

1 **Concerted action to develop a European Framework for**
2 **probabilistic risk assessment of the environmental impacts of**
3 **pesticides (EU QLRT-2001-01346)**
4

5 **EUFRAM**

6
7 **Work Package 9**
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9 **Practical approaches for validation**

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1. Introduction

The EUPRA workshop agreed that it is highly desirable to find robust ways of validating or verifying probabilistic approaches, but it also recognised that this might not be wholly achievable due to the difficulty of confirming probabilistic risk estimates experimentally. For example, extensive monitoring or field studies would be needed to test the accuracy of a prediction that effects were expected in 5% of cases. The workshop recognised that validating existing, deterministic approaches is also difficult also because often little is known about the original undisturbed populations as most field populations are exposed to some kind of stress. Also very little is known about the natural variability of the ecosystems we are trying to protect. It is also envisaged that stakeholders think that validating a probabilistic risk assessment (PRA) needs lots of data and that if one single result as observed in real practice does not match the PRA, the complete PRA is invalid. Similarly one result that agrees does not imply validity.

A common point is that no model can be fully validated in a literal sense, because all models are necessarily simplifications of reality. Suter et al. (1993) discusses this and argues that credibility is a more realistic goal, and can be pursued in 3 ways: experimental testing, publication in peer-reviewed journals, use in regulatory practice. To be able to validate PRA methods using experimental testing, validation criteria in terms of the distributions for assessment endpoints (e.g. fraction affected) or intermediate variables (e.g. PEC) should be set. If our probabilistic assessment has quantified uncertainty then this broadens the range within which the measured distribution can fall. Likewise, one should take account of uncertainty affecting the measured distribution (e.g. measurement uncertainty in chemical analyses, sampling uncertainty due to limited sample numbers, possible biases due to non-detection, etc) in our experimental testing. So validation may be a matter of assessing the degree of overlap between two 2D distributions, one for predictions and one for measurements. It is also important that any guidance and tools developed for probabilistic assessments are designed so as to avoid inconsistencies occurring when they are used. This could be reviewed, by publication in peer-reviewed journals and arranging for different assessors (e.g. different countries, different stakeholders) to conduct the same assessment in parallel and compare the results.

Dee (1995) recognised three major aspects of model validation (adapted using Law and Kelton, 2000):

- Conceptual validation
- Verification (validation of algorithms and software code)
- Functional validation

Conceptual validation concerns the question whether the model accurately represents the system under study. Was the simplification of the underlying biological process in model steps realistic; were the model assumptions credible? Usually, conceptual validation is largely qualitative and is best tested against the opinion of experts with different scientific backgrounds. Experimental or observational data in support of the principles and assumptions should be presented and discussed (EU, 2003).

Verification. Validation of algorithms concerns the translation of model concepts in mathematical formulas. Do the model equations represent the conceptual model, under which conditions are simplifying assumptions justified, what is the effect of the choice of (numerical) methods for model solving on the results, do results from different methods to solve the model agree? Validation of software code concerns the implementation of mathematical formulas in computer language (EU, 2003).

Functional validation concerns checking of the model against independently obtained observations. Ideally, it is evaluated by obtaining pertinent real world data, and to perform a statistical comparison of simulated outcomes and observations. It may be very difficult, or even impossible to obtain such data (EU, 2003).

Despite the above stated difficulties, the EUPRA workshop recommended that urgent consideration should be given to identifying and initiating practical strategies for evaluating

94 the suitability of proposed approaches and assessing the degree of confidence that can be
95 placed in probabilistic risk estimates. For this it is important that the extent to which validation
96 studies should cover the range of scenarios and problems that the models are intended for is
97 established. For example, is it sufficient to validate a model for one type of pesticide
98 (chemistry or mode of action), or in one country? Although it may not be possible to validate a
99 probabilistic assessment procedure in its entirety, many of the underlying models and
100 assumptions can be validated, calibrated or verified. Such parts should be identified, and
101 appropriate research should be implemented. This should include comparing the results of
102 probabilistic assessments to other lines of evidence (e.g. field studies and incident
103 monitoring).

104
105 Lastly it should be mentioned that validation is of course only as good as the data you
106 validate with. Besides uncertainty in the measurements as mentioned above, the use existing
107 data also encounters problems. For example, your paper already mentions the problem of
108 monitoring data for pesticide concentrations in water, that do not relate to the peak
109 concentrations which are predicted by regulatory models (and which are needed to assess
110 the risk of acute effects). Similarly, historical data on the frequency of impacts (e.g. fish and
111 bird kills) is likely to include only a small proportion of the impacts that actually occurred, it
112 only reflects acute mortality (delayed, chronic and reproductive effects are not identified) and
113 it is biased towards the more conspicuous impacts (large numbers of large, colourful animals
114 in places frequented by the public). So although the use of existing data should be
115 encouraged, they have to be used with caution.

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117

118 **2. Possible approaches to validate major components of** 119 **probabilistic approaches.**

120
121

122 **2.1 Exposure**

123

124 Commission Directive 91/414 requires data to conduct environmental risk assessments in the
125 following main environmental compartments:

- 126 • Terrestrial Compartment (soil dwelling organisms, plant dwelling organisms, avian,
127 organic Matter Breakdown)
- 128 • Aquatic environment (fresh water organisms, groundwater organisms, sediment
129 organisms, avian)
- 130 • Atmospheric environment (testing required is limited and no simulation program has
131 been recommended for use)

132

133 Exposure is thus estimated for leaching to groundwater, soil, and surface waters. Several
134 pesticide fate models have been evaluated to establish if they are suitable at the EU level.
135 This has been the work of the FOCUS (Forum for the Co-ordination of pesticide fate models
136 and their USE) since 1994. For each of the above-mentioned environmental compartments a
137 FOCUS Working Group was established. Three reports have been produced where the state-
138 of-the-art available simulation programs were compiled and tested for applicability (Boesten et
139 al. 1995; Boesten et al. 1996, Adriaanse et al. 1996). In these publications, simple models are
140 proposed for a first evaluation of the PECs. It is proposed that should a calculated TER trigger
141 higher tier testing, then further refinement of the exposure may be achieved by application of
142 the more sophisticated models proposed in these reports. A detailed discussion of all models
143 is out of the scope of this contribution but since the publication of these reports the PESTLA
144 model has been replaced by the PEARL (Pesticide Emission Assessment at Regional and
145 Local Scales)-model which apparently includes the most adequate descriptions of chemical
146 and biological processes (Leistra, et al., 2000; Tiktak et al., 2000). This program provides for
147 the simulation of all FOCUS scenarios and the so called Dutch Standard Scenario. It is worth
148 mentioning that the target variable for the Dutch registration procedure is the absolute
149 maximum of the average substance concentration in the upper meter of the groundwater.
150 Because the water table is kept variable in this model, the concentrations obtained are
151 expected to be somewhat higher.

152

153 Several European scenarios (9 locations and approximately 14 crops per location) have been
154 proposed by the FOCUS modelling working group (FOCUS, 2000). These scenarios are a
155 combination of: crop, location, long-term application schedule (i.e. annual, biennial or triennial
156 applications), and agronomic parameters (particularly irrigation data).

157
158 In developing ways to validating PRA outputs, it should be borne in mind that in FOCUS
159 scenarios, most model inputs are fixed. Only the following information can be edited by the
160 user:

- 161 - All pesticide properties, including the depth dependence of pesticide degradation
- 162 - Application schedules. Application times can be set relative to the time of crop
163 emergence to avoid the necessity of creating different application schedules for
164 different locations

165 It follows that data related to the hydrodynamic flow conditions in the soil profile, specific soil
166 types and their structure are not directly accessible and it would thus take an experienced
167 user to create a scenario that would represent any validation effort. The opposite approach is
168 to select one of the locations included in the FOCUS scenarios to proceed to a validation
169 procedure.

170
171 It should also be mentioned that efforts have been made to assess the usefulness of the
172 FOCUS selected simulation programs. Along these lines, Vanclooster (2001) reported a
173 pesticide leaching modelling validation effort undertaken in behalf of the EU by FOCUS. It is
174 thus very important to apply the results obtained by this group.

175
176 Moreover, there is the Apocop project which is intended to harmonise pesticide registration
177 within the EU i.e. by developing an agreed methodology on PEC calculation. The Apocop
178 project aims at validating the FOCUS procedure for PEC groundwater and identifying
179 alternative procedures which allow reducing the uncertainty associated with the actual
180 decision making rules.

181
182 The validation of local scale models, which are at the core of the risk analysis procedure, is to
183 be done by comparing results of local scale PEC models with data collected at six
184 experimental field sites. New procedures for preferential flow and pesticide volatilisation will
185 further be included in the models and thoroughly be validated. An attempt will be made to
186 validate scenarios, adopting a spatial approach, using simplified meta-models of pesticide
187 leaching in soils. It is envisaged to compile meta-models compatible with data available at the
188 European scale. For the PRA objectives a close collaboration with this group ought to be
189 proposed.

190
191 Last but not least another approach has been the development of GeoPEARL, which is the
192 regional-scale version of Pearl. Hydrological characteristics, land use, soil type, climate
193 district and soil chemical properties are considered. GeoPEARL may be soon available for
194 general use; this would have to be checked. It is expected that GeoPEARL will play an
195 important role in the new Dutch decision tree for evaluating the leaching of pesticides i.e. to
196 establish a more complete tier approach. It is, for example, possible to calculate the 80th
197 percentile of the leaching concentration from the simulated maps. Work with this program has
198 indicated that the traditionally used parameters (Koc and DT-50) cannot be considered as the
199 master variables in predicting the leaching fractions as it would be obtained from all FOCUS
200 recommended simulation models.

201
202 It has been reported that results with the following active ingredients have been conducted:
203 Atrazine (moderately mobile and moderately degradable), Bentazone (mobile and fairly
204 degradable), Dinoseb (mobile under basic conditions; immobile under acidic conditions;
205 moderately degradable), and Dichloropropene (fairly mobile; fairly degradable; volatile)

206
207 Considering the above information the following validation procedures are proposed for the
208 exposure part of the PRA

- 209 - select parameters according to their variability, uncertainty and influence on the
210 model outcome
- 211 - select geographical regions, and soils, similar to those proposed by FOCUS
- 212 - use FOCUS validation efforts to reduce the scope of work

- 213 - the product of a simulation program is the PEC in soil, surface and ground water.
 214 PECs obtained by PRA should encompass any monitoring or measured
 215 concentrations in high tier studies such as field dissipation studies, lysimetry, aquatic
 216 microcosm studies, other field studies. Thus selection of substances which include
 217 these high tier studies, and monitoring should be selected.
- 218 - Any PRA that is generated with 'worst-case' parameter values indicating a loss of wild
 219 life should be checked against event reports e.g. fish mortality, reduce diversity etc.
 220 since high level concentrations do imply that a clear effect would have been
 221 observed. This presupposes the use of simple GIS to match high probability exposure
 222 with clear effects.
- 223 - Even the most complex simulation program will be an approximation and thus we
 224 should just go for indications that support the use of the most sensitive parameters to
 225 assess exposure, the ultimate measurement is really the observed effects. That is, a
 226 simulation program may indicate high exposure but no effects can be observed or the
 227 other way around. This may be due to the chemical species which are actually toxic
 228 or which find their way to active sites. Since very little chemistry is applied in all these
 229 simulation programs they can only give an indication of the actual exposure.

230
 231 Another attempt to (functionally) validating fate models is the direct comparison of modelled
 232 and measured outcomes as described by Liess et al. (1999). The exposure through surface
 233 runoff can be modelled with an extended version of the "simplified formula for indirect
 234 loadings caused by runoff" (available from the OECD). Liess et al. (1999) used 18 small
 235 streams as the base of the simulation. and validation was performed contrasting the
 236 simulation to measured peak concentrations in the field.

237
 238 Exposure assessment for terrestrial vertebrates (mammals and birds) usually focuses on
 239 dietary exposure, which is calculated by combining estimates of the amounts of food
 240 consumed, the proportions of this food that are contaminated, and estimates of the levels of
 241 contamination (EU, 2002). Currently, estimates of contamination levels are obtained from
 242 empirical data (either generic, or specific to the pesticide in question) and are not predicted
 243 from environmental chemistry. There have been no attempts yet to validate these methods,
 244 but several possibilities exist:

- 245 • Food consumption is generally by combining allometric equations to estimate energy
 246 requirement with empirical data on the energy content of foods and the efficiency of
 247 energy assimilation by animals. In some cases, especially for low energy foods, animals
 248 are estimated to eat several times their body weight per day. There are concerns that
 249 these estimates are exaggerated, although they are confirmed in a few cases by more
 250 direct measurements. It would be desirable to test these estimation methods more
 251 thoroughly by conducting more experiments in which consumption is measured directly
 252 under controlled conditions.
- 253 • The most direct equivalent to measuring PECs for terrestrial vertebrates would be to
 254 measure ETE (estimated theoretical exposure, used as the numerator in the TER). This is
 255 challenging but possible, at least in certain scenarios. One possibility is to sample food
 256 items fed by parent birds to their young: this can be done without harm to the birds by a
 257 long-established technique using temporary neck collars to prevent swallowing. The food
 258 samples are very small but it has been shown that residues in single food items (insects
 259 or seeds) can be quantified using large-volume injection GC techniques (Brown et al., in
 260 prep.). The results could be used to derive a more direct estimate of ETE, which could be
 261 compared with estimates used in the normal regulatory assessment. This approach could
 262 also be used for more sophisticated investigations, such as testing whether the proportion
 263 of food obtained in treated areas is proportional to the time spent there (as is assumed
 264 when using radio-tracking data in the estimation of ETE). Implementing these types of
 265 validation studies would require a major field study (probably on multiple sites) for each
 266 pesticide/scenario to be investigated.
- 267 • Different methods are required to estimate ETE for granular pesticides. Birds may ingest
 268 granules accidentally with food, or intentionally as grit. Various methods have been
 269 proposed for modelling these intakes, but due to simplifying assumptions and limited input
 270 data the resulting estimates are highly uncertain. This may be critical to the assessment
 271 outcome because, in some cases, very small numbers of granules may be required to
 272 reach a lethal dose. It would therefore be desirable to test the predictions in semi-field or

273 field conditions, by measuring either ingestion or effects. Again this is challenging but
274 possible.

275

276 **2.2 Effects (validation on a community level using SSD)**

277

278 This chapter aims at the validation of the output of the SSD concept that is used in the
279 ecological risk assessment. For a thorough overview on the influence of species number,
280 identity and type of distribution model type on the validity of the outcome of SSD is referred to
281 Maltby et al. (2002) and Wheeler et al. (2002)

282

283 *2.2.1. Linking output of SSD with field observations*

284

285 This paragraph focuses on the field relevance of the output data of Species Sensitivity
286 Distribution (SSD) curves by seeking confirmation of lab-based SSD curves with population
287 responses observed in (semi-)field experiments. Two types of comparisons are made, namely
288 between full curve SSDs of laboratory and field toxicity data, and between SSDs based on
289 laboratory data and the magnitude and nature of effects in exposed field communities.
290 Attention is paid to the aquatic and terrestrial compartments and to chemicals with a specific
291 and a specific Toxic Mode of Action (TMOA). The chlorpyrifos and copper examples as given
292 in this paragraph are described in more detail by Van den Brink et al. (2002a).

293

294 Comparison between lab and field based SSD curves

295

296 Chlorpyrifos is an organophosphorus compound that displays broad-spectrum insecticidal
297 activity against a number of important arthropod pests. Because its molecule is nonpolar, it
298 has a low water solubility (2 mg/L) and a relatively high lipophilicity ($\log K_{ow}=4.7-5.3$).
299 Chlorpyrifos is a degradable compound, with hydrolysis as the most important process
300 (Racke, 1993). Because chlorpyrifos shows a field half life in water of < 0.08 to 2.4 days
301 (Racke, 1993), the SSDs were compared using acute toxicity data.

302

303 An overview of the ecotoxicological profile of the test substance was obtained using the
304 database AQUIRE (AQUIRE, 1998). Acute fish, algae and invertebrate EC50 values were
305 used to construct the lab SSD. Compared to chronic NOEC values, these data were available
306 in a much larger quantity. The EC50 values that we selected were all based on tests that
307 lasted between 2 and 4 days and having mortality, growth inhibition or immobility as effect
308 endpoint. When more than one value was available for the same species, the geometric
309 mean was calculated.

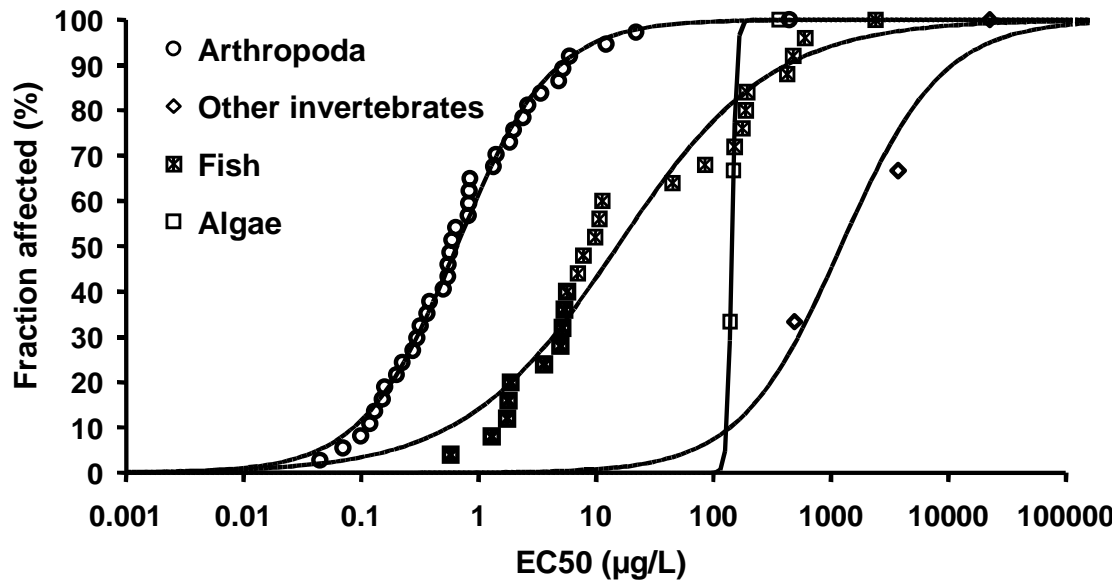
310

311 For compounds with specific modes of action, species may not be part of the same
312 distribution. For instance when considering an insecticide it can be expected that arthropods
313 will be much more sensitive compared to primary producers. Maltby et al., 2002 proposed that
314 therefore they need to be analysed separately. This separation is generally based on a
315 combination of information on TMOA and visual interpretation after plotting the data. Also the
316 endpoints are comparable for fish and inverts, but not for primary producers for which often
317 growth or photosynthesis inhibition is the endpoint.

318

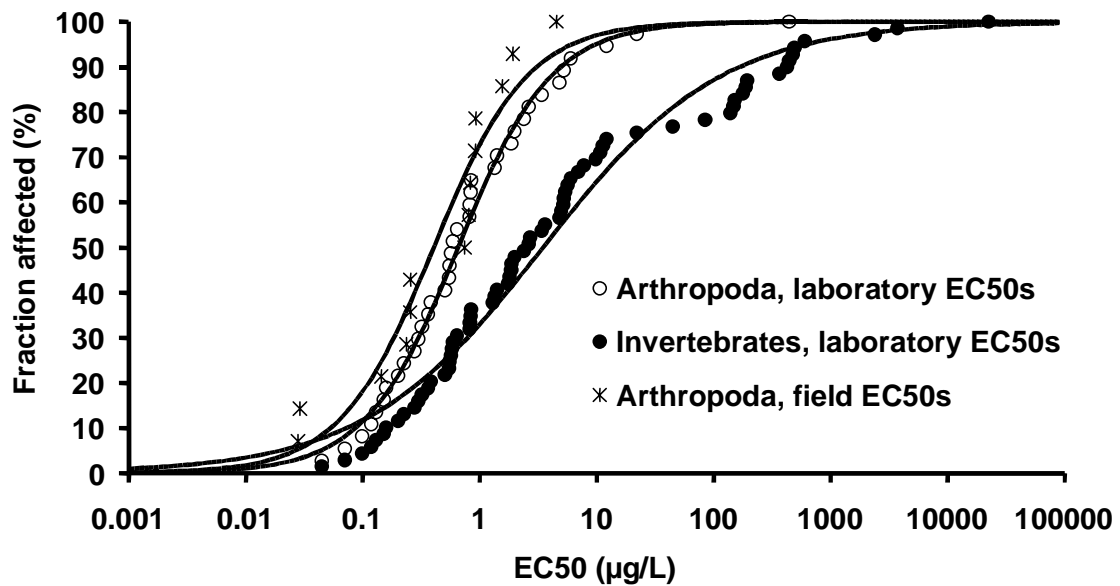
319 As could be expected from the specific TMOA of the compound, the lab SSD curves given in
320 Figure 1 show that arthropods are the most sensitive group, followed by fish. Algae and other
321 invertebrates are the least sensitive. The SSD curves for the various groups differ by two or
322 three orders of magnitude. This suggests that comparisons between lab SSDs and field SSDs
323 should be based on the taxon-specific curves, since the contributions of the species groups to
324 the local community may differ depending upon the circumstances (laboratory or field).
325 Moreover, this analysis tells us that it can be profitable in practical SSD applications to
326 distinguish between species groups prior to constructing a SSD curve.

327



328
 329 *Figure 1 Lab-based SSD curves for acute toxicity (individual points are EC50 in µg/L) for the insecticide*
 330 *chlorpyrifos, following the distinction of four groups of water organisms. Lines represent the logistic*
 331 *regressions on these data. For reasons of clarity, Figure 2 displays the SSD that has been constructed*
 332 *for all species simultaneously. Data obtained from AQUIRE (AQUIRE, 1998).*
 333

334 The experiment consisted of 8 experimental ditches that were sprayed with the insecticide
 335 chlorpyrifos, and of 4 other ditches that served as controls. The treatment levels 0.1, 0.9, 6
 336 and 44 µg/L were each applied to two experimental ditches. The ditches were 40 m long, 3.4
 337 m wide and 0.5 m deep, resulting in a water volume of 60 m³. The ditches were macrophyte-
 338 dominated. Detailed results of this experiment are described by Van Wijngaarden et al. (1996)
 339 and Van den Brink et al. (1996). The field EC50 values were calculated using the data
 340 obtained from samples taken 1 week after the application. A total of 59 different invertebrate
 341 taxa were identified at that time, 36 of which were arthropod taxa. It was possible to
 342 determine an EC50 for 14 arthropod taxa. These were subsequently used to construct a field-
 343 based SSD (see Figure 2). It was not possible to calculate EC50 values for the remaining
 344 arthropod taxa. In most cases, this was due to low abundance values and/or a high variability
 345 between replicates.
 346



347
 348 *Figure 2 SSD curves for chlorpyrifos based on arthropod sensitivity expressed by acute EC50 values*
 349 *collected from both laboratory tests and a semi-field test. The figure also shows the lab SSD curve*
 350 *based on all available EC50 values of aquatic invertebrates.*
 351

352 Figure 2 allows for a comparison between the lab-based and field-based SSD curves thus
353 derived for the arthropods. Arthropod taxa are almost equally susceptible to chlorpyrifos in the
354 laboratory and in the field, notwithstanding (amongst others) different exposure conditions
355 and the species contributing to the SSD curves. The 50th percentiles and the slopes are
356 almost the same (0.65 versus 0.40, and 1.09 versus 1.08, respectively). The latter indicates
357 an equal width of the distribution of the data. The difference of a factor 1.6 between the 50th
358 percentiles is well within the normal inter-experimental range (Rand and Petrocelli, 1985). In
359 this instance, lab-based and field-based SSDs can show a high similarity, one that may be
360 unexpected in view of the large number of unknown and uncontrolled variables. Note that this
361 pertains to a SSD comparison on laboratory and field data for a readily bioavailable
362 compound in water and acute toxicity to a specific group of sensitive organisms.

363
364 Figure 2 also shows the SSD curve based on all available laboratory data of invertebrates.
365 This curve is clearly distinct from both arthropod-based curves. The composite curve, based
366 on data from both sensitive and insensitive invertebrate groups, fits to a reasonable extent to
367 the data (statistically) in the lower tail of the curve, but it would clearly yield an under-
368 estimation of the field effects for expected sensitive endpoints beyond concentrations of
369 approximately 0.1-0.5 µg/L.

370
371 In conclusion, this case study can be interpreted as a confirmation of the usefulness of the
372 SSD concept based on laboratory data to represent field sensitivity. This means that for this
373 chemical, under more or less ideal conditions (i.e. minimal uncertainties) lab SSDs and field
374 SSDs are similar. It should be noted that optimal predictions are obtained when a sensible
375 division into taxonomic groups is made, one that reflects the knowledge on the TMOA of the
376 chemical.

377
378 Comparison between lab based SSD curves and the magnitude and nature of effects
379 observed in semi-field experiments

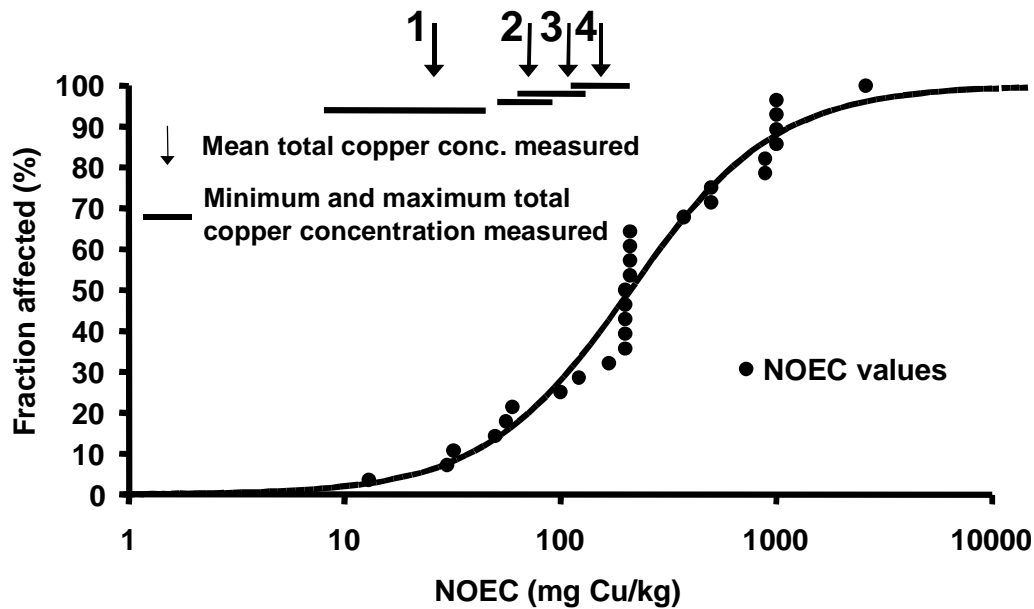
380
381 The most simple way to link laboratory based SSDs with effects observed in semi-field
382 experiments is the comparison of a threshold values based on this SSD curve (e.g. HC5) with
383 the safe concentration calculated from microcosm and mesocosm experiments
384 (NOEC_{ecosystem}). In 2000 Alterra has published an extensive review on the effects observed in
385 microcosm and mesocosm experiments (Brock et al., 2000a; b) which is used by several
386 authors to compare the outcome of this review with SSD derived safe-threshold values (Van
387 den Brink, 2001, 2002a; Brock et al., 2003; Maltby et al., 2002; Traas et al., 2002). All these
388 papers focus on the question whether the HC5 (originating from an SSD-analysis) was
389 sufficiently protective for community structure in aquatic mesocosms. Although partly based
390 on the same data these studies yielded the general pattern, that the HC5 was indeed lower
391 than the NOEC for community-level toxicity endpoints. This conclusion is also supported by
392 Emans et al. (1992), Okkerman et al. (1993), Versteeg et al. (1999) and Posthuma et al.
393 (1998 and 2002) using different studies. Below an example of a comparison also taking the
394 nature and magnitude of the effects into account. Other examples can be found in Van den
395 Brink et al. (2002a), Posthuma (1997) and Smit et al. (2002).

396
397 Copper is a heavy metal with many biological functions and its biological availability is also
398 determined by both substrate characteristics and the organisms' regulatory characteristics
399 (Peijnenburg et al., 1999). In view of the multi-year field study used in this paragraph, chronic
400 toxicity laboratory data were collected. All available laboratory toxicity data were used in a
401 single sensitivity distribution since it is hypothesized that Cu has no specific TMOA.

402
403 The data for the construction of the SSD curve for the effects of copper on terrestrial
404 organisms was obtained from the compilation of Crommentuijn et al. (1997). NOEC toxicity
405 values were found for 5 macrophytes, 1 nematode, 5 oligochaetes, 1 collembolan and 1
406 acarid mite. No geometric mean NOEC values were calculated from the multiple entries for
407 some species, in view of the range of soil characteristics present in the tests. Terrestrial
408 invertebrates and plants have apparently a similar sensitivity to copper (mean NOEC values
409 412 for invertebrates and 500 mg Cu/kg for plants), therefore a "mixed" curve was
410 constructed.

411

412 The resulting SSD curve is given in Figure 3. The 10th and 50th percentiles of the curve based
 413 on all NOECs available are 38 (95% CI: 32-44) and 210 (196-226) mg Cu/kg dry wt,
 414 respectively. The NOEC for the nematode *Caenorhabditis elegans* was on average 374 mg
 415 Cu/kg dry wt, although the NOEC ranges from 210 to 890 mg Cu/kg soil, depending on soil
 416 type and pH. The NOECs of this species fall well within the centre of the SSD curve, but are
 417 mentioned separately because the case-study pertains to copper effects on a nematode
 418 community.
 419



420
 421 Figure 3. Species Sensitivity Distribution (SSD) curves for copper chronic toxicity (individual points are
 422 NOECs) of terrestrial organisms. The sigmoid line represent the results of the logistic regression on
 423 these data. Arrows indicate the geometric mean copper concentrations of the copper treatment levels in
 424 the field study, the stripes indicate the minimum and maximum values measured at different pH levels
 425 in separate replicates (1 = nominal treatment equivalent to an original application of 0 kg Cu/ha 10 years
 426 before sampling; 2 = 250 kg/ha; 3 = 500 kg/ha; 4 = 750 kg/ha, see ranges in figure for measured levels
 427 in mg CU/kg).
 428

429 The field experiment with copper is described in detail by Korthals et al. (1996), who kindly
 430 provided the original data for the further statistical analyses required for this confirmation
 431 study. The case-study data pertains to a multi-year study on the chronic effects of copper on
 432 a natural nematode species assemblage in an agricultural field plot. The experiment
 433 consisted of a factorial design, studying the effects of copper treatment (0, 250, 500 or 750 kg
 434 Cu/ha) and pH manipulation (4.0, 4.7, 5.4 and 6.1). Each combination of the two treatments
 435 was applied to 8 agricultural plots (6*11 m), resulting in a total of 128 plots. Ten years after
 436 the treatment, the nematode community was sampled to investigate the results of long-term
 437 exposure. Exposure measurements were made at the time of sampling (Figure 3). Evident
 438 effects of both the copper treatment and the pH were found (Korthals et al., 1996), indicating
 439 adverse effects of copper on nematodes, and indicating that these effects are strongly
 440 influenced by the pH. Soil acidity probably influenced the response of the nematodes to
 441 copper, but probably also induced effects when no copper was added, as shown in the
 442 copper control treatments. For confirmation of the SSD concept, further analyses are needed,
 443 so as to disentangle the effects of copper and pH and to try to calculate a NOEC_{community},
 444 taking into account the pH regime. Furthermore, this NOEC has to be expressed as a total
 445 copper concentration, because the SSD curve could only be derived using total copper
 446 concentrations. To these ends, multivariate techniques, Principal Component Analysis and
 447 Monte Carlo permutation tests were applied (see Van den Brink et al., 2002a for a description
 448 of the methods).
 449

450 The comparison of the lab SSD with the field data shows that the control field soils apparently
 451 contained a total copper concentration that relates to the 1.5th and 12th percentile range of the
 452 lab SSD. This is associated with the background concentration of copper that was originally
 453 present, and that may have become available following reduced pH treatments (right side of

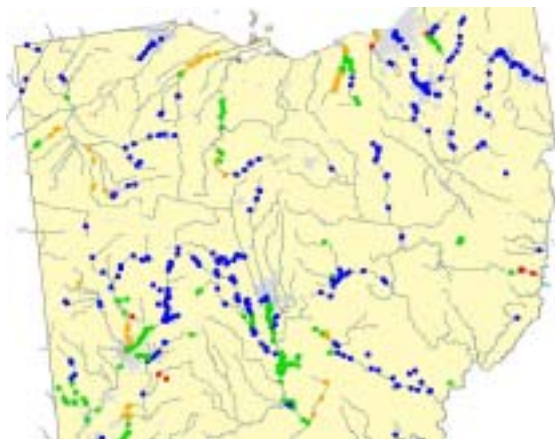
454 the control bar). In the control, at low pH, the effects would be predicted from the lab SSD.
455 Although a density reduction in the control was indeed found at a low pH, it is not clear
456 whether this directly (and only) relates to the release of copper or other metals, as direct pH
457 effects cannot be excluded as an alternative explanation.

458
459 The second treatment level indicates a concentration slightly higher than the community
460 NOEC. Comparison of the community NOEC (somewhat lower than 64-77 mg total Cu/kg)
461 and the lab SSD curve, irrespective of pH, suggests that community effects are evident and
462 concurrent with the steep rise of the SSD curve, in the same concentration range. The
463 concentrations of 64 and 77 mg Cu/kg, at which transient effects were recorded, correspond
464 with the 18th and 22nd percentile of the SSD curve based on all NOEC data. Apparently,
465 despite the large uncertainties regarding the representativeness of the laboratory data for the
466 field community with respect to actual exposure, the start of the community response is
467 relatively well predicted by a low percentile of the lab SSD curve.

468
469 Comparison between lab based SSD curves and the presence of species in the field.

470
471 In this study, based in Ohio and worked out by RIVM, P&G and the University of Utah, a large
472 data set on abiotic characteristics of surface waters and the occurrence of fish species in the
473 US-state of Ohio is being investigated by predictive models. One of the models is the SSD-
474 approach, which is used to compile the concentration data of an array of compounds into so-
475 called ms-PAF values (ms-PAF = multi-substance Potentially Affected Fraction, a
476 dimensionless value). These ms-PAFs can be considered estimates of the relative toxic
477 pressure of the local mixture at each of the sampling sites. The first result was that the ms-
478 PAF shows a signal in the data set: fish species that occur at the different sampling sites are
479 likely differently exposed to truly effective toxic mixtures of different strength. This first result is
480 being considered as to its relevance in predicting fish occurrence and density in relation to all
481 other stress factors, such as geological parameters (e.g., latitude, longitude, slope), other
482 water chemistry attributes (e.g., pH) and proportion effluent. The presence of these factors
483 means that the effects of the toxic compounds (the signal) are not the sole stress factor
484 determining the local communities. To address this, it was investigated whether the fish
485 occurrences and densities were statistically associated to all stress factors (including ms-
486 PAFs as estimates of toxic pressure). As a second result, it appeared that (amongst the other
487 factors) the local toxicant mixtures influenced species densities. This means that the
488 dimensionless estimate of risk (ms-PAF) contributes to species occurrence and density in
489 field communities.

490
491 Currently (march 2003), the data and findings are summarised in a draft manuscript (De
492 Zwart et al., in prep.), in which the types of graphs will show up as provided in Figure 4 and 5
493 (example Figures only shown for illustrating the general idea):
494



495
496 *Figure 4. Geographical Information System (GIS) map of ms-PAF values over Ohio (colours*
497 *indicate ms-PAF level)*
498



499
500

501 *Figure 5. GIS-map of pie sizes (indicating the magnitude of effects compared to a set of*
502 *reference sites) and slice sizes (identifying the contribution of different stress factors to the*
503 *local community composition, colours indicating the stress factor of importance).*

504

505 When the analyses are finalised, the research team will further investigate the statistical
506 association between ms-PAF and the occurrence of fish species, and whether this statistical,
507 eco-epidemiological association makes sense when confronted with the species'
508 autecological features.

509

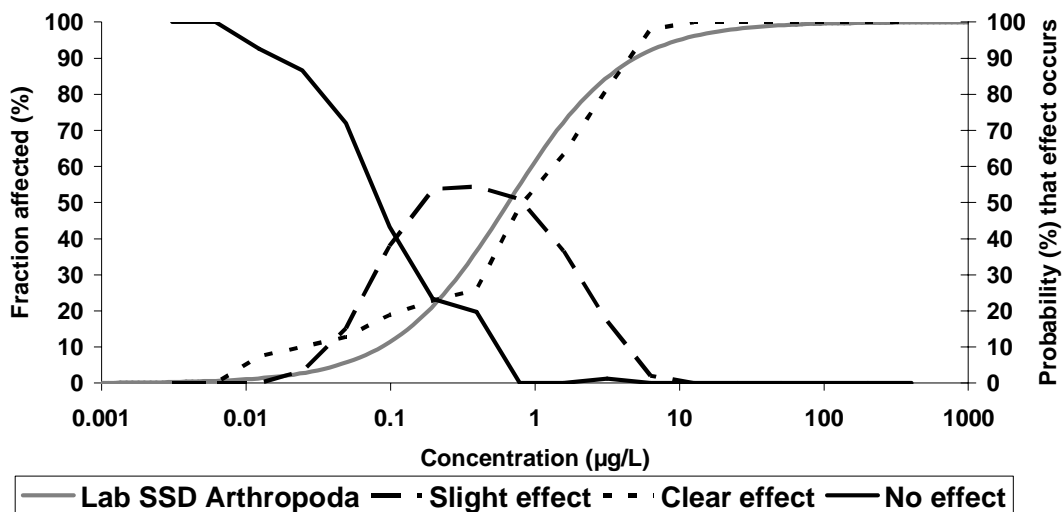
510 In summary, the work aims to elucidate the role of toxic compounds in shaping field
511 community composition, with emphasis on validating the SSD-concept, with emphasis on the
512 idea that the data can be summarised in a way that can easily be understood by risk
513 managers (pies and slices).

514

515 *2.2.2 Linking the output of SSD with prediction of the probabilistic model* 516 *PERPEST*

517

518 PERPEST (Predict the Ecological Risks of PESTicides) is an effect model that predicts the
519 effects of a certain pesticide on various (community) endpoints (Van den Brink et al., 2002b).
520 In contrast to most effect models PERPEST is based on real data. For this a literature review
521 of freshwater model ecosystem studies with insecticides and herbicides was performed to
522 assess the NOECecosystem for individual compounds, and to evaluate the ecological
523 consequences of exceeding these standards (Brock et al., 2000a, b). Effects on various
524 endpoints (e.g. community metabolism, phytoplankton, macro-invertebrates) were classified
525 according to their magnitude and duration. This literature review resulted in a case base
526 containing the effects of 18 herbicides and 21 insecticides. In total 90 experiments (42
527 herbicide, 48 insecticide) were evaluated, resulting in 317 cases (155 herbicide, 162
528 insecticide). This case base was used to construct the PERPEST model. In this model one
529 can enter the relevant properties of the compound, concentration and type of ecosystem to be
530 evaluated. The PERPEST model searches for analogous situations in the case base and
531 calculates a prediction using weighted averaging of the effects reported in most relevant
532 literature references. PERPEST results in a prediction showing the probability of effects on
533 the various groups. The model is integrated in a user-friendly interface and its first version is
534 available via the Internet (check www.perpest.alterra.nl after 1 July 2003). For this Work
535 Package the outcome of PERPEST can be compared with the risk assessment based on
536 uniform principles and the Species Sensitivity Distribution concept (see Figure 6 as an
537 example).



538
539 *Figure 6. Laboratory SSD for Arthropods (see Figure 1 for explanation) and the probability of*
540 *no, slight or clear effects on micro-crustaceans as predicted by the effect model PERPEST.*

541
542 **2.2.3 Validation attempts for terrestrial vertebrates**

543
544 In principle, data from field studies and wildlife incident reports could be compared with
545 estimates of acute lethal effects for birds and mammals. Previous attempts to do this have
546 been mostly comparisons of deterministic risk assessments with field data for particular
547 pesticides. The most sophisticated analysis to date was by Mineau (2002). He compiled a
548 large database of field studies and incident reports (mostly from North America) and derived
549 for each one an index of effects. He then used regression analysis to estimate the
550 relationships between the index of effects and various predictors including oral and dermal
551 toxicity. This is empirical derivation of assessment models, rather than validation. However,
552 the same dataset could be used for validation, by comparing the index of effects in the field
553 studies with risks predicted by probabilistic models. This approach will be attempted in the
554 "WEBFRAM 3" project funded by Defra. In making comparisons of this type, it is important to
555 take account of the limitations of the field data (e.g. under-reporting) and to ensure that the
556 model predictions are relevant to the field scenarios to which the data refer.

557
558 Existing data are not suitable for validating predictions of chronic or sublethal effects on birds,
559 or any effects on mammals, because very few field studies have attempted to quantify these
560 effects and they are not detected by existing monitoring schemes. Although extensive data
561 exist on population trends for birds, these are not useful for validation because of the extreme
562 difficulty of separating any influence of pesticides from the many other stressors affecting
563 birds. Although new studies could be designed specifically to validate probabilistic pesticide
564 assessments, these would be extremely expensive (because of the methodology needed to
565 detect effects reliably, and the large numbers of sites and scenarios that would be needed).

566
567 **3. Opportunities for validation in the ongoing research of the**
568 **authors.**

569
570 Alterra Green World Research is currently expanding the 2.2.2.1 validation studies with
571 fungicides (financed by DEFRA, UK in co-operation with Cranfield University, UK; Ponds
572 Conservation Trust, UK; UFZ Centre for Environmental Research, Germany and University of
573 Sheffield, UK), and writing several papers using the validation procedures described in 2.2.2.1
574 and 2.2.2.2

575
576 UFZ Centre for Environmental Research, Germany is currently working to extrapolate the
577 approach described in 2.2.1 towards a higher level of geographical scale:

- 578 1. Using data of measured exposure and effect (invertebrates) on 19 streams in an
579 agricultural landscape. Investigations have been carried out during several

580 successive years. Spatial variation of exposure and recovery is addressed.
 581 Landscape properties (like recovery potential due to existence of less effected
 582 populations) are adding to an integrated risk assessment.

583 2. Using data on modelled exposure and measured effect (invertebrates) on 110
 584 streams in an agricultural landscape. Investigations have been carried out during
 585 several successive years. Spatial variation of exposure and recovery is addressed.
 586 Landscape properties (like recovery potential due to existence of less effected
 587 populations) are adding to an integrated risk assessment.

588 3.

589

590 The RIVM is exploring work, subsequent to the Ohio case, regarding the eco-epidemiological
 591 association between stress factors (amongst which toxicants) and the occurrence of species.
 592 RIVM is engaged in ongoing research for RIVM and DEFRA develop and validate statistical
 593 methods to calculate assessment factors for risk assessment (Luttik and Aldenberg, 1997 and
 594 Aldenberg and Luttik 2002). For risk assessment based on a few toxicity data, it makes
 595 statistical sense to 'borrow' the characteristics of the sensitivity distribution from well-studied,
 596 similar compounds. The distribution characteristics can be calculated from toxicity data from
 597 large databases such as EPA's Aquire and RIVM's Ecotox-DB. Validation research will focus
 598 on validating extrapolation factors with new datasets not incorporated in those used for
 599 calculating the statistical constants.

600

601 Recently the DEFRA funded Web-integrated framework for addressing uncertainty and
 602 variability in pesticide risk assessment (WEBFRAM) project started. The objectives of this
 603 project are:

604 1. To review, evaluate and recommend the most appropriate existing
 605 mathematical/statistical approaches to addressing uncertainty and variability.

606 2. To develop and evaluate a generic model framework that incorporates uncertainty
 607 and variability into the assessment of pesticide risks to nontarget species.

608 3. To develop fully web-integrated software, by web-enabling the generic framework and
 609 a suite of specific models. The mathematical models and their supporting data will be
 610 provided by other projects addressing risks to different taxonomic groups (aquatic
 611 organisms, terrestrial vertebrates and invertebrates).

612 4. To establish a Coordinating Committee and effective means of electronic
 613 communication to ensure efficient interaction and exchange of results between this
 614 project and the other projects which will provide the specific models and data for web-
 615 enabling.

616 This approach will enable all the models to be web-enabled to a consistently high quality, and
 617 provide a fully harmonised interface for end-users. It will be much more effective than a
 618 collection of models brought together after being web-enabled in separate projects. It
 619 proposes to use the Microsoft .Net. programming platform, commonly referred to as "DotNet",.
 620 This currently defines the state of the art in programming platforms and offers unparalleled
 621 control, power, and flexibility in the development of web applications. Other platforms could
 622 be used if required by DEFRA. The results of this research will be used in the statutory
 623 pesticides approvals scheme and will contribute directly to the Government's objectives of
 624 assessing and minimising the risks that pesticides present to the environment and non-target
 625 species.

626

627 **3. Plan for testing consistency in the use of draft guidance by different users.**

628

629 As mentioned in the Introduction, it is also important that any guidance and tools developed
 630 for probabilistic assessments are designed to maximise consistency between different users,
 631 which may include users in different countries and individuals with differing levels of specialist
 632 expertise. This will be addressed during the end-user testing phase of EUFRAM. First
 633 proposals for the approach are as follows:

634

| | |
|---------------|---|
| December 2004 | End-user workshop 1, attended by about 70 prospective end-users including regulatory scientists from all EU-member states and industry scientists. The workshop will include basic training in the use of the draft guidance produced by EUFRAM, and any software and database tools recommended by EUFRAM. |
|---------------|---|

| | |
|---------------------------|---|
| January - September 2005 | End-users will be encouraged to try out the guidance and tools in their own organisations. By May 2005 (?), end-users will be sent a small number of example assessments (probably fictional) developed by WP9, and asked to carry out the assessments and return the results. WP9 will carry out a simple analysis of the results, focussing on (a) consistency of assessment outcomes and (b) consistency in applying key aspects of the guidance from other WPs. Reasons for inconsistencies will be identified. |
| October 2005 | End-user workshop 2. As part of this, WP9 will present their analysis of consistency. Modifications to improve consistency will be agreed if necessary, and implemented in a revised version of the EUFRAM guidance. |
| November 2005 - June 2006 | End-users will again be encouraged to try out the (revised) guidance and tools in their own organisations. The consistency assessment exercise will be repeated with new examples if necessary (either to gain additional responses, or to test improvements to the framework). |
| July 2006 | End-user workshop 3. As part of this, WP9 will present their finalised analysis of consistency. Any further modifications to improve consistency will be agreed if necessary, and implemented in the final version of the EUFRAM guidance. |

635

636

637

4. Specific recommendations for new validation studies for future funding.

638 Although it was felt by some members that the set-up and interpretation would be very
639 difficult, a possible new validation study could consist of the collation on field monitoring
640 studies within Europe. To complement such a dataset (which will be incomplete to a high
641 degree) it is suggested to conduct monitoring studies in selected regions within Europe. Such
642 regions should be selected according their ability to characterize landscapes typical for the
643 diversity of European landscapes. A first hint on such a selection might be given by the
644 scenarios identified by the FOCUS surface water group (see also paragraph 2.1). Certainly
645 geographic specialities should be included. Examples are far northern landscapes with short
646 summers and low temperatures, Mediterranean landscapes with high temperatures and
647 several streams drying in summer, near natural landscapes which can be found frequently in
648 several new member states from the east of Europe. These data on field communities should
649 be linked to the exposure measured and modelled in these areas, taking into account the
650 specific environmental parameters for each region. It was felt that it might be wise not to do
651 large monitoring programmes but to choose 1 to 3 substances with different modes of action,
652 2 different uses (crops), and different physical-chemical properties and monitor these
653 scenarios during one or two seasons. The main problem raised by the work package
654 members is that peak concentrations are important but rarely seized by monitoring that is
655 done weekly and the amount of resources needed to perform this monitoring program.

656

657 Other proposed experiments building on current research is that is focussed on validating
658 predictions of community effects in the (semi-)field from laboratory based SSDs, but that the
659 studies are especially set up for the purpose.

660

661 For terrestrial invertebrates the PRA approach can be validated in several different ways. As
662 mentioned in paragraph 2.2 the obvious way is to generate laboratory SSD's for a range of
663 taxa and then compare with field observed effects. A considerable body of data exists within
664 the industry and some of this could be made available. In addition to this the 'Ecotoc limited'
665 facility has toxic reference data with dimethoate and some with cypermethrin (LR50 values
666 with many different invertebrate species) and field results from about 8 full fauna studies in
667 cereals and orchards showing effects at different rates. These could be collated and put
668 together to show whether they support the validity of PRA given what is available. Generating
669 data to fill the gaps in such data, either with additional taxa LR50 values or with multi-rate full
670 fauna field studies is needed to expand this approach.

671

672

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