

## PART 3

### Section 6      Ecotoxicological Studies and risk assessment

The example of a summary and assessment of data which follows is intended to illustrate the approach recommended for the preparation of *Tier II* summaries and assessments. The material included has not been critically assessed for its technical content. Although based on a real submission, the data included in the following summary and evaluation have been amended to protect the commercial interests of the owner of the data.

Applicants should be aware that these guidelines are intended to provide a degree of flexibility. Where in particular cases, it is more appropriate to present the data and information in another format, applicants may do so. In such cases it is recommended that the applicant discuss the format proposed with the Regulatory Authority of the Country to which application is to be made.

For the purposes of calculating toxicity exposure ratios (TER values), distances and scenarios used as a basis for estimation of predicted environmental concentrations (PEC values) should reflect the results of risk assessments carried out *i.e.* where a calculation based on overspray is provided to illustrate the worst case likely to arise, it should be followed by a calculation reflecting risk mitigation measures proposed, such as use of buffer zones.

#### IIIA 10.1.1      Acute toxicity exposure ratio (TER<sub>A</sub>) for birds

OEC 2222 is a wheat herbicide intended for use post-emergence at GS 13-39 (expanded true leaf stage onwards to flag leaf ligule expansion). The recommended application rate is 25 g product/ha (20 g as/ha). One application per season is proposed. Avian exposure arises as a result of contact with treated foliage and soil and as a result of ingestion of avian food sources contained therein.

A summary of the acute toxicity of chemx to birds is provided in Table IIIA 10.1.1-1. Details of the studies concerned are provided at point IIA 8.1.1.

**Table IIIA 10.1.1-1      Acute avian toxicity data of chemx**

| Species        | Vehicle  | NOEL<br>mg as/kg bw | LLD<br>mg as/kg bw | LD <sub>50</sub><br>mg as/kg bw | Reference                   |
|----------------|----------|---------------------|--------------------|---------------------------------|-----------------------------|
| Bobwhite Quail | corn oil | Xxx                 | > xxxx             | > xxxx                          | Smith <i>et al</i> 1994 (a) |
| Mallard Duck   | corn oil | Xxxx                | > xxxx             | > xxxx                          | Smith <i>et al</i> 1995     |

On the basis of the recommendations for use of OEC 2222, the greatest levels of exposure for birds is likely to arise in the case of grazing birds, following treatment of cereals at early growth stages. Other possible exposure routes may be through ingestion of insects, large and small. For the purposes of calculations presented here under, birds are assumed to feed exclusively on contaminated material.

The residue level of chemx likely to arise in various food sources can be estimated in accordance with the method proposed by Hoerger and Kenaga (1972). Using that method, the maximum residue likely to occur on the various plant parts immediately following spraying and the maximum residue levels likely to be present in or on exposed invertebrate species are estimated on the basis of surface area to mass ratios.

The toxicity/exposure ratios calculated in respect of the acute exposure of birds feeding on a range of possible food sources, assuming worst case exposure (feeding exclusively on contaminated material), demonstrates that there is no significant practical risk to avian species (Table IIIA 10.1.1-2).

**Table IIIA 10.1.1-2      Estimated food residues, maximum daily intake and acute toxicity/exposure ratios for birds following maximum field use of chemx (0.02 kg as/ha)**

| Bird Type  | Body wt (kg)<br><sup>1</sup> Food Uptake | Food Consumed | <sup>2</sup> Estimated Food Residue (mg as/kg food) | <sup>3</sup> Estimated Theoretical Exposure (mg as/kg bw/day) | <sup>4</sup> TER <sub>A</sub> |
|------------|--|---------------|---|---|-------------------------------|
| Small Bird | 0.01<br>30 %                             | short grass   | 2.240   | 0.67  | xxxx                          |
|            |  | leaves        | 0.626   | 0.19  | xxxxx                         |
|            |  | seeds/grains  | 0.054   | 0.02  | xxxxxx                        |
|            |  | small insects | 0.590   | 0.18  | xxxxx                         |
|            |  | large insects | 0.060   | 0.02  | xxxxx                         |
| Large Bird | 0.1<br>10 %                              | short grass   | 2.240   | 0.23  | xxxx                          |
|            |  | leaves        | 0.626   | 0.06  | xxxxx                         |
|            |  | seeds/grains  | 0.054   | 0.01  | xxxxxx                        |
|            |  | small insects | 0.590   | 0.06  | xxxxx                         |
|            |  | large insects | 0.060   | 0.01  | xxxxxx                        |

<sup>1</sup> Food uptake in % of body weight

<sup>2</sup> Kg as/ha x Hoerger and Kenaga factors (1972)

<sup>3</sup> ETE = Estimated food residue X daily intake/body weight

<sup>4</sup> TER<sub>A</sub> = LD<sub>50</sub>/ETE (mg as/kg bw/day)

### IIIA 10.1.2      Short-term toxicity exposure ratio (TER<sub>ST</sub>) for birds

A summary of the short-term and sub-chronic toxicity of chemx to birds is provided in Table IIIA 10.1.2-1. Details of the studies concerned are provided at points IIA 8.1.3 and IIA 8.1.4.

The toxicity/exposure ratios calculated in respect of the short-term and sub-chronic exposure of birds feeding on a range of possible food sources, assuming worst case exposure (feeding exclusively on contaminated material), demonstrate that there is no significant practical risk to avian species (Table IIIA 10.1.2-2).

**Table IIIA 10.1.2-1      Short term and sub-chronic avian toxicity data for chemx**

| Species         | Exposure | Vehicle              | NOEC<br>mg as/kg diet | LLC<br>mg as/kg diet | LC <sub>50</sub><br>mg as/kg diet | Reference                      |
|-----------------|----------|----------------------|-----------------------|----------------------|-----------------------------------|--------------------------------|
| Bobwhite Quail* | 5 day    | acetone/<br>corn oil | xxxx                  | > xxxx               | > xxxx                            | Smith <i>et al</i> 1994<br>(b) |
| Mallard Duck*   | 5 day    | acetone/<br>corn oil | xxxx                  | > xxxx               | > xxxx                            | Smith <i>et al</i> 1994<br>(c) |
| Bobwhite Quail  | 21 weeks | acetone/<br>corn oil | xxxx                  | -                    | -                                 | Jones <i>et al</i> 1996<br>(a) |
| Mallard Duck    | 22 weeks | acetone/<br>corn oil | xxx                   | -                    | -                                 | Jones <i>et al</i> 1996<br>(b) |

\* Based on day zero measured values

**Table IIIA 10.1.2-2      Estimated food residues, maximum daily intake, short-term and sub-chronic toxicity/exposure ratios for birds following maximum field use of chemx (0.02 kg as/ha)**

| Bird Type  | Body wt (kg)<br><sup>1</sup> Food Uptake | Food Consumed | <sup>2</sup> Estimated Food Residue<br>(mg as/kg food) | <sup>3</sup> Estimated Theoretical Exposure<br>(mg as/kg bw/day) | <sup>5</sup> TER <sub>ST</sub> | <sup>6</sup> TER <sub>LT</sub> |
|------------|--|---------------|--|--|--------------------------------|--------------------------------|
| Small Bird | 0.01<br>30%                              | Short grass   | 2.240  | 0.67   | xxxx                           | xxx                            |
|            |  | Leaves        | 0.626  | 0.19   | xxxx                           | xxxx                           |
|            |  | Seeds/grains  | 0.054  | 0.02   | xxxxx                          | xxxxx                          |
|            |  | Small insects | 0.590  | 0.18   | xxxx                           | xxxx                           |
|            |  | Large insects | 0.060  | 0.02   | xxxxx                          | xxxxx                          |
| Large Bird | 0.1<br>10%                               | Short grass   | 2.240  | 0.23   | xxxx                           | xxx                            |
|            |  | Leaves        | 0.626  | 0.06   | xxxx                           | xxx                            |
|            |  | Seeds/grains  | 0.054  | 0.01   | xxxxx                          | xxxx                           |
|            |  | Small insects | 0.590  | 0.06   | xxxx                           | xxx                            |
|            |  | Large insects | 0.060  | 0.01   | xxxxx                          | xxxx                           |

<sup>1</sup> Food uptake in % of body weight

<sup>2</sup> Kg as/Ha x Hoerger and Kenaga factors (1972)

<sup>3</sup> ETE = Estimated food residue X daily intake/body weight

<sup>4</sup> TER<sub>A</sub> = LD<sub>50</sub>/ETE (mg as/kg bw/day)

<sup>5</sup> TER<sub>ST</sub> = LC<sub>50</sub>/ETE (mg as/kg food) [Toxicity data similar for both indicator species]

<sup>6</sup> TER<sub>LT</sub> = NOEC/ETE (mg as/kg food)



### IIIA 10.1.9      Effects of secondary poisoning

Due to the low residue burden likely to arise in soil and foliage inhabitants, following use of OEC 2222 and the low risk of bio-accumulation in tissues, there is no significant risk to terrestrial vertebrates through secondary poisoning.

### IIIA 10.2.1      Toxicity exposure ratios for aquatic species

Chemx was tested on a range of aquatic species in accordance with established test guidelines (see points IIA 8.2.1 through 8.6.1). Levels found to cause effects were analytically determined in all studies except where exposure concentrations were below the limit of quantification. Testing for effects on sediment dwelling organisms was not carried out because effects were not observed in invertebrate species at the exposure levels likely to arise following spray drift or runoff - direct application to water bodies is not proposed. A bio-concentration test was not carried out since Log Pow value for chemx is < 1, the trigger value used to determine when such testing is required, and furthermore repeated exposure does not occur.

A summary of the aquatic toxicity profile of chemx is provided in Table IIIA 10.2.1-1.

**Table IIIA 10.2.1-1      Acute and chronic toxicity of chemx to aquatic organisms**

| Test Species                     | Test Duration | Test Conc.           | No Effect Conc. (mg/l) | 50 % Effect Conc. (mg/l) | Effect Parameter                | Reference       |
|----------------------------------|---------------|----------------------|------------------------|--------------------------|---------------------------------|-----------------|
| <i>Oncorhynchus mykiss</i>       | 4 day         | Measured             | xx                     | > xx                     | Survival/growth                 | Fisk R 1994 (a) |
| <i>Lepomis macrochirus</i>       | 4 day         | Measured             | xx                     | > xx                     |                                 | Fisk R 1994 (b) |
| <i>Cyprinus carpio</i>           | 4 day         | Measured             | xx                     | > xx                     |                                 | Fisk R 1995 (a) |
| <i>Cyprinodon variegatus</i>     | 4 day         | Measured             | xxx                    | > xxx                    |                                 | Fisk R 1995 (b) |
| <i>Oncorhynchus mykiss</i>       | 87 day        | Measured             | xxx                    | -                        | Immobilisation/<br>Reproduction | Fisk R 1996 (a) |
| <i>Daphnia magna</i>             | 2 day         | Measured             | xx                     | > xx                     |                                 | Fisk R 1994 (c) |
| <i>Daphnia magna</i>             | 21 day        | Measured             | xxx                    | > xxx                    | Biomass*                        | Fisk R 1996 (6) |
| <i>Selenastrum capricornutum</i> | 3 day         | Nominal              | < x.xxx                | x.xxx                    |                                 | Rose A 1995 (a) |
|                                  | 3 day         | Nominal              | x.xxx                  | x.xxx                    |                                 | Growth rate     |
|                                  | 5 day         | Nominal              | x.xxx                  | x.xxx                    |                                 | Biomass**       |
|                                  | 5 day         | Nominal              | x.xxx                  | x.xxx                    |                                 | Growth rate     |
| <i>Scenedesmus subspicatus</i>   | 3 day         | Nominal/<br>Measured | x.xx                   | x.x                      | Biomass**                       | Rose A 1995 (b) |
| <i>Anabaena flos-aquae</i>       | 5 day         | Nominal              | x.xx                   | x.xx                     | Biomass**                       | Rose A 1996 (a) |
| <i>Navicula pelliculosa</i>      | 5 day         | Measured             | xx                     | > xx                     | Biomass**                       | Rose A 1996 (b) |
| <i>Skeletonema costatum</i>      | 5 day         | Measured             | xxx                    | > xxx                    | Biomass**                       | Rose A 1996 (c) |
| <i>Lemna gibba</i>               | 14 day        | Measured             | x.xxxx                 | > x.xxx                  | FronD Inhibition                | Rose A 1996 (d) |

\* Biomass calculated as area under growth curve

\*\* Biomass calculated from cell density

Chemco      September 1997      chemx (proposed ISO name)      page of

Aquatic organisms may be exposed to chemx as a consequence of the accidental entry of the compound into the environmental compartments occupied by organisms or as a consequence of run-off events. Since the recommended method for application of chemx involves application using tractor driven hydraulic equipment, the most realistic exposure route is through spray drift. Details of the predicted environmental concentrations for chemx in surface water, arising as a consequence of over-spraying, drift and run-off, are provided at points IIIA 9.7.1 through IIIA 9.10.2.

A summary of the predicted environmental concentrations of relevance for the purposes of calculating toxicity exposure ratios for aquatic species is provided in Table IIIA 10.2.1-2.

The highest PEC<sub>SW</sub> values estimated relate to initial water concentrations and range from 0.012 to 2.0 µg as/l. The corresponding time weighted average PEC<sub>SW</sub> values range from 0.0102 to 0.049 µg as/l depending on exposure route and captive water depth.

**Table IIIA 10.2.1-2      Initial and long-term PEC<sub>SW</sub> values for chemx (µg as/l)**

| Exposure Route *           | Initial Concentration |              |         |              |         | TWA Concentration |         |                         |
|----------------------------|-----------------------|--------------|---------|--------------|---------|-------------------|---------|-------------------------|
|                            | Over-spray            | Drift at 1 m |         | Drift at 5 m |         | Drift at 5 m      |         | Run-off<br>0.02 ha Pond |
| Water Depth                | 1 m                   | 1 m          | 0-30 cm | 1 m          | 0-30 cm | 1 m               | 0-30 cm | 1 m                     |
| initial PEC <sub>SW</sub>  | 2.0                   | 0.08         | 0.27    | 0.012        | 0.04    | 0.0117            | 0.0391  | 0.049                   |
| PEC <sub>SW</sub> - 14 day |                       |              |         |              |         | 0.011             | 0.037   | 0.046                   |
| - 21 day                   |                       |              |         |              |         | 0.011             | 0.037   | 0.046                   |
| - 60 day                   |                       |              |         |              |         | 0.0102            | 0.0340  | 0.0425                  |

\* The spray drift model, run-off model and scenario used were .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

### IIIA 10.2.1.1    TER<sub>A</sub> for fish

On the basis of the worst case toxicity values (Table IIIA 10.2.1-1) and the relevant worst case PEC<sub>SW</sub> values (Table IIIA 10.2.1-2), toxicity exposure ratios for acute exposure of fish were calculated (Table IIIA 10.2.1-3).

The TER<sub>A</sub> values estimated indicate that the potential risks arising for fish are extremely low. As the acute TER is >100, use of chemx is not considered to involve risks for fish.

**Table IIIA 10.2.1-3      Acute toxicity exposure ratios (TER<sub>A</sub>) for fish, exposed to chemx**

| Exposure Route         | Initial Concentration  |                         |                        |                         |                         | TWA Concentration       |                         |                            |
|------------------------|------------------------|-------------------------|------------------------|-------------------------|-------------------------|-------------------------|-------------------------|----------------------------|
|                        | Over-spray             | Drift at 1 m            |                        | Drift at 5m             |                         | Drift at 5 m            |                         | Run-off<br>0.02 ha<br>Pond |
| Water Depth            | 1 m                    | 1 m                     | 0-30 cm                | 1 m                     | 0-30 cm                 | 1 m                     | 0-30 cm                 | 1 m                        |
| <i>Cyprinus carpio</i> | x.xx x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup>    |

### IIIA 10.2.1.2    **TER<sub>LT</sub> for fish**

On the basis of the worst case toxicity values (Table IIIA 10.2.1-1) and the relevant worst case PEC<sub>SW</sub> values (Table IIIA 10.2.1-2), toxicity exposure ratios for acute exposure of fish were calculated (Table IIIA 10.2.1-4). The TER<sub>LT</sub> values estimated indicate that the potential risks arising for fish are extremely low. As the chronic TER >10 for fish, use of chemx is not considered to involve risks for fish.

**Table IIIA 10.2.1-4      Long-term toxicity exposure ratios (TER<sub>LT</sub>) for fish, exposed to chemx**

| Exposure Route             | Initial Concentration |  | TWA Concentration                               |                         |
|----------------------------|-----------------------|--|---|-------------------------|
|                            | Over-spray            |  | Drift at 5 m                                    | Run-off<br>0.02 ha Pond |
| Water Depth                | 1 m                   |  | 1 m      0-30 cm                                | 1 m                     |
| <i>Oncorhynchus mykiss</i> | x.x x 10 <sup>4</sup> |  | xxx.x x 10 <sup>4</sup> xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> |

### IIIA 10.2.1.3    **TER<sub>A</sub> for *Daphnia***

On the basis of the worst case toxicity values (Table IIIA 10.2.1-1) and the relevant worst case PEC<sub>SW</sub> values (Table IIIA 10.2.1-2), toxicity exposure ratios for acute exposure of aquatic invertebrates (*Daphnia magna*) were calculated (Table IIIA 10.2.1-5). The TER<sub>A</sub> values estimated indicate that the potential risks arising for *Daphnia magna* are extremely low. As the acute TER is > 100, use of chemx is not considered to involve risks for *Daphnia magna*.

**Table IIIA 10.2.1-5      Acute toxicity exposure ratios (TER<sub>A</sub>) for *Daphnia magna*, exposed to chemx**

| Exposure Route       | Initial Concentration |                         |                        |                         | TWA Concentration       |                         |                            |                         |
|----------------------|-----------------------|-------------------------|------------------------|-------------------------|-------------------------|-------------------------|----------------------------|-------------------------|
|                      | Over-spray            | Drift at 1 m            |                        | Drift at 5m             |                         | Drift at 5 m            | Run-off<br>0.02 ha<br>Pond |                         |
| Water Depth          | 1 m                   | 1 m                     | 0-30 cm                | 1 m                     | 0-30 cm                 | 1 m                     | 0-30 cm                    | 1 m                     |
| <i>Daphnia magna</i> | x.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup>    | xxx.x x 10 <sup>4</sup> |

### IIIA 10.2.1.4    **TER<sub>LT</sub> for *Daphnia***

On the basis of the worst case toxicity values (Table IIIA 10.2.1-1) and the relevant worst case PEC<sub>SW</sub> values (Table IIIA 10.2.1-2), toxicity exposure ratios for long-term exposure of aquatic invertebrates (*Daphnia magna*) were calculated (Table IIIA 10.2.1-6). The TER<sub>LT</sub> values estimated indicate that the potential risks arising for *Daphnia* are extremely low.

**Table IIIA 10.2.1-6      Long-term toxicity exposure ratios (TER<sub>LT</sub>) for *Daphnia magna*, exposed to chemx**

| Exposure Route       | Initial Concentration | TWA Concentration       |                         |                         |
|----------------------|-----------------------|-------------------------|-------------------------|-------------------------|
|                      | Over-spray            | Drift at 5 m            |                         | Run-off<br>0.02 ha Pond |
| Water Depth          | 1 m                   | 1 m                     | 0-30 cm                 | 1 m                     |
| <i>Daphnia magna</i> | x.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> |

**IIIA 10.2.1.5    TER<sub>A</sub> for an aquatic insect species**

The acute toxicity of chemx to aquatic insect species, was not determined, since .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

**IIIA 10.2.1.6    TER<sub>LT</sub> for an aquatic insect species**

The chronic toxicity of chemx to aquatic insect species, was not determined, since .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

**IIIA 10.2.1.7    TER<sub>A</sub> for an aquatic crustacean species**

The acute toxicity of chemx to aquatic crustacean species, was not determined, since .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

**IIIA 10.2.1.8    TER<sub>LT</sub> for an aquatic crustacean species**

The chronic toxicity of chemx to aquatic crustacean species, was not determined, since .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

**IIIA 10.2.1.9    TER<sub>A</sub> for an aquatic gastropod species**

The acute toxicity of chemx to aquatic gastropod species, was not determined, since .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

### IIIA 10.2.1.10 TER<sub>LT</sub> for an aquatic gastropod species

The chronic toxicity of chemx to aquatic gastropod species, was not determined, since .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

### IIIA 10.2.1.11 TER<sub>LT</sub> for algae

On the basis of the worst case toxicity values (Table IIIA 10.2.1-1) and the relevant worst case PEC<sub>SW</sub> values (Table IIIA 10.2.1-2), toxicity exposure ratios for exposure of algae were calculated (Table IIIA 10.2.1-7).

**Table IIIA 10.2.1-7      Toxicity exposure ratios for algae, exposed to chemx**

| Exposure Route                   | Initial Concentration  |                         |                        |                         | TWA Concentration       |                         |                         |                            |
|----------------------------------|------------------------|-------------------------|------------------------|-------------------------|-------------------------|-------------------------|-------------------------|----------------------------|
|                                  | Over-spray             | Drift at 1 m            |                        | Drift at 5m             |                         | Drift at 5 m            |                         | Run-off<br>0.02 ha<br>Pond |
| Water Depth                      | 1 m                    | 1 m                     | 0-30 cm                | 1 m                     | 0-30 cm                 | 1 m                     | 0-30 cm                 | 1 m                        |
| <i>Selenastrum capricornutum</i> | Xxx.x                  | xxxx.x                  | xxx.x                  | x.xx x 10 <sup>4</sup>  | xxxx                    | x.xx x 10 <sup>4</sup>  | xxxx                    | xxxx                       |
| <i>Scenedesmus subspicatus</i>   | xxxx                   | x.x x 10 <sup>4</sup>   | x.x x 10 <sup>4</sup>  | xx.x x 10 <sup>4</sup>  | x.x x 10 <sup>4</sup>   | xx.x x 10 <sup>4</sup>  | x.x 10 <sup>4</sup>     | x.x x 10 <sup>4</sup>      |
| <i>Anabaena flos-aquae</i>       | xxx                    | xxxx                    | xxxx                   | x.x x 10 <sup>4</sup>   | x.x x 10 <sup>4</sup>   | x.x x 10 <sup>4</sup>   | x.x x 10 <sup>4</sup>   | x.x x 10 <sup>4</sup>      |
| <i>Navicula pelliculosa</i>      | x.xx x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup> | xxx.x x 10 <sup>4</sup>    |

The TER values estimated indicate that the potential risks arising for algae are extremely low. As the algal growth inhibition TER > 10, use of chemx is not considered to involve risks for algae species.

### IIIA 10.2.1.12 TER for aquatic plants

On the basis of the worst case toxicity values (Table IIIA 10.2.1-1) and the relevant worst case PEC<sub>SW</sub> values (Table IIIA 10.2.1-2), toxicity exposure ratios for exposure of *Lemna gibba* were calculated (Table IIIA 10.2.1-8). The TER values estimated indicate that practical risks arise for *Lemna gibba*. The worst case TER<sub>A</sub> for over-spray was x.x, that for spray drift at 1 meter distance was x.x, while that for spray drift at 5 meter distance (0-30 cm water depth) was xx, based on initial water concentrations, and was xx, based on time weighted average concentrations. As chemx is not recommended for direct application to water bodies, the over-spray assessment is of value only for reference purposes. Spray drift is a more realistic route of exposure and could occur following use of OEC 2222 under practical field conditions.

**Table IIIA 10.2.1-8      Toxicity exposure ratios for *Lemna gibba*, exposed to chemx**

| Exposure Route     | Initial Concentration |              |         |             | TWA Concentration |              |         |                            |
|--------------------|-----------------------|--------------|---------|-------------|-------------------|--------------|---------|----------------------------|
|                    | Over-spray            | Drift at 1 m |         | Drift at 5m |                   | Drift at 5 m |         | Run-off<br>0.02 ha<br>Pond |
| Water Depth        | 1 m                   | 1 m          | 0-30 cm | 1 m         | 0-30 cm           | 1 m          | 0-30 cm | 1 m                        |
| <i>Lemna gibba</i> | x.x                   | xx.x         | x.x     | xx.x        | xx                | xx.x         | xx.x    | xx.x                       |

### IIIA 10.2.2      Acute toxicity (aquatic) of the preparation

The acute TER for chemx is > 100 for fish and *Daphnia* and the algal growth inhibition TER is > 10. Since chemx is a herbicide, it is not surprising that the TER value for *Lemna gibba* is < 100. Those TER values relate to established risks using worst case assumptions.

OEC 2222 is an 80 % WG formulation containing only 20 % w/w of formulants other than as - full details are provided in Document J. Given the composition of the formulation, the results of testing with chemx can be used to predict the toxicity of the formulation.

Accordingly, testing of the formulated product is not .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

### IIIA 10.2.3      Microcosm and mesocosm study

Since the TER values for acute exposure of aquatic organisms are > 100 and the TER values for long-term exposure are > 10, other than for the higher plant *Lemna gibba*, it is suggested that an aquatic microcosm or mesocosm study is .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

### IIIA 10.2.4      Residues data in fish (long term)

The octanol / water partition coefficient Log Pow is < 1, for chemx, which is well below the trigger value at which a fish bio-concentration study is required in accordance with the data requirements specified by .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

In addition, the predicted environmental concentrations in surface water following use as recommended are extremely low. Accordingly it can be concluded that the potential for .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)



**Table IIIA 10.3.1-1      Toxicity of chemx to terrestrial vertebrates**

| Species | Exposure                      | Toxicity Value<br>(mg as/kg bw) | Reference      |
|---------|-------------------------------|---------------------------------|----------------|
| Rat     | Acute - Oral LD <sub>50</sub> | > 5000                          | Jones KL 1993a |

The residue level of chemx likely to arise in various food sources can be estimated in accordance with the method proposed by Hoerger and Kenaga (1972). The exposure routes considered significant are ingestion of short grass, leaves, small insects, large insects and grains. For the purposes of these calculations, mammals are assumed to feed exclusively on contaminated food sources, under worst case exposure conditions.

**Table IIIA 10.3.1-2      Estimated food residues, maximum daily intake and toxicity/exposure ratios for mammals following maximum field use of chemx (0.02 kg as / ha)**

| Mammal Type  | Body weight (kg) Food Intake <sup>1</sup> | Food Consumed | Estimated Food Residue <sup>2</sup><br>(mg as/kg/food) | Estimated Theoretical Exposure <sup>3</sup><br>mg as/kg bw/day) | TER <sub>A</sub> <sup>4</sup> |
|--------------|---|---------------|--|---|-------------------------------|
| Small Mammal | 0.01<br>30 %                              | Short grass   | 2.240  | 0.67  | 7463                          |
|              |   | Leaves        | 0.626  | 0.19  | 26316                         |
|              |   | Seeds/grains  | 0.054  | 0.02  | 250000                        |
|              |   | Small insects | 0.590  | 0.18  | 27778                         |
|              |   | Large insects | 0.060  | 0.02  | 250000                        |
| Large Mammal | 0.1<br>10 %                               | Short grass   | 2.240  | 0.23  | 21739                         |
|              |   | Leaves        | 0.626  | 0.06  | 83333                         |
|              |   | Seeds/grains  | 0.054  | 0.01  | 500000                        |
|              |   | Small insects | 0.590  | 0.06  | 83333                         |
|              |   | Large insects | 0.060  | 0.01  | 500000                        |

<sup>1</sup> Food uptake in % of body weight

<sup>2</sup> kg as/ha x Hoerger and Kenaga Factors (1972)

<sup>3</sup> ETE = Estimated food residue x daily intake/body weight

<sup>4</sup> TER<sub>A</sub> = LD<sub>50</sub>/ETE (mg as/kg bw/day)

As the TER<sub>A</sub> values are greater than 100 for all possible exposure routes, chemx involves a low risk potential for non-target mammal species following acute exposure.

### IIIA 10.3.1.2    Short-term toxicity exposure ratio

On the basis of the limited mammalian toxicity of chemx, it was considered that .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

### IIIA 10.3.1.3 Long-term toxicity exposure ratio

The main route of exposure for terrestrial vertebrates (*e.g.* mammals) to chemx will be through consumption of vegetation containing residues. Toxicity to terrestrial vertebrate species can be estimated from the mammalian toxicity studies conducted with chemx. For the purposes of risk assessment, the rat and mouse can be used as surrogate species.

**Table IIIA 10.3.1-3 Toxicity of chemx to terrestrial vertebrates**

| Species | Exposure                | Toxicity Value<br>(mg as/kg bw) | Reference                   |
|---------|-------------------------|---------------------------------|-----------------------------|
| Rat     | Sub Acute - 90 day NOEL | Xxx                             | White MW and KL Jones 1995a |
| Mouse   | Sub Acute - 90 day NOEL | Xxx                             | White MW and KL Jones 1995b |

The residue level of chemx likely to arise in various food sources can be estimated in accordance with the method proposed by Hoerger and Kenaga (1972). The exposure routes considered significant are ingestion of short grass, leaves, small insects, large insects and grains. For the purposes of these calculations, mammals are assumed to feed exclusively on contaminated food sources, under worst case exposure conditions.

**Table IIIA 10.3.1-4 Estimated food residues, maximum daily intake and toxicity/exposure ratios for mammals following maximum field use of chemx (0.02 kg as / ha)**

| Mammal Type  | Body weight (kg) Food Intake <sup>1</sup> | Food Consumed | Estimated Food Residue <sup>2</sup><br>(mg as/kg/food) | Estimated Theoretical Exposure <sup>3</sup><br>(mg as/kg bw/day) | TER <sub>LT</sub> <sup>4</sup> |
|--------------|---|---------------|--|--|--------------------------------|
| Small Mammal | 0.01<br>30%                               | Short grass   | 2.240  | 0.67   | xx                             |
|              |   | Leaves        | 0.626  | 0.19   | xxx                            |
|              |   | Seeds/grains  | 0.054  | 0.02   | xxxx                           |
|              |   | Small insects | 0.590  | 0.18   | xxx                            |
|              |   | Large insects | 0.060  | 0.02   | xxxx                           |
| Large Mammal | 0.1<br>10%                                | Short grass   | 2.240  | 0.23   | xxx                            |
|              |   | Leaves        | 0.626  | 0.06   | xxx                            |
|              |   | Seeds/grains  | 0.054  | 0.01   | xxxx                           |
|              |   | Small insects | 0.590  | 0.06   | xxx                            |
|              |   | Large insects | 0.060  | 0.01   | xxxx                           |

<sup>1</sup> Food uptake in % of body weight

<sup>2</sup> kg as/ha x Hoerger and Kenaga Factors (1972)

<sup>3</sup> ETE = Estimated. food residue x daily intake/body weight

<sup>4</sup> TER<sub>LT</sub> = NOEL/ETE (mg as/kg food)

As the TER<sub>LT</sub> values are greater than 5 for all possible exposure routes, continued exposure to chemx involves a low risk potential for non-target mammalian species.

**IIIA 10.3.2    Effects on terrestrial vertebrates other than birds, where the required information is not provided by testing in accordance with points IIA 5 and IIIA 7, and where exposure is likely**

On the basis of the magnitude of the TER values calculated, it is concluded that no further testing of the chemx formulation is required. Chemx, when used as recommended, will pose minimal risk to non-target vertebrate species, and risks associated with secondary poisoning will be negligible.

**IIIA 10.4.1    Hazard Quotients for bees**

**IIIA 10.4.1.1    Oral exposure  $Q_{HO}$**

Chemx is a herbicide for control of grasses and weed species in winter wheat crop as a single application of 20 g as/ha crop growth stage 13 to 39. The hazard quotient for oral exposure of honeybees based on the maximum recommended field rate of use is presented in Table IIIA 10.4.1-1. The LD<sub>50</sub> figure used is that generated for the active substance in accordance with point IIA 8.7.1. The hazard quotient (application rate in g as/ha ÷ LD<sub>50</sub> in µg as/bee) is substantially below 50, indicating that at the maximum recommended field rate, an unacceptable risk to honey bees does not arise.

**Table IIIA 10.4.1-1      Oral toxicity of chemx to honey bees (*Apis mellifera*)**

| Exposure Route | Application Rate (g as/ha) | LD <sub>50</sub> (µg as/bee) | Hazard Quotient $Q_{HO}$ |
|----------------|----------------------------|------------------------------|--------------------------|
| Oral           | 20                         | > xx                         | < x.xx                   |

**IIIA 10.4.1.2    Contact exposure  $Q_{HC}$**

The hazard quotient for contact exposure of honeybees based on maximum recommended field rate of use is presented in Table IIIA 10.4.1-2. The LD<sub>50</sub> figure used is that generated for the active substance in accordance with point IIA 8.7.2. The hazard quotient (application rate in g as/ha ÷ LD<sub>50</sub> in µg as/bee) is substantially below 50, indicating that at maximum recommended field rate, an unacceptable risk to honey bees does not arise.

**Table IIIA 10.4.1-2      Contact toxicity of chemx to honey bees (*Apis mellifera*)**

| Exposure Route | Application Rate (g as/ha) | LD <sub>50</sub> (µg as/bee) | Hazard Quotient $Q_{HC}$ |
|----------------|----------------------------|------------------------------|--------------------------|
| Contact        | 20                         | xx                           | < x.xx                   |

### IIIA 10.4.2      **Acute toxicity of the preparation to bees**

#### IIIA 10.4.2.1      **Acute oral toxicity**

Testing of the formulation is not warranted since the data presented provides an adequate basis for predicting the significance of risks arising for honeybees. On the basis of the hazard quotient calculated the risks arising for honeybees under field conditions are minimal.

#### IIIA 10.4.2.2      **Acute contact toxicity**

Testing of the formulation is not warranted since the data presented provides an adequate basis for predicting the significance of risks arising for honeybees. On the basis of the hazard quotient calculated the risks arising for honeybees under field conditions are minimal.

### IIIA 10.4.3      **Effects on bees of residues on crops**

Since the  $Q_{HC} \ll 50$ , .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

### IIIA 10.4.4      **Cage tests**

In the light of the low hazard quotients calculated, .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

### IIIA 10.4.5      **Field Tests**

In the light of the low hazard quotients calculated, .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

### IIIA 10.4.6      **Investigation of special effects**

In the light of the low hazard quotients calculated, .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

## IIIA 10.5      **Effects on arthropods other than bees**

### IIIA 10.5.1      **Effects on sensitive species already tested, using artificial substrates**

Testing for effects on arthropod species other than bees was carried out using the formulated product OEC 2222 rather than the active substance. Laboratory studies were conducted to assess effects on the carabid beetle,

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*Bembidion tetracolum*, the Lycosid spider, the Phytoseiid mite, *Typhlodromus pyri*, and the parasitic wasp, *Aphidius rhopalosiphi*.

**Report** (first of four):      IIIA 10.5.1/01 Burke H 1994, An evaluation of the side-effects of the herbicide OEC 2222 on adults of the carabid beetle, *Bembidion tetracolum*, Report Number CCC-15234

### Guidelines

BBA Guideline VI, 23-2.1.8 which is equivalent to the that contained in the SETAC Guidance document on regulatory testing procedures for pesticides with non-target arthropods

### GLP

Fully GLP compliant - laboratory certified by the UK Department of Health and Social Security.

### Executive Summary

In an acute toxicity laboratory study, two to three week old, laboratory-bred adult *Bembidion tetracolum* (*Coleoptera* : *Carabidae*) were exposed to OEC 222, when placed in groups of six in arenas containing moist sand.

Five replicate arenas were treated with OEC 2222 at a rate equivalent to the maximum recommended rate of 37.5 g product/ha and five with water as a control. An additional two arenas were treated with a toxic reference product (*Afugan* 1L product/ha).

After treatment, the condition of the beetles was monitored for two weeks and their feeding activity was assessed by the provision of fruit fly pupae at regular intervals.

All of the beetles treated with the toxic standard died within 1 day. No beetles died in either the OEC 2222 treatment or the control throughout the study. There was no indication of a reduction in the feeding activity of beetles in the OEC 2222 treatment, when compared with beetles in the control.

These results indicated that OEC 2222 exposure was not harmful to adult *Bembidion tetracolum* when the beetles were treated topically with the product at its maximum recommended application rate.

## I. MATERIALS AND METHODS

### A. MATERIALS:

1. **Test Material:** OEC 2222  
**Description:** beige coloured water dispersible granule (80 % chemx)  
**Lot/Batch #:** NPD-9402-5737-F  
**Purity:** 80.7 % as <sup>23</sup>  
**Stability of test compound:** shown to be stable in an accelerated storage stability test (14 days at 54 °C)
2. **Vehicle and/or positive control:** tap water; *Afugan* in tap water

**3. Test animals -**

|                                   |  |
|-----------------------------------|--|
| <b>Species:</b>                   | <i>Bembidion tetracolum</i> (Coleoptera : Carabidae)   |
| <b>Age:</b>                       | Less than 3 weeks                                      |
| <b>Source:</b>                    | Bio-Test Labor GmbH, Sagerheide, Germany               |
| <b>Acclimation period:</b>        | 3 days   |
| <b>Environmental conditions -</b> |  |
| <b>Temperature:</b>               | 20 °C  |
| <b>Photoperiod:</b>               | 16-hour photoperiod of low intensity light (< 300 lux) |

**B. STUDY DESIGN AND METHODS:**

**1. In life dates:**      5 to 22 May 1994

**2. Experimental treatments**

Adult beetles (*Bembidion tetracolum*), 2 to 3 weeks old at treatment, were acclimatised to the test conditions without food for 3 days prior to treatment, in groups of 6 (3 males and 3 females), at circa 20 °C with 16 hour photoperiod of low intensity light (< 300 lux). The test arenas consisted of pots (diameter 9 cm, height 4 cm) containing sand at 70 % of water holding capacity. Just prior to application, a group of beetles was transferred to a test arena and provided with 2 *Drosophila melanogaster* pupae per beetle. OEC 2222 was sprayed onto the sand, beetles, and food in the test arena at an application rate equivalent to 37.5 g/ha. *Afugan*, a reference standard, was applied to separate test arenas at a rate of 1 L/ha. Five replicate chambers were used for the control treatments and a further five were used for the OEC 2222 treatments, while 2 replicate chambers were used for *Afugan* treatment. Immediately following treatment ventilated lids were placed on the test arenas. The treated pots were maintained at 18 - 22 °C with a 16-hr photoperiod (< 300 lux) for 14 days. Beetles were fed and the moisture content of the sand was adjusted at day 2, 4, 7 and 10.

**3. Observations**

Beetles were observed at 2 and 4 hours and 1, 2, 4, 7, 10 and 14-day intervals after treatment.

**II. RESULTS AND DISCUSSION**

**A. FINDINGS**

All of the beetles treated with the toxic standard died within 1 day. No beetles died in either the OEC 2222 treatment or the control throughout the study. There was no indication of a reduction in the feeding activity of beetles in the OEC 2222 treatment, when compared with beetles in the control.

**III. CONCLUSIONS**

OEC 2222 was found not to be toxic to carabid beetles at an application rate higher than the maximum field application rate.

**Table IIIA 10.5.1-1 Direct effect of OEC 2222 on the carabid beetle (*Bembidion tetracolum*)**

| Time after Treatment | Control* (n=30) |   |   |   | OEC 2222* (n=30) |   |   |   | <i>Afugan</i> * (n=12) |   |    |    |
|----------------------|-----------------|---|---|---|------------------|---|---|---|------------------------|---|----|----|
|                      | L               | A | M | D | L                | A | M | D | L                      | A | M  | D  |
| 2 h                  | 28              | 1 | 1 | 0 | 29               | 0 | 1 | 0 | 0                      | 0 | 12 | 0  |
| 4 h                  | 28              | 1 | 1 | 0 | 30               | 0 | 0 | 0 | 0                      | 0 | 8  | 4  |
| 1 d                  | 30              | 0 | 0 | 0 | 30               | 0 | 0 | 0 | 0                      | 0 | 0  | 12 |
| 2 d                  | 30              | 0 | 0 | 0 | 30               | 0 | 0 | 0 |                        |   |    |    |
| 4 d                  | 30              | 0 | 0 | 0 | 30               | 0 | 0 | 0 |                        |   |    |    |
| 7 d                  | 30              | 0 | 0 | 0 | 30               | 0 | 0 | 0 |                        |   |    |    |
| 10 d                 | 30              | 0 | 0 | 0 | 30               | 0 | 0 | 0 |                        |   |    |    |
| 14 d                 | 30              | 0 | 0 | 0 | 30               | 0 | 0 | 0 |                        |   |    |    |

\* Beetles categorised as alive (L), affected (A), moribund (M), or dead (D)

**Table IIIA 10.5.1-2 Effect of OEC 2222 on the food consumption of carabid beetles**

| Days after Treatment | Mean Number of <i>Drosophila</i> pupae eaten / surviving beetle |          |               |
|----------------------|---|----------|---------------|
|                      | Control   | OEC 2222 | <i>Afugan</i> |
| 0-2                  | 1.97  | 2.00     | 0             |
| 2-4                  | 1.73  | 1.67     |               |
| 4-7                  | 1.53  | 1.43     |               |
| 7-10                 | 1.30  | 1.67     |               |
| 10-14                | 1.07  | 1.30     |               |

(Burke H 1994)

**Report** (second of four): IIIA 10.5.1/02 Rohan T 1994, A laboratory evaluation of the side-effects of the herbicide OEC 2222 WG on Lycosid spiders, Agrochemical Evaluation Unit, The University of Southampton, unpublished report No. US-94-098.

**Guideline**

In-house protocol developed for this species generally following BBA VI, 23-2.1.9 (Draft Guideline). Due to difficulties in collecting large numbers of male spiders, it was not possible to have a 1 : 1 sex ratio in the treatment - an equal sex ratio was possible in some treatments while just female spiders were used in others. The temperature and humidity conditions in the test room during the study were of a wider range than specified in the protocol, being 16.4 - 26.2 °C (mean of 20.4 °C) instead of 19 - 23 °C and 36 - 72 % relative humidity (mean of 54 %) instead of 50 - 80 %.

These deviations did not affect the scientific integrity of the study.

## GLP

Fully GLP compliant - laboratory certified by the UK Department of Health and Social Security.

## Executive Summary

In an acute toxicity study, the effects of exposure to OEC 2222 on field collected adult Lycosid spiders (Araneae : Lycosidae) were assessed in a laboratory study. Individual spiders were placed in arenas containing moist sand, either before (topical exposure) or after (residual exposure) they had been treated using a laboratory sprayer. The test material was applied at a rate equivalent to 37.5 g/ha. *Decis* (100ml/ha) served as a positive control treatment. Twenty spiders were treated either topically or residually with OEC 2222, or with *Decis* and twenty were treated topically with water as a control.

After treatment, the condition of the spiders was monitored for two weeks and their feeding activity was assessed following provision of aphids at regular intervals.

Over 14 days, two spiders (10 %) treated topically with OEC 2222 died, but the remainder were unaffected. In the case of spiders exposed to dry residues of OEC 2222, no mortality and no side effects were observed. One spider died in the control treatment during the study.

Some 18 (90 %) of the spiders exposed to the reference product *Decis*, were killed following treatment topically. Following exposure to dry residues, 80 % of the spiders were knocked down within two hours but these effects were short-term and only 3 (15 %) of the spiders died.

There was no indication of a reduction in the feeding activity of spiders exposed to OEC 2222, when compared with those in the control treatment. The feeding activity of spiders exposed to *Decis* residues was greatly reduced for several days after treatment. It was concluded that OEC 2222 was not harmful to adult Lycosid spiders (*Pardosa species*.) under the conditions of testing.

## I. MATERIALS AND METHODS

### A. MATERIALS:

1. **Test Material:** OEC 2222  
**Description:** beige coloured water dispersible granule (80 % chemx)  
**Lot/Batch #:** NPD-9402-5737-F  
**Purity:** 80.7 % as <sup>23</sup>  
**Stability of test compound:** shown to be stable in an accelerated storage stability test (14 days at 54 °C)
2. **Vehicle and/or positive control:** tap water; *Decis* in tap water
3. **Test animals -**  
**Species:** *Pardosa species* (*P pullata*, *P proxima*, *P hortensis*, *P palustris*, and *P prativage*)  
**Age:** adult

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**Source:** collected from a garden in Southampton  
**Acclimation period:** 3 days  
**Environmental conditions -**  
**Temperature:** 16.4 to 26.2 °C  
**Humidity:** 36 to 72 % relative humidity  
**Photoperiod:** 16-hour photoperiod of low intensity light (417 - 500 lux)

## B. STUDY DESIGN AND METHODS:

1. **In life dates:** 6 to 23 June 1994

### 2. Experimental treatments

Adult Lycosid spiders collected from a garden, were placed singly in test arenas and stored on the lab bench at 16 - 21 °C with a natural photoperiod for 1 - 2 days. The spiders were then deprived of food and acclimatised to the test conditions for 3 days. The test arenas consisted of plastic pots (diameter 9 cm, height 5 cm) containing sand at 70 % of water holding capacity. The sides of the pots were treated with *Fluon* (obtained from ICI) to prevent spiders from climbing out. Five treatments were administered; control (spiders sprayed with tap water), topical / residual exposure with OEC 2222 at 37.5 g/ha, residual exposure with OEC 2222 at 37.5 g/ha, topical / residual exposure of *Decis* (reference standard) at 100 ml/ha, residual exposure of *Decis* (reference standard) at 100 ml/ha. Spiders were present at treatment for the topical / residual treatment and were removed from the test chambers (returned 1 hr after treatment) for the residual treatment. Ten male and ten females spiders were treated in control and topical / residual OEC 2222 treatments. All other treatments were limited to 20 female spiders due to a shortage in the number of males available. Spiders were fed 3 live pea aphids 1, 3, 7, and 10 days after treatment.

### 3. Observations

Spiders were observed at 2 hour and 1, 2, 3, 7, 10 and 14-day intervals after treatment. A record was kept of whether or not the aphids fed to spiders were consumed within 30 minutes of introduction.

## II. RESULTS AND DISCUSSION

### A. FINDINGS

Over 14 days, two spiders (10 %) treated topically with OEC 2222 died, but the remainder were unaffected (Table IIIA 10.5.1-3). In the case of spiders exposed to dry residues of OEC 2222, no mortality and no side effects were observed. One spider died in the control treatment during the study.

Some 18 (90 %) of the spiders exposed to the reference product *Decis*, were killed following treatment topically. Following exposure to dry residues, 80 % of the spiders were knocked down within two hours but these effects were short-term and only 3 (15 %) of the spiders died.

There was no indication of a reduction in the feeding activity of spiders exposed to OEC 2222, when compared with those in the control treatment (Table IIIA 10.5.1-4). The feeding activity of spiders exposed to *Decis* residues was greatly reduced for several days after treatment.

**Table IIIA 10.5.1-3      Toxicity of OEC 2222 to Lycosid spiders**

| Time after Treatment | Control* (n=20) |   |   |   | OEC 2222* (n=20)<br>Topical / Residual |   |   |   | <i>Decis</i> * (n=20)<br>Topical / Residual |    |   |    |
|----------------------|-----------------|---|---|---|--|---|---|---|---|----|---|----|
|                      | L               | A | M | D | L                                      | A | M | D | L   | A  | M | D  |
| 2 h                  | 20              | 0 | 0 | 0 | 20                                     | 0 | 0 | 0 | 0   | 1  | 8 | 11 |
| 1 d                  | 20              | 0 | 0 | 0 | 20                                     | 0 | 0 | 0 | 1   | 1  | 0 | 18 |
| 2 d                  | 20              | 0 | 0 | 0 | 20                                     | 0 | 0 | 0 | 2   | 0  | 0 | 18 |
| 3 d                  | 20              | 0 | 0 | 0 | 20                                     | 0 | 0 | 0 | 2   | 0  | 0 | 18 |
| 7 d                  | 20              | 0 | 0 | 0 | 19                                     | 0 | 0 | 1 | 2   | 0  | 0 | 18 |
| 10 d                 | 20              | 0 | 0 | 0 | 18                                     | 0 | 0 | 2 | 2   | 0  | 0 | 18 |
| 14 d                 | 19              | 0 | 0 | 1 | 18                                     | 0 | 0 | 2 | 2   | 0  | 0 | 18 |
|                      |                 |   |   |   | OEC 2222* (n=20)<br>Residual           |   |   |   | <i>Decis</i> * (n=20)<br>Residual           |    |   |    |
| 2 h                  |                 |   |   |   | 20                                     | 0 | 0 | 0 | 4   | 13 | 3 | 0  |
| 1 d                  |                 |   |   |   | 20                                     | 0 | 0 | 0 | 16  | 1  | 0 | 3  |
| 2 d                  |                 |   |   |   | 20                                     | 0 | 0 | 0 | 17  | 0  | 0 | 3  |
| 3 d                  |                 |   |   |   | 20                                     | 0 | 0 | 0 | 17  | 0  | 0 | 3  |
| 7 d                  |                 |   |   |   | 20                                     | 0 | 0 | 0 | 17  | 0  | 0 | 3  |
| 10 d                 |                 |   |   |   | 20                                     | 0 | 0 | 0 | 17  | 0  | 0 | 3  |
| 14 d                 |                 |   |   |   | 20                                     | 0 | 0 | 0 | 17  | 0  | 0 | 3  |

\* Spiders categorised as alive (L), affected (A), moribund (M), or dead (D)

**Table IIIA 10.5.1-4      Effect of OEC 2222 on food consumption of Lycosid spiders**

| Food Provided | Mean Number of Aphids fed upon over a 24 hour period*       |                              |                      |                                  |                          |
|---------------|---|------------------------------|----------------------|----------------------------------|--------------------------|
|               | Control   | OEC 2222<br>Topical/Residual | OEC 2222<br>Residual | <i>Decis</i><br>Topical/Residual | <i>Decis</i><br>Residual |
| 1             | 2.05  | 1.70                         | 1.95                 | 0.00 (2)                         | 0.12 (17)                |
| 3             | 1.65  | 1.85                         | 1.60                 | 0.50 (2)                         | 1.24 (17)                |
| 7             | 2.25  | 2.58 (19)                    | 2.35                 | 2.00 (2)                         | 2.00 (17)                |
| 10            | 2.35  | 2.78 (18)                    | 2.55                 | 3.00 (2)                         | 2.29 (17)                |
| Mean          | 2.08  | 2.23                         | 2.11                 | 1.38                             | 1.41                     |
| Food Provided | Number of Spiders which fed within 30 min of food provided* |                              |                      |                                  |                          |
| 1             | 16  | 13                           | 14                   | 0 (2)                            | 2 (17)                   |
| 3             | 8   | 4                            | 7                    | 0 (2)                            | 4 (17)                   |
| 7             | 9   | 9 (19)                       | 8                    | 1 (2)                            | 6 (17)                   |
| 10            | 15  | 13 (18)                      | 10                   | 2 (2)                            | 10 (17)                  |

\* Numbers in parenthesis are number of surviving spiders if less than 20

### III. CONCLUSIONS

OEC 2222 can be considered as being harmless to adult Lycosid spiders when applied at an application rate higher than the maximum rate recommended.

(Rohan T 1994)

**Report** (third of four):      IIIA 10.5.1/03    Evans R 1996, A laboratory evaluation of the side-effects of the herbicide OEC 2222 WG on the phytoseiid mite *Typhlodromus pyri*, Report Number CCC-15514.

#### Guideline

IOBC Guideline, which is equivalent to that contained in the SETAC Guidance document on regulatory testing procedures for pesticides with non-target arthropods

#### GLP

Fully GLP compliant - laboratory certified by the UK Department of Health and Social Security.

#### Executive Summary

In an acute toxicity study, predatory mites, species *Typhlodromus pyri* Scheuten (*Acarina* : *Phytoseiidae*) were exposed to OEC 2222. The test product was applied to the central area of glass dishes at a rate equivalent to an application rate of 37.5 g product/ha. Additional dishes were treated with *Dimethoate 40 EC* (reference standard) at 0.17 mL/ha or with tap water (equivalent to 200 L/ha) as a control treatment.

Once dry, protonymph mites were placed on the treated area at the centre of the glass dishes and were confined using a barrier of damp filter paper and a sticky non-toxic gel. The mites were provided with untreated food (broad bean pollen). Five replicates were prepared for each treatment, each containing 20 mites. Assessments of mortality were made 1 and 7 days after treatment.

After 7 days, adult mites were transferred to new arenas that had been sprayed 7 days earlier. They were observed for a further 7 days while their fecundity was being assessed.

In the OEC 2222 treatment 79 % of the mites survived the initial 7 days. In the control treatment the survival rate was 93 %. There was 100 % mortality in the positive control treatment. During the second week of the study, mean egg production was 7.2 eggs per surviving female in the OEC 2222 treatment and 5.5 eggs per female in the control treatment.

It was concluded that OEC 2222 could be considered harmless to the predatory, mite *Typhlodromus pyri*.

### I. MATERIALS AND METHODS

#### A. MATERIALS:

- 1. Test Material:**      OEC 2222  
**Description:**      beige coloured water dispersible granule (80 % chemx)



**Table IIIA 10.5.1-5      Effect of OEC 2222 on mortality of *Typhlodromus pyri***

| Time after Treatment | Control* (n=100) |   |   |    | OEC 2222* (n=100) |   |   |    | Dimethoate* (n=101) |    |   |    |
|----------------------|------------------|---|---|----|-------------------|---|---|----|---------------------|----|---|----|
|                      | L                | D | S | Mi | L                 | D | S | Mi | L                   | D  | S | Mi |
| 1 d                  | 100              | 0 | 0 | 0  | 100               | 0 | 0 | 0  | 6                   | 90 | 0 | 5  |
| 7 d                  | 93               | 3 | 2 | 2  | 79                | 4 | 1 | 16 | 0                   | 95 | 0 | 6  |
| 14 d                 | 83               | 4 | 9 | 4  | 77                | 4 | 3 | 16 | **                  | -  | - | -  |

\* L-alive, D-dead, S-dead in sticky barrier, Mi-missing, presumed dead,

\*\* no living mites to assess

**Table IIIA 10.5.1-6      Effect of OEC 2222 on fecundity of *Typhlodromus pyri***

| Parameter Observed      | Control** | OEC 2222 | Dimethoate |
|-------------------------|-----------|----------|------------|
| No. live adult females* | 26        | 21       | 0          |
| No. eggs                | 142       | 152      | 0          |
| No. eggs per female     | 5.5       | 7.2      | 0.0        |

\* On Day 7

\*\* Only 21 live females remained on Day 14

### III. CONCLUSIONS

OEC 2222 can be considered to be harmless to the phytoseiid mite *Typhlodromus pyri* when applied at an application rate higher than the maximum rate recommended.

(Evans R 1996a)

**Report** (fourth of four):      IIIA 10.5.1/04 Evans R 1996, A laboratory evaluation of the side effects of the herbicide OEC 2222 WG on the parasitic wasp *Aphidius rhopalosiphi*, Report Number CCC-15506.

#### Guideline

In-house protocol for Tier I test with *Aphidius rhopalosiphi* - equivalent to the guideline contained in the SETAC Guidance document on regulatory testing procedures for pesticides with non-target arthropods

#### GLP

Fully GLP compliant - laboratory certified by the UK Department of Health and Social Security.

## Executive Summary

In an acute toxicity study, parasitic wasps of the species *Aphidius rhopalosiphi* (Hymenoptera : Braconidae) were exposed to OEC 2222. The test product was applied to glass plates at a rate equivalent to an application rate of 37.5 g product/ha. Additional dishes were treated with *Dimethoate 40 EC* (reference standard) at 0.2mL/ha or with tap water (equivalent to 200 L/ha) as a control treatment. Once dry, the treated glass plates were used to form the floor and ceiling of arenas into each of which 10 adult wasps were introduced. The wasps were of equal sex ratio and were less than 48 hours old. Three replicate units were prepared for the test and control treatments, and one for the toxic reference standard. The condition of the wasps was assessed over a 48-hour period.

To assess sub-lethal effects of treatment on the fecundity of the test insects, 10 female wasps from the OEC 2222 treatment and 10 from the control were individually confined over pots of aphid-infested barley seedlings for 24 hours before being removed. The numbers of mummies (parasitised aphids) that developed on the barley seedlings was assessed 11 days later.

After 48-h exposure no wasps had died in the OEC 2222 treatment, although two wasps (7 %) were left moribund. None of the wasps were affected in the control treatment, while all those in the toxic reference treatment died. In the fecundity test, the mean numbers of mummies produced was 11.9 in the OEC 2222 treatment and 14.9 in the control treatment. The response was not statistically significant (t-test,  $P > 0.05$ ).

It was concluded that OEC 2222 exposure was not harmful to *Aphidius rhopalosiphi*.

## I. MATERIALS AND METHODS

### A. MATERIALS:

1. **Test Material:**
  - Description:** OEC 2222
  - beige coloured water dispersible granule (80 % chemx)**
  - Lot/Batch #:** NPD-9501-6384-F
  - Purity:** 79.3 % as<sup>23</sup>
  - Stability of test compound:** shown to be stable in an accelerated storage stability test (14 days at 54 °C)
2. **Vehicle and/or positive control:** tap water; *Dimethoate 40 EC* in tap water
3. **Test animals -**
  - Species:** *Aphidius rhopalosiphi*
  - Source:** in-house culture maintained on the rose-grain aphid *Metopolophium dirhodum* (Walk.)
  - Food:** 1 : 2 solution of honey and water
  - Environmental conditions -**
    - Temperature:** 17 to 22 °C
    - Humidity:** 44 to 64 % relative humidity
    - Photoperiod:** 16-hour photoperiod (6500 lux)

### B. STUDY DESIGN AND METHODS:

1. **In life dates:** 22 October to 6 November 1995

## 2. Experimental treatments

Adult wasps (*Aphidium rhopalosiphi*) less than two days old were exposed to fresh product residues in test units comprised of treated glass plates. To concentrate the activity of the wasps on the treated surfaces, the glass plates were covered with black paper with a circular hole in the centre. The glass plates were sprayed with the equivalent of 37.5 g/ha OEC 2222. Further plates were treated with tap water (control), or 0.2 ml/ha *Dimethoate 40 EC* (positive control). Plates were allowed to dry for about an hour, after which the test chambers were assembled. Three replicate chambers were used for the control and OEC 2222 treatments and 1 replicate for *Dimethoate 40 EC* treatment. Adult wasps (5 males and 5 females) were transferred into each test arena. Wasps were fed a 1 : 2 solution of honey and water through an access hole.

In order to determine effects on the fecundity of surviving wasps, 10 female wasps from the control treatment and from the OEC 2222 treatment were individually confined over pots of untreated seedling barley, previously infested with aphids so that there were > 100 nymphs per pot. Pots were enclosed in acrylic cylinders closed at the top with nylon netting. Adult wasps were removed and parasitised aphids were left to develop.

## 3. Observations

Wasps were observed at intervals of approximately 1, 2, 24, and 48 hours after being placed in the chambers to assess toxicity. To assess effects on fecundity, the number of mummies present was counted 11 days after adult removal.

# II. RESULTS AND DISCUSSION

## A. FINDINGS

After 48-h exposure no wasps had died in the OEC 2222 treatment, although two wasps (7 %) were left moribund (Table IIIA 10.5.1-7). None of the wasps were affected in the control treatment, while all those in the toxic reference treatment died. In the fecundity test, the mean numbers of mummies produced was 11.9 in the OEC 2222 treatment and 14.9 in the control treatment (Table IIIA 10.5.1-8). The response was not statistically significant (t-test,  $P > 0.05$ ).

**Table IIIA 10.5.1-7 Toxicity of OEC 2222 to the parasitic wasp (*Aphidius rhopalosiphi*)**

| Time after Treatment | Rep | Control* (n=30) |   |   |   | OEC 2222* (n=30) |   |   |   | Dimethoate* (n=10) |   |   |    |
|----------------------|-----|-----------------|---|---|---|------------------|---|---|---|--------------------|---|---|----|
|                      |     | L               | A | M | D | L                | A | M | D | L                  | A | M | D  |
| 1 h                  | a   | 10              | 0 | 0 | 0 | 10               | 0 | 0 | 0 | 10                 | 0 | 0 | 0  |
|                      | b   | 10              | 0 | 0 | 0 | 10               | 0 | 0 | 0 |                    |   |   |    |
|                      | c   | 10              | 0 | 0 | 0 | 10               | 0 | 0 | 0 |                    |   |   |    |
| 2 h                  | a   | 10              | 0 | 0 | 0 | 10               | 0 | 0 | 0 | 10                 | 0 | 0 | 0  |
|                      | b   | 10              | 0 | 0 | 0 | 10               | 0 | 0 | 0 |                    |   |   |    |
|                      | c   | 10              | 0 | 0 | 0 | 10               | 0 | 0 | 0 |                    |   |   |    |
| 24 h                 | a   | 10              | 0 | 0 | 0 | 10               | 0 | 0 | 0 | 0                  | 2 | 3 | 5  |
|                      | b   | 10              | 0 | 0 | 0 | 10               | 0 | 0 | 0 |                    |   |   |    |
|                      | c   | 10              | 0 | 0 | 0 | 10               | 0 | 0 | 0 |                    |   |   |    |
| 48 h                 | a   | 10              | 0 | 0 | 0 | 9                | 0 | 1 | 0 | 0                  | 0 | 0 | 10 |
|                      | b   | 10              | 0 | 0 | 0 | 10               | 0 | 0 | 0 |                    |   |   |    |
|                      | c   | 10              | 0 | 0 | 0 | 9                | 0 | 1 | 0 |                    |   |   |    |
| Overall              |     | 30              | 0 | 0 | 0 | 28               | 0 | 2 | 0 | 0                  | 0 | 0 | 10 |

\* Wasps categorised as alive (L), affected (A), moribund (M), or dead (D)

**Table IIIA 10.5.1-8 Effects of OEC 2222 on the fecundity of the parasitic wasp (*Aphidius rhopalosiphi*)**

| Replicate      | Number of mummies produced per female wasp |          |
|----------------|--|----------|
|                | Control                                    | OEC 2222 |
| a              | 5  | 13       |
| b              | 21   | 11       |
| c              | 7  | 16       |
| d              | 15   | 5        |
| e              | 16   | 15       |
| f              | 13   | 9        |
| g              | 19   | 15       |
| h              | 8  | 18       |
| I              | 21   | 12       |
| j              | 24   | 5        |
| Mean           | 14.9                                       | 11.9     |
| $\sigma_{n-1}$ | 6.6  | 4.5      |

### III. CONCLUSIONS

OEC 2222 can be considered to be harmless to adult *Aphidius rhopalosiphi* when applied at an application rate higher than the maximum rate recommended.

(Evans R 1996b)

#### IIIA 10.5.2      Effects on non-target terrestrial arthropods in extended laboratory tests

The overall results obtained in the Tier I testing of the effects of OEC 2222 on arthropod species, indicated that its toxicity to the various non-target species tested is very low. On that basis it unlikely that the use of OEC 2222 will result in .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

#### IIIA 10.5.3      Effects on non-target terrestrial arthropods in semi-field tests

On that basis it unlikely that the use of OEC 2222 will result in significant adverse effects on non-target terrestrial arthropods, semi-field testing was not carried out.

#### IIIA 10.5.4      Field tests on terrestrial arthropod species

On that basis it unlikely that the use of OEC 2222 will result in significant adverse effects on non-target terrestrial arthropods, field testing .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

#### IIIA 10.6.1      Toxicity exposure ratios for earthworms TER<sub>A</sub> and TER<sub>LT</sub>

The predicted initial environmental concentrations of chemx in soil, based on the recommended use rate of 20 g as/ha and exposure based on worst case scenarios, are given in Table IIIA 10.6.1-1 (full details are provided at point IIIA 9.4.1). The predicted initial environmental concentration in soil range from 0.0267 to 0.001 mg as/kg.

**Table IIIA 10.6.1-1      Initial PEC<sub>S</sub> for chemx**

| % Spray Reaching soil | Application Rate |         | As reaching soil mg/m <sup>2</sup> | PEC <sub>S</sub> (mg as/kg) |                      |                     |                      |
|-----------------------|------------------|---------|------------------------------------|-----------------------------|----------------------|---------------------|----------------------|
|                       | kg as/ha         | mg as/m |                                    | 0-5 cm <sup>2</sup>         | 0-10 cm <sup>2</sup> | Off Target          |                      |
|                       |                  |         |                                    |                             |                      | 0.5 cm <sup>3</sup> | 0-10 cm <sup>3</sup> |
| <sup>1</sup> 100%     | 0.02             | 2.0     | 2.0                                | 0.0267                      | 0.0133               | 0.0002              | 0.0001               |
| <sup>1</sup> 50%      | 0.02             | 2.0     | 1.0                                | 0.0133                      | 0.0067               |                     |                      |

<sup>1</sup> 100 % of spray reaching soil based on bare soil application and 50 % based on field results (50 % crop interception)

<sup>2</sup> Based on EPPO guidelines (1993) assumed soil density of 1.5

<sup>3</sup> Off target deposition based on 5 m distance from cereal fields, wind speed 2.5 - 3.5 m/s, giving maximum drift of 0.6 % (Ganzelmeier 1993 a & b)



### IIIA 10.6.5      **Residue content of earthworms**

Given the low toxicity of chemx to birds, it is .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

### IIIA 10.6.6      **Effects on other soil non-target macro-organisms**

The results of testing with the active substance at dose rates comparable to or exceeding the maximum recommended field rate indicated that .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

On the basis of the worst case exposure estimates reported, it is clear that the margin of safety in respect of earthworm toxicity is such that there is .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

Similarly, it is probable that risks do not arise for non-target soil macro-organisms that contribute to the breakdown of dead plant and animal organic matter, as a result of field use of chemx as recommended. Accordingly testing is not required.

### IIIA 10.7.1      **Laboratory test to investigate impact on soil microbial activity**

Testing for effects on the metabolic activity of soil microbial biomass was carried out in two soils using the formulated product OEC 2222.

**Report:**      IIIA 10.7.1/01 Mohan H 1995, A study on the influence of OEC 2222 on soil micro-flora, Report Number CCC-94-099

#### **Guidelines**

BBA Guideline VI, Section 1-1 ≡ Procedures for assessing the environmental fate and behaviour of pesticides, SETAC-Europe, 1995, ISBN Number 90-5607-002-9.

#### **Deviations**

The soils used contained 54 and 61 % sand, instead of the > 70 % specified in the guideline<sup>27</sup>. No information is provided concerning soil history – the previous pesticide and fertiliser history of the soils is not known. The test compound was applied to the soil surface, rather than being mixed into the soil as specified. Sampling intervals beyond 28 days were at intervals of 35 and 37 days, rather than 14 days as specified in the guidelines. None of these deviations impact the scientific validity of the study - the application method and use on pesticide and fertiliser treated soils are typical of conditions under which the product will be used.

#### **GLP**

Fully GLP compliant - laboratory certified by the UK Department of Health and Social Security.

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<sup>27</sup> There is a typographical error in the SETAC guideline - it should read < 70 % not > 70 %

## Executive Summary

In a soil microbial activity study, the effects of OEC 2222 on metabolic activity, nitrogen metabolism and microbial biomass were investigated in a sandy loam soil and a loamy sand soil of UK origin. OEC 2222 was applied to samples of each soil type at nominal application rates of 0.038 mg/kg and 0.19 mg/kg of soil (dry weight equivalent), equivalent to 1.15 and 7.6 times the maximum recommended application rate of 20 g as/ha. Dinoseb acetate was applied to samples of each soil type at an application rate of 12.5 mg/kg of soil (dry weight equivalent), equivalent to an application rate of 12.5 kg/ha to serve as a positive control treatment. OEC 2222 treated soils, dinoseb acetate treated soils and control soil treatments fortified with the blank dose vehicle, were incubated under a continuous, CO<sub>2</sub>-free humid air supply at 20 °C in the dark for the duration of the study.

Triplicate samples of each soil type and each treatment were removed for analysis of microbial biomass and mineral nitrogen content after 3 hours, 14, 28, 63 and 100 days. There were no persistent significant effects on soil microbial biomass or on rate of conversion of NH<sub>4</sub>-N to NO<sub>3</sub>-N at either application rate, in either soil type. Dinoseb acetate treatment caused a significant reduction in the biomass of the sandy loam soil, and had a significant effect on nitrogen transformation in both soil types.

## I. MATERIALS AND METHODS

### A. MATERIALS:

1. **Test Material:** OEC 2222  
**Description:** beige coloured water dispersible granule (80 % chemx)  
**Lot/Batch #:** NPD-9402-5737-F  
**Purity:** 80.7 % as <sup>23</sup>  
**Stability of test compound:** shown to be stable in an accelerated storage stability test (14 days at 54 °C)
  
2. **Soil:** Two soils were used in this study, a sandy loam soil (54 % sand, pH 6.3, 1.9 % organic carbon) and a loamy sand soil (61 % sand, pH 5.9, 0.9 % organic carbon).

**Table IIIA 10.7.1-1      Soil physicochemical properties**

| Soil Origin | Soil Type  | pH  | OC<br>% | Sand<br>% | Silt<br>% | Clay<br>% | CEC<br>(meg/100g) | NO <sub>3</sub> -N<br>(mg/kg) |
|-------------|------------|-----|---------|-----------|-----------|-----------|-------------------|-------------------------------|
| UK          | Sandy loam | 6.3 | 1.9     | 54        | 35        | 11        | 14.7              | 13.4                          |
| UK          | Loamy sand | 5.9 | 0.9     | 61        | 27        | 12        | 10.2              | 3.8                           |

### B. STUDY DESIGN

1. **In life dates:** 17 May to 1 December 1994
  
2. **Experimental conditions**

The test material was applied in aqueous solution to the soil surface at application rates of 0.038 mg/kg and 0.19 mg/kg of soil. Assuming incorporation to a depth of 5 cm and a soil bulk density of 1.5, these rates are equivalent to 1.15 and 5.76 times the maximum recommended application rate of 20 g as/ha. Dinoseb was used as a positive control at a rate equivalent to 12.5 kg/ha. Soils (100 g/flask) were adjusted to 40 % of maximum water holding capacity. Control treatments for each soil were fortified with the blank dose vehicles. Ground lucerne (0.5 % w/w) was added to the samples for nitrogen determination (ammonium-nitrogen source) prior to incubation. Soils were incubated at  $20 \pm 1$  °C in the dark under a continuous stream of carbon dioxide-free air.

### 3. Sampling

Samples were taken for biomass and nitrogen consumption determination at 3 hr, 14, 28, 63, and 100 days.

### 4. Description of analytical procedures

Ammonium-nitrogen, nitrate-nitrogen plus nitrite-nitrogen, and nitrite-nitrogen were measured colourimetrically. Nitrate-nitrogen was determined by subtraction. Metabolically active soil biomass was determined using a modified version of the Anderson and Domsch method using [<sup>14</sup>C] glucose. The minimum rate of glucose required to produce the maximum rate of respiration was determined in advance in a preliminary test.

## II. RESULTS AND DISCUSSION

### A. BIOMASS

In sandy loam soil the test compound had no significant effect on soil microbial biomass at either treatment level compared to the control treatment (Table IIIA 10.7.1-2). At the initial sampling point (3 hours), biomass levels were highly variable as predicted by the BBA guideline (1990), but recovered to < 2 % deviation from the control treatment at both application rates by day 28. There were no significant effects at the equivalent of the field rate of application or the equivalent of five times the field application rate after 28 or 100 days.

In the loamy sand soil microbial biomass levels were significantly lower for both application rates than for the control treatment after 14 days (- 17.8 % to - 19.1 %). By day 28 only the five-fold field rate was significantly different from the control soil but the microbial biomass of this soil had recovered by day 63.

The positive control treatment (dinoseb acetate treated) sandy loam soil showed a significant decline in biomass levels from day 14 to day 100, while the similarly treated loamy sand soil showed higher biomass levels on day 28 (16.7 %) and day 100 (10.9 %) as compared to control values but were not significantly different.

### B. NITROGEN TRANSFORMATION

The effect of OEC 2222 on nitrogen transformation in soil was determined at each sampling point as relative amounts of NH<sub>4</sub>-N, NO<sub>2</sub>-N, and NO<sub>3</sub>-N and was expressed as percent deviation from control values both in respect of OEC 2222 treatments and dinoseb acetate treatment (Table IIIA 10.7.1-3). Statistical analysis was only carried out for NO<sub>3</sub>-N as the NH<sub>4</sub>-N levels fell rapidly below the limit of determination.

**Table IIIA 10.7.1-2      Effects of OEC 2222 on biomass**

| Soil Type  | Treatment rate<br>(mg OEC 2222 / kg soil) | Percent deviation from control samples <sup>1</sup> |         |         |         |          |
|------------|---|---|---------|---------|---------|----------|
|            |   | 3 hour  | 14 days | 28 days | 63 days | 100 days |
| Sandy loam | Control <sup>1</sup>                      | 34.11   | 50.78   | 42.77   | 35.65   | 36.28    |
|            | 0.038                                     | - 27.7  | - 7.1   | 0.3     | 8.7     | 11.9     |
|            | 0.190                                     | - 38.9  | - 4.8   | - 1.6   | - 2.4   | - 6.7    |
|            | Positive Control                          | 2.4   | - 22.1* | - 36.1* | 37.9*   | 40.9*    |
| Loamy sand | Control <sup>1</sup>                      | 24.60   | 31.85   | 27.20   | 23.66   | 20.52    |
|            | 0.038                                     | 5.4   | - 17.8* | - 9.5   | - 4.3   | 7.8      |
|            | 0.190                                     | 19.4  | - 19.1  | - 13.9* | 0.1     | - 9.5    |
|            | Positive Control                          | 8.0   | 7.6     | 16.7    | - 2.5   | 10.9     |

<sup>1</sup> mg biomass C/100g soil (dry weight equivalent)

\* Result vary from control at level a by Dunnetts test (1955/1964) (0.05 > P > 0.01)

**Table IIIA 10.7.1-3      Effects of OEC 2222 on nitrogen transformation**

| Soil Type           | Treatment Rate<br>(mg OEC 2222 / kg soil) | Percent deviation from Control Samples <sup>1</sup><br>(Nitrogen determination) |         |         |         |          |
|---------------------|---|---|---------|---------|---------|----------|
|                     |   | 3 Hour  | 14 Days | 28 Days | 63 Days | 100 Days |
| <b>Sandy Loam</b>   |   |   |         |         |         |          |
| NH <sub>4</sub> -N  | Control <sup>1</sup>                      | 8.98  | < 0.5   | < 0.5   | < 0.5   | < 0.5    |
|                     | 0.038                                     | 9.3   | 0.0     | 0.0     | 0.0     | 0.0      |
|                     | 0.190                                     | 14.2  | 0.0     | 0.0     | 0.0     | 0.0      |
|                     | Positive Control                          | 6.4   | 79.1    | 121.3   | 0.0     | #        |
| NO <sub>3</sub> -N  | Control <sup>1</sup>                      | 15.57   | 37.40   | 58.87   | 87.73   | 111.17   |
|                     | 0.038                                     | 14.6  | 12.7*   | 4.3     | - 4.3   | - 2.5    |
|                     | 0.190                                     | - 1.5   | 3.4     | 9.2     | - 2.8   | 4.5      |
|                     | Positive Control                          | 22.9*   | 0.4     | 7.4*    | 44.1*   | 26.5*    |
| <b>Loamy Sand</b>   |   |   |         |         |         |          |
| NH <sub>4</sub> -N  | Control <sup>1</sup>                      | 19.2  | 15.93   | 2.90    | < 0.5   | < 0.5    |
|                     | 0.038                                     | - 8.2   | 4.4     | - 13.8  | 0.0     | 0.0      |
|                     | 0.190                                     | - 6.4   | 41.6    | 128.7   | 0.0     | 0.0      |
|                     | Positive Control                          | - 11.8  | 24.0    | 73.6    | #       | #        |
| NO <sub>3</sub> - N | Control <sup>1</sup>                      | 0.57  | 25.17   | 47.03   | 65.27   | 71.33    |
|                     | 0.038                                     | - 29.4  | - 6.7   | 3.3     | 5.6     | - 1.4    |
|                     | 0.190                                     | 17.6  | - 31.3* | - 7.9*  | 4.8     | 1.3      |
|                     | Positive Control                          | 16.7  | - 41.9* | - 35.9* | 14.6*   | 26.2*    |

<sup>1</sup> mg/kg soil (dry weight equivalent)

# Below limit of detection

\* Result varies from control at level a by Dunnetts test (1955/1964) (0.05 > P>0.01)

In the sandy loam soil the test compound had no pronounced effect on the rate of mineralization of NH<sub>4</sub>-N to NO<sub>3</sub>-N. An initial effect on NO<sub>3</sub>-N (12.7 %) in comparison to the control treatment was observed but after 28 days the difference was < 10 % and after 100 days the difference was < 5 %, for both application at a rate equivalent to the field rate and five times that rate.

In the loamy sand soil, the NO<sub>3</sub>-N level at the recommended field rate was not significantly different from the control treatment at any time during the study. The NO<sub>3</sub>-N level at the five fold field application rate was significantly lower at days 14 and 28 (31.3 % and 7.9 %) in comparison to the control treatment, but had recovered by day 63. NH<sub>4</sub>-N was not effected by OEC 2222 treatment.

The large variation in the NH<sub>4</sub>-N results in comparison to the control treatment values at for instance day 28 (five fold field rate) is ascribed to the low levels of NH<sub>4</sub>-N present rather than to a large difference in the absolute level of NH<sub>4</sub>-N present. In the dinoseb acetate positive control treatments, a significant variation in levels of NO<sub>3</sub>-N was observed in comparison to the control treatment values after 28, 63 and 100 days.

### III. CONCLUSIONS

Chemx applied at the recommended field rate and five times that rate of application had no significant effect on soil biomass or mineral nitrogen level in sandy loam soil. In loamy sand soil treated at the recommended field rate, a similar result was obtained. However, in loamy sand soil treated at five times the recommended rate of application in the field, significant effects on microbial biomass and nitrogen transformation was recorded on days 14 and 28. This treated soil had recovered by day 63.

Dinoseb acetate, a known respiratory inhibitor, which was used as a positive control treatment, caused a significant negative effect on biomass in the sandy loam soil but had little effect on the microbial population of the loamy sand soil, suggesting the presence of different microbial populations. Nitrogen transformation was significantly different in both dinoseb acetate treated soils after 28, 63 and 100 days.

**Table IIIA 10.7.1-4      Summary of effects of OEC 2222 on soil microbial activity**

| Soil Type  | Treatment<br>(mg OEC<br>2222/<br>kg soil) | Equivalent<br>Field Rate<br>(kg/ha) | Recommended<br>Field Rate<br>(kg/ha) | Effects on  |                       |
|------------|---|-------------------------------------|--------------------------------------|---|-----------------------|
|            |   |                                     |                                      | Soil Biomass  | Mineral Nitrogen      |
| Sandy loam | 0.038                                     | 0.023                               | 0.02                                 | No significant effect                                     | No significant effect |
|            | 0.190                                     | 0.115                               | 0.02                                 | No significant effect                                     | No significant effect |
| Loamy sand | 0.038                                     | 0.023                               | 0.02                                 | No significant effect                                     | No significant effect |
|            | 0.190                                     | 0.115                               | 0.02                                 | Transient effect on day 14 and 28 with recovery by day 63 |                       |

OEC 2222, when applied to two UK soil types at application rates equivalent to the recommended field rate and at a rate more than five times that rate, elicited only transient effects on soil micro-flora activity at the five-fold field rate. It can thus be concluded that OEC 2222 applied at the normal recommended field rate will not involve any significant risk for soil microbial communities under the proposed practical conditions for use of the product.

(Mohan H 1995)

### IIIA 10.7.2      **Further laboratory, glasshouse or field testing to investigate impact on soil microbial activity**

In the laboratory studies with OEC 2222 conducted using two UK soils and treatment at the equivalent of the recommended field rate and five times that rate of application, there were no long-term effects on soil microbial populations - the test was continued for 100 days. Since in laboratory testing microbial activity (microbial biomass and nitrogen transformation did not deviate by more than 25 % from that of the control treatment after 100 days, .....*(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)*

### IIIA 10.8.1      **Effects on non-target terrestrial plants**

The effects of chemx on the germination, seedling emergence, vegetative vigour and phytotoxicity of a range of terrestrial non-target plants was assessed in laboratory studies.

**Report** (first of two):      IIIA 10.8.1/01 Smith RS and CJ Seagrave 1995, Tier 2  
seed germination / seedling emergence / non-target phytotoxicity using chemx, Report Number CCC-94-320

**Guidelines:**      US EPA FIFRA Guideline § 123-1

**GLP:**      Fully GLP compliant<sup>22</sup>

#### **Executive Summary**

In a terrestrial plant study, seed germination and seedling emergence test, were assessed in a preliminary test following application of OEC 2222 at 1.1, 2.2, 4.4, 8.9, 36 and 71 g/ha to soybean, lettuce, radish, tomato, cucumber, cabbage, oat, ryegrass, corn and onion seed. Each species was tested using four replicates (10 seeds per replicate) per treatment level. OEC 2222 was applied to seeds placed on absorbent paper in petri plates and the seeds were incubated in continuous darkness. Germination was recorded seven days after treatment.

In a second test, OEC 2222 seeds were planted in a sandy soil in pots. OEC 2222 was applied pre-emergence to the soil in deionised water. Pots were hand watered with 21.4 mL water per pot to ensure movement into the seed zone. Emerging plants were observed for 21 days following treatment. Emergence was recorded on days 10 and 14, and survival on day 21. Phytotoxicity observations were recorded on days 10, 14, and 21. Plant height and dry weight effects were determined on day 21.

No-observable effect concentrations (NOEC) and the concentrations at which 25 % and 50 % detrimental effects (EC<sub>25</sub> and EC<sub>50</sub>) occur were estimated. The NOEC level was taken to be the highest dosing level not statistically different from controls. EC<sub>25</sub> and EC<sub>50</sub> values were determined by regression of the percent effect data when a true dose response was observed.

At the rates tested, chemx had no effect on seed germination with the exception of oats where the no observed effect concentration was 36 g/ha. Pre-emergence application of chemx at a rate of application of 71 g/ha resulted in a significant reduction in seedling emergence in the case of ryegrass and onion (NOEC of xx g/ha). Maize, ryegrass, and onion seedling survival were also affected at 71 g/ha. Phytotoxicity was observed at some of the rates tested for all ten species. Plant height was the most sensitive of the parameters measured to chemx treatment, for all species but cabbage.

## I. MATERIALS AND METHODS

### A. MATERIALS:

- |                                    |                         |
|------------------------------------|-------------------------|
| <b>1. Test Material:</b>           | chemx                   |
| <b>Description:</b>                | white powder            |
| <b>Lot/Batch #:</b>                | NPD-9307-5385-T         |
| <b>Purity:</b>                     | 98.5 % as <sup>23</sup> |
| <b>CAS #:</b>                      | 16335-17-2              |
| <b>Stability of test compound:</b> | not determined          |
- 2. Vehicle and/or positive control:** deionised water to which 3.3 mL/L of 0.1M KOH was added (solubiliser)

### B. STUDY DESIGN AND METHODS:

- 1. In life dates:** The study was conducted during the period December 1994 to January 1995

#### 2. Experimental conditions

The study was carried out to assess the effects of application of chemx at rates of 1.1, 2.2, 4.4, 8.9, 18, 36 and 71 g/ha on the germination and emergence of lettuce, radish, tomato, cucumber, cabbage, ryegrass, maize, onion, soybean and oats. In a preliminary test, application was made to seeds placed on absorbent paper in Petri dishes, which were then placed in plastic boxes to maintain a high relative humidity and were maintained in a temperature controlled incubator in continuous darkness. The percentage germination was assessed seven days after treatment.

In a second test, seeds were planted in a sandy soil in pots and a pre-emergence application of test substance was made to the soil surface. Pots were then hand-watered with 21.4 mL of water to move the test substance into the seed zone. The pots were maintained in a greenhouse under controlled conditions (18 - 29 °C, 43 - 87 % relative humidity) and assessments were made of the percentage emergence of the seedlings after 10 and 14 days, percentage survival after 21 days and phytotoxicity after 10, 14 and 21 days. In addition, the height and the dry weight of the seedlings after 21 days were measured.

## II. RESULTS AND DISCUSSION

### A. FINDINGS

Since the data from the test are extensive only example data for onion have been included here to demonstrate the types of observations made (Table IIIA 10.8.1-1). Onion was selected since it was one of the most sensitive species tested. At the rates tested, chemx had no effect on seed germination with the exception of oats where the no observed effect concentration was 36 g/ha. Pre-emergence application of chemx at a rate of application of 71 g/ha resulted in a significant reduction in seedling emergence in the case of ryegrass and onion. Maize, ryegrass, and onion seedling survival were also affected at 71 g/ha. Phytotoxicity was observed at some of the rates tested for all ten species. Plant height was the most sensitive of the parameters measured to chemx treatment, for all species but cabbage. The no observed effect, 25 % effect, and 50 % effect concentrations for each species for all parameters measured are listed in Table IIIA 10.8.1-2.

**Table IIIA 10.8.1-1      Effects of chemx on onions**

| Data for onion selected as an example |     |                                  |  |  |                               |                                  |                                     |
|---------------------------------------|-----|----------------------------------|--|--|-------------------------------|----------------------------------|-------------------------------------|
| Treatment rate g as/ha                | Rep | Seeds Germinated / Total Planted | Seeds Emerged <sup>1</sup> / Total Planted | Seeds Surviving <sup>2</sup> / Total Planted | Phyto-toxicity <sup>2,3</sup> | Plant Height <sup>2,4</sup> (mm) | Plant Dry Weight <sup>2,4</sup> (g) |
| 0.0                                   | 1   | 9/10                             | 9/10                                       | 9/10   | 0.0                           | 65                               | 0.028                               |
|                                       | 2   | 9/10                             | 10/10                                      | 10/10  | 0.0                           | 72                               | 0.042                               |
|                                       | 3   | 10/10                            | 9/10                                       | 9/10   | 0.0                           | 67                               | 0.028                               |
|                                       | 4   | 10/10                            | 9/10                                       | 9/10   | 0.0                           | 65                               | 0.034                               |
| 1.1                                   | 1   | 10/10                            | 10/10                                      | 9/10   | 0.0                           | 69                               | 0.026                               |
|                                       | 2   | 10/10                            | 10/10                                      | 10/10  | 0.0                           | 66                               | 0.026                               |
|                                       | 3   | 10/10                            | 9/10                                       | 9/10   | 0.0                           | 74                               | 0.020                               |
|                                       | 4   | 9/10                             | 10/10                                      | 10/10  | 0.0                           | 65                               | 0.038                               |
| 2.2                                   | 1   | 10/10                            | 9/10                                       | 9/10   | 0.18 *                        | 47 *                             | 0.019 *                             |
|                                       | 2   | 10/10                            | 9/10                                       | 9/10   | 0.13 *                        | 55 *                             | 0.024 *                             |
|                                       | 3   | 9/10                             | 10/10                                      | 10/10  | 0.48 *                        | 41 *                             | 0.020 *                             |
|                                       | 4   | 10/10                            | 9/10                                       | 9/10   | 0.11 *                        | 59 *                             | 0.019 *                             |
| 4.4                                   | 1   | 10/10                            | 8/10                                       | 9/10   | 0.69 *                        | 32 *                             | 0.011 *                             |
|                                       | 2   | 10/10                            | 8/10                                       | 8/10   | 0.55 *                        | 38 *                             | 0.010 *                             |
|                                       | 3   | 10/10                            | 9/10                                       | 9/10   | 0.73 *                        | 24 *                             | 0.010 *                             |
|                                       | 4   | 9/10                             | 9/10                                       | 9/10   | 0.49 *                        | 36 *                             | 0.015 *                             |
| 8.9                                   | 1   | 10/10                            | 9/10                                       | 8/10   | 0.63 *                        | 39 *                             | 0.015 *                             |
|                                       | 2   | 10/10                            | 8/10                                       | 8/10   | 0.83 *                        | 23 *                             | 0.006 *                             |
|                                       | 3   | 10/10                            | 8/10                                       | 8/10   | 0.60 *                        | 37 *                             | 0.011 *                             |
|                                       | 4   | 10/10                            | 9/10                                       | 9/10   | 0.62 *                        | 35 *                             | 0.013 *                             |
| 18                                    | 1   | 10/10                            | 7/10                                       | 7/10   | 0.86 *                        | 28 *                             | 0.004 *                             |
|                                       | 2   | 10/10                            | 9/10                                       | 7/10   | 0.80 *                        | 28 *                             | 0.008 *                             |
|                                       | 3   | 10/10                            | 10/10                                      | 10/10  | 0.76 *                        | 33 *                             | 0.011 *                             |
|                                       | 4   | 9/10                             | 8/10                                       | 9/10   | 0.80 *                        | 30 *                             | 0.005 *                             |
| 36                                    | 1   | 10/10                            | 8/10                                       | 8/10   | 0.80 *                        | 20 *                             | 0.009 *                             |
|                                       | 2   | 10/10                            | 8/10                                       | 8/10   | 0.80 *                        | 13 *                             | 0.007 *                             |
|                                       | 3   | 10/10                            | 9/10                                       | 9/10   | 0.84 *                        | 18 *                             | 0.004 *                             |
|                                       | 4   | 10/10                            | 9/10                                       | 9/10   | 0.80 *                        | 15 *                             | 0.005 *                             |
| 71                                    | 1   | 10/10                            | 4/10 *                                     | 4/10 *                                       | 0.85 *                        | 17 *                             | 0.000 *                             |
|                                       | 2   | 9/10                             | 4/10 *                                     | 4/10 *                                       | 0.80 *                        | 12 *                             | 0.003 *                             |
|                                       | 3   | 10/10                            | 3/10 *                                     | 3/10 *                                       | 0.87 *                        | 18 *                             | 0.001 *                             |
|                                       | 4   | 10/10                            | 10/10 *                                    | 10/10 *                                      | 0.86 *                        | 16 *                             | 0.004 *                             |

<sup>1</sup> 14 days after treatment

<sup>2</sup> 21 days after treatment

<sup>3</sup> phytotoxicity assessed using a 0 - 5 scale with 0 = no effect and 5 = dead plant, the mean value for all surviving plants, expressed as a proportion of the maximum value of 5, is shown for each replicate

<sup>4</sup> mean values for weight and height for the surviving plants is provided.

\* indicates that the mean value of replicates is different from mean value of negative control replicates

**Table IIIA 10.8.1-2 Effects of the chemx on the germination, emergence, survival and growth of non-target plants following pre-emergent application**

| No Observed Effect Concentration (NOEC), EC <sub>25</sub> and EC <sub>50</sub> (g /ha) of Chemx<br>for Non-target Plants |                        |                  |                  |                      |                      |                  |                     |                  |                  |
|--|------------------------|------------------|------------------|----------------------|----------------------|------------------|---------------------|------------------|------------------|
| Plants   | Percentage Germination |                  |                  | Percentage emergence |                      |                  | Percentage survival |                  |                  |
|  | NOEC                   | EC <sub>25</sub> | EC <sub>50</sub> | NOEC                 | EC <sub>25</sub>     | EC <sub>50</sub> | NOEC                | EC <sub>25</sub> | EC <sub>50</sub> |
| Lettuce  | xx                     | > xx             | > xx             | Xx                   | > xx                 | > xx             | Xx                  | > xx             | > xx             |
| Radish   | xx                     | > xx             | > xx             | xx                   | > xx                 | > xx             | Xx                  | > xx             | > xx             |
| Tomato   | xx                     | > xx             | > xx             | xx                   | > xx                 | > xx             | Xx                  | > xx             | > xx             |
| Cucumber   | xx                     | > xx             | > xx             | xx                   | > xx                 | > xx             | Xx                  | > xx             | > xx             |
| Cabbage  | xx                     | > xx             | > xx             | xx                   | > xx                 | > xx             | Xx                  | > xx             | > xx             |
| Oat  | xx                     | > xx             | > xx             | xx                   | > xx                 | > xx             | Xx                  | > xx             | > xx             |
| Soya   | xx                     | > xx             | > xx             | xx                   | > xx                 | > xx             | Xx                  | > xx             | > xx             |
| Maize  | xx                     | > xx             | > xx             | xx                   | > xx                 | > xx             | Xx                  | > xx             | > xx             |
| Ryegrass   | xx                     | > xx             | > xx             | xx                   | > xx                 | > xx             | Xx                  | > xx             | > xx             |
| Onion  | xx                     | > xx             | > xx             | xx                   | xx                   | > xx             | Xx                  | > xx             | > xx             |
| Plants   | Phyto-toxicity         | Height of plants |                  |                      | Dry weight of plants |                  |                     |                  |                  |
|  | NOEC                   | NOEC             | EC <sub>25</sub> | EC <sub>50</sub>     | NOEC                 | EC <sub>25</sub> | EC <sub>50</sub>    |                  |                  |
| Lettuce  | x.x                    | x.x              | x.x              | xx                   | x.x                  | x.x              | Xx                  |                  |                  |
| Radish   | x.x                    | x.x              | x.x              | x.x                  | x.x                  | x.x              | x.x                 |                  |                  |
| Tomato   | x.x                    | x.x              | xx               | > xx                 | x.x                  | xx               | xx                  |                  |                  |
| Cucumber   | x.x                    | x.x              | x.x              | x.x                  | x.x                  | > x.x            | > x.x               |                  |                  |
| Cabbage  | x.x                    | x.x              | x.x              | x.x                  | x.x                  | x.x              | x.x                 |                  |                  |
| Oat  | x.x                    | x.x              | xx               | xx                   | x.x                  | xx               | xx                  |                  |                  |
| Soya   | x.x                    | x.x              | x.x              | xx                   | xx                   | > xx             | > xx                |                  |                  |
| Maize  | x.x                    | x.x              | x.x              | xx                   | x.x                  | x.x              | xx                  |                  |                  |
| Ryegrass   | x.x                    | x.x              | x.x              | xx                   | x.x                  | x.x              | xx                  |                  |                  |
| Onion  | x.x                    | x.x              | x.x              | x.x                  | x.x                  | x.x              | x.x                 |                  |                  |

### III. CONCLUSIONS

Seed germination, emergence and survival of the non-target plant species tested are not affected by a pre-emergence application of chemx at the maximum rate of application recommended for use of chemx. However, plant height and dry weight of most species tested are affected at the maximum field rate of application and phytotoxicity is observed. These effects are not observed after soil application of 1 g/ha (5 % of the maximum application rate).

(Smith RS and CJ Seagrave 1995a)

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Chemco      September 1997      chemx (proposed ISO name)      page of

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**Report** (second of two):      IIIA 10.8.1/02 Smith, R.S., and C.J. Seagrave, 1995, Vegetative vigour non-target phytotoxicity study using chemx, Report Number CCC-94-319

**Guidelines:**      US EPA FIFRA Guideline § 123-1

**Deviations**

During testing, the temperature fell below the specified range of 18 - 33 °C by 3 - 4 °C for 2 days and by 1 °C for 3 days. The deviations did not affect the scientific integrity of the study as plants exhibited normal growth.

**GLP:**      Fully GLP compliant<sup>22</sup>

**Executive Summary**

In a terrestrial plant study, the effects of application of chemx on the vegetative vigour of and phytotoxicity to non-target plants following a post-emergence application was assessed. Seedlings of lettuce, radish, tomato, cucumber, cabbage, ryegrass, maize, onions, soybean and oats, were grown in pots maintained in a greenhouse under controlled conditions - temperature range 14 - 35 °C, relative humidity of 32 - 98 % (mean ≈ 60 %). There were 4 replicates and 5 seedlings per treatment. Chemx was applied as a foliar spray at the 1 - 3 true leaf at the following rates of application, 1.1, 2.2, 4.4, 8.9, 18, 36 and 71 g/ha.

The percentage survival, height and dry weight of the seedlings were recorded 21 days after application. Visual observations of phytotoxicity were made on days 7, 14, and 21 using a 0 - 5 scale, with 0 = no effect and 5 = dead plant. The mean value for all surviving plants, expressed as a proportion of the maximum value of 5, was calculated for each replicate.

Following post-emergence application, only radish did not survive application at a rate of 36 g/ha chemx. Phytotoxic effects and reduction in plant height and dry weight were observed at rates below 20 g/ha for all species except tomato. Since chemx is a herbicide, phytotoxic effects on a range of plant species are not unexpected. Of the species tested, however, only radish displayed visual signs of phytotoxicity at rates below 10 % of the maximum application rate and only radish plants were reduced in weight or height by more than 25 % by post-emergence application of chemx at a rate less than 5 % of the maximum application rate.

**I. MATERIALS AND METHODS**

**A. MATERIALS:**

- |  |  |
|--|--|
| <b>1. Test Material:</b>                   | chemx  |
| <b>Description:</b>                        | white powder   |
| <b>Lot/Batch #:</b>                        | NPD-9307-5385-T  |
| <b>Purity:</b>                             | 98.5 % as <sup>23</sup>  |
| <b>CAS #:</b>                              | 16335-17-2   |
| <b>Stability of test compound:</b>         | not determined   |
| <b>2. Vehicle and/or positive control:</b> | deionised water to which 12 mL/L of 0.1M KOH was added (solubiliser) |

## B. STUDY DESIGN AND METHODS:

**1. In life dates:**      The study was conducted during the period January to March 1995

### 2. Experimental conditions

The study was carried out to assess the effects of application of chemx on the vegetative vigour of and phytotoxicity to non-target plants following a post-emergence application. Seedlings of lettuce, radish, tomato, cucumber, cabbage, ryegrass, maize, onion, soybean and oats, grown in pots maintained in a greenhouse under controlled conditions - temperature range 14 - 35 °C, relative humidity of 32 - 98 % (mean ≈ 60 %) - were treated with chemx as a foliar spray at the following rates of application, 1.1, 2.2, 4.4, 8.9, 18, 36 and 71 g /ha. Plants were treated at the 1 - 3 true leaf stage. In addition, cabbage and radish were tested at application rates below 1.1 g/ha in order to determine the no effect level for at least one parameter.

### 3. Observation

The percentage survival and the height and dry weight of the seedlings were recorded 21 days after application. Visual observations of phytotoxicity were made on days 7, 14, and 21 using a 0 - 5 scale, with 0 = no effect and 5 = dead plant. The mean value for all surviving plants, expressed as a proportion of the maximum value of 5, was calculated for each replicate.

## II. RESULTS AND DISCUSSION

### A. FINDINGS

Post-emergence application of chemx resulted in significant phytotoxicity to all species except tomato at one or more of the rates of application tested. Because the data from the study are extensive, the results for two species have been selected to illustrate the nature of the observations made (Table IIIA 10.8.1-3).

**Table IIIA 10.8.1.-3      Effects of chemx on plant height and weight of tomatoes and radishes**

| Treatment Rate<br>g as/ha | Rep | Plant Height (mm) <sup>1</sup>                   |   | Plant Weight (g) <sup>1</sup>                    |                                       |
|---------------------------|-----|--|---|--|---------------------------------------|
|                           |     | Tomato<br>( <i>Lycopersicon<br/>esculentum</i> ) | Radish<br>( <i>Raphanus<br/>sativus</i> ) | Tomato<br>( <i>Lycopersicon<br/>esculentum</i> ) | Radish<br>( <i>Raphanus sativus</i> ) |
| 0.0                       | 1   | 392  | 160, 144                                  | 4.137  | 1.906, 1.330                          |
|                           | 2   | 430  | 146, 168                                  | 5.330  | 1.850, 1.593                          |
|                           | 3   | 404  | 143, 174                                  | 4.208  | 1.756, 1.708                          |
|                           | 4   | 414  | 158, 157                                  | 4.304  | 1.710, 1.444                          |
| 0.018                     | 1   |  | 162                                       |  | 2.154                                 |
|                           | 2   | --   | 152                                       | --   | 1.640                                 |
|                           | 3   |  | 145                                       |  | 1.937                                 |
|                           | 4   |  | 145                                       |  | 1.974                                 |

<sup>1</sup> Values shown are mean values for all surviving plants within a replicate, where two sets of values are listed the second set of values is associated with a higher treatment rate range.

\* Mean of replicates is significantly different from mean of negative control replicates

Table IIIA 10.8.1-3      Continued

| Treatment Rate<br>g as/ha | Rep | Plant Height (mm) <sup>1</sup>                   |   | Plant Weight (g) <sup>1</sup>                    |                                       |
|---------------------------|-----|--|---|--|---------------------------------------|
|                           |     | Tomato<br>( <i>Lycopersicon<br/>esculentum</i> ) | Radish<br>( <i>Raphanus<br/>sativus</i> ) | Tomato<br>( <i>Lycopersicon<br/>esculentum</i> ) | Radish<br>( <i>Raphanus sativus</i> ) |
| 0.035                     | 1   |  | 139                                       |  | 1.759                                 |
|                           | 2   | --   | 133                                       | --   | 1.960                                 |
|                           | 3   |  | 145                                       |  | 1.765                                 |
|                           | 4   |  | 138                                       |  | 2.023                                 |
| 0.071                     | 1   |  | 140 *                                     |  | 1.531 *                               |
|                           | 2   | --   | 115                                       | --   | 1.506                                 |
|                           | 3   |  | 128                                       |  | 1.390                                 |
|                           | 4   |  | 129                                       |  | 1.697                                 |
| 0.15                      | 1   |  | 134 *                                     |  | 1.506 *                               |
|                           | 2   | --   | 98  | --   | 1.059                                 |
|                           | 3   |  | 100                                       |  | 1.072                                 |
|                           | 4   |  | 117                                       |  | 1.393                                 |
| 0.28                      | 1   |  | 122 *                                     |  | 1.115 *                               |
|                           | 2   | --   | 104                                       | --   | 1.109                                 |
|                           | 3   |  | 88  |  | 0.699                                 |
|                           | 4   |  | 97  |  | 0.898                                 |
| 0.56                      | 1   |  | 84 *                                      |  | 0.802 *                               |
|                           | 2   | --   | 88  | --   | 0.665                                 |
|                           | 3   |  | 81  |  | 0.548                                 |
|                           | 4   |  | 87  |  | 0.794                                 |
| 1.1                       | 1   | 416  | * 54, 72 *                                | 4.290  | * 0.507, 0.430 *                      |
|                           | 2   | 417  | 54, 87                                    | 4.105  | 0.441, 0.396                          |
|                           | 3   | 373  | 52, 82                                    | 4.678  | 0.546, 0.419                          |
|                           | 4   | 421  | 56, 81                                    | 4.729  | 0.557, 0.533                          |
| 2.2                       | 1   | 356  | 70 *                                      | 3.839  | 0.425 *                               |
|                           | 2   | 400  | 79  | 3.413  | 0.454                                 |
|                           | 3   | 386  | 81  | 3.064  | 0.307                                 |
|                           | 4   | 429  | 72  | 4.996  | 0.353                                 |
| 4.4                       | 1   | 380  | 68 *                                      | 3.554  | 0.361 *                               |
|                           | 2   | 457  | 61  | 3.880  | 0.368                                 |
|                           | 3   | 437  | 65  | 4.570  | 0.343                                 |
|                           | 4   | 361  | 60  | 3.639  | 0.388                                 |
| 8.9                       | 1   | 371  | 12 *                                      | 3.288  | 0.064 *                               |
|                           | 2   | 398  | 45  | 4.118  | 0.266                                 |
|                           | 3   | 370  | 55  | 4.988  | 0.280                                 |
|                           | 4   | 427  | 31  | 4.514  | 0.269                                 |
| 18                        | 1   | 387  | 29 *                                      | 3.342  | 0.219 *                               |
|                           | 2   | 402  | 23  | 4.176  | 0.161                                 |
|                           | 3   | 386  | 34  | 4.153  | 0.236                                 |
|                           | 4   | 392  | 42  | 3.842  | 0.214                                 |
| 36                        | 1   | 434  | 0 *                                       | 2.891  | *                                     |
|                           | 2   | 399  | 0   | 3.798  | no survivors                          |
|                           | 3   | 410  | 0   | 4.535  |                                       |
|                           | 4   | 442  | 0   | 4.756  |                                       |

<sup>1</sup> Values shown are mean values for all surviving plants within a replicate, where two sets of values are listed the second set of values is associated with a higher treatment rate range.

\* Mean of replicates is significantly different from mean of negative control replicates

**Table IIIA 10.8.1-3      Continued**

| Treatment Rate<br>g as/ha | Rep | Plant Height (mm) <sup>1</sup>                   |   | Plant Weight (g) <sup>1</sup>                    |                                       |
|---------------------------|-----|--|---|--|---------------------------------------|
|                           |     | Tomato<br>( <i>Lycopersicon<br/>esculentum</i> ) | Radish<br>( <i>Raphanus<br/>sativus</i> ) | Tomato<br>( <i>Lycopersicon<br/>esculentum</i> ) | Radish<br>( <i>Raphanus sativus</i> ) |
| 71                        | 1   | 383  | 0 *                                       | 3.117 *  | *                                     |
|                           | 2   | 374  | 0   | 3.443  | no survivors                          |
|                           | 3   | 369  | 0   | 2.545  |                                       |
|                           | 4   | 404  | 0   | 3.510  |                                       |

<sup>1</sup> Values shown are mean values for all surviving plants within a replicate, where two sets of values are listed the second set of values is associated with a higher treatment rate range.

\* Mean of replicates is significantly different from mean of negative control replicates

Following post-emergence application, only radish did not survive application at a rate of 36 g/ha chemx (Table IIIA 10.8.1-4). Phytotoxic effects and reduction in plant height and dry weight were observed at rates below 20 g/ha for all species except tomato.

Since chemx is a herbicide, phytotoxic effects on a range of plant species are not unexpected. Of the species tested, only radish displayed visual signs of phytotoxicity at rates below 10 % of the maximum application rate and only radish plants were reduced in weight or height by more than 25 % by post-emergence application of chemx at a rate less than 5 % of the maximum application rate.

**Table IIIA 10.8.1-4      Effects of chemx applied post-emergence on the survival and growth of non-target plants**

| No Observed Effect Concentration (NOEC), EC <sub>25</sub> AND EC <sub>50</sub> (g/ha) of Chemx<br>for Non-target Plants 21 days after treatment |                     |                  |                  |
|---|---------------------|------------------|------------------|
| Plants  | Percentage Survival |                  |                  |
|   | NOEC                | EC <sub>25</sub> | EC <sub>50</sub> |
| Lettuce   | xx                  | > xx             | > xx             |
| Radish  | x.x                 | x.x              | xx               |
| Tomato  | xx                  | > xx             | > xx             |
| Cucumber  | xx                  | > xx             | > xx             |
| Cabbage   | xx                  | > xx             | > xx             |
| Oat   | xx                  | > xx             | > xx             |
| Soya  | xx                  | > xx             | > xx             |
| Maize   | xx                  | xx               | > xx             |
| Ryegrass  | xx                  | > xx             | > xx             |
| Onion   | xx                  | > xx             | > xx             |

**Table IIIA 10.8.1-4      Continued**

| No Observed Effect Concentration (NOEC), EC <sub>25</sub> AND EC <sub>50</sub> (g/ha) of Chemx<br>for Non-target Plants 21 days after treatment |                    |                  |                  |                  |                      |                  |                  |
|---|--------------------|------------------|------------------|------------------|----------------------|------------------|------------------|
| Plants  | Phyto-<br>toxicity | Height of plants |                  |                  | Dry weight of plants |                  |                  |
|   | NOEC               | NOEC             | EC <sub>25</sub> | EC <sub>50</sub> | NOEC                 | EC <sub>25</sub> | EC <sub>50</sub> |
| Lettuce   | x.x                | x.x              | xx               | xx               | x.x                  | x.x              | xx               |
| Radish  | x.xxx              | x.xxx            | x.xx             | x.xx             | x.xxx                | x.xx             | x.xx             |
| Tomato  | xx                 | xx               | > xx             | > xx             | xx                   | xx               | > xx             |
| Cucumber  | xx                 | x.x              | xx               | > xx             | xx                   | xx               | xx               |
| Cabbage   | x.x                | x.xxx            | x.x              | xx               | x.xx                 | x.x              | x.x              |
| Oat   | x.x                | x.x              | xx               | xx               | x.x                  | x.x              | xx               |
| Soya  | x.x                | x.x              | x.x              | x.x              | x.x                  | x.x              | x.x              |
| Maize   | x.x                | x.x              | x.x              | xx               | x.x                  | x.x              | xx               |
| Ryegrass  | x.x                | x.x              | x.x              | xx               | x.x                  | x.x              | xx               |
| Onion   | x.x                | x.x              | x.x              | > x.x            | x.x                  | x.x              | xx               |

### III. CONCLUSIONS

Since spray application with ground equipment has been found to result in deposition of 5 % or less of the treatment at a distance of one meter outside the treated area, these results indicate that the great majority of plants outside a one meter buffer area around the treatment area should be unaffected by chemx that might drift from the treatment area. Within the treatment area, degradation of chemx would be required to avoid effects on most plant species grown in following seasons.

(Smith RS and CJ Seagrave 1995b)

#### IIIA 10.9.1      **Summary of available data from preliminary tests used to assess biological activity and dose range finding, which may provide information on other non-target species (flora and fauna)**

None of the data generated in the course of preliminary screening tests are available to the applicant.

#### IIIA 10.9.2      **A critical assessment as to the relevance of the preliminary test data to potential impact on non-target species**

Not relevant since .....(in the interest of brevity and to avoid the inference that particular justifications have universal application, the remainder of the text is omitted)

