<u>Outcome of the Insecticide Working Group B</u>

Although centred on the fictitious dossier for 'Lepticide', the discussion also covered more general aspects of resistance risk assessment (highlighted **in bold below**). The dossier was unsatisfactory in several respects, but especially since data on cross-resistance in *Myzus persicae* presented in the Annex bore no relation to figures summarised in the main text. Because of unclear data, a final risk assessment for *M. persicae* was impossible. Some other inconsistencies and criticisms are presented below.

The dossier presents a comparable easy case because pyrethroids are a well known class of insecticides and resistance to this group has been characterised in many important pest species. The two primary targets, the aphid *Myzus persicae* and the Colorado beetle *Leptinotarsa decemlineata*, are well known to have developed resistance to a number of insecticide classes including pyrethroids. Emphasis on these species as 'high risk' targets is therefore correct, but a more detailed account including reference to some key publications is needed to justify these inherent risks to a regulator not necessarily well versed in insecticide resistance research. The inherent risk for the other aphids present in potato is not addressed in the dossier, and should have been considered to justify the exclusion of other species (eg. *Aphis frangulae*) that are also known to present resistance problems

Evidence for the presence or absence of cross-resistance between Lepticide and other pyrethroids is poorly presented. Data in Tables 1 and 3 are contradictory and clearly relate to different bioassays done for different purposes. The presentation of probit parameters is very imprecise, and on this basis it is not clear if the pyrethroid-resistant strain of *M. persicae* is a really a resistant one because values for deltamethrin have not been determined. In reality, cross-resistance is extremely likely but a non-specialist is unable to reach this conclusion. For this reason, no decisions and management options are possible. Data for cross resistance in Colorado beetle in Tables 2 and 4 do not represent the levels of pyrethroid resistance that have been reported by several laboratories.

This emphasises the value of applicants having access to resistant strains with known resistance mechanisms that are kept available as a common resource, so that testing for cross resistance with different resistance mechanisms is possible for all interested parties. If this is not possible much more effort has to be put into cross resistance research because many potentially resistant strains need to be tested which finally allow the conclusion if there is a threat of cross-resistance in contemporary field populations. Many such 'standard' resistance cultures reside in European laboratories (both in the public and private sectors) and opportunities for exploiting these to facilitate implementation of PP 1/213 should be explored further.

Sensitivity data were presented for both target species. Data for Colorado beetles (except some clearly wrong or unclear numbers in the tables) were generally satisfactory. For *M. persicae* only 1 sensitive strain from 1982 and 2 strains with well described resistance mechanisms were tested. An explanation is missing of why these strains were considered adequate without use of more recently collected field samples.

Locations for sampling of sensitivity data were considered to be decided not necessarily under regional aspects but more under European ones. It was suggested that less sensitivity data are needed for new molecules unlikely to suffer from cross-resistance than for old molecules in well established insecticide classes. For new molecules with novel modes of action, absence of cross-resistance can sometimes be established by

testing few strains representing the 'worst-case scenario' (i.e. exhibiting multiple resistance across insecticide classes and encompassing most or all mechanisms known to occur). In all cases, applicants should take advantage of published data and the sources of such information should be clearly cited.

Many regulators, and some company personnel involved in compiling risk assessment dossiers are not specialists in resistance. Co-operation with independent scientists to formulate cases and assist with their interpretation should be encouraged, although issues relating to confidentiality need to be addressed further. Dossiers need to be detailed enough to justify decisions on sampling, testing and interpretation for non experts.

The test methods were described but their suitability for the task in hand was not discussed or justified. **Guidance for less experienced applicants, regulators and those carrying out tests would be helpful for reviewing sampling procedures (time and location) as well as bioassay techniques, to foster confidence that the optimal methods have been adopted.**

The proposal for a discriminating dose is welcomed, but the decision for this rate in the dossier is not explained and seems to be clearly wrong especially in the case of Colorado beetle. The label advice given in the dossier seems not to be appropriate for Colorado beetle, where a more distinct reduction in the number of applications seems necessary.

Label advises or other means of communication of avoidance strategies would be most helpful if they could take into account the different agricultural situations also within a region of concern and should be more flexible (e.g. instead of "only 2 application" (of 4) asking for e.g. 50 % of applications with a given product and by this also providing strategies for years or regions where usually only 2 applications are needed.) There is a potential conflict in imposing restrictions for Lepticide that are more stringent than for pyrethroids already used in the fields, which are likely to select for the same mechanism(s). It is unclear how such a conflict will be handled by regulators. For resistance management purposes it is desirable to impose the same restrictions across all pyrethroids, but there may be some legal restriction for label changes in different countries and a considerable time-lag in changes between labels of newly registered and old products.

Appendix 1

Product Y (insecticide)

Background information

- Lepticide is a new pyrethroid insecticide for use on potatoes.
- Target pests are aphids to prevent virus transmission in seed potato productionand Colorado potato beetle *Leptinotarsa decemlineata* in all types of potato production
- Pyrethroids are well known and widely used insecticides and resistance has been shown by several insects and mite species for many years, (see IRAC web page) but resistance management strategies have shown to be able to handle the problems, e.g. in France and Italy. Where pyrethroids have been used without any resistance management strategy then resistance to that group (and other product groups) has steadily been increasing. E.g Poland, Croatia, Hungary, Romania and Russia. Even where some strategies have been in place then sucking insects in particular have evolved complex resistance mechanisms.

There are several aphid species on potatoes, but only in *Myzus persicae* are resistance problems known and likely. Peach-potato aphid, *Myzus persicae* is a polyphagous feeder which can transmit plant viruses. The main food plants are sugar beet, potatoes, brassicas, oilseed rape and as well as vegetable, ornamentals and fruit trees. Various aphid strains are known to be resistant to OPs, carbamates and pyrethroids. Three different resistance mechanisms are involved including metabolic resistance. In field crops selection of resistant strains comes from protective sprays used for virus prevention and also following treatments aimed at other target pests as well as from treatments in the horticultural area such as greenhouses.
Colorado potato beetle *Leptinotarsa decemlineata* is a major pest of potato crops in both southern and northern Europe. Both larval and adult forms are prodigious and destructive leaf feeders often present in high numbers and if not controlled can defoliate large areas of the potato crop. Colorado potato beetle can be a serious secondary pest of other Solanaceous crops such as tomatoes, eggplants, and peppers. The beetles over winter in the soil as adults and become active in the spring as temperatures rise and begin to feed on weeds and volunteer or early planted potatoes, even entering the soil to attack emerging foliage. Female beetles lay orange-yellow eggs in batches of about two dozen on the underside of the leaves. Each female can lay 500 or more eggs over a four to five week period. Eggs hatch in four to nine days and the larvae begin to feed on potato foliage. There can be one to two (in warm climate also 3) generations a year and it is possible to have all stages of the insect present in the crop at any one time.
Colorado beetle have shown resistance to several groups of insecticides including pyrethroids. Resistance in France and Italy has not been observed whilst in many Eastern European countries it is an issue.

Background - Agricultural situation in which the product will be used.

The Northern zone country for which the application is sought has about 250000 ha of potatoes growing in one main area in the north typified by a colder climate. Mainly late ripening potatoes are grown there with intensive and dense potato production. In the north applications are not needed every year and usually not more than once per year.

In the south another main growing area has a warm and dry climate and with early and late potatoes. Seed potatoes are grown in all regions and also commercial fields are scattered everywhere but not very dense. Treatments for the control of Colorado beetle are applied in the south up to 3 times and there is nearly no year without applications.

Seed potato production covers about 20000 ha and seed potatoes are produced in several regions. Aphids in seed potatoes are treated on average with insecticides up to ten times a year; some years have fewer and others have more applications. Early ripening varieties need fewer applications than late ones. In one of the main seed potato production areas fewer applications are needed, because there a less aphids active there.
Usually contact insecticides have to be used at early growing stages, later systemic compounds should be used. There is registration for 2 other pyrethroids but it has been shown that a small proportion of the population is highly resistant to pyrethroids and also cross resistant to carbamates. Recently registration for OPs have been withdrawn so that at the moment only a new systemic action compound is available for control of aphids.
Aphid infesting potatoes for consumption or industry are less important for growers and applications are carried out only if very high populations appear. Aphids are mainly a problem on late ripening varieties but at most only 2 applications are needed to control them.

Other products are registered for the control Colorado beetle; many are pyrethroids which have already shown some signs of resistance and also cross resistance to each other as well as older OP and carbamates. There is also a natural product and Bacillus thuringiensis registered but both have reduced efficacy and are also more expensive than other products so that farmers use them only on a limited scale. Alternative control mechanisms are not really practicable because they are too expensive and can be financed only by biofarmers.

Dossier for product Y

4. Resistance (Annex III. 6.3)

4.1 Background

Proposed label text:
Resistance Management
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To prevent the development of resistance, Lepticide should not be used continuously or as the sole method of control. Consideration should be given to alternation with products having a different modes of action and no cross resistance and where possible using biological control methods.

As it is a pyrethroid the mode of action of Lepticide is well known. This product is a single, potent isomer. Although this activity is not novel it is anticipated that the application rate of Lepticide will be up to 4 times less than current products but still deliver commercial levels of control

4.2 Laboratory work

The general performance of Lepticide in the field is presented in the efficacy section of this dossier. In order to develop the assessment of the risk of resistance developing the performance of Lepticide against 2 OP- and pirimicarb-resistant strains of *Myzus persicae* was tested in the laboratory. The product was not affected by any cross resistance. Therefore, Lepticide can also be used where other compounds have become ineffective.

In the laboratory, the performance of Lepticide against organophosphorous and pirimicarb resistant strains of *Myzus persicae* (R3) was compared to a normal sensitive strain (NS) (Table 1) to define sensitivity and confirm methodology. The product showed no sign of any cross resistance.

(figures in ppm)	US1LNormal sensitive strain (NS)	794JZ OP, pyrethroid and carbamate resistant strain (R3)	926B Highly OP and carbamate resistant strain (R3)
dimethoate	<20	>200	>200
pirimicarb	10 to 20	30-50	50 to 200
deltamethrin	12	>40	>20
Lepticide	4	8	10

Table 1: LC_{90} values (ppm) determined in several series of bioassays with *Myzus persicae*

The performance of Lepticide was tested in the laboratory against a sensitive and an OP / pyrethroid resistant strain of *Leptinotarsa decemlineata* to definesensitivity and to confirm the methodology. Table 2) The product performance was not affected by any cross resistance with OPs' although some impact on activity was found on pyrethroids resistant strains.

(figures in ppm) Product	Sens 1-Normal sensitive strain (NS)	Ponski 1 OP resistant strain (R3)	Ponski 2 OP and carbamate resistant strain (R3)	Ponski 3 OP and pyrethroid resistant strain (R3)
triazofos	50	>300	>250	60
carbosulfan	65	70	>300	72
deltamethrin	15	15	>20	>75
Lepticide	2.6	2.8	7.9	11.8

Table 2: LC_{90} values (ppm) determined in several series of bioassays with *Leptinotarsa sp*

4.3 **Results** (Appendices 1 and 2).

The study showed the following:

- Lepticide was effective against OP and carbamate resistant *Myzus persicae* and did not show any cross resistance.
- Lepticide was effective against OP resistant *Leptinotarsa decemlineata* but showed some reduction in performance against pyrethroid

4.4 **Summary**

Lepticide provides growers with a broad spectrum insecticide for the control of aphids and Colorado beetles at extremely low dose rates compared to existing products. The above studies demonstrate no cross resistance to existing aphicides and only a slight reduction in activity on pyrethroids resistant Colorado beetle. The low rate of application would indicate that this product is of value in controlling the pest where resistance is not an issue or where only occasional applications are needed. Lepticide can be of value as part of a programme of treatments and therefore the proposed strategy is to use it in alternation with products of different modes of action. This approach is considered to be the most technically sound and responsible in order to protect the future effectiveness of the product.

4.5 Management strategy

- Prior to launch a good communication programme with advisors and growers is planned via technical leaflets indicating the potential resistance problems when controlling Colorado beetles and outlining anti-resistance strategies.
- A leaf-dip bioassay has been developed and dose response has been established for both peach-potato aphid and Colorado beetles.
- In areas of risk, monitoring for the development of resistance or shifts in sensitivity may form part of the resistance management programme.
- Field performance will be regularly reviewed and any changes investigated and reported promptly.

Appendix 1

Evaluation of the response of insecticide-susceptible and -resistant clones of *Myzus persicae* to Lepticide in leaf-dip bioassays

Summary

Leaf-dip bioassays with Lepticide against nymphs of a fully susceptible laboratory clone of *Myzus persicae* and two resistant clones with and without insecticide-insensitive acetylcholinesterase yielded very similar LC_{90} estimates with no evidence of cross-resistance to insecticides used in the past. The bioassay method is considered well suited for monitoring the response of *M persicae* to Lepticide and diagnosing any incipient resistance resulting from use of this chemical in practice.

1. Objectives

The objectives of this research were as follows:

(i) To exploit a leaf-dip bioassay to quantify the sensitivity of a laboratory susceptible clone of *Myzus persicae* to Lepticide. The main resistance mechanisms are described with the strains below.

(ii) to assess the response to Lepticide of *M. persicae* clones possessing all known mechanisms of resistance to other insecticides. These mechanisms include a newly-detected mutant form of acetylcholinesterase (AChE) that is presently spreading rapidly in the field throughout Europe including the UK as well as metabolic resistance.

2. Materials and methods

2.1 Aphid strains

US1L A reference laboratory clone collected from sugar beet in the UK in 1974, and since maintained as a clone exhibiting full susceptibility to all insecticides.

794JZ Collected from tobacco in the UK in 1982. This clone exhibits very high (R3) levels of carboxylesterase E4 conferring broad-spectrum resistance to organophosphates, pyrethroids and carbamates, but lacks the mutant AChE that greatly enhances E4-based resistance to pirimicarb and triazamate.

926B Collected from tobacco in southern Europe in 1990. This was one of the first clones in which mutant AChE was detected and characterised. Its biochemical characteristics are identical to those of aphids with insensitive AChE that caused major control problems of potatoes, sugar beet and other crops in the UK during 1996. 926B is immune to pirimicarb in standard laboratory bioassays.

All three clones are maintained on Chinese cabbage and their genetic integrity confirmed at regular intervals using biochemical tests for diagnosing E4 levels and AChE insensitivity.

2.2 Bioassay method

Apterous adults were placed onto leaf discs (35mm diameter) cut from Chinese cabbage that had previously been dipped into aqueous solutions of formulated Lepticide, and placed on an agar bed inside plastic Petri-dishes. 24h later, adults and surplus nymphs were removed by suction to leave ca. 20 nymphs per disc. Mortality of nymphs was recorded at 24h intervals thereafter. In keeping with previous results a holding period of at least 96h following removal of adults proved essential to obtain a consistent endpoint. A minimum of three replicate discs were used per concentration, and each dose-response assay was conducted twice to ensure the repeatability of results.

3. Results

Repeat bioassays yielded very similar results, and LC_{90} estimates for all clones collectively varied only three-fold at most. LC_{90} estimates in particular were very similar, disclosing no increased tendency for any of the clones to tolerate higher concentrations of Lepticide. Hence it can safely be concluded that none of the resistance mechanisms currently known to occur in field populations have implications for the performance of Lepticide. As a consequence, Lepticide appears to exhibit excellent potential for combating the rapidly worsening extent of *M. persicae* resistance to conventional insecticide classes.

4. Implications for resistance monitoring

Lack of cross-resistance to Lepticide in existing populations of *M. persicae* is no guarantee that resistance will not develop following use of this chemical in practice. One important component of resistance management is to implement resistance monitoring, in areas of risk using a method of proven applicability and reliability. The leaf-dip bioassay exploited here meets these criteria for aphid species in general and is therefore strongly advocated for monitoring the efficacy of Lepticide in the UK and elsewhere. Two concentrations 2.5 and 25ppm should suffice for monitoring purposes. Survival at 25ppm in particular would present a cause for concern and require follow-up studies to confirm the occurrence of resistance and to characterise it further.

Table 3. Probit statistics for three clones of *Myzus persicae* tested with Lepticide in leaf-dip bioassays. (ppm)

Clone	LC_{50}	C.I.	LC_{90}	Slope
US1L A	0.92	0.54-0.82	3.9	2.5
US1L B	0.87	0.32-0.47	4.2	2,7
794JZ A	1.1	0.07-0.60	5	1.7
794JZ B	1.2	0.20-0.74	5.6	1.7
926B A	0.88	0.21-1.4	3.8	2.4
926B B	0.89	0.71-1.2	4.1	2.3

'A' and 'B' refer to replicate bioassays

Appendix 2

Evaluation of the response of populations of *Leptinotarsa decemlineata* from 3 European countries to Lepticide in leaf-dip bioassays

Summary

Leaf-dip bioassays with Lepticide against nymphs and imagos of laboratory and field strains of *Leptinotarsa decemlineata* collected from Poland, Germany, Hungary and France showed slight evidence of cross-resistance to other pyrethroids. However Lepticide showed a lower resistance ratio compared with current standards. The IRAC 7 bioassay method is considered well suited for monitoring the response of *L. decemlineata* to Lepticide and is excellent for monitoring shifts in population sensitivity.

1. Objectives

The objectives of this research were as follows:

(i) To confirm the suitability of the IRAC 7 leaf dip bioassay to measure shifts in dose response. (This method has been validated extensively on this pest in Poland.)

(ii) To determine the variation in response of populations where control with pyrethroids has been less than optimal.

2. Materials and methods

2.1 Strains

The laboratory strains were obtained from the PPI, Poznan, Poland and consisted of a susceptible population kept without insecticide pressure (Sens 1) and a second strain which has been pressured with commonly used standards (Ponski 3)

The field strains were collected from sites around Poznan, Poland, Meissen, Germany, Szolnok, Hungary and Nimes, France.

2.2 Bioassay method (IRAC 7)

(a) Field strains were collected as L_1 larvae for rearing to the appropriate stage or material from which an F1 population for testing can be reared.
(b) Prepare accurate dilutions of the test compound from identified commercial product. For initial studies, five widely spaced rates are recommended. The use of additional wetter is only recommended for highly waxed leaf material, in which case this wetter solution is used for the 'untreated' (control) solution on place of water alone.
(c) Individual potato leaves were dipped in the test liquid for 5 s with gentle agitation and place to surface-dry on paper towelling. The 'untreated' leaves were dipped first in water followed by the remainder in different concentrations through the test liquids.
(d) Place the treated surface-dry leaves in the labelled test containers, which must be suitable for holding enough leaf material in good condition for up to 3 days.
(e) Equal numbers of larvae were added to each container, so that a minimum total of 40 larvae are used per treatment, divided between at least four replicate containers.
(f) The containers were stored in an area where they were not exposed to direct sunlight or extremes of temperature. Record maximum and minimum temperatures. If possible a mean temperature of 25°C is preferred.
(g) larval mortalities were assessed after 1, 24 and 48 hours.

3. Results

3.1 Sens 1 strain

Repeated bioassays using Lepticide against larvae of the Sens 1 strain showed very consistent dose-response relationships after 48h exposure to Lepticide). The pooled LC_{90} data from these bioassays (3 ppm; Table 4) was consistent and confirmed the applicability of the IRAC 7 method. Tests on the pressurised Ponski strain produced steeper dose response curves especially with deltamethrin (no data provided) but less so with Lepticide.

3.2 Field strains

The responses from the field strains were more variable, some were similar to the Sens 1 strain whilst other approached those seen on the Ponski strain indicating very mixed field populations.

4. Implications for resistance monitoring

The variability on response to Lepticide reflects the potential development of pyrethroids resistance. However the resistance ratio of Lepticide (1:3) compared to the deltamethrin standard (1:6) would indicate the utility of Lepticide on such diverse populations. One important component of resistance management is to implement resistance monitoring as quickly as possible using a method of proven applicability and reliability. The IRAC 7 leaf-dip bioassay meets these criteria and is strongly advocated for monitoring the efficacy of Lepticide. Based on our findings, two concentrations, 10 and l00 ppm, should suffice for monitoring purposes. Survival at 100 ppm in particular would be a cause for concern and require follow-up studies to confirm the occurrence of resistance and to characterise it further.

Table 4. Probit statistics for eight field strains of Colorado beetles tested with Lepticide in leaf-dip bioassays. (ppm)

Strain	LC_{50}	C.I.	LC_{90}	Slope
Sens 1	0.89	0.55-1.1	2.6	2.9
Ponski 1	0.86	0.50-1.2	2.8	2.9
Ponski 2	2.7	1.4-3.2	7.9	5.1
Ponski 3	3.3	1.3-7.2	11.8	9.9
Poz 1	1.3	0.9-1.5	5.2	4.9
Poz 2	2.5	1.1-2.9	8.3	7.7
Meis 1	1.1	0.75-1.7	3.1	3.2
Meis 2	2.3	1.8-2.5	6.7	6.3
Szol 1	2.9	1.1-3.3	8.0	5
Szol 2	2.2	1.3-3.1	7.4	5.1
Nimes 1	1.4	0.75-1.6	3.3	3.1
Nimes 2	2.4	1.8-2.5	6.8	6.6

Draft label for - LEPTICIDE®

- Lepticide is a new pyrethroid insecticide for use on potatoes.
- Lepticide can be used to control aphids to prevent virus transmission in seed potatoes, and Colorado potato beetle *Leptinotarsa decemlineata in all types of potato production.*

- **Aphid control**

Lepticide is a pyrethroid insecticide for the control of organophosphorus and carbamate susceptible and resistant strains of peach potato aphids. It has good knockdown and residual action.

Crop – Potato

Apply as soon as aphids are seen in the crop. Repeat as necessary up to a maximum of 3 applications.
Maximum Individual Dose: 5 g as / ha
Maximum Total Dose per Year 15 g as / ha

Resistance Management Strategy

Aphid control may be reduced if strains resistant to **Lepticide** develop. To prevent the development of resistance, **Lepticide** should be used in alternation with products having different modes of action and not continuously or as the sole method of control. Because of *Myzus persicae* has several resistance mechanisms contact your advisory bodies to identify which products are effective.

- **Colorado beetle control**

Lepticide is a pyrethroid insecticide for the control of organophosphorus and carbamate susceptible and resistant strains of Colorado beetles. It has good knockdown and residual action. Some populations of this pest are showing increased tolerance to pyrethroids products so Lepticide should be used as part of a control programme in conjunction with products having different modes of action.

Crop – Potato

Apply as soon as the pest is seen in the crop. No more than 2 applications of Lepticide or other pyrethroids should be made per season

Maximum Individual Dose:	5 g as / ha
Maximum Total Dose per Year	10 g as / ha

Resistance Management Strategy

To prevent the development of resistance, **Lepticide** should be used in alternation with products having different modes of action and not continuously or as the sole method of control. The registered numbers of treatments should not be exceeded. Monitoring of representative populations with suspected pyrethroid resistance should be made at the beginning and end of the season.