and ECCO 31 and were previously considered acceptable, only the data on *Lemna* and sediment dwelling invertebrates were required for clarification. However, the newly submitted studies on algae summarised in 8.3.1.1 give a 120h $E_bC50$ of 0.0049 mg paraquat/l for *Anabaena flos-aquae* and a 96h $E_bC50$ of 0.00023 mg a.s./l for *Navicula pelliculosa*. These figures are significantly below the previously considered EC50 for algae of 0.075 mg a.s./l and require that the risk to algae be reassessed. Regarding the risk to higher aquatic plants, the study summarised at 8.3.1.1 gives an $E_bC50$ for *Lemna gibba* of 0.037 mg paraquat/l.

#### B.8.3.2.1 Risk to algae and higher aquatic plants

Risk to algae:

Based on spray drift (5%) at 1 m into a static 30 cm deep water body, the initial PEC was previously calculated to be 0.018 mg a.s./l for maximum rate arable/orchard use at 1100 g a.s./ha (Section B.7.5, Table B.7.8 of Monograph). Comparing this value with the lowest EC50 for algae (0.00023 mg a.s./l for *Navicula pelliculosa*) gives a TER of 0.013, this is below the Annex VI trigger of 10 for algae and indicates a potential high risk. However, as discussed in the

Monograph, the actual risk to algae is likely to be reduced owing to the rapid dissipation of paraquat from the water body due to adsorption to suspended particulates, organic material and the sediment. It was previously considered appropriate to use a Time Weighted Average (TWA) concentration in water based on a DT50 of ≤1 day in the water phase. The TWA using the same exposure scenario as above but spread over 96 hours would be 0.0062 mg a.s./l (see Table B.7.9 of Monograph), comparing this with the 96 hour EC50 of 0.00023 mg a.s./l would give a TER of 0.04. This is still below the trigger of 10 and requires that the risk to algae be refined further.

A study on the most sensitive algal species studied (*Navicula pelliculosa*) has been conducted in the presence of sediment. This is intended to replicate the dissipation processes which would occur following contamination of a natural water body and is considered appropriate for use in a refined risk assessment. The EC50 from this study (see Table 8.3) was >290 µg or >0.29 mg paraquat/l. Comparing this with the initial PEC of 0.018 mg a.s./l gives a TER of >16, alternatively comparing it with the TWA PEC for 96 hours of 0.0062 mg a.s./l gives a TER of >46. These TERs are above the Annex VI trigger of 10 and indicate a low risk to algae under more realistic conditions. It is also worth noting that *Navicula pelliculosa* was by far the most sensitive species of those tested and so the level of uncertainty regarding variation in species sensitivity could be reduced.

Risk to higher aquatic plants:

Using the above initial PEC of 0.018 mg a.s./l and the EC50 for *Lemna* of 0.037 mg a.s./l gives a TER of 2.06, this is also below the trigger of 10 used for higher aquatic plants and indicates a potential high risk. However, as with the risk to algae, the actual risk to higher aquatic plants is likely to be reduced due to the rapid dissipation of paraquat from the water . It is not clear whether the effect of paraquat on aquatic plants occurs from an initial dose or progressively over constant exposure but, considering that the study on *Lemna* was run over 14 days with renewal and reasonable maintenance of test concentrations, the exposure and toxicity experienced in the test are more worst case than likely to be experienced in the field. Therefore, the use of TWA concentrations is considered appropriate over this timescale. The TWA using the same exposure scenario as above but spread over 14 days would be 0.0019 mg a.s./l (see Table B.7.9 of Monograph), comparing this with the 14-day EC50 of 0.037 mg a.s./l would give a TER of 19.5. This is above the trigger of 10 and indicates a low risk to higher aquatic plants from spray drift of paraquat applied during normal arable/orchard use at up to 1100 g a.s./ha.

### B.8.3.2.2 Risk to sediment dwelling organisms

The rapid partitioning of paraquat to sediments may pose a risk to sediment dwelling organisms and a confirmatory data requirement was proposed to address this risk. Data have now been submitted from two studies on *Chironomus riparius* to address this concern. The studies evaluated at 8.3.1.2 gave a NOEC of either 100 mg paraquat./kg of sediment or 0.367 mg a.s./l of water. The first figure may be compared with the $PEC_{sed}$ from Section B7.5 of the Monograph of 0.733 mg a.s./kg to give a TER of 136. The second figure may be compared with the initial worst case PEC of 0.018 mg a.s./l to give a TER of 20.4. Both of these TERs are above the Annex VI trigger of 10 proposed in the Draft Guidance Document on Aquatic Ecotoxicology in the frame of the Directive 91/414/EEC. The risks to sediment dwelling organisms from use of paraquat as directed, is therefore considered to be low and no further data or risk mitigation are required.

## B.8.4 Effects on bees (IIA 8.3.1, IIIA 10.4.1)

Data previously evaluated on the toxicity of paraquat to bees indicated that the acute (120-hour) oral and contact LD50s were 11 and 51 µg a.s./bee using the technical material or 9.06 and 9.26 µg a.s./bee using a 200 g/l SC formulation. Based on a maximum application rate of 1100 g a.s./ha, these gave Hazard Quotients (HQs) of either 100 and 22 or 121 and 119 respectively. A number of these HQs are greater than the Annex VI trigger of 50 indicating a potential, although only moderate, risk to bees from this use. ECCO 31 concluded that suitable risk management/labelling should be considered by MS, with the notifier being given the opportunity to submit further data to remove any restrictions.

Further data have now been submitted from German semi-field trials (to a BBA protocol). These are have been evaluated and are summarised below:

B.8.4.1 Toxicity to bees

A total of nine semi-field tent studies were conducted in Bonn, Celle, Munster and Erlangen, Germany between 1987 and 1989. The test methodology followed was in accordance with a BBA guideline.

Methodology

In each trial, the effect of paraquat. applied at twice the recommended application rate (as a 100 g/l SC formulation), was tested on small hives in tents with flowering Phacelia (*Phacelia tanacetifolia* Benth.) or wild mustard. Effects were compared to a water treated control group and a toxic standard. Applications were made directly onto foraging bees and each test was carried out using two test runs performed at different dates. Assessments included mortality before and after application of the test substance (at the edge of the treated area and at the entrance of the colonies), foraging activity, behaviour of the bees prior to. during and after application and development of the brood.

Results

A summary of the main conclusions from each study is given in the following Table 8.4:

Table 8.4: The effects of paraquat on bees in semi-field tent tests

| Trial No. | Location | Date | Conclusions |
|---|---|---|---|
| 1 | Bonn | July 1987 | Inconclusive, some reduced foraging, wilting of flowers at 18:00h on application day |
| 2 | Celle | July 1987 | Not harmful to bees. foraging activity reduced, blossoms clearly damaged in evening after spray application day |
| 3 | Celle | July 1987 | Not harmful to bees, marked reduction in foraging activity, no effects on brood |
| 4 | Munster | August 1987 | Not harmful to bees, no effects on brood |
| 5 | Munster | August 1987 | Not harmful to bees, no effects on brood |
| 6 | Bonn | July 1988 | Slightly increased mortality, no effects on brood |
| 7 | Bonn | July 1988 | Slightly increased mortality, marked reduction in bee visits to blossoms in afternoon of spraying, no brood effects |
| 8 | Erlangen | September 1989 | Not harmful to bees, no effects on brood |
| 9 | Erlangen | September 1989 | Not harmful to bees, no effects on brood |

The overall conclusions from the nine tent tests are that paraquat, sprayed directly onto foraging honeybees at twice the recommended application rate, demonstrates a low risk to bees. It was apparent that foraging activity can be reduced after application and that blooms were only attractive to bees for two days after application, therefore reducing exposure. The plants themselves are likely to die at that stage No effects on bee brood were recorded in any of the trials.

The recommended uses and application timings of paraquat-containing products are unlikely to result in direct application to crops on which bees may be foraging. However, these semi-field trials data demonstrate that, even if exposure does occur, there is a low risk to bees. As further confirmation, the main notifier has also noted that the UK monitoring scheme (WIIS) has only reported a single poisoning incident following the use of paraquat over many years. Similarly in Germany (the only other MS with a detailed honeybee poisoning monitoring scheme) no incidences of poisoning following paraquat use have been reported.

These data are considered sufficient to remove the need for any specific bee hazard or risk labelling at MS level.

## B.8.5 Effects on arthropods other than bees (IIA 8.3.2, IIIA10.5)

The risk to non-target terrestrial arthropods (other than bees) from use of paraquat as directed was considered in the Monograph and at ECCO 31 to be low. This was based on laboratory and field studies on a range of crop relevant species, however, neither of the two standard sensitive species (*Aphidus rhopalosiphi* and *Typhlodromous pyri*) had been tested. At ECCO 31 it was concluded that neither of the two standard species were relevant to the use patterns and risks posed by this total herbicide. However a data requirement was established and this was confirmed at ECCO 36, the regulatory decisions meeting. This has been addressed by the main notifier and data on *Aphidus rhopalosiphi* have also been submitted. The studies on both of these species have been evaluated and are summarised below.

### B.8.5.1 Toxicity to terrestrial arthropods (IIA 8.3.2, IIIA 10.5.1, 10.5.2)

Table 8.5 The effects of formulated paraquat on non-target terrestrial arthropods

| Species | Test substance | Test type, &duration | Applied dose | Effect(s) | Test guideline* | Reference |
|---|---|---|---|---|---|---|
| *Typhlodromus pyri* | 200 g/l SC form.n | laboratory, on glass slide over 3 days | ≡1100 g a.s./ha | 99% mortality after 1 day 100% mortality after 3 days | IOBC, based on Overmeer, 1988 | A Gill and H M Austin, 1996 |
| *Typhlodromus pyri* | 100 g/l SC form.n | laboratory, dose: response on glass over 7 days plus fecundity test over 14 days | dose: response, fecundity test at 0.5 and 1.0 g a.s./ha | 7 day control mortality 15%, corrected LC50: 1.9 g a.s./ha, LC30: 1.1 g a.s./ha, no effect on fecundity at 0.5 or 1.0 g a.s./ha | based on Overmeer, 1988 and Lois and Ufer, 1995 plus in-house protocol | H M Austin and V L Elcock, 1999, Report No.: ER-99-12 |
| *Typhlodromus pyri* | 100 g/l SC form.n | extended laboratory, dose: response on bean leaf over 7 days plus fecundity test on leaf over 14 days | dose: response, fecundity test at 2.0 and 4.0 g a.s./ha | 7 day control mortality 10%, corrected LC50: 8.2 g a.s./ha, LC30: 5.8 g a.s./ha, no effect on fecundity at 2.0 or 4.0 g a.s./ha | in-house protocol | H M Austin 1999, Report No.: ER-99-25 |

continued over

Table 8.5 continued

| Species | Test substance | Test type, &duration | Applied dose | Effect(s) | Test guideline* | Reference |
|---|---|---|---|---|---|---|
| *Aphidius rhopalosiphi* | 100 g/l SC form.n | laboratory, dose: response on glass over 48 hours plus fecundity test on aphid-infested barley over 12 days | dose: response, fecundity test at 1.0 and 2.0 g a.s./ha | 48h control mortality 16.7%, corrected LC50: 2.5 g a.s./ha, LC30: 1.4 g a.s./ha, NOEC: 1.0 g a.s./ha, no effect on fecundity at 1.0 or 2.0 g a.s./ha | based on Mead-Briggs, 1992 | H M Austin, 1999, Report No.: ER-99-14 |
| *Aphidius rhopalosiphi* | 100 g/l SC form.n | extended laboratory, dose: response on barley plants over 48 hours plus fecundity test on aphid-infested barley over 12 days | dose: response, fecundity test at 100 and 600 g a.s./ha | 48h control mortality 12.5%, corrected LC50: 796.3 g a.s./ha, LC30: 497.7 g a.s./ha, no effect on fecundity at 100 or 600 g a.s./ha | based on Mead-Briggs, pers. comm. | H M Austin and V L Elcock, 1999, Report No.: ER-99-HMA310 |

*Test conducted without deviation and in accordance with GLP unless otherwise stated.

### B.8.5.2 Risk to non-target terrestrial arthropods other than bees

The risk to crop relevant non-target terrestrial arthropods has previously been considered to be low. The additional studies above are of limited relevance to assessing the risk in-crop. A methodology for using dose:response data in non-target arthropod risk assessments has also yet to be agreed. However, individual MS may find the results for these sensitive indicator species relevant if undertaking an off-crop risk assessment for non-target arthropods in general. Otherwise, at EU level the requirement for data on this group is considered fulfilled.

### B.8.5.3 Overall conclusions regarding ecotoxicological risks

The data evaluated above have satisfied the outstanding ecotoxicological requirements set previously during the ECCO process. The assessments have concluded a low risk to terrestrial vertebrates, aquatic life, bees and other non-target arthropods from the use of paraquat. The Rapporteur considers that no specific risk mitigation measures or further data are required.