International Journal of Occupational Safety and Ergonomics

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tose20

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Jolanta Skowroń[a], Lidia Zapór[a] & Katarzyna Miranowicz-Dzierżawska[a]

[a] Central Institute for Labour Protection, Poland

Published online: 08 Jan 2015.

To cite this article: Jolanta Skowroń, Lidia Zapór & Katarzyna Miranowicz-Dzierżawska (1998) Classification of the Substances on the Basis of the Acute-Toxic-Class Method (ATC), International Journal of Occupational Safety and Ergonomics, 4:1, 107-115

To link to this article: http://dx.doi.org/10.1080/10803548.1998.11076384

INTERNATIONAL JOURNAL OF
OCCUPATIONAL SAFETY AND ERGONOMICS 1998, VOL. 4, NO. 1, 107–115

NOTES

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Jolanta Skowroń
Lidia Zapór
Katarzyna Miranowicz-Dzierżawska

Central Institute for Labour Protection, Poland

The acute-toxic-class method (ATC) is an alternative to the classical LD_{50} test. Four substances were tested with an ATC testing procedure. The results were compared with LD_{50} data obtained from the literature. Great importance was attached to the observations of toxic signs following administration. The results of this study have shown that the ATC method allows allocation to toxicity classes in the same manner as on the basis of the classical LD_{50} tests. The ATC method uses fewer animals and yields the same information on toxic signs. Introducing the ATC method into the quality system allows estimating the acute oral toxicity of chemicals according to the Organisation for Economic Cooperation and Development (OECD; OECD, 1992, 1996).

acute-toxic-class method alternative LD_{50} test animal welfare classification

1. INTRODUCTION

The determination of the mean lethal dose (LD_{50}) for the evaluation of the acute oral toxicity of a chemical or any other test material is usually the first step in a series of follow-up toxicological studies. An LD_{50} value is also used for classifying chemicals, which in turn leads to specific labelling and packaging. With the LD_{50} test, groups of experimental animals are treated with graduated doses of a test substance with the aim of obtaining a 50% or even higher mortality at the highest doses. In addition, the test gives information on the dose response of toxic signs and, to some extent, on pathological findings. The scientific significance of the classical LD_{50} test has been questioned on the basis of the relatively broad variability of the results and for animal welfare reasons (Zbinden & Flury-Roversi, 1981). For the evaluation of the acute oral toxicity of substances, it is not necessary to derive this information with an LD_{50} test using large numbers of animals but instead only a few animals carefully monitored for signs of toxicity and signs of recovery or mortality can be used.

Correspondence and requests for reprints should be sent to Jolanta Skowroń, Central Institute for Labour Protection, Department of Chemical and Aerosol Hazards, Laboratory of Toxicology, Czerniakowska 16, 00-701 Warsaw, Poland. E-mail: <josko@ciop.waw.pl.>.

Numerous alternatives to the LD_{50} test have been published, with the aim of reducing the number of animals and gaining detailed information on toxic signs, mortality ranges, and mortality (Rydzyński & Krysiak, 1995). Some of them have been recommended by the Organisation for Economic Cooperation and Development (OECD) and by the European Union (EU). The fixed dose method was accepted by the OECD in 1992. In principle, this method relies on signs of toxicity rather than on mortality for the evaluation of the toxicity of a test material and it uses 50% fewer animals when compared to the LD_{50} test (Huvel et al., 1990; OECD, 1992). Another test, called the up-and-down method, is now validated in an international collaborative study. The number of animals was reduced to 6–10 in this test (Bruce, 1985). In Germany, another alternative test has been developed: the acute-toxic-class method (ATC).

The ATC method (oral) is a stepwise procedure with the use of three animals per step. It has been designed with three fixed doses (25, 200, and 2000 mg/kg). It is not necessary to perform a preliminary sighting study. In general, this testing is sufficient for the allocation to toxicity classes of the majority of the international classification systems currently in use. With the ATC method, substances can be marked in a similar or even better manner than with an LD_{50} test but this method uses up to 90% fewer animals, the average being 70% fewer. This also results in substantially fewer moribund and dead animals (Schlede, Mischke, Diener, & Kayser, 1995). The ATC method is based on biometric evaluations that, together with the experimental results, demonstrate that this method is a sensitive and reliable alternative to the LD_{50} test (Diener, Siccha, Mischke, Kayser, & Schlede, 1994).

The method has been tested in a national German validation study for the classification system of chemicals of the EU. Six laboratories from Germany participated in this study and 30 substances were tested. The comparison between the results obtained with ATC method and the LD_{50} values documented in the literature showed that in 86% the results were identical, 5% produced a lower, and 9% a higher classification. The average number of rats used was 7.7 per test, and the average number of dead animals was only 2.7 rats per test. The results of the national validation study with the ATC method demonstrated that (a) reliable results were obtained for the evaluation of toxicity and for the classification system of the European Union, (b) a substantially smaller number of animals was used in comparison to the LD_{50} tests, and (c) the method produced sufficient information on the signs of toxicity (Schlede, Mischke, Roll, & Kayser, 1992).

The acute-toxic-class method was also validated with 20 substances in an international study with nine laboratories in five countries, taking into consideration all currently used international classification systems. The use of additional fixed doses of 5, 50, and 5000 mg/kg produced identical classification results because they are, in fact, independent of the starting dose. With the ATC method, the tested substances were ranked in a similar or even better manner than with an LD_{50} test (Schlede et al., 1995). The ATC method was accepted by the OECD in 1996 (OECD, 1996).

The aim of the study was to adapt the ATC method and to introduce it into the quality system according to standard EN 45001 (Comité Européen de Normalisation, 1989). The tested substances were selected to get the whole range of toxicity: from

very toxic to unclassified. Careful clinical observation and pathological changes typical of a toxicity profile were allowed to prepare standard individual records.

2. MATERIALS AND METHODS

2.1. Animals

Male and female Wistar rats, approximately 3 months old, were used. The weight variation of animals did not exceed $\pm 20\%$ of the mean weight for each sex. The animals were housed 3 per metal cage in a room kept at a constant temperature of $22 \pm 3°C$ with the relative humidity of 45–55%. They were fed a standard pellet diet (LSM) with unlimited drinking water. Lighting was artificial, the sequence being 12 hrs of light, 12 hrs of darkness.

2.2. Tested Substances

The substances—sodium cyanide 98% (Riedel-de Haën, Germany), allylalcohol $\geqslant$ 98% (Fluka Chemie AG, Germany), aniline (POCh, Poland), and ethylene glycol (POCh, Poland)—were selected on the basis of their physico-chemical properties, toxicity profile, and LD_{50} values from the literature. Due to the board toxicity profile of the test substances, it was decided for animal welfare reasons to use the starting dose according to their toxicity classes following the classification criteria of the EU (Table 1).

Sodium cyanide, allylalcohol, and ethylene glycol were prepared as water solutions, whereas aniline as a solution in oil. Variabilities in test volume were minimised by adjusting the concentration to ensure a constant volume at all dose levels.

TABLE 1. LD_{50} Values (in mg/kg Body Weight) Cited in the Literature and the European Union (EU) Classification

Substance	CAS No.	LD_{50}	EU Classification	Literature
Sodium cyanide	143-33-9	5.1	very toxic	Ballantyne (1988)
Allylalcohol	107-18-6	64.0	toxic	Smyth, Carpenter, and Weil (1951)
Aniline	62-53-3	450.0	harmful	Czajkowska, Krysiak, and Stetkiewicz (1977)
Ethylene glycol	107-21-1	8540.0	unclassified	Smyth, Carpenter, and Weil (1950)

Notes. CAS—Chemical Abstracts Service.

2.3. Method

The ATC test procedure for the classification system of the EU (Table 2) for chemicals is shown in Figure 1. The animals were fasted prior to dosing, food but not water were withheld overnight. Following the period of fasting, the animals were

weighed and the test substance was administered. It was administered in a single dose using a stomach tube. The maximum volume of the solution was 10 ml/kg body weight. After the substance had been administered, food was withheld for a further 3–4 hrs. Three animals of one sex were used for each step. The dose level to be used as a starting dose was selected from one of three fixed levels: 25, 200, and 2000 mg/kg body weight. The time interval between treatment groups was determined by the onset, duration, and severity of toxic signs. The treatment of animals of the other sex, or at the next dose, was delayed until one was confident of the survival of the previously dosed animals.

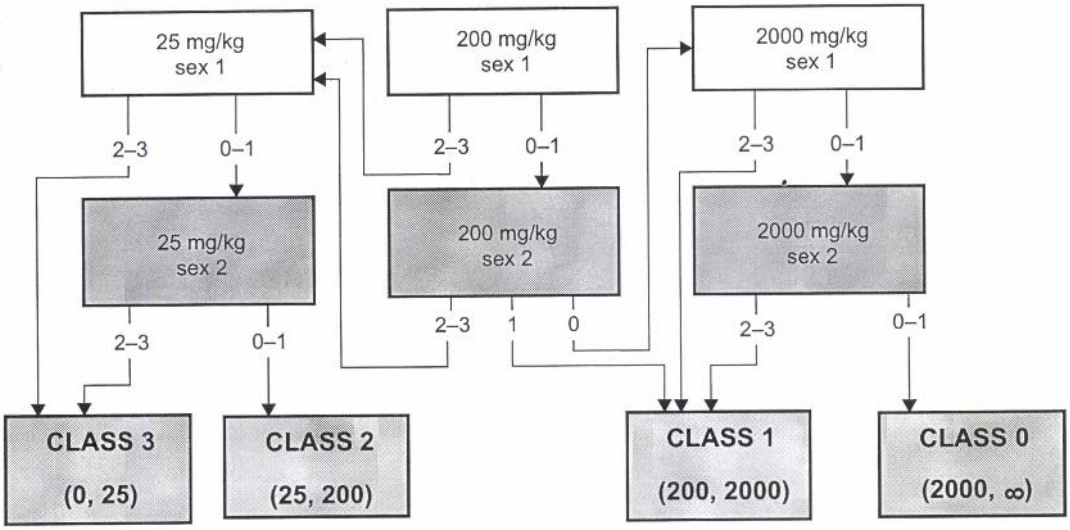

Figure 1. The acute-toxic-class test procedure for the classification system of the European Union for chemicals with three animals per sex and dose level (Schlede et al., 1995). *Notes.* 25, 200, 2000—doses and class limits in mg/kg body weight; 0, 1, 2, 3—the number of moribund and dead animals.

TABLE 2. Toxicity Classes of the European Union (Council Directive 92/32/EEC, 1992)

Toxicity Classes	LD_{50} Range (mg/kg Body Weight)
Very toxic	$LD_{50} \leqslant 25$
Toxic	$25 < LD_{50} \leqslant 200$
Harmful	$200 < LD_{50} \leqslant 2000$
Unclassified	$2000 < LD_{50}$

The absence or presence of compound-related mortality of the animals dosed at one step determined the next step, that is (a) no further testing was needed, (b) the next step was performed with the same dose but with animals of the other sex, or (c) the next step was performed at the next higher or the next lower dose level.

2.4. Observations

Careful clinical observation of dosing was made at least twice on the day and once daily thereafter. Observations included changes in skin and fur; eyes and mucous membranes; respiratory, circulatory, autonomic, and central nervous systems; somatomotor activity; and behaviour pattern. Attention was directed at observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, and coma. Individual weights of animals were determined shortly before the test substance was administered and at least weekly thereafter. Weight changes were calculated and compared with Student's *t*-test (homogenous variances; Oktaba, 1977). All observations were systematically recorded with individual records maintained for each animal. Animals found

TABLE 3. Dose, Mortality, and the Interpretation of the Results (Schlede et al., 1992; Values in mg/kg Body Weight)

Starting Dose	Last Dose Tested	Mortality at the Last Dose and Last Group Tested (on Three Animals)	Interpretation
			Mortality range is greater than 2000
25	2000	0	**Unclassified**
			Mortality range is greater than 200 but less or equal to 2000
25	2000	1–3	**Harmful**
			Mortality range is greater than 25 but less or equal to 200
25	200	2–3	**Toxic**
			Mortality range is less or equal 25*
25	25	2–3	**Very toxic**
			Mortality range is greater than 2000
200	2000	0	**Unclassified**
			Mortality range is greater than 200 but less or equal to 2000
200	2000	1–3	**Harmful**
			Mortality range is greater than 25 but less or equal to 200
200	25	0–1	**Toxic**
			Mortality range is less or equal 25*
200	25	2–3	**Very toxic**
			Mortality range is greater than 2000
2000	2000	0	**Unclassified**
			Mortality range is greater than 200 but less or equal to 2000
2000	200	0–1	**Harmful**
			Mortality range is greater than 25 but less or equal to 200
2000	25	0–1	**Toxic**
			Mortality range is less or equal 25*
2000	25	2–3	**Very toxic**

Notes. * The acute toxicity of the substance may need to be further investigated.

in a moribund condition and animals showing severe pain or enduring signs of severe distress were humanely killed. When animals were killed for humane reasons or they were found dead, the time of death was recorded. The animals were observed for 14 days, except in cases when an animal had to be removed from the study and humanely killed for animal welfare reasons or it was found dead. The time at which signs of toxicity appeared and disappeared were also recorded. At the end of the test, surviving animals were weighed and then humanely killed.

All test animals were subjected to gross necropsy. All gross pathological changes were recorded for each animal. A microscopic examination of organs showing evidence of gross pathology in animals was made.

2.5. Interpretation of Results

On the basis of the mortality of the animals at various steps, test substances were classified according to criteria given in Table 3.

3. RESULTS

The test reports were evaluated according to the following criteria: (a) classification of the substances into any of the four toxicity classes in comparison to the LD_{50} data and their allocated toxicity class, (b) tabulation of the signs of toxicity, (c) autopsy findings, and (d) the number of animals used and the number of dead animals.

Table 4 summarises the results of the acute-toxic-class tests and Table 5 compares LD_{50} tests with the results of the ATC tests. The results were 100% identical.

TABLE 4. Results of the Acute-Toxic-Class Tests (Values in mg/kg Body Weight)

Substance	Starting Dose	Last Dose Tested	Mortality at the Last Dose and Last Group Tested (on Three Animals)	Interpretation
Sodium cyanide	25	25	3	Mortality range is less or equal 25 **Very toxic** Mortality range is greater than 25 but less or equal to 200
Allyalcohol	200	25	0	**Toxic** Mortality range is greater than 200 but less or equal to 2000
Aniline	200	2000	3	**Harmful** Mortality range is greater than 2000
Ethylene glycol	200	2000	0	**Unclassified**

Table 6 lists the signs of toxicity observed after dosing the test substances. Apathy, tremor, and respiratory distress were the signs detected most frequently. Salivation and lacrimation were very characteristic for allylalcohol, whereas cyanosis

TABLE 5. Comparison of the Classification of Substances Between the LD_{50} Tests and the Acute-Toxic-Class Method (Values in mg/kg Body Weight)

Substance	Classification of the LD_{50} Data	Classification of the Acute-Toxic-Class Tests
	5.1	Mortality range is less or equal 25
Sodium cyanide	**Very toxic**	**Very toxic**
	64	Mortality range is greater than 25 but less or equal to 200
Allylalcohol	**Toxic**	**Toxic**
	450	Mortality range is greater than 200 but less or equal to 2000
Aniline	**Harmful**	**Harmful**
	8540	Mortality range is greater than 2000
Ethylene glycol	**Unclassified**	**Unclassified**

and pilorection—for aniline. The duration of the toxic signs until the end of the study (or death) varied widely from a few minutes (for sodium cyanide) to a few hours (for allylalcohol and aniline).

TABLE 6. Signs of Toxicity Observed With Acute-Toxic-Class Tests

	Signs of Toxicity									
Substance	Apathy	Hunched Posture	Tremor	Convulsion	Reduced Motor Activity	Respiratory Disturbance	Cyanosis	Piloerection	Salivation	Lacrimation
Sodium cyanide	+	+	+	+	+	+	–	–	–	–
Allylalcohol	+	+	–	+	–	+	–	–	+	+
Aniline	+	–	+	+	–	+	+	+	–	–
Ethylene glycol	+	–	–	–	–	–	–	+	–	–

Notes. +—observed toxic sign, ––sign not observed.

Autopsies were conducted on all the animals. Three substances—sodium cyanide, allylalcohol, and aniline—showed general findings consistent with the findings after acute oral toxicity testing: reddening and oedema of the lung and the gastrointestinal tract (toxic shock). For sodium cyanide, bright red colouring of the skin was very characteristic, for aniline—formation of methemoglobin, cyanosis, and asphyxiation. Leukocyte infiltrates were observed in the stomachs of the rats that received sodium cyanide. Allylalcohol produced stimulation of the reticuloendothelial system in the liver, which should testify to the disturbance activity of this organ. The histopathologic changes in the stomachs of rats that received aniline pointed to corrosive (necrosis) and irritation (leukocyte infiltrates) activity of this substance.

The weight changes seen in one male rat that survived the administration of allylalcohol at a dose of 200 mg/kg body weight pointed to delayed action of this

substance (Table 7). The female that received aniline at a dose of 200 mg/kg showed slow growth body weight in comparison with the male group, which should indicate more sensitivity of this sex to the toxicity of aniline. No special findings were observed for ethylene glycol (Table 8).

TABLE 7. Change of Rat Weight (in kg) After the Administration of Allylalcohol at a Dose of 200 mg/kg Body Weight

	Week		
	0	1	2
Weight	0.282	0.204 ↓	0.211

Notes. ↓—decrease of body weight.

TABLE 8. Change of Rat Weight (in kg) After the Administration of Aniline at a Dose of 200 mg/kg Body Weight

Number of Animals	Sex	Week		
		0	1	2
1	♂	0.279	0.303[a]	0.322[b, c]
2	♂	0.303	0.324[a]	0.352[b, c]
3	♂	0.275	0.300[a]	0.319[b, c]
1	♀	0.220	0.232[a]	0.233[c]
2	♀	0.213	0.232[a]	0.234[c]
3	♀	0.210	0.225[a]	0.230[c]

Notes. ♂—male, ♀—female, a—value obtained in week 1 significantly different from the previous value at $p < .05$, b—value significantly different from the value obtained in week 1 at $p < .05$, c—value significantly different from the previous value at $p < .05$.

The number of moribund and dead animals depended on the starting dose. The lowest number was 3 (sodium cyanide) and the highest number was 12 (ethylene glycol).

4. DISCUSSION

Various methods for the determination of acute oral toxicity were elaborated by the OECD according to the principles of the "3R" (Russell & Burch, 1959):

1. *reduction* in the number of living animals used to experiments,
2. *refinement*—introduction of new methods to reduce animal suffering to an absolute minimum,
3. *replacement* of animal experimentation with new techniques in vitro.

An alternative to the oral LD_{50} test, the acute-toxic-class method (ATC) was validated in a national German (Schlede et al., 1992) and international (Schlede et

al., 1995) collaborative study. With the ATC method, substances can be ranked in a similar or even better manner than with the classical LD_{50} test. The ATC method uses fewer animals and subjects fewer animals to pain and distress, at the same time yielding the same information on toxic signs.

To adapt the ATC method and introduce it to the quality system, four substances were tested according to a test procedure (Figure 1). All signs of toxicity listed in Table 6 were reported during the course. Autopsies were conducted on all the animals.

With the acute-toxic-class method, no LD_{50} value is determined but the range of mortality between defined doses, in this case < 25, 25–200, 200–2000, and > 2000 mg/kg body weight. This means that with any given results the true LD_{50} is between the defined dose levels. The mortality is the main endpoint in the ATC method, because our present knowledge of the signs of toxicity of substances with a completely difficult chemical structure is limited.

The results of the ATC method were related to LD_{50} data in Table 5. The tested substances were allocated to the four toxicity classes of the European Union. The same classification was obtained with the ATC method and with the use of substantially fewer animals. The number of experimental animals depends on the choice of the starting dose. In this study, the lowest number was 3 and the highest number was 12. In the classical LD_{50} test, it would have never been possible to evaluate the toxicity of the tested substances and to make a correct classification with such a low number of animals.

The results of this study with the ATC method demonstrate that with this method the substances can be ranked into toxicity classes in the same manner as with data obtained with the LD_{50} tests. The use of the ATC procedure produces sufficient information of the signs of toxicity of the tested substances.

5. CONCLUSIONS

1. The acute-toxic-class method (ATC) is sufficient for the allocation of a test substance to a toxicity class according to the OECD and the classification system of the European Union.
2. The ATC method allows a reduction of the total number of animals used in an experiment, their pain, and distress.
3. The precise testing procedure of the ATC method, the use of three fixed doses, and the detailed principles of interpreting the results increase consistency from laboratory to laboratory.

REFERENCES

Ballantyne, B. (1988). Toxicology and hazard evaluation of cyanide fumigation powders. *Clinical Toxicology, 26*(5 & 6), 325–335.

Bruce, R.D. (1985). An up-and-down procedure for acute toxicity testing. *Fundamental and Applied Toxicology, 5*, 151.