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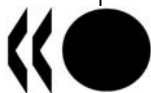
English - Or. English

**ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**POSSIBLE APPROACH FOR DEVELOPING VALIDATED DATA FOR ESTIMATING EXPOSURE
OF USERS OF BIOCIDAL PRODUCTS
FACTORS, ORTHOGONAL EXPERIMENTS AND PROBABILISTIC MODELLING**

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OECD Environment, Health and Safety Publications

Series on Biocides

No. 2

**POSSIBLE APPROACH FOR DEVELOPING
VALIDATED DATA FOR ESTIMATING EXPOSURE
OF USERS OF BIOCIDAL PRODUCTS
FACTORS, ORTHOGONAL EXPERIMENTS AND
PROBABILISTIC MODELLING**

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**INTER-ORGANISATION PROGRAMME FOR THE
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Paris 2009**

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- harmonise their pesticide review procedures,
- share the work of evaluating pesticides, and
- reduce risks associated with pesticide use.

The Pesticide Programme is directed by the Working Group on Pesticides, composed primarily of delegates from OECD Member countries, but also including representatives from the European Commission and other international organisations (*e.g.* United Nations Food and Agriculture Organization, United Nations Environment Programme, World Health Organization, Council of Europe), and observers from the pesticide industry and public interest organisations (NGOs).

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The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, FAO, WHO, UNIDO and the OECD (the Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. UNITAR joined the IOMC in 1997 to become the seventh Participating Organization. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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or contact:

**OECD Environment Directorate,
Environment, Health and Safety Division**

**2, rue André-Pascal
75775 Paris Cedex 16
France**

Fax: (33-1) 45 24 16 75

E-mail: ehscont@oecd.org

**Possible Approach for Developing Validated Data for Estimating Exposure
of Users of Biocidal Products**

Factors, Orthogonal Experiments and Probabilistic Modelling

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1. INTRODUCTION

1. This document elaborates on a procedure how data in a validated form can be gathered by companies who need estimating the exposure of users of biocidal products for regulatory purposes. The validity of data is - as it has been made clear in the European Commission Report "Technical Notes for Guidance: Human exposure to biocidal products - Guidance on exposure estimation"- a substantial requirement in order to be able to adequately evaluate risks for human health through the use of biocidal products or as a consequence thereof. This document should be seen as a 'living' document that can be complemented to take account of new information that becomes available.

2. The method described in this document represents only one approach for generating data and estimating exposure of users to biocidal products. An orthogonal factorial study design in combination with a probabilistic approach is in certain cases preferable. With regard to biocides there is a tendency to collect exposure data by means of experiments with an orthogonal design to be efficient in the way measurements are done and in the collection of information about (possible) important influencing factors. This approach may not be appropriate in all circumstances and the present document should not be considered as a recommendation from OECD to use this method exclusively.

3. The procedure described in the document was initially developed for estimating the exposure of users of wood preservatives painting a fence. This use was chosen just as an example of the many biocides on the market because of the variety of different use patterns and types of users. However, the procedure is equally applicable to all products and users.

4. Many different factors may contribute to the variability of exposure, including the handling of the product/substance by the user, the equipment used, the environmental conditions and also the time elapsed between jobs. Therefore generating statistically valid data in a randomised way without controlled factorial variation requires a very high number of single experiments and volunteers/users.

5. The goal of the document is to assist researchers performing human exposure assessment for biocides. It specifically aims at designing statistically sound studies with fewer measurements. Using an orthogonal factorial design in combination with a probabilistic modelling approach allows fewer experiments to be performed with all relevant variables explored, thus resulting in lower costs and thereby permits an economical and scientifically sound gathering of exposure data. The principles of the experimental design and of the statistical analyses for such a procedure are described in this document.

6. This document describes a procedure which could be used for conducting research on human subjects. It is important for the investigator to treat all subjects in the research ethically. Many individual countries will have detailed ethical requirements applicable to the conduct and reporting of such research. For further information, the investigator is encouraged to contact the appropriate regulatory authorities of any country in which such research may be conducted or to whom the results may be submitted.

2. BACKGROUND

7. The actual exposure depends on many influencing factors (determinants of exposure). Valid data representing the exposure has to take into account all possible realisations (combinations) of these determinants of exposure. When measuring exposure in a randomised way, the following limitations show up:

- Many realisations are necessary.

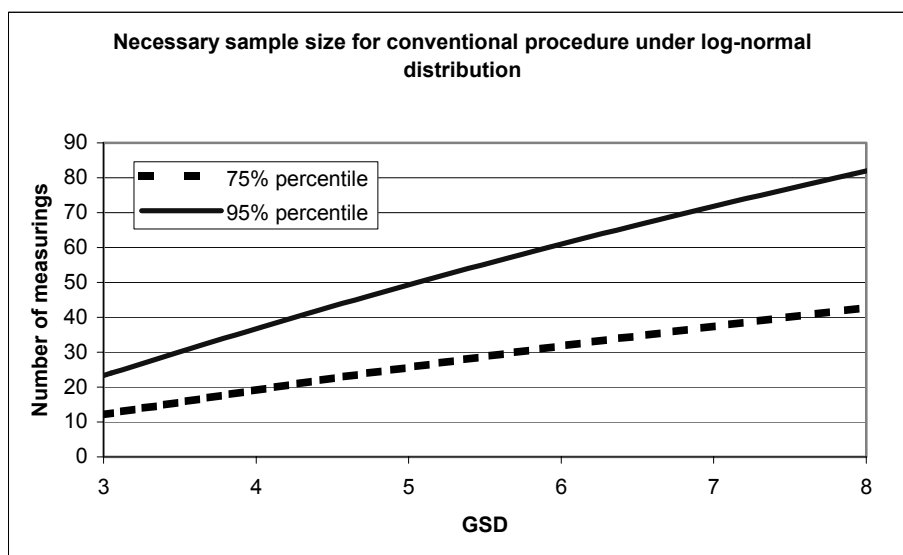
- Available realisations may not agree with the distribution of the ones possible; therefore, the data are not valid.
- Boundary conditions are unknown; therefore, the data are not reproducible.

8. A factorial statistical design offers ways to counteract these limitations:

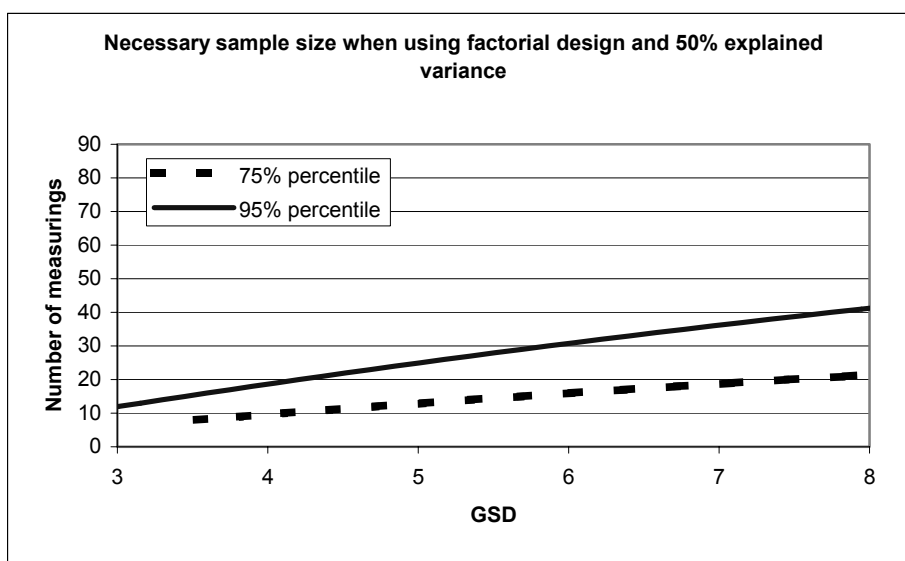
- The realisations are divided systematically into subsets (stratification); thereby the data become reproducible. However, this is only possible when influencing factors are known.
- The influencing factors are analysed at only a small number of levels (often two) and these levels are selected in the form that the complete parameter range is covered; thereby the data become valid. Dichotomizing data, however, results in loss of information.
- Statistical analysis of the data reveals the relevant influencing factors and an empirical model for the exposure estimation can be derived. However, all relevant influencing factors should be measured. When one (or multiple) relevant factors are not measured, a proper empirical model cannot be derived.

9. A factorial statistical design can reduce the number of necessary measurements considerably. As an example for determining the minimum sample size, it is assumed that the deviation of the empirical percentile value from the true value by more than 100% upwards and 50% downwards should be avoided with a statistical certainty of 95%. The statistical analysis shows that the number of necessary measurements depends on the standard deviation. The higher the dispersion, the higher the number of measurements has to be: in the typical interval of 3 to 6 of GSD (Geometric Standard Deviation: $GSD = \exp(\text{standard deviation of the logs})$), e.g. for 75th percentile at the most 32 measurements are necessary to fulfil the requirements. To determine the 95th percentile with the same precision even 60 measurements at the most are necessary, as is shown in Figure 2-1.

Figure 2-1: Sample size for conventional procedure



10. Based on an orthogonal factorial design, a decrease of the variance by approx. 50% is possible with an adequate choice of factors even under unfavourable circumstances; in this way the number of measurements can be reduced by about a half. In the above example, 30 measurements would be sufficient to obtain the desired precision for the 95th percentile, as is shown in Figure 2-2.

Figure 2-2: Sample size for *factorial* design of experiments

11. According to these relationships, an orthogonal factorial design may even reduce the required sample size by more than 50%. Furthermore, considerable reduction of the sample size is possible when several products/substances are investigated in one study.

3. DESCRIPTION OF THE PROCEDURE

12. The procedure can be roughly divided into the following steps:

1. Definition of the experimental design (section 3.1)
2. Selection of volunteers/user and products/substances (section 3.2)
3. Realisation of experiments (section 3.3)
4. Statistical analysis of results (section 3.4)
5. Probabilistic modelling (section 3.5)

13. These steps are explained in the following sections. For each section an example taken from selected studies on human exposure on wood preservatives is added (see Annex 1). For more details, the reader is referred to the reports in the references.

3.1 Definition of the experimental design

3.1.1 Selection of measurands (exposure figures) and analytical methods

14. An expert panel should discuss and select relevant measurands (to record and report exposure in relation to other units, e.g. time, weight, volume or area) for the assessment of the exposure. The definition of the measurands comprises the exposure related to the selected item (volume, body part) to be tested. At the same time a comprehensively validated analytical method should be available. An example of the assessment of dermal exposure is presented in Annex 1, example 1.

15. Feasibility of the analytical method should be studied. The analytical method should be validated under Good Laboratory Practice (GLP). In order to validate the analytical method, samples are analysed under different conditions at different concentration levels (e.g. 0.05, 1, 5 and 50 µg/kg). These levels cover the range of measured values expected in the exposure experiments. An example of the factorial design of the experiment and the results for Propiconazole are presented in example 2 of Annex 1.

16. The validation parameters recovery, relative in-house reproducibility and repeatability standard deviations can be calculated with the validation software InterVal (6). As an example, the results of the calculations with the validation software InterVal (6) are shown in Annex 1, example 3.

3.1.2 Selection of factors and factor levels

17. An expert panel should discuss and select relevant factors and factor levels influencing the exposure.

18. Factors should be defined in a reproducible way and characterise:

- the application form (kind of objects, where/on which the product/substance is intended to be used, what kind of applicator, workplace conditions etc.);
- the product/substance (physical and chemical properties, etc.);
- the volunteer/user (sex, height, physical conditions, etc.);
- a set of variable conditions for the behaviour of volunteers/users (e.g. one condition is to focus on speediness, another one on clean working).

19. Factor levels are selected according to the criterion of representativeness, i.e. the levels need to be chosen in order that a representative empirical model can be derived from the results of the study. In this way, the factor levels should characterize the whole range of realisations for a realistic worst case that is to be expected. Factors with two levels only are to be preferred.

20. Factors which are controlled in the experiment are also referred to as primary factors. In addition to the primary factors, quantitative and qualitative secondary factors may be defined. Secondary factors are not planned and vary within the study like primary factors, but they are useful for getting a better understanding of the dependency of the exposure on different factors, e.g.

- the amount of the product/substance used;
- the duration of each job;
- physical status of the volunteers/users (e.g. heart rate);
- environmental conditions not controlled in the experiment.

21. These secondary factors cannot be controlled directly in the experiment, but may affect the exposure considerably. Secondary factors can especially be important for calculating different response variables like exposure level per time unit, exposure level per volume of product/substance. The accurate determination of secondary factors within the experiments is crucial to be able to use them adequately in the statistical analysis.

22. If necessary, preliminary tests should be carried out, e.g. in order to determine the levels of exposure to be expected, how long one job (i.e. realisation of the work to be done) will take, if the experimental arrangements are feasible and if the determination of the secondary factors can be realised in the way it was planned.

23. For the analysis of exposure levels, the primary factors and factor levels which have been surveyed for relevance within the preliminary tests are used in the main study. In Annex 1, example 4 some examples are presented taken from the studies already referred to regarding

1. the application,
2. the product,
3. the volunteer/user,
4. the settings for the volunteers/users and
5. the list of selected secondary factors.

3.1.3 Definition of the orthogonal experimental design

24. Even if the number of primary factors, k , in a design is small, the 2^k experiments specified for a full factorial design can quickly become very large. For example, for a two-level, full factorial design with 10 factors $2^{10} = 1024$ experiments would be needed.

25. The solution to this problem is to use only a fraction of the experiments specified by the full factorial design. This fraction should be chosen so that all main effects and all relevant interaction effects are orthogonal. With 10-15 factors not more than 32 or 64 experiments are required. In order to guarantee a minimum level of confidence, the total number of experiments should not be below 32.

26. Orthogonal experimental designs are particularly suitable, since they provide a maximum of information regarding factorial effects while requiring a minimum effort of experiments. An orthogonal design exhibits for two factors exactly the same number of all possible factor level combinations. E.g., for two factors A and B, the combination A+ and B+ appears as often as the combinations A+ and B-, A- and B+, as well as A- and B-.

27. After defining the experimental design, it should be randomised in order to avoid time trends. An example of an orthogonal experimental design is given in Annex 1 (example 5)

3.2 Selection of volunteers/users and products/substances

3.2.1 Selection of volunteers/users

28. Potential volunteers/users are divided into subpopulations which meet the criteria defined by the

chosen factors and factor levels. Volunteers/users of the different subpopulations have to be selected randomly (stratified random sampling). Volunteers/users should not only come from the local area, but be a cross-section of the population.

29. The minimum number of volunteers/users required is 8. If the minimum number of experiments (32) is carried out, this means that each volunteer/user has to carry out 4 jobs, i.e. each volunteer/user carries out the same job under different conditions. In this case it is crucial that these different conditions comprise a relevant set of variable conditions for the behaviour of the volunteers/users, in other words, appropriate factors and factor levels for the control of the behaviour of the volunteers/users (e.g. one condition is to focus on speediness, another one on clean working). If no factors for the behaviour of the volunteers are taken into account, the minimum number of volunteers/users required is 16.

30. In all steps, the ethical principles of the Helsinki Declaration for research involving human subjects have to be taken into account (9). An example of the criteria for selecting volunteers is given in Annex 1, example 6.

31. Instruction to volunteers is an essential part of the study protocol. In observational studies it is important that the volunteers do not know the reason for the observation.

32. The volunteers/users are invited for a personal interview, in which intention and procedure of the study as well as the product and its safe handling are explained in detail.

33. The ethical principles of the Helsinki Declaration are applied. If all the criteria are met, the volunteers are accepted for the study after special instructions regarding safety at work and confidentiality.

3.2.2 Selection of products/substances

34. The ideal case is that the primary factors are defined before characterising the products/substances completely. For test products to be tested only real marketed products are used. Thereby, it will not be possible to select several products that differ only regarding the primary factors but not in other properties. However, it is very important to gather as much information as possible on the properties of the products/substances, like density, viscosity and solid body content. In Annex 1, an example is given (example 7).

3.3 Realisation of experiments

35. The form of the jobs as well as the experimental design shall not be changed during the study. Each job should be observed for noticeable incidents to be documented (e.g. spilling of the product). Additionally, it is recommended to record each experiment on video in order to be able to detect individual performance attitudes and potential outliers retrospectively. An example is given in Annex 1 (example 8).

3.4 Statistical analysis of results

3.4.1 Determine response

36. Generally, exposure levels are being logarithmised for the statistical analysis, although this is not mandatory. An example is given in Annex 1 (example 9).

3.4.2 Explorative analysis

37. In a first explorative analysis, statistical parameters as arithmetic mean, median, standard deviation, minimum, maximum, geometric mean and geometric standard deviation should be determined separately for each body part analysed. Also – again separately for each body part analysed - a histogram of the measured values can be taken into account in order to obtain a first impression of the distribution of experimental data.

38. The statistical parameters mentioned cannot be used to characterise the exposure distribution under actual conditions but describe only the exposure figures under specifically designed experimental conditions. The purpose of the explorative analysis is to identify outliers and implausible values. Discrepancies in the data and outliers should be re-checked (see section 41 for further information).

39. If several parts of the body have been analysed separately, the scatter plots between the measured values of those parts should be determined. These scatter plots can give hints on the causes of outliers and discrepancies. An example is given in Annex 1 (example 10).

3.4.3 Statistical model

40. The statistical model describes the relationship between the response (e.g. the log exposure per minute) and the relevant factors and the distribution to be expected. As the volunteers/users in most cases affect the response randomly, a linear model with stochastic design has to be established. Such a model considers not only systematic effects from primary or secondary factors, but also random effects (effects that are not covered by primary factors). The volunteer/user contributes to the exposure not only by systematic effects, but also by random effects.

41. The statistical model should be selected according to the criteria of sparsity and parsimony, i.e. the number of parameters to be estimated in the model should be as small as possible. Testing of parameters should take into account the stochastic nature of the model (mixed model). The same approach should be used for estimating the parameters. Outliers should only be eliminated from the data when there is an analytical error or an error in performing the experiment. They should not be eliminated only on the basis of an outlier test. An example is given in Annex 1 (example 11).

3.4.4 Significant differences

42. If different products/substances with different characteristics are used in the experiments and if these characteristics are not considered in the primary factors, the exposure data of each of the products/substances should be analysed separately. After statistical models have been established for each product, a t-test or a Welch-test should be carried out in order to identify significant differences of effects of primary factors.

43. If no differences can be identified, the data can be combined and a statistical analysis of all data has to be carried out.

44. In the joint statistical analysis it has to be checked whether the exposure level is significantly different for the different products. If this is the case, a random product/substance factor has to be incorporated in the model. An example is given in Annex 1 (example 12).

3.4.5 Interactions

45. Apart from the assessment of the main effects of the factors, also 2-factor interactions have to be examined. These interactions should be judged critically for their relevance. If the two factors involved in the interaction are not statistically significant themselves, in most cases the respective interaction should be excluded from the statistical model (see Annex 1, example 13). For assessing the relevance of significant interactions, an expert panel should be consulted.

3.4.6 Influence of primary factors on secondary factors

46. By the same generalised linear model approach, the effects of primary factors on secondary factors can be estimated. This may lead to a simplification of the model if the individual secondary factor is relevant for exposure. An example is given in Annex 1 (example 14).

3.4.7 Validation of model

47. Within the validation of the statistical model, the residuals (deviations between exposure figures for the single jobs and the respective exposure figures calculated from the model) of the model have to be inspected by means of a residual analysis.

48. Scatter plots of the residuals versus the factors in the model and versus potential factors that are not included in the model are the primary plots used to assess sufficiency of the functional part of the model (see figure 3-2 in example 15, Annex 1). Plots in which the residuals do not exhibit any systematic structure indicate that the model fits the data well. Plots of the residuals that exhibit systematic structure indicate that the form of the function can be improved in some way.

49. Similar to their use in checking the sufficiency of the functional form of the model, scatter plots of the residuals are also used to check the assumption of constant standard deviation of random errors. Scatter plots of the residuals versus the factors and versus the predicted values from the model allow comparison of the amount of random variation in different subsets of the data.

50. The histogram of the residuals are used to check whether or not it is reasonable to assume that the random errors inherent in the exposure process have been drawn from a normal distribution or at least from a symmetric distribution. If the random errors are not symmetric, exposure levels computed with the model can be biased.

51. In addition to the above-mentioned scatter plots, scatter plots of the residuals of the different body parts analysed are used to assess the correlation. High correlation of residuals between different body parts indicate that a factor is missing in the model.

52. The statistical model can be considered validated if the following conditions fulfilled (see examples 15 and 16 in Annex 1):

1. No apparent systematic structure in the residuals (i.e. model fits the data well)
2. Constant standard deviation of random errors in different subsets of the data

3. Symmetric distribution of random errors
4. Correlation between residuals of different body parts considerably smaller than the correlation of the exposure level itself.
5. Explained variance of the model should not be below 40% of the total variance. (Otherwise the model is possibly correct but the variability of exposure is mainly due to unknown factors not included in the model.)

3.4.8 *Application range of the model*

53. The model is valid for the products/substances tested. If it can be demonstrated in the experiment that (a) the product/substance effect is negligible, the model can directly be used also for other products/substances with similar properties; (b) the product/substance effect can be explained by specific properties of the products/substances, the model can be complemented by the respective factors in order to be used for other products/substances with similar properties; (c) the product/substance effect can be modelled as a random effect, the model can be complemented by this random effect in order to be used for other products/substances with similar properties. Then a realistic worst case approach has to be used that takes into account possible effects of the product to the exposure. An example is given in Annex 1 (example 17).

3.4.9 *Extension of the application area*

54. If the model shall be used for other products/substances or for other types of applications, another factorial experiment can be performed. In a joint analysis of the original dataset and of an “add-on” experiment it has to be examined whether there are significant differences of effects of the primary factors. If this is not the case or if the differences can be explained in the statistical model, an extended model can be established. An example is given in annex 1, example 18.

3.5 Probabilistic modelling

3.5.1 *Objective of probabilistic modelling*

55. The main objective of probabilistic modelling of exposure by means of factorial experiments is to explore the variability, distribution and uncertainty of exposure. Probabilistic modelling uses the statistical model of the experimental data for the assessment of the exposure variability and distribution under realistic worst case scenarios.

56. Primary and secondary factors affecting the exposure may be different in the different scenarios to be considered. In probabilistic modelling, each of these factors can be modelled by the distribution of its realisations or can be considered as fixed.

57. As the statistical model of the experimental data regarding the exposure level for the different body parts consists of estimated parameters for systematic factorial effects and of estimated parameters for

the distribution of random factors, all model components are affected by an estimation error. In the assessment of the exposure levels the resulting statistical uncertainty has to be taken into account.

58. The result of probabilistic modelling is the characterisation of the variability, distribution and uncertainty in the exposure assessment, and a quantification of the relationship between exposure conditions and exposure levels.

3.5.2 *The bootstrap method*

59. With the bootstrap method multiple replications of the input parameters and observed random deviations are being created in order to obtain an adequate copy of their distributions. Thereby the simulation of the statistical distribution yields equally multiple replicates of the response variable which again serve as a copy of their distribution. However it should be noted that the bootstrap technique is not directly applied to the data measured but to the deviations between statistical model and measured data. Both parametric bootstrap techniques and non-parametric approaches can be applied. Not only the measured data, but also the factors, that are modelled by a random distribution, will be varied according to their distribution. There are several statistical programs available for probabilistic modelling.

60. The outcome of the bootstrap method is finally the estimated distribution of exposure. From the resulting distribution of the exposure, the percentiles of exposure can be derived. This bootstrap method also allows for the calculation of confidence intervals of the percentiles.

3.5.3 *Total Exposure*

61. The total exposure can be derived from the exposure figures obtained for the different body parts. Percentiles and confidence intervals for the total exposure can be obtained by the bootstrap by adding the exposure of the different body parts. An example is given in Annex 1 (example 19). Exposures of various body parts may be correlated, therefore correlations between exposure levels on different body parts have to be considered when summing bootstrapped exposure. Otherwise, the upper percentiles of the exposure distribution will be underestimated. The Technical Notes on Guidance on human exposure to biocidal products has to be respected on use of percentiles.

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ANNEX 1: EXAMPLES

Example 1:

For the assessment of the dermal exposure of the volunteers/users, 6 body parts are distinguished: face, arms, hands, corpus, legs and feet. During the experiments the volunteers/users wear protective clothing: overall and shoe cover, mask, and cotton gloves of thin untreated cotton. Immediately after each job the arms, the front of the legs and the front of the corpus including the shoulders are cut off from the overalls. These overall-parts, the gloves, the outer fleece of the mask and the top side of shoe covers are transferred into glass bottles. After adding a solvent these pieces are extracted. An aliquot of the extraction solvent is analysed with high performance liquid chromatography (LC-MS/MS).

Example 2:

An example of the factorial design of the experiment and the results for Propiconazole are presented in the following table:

Table A-1: Factorial Design and Results of Validation Experiment

Matrix	Storage extract	Routine of Operator	Base	Viscosity	CL1 ¹ 0.05	CL2 1.00	CL3 5.00	CL4 50.0	Intercept	Slope
Mask	4-8d	daily	spirit	low	0.43	0.83	3.59		0.30	0.65
Gloves	4-8d	daily	spirit	low	0.27	2.45		52.6	0.81	1.04
Mask	4-8d	daily	water	low	0.27	1.64	7.18		0.22	1.39
overall XL	4-8d	low	spirit	high		1.59	6.73		0.30	1.29
Gloves	1-2d	daily	spirit	high	0.96	1.40		60.7	0.55	1.20
overall XXL	1-2d	low	water	Low	0.36	1.80	6.07		0.47	1.13
overall M	1-2d	low	spirit	Low	0.46	1.86	6.51		0.52	1.20
Mask	1-2d	low	spirit	Low	0.54	2.17	6.28		0.74	1.12
Mask	4-8d	low	water	High	0.69	1.20	5.55		0.44	1.01
overall M	4-8d	low	water	High	0.30	1.43	6.74		0.19	1.31
Mask	4-8d	low	spirit	High	0.93	2.12	6.90		0.89	1.20
Gloves	4-8d	daily	water	Low	0.60	2.86		56.4	1.15	1.11
Mask	1-2d	daily	spirit	High	0.68	1.96	4.97		0.86	0.83
Gloves	1-2d	low	water	Low	0.19	1.84		57.2	0.41	1.14
overall XXL	1-2d	daily	water	High	0.41	1.33	5.64		0.31	1.06
Gloves	1-2d	daily	water	High	0.33	1.36		48.7	0.34	0.97

¹ CL= concentration level (µg/kg)

Example 3:

The results of the calculations with the validation software InterVal (6) are shown in the following table.

Table A-2: Validation parameters

Concentration [µg/kg]	Recovery [%]	Relative In house reproducibility s.d. [%]	Relative In house repeatability s.d. [%]
1.00	163.4	25.5	24.5
3.00	128.0	16.4	10.4
5.00	120.9	14.8	6.6
27.50	112.2	13.7	1.3
50.00	111.4	13.6	0.7

Example 4:

The primary factors and factor levels which have been surveyed for relevance within the preliminary tests taken from the studies already referred to regarding

6. a. the application,
7. b. the product,
8. c. the volunteer/user,
9. d. the settings for the volunteers/users and
10. e. the list of selected secondary factors.

a. the application:

Factor	+	-	Description
Object type	lattice fence	trellis fence	A trellis fence represents an object difficult to handle and a lattice fence is a substitute for a laminar object.
Wind	Windy	no wind	Windy means the average maximum wind speed at which volunteers would still brush fence.

b. the product:

Factor	+	-	Description
Base	water-based	spirit-based	Base of the used wood preservative
Type	glaze	primer	Type of the used wood preservative

c. the volunteer/user:

Factor	+	-	Description
Size in cm	tall	small	The size is regarded as depending on the sex. For male volunteers/users ≤ 180 cm is considered as being small and >180 cm as being tall. For female volunteers/users ≤ 170 cm is considered as being small and >170 cm as being tall.
Experience	experienced	inexperienced	A volunteer/user that had privately brushed fences several times before (no professional-user!) is being considered as experienced while a volunteer/user that has never brushed a fence before or only brushed a smaller object a long time ago is being considered as inexperienced.

d. the settings for the volunteers/users:

Factor	+	-	Description
Exhaustion	yes	no	Every volunteer/user has to brush two fences in a row with only changing the overall in-between (in order to do a separate examination of the exposure of both jobs).
Speediness	speedily	neatly	To give an incentive to speed up or work as neatly as possible, a bonus is promised to the 50% most neatly done jobs and to the 50% speediest ones.

e. the list of selected secondary factors:

Data	Unit/Category
Job-No.	[n]
Date	[day]
Time of beginning	[hour]
Time of end	[hour]
Duration of one job	min
Initial weight	g
Output weight	g
Consumption of wood preservative	g
Consumption / m ²	g/m ²
Number of dippings of the brush into the wood preservative can	[m]
Number of dippings per minute	min ⁻¹
Accuracy / Brushing outcome	3 levels of assessment: +, +/-, -
Drip loss	3 levels of assessment: +, +/-, -
Duration until changing fence sides	min

Example 5:

For the realisation of the series of jobs (no 1) altogether 32 experiments are performed. The experimental design realised in this series is presented in the following table. The order of experiments is randomised. Additionally, it is an orthogonal design in that way that for each pair of factors each combination of factor levels appears 8 times

Table A-3: Experimental design of the series of jobs (no 1)

Date	Volunteer/user	Job of volunteer/user	Sex	BMI	Size	Experience	Exhaustion	Wind	Speediness	Object type	Brush	Base	Type
24.11.2004	1	1	male	>=24	>=180	experienced	no	yes	no	trellis	long	water	primer
24.11.2004	1	2	male	>=24	>=180	experienced	yes	yes	yes	lattice	long	water	glaze
14.12.2004	1	3	male	>=24	>=180	experienced	no	no	yes	lattice	short	spirit	primer
06.12.2004	1	4	male	>=24	>=180	experienced	yes	no	no	trellis	short	spirit	glaze
07.12.2004	2	1	male	>=24	<180	inexperienced	no	no	no	trellis	long	spirit	glaze
07.12.2004	2	2	male	>=24	<180	inexperienced	yes	no	yes	lattice	long	spirit	primer
08.12.2004	2	3	male	>=24	<180	inexperienced	no	yes	yes	lattice	short	water	glaze
08.12.2004	2	4	male	>=24	<180	inexperienced	yes	yes	no	trellis	short	water	primer
13.12.2004	3	1	male	< 24	<180	experienced	no	yes	yes	trellis	long	spirit	glaze
13.12.2004	3	2	male	< 24	<180	experienced	yes	yes	no	lattice	long	spirit	primer
15.12.2004	3	3	male	< 24	<180	experienced	no	no	no	lattice	short	water	glaze
15.12.2004	3	4	male	< 24	<180	experienced	yes	no	yes	trellis	short	water	primer
08.12.2004	4	1	male	< 24	>=180	inexperienced	no	no	yes	trellis	long	water	primer
08.12.2004	4	2	male	< 24	>=180	inexperienced	yes	no	no	lattice	long	water	glaze
14.12.2004	4	3	male	< 24	>=180	inexperienced	no	yes	no	lattice	short	spirit	primer
14.12.2004	4	4	male	< 24	>=180	inexperienced	yes	yes	yes	trellis	short	spirit	glaze
13.12.2004	5	1	female	>=24	>=170	experienced	no	yes	no	lattice	long	spirit	glaze
13.12.2004	5	2	female	>=24	>=170	experienced	yes	yes	yes	trellis	long	spirit	primer
16.12.2004	5	3	female	>=24	>=170	experienced	no	no	yes	trellis	short	water	glaze
16.12.2004	5	4	female	>=24	>=170	experienced	yes	no	no	lattice	short	water	primer
07.12.2004	6	1	female	>=24	<170	inexperienced	no	no	no	lattice	long	water	primer
07.12.2004	6	2	female	>=24	<170	inexperienced	yes	no	yes	trellis	long	water	glaze
09.12.2004	6	3	female	>=24	<170	inexperienced	no	yes	yes	trellis	short	spirit	primer
09.12.2004	6	4	female	>=24	<170	inexperienced	yes	yes	no	lattice	short	spirit	glaze
09.12.2004	7	1	female	< 24	<170	experienced	no	yes	yes	lattice	long	water	primer
09.12.2004	7	2	female	< 24	<170	experienced	yes	yes	no	trellis	long	water	glaze
10.12.2004	7	3	female	< 24	<170	experienced	no	no	no	trellis	short	spirit	primer
10.12.2004	7	4	female	< 24	<170	experienced	yes	no	yes	lattice	short	spirit	glaze
24.11.2004	8	1	female	< 24	>=170	inexperienced	no	no	yes	lattice	long	spirit	glaze
24.11.2004	8	2	female	< 24	>=170	inexperienced	yes	no	no	trellis	long	spirit	primer
06.12.2004	8	3	female	< 24	>=170	inexperienced	no	yes	no	trellis	short	water	glaze
06.12.2004	8	4	female	< 24	>=170	inexperienced	yes	yes	yes	lattice	short	water	primer

Example 6:

In the study, volunteers are selected according to the following criteria:

- Minimum age 18 years;
- Pregnant or nursing women excluded;
- Non-professional user, i.e. no painter or the like;
- Good status of health (some volunteers with normal weight, others with overweight);
- Interest in study and willingness to cooperate.

Example 7:

In the study, four different wood preservative formulations containing Propiconazole and wood preservative formulations each with one of the active substances Tolyfluanid, Permethrin and IPBC are used. For the selection of these formulations with the different active substances their respective relevance on the market is considered.

To characterise the products available on the market, a number of relevant parameters as density, viscosity and solid body content are considered. For the factorial characterisation the following two parameters are regarded as reasonable:

- base of wood preservative: spirit-based or water-based
- type of wood preservative: primer or glaze

Example 8:

During the experiments the volunteers/users wear the following protective clothing: overall and shoe cover, mask, and cotton gloves of thin untreated cotton. The jobs are carried out in a testing hall with the possibility of ventilation between the jobs. Since concerning the dermal exposure of the body, not the mist but the drops of the wood preservative are playing a decisive role, the results from the experiments in the testing hall with huge dimensions can be transposed to confined places. The wind is generated artificially by a ventilator.

The brushing of a fence includes front and back side by each volunteer. The volunteer can decide independently with which side to start. During an experiment, noticeable incidents as touching the fence during brushing or spilling the wood preservative are documented as well.

Additionally, every job is recorded on video.

After every job, the protective clothing is changed and the testing hall is aired for about 15 minutes, while the study personnel are preparing the clothing already used for chemical analysis. In the calculation of the exposure figures for the different body parts, clothing is not taken into account (potential dermal exposure). If necessary, clothing can easily be taken into account by assuming specific reduction factors (to estimate the actual dermal exposure).

Example 9:

In the study, several possible exposure variables are examined. As the duration of brushing depends very much on the individual volunteer and on the wood preservative used, the dependency of the exposure level per minute on the several primary and secondary factors will become very complex. A simpler model can be derived for the exposure level per m² of fence surface. Also – due to different amounts of the active substances in the products – all exposure levels are normalised to 1% of active substance. Therefore, the “final” response variable in the study is the logarithmised exposure per m² fence surface for 1% content of active substance.

Example 10:

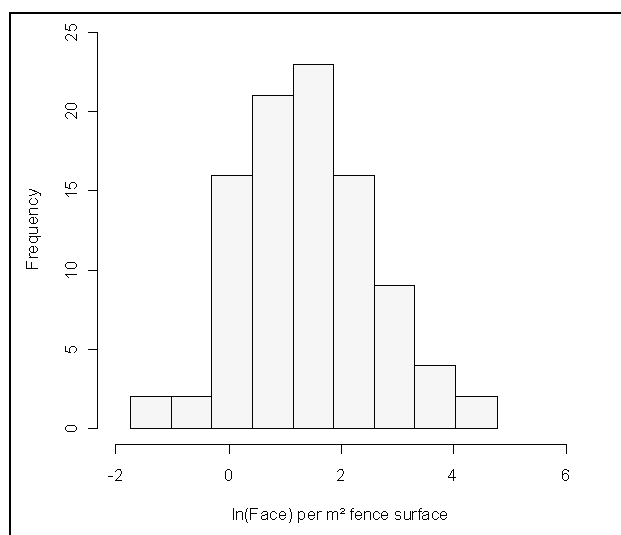
The following table shows the statistical parameters for the distribution of experimental data.

Table A-4: Distribution of experimental data (in µg/m²) for 1% content of active substance

Part of body	Mean	Median	Minimum	Maximum	GM	GSD
Face	9	4	0.0	116.8	4.1	3.3
Arms	57	20	1.0	670.0	22.2	4.1
Corpus	28	10	0.5	318.4	10.6	4.2
Legs	47	17	0.6	553.2	16.1	4.7
Hands	772	187	12.2	6638.2	211.7	5.2
Feet	50	16	0.8	498.5	19.8	4.1

The distribution of the experimental data for the face is shown in the following histogram.

Figure A-1: Histogram of logarithmised exposure (in µg/m²) of the face for 1% content of active substance



Example 11:

The following formula shows the results of the mixed model approach for the exposure of the Corpus:

$$\begin{aligned} &\text{Exposure of Corpus in } \mu\text{g/m}^2 \text{ for 1\% content of active substance =} \\ &3.82 * \exp\{ 0.56 * \text{wind} - 0.53 * \text{object type} + 0.47 * \text{base of wood preservative} + 0.32 * \\ &\text{speediness} + 1.03 * \ln(1.07 + 0.09 * \text{attitude of volunteer/user} + \text{formulation effect} + N(0;0.38^2))\} \\ &* \log N(0;0.99) \end{aligned}$$

Within this model, the attitude of volunteer/user and the formulation effect can be considered as random effects.

Example 12:

In the study, no fundamental differences between the models of the single series of jobs can be observed. Only in case of the exposure of the arms the factor “speediness” and in case of the hands the factor “size” exhibit significant differences.

However, when assessing these results it has also to be considered that for both cases only one of the six parts of the body exhibit a significant difference. The assumed significance level for the analysis is 5%. As every test is carried out 6 times, the probability for a false positive decision (i.e. a decision that a significant difference exists, although this is not true) for at least one of the analysed parts of the body is $1 - 0.95^6 = 0.26$ and thus equals 26%. Therefore it should not be assigned too much importance to the t-values for the exposure of the arms and the hands.

Example 13:

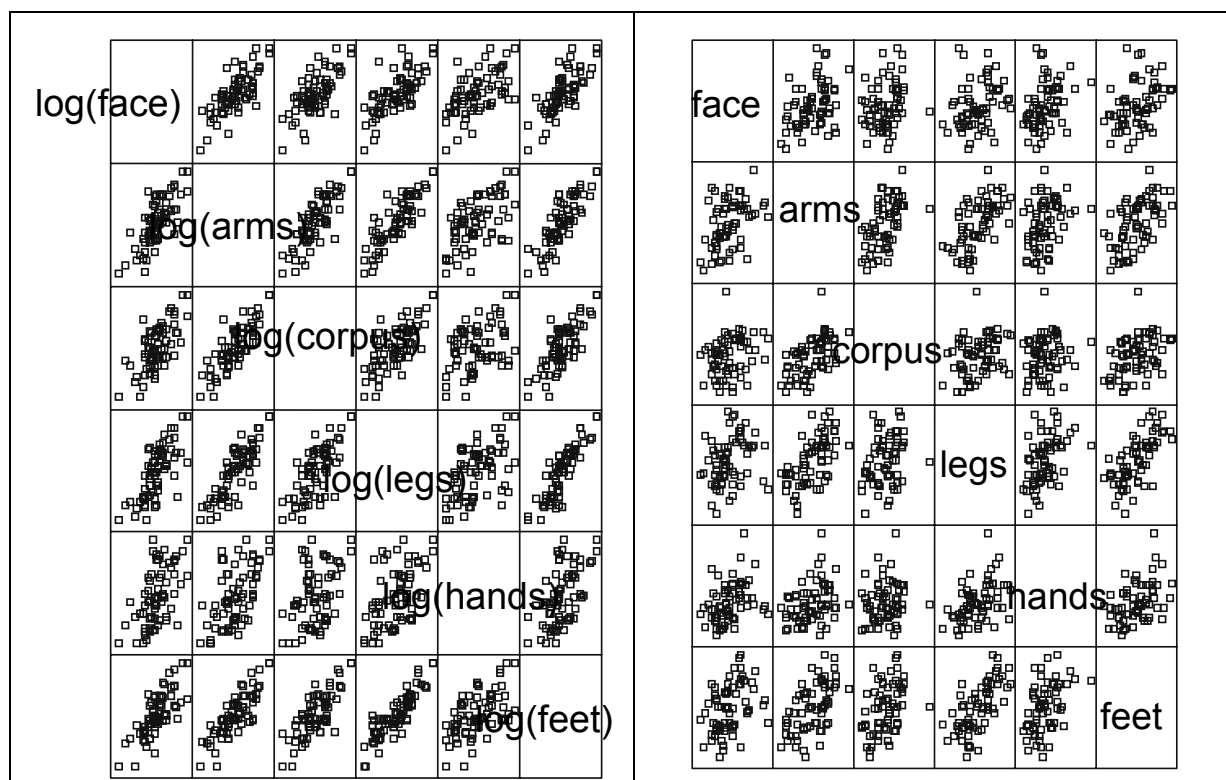
The interaction *Brush x Object type* proved to be significant. This interaction might be explained as follows: Due to the larger surfaces, a lattice fence can be brushed considerably easier with a longer brush than with the shorter one. Whereas a long brush might be unsuitable for the trellis fence, because of the complicated structure of the fence.

Example 14:

For several parts of the body, the logarithmised amount of use of wood preservative per dipping (in g) has a significant influence on the exposure. In a further analysis, the influence of the primary factors on the logarithmised amount per dipping is examined. The base and the type of wood preservative as well as the interaction of these two factors have a significant effect.

Example 15:

Figure A-2: Scatter plot of logarithmised exposure figure (left) and of the residuals (right) of the different body parts analysed

*Example 16:*

In the model no apparent systematic structure and no heterogeneity of the standard deviation of the residuals can be observed. The distribution of residuals is close to the normal distribution. The explained variance of the model for the different body parts varies between 45% and 55%. The correlation of the exposure figures of the different parts of the body varies between 40% and 89%. The correlation of the residuals is between 13% and 68% and in most cases it varies between 20% and 40%.

Example 17:

The strong effect of the interaction between base and type of a wood preservative on the amount of its use per dipping might be caused by the specific formulation which does not only differ in its type and base but also with regard to other physical and chemical properties. The determination of the actual cause of this interaction is impossible. To solve this issue, specifically manufactured products would be necessary. Thus, the factors “type” and “base” of the wood preservative are replaced by the new factor “formulation”. This factor is a random factor which assigns a factor level to each of the 7 formulations analysed in the study.

Table A-5 shows the mean amount of use of a wood preservatives per dipping (in g) for each of the 7 formulations as well as their individual effect on this amount. Apparently, there is an effect of the formulation on the amount of use of a wood preservative per dipping which of course also affects the exposure levels.

In further analyses, the factor “formulation” is considered to be a normally distributed random factor with zero mean and a standard deviation of 0.19 (empirical standard deviation of the 7 used formulations).

Table A-5: Mean amount of use of the wood preservative per dipping (in g) for seven formulations

Formulation	Base	Type	Active substance	Consumption of wood preservative per dipping (in g)	Influence of the formulation on the Consumption of wood preservative per dipping (log scale)
1	water	glaze	Propiconazole	3.09	0.11
2	spirit	glaze	Propiconazole	2.23	-0.22
3	water	primer	Propiconazole	2.65	-0.05
4	spirit	primer	Propiconazole	3.23	0.15
5	spirit	primer	Tolyfluanid	3.20	0.14
6	spirit	primer	Permethrin	3.99	0.36
7	water	primer	IPBC	2.60	-0.07

Example 18:

In the study, wood preservatives with 7 different formulations are used. For each of the formulation a separate statistical analysis of the exposure levels is carried out. The obtained models are compared to each other in order to detect significant differences (see also section 0). There are no differences in the factorial effects found but slight differences in the mean exposure level. Based on these differences a random factor “formulation” is defined and incorporated in the model.

These depend on sample size, GSD and confidence intervals. An example is given in annex 1, example 20.

Example 19:

Table A-6 shows the 75% and 95% percentiles as well as the respective 90% confidence intervals for the total exposure (including the inhalative exposure) in series 1 and 2 for each of the 4 formulations. All values refer to 1m² fence surface.

The 75th percentile may be used as a proxy for the mean value, whereas the 95th percentile may represent a proxy for the worst-case-scenario. Under worst conditions (trellis fence, wind, brushing speedily, inexperienced and small-sized volunteer/user, last job of volunteer/user, water-based glaze) the total exposure might even be about 50 times higher than under optimal conditions (lattice fence, no wind, brushing neatly, experienced and tall volunteer/user, first job of the volunteer/user, spirit-based primer): under optimal conditions the 75th and the 95th percentile equal 80 µg and 235 µg, respectively, whereas under worst case conditions the 75th and the 95th percentile equal 4475 µg and 11933 µg, respectively, based on 1m² fence surface.

Table A-6: 75% and 95% percentile with respective 90% confidence intervals for total exposure of 4 products containing Propiconazole [$\mu\text{g}/\text{m}^2$]

Formulation	percentiles		90 % confidence intervals	
water-based glaze (Propiconazole)	75 %	794	693	961
	95 %	2523	2064	3358
spirit-based glaze (Propiconazole)	75 %	664	570	824
	95 %	2392	1935	3348
water-based primer (Propiconazole)	75 %	779	678	946
	95 %	2516	2067	3445
spirit-based primer (Propiconazole)	75 %	650	545	818
	95 %	2368	1892	3292