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LETTER TO THE EDITOR

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The biometrical evaluation of the OECD modified version of the acute toxic class method (oral)

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Dear Sir,

The acute toxic class method (ATC method) is an alternative to the classical LD_{50} test (Schlede et al. 1995). Previous test procedures (Diener et al. 1994) have been slightly modified. These modifications were agreed upon at the OECD Expert Meeting held in Berlin on January 26–28, 1994. In this letter the changes are shown and discussed with respect to the consequences for classification probabilities and animal numbers. In summary, it can be said that the new procedures do not or only very slightly influence the previously published results.

Two different, equally usable options are available, both with advantages and disadvantages. The following changes have been made.

Option 1

Option 1 is comparable to the previous procedures (Diener et al. 1994), and it is part of the new Guideline. For reasons of animal welfare, the dose of 5000 mg/kg is not part of the Guideline, and for formal reasons the dose of 5 mg/kg is not part of option 1. Therefore, classification systems[1] 6, 11, and 12 had not been considered by the OECD experts, and systems 3, 4 and 5 could only be tested by option 2.

One modification has been made by testing the dose of 2000 mg/kg, which must be repeated now when no or one animal dies at the first step. Therefore, the allocation of a substance is slightly changed when using the new test schemes. It results in other classification probabilities and animal numbers for systems 1, 2 and 13. For classification systems 7, 8, 9, and 10 this modification only has consequences for the starting dose of 2000 mg/kg.

Furthermore, a substance is to be allocated now if one animal dies at the second step of the identical dose. This substance can be classified in that toxicity class as though at the first step of the next higher dose level (if it exists) all three animals had died. It results in a lower number of used and dead animals for classification systems 1, 2, 9, 10, and 13, when the test procedure starts with 25 or 200 mg/kg. For systems 4 and 8 this occurs only with a starting dose of 25 mg/kg. All these cases lead to only slightly changed classification probabilities.

To allocate a substance to all possible classification systems exclusively by means of option 1, it is necessary to include doses of 5 and 5000 mg/kg into the test procedures. This is demonstrated for a starting dose of 200 mg/kg and defined as the first procedure (Schlede et al. 1995). Then classifications are also possible for systems 3, 4, 5, 6, 11, and 12. For the other two starting doses the test procedures are similar.

The formulae of the classification probabilities and of the expected animal numbers as published by Diener et al. (1994) are changed to the following:

This is an abridged report. Detailed results are available from the authors on request.

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[1] List of classification systems: 1 European Union (chemicals), 2 European Union (liquid pesticides), 3 European Union (solid pesticides), 4 United Nations (solids), 5 United Nations (liquids), 6 Switzerland, 7 US Environmental Protection Agency (community right-to-know), 8 Japan (Poisonous and Deliterious Substances Control Act), 9 Canada (Workplace Hazardous Materials Information System), 10 US Occupational Safety and Health Administration, 11 US Environmental Protection Agency (pesticides), 12 US Consumer Product Safety Commission, 13 Canada (pesticides).

$$P_{EU}(25)^4 = \begin{cases} p_{23}(25)^2(1+p_{01}(25)), & LD_{50}{}^3 \leq 25 \\ p_{01}(25)(p_1(25)+p_0(25)p_{23}(200)(1+p_{01}(200))), & 25 < LD_{50} \leq 200 \\ p_{01}(25)p_0(25)p_{01}(200)(p_1(200)+p_0(200)p_{23}(2000)(1+p_{01}(2000))), & 200 < LD_{50} \leq 2000 \\ p_{01}(25)p_0(25)p_{01}(200)p_0(200)p_{01}{}^2(2000), & LD_{50} > 2000 \end{cases}$$

$$P_{EU}(200) = \begin{cases} p_{23}(25)(1+p_{01}(25))p_{23}(200)(1+p_{01}(200)), & LD_{50} \leq 25 \\ p_{01}{}^2(25)p_{23}(200)(1+p_{01}(200)), & 25 < LD_{50} < 200 \\ p_{01}(200)(p_1(200)+p_0(200)p_{23}(2000)(1+p_{01}(2000))), & 200 < LD_{50} \leq 2000 \\ p_{01}(200)p_0(200)p_{01}{}^2(2000), & LD_{50} > 2000 \end{cases}$$

$$P_{EU}(2000) = \begin{cases} p_{23}(25)(1+p_{01}(25))p_{23}(200)(1+p_{01}(200))p_{23}(2000)(1+p_{01}(2000)), & LD_{50} \leq 25 \\ p_{01}{}^2(25)p_{23}(200)(1+p_{01}(200))p_{23}(2000)(1+p_{01}(2000)), & 25 < LD_{50} \leq 200 \\ p_{01}{}^2(200)p_{23}(2000)(1+p_{01}(2000)), & 200 < LD_{50} \leq 2000 \\ p_{01}{}^2(2000), & LD_{50} > 2000 \end{cases}$$

$$N_{EU}(25)^5 = 3(1+p_{01}(25)+p_{01}(25)p_0(25)(1+p_{01}(200))+p_{01}(25)p_0(25)p_{01}(200)p_0(200)(1+p_{01}(2000)))$$

$$T_{EU}(25)^6 = 3(p(25)^7(1+p_{01}(25))+p(200)p_{01}(25)p_0(25)(1+p_{01}(200)) +p(2000)p_{01}(25)p_0(25)p_{01}(200)p_0(200)(1+p_{01}(2000)))$$

$$N_{EU}(200) = 3(1+p_{01}(200)+p_{01}(200)p_0(200)(1+p_{01}(2000))+p_{23}(200)(1+p_{01}(200))(1+p_{01}(25)))$$

$$T_{EU}(200) = 3(p(200)(1+p_{01}(200))+p(2000)p_{01}(200)p_0(200)(1+p_{01}(2000)) +p(25)p_{23}(200)(1+p_{01}(200))(1+p_{01}(25)))$$

$$N_{EU}(2000) = 3(1+p_{01}(2000)+p_{23}(2000)(1+p_{01}(2000))(1+p_{01}(200)) +p_{23}(2000)(1+p_{01}(2000))p_{23}(200)(1+p_{01}(200))(1+p_{01}(25)))$$

$$T_{EU}(2000) = 3(p(2000)(1+p_{01}(2000))+p(200)p_{23}(2000)(1+p_{01}(2000))(1+p_{01}(200)) +p(25)p_{23}(2000)(1+p_{01}(2000))p_{23}(200)(1+p_{01}(200))(1+p_{01}(25)))$$

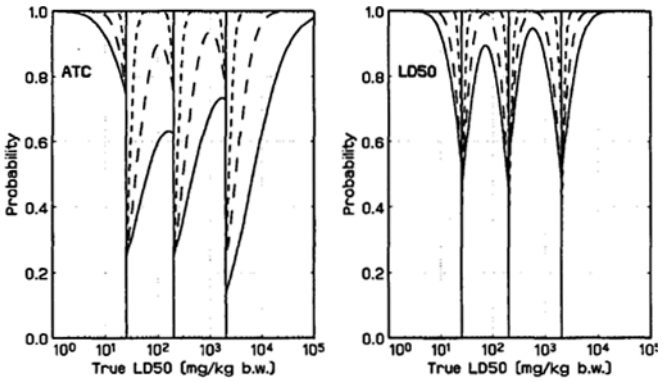


Fig. 1 Probabilities of correct classification to the four classes for chemicals and liquid pesticides in the European Union by using the ATC method with a starting dose of 200 mg/kg body weight versus the LD_{50} test (ten animals with doses of 25, or 200, or 2000 mg/kg) depending on the true LD_{50} for different slopes β (*solid line:* $\beta = 1$, *long dashed line:* $\beta = 2$, *short dashed line:* $\beta = 6$)

Option 2

The procedures of option 2 are illustrated in the new Guideline. The doses of 5, 50 and 500 mg/kg are included, and the test schemes are comparable to the procedure of the international ring study. Especially for classification systems containing these additional doses, and for substances with LD_{50} values in the neighbourhood of these doses, option 2 can be useful to increase the precision of the classification.

One special feature of option 2 is that, for example, at a starting dose of 200 mg/kg the test procedure is continued immediately by testing the doses of 50 mg/kg, when at the second step of the dose of 200 mg/kg two or three animals die. Therefore classification of a substance with respect to other classification systems is often not possible afterwards. This option is only sensible and feasible for systems 3, 4, 5, 9, 10, and 13. Compared to option 1, the probabilities of correct classification are generally slightly better with similar numbers of used and dead animals.

In order to include classification systems containing $LD_{50} = 5000$ mg/kg as a class limit (nos 6, 11, and 12), the dose of 5000 mg/kg is added, and the scheme is marked as second procedure (Schlede et al. 1995).

Options 1 and 2

In the so-called third procedure, option 1 is completely finished first before testing doses 5, 50, 500, or 5000 mg/kg of the second procedure (Schlede et al. 1995). The main difference to the second procedure involves the continuation after step 2 of the 200 mg/kg dose, when two or three animals die at this dose. The procedure is continued by testing the first gender of the dose 25 mg/kg instead of 50 mg/kg earmarked by option 2. For all starting doses the test procedure is valid, whereby the second and the third procedures are identical, when the test starts with the dose of 25 mg/kg.

The third procedure is useful for all classification systems. However, it is only sensible when there are class limits of $LD_{50} = 50$ and/or $LD_{50} = 500$ mg/kg.

Consequences of the modifications and conclusion

All formulae can be developed analogously to the previous calculations (Diener et al. 1994). The corresponding functions, graphics and tables also are computed and are available for all systems and a great number of different conditions, and only a few of them can be demonstrated here as an example. In general, the modifications result into the following changes.

[2] Probability that more than one out of three animals will be dead at the dose of 25 mg/kg, analogously $p_{23}(200)$, $p_{23}(2000)$, $p_{01}(25)$, $p_{01}(200)$, $p_{01}(2000)$, $p_0(25)$, $p_1(25)$, $p_0(200)$, $p_1(200)$.

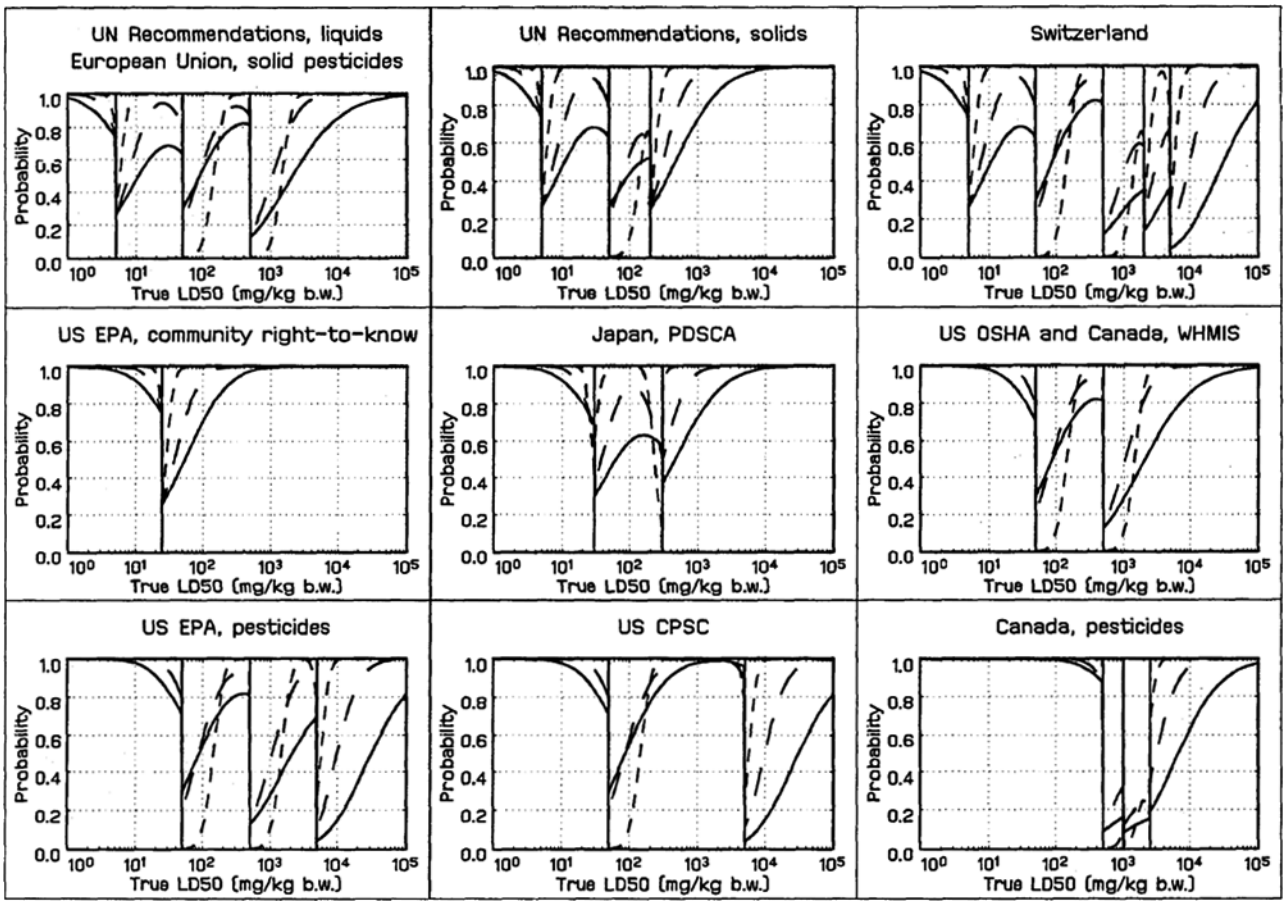
[3] Lethal dose for 50% of the animals.

[4] Probability that a substance has been allocated to the correct toxicity class with respect to system 1 with a starting dose of 25 mg/kg, analogously $P_{EU}(200)$, $P_{EU}(2000)$.

[5] Expected number of used animals for a substance according to system 1 with a starting dose of 25 mg/kg, analogously $N_{EU}(200)$, $N_{EU}(2000)$.

[6] Expected number of dead animals for a substance according to system 1 with a starting dose of 25 mg/kg, analogously $T_{EU}(200)$, $T_{EU}(2000)$.

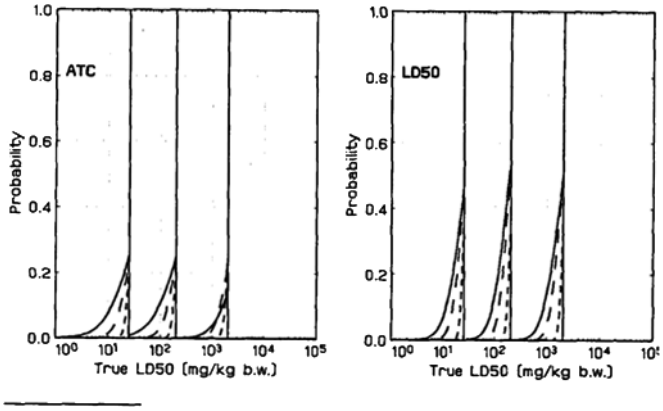
[7] Probability that the animal will die at a dose of 25 mg/kg; analogously p(200) and p(2000).



First procedure

- The probabilities of correct classification have only been changed very slightly. They are better for classification systems containing the LD_{50} class limit of 2000 or 2500 mg/kg (systems 1, 2 and 13).

Fig. 3 Probabilities of classification to a less toxic class for chemicals and liquid pesticides in the European Union by using the ATC method with a starting dose of 200 mg/kg body weight versus the LD_{50} test (ten animals with doses of 25, or 200, or 2000 mg/kg) depending on the true LD_{50} for different slopes β (*solid line:* $\beta = 1$, *long dashed line:* $\beta = 2$, *short dashed line:* $\beta = 6$)



[8] Area above the probabilities as shown in Figs 1, 2, 6 and 7.

Fig. 2 First procedure. Probabilities of correct classification for additional classification systems with a starting dose of 200 mg/kg body weight and different β ($\beta = 1$, *solid line*, $\beta = 2$, *long dashed line*, $\beta = 6$, *short dashed line*)

These probabilities are represented in Fig. 1 for the system of chemicals and liquid pesticides of the EU, and are compared with the classical LD_{50} test. It can be seen that, especially for substances with an LD_{50} value of about 2000 mg/kg, the probabilities are better than those calculated from the previous version (Diener et al. 1994). For example, the area A[8] has a value of 0.80 for $\beta = 2$ and a starting dose of 200 mg/kg (previously 1.17). This effect is detectable for system 13 also (Fig. 2), whereby the areas A are 0.74 versus 1.03 (Diener et al. 1994). Other classification systems lead to very low effects in their probabilities of correct classification (Fig. 2).

- The probabilities to classify a substance into a lower toxicity class have been slightly increased for systems 1, 2, and 13 (Fig. 3 as an example for systems 1 and 2).
- The probabilities to classify a substance to a stronger toxicity class have been slightly decreased for those systems.
- In general, the mean expected numbers of the used and dead animals are slightly decreased.

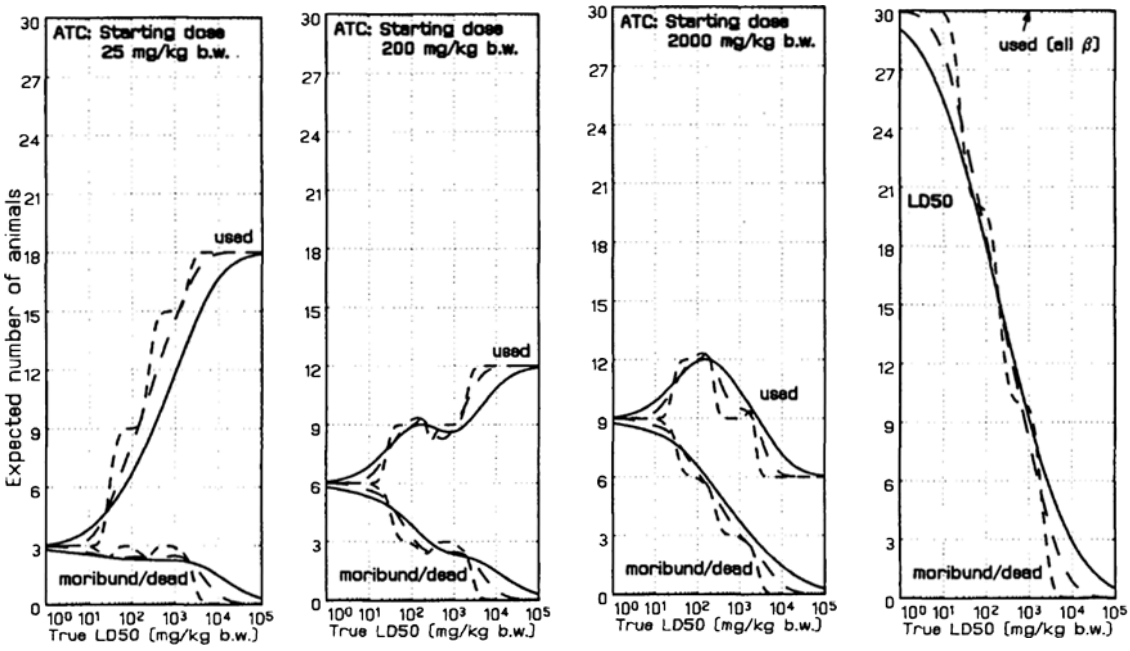


Fig. 4 Expected numbers of animals (used and moribund/dead) by using the ATC method for chemicals and liquid pesticides in the European Union versus the LD_{50} test depending on the true LD_{50} for slopes $\beta = 1$ (*solid line*), 2 (*long dashed line*), and 6 (*short dashed line*). Starting doses are 25, 200, and 2000 mg/kg

The number of animals are shown in Fig. 4 (see also Diener et al. 1994) for systems 1 and 2, as examples. The mean numbers of experimental animals with $\beta = 2$ and the three starting doses are 11.10, 9.04, and 9.26 with the use of the previous procedure, whereas the numbers are by the new procedure 10.96, 9.10, and 8.98, respectively. The values of the mean numbers of dead animals are 2.12, 3.05, and 4.53 versus 1.90, 2.98, and 4.52, respectively. Special substances can lead to an increased animal number. However, these changes are small.

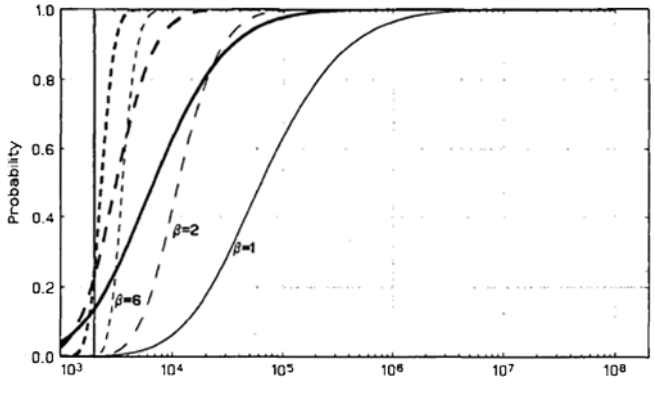
- The dependence on the starting dose has been slightly increased for the classification probabilities, especially for substances with a small slope. However, this dependence is weak.

The greatest difference of the probabilities of correct classification among the three starting doses for the previous procedure is 0.002 and 0.028 for the new procedure, taking into account the system of EU, chemicals with $\beta = 2$.

- The limit test of 2000 mg/kg with six animals can be derived from the procedure.

The limit test implies that no animal dies at the two steps tested at this dose. Figure 5 shows the probabilities to allocate a substance as "unclassified" for the system of chemicals of the EU compared with those of the classical limit test with ten animals.

Fig. 5 Probabilities to classify a substance "unclassified" by using the ATC method for chemicals and liquid pesticides in the European Union (*thick lines*) versus the limit test with ten animals of dose 2000 mg/kg (*thin lines*) in dependence on the true LD_{50} for different slopes β



Second procedure

- In comparison to the first procedure, the probabilities of correct classification are slightly better for systems 3–6 and 9–13.

Figure 6 shows these probabilities for the seven different classification systems relevant for the use of option 2. The areas A of the system UN (liquids) with a starting dose of 200 mg/kg are 1.47, 0.79, and 0.27 for $\beta = 1$, $\beta = 2$, and $\beta = 6$, respectively. In the first procedure these values are larger with 1.58, 0.94, and 1.01, respectively. A similar effect for other systems can be observed.

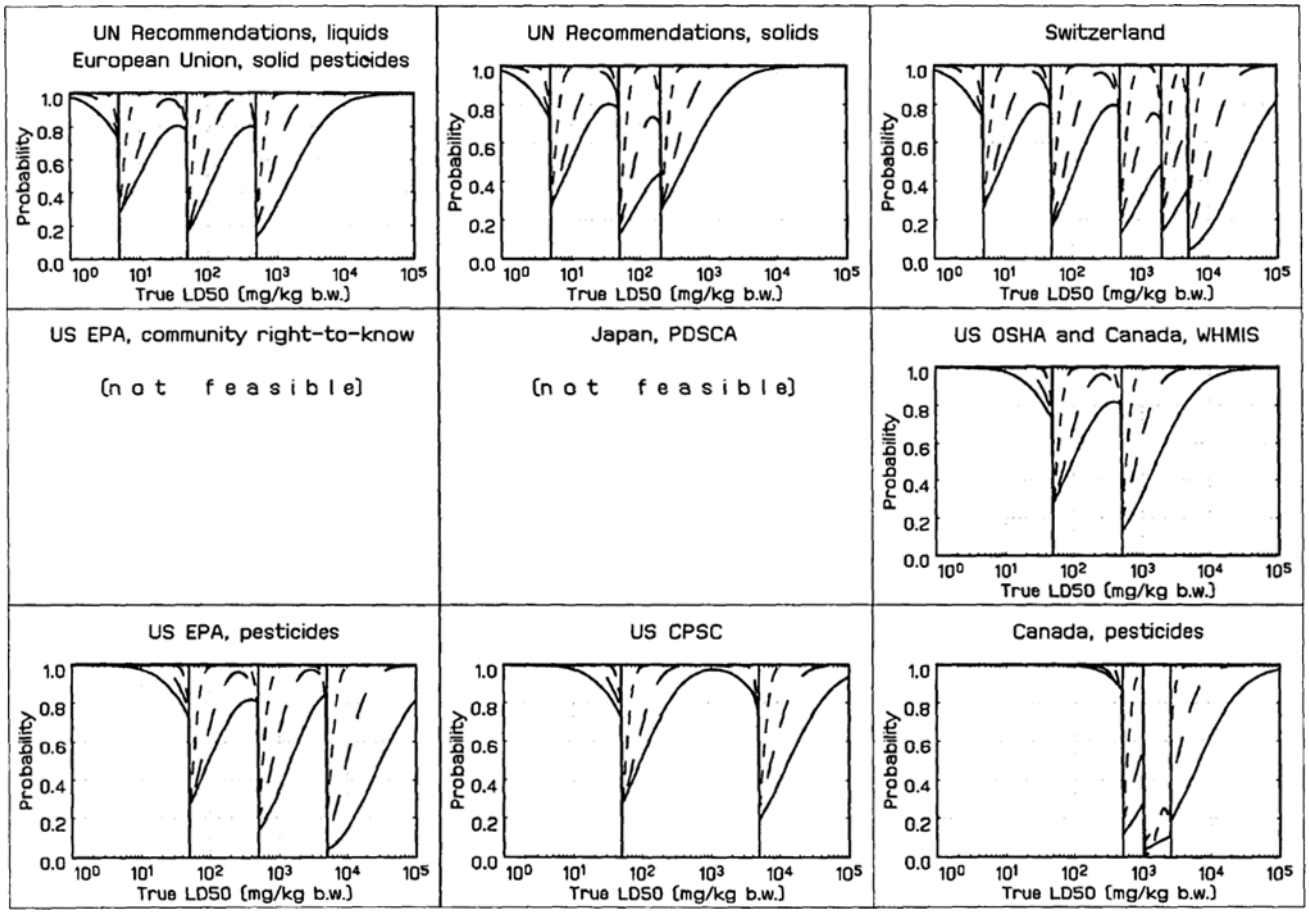


Fig. 6 Second procedure. Probabilities of correct classification for additional classification systems with a starting dose of 200 mg/kg and different β (β = 1, *solid line*, β = 2, *long dashed line*, β = 6, *short dashed line*)

- The mean expected numbers of animals are approximately similar to those of the first procedure.

For the system UN (liquids), β = 2, and the three starting doses, the mean expected numbers of used animals varied from 13.90 to 11.81 to 11.98, whereas the first procedure leads to the values of 11.44, 9.59, and 10.46, respectively. Compared to the mean expected numbers of dead animals, the values are 2.53, 3.62, and 5.70 versus 2.32, 3.41, and 5.18, respectively.

- Without a new test, a classification of a substance with respect to other classification systems is often not possible after finishing the test procedure relating to one system.

Since one or more doses can often be deleted, the test results with these doses are not always available for an allocation of a substance to other classification systems. For example, the dose of 25 mg/kg will not be tested after starting with a dose of 200 mg/kg, when two or three animals die at the second step of dose 200 mg/kg. This effect does not occur with a starting dose of 25 mg/kg. However, in this case the animal numbers are often very large.

Third procedure

- In general, this procedure leads to slightly better results according to the probabilities of correct classification in comparison to the first procedure.

Figure 7 shows these probabilities for the seven different classification systems. The areas A of the system UN (liquids) with a starting dose of 200 mg/kg are 1.44, 0.78, and 0.27 for β = 1, β = 2, and β = 6, respectively. These values, compared with those of the second procedure, are only slightly decreased or even equal.

- In comparison to the other two procedures, the mean expected animal numbers are increased.

For the above classification system, β = 2, and the three starting doses the mean expected numbers of used animals varied from 14.43 to 12.57 to 12.43, respectively, and are larger than those of the other procedures. The mean expected numbers of dead animals are 2.99, 4.22, and 5.72, and thus also larger.

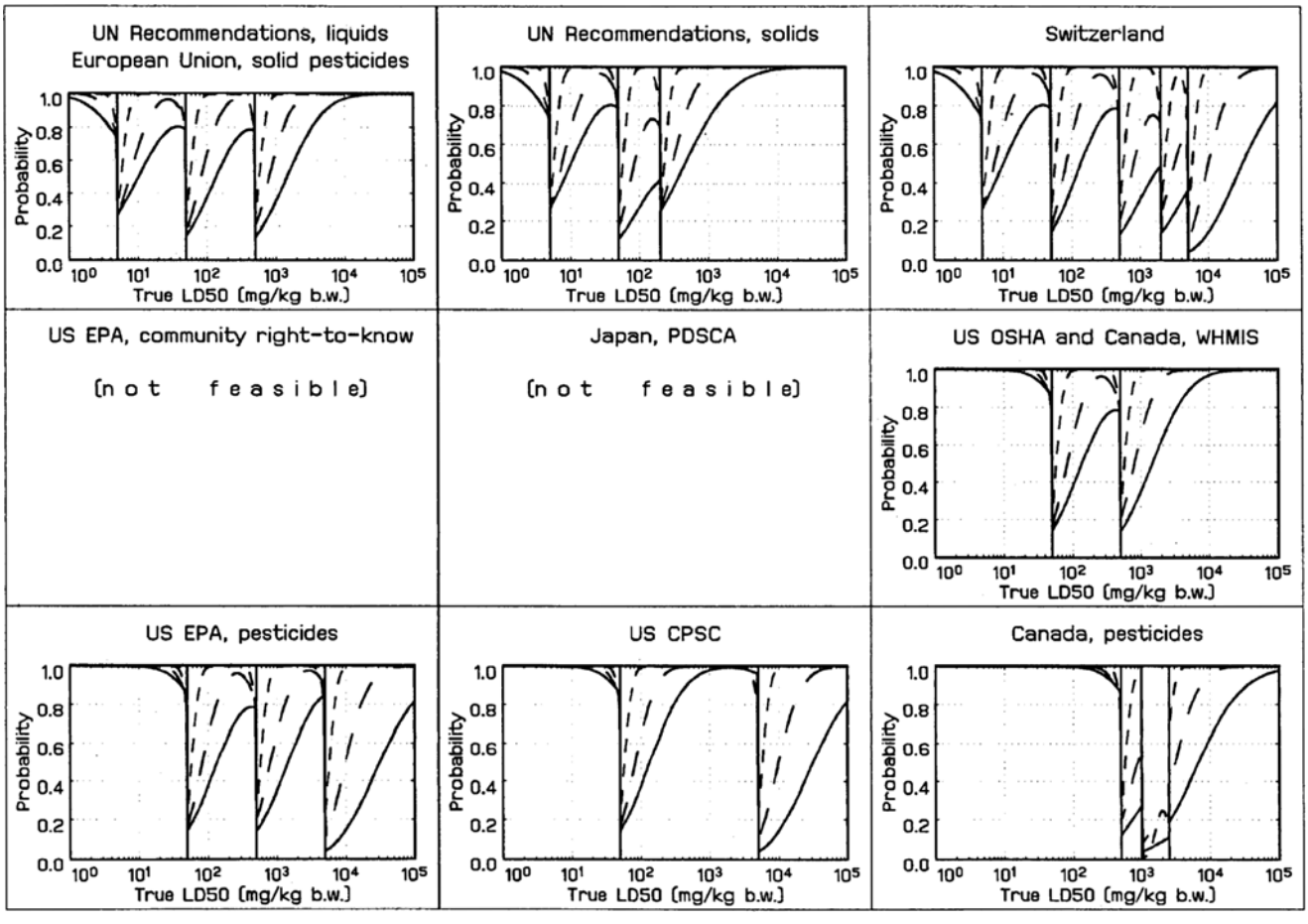


Fig. 7 Third procedure. Probabilities of correct classification for additional classification systems with a starting dose of 200 mg/kg and different β (β = 1, *solid line*, β = 2, *long dashed line*, β = 6, *short dashed line*)

- Often a substance can be allocated subsequently to other classification systems, similar to the first procedure.

However, this is not always possible, when an allocation according to a special classification system has already been made. For example, a substance can be classified without testing the dose of 2000 mg/kg by using the third procedure according to the system of UN (solids).

Rather low correct classification probabilities for Canada (pesticides) can be observed for all three procedures. This effect also occurs by using the previous procedure (Diener et al. 1994), and also with the classical LD_{50} test. Therefore the use of the ATC method for this system is not recommended. These classes have a very small range, and they do not have a factor of 4 between two neighbouring class limits as recommended previously (Diener et al. 1994).

In summary, the ATC method is a sensitive and reliable alternative to the LD_{50} test with the use of substantially fewer animals. Substances can be ranked to the currently used classification systems in the same or even better manner than with an LD_{50} test.

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