APPENDIX 7

FORMAT FOR COMPILATION OF *TIER II* SUMMARIES - ACTIVE SUBSTANCE

PART 1

Section 1 **Identity of the Active Substance; Physical and Chemical Properties of the Active Substance; Further Information on the Active Substance; Proposals including Justification of the Proposals for the Classification and Labelling of the Active Substance**

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. The data included in the following summary and evaluation are not based on a real submission.

Applicant 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.

1 **Identity of the active substance**

IIP 1.1	**Applicant**	Contact person:	Dr John Jones
		Address:	Pheroco 36 -39 Plant Street Marlborough Wiltshire England
		Telephone:	+44 (0) 1345 6789112
		Fax:	+44 (0) 1345 4567890
IIP 1.2	**Manufacturer**	Contact Person:	Dr S. Smith
		Address:	As above
		Telephone:	as above
		Fax:	as above

IIP 1.3 **Common name** PHEROMX (proposed ISO name)

The active substance consists of three active ingredient components.

IIP 1.4 **Chemical Name**

The active substance consists of three active ingredient components.

Active ingredient component 1:

Content in technical active substance: 600 g/kg

IUPAC: 2-hexenyl-acetate

CA:

Active ingredient component 2:

Content in technical active substance: 300 g/kg

IUPAC: 10-undecyl acetate

CA:

Active ingredient component 3:
Content in technical active substance: 100 g/kg
IUPAC: 3-heptenyl-acetate
CA:

IIP 1.5 **Manufacturer's development code numbers**

OEC_PHE_AS is used as code number for the active substance (technical and pure material).

IIP 1.7 **Molecular and structural formula, molecular mass**

Active ingredient component 1
Molecular Formula:

Structural Formula:

Molecular Mass : 433.37

Active ingredient component 2
Molecular Formula:

Structural Formula:

Molecular Mass : 455.37

Active ingredient component 3
Molecular Formula:

Structural Formula:

Molecular Mass : 440.37

IIP 1.8 **Method of manufacture of the active substance**
the information concerned is included with all other confidential information in Document J.

IIP 1.9 **Specification of purity of the active substance**
Detailed information concerned is included with all other confidential information in Document J. Active ingredient component 1 is purchased from a second manufacturer. Components 2 and 3 are synthetised by PHEROCO.

Active ingredient component 1: 600 g/kg +/- 10 g/kg
Active ingredient component 2: 300 g/kg +/- 10 g/kg
Active ingredient component 3: 100 g/kg +/- 10 g/kg

TOTAL active components in technical grade active substance: 985 g/kg +/- 15 g/kg

IIP 1.10 **Identity of isomers, impurities and additives together with the structural formula**

The information concerned is included with all other confidential information in Document J. None of the impurities exceeds 10 g/kg in the technical grade active substance.

IIP 1.11 **Analytical profile of batches**

The information concerned is included with all other confidential information in Document J.

IIP 1.12 **Special studies**

None

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2 Physical and chemical properties of the technical grade active substance

Test or Study & Data point	Guideline and method	Test material purity and specification	Findings	Comments	GLP Y/N	Reference
Melting point, freezing point or solidification point (IIP 2.1.1)				No data presented. Waived on basis of information on individual active ingredient components		
Boiling point (IIP 2.1.2)	EEC A2	97 % pure, lot number GHQ-9209-4531-A (Document J)	168°C (mean of 3 measurements; accuracy, ± 0.1 °C max)		Y	White T 1996a p 39
Relative density (IIP 2.2)			no data presented	Waived on basis of information on individual active ingredient components		Waiving statement provided
Vapour pressure (IIP 2.3.1)			no data presented	Waived on basis of information on individual active ingredient components		Waiving statement provided
Henry's law constant (IIP 2.3.2)			no data presented	Waived on basis of information on individual active ingredient components		Waiving statement provided
Colour and physical state (IIP 2.4.1)		97 % pure, lot number GHQ-9209-4531-A (Document J)	Colourless liquid		N	White T 1996a p 13
Odour (IIP 2.4.2)		97 % pure, lot number GHQ-9209-4531-A (Document J)	no odour		N	White T 1996a p 13
UV/VIS, IR, NMR, MS spectra (as) (IIP 2.5.1.1)			no data presented	Waived on basis of information on individual active ingredient components		Waiving statement provided
UV/VIS, IR, NMR, MS spectra (impurities) (IIP 2.5.2)			no data presented	Waived on basis of information on individual active ingredient components		Waiving statement provided
Solubility in water (IIP 2.6)	EEC A.8 (flask method)	97 % pure, lot number GHQ-9209-4531-A (Document J)	pH 5: 1.60 ± 0.71 ppm pH 7: 1.8 ± 0.8 ppm pH 9: 4.44 ± 1.35 ppm all at 20°C (all results are given at the 95% confidence	PHEROMX is insoluble in water at environmental pH values.	Y	White T 1996a p 43-63

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Test or Study & Data point	Guideline and method	Test material purity and specification	Findings	Comments	GLP Y/N	Reference
			interval, n = 3)			
Solubility in organic solvents (IIP 2.7)	OECD 105 (flask method)	97 % pure, lot number GHQ-9209-4531-A (Document J)	n-heptane: 850 g/l at 20°C xylene: 860 g/l at 20°C 1,2-dichloroethane: 885 g/l at 20°C methanol: 866 g/l at 20°C acetone: 844 g/l at 20°C ethyl acetate: 822 g/l at 20°C	PHEROMX is highly soluble in organic solvents	N	White T 1996a p 175-177
n-octanol/water partition co-efficient (IIP 2.8)			No data presented.	Waived on basis of information on individual active ingredient components		Waiving statement provided
Hydrolysis rate at pH 4,7 and 9 under sterile conditions in the absence of light (IIP 2.9.1)			No data presented.	Not determined. Low solubility in water. Photodegradation is the most likely and rapid route of degradation in the environment		Waiving statement provided
Direct photo-transformation (IIP 2.9.2)			No data presented	Based on literature data for all components, rapid photodegradation can be expected.		Waiving statement provided, based on White T 1993b
Estimated photochemical oxidative degradation (IIP 2.10)			No data presented	Based on literature data for all components, rapid photodegradation can be expected.		Waiving statement provided, based on White T 1993b
Flammability (IIP 2.11.1)			No data presented	Not required		Waiving statement provided
Auto-flammability (IIP 2.11.2)			No data presented	Not required		Waiving statement provided
Flash point (IIP 2.12)	EEC A 9		678 °C			White T 1996a p 238-329
Explosive properties (IIP 2.13)			No data presented	Not required		Waiving statement provided

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Physical and chemical properties of the active substance components
Component 1
Data are based on information from commercial supplier (see Document J)

Test or Study & Data point	**Guideline and method**	**Test material purity and specification**	**Findings**	**Comments**	**GLP Y/N**	**Reference**
Melting point, freezing point or solidification point (IIP 2.1.1)	OECD 102	99 % pure, lot number xyz (Document J)	Mean range: 1.1 - 2.7 °C (mean of 3 measurements; accuracy, ± 0.1 °C max)		Y	White T 1996a p 39
Boiling point (IIP 2.1.2)	EEC A2	99 % pure, lot number xyz (Document J)	168°C (mean of 3 measurements; accuracy, ± 0.1 °C max)		Y	White T 1996a p 39
Relative density (IIP 2.2)	CIPAC MT3. [Capillary stoppered Pycnometer]	99 % pure, lot number xyz (Document J)	1.518 g/cm^3 at 20.0 °C		Y	White T 1996a p 173-175
Vapour pressure (IIP 2.3.1)	OECD 104 (Gas saturation method)	97 % pure, lot number GHQ-9209-4531-A (Document J)	7.22 x 10^{-3} Pa at 35_C 1.87 x 10^{-1} Pa at 40_C Conclusion: 3.05 x 10^{-2} Pa at 20 _C and 8.81 x 10^{-2} Pa at 25_C (by extrapolation)	Pheromx is volatile at ambient temperatures.		White T 1996a p 177-179
Henry's law constant (IIP 2.3.2)	Calculated using solubility at 3 pH values and vapour pressure		Henry's Law constant at 20°C (calculated): pH 5: 8.15 x 10^{-07} Pa/m^3/mol pH 7: 8.83 x 10^{-09} Pa/m^3/mol pH 9: 2.97 x 10^{-08} Pa/m^3/mol			White T 1996a p 181-183
Colour and physical state (IIP 2.4.1)		99 % pure, lot number xyz (Document J)	Colourless liquid			White T 1996a p 13
Odour (IIP 2.4.2)		99 % pure, lot number xyz (Document J)	no odour			White T 1996a p 13

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Test or Study & Data point	Guideline and method	Test material purity and specification	Findings	Comments	GLP Y/N	Reference
UV/VIS, IR, NMR, MS spectra (as) (IIP 2.5.1.1)		99 % pure, lot number xyz (Document J)	UV (aqueous methanol, pH > xx) IR (KBr) ^{1}H-NMR (300MHz, IBM AF-300 FT NMR) ^{13}C-NMR (75MHz IBM AF-300 FT NMR) UV Absorption Characteristics: Basic (aqueous methanol, pH > 10) λ_{max} = 208 nm, ε = 187,150 L.mol^{-1}.cm^{-1}	Based on literature data	N	White T 1996a p 22
Solubility in water (IIP 2.6)			Insoluble in water	Based on literature data, component 1 is insoluble in water at environmental pH values.	N	White T 1996a p 43-63
Solubility in organic solvents (IIP 2.7)			Highly soluble	Based on literature data, component 1 is insoluble in water at environmental pH values.	N	White T 1996a p 43-63
n-octanol/water partition co-efficient (IIP 2.8)			no data presented			Waiving statement provided
Hydrolysis rate at pH 4,7 and 9 under sterile conditions in the absence of light (IIP 2.9.1)			No data presented	Not determined. Low solubility in water. Photodegradation is the most likely and rapid route of degradation in the environment		Waiving statement provided
Direct photo-transformation (IIP 2.9.2)			Rapid photodegradation	Based on literature data, rapid photodegradation can be expected.		White T 1993b
Flammability (IIP 2.11.1)			no data presented	Not required		Waiving statement provided

Auto-flammability (IIP 2.11.2)			no data presented	Not required		Waiving statement provided
Flash point (IIP 2.12)	EEC A 9		678 °C		N	White T 1996a p 238-329
Explosive properties (IIP 2.13)			no data presented	Not required		Waiving statement provided

3 Further information on the active substance

IIP 3.1 Function

Pheromone. Mating disruption of female months *(Insect anonymia)* PHEROMX is not taken up or translocated in plants

IIP 3.3 Field of use envisaged

Forestry

IIP 3.4 Harmful organisms controlled and crops or products protected or treated

IIP 3.4.1 Details of existing and intended use

Table IIA 3.4.1-1 Effect of timing and application rate on the effectiveness of PHEROMX applied in forest to control moths *(Insect anonymia)*[1]

Application Rate g as / ha [No of Applications]	Timing Of Application	Mean % InsectControl {no. of results}	Number of Seasons tested
1. Ireland			
xx g	May	25 (3)	2
xx g	May	23 (9)	2
xx	May	10 (3)	2
2. UK			
xx g	May	25 (3)	3
xx g	May	23 (9)	3
xx g	May	10 (3)	3
3. Germany in-house trials + contract trials			
xx g	May	25 (3)	3
xx g	May	23 (9)	3
xx g	May	10 (3)	3
xx g	May	no data (7)	2

(1) Prepared on the basis of trials over three years in the Northern region of the EU (Ireland, UK, Germany).

Observation : Where infestation levels are high an application of xx g as / ha is required to achieve satisfactory. Such conditions occur in most seasons in the Northern region of the EU. Full details are given in the Biological Dossier.

IIP 3.5 **Mode of action**

IIP 3.5.1 **Details of biochemical and physiological mechanisms and biochemical pathways**

PHEROMX is a pheromone, consisting of three components, which all belong to the class of straight chain lepidopteran pheromones. The substances, in the composition provided in PHEROMX, effectively disrupts mating by disorienting the females so they can not locate their mating partners.

Following application the active substance evaporates slowly from its microcapsule and through the polyethylene container. Overall, the aerial concentrations reached in the treated area are comparable to high natural infestation events. No ecological side effects are expected from the treatment. (Beyer EM *et al* 1987).

The intended effect is exerted by the active ingredient, PHEROMX. Its components are rapidly degraded to ineffective breakdown products, once the active substance is released from its microcapsule.

IIP 3.7 **Recommended methods and precautions concerning handling, storage, transport or fire**

A safety data sheet for PHEROMX is provided in Document KII (IIA 3.7/01)

Hazards identification: On the basis of available information PHEROMX is not expected to produce any significant adverse health or environmental effects when the recommended use instructions are followed.

Fire Fighting Measures:

Flash Point: NA

Hazardous Products of
Combustion: None known

Extinguishing Media: In case of fire, use water (flood with water), dry chemical, CO_2, or alcohol foam.

Unusual Fire and
Explosion Hazards: None

Fire Fighting Equipment: Fire fighters and others exposed to products of combustion should wear self-contained breathing apparatus. Equipment should be thoroughly decontaminated after use.

Transport : Not classified for IMO or IATA.

Handling & Storage: Good industrial practice in housekeeping and personal hygiene should be followed. When using do not eat, drink or smoke. Wash hands thoroughly after handling. Store only in original container.

IIP 3.8 **Procedures for destruction or decontamination**

Like other straight chain lepidopteran pheromones PHEROMX is insoluble in water. It will form a layer on top of water bodies, which will be rapidly removed by evaporation and photodegradation. Any chemical or additive, which could be used to decontaminate water is likely to be more harmful than PHEROMX itself

On this basis precautions must be taken to avoid contamination for example:

Minimise spread - products containing PHEROMX should not be dumped, spilled, rinsed or washed into sewers or public waterways

When off-loading, ensure vehicle is in a bunded area, and products containing PHEROMX should be stored in a bunded area

If in a worst case situation, PHEROMX were to contaminate water in excessive volumes (*i.e.* several hundred fold increase in exposure from its normal use), as a consequence of its very low toxicity (see Sections 3 and 6) it is unlikely to have any effect. In addition the half-life as a consequence of aqueous photolysis (see point 2.9) is fairly short and so breakdown in the top 30 cm will be relatively fast

IIP 3.8.1 **Controlled incineration**

As PHEROMX does not have a halogen content, an assessment of its pyrolytic behaviour under controlled conditions is not required.

The recommended means of safe disposal is by controlled incineration at an approved chemical waste facility. This is a standard process and no further detailed instructions are required.

IIP 3.8.2 **Others**

No other means of safe disposal are proposed.

IIP 10 **Other special studies**

None

Appendix 7 **Format for the compilation of *Tier II* summaries - Active Substance**

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PART 2

Section 2 Analytical methods

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. The data included in the following summary and evaluation are not based on a real submission.

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IIP 4.1 **Analytical standards for the active substance**

Analytical standards for all three components of the active substance are available.

IIP 4.2.1; 4.2.3 **Methods for the analysis of the active substance as manufactured and of impurities**

A gas chromatography (GC) method with flame ionization detector was used for the determination of the active substance and significant impurities (content $\geq$ 1%) in the technical product. The method fulfills the requirements for specificity and limit of determination. Validation data for linearity and repeatablity of the method were waived, as there are no cleanup procedures involved in the sample preparation, and the flame ionization detector usually has a wider linear range.

The formulation process introduces or enhances the presence of impurities of toxicological concern. An enforcement analytical method with upper limits has been provided to identify these impurities.

A gas chromatography (GC) method with flame ionization detector was used for the determination of active substance in the formulation. The method has been validated for specificity, linearity, repeatability and limit of determination.

IIP 4.3 **Methods for the determination of residues in and/or on plants, plant products, foodstuffs (of plant and animal origin), feedingstuffs**

Due to the nature of the active substance and its application technique no residues can be expected on or in

any food or feeding stuff, which might be related to the use of PHEROMX. Release into the environment after application remains within the range of release from moths during naturally occurring infestation events.

Data requirements are therefore waived.

IIP 4.7 **Residues in air**

On the basis of PHEROMX's vapour pressure and Henry's Law constant, it is clear that it will evaporate after release from its microcapsule and penetrate through the polyethylene dispenser. Exposure via the air will therefore be the most likely route of exposure for operators and bystanders. Due to the fact that exposure of operators workers and bystanders by the inhalation route is unlikely to exceed natural background levels, an analytical method for air is not required. Moreover, the active substance is of no toxicological concern and degrades rapidly by photolysis.

IIP 4.9 **Other special studies.**

None

Appendix 7 **Format for the compilation of *Tier II* summaries - Active Substance**

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PART 3

Section 3 Toxicological and Metabolism Studies on the Active Substance

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. The data included in the following summary and evaluation are not based on a real submission.

Applicant 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.

IIP 5.1.1 **Absorption, distribution, metabolism and excretion (ADME) in the rat**

PHEROMX consists of straight chain lepidopteran pheromones , which occur also naturally as a result of their excretion by male moths (*Insect anonymia)* The application technique prevents that any residues will occur on food or feed commodities which might exceed naturally occurring contamination levels. Due to the known low toxicity of the active substance, no residue limits are required in agricultural commodities used for food or animal feed.

All data requirements are therefore waived.

IIP 5.2. **Acute toxicity**

General remark:

PHEROMX consists of straight chain lepidopteran pheromones , which occur also naturally. Reduced toxicological data requirements have been established for these pheromones, which contain only carbon, hydrogen and oxygen and are poorly soluble in water. They are products of fatty acid metabolism and are biodegradable by enzyme systems present in most living organisms. Health studies available in the literature have established that these substances pose no risk.

IIP 5.2.1 **Acute oral toxicity**

Report: II A 5.1/01 Smith H 1993, The acute toxicity of PHEROMX in rats following oral administration, Report No.: CCC-13156

Executive Summary: In an acute oral toxicity study, groups of fasted, young adult Sprague-Dawley rats 5/sex/dose were given a single oral dose (gavage) of PHEROMX (98.5 % purity) in corn oil a single dose of 5,000 mg/kg bw and were observed for 14 days.

Oral LD_{50} rats = > 5000 mg/kg bw

PHEROMX was found to be of low acute toxicity following exposure of rats. Clinical signs on the day of dosing or within two days after dosing included faecal staining and soft stools. All animals had gained weight 7 and 14 days following dosing. On the basis of this study, PHEROMX does not warrant classification as being harmful or toxic.

Guidelines: OECD 420

GLP: yes (certified laboratory).

I - MATERIALS AND METHODS

A MATERIALS:

1 Test Material: PHEROMX

Description:	Colourless liquid
Lot/Batch #:	NPD-9209-4523-T
Purity:	98.5 % as
Stability of test compound:	not determined

2 Vehicle and/or positive control: Corn oil

3 Test animals -

Species:	Rat
Strain:	Crl:CD(SD)BR, albino
Age:	Young adult
Weight at dosing:	217 - 286 g males
Source:	Charles River Laboratories, Portage, MI
Acclimation period:	7 days
Diet:	Chow (#5001), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were individually housed in stainless steel suspended cages
Environmental conditions -	
Temperature:	Temperature was not specified
Humidity:	Relative humidity ranged from 35 to 84 %
Air changes:	Not recorded
Photoperiod:	Alternating 12-hour light and dark cycles

B STUDY DESIGN AND METHODS:

1 In life dates: 15 January to 5 February 1993

2 **Animal assignment and treatment:**
A single dose of 5000 mg/kg bw was selected for the sighting study (using one animal) and for the main study (using four animals). One animal was dosed in the sighting study, the other four individuals were exposed to the test substance 24 hours later. Following an overnight fast (17 - 22 hours), rats were given a single dose of PHEROMX (98.5 % pure) by gavage. The test substance was administered in corn oil at a volume of 10 ml/kg bw. Animals were observed for gross toxicity, behavioural changes and/or mortality at approximately 1, 2.5 and 4 hours after dosing and at least once daily for the remainder of the 14-day study. Body weights were recorded at day 0 (prior to dosing), 7 and 14. On day 14, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Table IIA 5.2.1-1 **Doses, mortality / animals treated**

Dose (mg/kg bw)	Males
0	0/5
5,000	0/5

3 **Statistics** - The data did not warrant statistical analysis.

II - RESULTS AND DISCUSSION

A **Mortality:** No mortalities occurred.
The oral LD_{50} was > 5,000 mg/kg bw

B **Clinical observations:** Clinical signs on the day of dosing or within two days after dosing included faecal staining and soft stools.

C **Body Weight:** All animals had gained weight 7 and 14 days following dosing.

D **Necropsy:** No internal abnormalities were observed at necropsy.

E **Deficiencies:** None

III - CONCLUSIONS

The oral LD_{50} of PHEROMX was found to be in excess of 5000 mg/kg bw and does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity.

(Smith H 1993)

IIP 5.2.2 **Acute percutaneous toxicity**

Report: IIA 5.2.2/01 Jones KL 1993, Acute dermal toxicity study in rats chemx, Report: SB-92-480

Executive Summary: In an acute dermal toxicity study, groups of young adult Sprague-Dawley rats, 5/sex were exposed by the dermal route to PHEROMX(98.5% purity). Test material was applied in distilled water for 24 hours to 10 % of each animals body surface at a dose of 5,000 mg/kg bw. Animals observed for the following 15 days.

Dermal LD_{50}	males	=	> 5000 mg/kg bw
	females	=	> 5000 mg/kg bw
	combined	=	> 5000 mg/kg bw

PHEROMX was found to be of a low order of acute toxicity following exposure of rats *via* the dermal route. No clinical signs were noted as a result of the treatment. On the basis of this study, PHEROMX does not warrant classification as being harmful or toxic.

Guidelines: US EPA FIFRA Guideline § 81-2, which is equivalent to OECD 402

GLP: yes (certified laboratory).

I- MATERIALS AND METHODS

A MATERIALS:

1 Test Material: PHEROMX
- **Description:** Colourless liquid
- **Lot/Batch #:** NPD-9209-4523-T
- **Purity:** 98.5 % as
- **Stability of test compound:** not determined

2 Vehicle and/or positive control: test material dosed as received

3 Test animals -
- **Species:** Rat
- **Strain:** Crl:CD(SD)BR, albino
- **Age:** Young adult
- **Weight at dosing:** 240 - 260 g males; 230 - 245 females
- **Source:** Charles River Laboratories, Portage, MI
- **Acclimation period:** 5 days
- **Diet:** Chow (#5002), *ad libitum*
- **Water:** Tap water, *ad libitum*
- **Housing:** Animals were individually housed in stainless steel suspended cages
- **Environmental conditions -**
- **Temperature:** Temperature was not specified
- **Humidity:** Relative humidity ranged from 35 to 84 %
- **Air changes:** Not recorded
- **Photoperiod:** Alternating 12-hour light and dark cycles

B STUDY DESIGN AND METHODS:

1 In life dates: 28 October to 16 December 1993

2 Animal assignment and treatment:
On the day prior to dosing, the fur was clipped from the dorsal area of the trunk of each animal. The

clipped area accounted for more than 10 % of each animals body surface. The test substance was administered as a single occluded dermal application and was applied moistened with distilled water. After an exposure period of 24 hours, the occlusion was removed and residual test material was removed with distilled water. Animals were observed for gross toxicity and behavioural changes on three occasions on the day of dosing and once daily thereafter for the duration of the study. Mortality checks were conducted twice daily. Individual body weights were measured and recorded on days 1, 8 and 15. On day 15, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Table IIP 5.2.2-1Doses, mortality / animals treated

Dose (mg/kg bw)	Males	Females	Combined
0	0/5	0/5	0/10
5,000	0/5	0/5	0/10

3 **Statistics** - The data did not warrant statistical analysis.

II - RESULTS AND DISCUSSION

A **Mortality:** No mortality occurred.

The dermal LD_{50} for males was > 5,000 mg/kg bw
for females was > 5,000 mg/kg bw
combined was > 5,000 mg/kg bw

B **Clinical observations:** No clinical signs were observed.

C **Body weight:** All animals gained weight during the study.

D **Necropsy:** No treatment related observations were noted.

E **Deficiencies:** None.

III - CONCLUSIONS

The percutaneous LD_{50} of PHEROMX was found to be in excess of 5,000 mg/kg bw. PHEROMX does not warrant classification as being harmful or toxic on the basis of its acute percutaneous toxicity.

(Jones KL 1993a)

IIP 5.2.3 **Acute inhalation toxicity**

Report: IIA 5.2.3/01 Smith CL 1994, Acute inhalation study of PHEROMXherbicide, Report: CCC-13880

Executive Summary: In an acute inhalation toxicity study, groups of young adult Sprague-Dawley rats (5/sex), were exposed by the inhalation route to PHEROMX (98.5 % purity) in air for 1 hour to nose only at a concentration of around 3 mg/L. Animals observed for the following 14 days.

Inhalation LC_{50}	males	=	> 3 mg/L
	females	=	> 3 mg/L
	combined	=	> 3 mg/L

PHEROMX was found to be of a low order of acute toxicity following exposure of rats *via* the inhalation route. No clinical signs were noted. All animals gained weight by the seventh day following treatment and continued to gain weight until sacrificed. The only gross necropsy abnormality observed was the occurrence of enlarged livers in two males, which was, however, not related to the treatment. On the basis of this study, PHEROMX does not warrant classification as being harmful or toxic.

Guidelines: Study not in conformity with current Guidelines

GLP: No. The following deficiencies are noted: 1.) Exposure period 1 hr only. 2.)Unclear documentation of analytical results. Concentration tested can therefore not be fully assessed.

I - MATERIALS AND METHODS

A MATERIALS:

1	**Test Material:**	PHEROMX
	Description:	Colourless liquid
	Lot/Batch #:	GHQ-9307-5385-T
	Purity:	98.5 % as
	Stability of test compound:	Stable for at least 4 weeks stored in darkness at room temperature
2	**Vehicle and/or positive control:**	PHEROMX aerosol
3	**Test animals -**	
	Species:	Rat
	Strain:	Crl:CD(SD)BR, albino
	Age:	Young adult
	Weight at dosing:	315 - 340 g males; 235 - 255 females
	Source:	Charles River Laboratories, Portage, MI
	Acclimation period:	8 days
	Diet:	Chow (#5002), *ad libitum*
	Water:	Tap water, *ad libitum*
	Housing:	Animals were individually housed in stainless steel suspended cages
	Environmental conditions -	
	Temperature:	Temperature was not specified
	Humidity:	Relative humidity ranged from 35 to 84 %
	Air changes:	Not recorded
	Photoperiod:	Alternating 12-hour light and dark cycles

B STUDY DESIGN AND METHODS:

1 **In life dates:** 13 February to 7 March 1994

2 **Animal assignment and treatment:**
Animals were observed twice during the 1-hour exposure period. Thereafter mortality and moribundity checks were conducted twice daily. Observations for signs of toxicity were conducted immediately following exposure and daily thereafter. Individual body weights were measured and recorded on days 2, 7 and 14. On day 14, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Table IIA 5.2.3-1 **Doses, mortality / animals treated**

Dose (mg/L)	Males	Females	Combined
0	0/5	0/5	0/10
3	0/5	0/5	0/10

3 **Generation of the test atmosphere / chamber description:**
An 80-L nose only exposure chamber was used. During exposure (1 hour), individual plastic tubes were positioned in two tiers around the outside of the chamber such that only the nose of test animals was exposed to the interior of the chamber. A JET-O-BLASTER⁻ atomisation device was used to generate the test aerosol. The test atmosphere was sampled at pretest but not during the exposure period for GC analysis, using flame ionization detection. The limit of detection (LOD) for PHEROMX was 0.1 _g as/L of air, while the limit of quantification (LOQ) was 0.2 _g as/L of air. The test atmosphere concentration was 3.0 mg as/L air in the one and only sample taken.

Mass median aerodynamic diameter: 2.1 microns; Deomatric standard deviation: 0.6;
% particles < 10 microns: 85
% particles < 1 micron: 1.8

4 **Statistics** - The data did not warrant statistical analysis.

II - RESULTS AND DISCUSSION

A **Mortality:** No mortality occurred.

The 1 hour inhalation LC_{50} for males was > 3 mg / L
for females was > 3 mg / L
combined was > 3 mg / L

B **Clinical observations:** All animals appeared normal.

C **Body weight:** All animals gained weight during by the seventh day following treatment and continued to gain weight until sacrificed.

D **Necropsy:** The only gross necropsy abnormality observed was the occurrence of enlarged livers

in two males.

E **Deficiencies:** The inhalation period was below current Guideline requirements and the test concentration was not analysed to the fullest extent necessary. The study is therefore regarded as circumstantial evidence of confirmatory information.

III - CONCLUSIONS

Although the inhalation period was below current Guideline requirements and the test concentration was not analysed to the fullest extent necessary, the study appears acceptable in view of the known, low toxicity of this class of substances. The study can therefore be regarded as circumstantial evidence of confirmatory information. The acute inhalation LC_{50} of PHEROMX for the combined sexes was found to be in excess of 3 mg/L and the compound does not warrant classification as being toxic or harmful on the basis of its acute inhalation toxicity.

(Smith CL 1994)

IIP 5.2.4 **Skin irritation**

Report: IIA 5.2.4/01 Jones KL 1993, Primary dermal irritation study in rats with PHEROMX Report: SB-92-480

Executive Summary: In a primary dermal irritation study, 6 young adult New Zealand rabbits, 2 males and 4 females, were exposed *via* the dermal route to 0.5 g of PHEROMX (98.5% purity) per animal. The test material was applied undiluted for 4 hours to x % of the body surface area of test animals. Animals then were observed for 3 days. Irritation was scored using the Draize scheme.

Slight erythema was noted in one site at one hour post treatment. This had resolved by 24 hours. No other effect was seen. In this study, PHEROMX was not a dermal irritant. On the basis of this study, PHEROMX does not warrant classification as being irritating to the skin.

Guidelines: US EPA FIFRA § 81-5, which is equivalent to OECD 404

GLP: yes (certified laboratory), fully compliant

I - MATERIALS AND METHODS

A **MATERIALS:**

1 **Test Material:** PHEROMX
- **Description:** Colourless liquid
- **Lot/Batch #:** NPD-9209-4523-T
- **Purity:** 98.5 % as
- **Stability of test compound:** not determined

2 **Vehicle and/or positive control:** test material dosed as received

3 **Test animals -**
- **Species:** Rabbit
- **Strain:** New Zealand
- **Age:** Young adult
- **Weight at dosing:** 2.1 – 2.3 kg males; 2.1 – 2.5 kg females
- **Source:** Chalk Cliff Rabbitry, Whitesville, MI

Acclimation period: 5 days
Diet: Chow (#5322), *ad libitum*
Water: Tap water, *ad libitum*
Housing: Animals were individually housed in stainless steel suspended cages

Environmental conditions -
Temperature: Temperature was not specified
Humidity: Relative humidity ranged from 35 to 84 %
Air changes: Not recorded
Photoperiod: Alternating 12-hour light and dark cycles

B STUDY DESIGN AND METHODS:

1 In life dates: 21 October to 1 November 1993

2 Animal assignment and treatment:
On the day prior to dosing, the fur was clipped from the dorsal area of the trunk of each animal using a small animal clipper. The test material was applied, semi-occluded, as a single dermal administration to two male and four female New Zealand White rabbits. The application rate was 0.5 gm per animal. The substance was applied undiluted. Two application sites were used. After an exposure period of 4 hours, the occlusion was removed and residual test material was removed with distilled water. The test sites were examined for signs of erythema and oedema at 1, 24, 48 and 72 hours following patch removal.

II - RESULTS AND DISCUSSION

A FINDINGS: Slight erythema was noted in one site at one hour post treatment. This had resolved by 24 hours. No other effect was seen.

Table IIA 5.2.4-1: Individual and mean skin irritation scores according to the Draize scheme

	Erythema						**Oedema**					
Animal no	44529	44530	44531	44478	44448	44286	44529	44530	44531	44478	44448	44286
after 4 hr	0	1	0	0	0	0	0	0	0	0	0	0
after 24 hr	0	0	0	0	0	0	0	0	0	0	0	0
after 48 hr	0	0	0	0	0	0	0	0	0	0	0	0
after 72 hr	0	0	0	0	0	0	0	0	0	0	0	0
mean score 24-72 h	0.0						0.0					
Additional criteria specified in Directive 93/21/EEC Point 3.2.6.1 fulfilled: Yes/<u>No</u>												

III - CONCLUSIONS

PHEROMX was non-irritant to rabbit skin. On the basis of this study, PHEROMX does not warrant classification as being irritating to the skin.

(Jones KL 1993b)

IIP 5.2.5 **Eye irritation**

Report: IIA 5.2.5/01 Jones KL 1993, Primary eye irritation study in rabbits with chemx, Report: SB-92-480

Executive Summary: In a primary eye irritation study, 0.1 ml of PHEROMX (98.5% purity) was instilled into the conjunctival sac of the right eye of 6 young adult New Zealand White rabbits (1 female and 5 males). Animals then observed for the following 7 days. Irritation was scored using the Draize scheme for unwashed eyes.

Moderate iritis was noted in 4/6 animals after one hour, an effect that had resolved by 24 hours. Slight to moderate conjunctival redness and slight to moderate swelling were observed in each of six rabbits at 1 hour after dosing, with ocular discharge in five of six rabbits. Conjunctival findings were resolved by 72 hours post-instillation in five rabbits and by day 7 in the sixth. In this study, PHEROMX induced slight to moderate ocular irritation that was reversed during the study period. On the basis of this study, PHEROMX does not warrant classification as being an eye irritant.

Guidelines: US EPA FIFRA Guideline § 81-4
GLP: yes (certified laboratory), fully compliant

I - MATERIALS AND METHODS

A MATERIALS:

1 Test Material: PHEROMX

Description:	colourless liquid
Lot/Batch #:	NPD-9209-4523-T
Purity:	98.5 % as
Stability of test compound:	not determined

2 Vehicle and/or positive control: test material was applied undiluted

3 Test animals -

Species:	Rabbit
Strain:	New Zealand
Age:	Young adult
Weight at dosing:	2.5 to 2.9 kg
Source:	Chalk Cliff Rabbitry, Whitesville, MI
Acclimation period:	5 days
Diet:	Chow (#5322), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were individually housed in stainless steel suspended cages
Environmental conditions -	
Temperature:	Temperature was not specified
Humidity:	Relative humidity ranged from 35 to 84 %
Air changes:	Not recorded
Photoperiod:	Alternating 12-hour light and dark cycles

B STUDY DESIGN AND METHODS:

1 In life dates: 11 November to 16 December 1993

2 Animal assignment and treatment:
Test material was instilled into the conjunctival sac of the right eye of each of six (one female and 5 males) NZW rabbits (0.1 ml test material per animal). The contralateral eyes served as controls for

the animals used. This was followed by a 7-day observation period. Both eyes of each animal were examined for signs of irritation at 1, 24, 48 and 72 hours and 7 days after dosing. Flourescein dye retention was assessed at 24 hours and at each subsequent interval until a negative response was obtained.

II - RESULTS AND DISCUSSION

A FINDINGS: Moderate iritis was noted in 4/6 animals after one hour, an effect which had resolved by 24 hours. Slight to moderate conjunctival redness and slight to moderate swelling were observed in each of six rabbits at 1 hour after dosing, with ocular discharge in five of six rabbits. Conjunctival findings were resolved by 72 hours post-instillation in five rabbits and by day 7 in the sixth.

Table IIA 5.2.5-1: Eye irritation scores according to the Draize scheme - unwashed eyes

	Cornea						Iris						Conjunctiva-redness						Conjunctiva-chemosis					
Time/ Rabbit	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1 hour	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
24 hours	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
48 hours	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
72 hours	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
mean scores 24-72 h	0.0						0.0						0.0						0.0					
Additional criteria in Directive 93/21/EEC Point 3.2.6.2 fulfilled: Yes/No																								

* individual animal scores deleted for the purposes of this document

III - CONCLUSIONS

Slight to moderate ocular irritation was observed which was reversed within the study period. On the basis of this study, PHEROMX does not warrant classification as being an eye irritant.

(Jones KL 1993c)

IIP 5.2.6 **Dermal sensitisation**

Experimental studies on dermal sensitisation are not available for the active substance PHEROMX but for two of its components, i.e. 2-hexenyl acetate and 10-undecyl acetate. A literature survey on structurally similar straight chain lepidopteran pheromones was also provided.

All this data showing no indication of a sensitizing potential of SCLP's, the available information appears to be sufficient to draw a conclusion on PHEROMX.

Report: IIA 5.2.6/01 Jones KL 1995, Guinea pig maximization test with 2-hexyl acetate (Method of Magnusson and Kligman), Report: PL-95-047

Executive Summary: In a dermal sensitization study, 2-hexyl acetate (99.8% purity) in Freund's Complete Adjuvant (FCA) Emulsion was tested using young adult Albino Dunkin Hartley Guinea pigs (10/sex). The treatment regime involved induction of sensitization by intradermal injection on day 1, induction of sensitization by topical administration on day 8 and challenge by topical administration on day 22.

One animal died of unknown causes on day 22. The death was not considered treatment-related. There was no dermal response to either induction or challenge applications. Appropriate historical control data using dinitrochlorobenzene

demonstrated a positive response. On the basis of this study, 2-hexyl acetate does not warrant classification as being a dermal sensitiser.

Guidelines: US EPA FIFRA Guideline § 81-6, which is equivalent to OECD 406

GLP: yes (certified laboratory), fully compliant

I - MATERIALS AND METHODS

A MATERIALS:

1 Test Material: 2-hexyl acetate

Description:	liquid
Lot/Batch #:	GHQ-0307-5385-T
Purity:	99.8 % as [18]
Stability of test compound:	not determined

2 Vehicle and/or positive control: polypropylene glycol, Freund's Complete Adjuvant (FCA) emulsion and saline 9 %

3 Test animals -

Species:	Albino Guinea Pigs
Strain:	Dunkin Hartley Haz:(DH)FBR
Age:	5 to 7 weeks at dosing
Weight at dosing:	345 to 420 g males; 270 to 435 g females
Source:	GTP, Gainsville, Pa
Acclimation period:	14 days
Diet:	Agway Prolab Purina Guinea Pig Diet, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were individually housed in stainless steel suspended cages with wire mesh bottoms
Environmental conditions -	
Temperature:	18 to 24 _C
Humidity:	Relative humidity ranged from 30 to 60 %
Air changes:	Not recorded
Photoperiod:	Alternating 12-hour light and dark cycles

B STUDY DESIGN AND METHODS:

1 In life dates: 11 November to 16 December 1993

2 Animal assignment and treatment:

The treatment regime involved induction of sensitization by intradermal injection on day 1, induction of sensitization by topical administration on day 8 and challenge by topical administration on day 22. The test levels for dermal and intradermal inductions and challenge were selected following preliminary irritancy testing. The sites were pre-treated with 10 % sodium lauryl sulphate to elicit some dermal response, because of the known non-irritancy of the test substance. Propylene glycol was used alone for intradermal induction and mixed with 2-hexyl acetate to produce a 5 % w/v

mixture for intradermal induction. Freund's Complete Adjuvant (FCA) Emulsion was mixed 50 % v/v in distilled water for intradermal induction and mixed with 2-hexyl acetate to produce a 5 % w/v mixture for intradermal induction. 0.9 % saline was used alone for topical induction and challenge and also used to moisten 2-hexyl acetate for topical induction and challenge. The test material was administered at 5 % for the intradermal induction and at 100 % for the topical induction to 20 Dunkin Hartley guinea pigs (10 male and 10 female).

II - RESULTS AND DISCUSSION

A FINDINGS: One animal died of unknown causes on day 22. The death was not considered treatment-related. There was no dermal response to either induction or challenge applications. Appropriate historical control data using dinitrochlorobenzene demonstrated a positive response.

III - CONCLUSIONS

2-hexyl acetate did not exhibit dermal sensitisation potential under the test conditions. On the basis of this study, PHEROMX does not warrant classification as being a skin sensitizer.

(Jones KL 1995)

Report: IIA 5.2.6/02 Sexsmith, W (1999), Sensitising potential of straight chain lepidopteran pheromones - a survey. Journal of semiochemicals, 6(23) pp 23-45, 1999

Executive Summary: A survey of available literature data on sensitisation of pheromones and semiochemicals was undertaken by the author. Among the substances which were investigated is 10-undecyl acetate, one of the components of PHEROMX. The substance was tested in adult Albino Guinea pigs (10/sex) using the Maximization test protocol with complete Freund's adjuvants. Further details on the test protocol were not reported.

None of the straight chain lepidopteran pheromones included in this survey showed a sensitizing potential in Guinea pigs.

Guidelines: not specified

GLP: not specified

I - MATERIALS AND METHODS

A MATERIALS:

1 Test Material: 10-undecyl acetate
6 other SCLP's **Purity:** not specified

Stability of test compound: not determined

2 Vehicle and/or positive control: Freund's Complete Adjuvant (FCA) ; no other details reported

3 Test animals -

Species: Albino Guinea Pigs

Strain: not specified

Other details on source of animals, housing and feeding conditions not specified.

B STUDY DESIGN AND METHODS:

The treatment regime is described as Maximisation Test, which involved induction of sensitization by intradermal injection, induction of sensitization by topical administration and challenge by topical administration. The test levels for dermal and intradermal inductions and challenge were not reported.

II - RESULTS AND DISCUSSION

A FINDINGS: None of the SCLP's tested led to any dermal response to either induction or challenge applications. Historical control data were not reported.

III - CONCLUSIONS

Although the literature report leaves several shortcomings with respect to the details of the treatment protocol employed, it indicates that none of the SCLP's investigated showed any sensitizing potential. On the basis of this study, PHEROMX does not warrant classification as being a skin sensitizer.

(Sexsmith 1999)

IIP 5.3. **Short term toxicity** (IIP 5.3.2, IIP 5.3.6, IIP 5.3.8)

PHEROMX consists of straight chain lepidopteran pheromones , which occur also naturally as a result of their excretion by male moths (*Insect anonymia)* The application technique prevents that any residues will occur on food or feed commodities which might exceed naturally occurring contamination levels. Due to the known low toxicity of the active substance, no residue limits are required in agricultural commodities used for food or animal feed.

All data requirements are therefore waived.

IIP 5.4 **Genotoxicity testing**

PHEROMX consists of straight chain lepidopteran pheromones. Many substances from this class were investigated for mutagenic properties without indication of any positive findings in the literature. The application technique prevents that any residues will occur on food or feed commodities which might exceed naturally occurring contamination levels. One study submitted by the notifier confirms the absence of mutagenic effects also for PHEROMX.

All further data requirements are therefore waived.

IIP 5.4.1 ***In vitro* genotoxicity testing (bacterial assay for gene mutation)**

Report: IIA 5.4.1/01 Smith A 1995, Ames / Salmonella mutagenicity assay of PHEROMX, Report No.: CC-94002

Executive Summary: In a reverse gene mutation assay in bacteria, strains TA98, TA100, TA102, TA1535, and TA1537 of *Salmonella. typhimurium* were exposed to PHEROMX(98.5% purity), using dimethylsulfoxide (DMSO)

solvent at concentrations of 50, 150, 500, 1,500 and x,xxx _g/plate in the presence and absence of S9 activation. A single plate was used, per dose, per condition.

PHEROMX was tested up to cytotoxic concentrations or limit concentration, x,xxx _g/plate. Based on the qualitative data generated, cytotoxicity (as indicated by decreased revertants/plate) was evident at the highest dose tested (HDT) with and without S9 activation. Hence, x,xxx _g/plate ±S9 was chosen as the highest dose tested for mutagenicity testing using both the plate incorporation and pre-incubation procedures. An additional six doses (down to 5 _g/plate ±S9) were included for statistical purposes. Reproducible cytotoxic effects were seen under both plate incorporation and pre-incubation test conditions in the majority of strains at doses _ 1,500 _g/plate ±S9. The positive controls induced the appropriate responses in the corresponding strains.

There were no significant (p _ 0.01) elevations in revertants / plate or in dose responses in any of the tests. It was concluded that PHEROMX was not mutagenic in the bacterial strains tested, either in the presence or absence of metabolic activation.

Guidelines: EEC B 14, equivalent to OECD Guideline 471

GLP: Fully GLP compliant [18].

I - MATERIALS AND METHODS

A MATERIALS:

1 Test Material: PHEROMX

Description: Colourless liquid
Lot/Batch #: NPD-9307-5385-T
Purity: 98.5 % as
Stability of test compound: Not determined Dosing solutions were prepared on the day of use and samples of the stock solution were analysed for achieved concentrations.
Solvent used: dimethylsulfoxide (DMSO).

2 Control Materials:
Negative: culture medium
Solvent/final concentration: DMSO at 0.1 mL/plate
Positive: non-activation:

4-nitroquinoline-N-oxide	0.02, 0.1 & 0.2	_g/plate TA98, TA100
sodium nitrite	0.5, 2.5 and 5	mg/plate TA1535
9-aminoacridine	10, 50 and 100	_g/plate TA1537
cumene hydroperoxide	10, 50 and 100	Tg/plate TA102

activation:

2-acetylaminofluorene	3, 15 and 30	_g/plate TA98
benzo(a)pyrene	0.2, 1 and 2	_g/plate TA100
2-aminoanthracene	1, 5 and 10	_g/plate TA1535, TA1537
Dantron	5, 25 and 50	_g/plate TA102

3 Activation: S9 derived from male Sprague-Dawley rats (Aroclor 1254 induced rat liver)

The rat liver S9 (Lot No. MolTox 0339; protein content = 39.2 mg/mL) was purchased from Molecular Toxicology, Inc., College Park, MD. The metabolic activation ability of the S9 was characterized using

varying S9 and positive control concentrations.

S9 mix composition:	**Component:**	**Concentration**
	sodium phosphate buffer (pH 7.4)	100 _moles
	glucose 6-phosphate	5 _moles
	NADP	4 _moles
	KCl	33 _moles
	$MgCl_2$	8 _moles
	S9	10 % (v/v)

4 **Test Organisms:** *S. typhimurium* strains: TA98, TA100, TA102, TA1535, TA1537 - test organisms were properly maintained and were checked for appropriate genetic markers (rfa mutation, R factor)

5 **Test Concentrations:**

(a) Preliminary cytotoxicity assay: Two preliminary assays were performed:

Plate incorporation assay: 50, 150, 500, 1,500 and x,xxx _g/plate were evaluated with and without S9 activation in S. typhimurium strain TA100. A single plate was used, per dose, per condition.

Pre-incubation assay: 50, 150, 500, 1,500 and x,xxx _g/plate were evaluated with and without S9 activation in S. typhimurium strain TA100. A single plate was used, per dose, per condition.

(b) Mutation assays:

Plate incorporation assay: 5, 15, 50, 150, 500, 1,500 and x,xxx _g/plate were evaluated in triplicate in the presence and absence of S9 activation; all test strains were used.

Pre-incubation assay: As above for the plate incorporation assay.

Re-tests: Owing to contamination, poor performance of the positive controls or unacceptable analytical data, portions of the plate incorporation assay were repeated with strains TA1535, TA1537, TA98 and TA100. Doses and assay conditions were comparable to those used with the initial plate incorporation assay.

B **TEST PERFORMANCE:** The study (Salmonella Assay - standard plate test, pre-incubation for 20 minutes) was conducted during the period January to October 1994 by the Chemco Research Laboratory, New York.

1 **Preliminary cytotoxicity/plate incorporation mutation assay:** In general, similar procedures were used for the preliminary cytotoxicity and the plate incorporation mutation assay.

A 0.1 mL aliquot of the appropriate test strain and 0.1 mL of the appropriate test material dose, positive controls (mutation test only) or solvent, were added to tubes containing 2.0-mL volumes of molten top agar. For the S9-activated tests, 0.5 mL of the S9-cofactor mix was also added. Test strains, and test and control solutions were added as described. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium E plates, and incubated at 37 ± 1_C for 48 hours. For the mutation assay, triplicate plates per strain, per dose, per condition were used for the test compound; nine replicate plates were prepared for the solvent controls, and single plates were used for the negative controls and each positive control concentration. Means and standard deviations were calculated for the mutation assay data.

2 Pre-incubation assay: The independently repeated mutation assay was conducted using the pre-incubation modification to the standard plate incorporation test. The pre-incubation assay was carried out as described above with the following two exceptions: 0.5 mL of buffer were added to cultures prepared for testing under non-activated conditions; prior to the addition of top agar, reaction mixtures were incubated for 20 minutes at 37 ± 1_C.

3 Statistics: Data were transformed using a $\log_{10}$ transformation and analysed using Bartlett's test for homogeneity of variance; test groups were compared with controls using within-levels pooled variance and a one-sided t-test. Grubbs' test was performed to determine outliers and dose response was evaluated by regression analysis. Significance was established at p _ 0.01.

4 Evaluation Criteria: The test material was considered positive for a particular strain and condition if it caused a statistically significant (p _ 0.01) dose-related increase in revertants over the solvent controls at three treatment levels.

II - RESULTS AND DISCUSSION

A ANALYTICAL DETERMINATIONS: Results of the analytical determinations indicated that with the exception of a single sample (78 % of target), actual concentrations (high dose only) used in valid tests ranged from 87 to 119 % of the intended levels. The overall percent difference from target for the nine samples was -1.76%

B PRELIMINARY CYTOTOXICITY ASSAY: Five doses of the test material ranging from 50 to x,xxx _g/plate _ S9 were evaluated in the plate incorporation and the pre-incubation cytotoxicity tests. No precipitation was observed up to the limit dose, x,xxx _g/plate. Based on the qualitative data generated, cytotoxicity (as indicated by decreased revertants/plate) was evident at the highest dose tested (HDT) with and without S9 activation (see study report, Appendix I, Table 1, p. 18). Hence, x,xxx _g/plate _ S9 was chosen as the HDT for mutagenicity testing using both the plate incorporation and pre-incubation procedures. An additional six doses (down to 5 _g/plate _ S9) were included for statistical purposes.

C MUTATION ASSAYS: Reproducible cytotoxic effects were seen under both plate incorporation and pre-incubation test conditions in the majority of strains at doses _ 1,500 _g/plate _ S9. Reverent counts in PHEROMX treated initial and repeat plate incorporation tests did not differ significantly from the DMSO solvent control or from the negative (culture medium) control data (see study report, Appendix I, Tables 2-9, pp. 19-26). Similar results were obtained in the initial and repeat pre-incubation tests (see study report, Appendix II, Tables 1-3, pp. 28-30). In contrast, positive controls responded appropriately with significant (p _ 0.01) reverent values 5-fold to 50-fold over background (see study report, Appendix III, Tables 2-9).

III - CONCLUSIONS

It was concluded that the test article was not mutagenic in this bacterial test system, either in the presence or absence of metabolic activation in the strains tested. Additionally, the sensitivity of both the plate incorporation and pre-incubation procedures to detect mutagenesis was adequately demonstrated by the results obtained with the positive controls.

(Smith A 1995a)

IIP 5.5 **Long term toxicity**

PHEROMX consists of straight chain lepidopteran pheromones , which occur also naturally as a result of their excretion by male moths (*Insect anonymia)* The application technique prevents that any residues will occur on food or feed commodities which might exceed naturally occurring contamination levels. Due to the known low toxicity of the active substance, no residue limits are required in agricultural commodities used for food or animal feed.

All data requirements are therefore waived.

IIP5.6 **Reproductive toxicity**

PHEROMX consists of straight chain lepidopteran pheromones , which occur also naturally as a result of their excretion by male moths (*Insect anonymia)* The application technique prevents that any residues will occur on food or feed commodities which might exceed naturally occurring contamination levels. Due to the known low toxicity of the active substance, no residue limits are required in agricultural commodities used for food or animal feed.

All data requirements are therefore waived.

IIP 5.9. **Medical data**

IIP 5.9.1 **Reports on medical incidents among manufacturing personnel**

Report: IIA 5.9.1/01 Smith A 1998, Occupational health report for PHEROMX, Report No.: CC-98002

Executive Summary: During research and development of PHEROMX a total of 33 male and female individuals came in contact with PHEROMX, which are under regular medical checks according to the occupational health policy of the notifier. Potential exposure situations included synthesis and formulation/packaging development as well as numerous small and medium scale laboratory and field trials over a period of 6 years.

An adverse effects reporting scheme is established in the company and all individuals were questioned concerning skin changes or any other local or systemic health effects during regular medical checks twice a year.

None of the individuals reported any adverse observations which might be related to their contact with PHEROMX.

IIP 5.10 **Special studies**
None

IIP 5. 11 **Summary of mammalian toxicity and overall conclusion**

The following results have been obtained in toxicology studies:

Appendix 7 **Format for the compilation of *Tier II* summaries - Active Substance**

Company name	**Month and year**	**Active Substance (Name)**	**page of**

Table IIP 5.11-1: Summary of acute toxicity data for chemx

Type of study	**Species**	**Results**
Oral route	Rat	LD50 > 5000 mg/kg bw
Dermal route	Rat	LD50 > 5000 mg/kg bw
Inhalation	Rat	LC50 at 1 hours > 3.0 mg/L
Primary skin	Rabbit	Non-irritating
Eye irritation	Rabbit	Slight to moderate eye irritation, but does not warrant classification as being an eye irritant
Skin sensitisation	Guinea pig	No sensitising properties expected

Appendix 7 **Format for the compilation of *Tier II* summaries - Active Substance**

Company name	**Month and year**	**Active Substance (Name)**	**page of**

PART 4

Section 4 Residues in or on Treated Products, Food and Feed

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. The data included in the following summary and evaluation are not based on a real submission.

Applicant 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.

IIP 6.3 **Residues trials (pre-harvest use on major crops)**

PHEROMX consists of straight chain lepidopteran pheromones , which occur also naturally as a result of their excretion by male moths (*Insect anonymia).* The application technique prevents that any residues will occur on food or feed commodities which might exceed naturally occurring contamination levels. Due to the known low toxicity of the active substance, no residue limits are required in agricultural commodities used for food or animal feed.

No residue trials, livestock feeding studies or plant metabolism studies are therefore required and all data requirements in Sections II P 6.3 - 6.6 are waived

IIP 6.4 **Livestock feeding studies**
IIP 6.6 **Residues in succeeding crops and metabolism in plants**

IIP 6.8.1 **Proposed pre-harvest intervals**

The notifier proposed a per-harvest interval of 60 days as an additional safety measure to exclude any detectable residues on commodities even if the substance would be misused.

IIA 6.10 **Special studies**

None

PART 5

Section 5 Fate and Behaviour in the Environment

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. The data included in the following summary and evaluation are not based on a real submission.

Applicant 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.

IIP 7.4.1 **Adsorption and desorption of pheromx**

PHEROMX consists of straight chain lepidopteran pheromones, which occur also naturally as a result of their excretion by male moths (*Insect anonymia*).

Data on the persistence of a semiochemical and its transport from the site of application to another site or medium are not required because ecotoxicity data and public literature indicate no hazard to biota. These data indicate that no significant persistence and transport of these agents in any part of the environment occurs.

The environmental fate of the semiochemical PHEROMX has been assessed, based on available information. Test data on the compound PHEROMX are not required because its use will not result in environmental contamination exceeding natural background levels.

All data requirements are therefore waived.

IIP 7.4.3 **Column leaching studies with the active substance**

Column leaching studies are not required because ecotoxicity data and public literature indicate no hazard to biota (point IIP 7.4.1) (Grenfel RG 1995b).

All data requirements are therefore waived.

IIP 7.4.9 **Volatility**

SC's are generally assumed to dissipate rapidly in the environment, primarily by volatilization and degradation; this is partly because persistence is counterproductive to a communication signal received by an olfactory system. Only the aerial compartment within and around the crop need to be loaded and concentrations in the air are unlikely to exceed several ng/m^3 (see e.g. Bäckman 1997; Koch et al 1997).

All data requirements are therefore waived.