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Critical review on acute ecotoxicity data for antimony

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EXECUTIVE SUMMARY

The aim of the current study was the compilation, review and evaluation of acute ecotoxicity data of antimony for the purposes of the acute Environmental Hazard Identification of Sb_2O_3. The collected data, a total number of 16 papers, studies and data sheets, were evaluated and rated according to the Guidelines set in the Technical Guidance Document (in support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No1488/94 on Risk Assessment for Existing Substances) and the experiences gained in the ongoing zinc and cadmium risk assessments.

A total of 13 publications have been rated as not reliable and should therefore not be used in hazard and risk assessment procedures. Three publications were found to be reliable. In order to perform a correct hazard classification, these data have been further evaluated according to their compatibility with the data originating from the transformation/dissolution research.

On this basis only a limited set of acute ecotoxicity data representing invertebrates and fishes are available. The effect levels for these species are within the 10-100 mg/L. range. The most sensitive species found in the evaluated database was the alga *S. capricornutum.* Although these test results were rated as unreliable it is recommended that an acute study be performed with $SbCl_3$ since algae are known to be quite sensitive for metal compounds.

The selected ecotoxicity reference values are clearly situated above the measured dissolved Sb^{3+} concentrations observed in the 24h transformation/dissolution screening test (1.86 mg/L. at a loading rate of 100 mg/L.). On the basis of this observation Sb_2O_3 can be classified as a sparingly soluble compound. For the acute hazard classification the acute ecotoxicity data have to be compared with the short-term (7 days) transformation/ dissolution endpoint of a full test. Therefore it is recommended to perform a full transformation/dissolution test for seven days at a pH (within the range of 6-8.5) which maximises ecotoxicity.

1. INTRODUCTION

Antimony trioxide can be considered as a sparingly soluble metal compound (solubility at 20 °C < 0.01 g/L.) for which its potential ecotoxic properties are related to the antimony ion. The Hazard Identification and the Risk Assessment of metals and sparingly soluble metal compounds in particular is hampered by the complexity of the processes involved in governing the bioavailability of these compounds. These constraints have recently been acknowledged by the EU resulting in the development of a new and improved Hazard Identification procedure for metals and sparingly soluble metal compounds which is being finalized and will be implemented in the near future.

In this procedure the evaluation of the environmental hazard of metals and sparingly soluble metal compounds, based on short term and long term toxicity, is to be accomplished by the comparison of (a) the concentration of bioavailable forms of Sb^{3+} ion, produced during transformation or dissolution in a standard aqueous medium with (b) appropiate standard ecotoxicity data as determined with the soluble metal salt where it can be assumed that this toxicity is related to the Sb^{3+} ion (acute and chronic values) (ENV/JM/HCL, 2000,14).

It is clear that the selection and evaluation of appropriate ecotoxicity data for antimony trioxide, needed to compare with transformation data, are crucial for performing a scientifically valid hazard identification. Therefore the main objective of this study is the critical evaluation of acute toxicity data on antimony compounds in order to recommend only the most reliable and scientifically sound data for use in the acute environmental hazard identification/classification.

2. DATA SEARCH AND COMPILATION

2.1 AQUIRE databank

The references on the acute ecotoxicological effects of antimony salts, which were made available to the consultant for a critical review, were extracted from The AQUIRE database (US-EPA).

AQUIRE is an aquatic toxicity database establihed in 1981 by the United States Environmental Protection Agency (US-EPA), Office of Pesticides and Toxic Substances. This database provides scientists with objective, comprehensive and rapid access to aquatic toxicity data (Murphy and Balogh, 1992). The information includes acute, chronic, sublethal and bioconcentration effect data on both freshwater and saltwater species. The following species are not included: bacteria, microscopic organisms, birds, many amphibians, reptiles, rooted semi-aquatic plants and aquatic mammals.

The following major data elements are provided in the AQUIRE-database: test chemical, test organism, lifestage, test water, test location, exposure time and type, water chemistry, concentrations and control, test endpoints, bioconcentration, remarks and citation. To each document a document code representing the completeness of the data is assigned. The used codes are summarised in Table 1.

Table 1: Document codes used in the AQUIRE-database

Documentation scoring	Code	Description
Complete	C	Thorough documentation of methods and results
Moderate	M	Documentation is generally satisfactory but one or more pieces of information are missing
Incomplete	I	Insufficient methods and results documentation

2.2 Data search and results

A search for toxicity data was performed for the following antimony compounds : antimony trioxide, chloride oxide, antimony and antimony trichloride. Only acute tests are being considered. Tests were considered acute when exposure duration was below or equal to 7 days. Embryo and sac-fry tests have been considered as sub-chronic and were not taken into account in this review.

A schematic overview of the number of papers retrieved for the different compounds and their respective AQUIRE-documentation codes is presented in Table 2.

Table 2: Overview of the retrieved toxicity data for different antimony compounds

Compound	No. of references	Documentation code		
		C	M	I
Antimony (salt form unknown)	1	1	--	--
Antimony trioxide	5	2	3	--
Antimony trichloride	6	1	5	--
Antimony pentachloride	1	--	1	--

All these papers were collected (if possible), reviewed and assigned a relevance and reliability score, according to the index set in the draft zinc and cadmium risk assessment documents. The evaluation methodology will be discussed in section 3.

2.3 Other sources

Two ecotoxicity studies with antimony oxide (Sb_2O_3) were available from Janssen Biotech. The tests were performed with *Brachydanio rerio* and *Daphnia magna* and were performed according to the OECD guidelines. Secondly, an ecotoxicity study performed by LISEC with antimony oxide and *Selenastrum capricornutum* was also available.

3. CRITERIA FOR THE RELIABILITY, RELEVANCE AND APPLICABILITY OF ECOTOXICITY DATA FOR HAZARD IDENTIFICATION AND ENVIRONMENTAL RISK ASSESSMENT

3.1 Introduction

The hazard identification and classification of dangerous substances is regulated by the directive EEC/DIR/67548/18th ATP (1993). The ecotoxicological criteria that are used to assign a substance to one of the classification categories are summarised in Annex C.

Criteria for evaluating the relevance and reliability of the ecotoxicity data have been proposed by several national and international authorities and organisations: USEPA (1995), IPCS (1996), RIVM (1997) and the Belgian Cd-expert group Ex. Subst. (1998). Although most of these approaches are similar, attempts to further develop and harmonise these approaches are currently being undertaken. According to the Guidelines set out in the Technical Guidance Document (TGD, 1996), in support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No1488/94 on Risk Assessment for Existing Substances, it is very important to evaluate ecotoxicity data with regard to their adequacy and completeness. The assessment of data adequacy involves a review of individual data elements with respect to how the study is conducted and how the results are interpreted in order to accept (or reject) a study in accordance with the purpose of the assessment.

The term adequacy covers both the reliability of the available data and the relevance of the data for environmental hazard and risk assessment. These two basic elements have been defined by the TGD as follows:

- Reliability: covering the inherent quality of a test relating to test methodology and the way that the performance and results of a test are described.
- Relevance: covering the extent to which a test is appropriate for a particular hazard or risk assessment

Since the latter criterion covers mainly the ecological relevance of a study, a third criterion, applicability, has been introduced and is being defined as:

- Applicability: the usefulness of the test data within the constraints of the transformation/dissolution test protocol.

Only ecotoxicity data that comply with all of the above-mentioned criteria can be considered valid and may be used in the Hazard Identification.

The experience gained within the data evaluation in the EU risk assessment process of metals, such as the data quality and reliability schemes used in the Zn and Cd risk assessment exercise, have been used as a framework for our evaluation. In addition, other reliability factors based on test organism history (culture conditions) and statistical robustness have been examined.

3.2 Data reliability

3.2.1 Introduction

Standardised tests, as prescribed by organisations such as OECD and USEPA, are used as a reference when test methodology, performance and data treatment/reporting are considered. Indeed, the thorough description of key requirements guarantees the (high) reliability of the reported ecotoxicity data. Non-standardised test data, however, may also have a high reliability, but require a more thorough check on their compliance with reliability criteria before being used in hazard identification and/or risk assessment.

Typically, four different categories of reliability are identified (USEPA, 1995 and RIVM, 1997). These categories are defined in Table 3.

Table 3: Reliability index, according to USEPA (1995) and RIVM (1997)

Reliability index	USEPA 1995	RIVM 1997
I (highly reliable)	high confidence	valid without restrictions
II (reliable)	moderate confidence	valid with restrictions
III (not reliable)	low confidence	invalid
IV (unknown reliability)	unknown confidence	not assignable

This classification system leads to a prioritisation of data, reflecting their level of reliability. For use in hazard identifications, PNEC-derivations, risk assessments or other analyses with legislative consequences, only data that are rated as I or II are being considered. The highest possible ranking for non-standardised tests is RI-II., only standardised tests (cfr-OECD and accordingly) can be ranked as RI-I.

In the present study this level of detail is not considered meaningful since both classes can be used for hazard identification if these studies comply with the other two criteria (relevance

and applicability). Therefore in this study selections have been made on a simple accept/reject basis and no further attempt for prioritisation within these categories has been made.

3.2.3 Criteria for data reliability

The term reliable can be assigned to a study if this study complies with a number of criteria. Some of the most important items that have to be considered are listed in Table 4.

Table 4: Checklist for the reliability evaluation of ecotoxicity studies criteria used for environmental risk assessment and/or hazard identification.

Type of test • standard test or non standard test • endpoint used • test duration
Description of test material and methods • test set-up, measuring chamber/device • test material, solutions, dilution water • test organism, including size (age), origin, acclimation • test design (# replicates) • physico-chemical test conditions
Physico-chemical test conditions • proper description of temperature, pH, hardness, dissolved oxygen • proper control of temperature, pH, hardness, dissolved oxygen • pH, dissolved oxygen should be within the required ranges of standardised tests:
Chemical analysis • test concentrations during the test are measured • test concentrations are not measured, but indication is given that the nominal concentrations are close to measured concentrations • evidence is given that concentrations were maintained during the test (< 30% variation)
Concentration-effect relationship • acceptable control mortality • sound statistics used, 95 % confidence limits reported • concentration range should be given • concentration series of =<0.5 log units (cfr. OECD) are applied • a concentration response should be clear

Type of test

Standard OECD approved tests and non standardised tests have been considered as suitable. Preference is given to data extracted from peer reviewed publications, but data from national environment agencies (US EPA, RIVM, …) are also retained. In the present study only acute tests have been considered. Tests have been considered acute when exposure duration was below or equal to 7 days. Seven-day embryo and sac-fry tests have been considered as sub-chronic and were not taken into account in this review.

Description of test material and methods

Tests should be performed according to standard operational procedures. A detailed description of methods employed in the study should be provided. This description should include, method of test solution (environment) preparation, timing of administration and observations recorded, etc.

Physico-chemical test conditions

General water characteristics such as temperature, oxygen, hardness, salinity and pH should be within the tolerance limits of the test organism. If these requirements are exceeded the test has to be considered invalid

Chemical analysis

Since the acute effect levels are well above reported antimony background concentrations and exposure durations are quite short, both effect levels based on actual (measured) and nominal concentrations have been found reliable as long as soluble antimony salts have been used. When the solubility of a metal salt (e.g. in the case of antimony oxide we are dealing with a sparingly soluble product) is exceeded the test has been considered as unreliable.

Actual measurement of the metal concentration, however, is preferred because this makes it possible to evaluate if the exposure concentrations are adequately maintained over the course of the test. If it is not mentioned wether the $L(E)C_{50}$ values are based on measured or nominal concentrations, they were considered as nominal concentrations.

Concentration-effect relationships

Minimal requirements for endpoints such as mortality, growth, reproduction (e.g.; control mortality < 10 %) are often given in standard procedures. These requirements should be met in order to prevent any influence of confounding factors.

Because effect concentrations are statistically derived values, information concerning the statistics should be used as a criterion for data selection. If no methodology is reported or if values are 'visually' derived, the data were considered unreliable. Effect levels derived from toxicity tests using only 1 test concentration always results in unbounded and therefore unreliable data.

Tests that do not comply with the above-mentioned stipulations are rated as not reliable and are not recommended for use in hazard identification and/or risk assessment exercises.

3.3 Data relevancy

Not all data that are rated as reliable can be used for hazard identification purposes. The relevancy of these data should be considered carefully, based on the items summarised in Table 5.

Table 5: Evaluation of data relevancy

Biological relevancy	The use of non-standardised endpoints (enzyme activity, morphological changes,...) should be done with caution. Preference is given to toxicological criteria that may affect the species at the population level (e.g. survival, growth and reproduction).
Relevancy of the test substance	Unless impurities can have an effect on the toxic properties of the substance under investigation or has a toxic effect itself, studies involving test substances in which impurity levels >1% should not be used.
Relevancy of the test medium	Used media should be representative for the environmental compartment(s) studied.
Ecological relevancy of data	The assessment of the ecological relevancy of a particular study requires that certain basic ecological considerations be taken into account (e.g; test organisms should be representative for the environment.

Because abiotic factors can influence speciation, and therefore bioavailability and toxicity of antimony, water characteristics of the test media have been taken into account for freshwater data selection. Both natural and artificial test solutions waters are accepted provided that the major physico-chemical characteristics (in particular pH and hardness) are similar to the ranges that would be encountered in natural freshwaters.

According to the Zn risk assessment, the following values for pH and hardness have been used for data selection, primarly departing from the current OECD guidelines: the pH values should be within 6 to 9 and the hardness should be within 24 and 250 mg/l $CaCO_3$

3.4 Applicability

Not all available reliable and relevant ecotoxicity data can be used for the purpose of the hazard identification of metals and sparingly soluble metal compounds. Tests, which could be applicable, should comply with the constraints of the transformation/dissolution test protocol. According to this protocol the evaluation of the environmental hazard of metals and sparingly soluble metal compounds, based on short term and long term toxicity, is to be accomplished by the comparison against two criteria. These are (a) the concentration of bioavailable forms, produced during transformation or dissolution in a standard aqueous medium , and (b) appropiate standard ecotoxicity data as determined with the soluble metal salt (acute and chronic values) (ENV/JM/HCL, 2000,14).

The standard aqueous medium is generally ISO 6341 medium with a mean water hardness of 250 mg/l as Ca CO_3. However, the most important restriction is that only the ecotoxicity results of soluble metal salts should be used.

In this study tests were considered applicable if tests were executed with a soluble form of antimony and in a medium similar to the medium used in the transformation/dissolution protocol.

4. RELIABILITY-EVALUATION OF THE COLLECTED DATA

A list of all evaluated studies and articles is presented in Annex A. Each of these publications has been studied carefully and evaluated based on its scientific merits. The data reliability evaluation sheet of each study is given in Annex B. Based on the results, data were either classified as reliable (accepted) or not reliable (rejected). An overview of the reliable articles is presented in paragraph 4.1. The rejected articles are presented in paragraph 4.2.

4.1 Reliable data

The studies which were found to be reliable are summarized in Table 6. All studies were performed on antimony trichloride. Only one study, Kimball G. (1978) can be rated as highly reliable. Although not performed under GLP, the study conforms to the specifications set in 3.2. Origin and condition of the test organisms are specified, physico-chemical parameters before, during and after the test are reported and results are reported based on actual measurements. The test results reveal that effect levels of antimony trichloride on fathead minnows (96 h LC_{50} = 21.9 mg $Sb3^{+}$/L.) and Daphnids (48h LC_{50} = 18.8 mg Sb^{3+}/L.) are quite similar.

Two other studies by Sauvant et al (1995a; 1995b) were missing some information on pH and hardness but are still considered reliable. Effect data are given for *Tetrahymnea pyriformis* and a cell culture of *L-929 fibroblasts*.

The reported values in these studies and the one from the Kimball study were for each species in the same order of magnitude.

Table 6: Selected data for acute antimony toxicity based on the reliabilty criteria set out in 3.2

Test substance	Test organism	Test medium	Test conditions	Nominal/ Measured	Dura-tion (h)	Endpoint	NOEC (mg L^{-1})	LOEC (mg L^{-1})	L(E)C_{50} (mg $Sb^{3+}L^{-1}$)	References
$SbCl_3$	*Tetrahymena pyriformis*	PPYSm medium (proteose peptone yeast-extract substrate modified)	Static T: 28	N	36	growth (Relative doubling time)			Microplate: 6 Flask: 16	Sauvant M.P., *et al.*, 1995
$SbCl_3$	*L-929 Fibroblasts*	Eagle's Minimum Essential Medium (MEM); 5% fetal calf serum; 1% L-glutamine, 1% non-essential amino acids; 1% vitamins; 0.1 g gentamicine/l	Static T: 37	N	24				22	Sauvant M.P. *et al.*, 1995
	Tetrahymena pyriformis GL	Proteose peptone/yeast extract medium enriched with inorganic salts	Static T: 28		3 6 9				60 38 20	
$SbCl_3$	*Pimephales promelas* *Daphnia magna*	Hard well water (tapping the Jordan sandstone stratum underlying the Minneapolis-St Paul metropolian area.		M (AAS)	96 192 48				21.9 20.2 18.8	Kimball G., 1978

4.3 Not-reliable data

A total of 13 publications have been rated as not reliable and should therefore not be used in hazard identification and risk assessment procedures. These studies and the toxicity data noted in the publications, are summarized in table 7. Most often results of these studies were rejected because exposure concentrations exceeded the solubility of the antimony salt used and/or the effect data were unbounded (= greater than values).

One study (Anderson, 1948) did not comply with the stipulations for reliable data for a limited number of parameters. No information is given on the statistics used to derive the effect concentration. Furthermore, no details are given on how the tests were performed (e.g. # replicates). The data (64h LC_{50} for *Daphnia magna* = 19.7 mg Sb^{3+}/L.) in this study can therefore not be used for hazard identification purposes.

Two studies, Tarzwell and Henderson (1960) and US-EPA (1978) were not considered reliable due to the lack of information on test methodology and test medium. For the latter only a summary table from Charles E. Stephan was available. According to Charles Stephan (personal communication, 2000) the contractor who carried out the tests was only obliged to publish the results but unfortunately not to write a final report.

Table 7: Rejected data for acute antimony toxicity based on the reliabilty criteria set out in 3.2

Test substance	Test organism	Test medium	Test conditions	Nominal/ Measured	Dura-tion (h)	Endpoint	NOEC (mg L^{-1})	LOEC (mg L^{-1})	L(E)C_{50} (mg $Sb^{3+}L^{-1}$)	References
Antimony (salt form unknown)	*Daphnia magna*	Reconstituted well water; H: 72±6; pH: 7.0 ±0.2; DO 6.5-9.1	Static T: 22±1	N	24 48	mortality	 530		> 530 > 530	LeBlanc G.A., 1980
Sb_2O_3	*Daphnia magna*	Filtered aerated tubewell water; H: 235-360; Alk: 390-415; pH: 7.2 –7.8;	Static T: 13±2	N	24 48	immobility			555.3 423.5	Khangarot B.S. and Ray P.K., 1989
Sb_2O_3	*Lepomis macrochirus*	Reconstituted water; H: 32-48; Alk: 28-34, pH; 6,5-7,9	Semi-static T: 22±1	N	24 96	mortality			> 443 > 443	Buccafusco R.J. *et al*, 1981
Sb_2O_3	*Tubifex tubifex*	Tubewell water; H: 230-250; Alk: 390-410; pH: 7.5-7.7	Static T: 30 ±1	N	24 48 96	immobility (mortality)			108 920 678	Khangarot B.S.,1991
$SbCl_3$	*Caenorhabditis elegans*	Nutrient-free medium, distilled H_20 with 1.23 g NaCl and 0.968 g KCl (K-medium)	Static T: 20	N	96	mortality			> 20	Williams P.L. and Desenbery D.B., 1990
$SbCl_3$	*Daphnia magna*	Natural water: Lake Erie, semi hard water; pH: 8.2-8.4	Static T: 25	N	64	immobility			19.7	Anderson B.G., 1948
$SbCl_3$ Sb_2O_3	*Pimephales promelas*	Hard water; H: 400; Alk: 360; pH 8.2 Soft water; H: 20; Alk: 18; pH 7.4 Hard water; H: 400; Alk: 360; pH 8.2 Soft water; H: 20; Alk: 18; pH 7.4			96 96 96 96	mortality			17 9 > 80 > 80	Tarzwell C.M. and Henderson C., 1960
$SbCl_3$	*Petromyzon marinus*	Natural water: Hammond Bay of Lake Huron.	Static T:12.8	N	24	mortality			>2.67	Applegate V.C., Howell J.H. Hall

Test substance	Test organism	Test medium	Test conditions	Nominal/ Measured	Dura-tion (h)	Endpoint	NOEC (mg L^{-1})	LOEC (mg L^{-1})	L(E)C_{50} (mg $Sb^{3+}L^{-1}$)	References
$SbCl_5$	*Salmo gairdnerii* *Lepomis macrochirus* *Petromyzon marinus* *Salmo gairdnerii* *Lepomis macrochirus*	pH: 7.5-8.2; DO: 8.6-13.7			24 24 24 24 24				>2.67 >2.67 >2.04 >2.04 >2.04	A.E., 1957
Antimony (salt form unknow)	*Selenastrum capricornutum*	unknown	unknown	unknown	96 96	Inhibition of chlorophyll (i) Reduction in cell number (r)			0.610 0.630	U.S. Environmental Protection Agency (1978)
Sb_2O_3	*Pimephales promelas*	Freshwater;	Static	M (AAS)	96	mortality			> 834	Curtis M.W. and Ward H.., (1980).
Sb_2O_3	*Selenastrum capricornutum*	Algal medium (recommended in OECD Guideline 201). Concentrations of macronutrients in mg/l : NH_4Cl (15); $MgCl_2.6H_2O$ (12) and $CaCl_2.2H_2O$ (18); $MgaSO_4.7H_2O$ (18); $NaHCO_3$ (50) and KH_2PO_4 (1.6) and several micronutrients in mg/l: $FeCl_3.6H_2O$ (0.08); H_3BO_3	Static T: 23.6-24.1	M (ICP)	72 72	growth (b) growth rate (r)	8.3		28.4 55.8	LISEC (1994)

Test substance	Test organism	Test medium	Test conditions	Nominal/ Measured	Dura-tion (h)	Endpoint	NOEC (mg L^{-1})	LOEC (mg L^{-1})	$L(E)C_{50}$ (mg $Sb^{3+}L^{-1}$)	References
		(0.185); $MnCl_2.4H_2O$ (0.415); $Na_2EDTA.2H_2O$ (0.10); $CuCl_2.2H_2O$ (0.00001); $ZnCl_2$ (0,003); $CoCl_2.6H_2O$ (0.0015); $Na_2MoO_4.2H_2O$ (0.007); pH: 7.96-8.80								
Sb_2O_3	*Daphnia magna*	Dilution water was prepared in accordance with the ISO norm "ISO-6341, 1982", to which mircronutrients were added (in mg/l): $NaHCO_3$ (64.75); KCl (5.75); $CaCl_2.2H_2O$ (294); $MgSO_4.7H_2O$ (123.25); K_2-$EDTA.2H_2O$ (1.086); $FeCl_3.6H_2O$ (1.5); $MnSO_4.H_2O$ (0.031); $Na_2MoO_4.2H_2O$ (0.013); $ZnSO_4.7H_2O$ (0.004); SeO_2 (0.001); vitamine B_{12} (0.001). pH: 7,91 and H: 240	Static T: 20±1	N	48	immobility			> 348	Janssen Biotech N.V. (1990)
Sb_2O_3	*Brachydanio rerio*	Dilution water was prepared in accordance with the ISO norm "ISO/DIS 7346/1, 1982". The constituents of this medium are (in ml/l): $NaHCO_3$ (5); KCl (5); $CaCl_2.2H_2O$ (5); $MgSO_4.7H_2O$ (5) pH: 7.85 and H: 241.8	Static T: 22-23	N	96	mortality			> 417	Janssen Biotech N.V.(1990)

H: hardness (mg/l $CaCO_3$),, Alk: alkalinity (mg/l $CaCO_3$), DO: Dissolved oxygen (mg/l), T: temperature (°C)

5. Relevance and applicability evaluation of accepted data

5.1 Introduction

As previously mentioned in section 3.3, only relevant ecotoxicological data should be used in hazard identification and risk assessment studies. The data used should therefore comply to the following stipulations:

- biological relevant (used endpoints,..)
- relevant test substances
- relevant test medium
- ecological relevant data

In addition if the results have to be used in the framework of the hazard identification (section 6) of sparingly soluble metal compounds such as antimony trioxide, these results should also meet the requirements (paragraph 3.4) set out by the hazard identification scheme used in this context. In this regard tests conducted with a soluble form of antimony and in a medium similar to the medium used in the transformation/dissolution protocol should be favoured (e.g.. standard OECD tests with daphnids and fish).

5.2 Selection of ecotoxicity data

The data, obtained for *D. magna* and the teleost *Pimephales promelas* (fathead minnow) in the study by Kimball G. (1978) are the only results which fulfill most of the above-mentioned restrictions/requirements. The followed procedures were not entirely in accordance with the OECD Guideline 202 “Daphnia sp. Acute immobilisation test and reproduction test” and OECD Guideline 203 “Fish, Acute Toxicity Test”. The tests were for example not performed in OECD test medium but in hard well water (an absolute hardness value was not given, but the OECD test medium can also be classified as hard water).

The two other tests with *Tetrahymena pyriformis* and a cell culture of *L-929 fibroblasts* are considered less relevant for the acute hazard identification process (in particular the used test medium is less relevant).

The selected data are summarised in Table 8

Table 8: Selected acute ecotoxity data on antimony reliable for the purpose of acute hazard identification/classification.

Test organism	Duration (h)	L(E)C_{50} ($mgSb^{3+}L^{-1}$)	Reference
Daphnia magna	48	18.8	Kimbal (1978)
Pimephales promelas	96	21.9	Kimbal (1978)
	196	20.2	

6. HAZARD IDENTIFICATION/CLASSIFICATION

6.1 Introduction

The hazard identification and classification of dangerous substances is regulated by the directive EEC/DIR/67548/18TH ATP (1993). The ecotoxicological criteria that are used to assign a substance to one of the classification categories are summarised in Annex C. The risk phrases are summarised in Table 9.

Table 9: Applied Risk-phrases for the aquatic environment

R50 :	Very toxic to aquatic organisms
R51 :	Toxic to aquatic organisms
R52 :	Harmful to aquatic organisms
R53 :	May cause long-term adverse effects in the aquatic environment

Recently a new approach has been suggested for the hazard classification of metals and sparingly soluble metal compounds. This classification is based on a comparison between a reference ecotoxicity value and the amount and rate of dissolution of the metal in standard test water (as determined in a standard transformation/ dissolution test).

The standard laboratory transformation/dissolution protocol is based on a simple experimental procedure of agitating various quantities of the test substance in a pH buffered aqueous medium, and sampling and analysing the solutions at specific time intervals to determine the concentrations of total dissolved metal. Two types of tests are available: a 24 h “screening” test and a 7 or 28 days “full” test. The function of the screening test (at a single loading of 100 mg/l) is to identify those metal compounds that undergo rapid transformation or dissolution such that they are not distinguishable from soluble forms. Metal compounds that do not behave in this way are then subjected to a full test. A full test is normally performed at three loadings: 1, 10 and 100 mg/. The short-term transformation/dissolution endpoints are based on the total dissolved metal concentrations obtained during a 7 days transformation/ dissolution period. The long-term transformation/dissolution endpoint is obtained during a 28 days transformation/dissolution period, using a single load of 1 mg/l (ENV/JM/HCL (2000) 14.

The results of the short-term transformation/dissolution tests are used for the acute hazard classification. Figure 1 gives an overview of the acute hazard classification scheme.

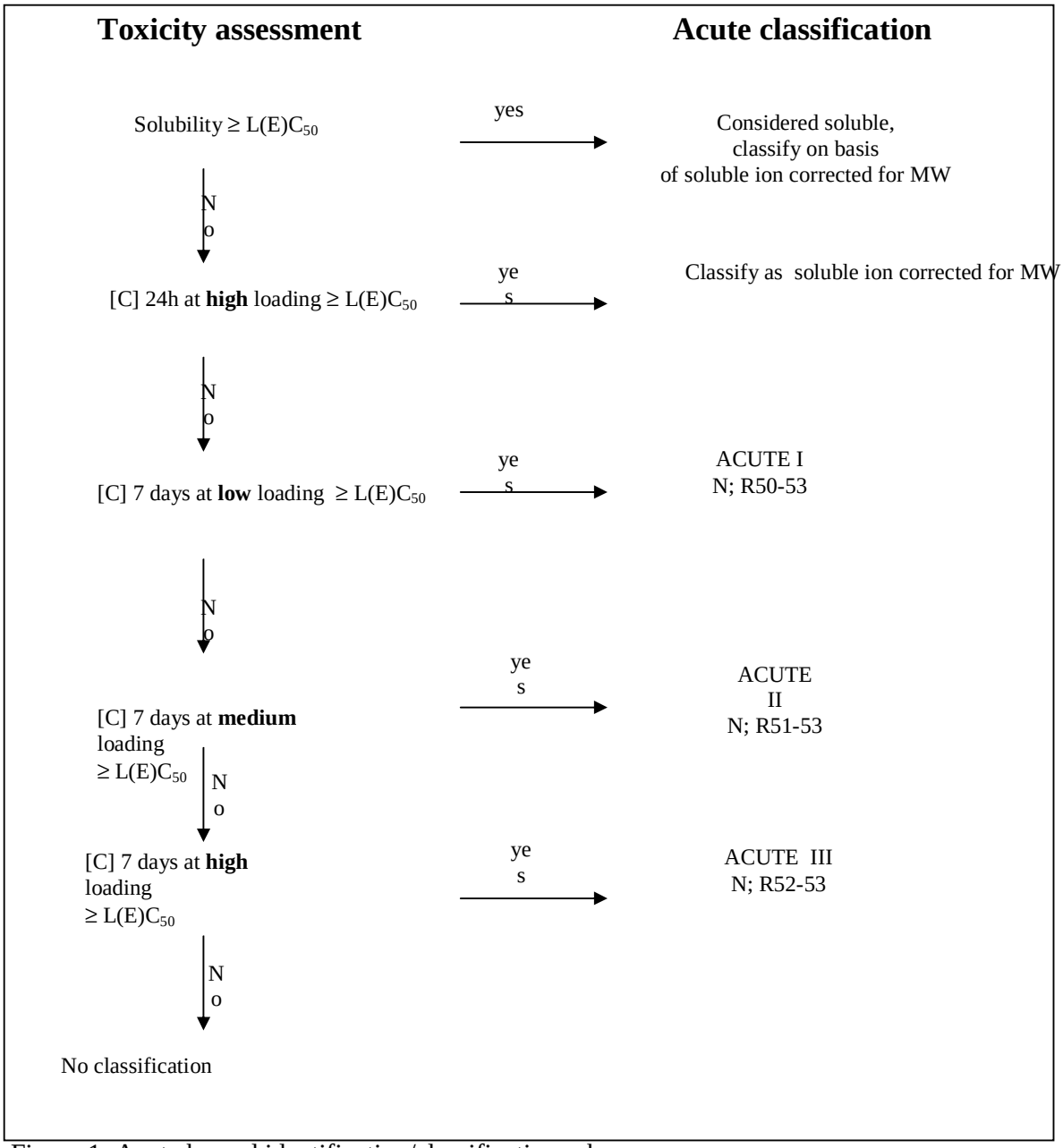

Figure 1: Acute hazard identification/classification scheme

6.2 Available transformation/dissolution data on antimony trioxide

Two transformation/dissolution studies are available for antimony trioxide. One transformation/dissolution test has been performed by LISEC (January 2000). In a 24 h screening test 100 mg Sb_2O_3 (average particle size ranged from 0.95-1.35 µm) was added to ISO 6341 medium at a pH of 8. The test was performed according the draft OECD document (1999) on transformation/dissolution of metals and metal compounds in aqueous media as presented in the Room Document ENV/JM/HCL (2000) 14. The average dissolved antimony concentration after 24 hours was 1.86 mg/l. A second study, on the transformation/dissolution kinetics of Sb_2O_3 , is reported by Umweltanalytik (1993). The solubility of the test substance was conducted at pH 5, 7 and 9. Ten g Sb_2O_3 was added to 100ml of distilled water. Solutions were buffered by adding the buffer system KH_2PO_4/Na_2HPO_4. The average dissolved antimony concentrations after 24 h were 16.4 mg/L., 21.2 mg/L. and 23.8 mg/L. for respectively a pH of 5, 7 and 9. A summary of the test conditions for both tests is given in Table 10.

Table 10: Transformation/dissolution results of Sb_2O_3 in aqueous media.

LISEC (2000)						
Substance	Medium	Time (h)	Loading (mg Sb_2O_3/L.)	Buffer system	pH	Dissolved Sb (mg/L.)
Sb_2O_3	ISO 6341	24	100	Air buffering	8	1.86
Umweltanalytik (1993)						
Substance	Medium	Time (h)	Loading (mg Sb_2O_3/L.)	Buffer system	pH	Dissolved Sb (mg/L.)
Sb_2O_3	Distilled water	24	100,000	KH_2PO_4/ Na_2HPO_4	5	16.4
					7	21.2
					9	23.8

The data from Umweltanalytik are not useful for the purpose of hazard identification. The test does not comply with the Guidance given by the OECD. Tests were conducted in distilled water instead of the standard ISO 6341 medium. The used pH range was outside the range recommended by the OECD: 6-8.5 (the data at pH 5 and 9 should therefore not be used. The test performed at pH 7 is still valid according to the pH criterion). Only air buffering or CO_2 buffering should be used to stabilize the pH. In this study a buffer KH_2PO_4/Na_2HPO_4 was added. The addition of a buffer should be avoided since doing so could alter the chemical composition of the aqueous medium. But above all tests were executed at a very high loading,

100 g/L, instead of the 100 mg/L. recommended by the OECD, probably resulting in increased Sb concentrations due to abrasion phenomena.

It is clear from the results above, screening test solubility of 1.86 mg/L., that antimony trioxide should be considered as a sparingly soluble metal compound as the solubility at 24h is below the lowest ecotox figure of Sb^{3+}.

6.3 Acute hazard classification of antimony trioxide

In order to perform a correct hazard classification, the selected ecotoxity data have to be compared with data originating from the transformation/dissolution research.

The following conclusions can be formulated from this comparison:

- Only a limited set of reliable acute ecotoxicity data representing invertebrates and fishes are available (paragraph 5.2). The effect levels for these species are within the 10-100 mg/L. range. The most sensitive species found in the evaluated database was the alga *S. capricornutum*. Although these test results were rated as unreliable it is recommended that an acute test be performed with $SbCl_3$ since algae are known to be quite sensitive to metal compounds.

- The selected ecotoxicity reference values are clearly situated above the measured dissolved Sb^{3+} concentrations observed in the 24h transformation/dissolution screening test (1.86 mg/L. at a loading rate of 100 mg/L.). On the basis of this observation Sb_2O_3 can be classified as a sparingly soluble compound. For the acute hazard classification the acute ecotoxicity data have to be compared with the short-term (7 days) transformation/ dissolution endpoint of a full test. Therefore it is recommended to perform a full transformation/dissolution test for seven days at a pH (within the range of 6-8.5) which maximises ecotoxicity.

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ANNEX A: Full references of the evaluated publications

Anderson B.G. 1948. The apparent tresholds of toxicity to *Daphnia magna* for chlorides of various metals when added to Lake Erie water. In Transactions-The American Fisheries Society. 78: 96-113

Applegate V.C., Howell J.H.and Hall A.E.Jr. and Smith M.A. 1957. Toxicity of 4,346 chemicals to larval lampreys and fishes. Special Scientific Report-Fisheries No. 207, Washington D.C.

Buccafusco R.J., Ells S.J. and LeBlanc G.A. 1981. Acute toxicity of priority pollutants to bluegill (*Lepomis macrochirus*). Bulletin of Environmental Contamination and Toxicology. 26: 446-452

Curtis M.W. and Ward C.H. 1981. Aqutatic toxicity of forty industrial chemicals: testing in support of hazardous substance spill prevention regulation. Journal of Hydrology 51: 37-44

Janssen Biotech. 1990. The acute toxicity of antimony trioxide in the water flea (*Daphnia magna*). Environmental assessment report ADK6/0009, pp. 13

Janssen Biotech. 1990. The acute toxicity of antimony trioxide in the zebra fish (*Brachydanio rerio*). Environmental assessment report AFBr/0007, pp. 14

Khangarot B.S. 1991. Toxicity of metals to a freshwater tubificid worm, *Tubifex tubifex* (Muller). Bulletin of Environmental Contamination and Toxicology. 46: 906-912

Khangarot B.S. and Ray P.K. 1989. Investigation of correlation between physicochemical properties of metals and their toxicity to the water flea *Daphnia magna* Straus. Ecotoxicology and Environmental Safety. 18: 109-120

Kimball G. 1978. The effects of lesser known metals and one organic to fathead minnows (*Pimephales promelas*) and *Daphnia magna*. Manuscript, Department of Entomology, Fisheries and Wildlife, University of Minnesota, Minneapolis

LeBlanc G.A. 1980. Acute toxicity of priority pollutants to water flea (*Daphnia magna*). Bulletin of Environmental Contamination and Toxicology. 24: 684-691

LISEC. 1994. Alga, growth inhibition test effect of Sb_2O_3 on the growth of *Selenastrum capricornutum*. Final report WE-06-088, pp. 17

Sauvant M.P., Pépin D., Bohatier J. and Grolière C.A. 1995. Microplate technique for screening and assessing cytotoxicity of xenobiotics with *Tetrahymena pyriformis.* Ecotoxicology and Environmental Safety. 32: 159-165

Sauvant M.P., Pépin D., Grolière C.A. and Bohatier J. 1995. Effects of organic and inorganic substnaces on the cell proliferation of *L-929 Fibroblasts* and *Tethrhymena pyriformis* Gl Protozoa used for toxicological bioassays. Bulletin of Environmental Contamination and Toxicology. 55: 171-178

Tarzwell C.M. and Henderson C. 1960. Toxicity of less common metals to fishes. Industrial Wastes. 5(1): 12

U.S. Environmental Protection Agency. 1978. In-depth studies on health and environmental impacts of selected water pollutants, contract no. 68-01-4646, U.S.EPA, Duluth (Table of data available from Charles E. Stephan., USEPA Duluth)

Williams P.L. and Dusenbery D.B. 1990. Aquatic toxicity testing using the nematode, *Caenorhabditis elegans*. Environmental Toxicology and Chemistry. 9: 1285-1290

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ANNEX B: Data reliability evaluation sheets

Data Reliability Evaluation Sheet

Title	The apparent tresholds of toxicity to *Daphnia magna* for chlorides of various metals when added to lake Erie water
Author	Anderson Bertil G. (1948)
Test substance	$SbCl_3$
Test species	*Daphnia magna* Straus. Origin: laboratory cultures. Neonates: 4 ± 4h were used.
Test medium	Natural water: Lake Erie water, semi-hard water, pH ranged from 8.2 to 8.4.
Test endpoint	Immobilisation
Test conditions	Static bioassays were conducted at 25 °C for 64h.
Comments	❑ Test concentrations were not measured. No evidence was given that the concentrations were maintained during the test. ❑ No concentration-effect relation is reported. ❑ No confidence limits are given. ❑ Used statistics are not reported. ❑ Acute toxicity (mg Sb^{3+}/L.) ▪ LC_{50} (64h): 19,7 mg/L.)
Reliability	This study is being classified as not reliable since no information is given on the statistics used to derive the effect concentration. In addition no further details are given on how the tests were performed (e.g. number of replicates)

Data Reliability Evaluation Sheet

Title	Toxicity of 4,346 chemicals to larval lampreys and fishes
Author	Applegate V.C., Howell J.H. Hall A.E. (1957)
Test substance	$SbCl_3$ and $SbCl_5$
Test species	❑ *Oncorhynchus mykiss (Salmo gairdnerii)* (rainbow trout): commercial supplier, fingerling size (4 inches or slightly less in lenght were used) ❑ *Lepomis macrochirus* (bluegill sunfish): commercial supplier, fingerling size (4 inches or slightly less in lenght were used)
Test medium	Natural water: Hammond Bay of Lake Huron. pH: 7,5-8,2; Dissolved oxygen 8.6-13.7 mg/l. Further data on physico-chemical characteristics has been presented in Ayers et al (1956)[a].
Test endpoint	Mortality
Test conditions	All tests were conducted by introducing 6 test organisms in 5 L. of test water in a 10-L glass battery jar. Tests were conducted at one concentration only.
Comments	❑ No hardness is reported. ❑ No alkalinity is reported. ❑ Only a preliminary screening method have been used (testing of only one concentration). ❑ No concentration-effect curve is reported. ❑ Test concentrations were not measured. No evidence was given that the concentrations were maintained during the test. ❑ Acute toxicity (mg Sb^{3+}/L.) ▪ *O. mykiss*: LC_{50}(24 h) for ($SbCl_3$): > 2,67 mg/L. ▪ *O. mykiss*: LC_{50}(24 h) for ($SbCl_5$) > 2,04 mg/L. ▪ *L. macrochirus*: LC_{50}(24 h) for ($SbCl_3$): >2,67 mg/L. ▪ *L. macrochirus*: LC_{50}(24 h) for ($SbCl_5$) > 2,04 mg/L.
Reliability	This study is being classified as not reliable since the reported effect data are unbounded. Furthermore only one exposure concentration has been tested.

Data Reliability Evaluation Sheet

Title	Acute toxicity of priority pollutants to bluegill (*Lepomis macrochirus)*
Author	Buccafusco R.J., Ells S.J. and LeBlanc G.A. (1981)
Test substance	Sb_2O_3
Test species	Lepomis macrochirus . *Origin: commercial supplier, Juvenile blue gill: wet weight ranged from 0.32-1.2 g.*
Test medium	Reconstituted water according to recommended procedures (U.S. EPA, 1975)[a]. the water had a total hardness of 32-48 mg/L. CaCO3, a pH of 6.7-7.8, a dissolved oxygen concentration of 7.0-8.8 mg/L. and a specific conductance as 93-190 µmhos/cm.
Test endpoint	Mortality
Test conditions	Tests were conducted in 19.6 l glass jarrs containing 15 L. of test solution. Ten fish per concentration. Tests were incubated at 22 ± 1 °C. Fish mortality and behavioral responses were observed and recorded at time 0 and every 24 h during exposure (max. 96 h).
Comments	❑ The authors draw the attention on the fact that antimony trioxide was undissolved. Therefore the LC_{50} values reported are high and do not reflect the actual concentrations of the chemical which was in solution in the diluent. No actual measurements were made. ❑ No concentration series were reported. ❑ No concentration-effect curves were reported. ❑ Acute toxicity of Sb^{3+}: ▪ LC_{50} (24h) > 443 mg/L. (> 530 mgSb_2O_3) ▪ LC_{50} (96h) > 443 mg/L. (> 530 mgSb_2O_3)
Reliability	This study is being classified as not reliable since the reported effect data are unbounded. Furthermore exposure concentrations exceeded the solubility of antimony trioxide.

[a] *US-EPA (1975): Methods for acute toxicity tests with fish, macroinvertebrates and amphibians. Ecological Research Series (EPA-660/3-75-009), 61 p*

Data Reliability Evaluation Sheet

Title	Aquatic toxicity of forty industrial chemicals: testing in support of hazardous substance spill prevention regulation
Author	Curtis M.W. and Ward C.H. (1980)
Test substance	Sb_2O_3
Test species	*Pimephales promelas*, Origin: fathead minnows were raised from controlled breeding stocks at the E.P.A. Newtown Fish Toxicology Station, Cincinnati, Ohio.
Test medium	Reconstituted soft water was used: pH: 7.2-7.9 ; hardness: 40-48 mg/L. as $CaCO_3$; alkalinity: 30-35 mg/L. as $CaCO_3$; temperature: 22 ± 1°C, conductivity: 120-160 µS/cm
Test endpoint	Mortality
Test conditions	Static 96-hour toxicity tests were conducted. Five organisms were placed in a bioassay container filled with 12 L. of dilution water. For each chemical, at least five concentrations were tested in duplicate.
Comments	❑ Test concentrations were monitored by atomic absorption spectometry (relative standard deviation ± 4.1) at the beginning and the end of the test. Initially, water samples were not filtered. Later it was decided that all water samples were filtered through 0.45 µm filters prior to analysis. ❑ No dissolved oxygen values are reported. ❑ No concentration ranges are reported. ❑ No concentration-effect curve is reported. ❑ Acute toxicity (mg Sb^{3+}/L.) ▪ *P. promelas*: LC_{50} (96h): > 696 mg/L. (830 mg/L. Sb_2O_3)
Reliability	This study is being classified as not reliable since the reported effect data are unbounded. Furthermore exposure concentrations exceeded the solubility of antimony trioxide.

Data Reliability Evaluation Sheet

Title	The acute toxicity of antimony trioxide in the zebra fish (*Brachydanio rerio*)
Author	Janssen Biotech N.V. (1990)
Test substance	Sb_2O_3
Test species	*Brachydanio rerio,* Origin:Zebra fish were obtained from a local hatchery (Huybrechts, Zoersel). The body weight and lenght of the fish at the start of the test was 0.28 ± 0.06 g and 2.87 ± 0.3 cm respectively.
Test medium	Dilution water was prepared in accordance with the ISO norm "ISO/DIS 7346/1, 1982[a]". The constituents of this medium are (in ml/l): $NaHCO_3$ (5); KCl (5); $CaCl_2.2H_2O$ (5); $MgSO_4.7H_2O$ (5) Temperature: 18.9°C; pH: 7.85 and hardness: 241.8 mg/L. as $CaCO_3$
Test endpoint	Mortality
Test conditions	The 96 h acute toxicity test was investigated following the OECD-guideline N° 203[b]. Ten fish were used per concentration. Two replicates per concentration. Observations were made every 24 h.
Comments	❑ Test concentrations were not measured. No evidence was given that the concentrations were maintained during the test. ❑ Concentration-effect curves have been reported ❑ Oxygen levels varied between 8.1 and 9.5 mg/L. ❑ pH levels varied between 7.5 and 7.8 ❑ Temperature varied between 22.3 and 22.7 °C ❑ Test concentrations of 100, 180, 320, 560 and 1,000 mg Sb_2O_3/L. are used ❑ Acute toxicity (mg Sb^{3+}/L.) ▪ EC_{50}(96h) > 834 mg/L. (>1,000 mg Sb_2O_3)
Reliability	This study is being classified as not reliable since the reported effect data are unbounded. Furthermore exposure concentrations exceeded the solubility of antimony trioxide.

[a] *ISO/DIS 7346/1 Water quality – Determination of the acute lethal toxicity of substances to a freshwater fish Brachydanio rerio, (Hamiton-Buchanan), Teleostei, Cyprinidae- part 1: static method, International Organization for standardizatiion, 1982.*

[b] *OECD Guidelines for Testing of Chemicals, Paris (1984). Guideline N° 203: Fish acute toxicity test.*

Data Reliability Evaluation Sheet

Title	The acute toxicity of antimony trioxide in the water flea *(Daphnia magna).*
Author	Janssen Biotech N.V. (1990)
Test substance	Sb_2O_3
Test species	*Daphnia magna* Straus, Origin:Laboratory culture (originally obtained from Ghent University, Laboratory for Biological Research in Aquatic Pollution. Juveniles < 24 h old.
Test medium	Dilution water was prepared in accordance with the ISO norm "ISO-6341, 1982[a]", to which micronutrients were added (in mg/L.): $NaHCO_3$ (64.75); KCl (5.75); $CaCl_2.2H_2O$ (294); $MgSO_4.7H_2O$ (123.25); K_2-EDTA.$2H_2O$ (1.086); $FeCl_3.6H_2O$ (1.5); $MnSO_4.H_2O$ (0.031); $Na_2MoO_4.2H_2O$ (0.013); $ZnSO_4.7H_2O$ (0.004); SeO_2 (0.001); vitamine B_{12} (0.001).T: 20.5°C; pH: 7.9 and hardness was 240mg/L. as $CaCO_3$.
Test endpoint	Immobility
Test conditions	*Daphnia magna* was investigated according to the OECD-guideline No. 202[b]. Each concentration (50 mL. test volume) consisted of four replicates. Tests were incubated at 20 ± 1°C. and scored after a 48 h exposure period.
Comments	❑ Test concentrations were not measured. No evidence was given that the concentrations were maintained during the test. ❑ Concentration-effect curves have been reported ❑ Oxygen levels varied between 8.3 and 9.2 mg/L. ❑ pH levels were 7.7 ± 0.2, ❑ Test concentrations of 100, 180, 320, 560 and 1,000 mg Sb_2O_3/L. are used. ❑ Acute toxicity for Sb^{3+}: ▪ EC_{50}(48h) > 834 mg/L. (>1,000 mg Sb_2O_3)
Reliability	This study is being classified as not reliable since the reported effect data are unbounded. Furthermore exposure concentrations exceeded the solubility of antimony trioxide.

[a] *ISO 6341, Water quality – Determination of the inhibition of the mobility of Daphnia magna Straus, International Organization for standardizatiion, 1982.*

[b] *OECD Guidelines for Testing of Chemicals, Paris (1984). Guideline N° 202: Daphnia sp., Acute Immobilisation Test and reproduction Test.*

Data Reliability Evaluation Sheet

Title	Toxicity of metals to a freshwater tubificid worm *Tubifex tubifex (Muller)*
Author	Khangarot B.S. (1991).
Test substance	Sb_2O_3
Test species	*Tubifex tubifex* (Muller). Origin: tubificid worms were obtained from a natural pond. Tests were initiated after a 7 day acclimatization period.
Test medium	Filtered aerated tubewell hard water: Reported hardness ranged from 230 to 250 mg/L. as $CaCO_3$. Alkalinity ranged from 390 to 410 mg/l as $CaCO_3$. pH values ranged from 7.5 to 7.7. Other parameters measured were: Ca: 151-167 mg/L., Mg: 80-98 mg/L. and Cl: 7-12 mg/L..
Test endpoint	Immobilisation (mortality)
Test conditions	Stock solutions were made in distilled water. Prior to this antimony trioxide was boiled in a small amount of dilute HCl. A concentration gradient series from each respective stock solution was prepared in tubewell water. Tests concentrations were selected on a logarithmic scale. Test were conducted in 200 mL. beakers containing 100 mL. test water. Ten tubificid worms were exposed to each concentration, and each concentration was tested in replicates of three. Test were terminated after 96h. Observations were made in the following time intervals: 30 min and 1, 2, 4, 8, 14± 2, 24, 33 ± 3, 48 and 96h. Test water was renewed every 24 h. Tests were conducted at 30 ± 1 °C.
Comments	❑ Field collected test organisms. ❑ Test temperature is quite high: ranged from 29.5 to 31 °C. ❑ Test concentrations were not measured. No evidence was given that the concentrations were maintained during the test. ❑ 95% confidence limits were calculated by the moving average-angle method. ❑ Tests were conducted without substratum (can cause additional stress for benthic organisms). ❑ No concentration series were reported. ❑ No concentration-effect curves are reported. ❑ Acute toxicity of Sb^{3+}: ▪ EC_{50} (24h) = 108 mg/L. (95 % C.L.:926-1330) ▪ EC_{50} (48h) = 920mg/L. (95 % C.L.: 840-1181) ▪ EC_{50} (48h) = 678mg/L. (95 % C.L.: 610-884)
Reliability	This study is being classified as not reliable since the exposure concentrations exceeded the solubility of antimony trioxide.

Data Reliability Evaluation Sheet

Title	Investigation of correlation between physicochemical properties of metals and their toxicity to the water flea *Daphnia magna* Straus
Author	Khangarot B.S. and Ray P.K.(1989)
Test substance	Sb_2O_3
Test species	*Daphnia magna* Straus. Origin : waterfleas were obtained from a natural pond.
Test medium	Filtered aerated tubewell hard water: Reported hardness ranged from 235 to 260 mg/L. as $CaCO_3$. Alkalinity ranged from 390 to 415 mg/L. as $CaCO_3$. pH values ranged from 7.2 to 7.8. Other parameters measured were: Ca: 145-165 mg/L., Mg: 85-96 mg/L. and Cl: 5-10 mg/L..
Test endpoint	Immobility
Test conditions	Stock solutions were made in distilled water. Prior to this antimony trioxide was boiled in a small amount of dilute HCl. Static bioassays were conducted in 200 mL. beakers containing 100 mL. of test solution. Ten daphnids were exposed to each concentration (selected on a logarithmic scale). Three replicates per concentration. Test were conducted at 13 ± 2 °C. Scoring of the test was done after 30 min and 1, 2, 4, 8, 14± 2, 24, 33 ± 3 and 48h.
Comments	❑ Field collected test organisms ❑ Test temperature is quite low: ranged from 11.5 to 14.5 °C. ❑ Test concentrations were not measured. No evidence was given that the concentrations were maintained during the test ❑ 95% confidence limits were calculated by the moving average-angle method ❑ Hardness level is relevant ❑ No concentration series were reported ❑ No concentration-effect curves are reported ❑ Acute toxicity (mg Sb^{3+}/L.) ▪ EC_{50} (24h) = 555,3 mg/L (95 % C.L.:453.8-726.3) ▪ EC_{50} (48h) = 423,5 mg/L (95 % C.L.: 361.5-496.0)
Reliability	This study is being classified as not reliable since exposure concentrations exceeded the solubility of antimony trioxide.

Data Reliability Evaluation Sheet

Title	The effects of lesser known metals and one organic to fathead minnows (*Pimephales promelas*) and *Daphnia magna.*
Author	Kimball G. (1978)
Test substance	$SbCl_3$
Species	❑ *Pimephales promelas.* Origin: 8 week-old juvenile organisms averaging 12-16 mm total length.were obtained from a hatchery maintained in the fisheries laboratories. The original source of stock for the hatchery came from the Duluth-Newton laboratory strain of fathead minnows. ❑ *Daphnia magna.* Origin: Laboratory culture, neonates 12 ± 12h were taken to start the testing.
Test medium	Hard well water (tapping the Jordan sandstone stratum underlying the Minneapolis-St. Paul metropolian area, Smith et al, 1976[a]).
Test endpoint	Mortality
Test conditions	❑ Acute tests with *P. promelas* were conducted using a flow-through system. Six concentrations (two replicates per concentration) and a control were used. Test chambers were all glass aquaria (5.1 L.) containing 4.1 L. of test volume. Ten fishes were introduced into each chamber. Tests were run for 8 days. Each day the fish were being fed and observations on mortalities, water chemistry and temperature were made. ❑ Acute tests with *D. magna* were performed by adding 10 daphnids in a series of 250 ml beakers each containing 200 mL. of water with food introduced at a concentration of 30 mg/L.. Each test consisted of four replicates with one control and six treatments per replicate. Two replicates were fed (96h) and the two others were not fed. These bioassays were conducted using a static system.

Comments	❑ Test concentrations were monitored by atomic absorption spectometry (Varian Techtron AA-6). ❑ Fish have being fed during acute tests. ❑ A mean total alkalinity of 232 mg/l for the fathead minnows acute test is reported; no alkalinity for the Daphnia test is reported ❑ Mean dissolved oxygen values of 6.88 and 7.87 mg/L. are reported for fathead minnows and daphnids tests respectively. ❑ Mean pH values of 8.02 and 8.16 are reported for fathead minnows and daphnids tests respectively (a moderate pH decrease with concentration increase is reported). ❑ Mean temperature values of 25.5 and 20.0°C are reported for fathead minnows and daphnids tests respectively ❑ Antimony concentration ranges of 1.0-27.6 and 1.65-44.15 mg/L. are reported for fathead minnows and daphnids tests respectively. ❑ LC_{50}-values were calculated using one of these four methods: binomial test, moving average method, probit method or square root product method (the method that gave the tightest confidence interval was used). ❑ 95 % Confidence limits not given. ❑ Acute toxicity ($mgSb^{3+}/l$) ▪ *P. promelas*: LC_{50} (96 h): 21,9 mg/L. ▪ *P. promelas*: LC_{50} (192 h): 20,2 mg/L. ▪ *D. magna*: LC_{50} (48 h): 18,8 mg/L (not fed) ▪ *D. magna*: LC_{50} (48 h): 12.1 mg/L. (fed)
Reliability	This study is being considered reliable. The methodology used is well described. In addition antimony concentrations were measured and water characteristics remained within the tolerance limits of the test species.

[a] *Smith L.L., Jr., D.M. Oseid, G.L. Kimball and S.G. El-Kandelgy, 1976. Toxicity of hydrogen sulfide to various life history stages of bluegill (Lepomis macrochirus). Trans. Am. Fish. Soc., 105 : 442-449.*

Data Reliability Evaluation Sheet

Title	Acute toxicity of priority pollutants to water flea (*Daphnia magna*)
Author	LeBlanc Gerald A. (1980).
Test substance	Antimony (salt form unknown)
Test species	*Daphnia magna* Straus (< 24 h old). Origin : laboratory stock culture.
Test medium	Reconstituted well water having a total hardness of 72 ± 6 mg/L. as $CaCO_3$ with a pH of 7.0 ± 0.2.
Test endpoint	Mortality
Test conditions	Stock solutions were prepared in distilled water and used to provide the desired concentrations for testing. Procedures used were based on US-EPA guidelines[a]. Five to eight nominal concentrations of the chemical were tested. Five daphnids were randomly placed in 150 mL. test solution. Each concentration was prepared in replicates of three. Tests were incubated at 22 ± 1 °C for 24h and 48 h.
Comments	❑ Only nominal concentrations have been reported. No evidence was given that the concentrations were maintained during the test. ❑ pH, O_2 and temperature were monitored at the initiation and the termination of the test. ❑ Oxygen concentrations ranged from 6.5-9.1 mg/L., pH ranged from 6.6-8.1 ❑ No concentration range is reported. ❑ No concentration-effect curve is reported. ❑ No significant differences with the control have been observed at the highest concentration tested. ❑ Acute toxicity (mg Sb^{3+}/L.): ▪ EC_{50} (24 h): > 530 mg/L. ▪ EC_{50} (48 h): > 530 mg/L. ▪ NOEC (48 h): 530 mg/L.
Reliability	This study is being classified as not reliable since the reported effect data are unbounded. Furthermore the salt form is not mentioned in the original article. According to US-EPA (1988) the salt form used would have been Sb_2O_3.

[a] *US-EPA (1975): Methods for acute toxicity tests with fish, macroinvertebrates and amphibians. Ecological Research Series (EPA-660/3-75-009), 61 p.*

Data Reliability Evaluation Sheet

Title	Alga, growth inhibition test effect of Sb_2O_3 on the growth of *Selenastrum capricornutum*
Author	Lisec, studiecentrum voor ecologie en bosbouw v.z.w (1994)
Test substance	Sb_2O_3
Test species	*Selenastrum capricornutum* (currently renamed *Raphidocelis subcapitata*): Origin: LISEC laboratory culture (ex. CCAP 278/4).
Test medium	Algal medium reconstituted according to the OECD Guideline 201[a]. Concentrations of macronutrients in mg/L. : NH_4Cl (15); $MgCl_2.6H_2O$ (12) and $CaCl_2.2H_2O$ (18); $MgSO_4.7H_2O$ (18); $NaHCO_3$ (50) and KH_2PO_4 (1.6) and several micronutrients in mg/l: $FeCl_3.6H_2O$ (0.08); H_3BO_3 (0.185); $MnCl_2.4H_2O$ (0.415); $Na_2EDTA.2H_2O$ (0.10); $CuCl_2.2H_2O$ (0.00001); $ZnCl_2$ (0.003); $CoCl_2.6H_2O$ (0.0015); $Na_2MoO_4.2H_2O$ (0.007), Temperature: 23.6-24.1°C and pH: 7.96
Test endpoint	Growth inhibition
Test conditions	Exponentially growing cultures of algae were exposed to various concentrations of the test substance for a period of 72h. Each concentration (50 mL. test volume) consisted of three replicates. Temperature ranged between 23.6-24.1 °C.
Comments	❑ Test concentrations were monitored by ICP analysis. Total concentrations were within 10 % of the nominal concentrations. Therefore the nominal concentrations have been used to calculate the effect levels. ❑ Concentration-effect curves have been reported. ❑ Concentration ranges of 100, 180, 320, 560 and 1,000 mg Sb_2O_3/L. were reported. ❑ Algal growth was determined by microscopic counting.at 48 and 72 h because of the insolubility of the test substance. ❑ pH varied in the controls from 7.96 to 8.8 during the test. ❑ pH values varied between 7.96 at the beginning to 9.65 at the end of the test. ❑ A moderate pH increase with concentration increase was observed. ❑ Acute toxicity (mg Sb^{3+}/L.) ▪ E_bC50(72h): 28.4 mg/L. (34 mg/L. Sb_2O_3) ▪ E_rC50(72h): 55.8 mg/L. (67 mg/L. Sb_2O_3 ▪ NOEC(72h): 8.3 mg/L. (10 mg/L. Sb_2O_3)
Reliability	This study is being classified not reliable since exposure concentrations exceeded the solubility of antimony trioxide. Furthermore the EC_{50} based on growth rate is invalid since only a 16 % inhibition in growth rate was observed in the highest test concentration. Hence the figure of 55.8 mg/L. is an extrapolated value and should not be used.

[a] *OECD Guidelines for Testing of Chemicals, Paris (1984). Guideline 201: Alga Growth Inhibition test*

Data Reliability Evaluation Sheet

Title	Microplate technique for screening and assessing cytotoxicity of xenobiotics with *Tetrahymena pyriformis*
Author	Sauvant M.P., Pepin D., Bohatier J. and Groliere C.A.(1995a).
Test substance	$SbCl_3$
Test species	*Tetrahymena pyriformis.* Origin: the ciliated protozoa *T. pyriformis*, strain GL was cultured in the laboratory. Tests were initiated with cells which were in the exponential growth phase.
Test medium	A PPYSm (proteose peptone yeast–extract substrate modified) medium was prepared by dissolving 7.5 g proteose peptone (Difco) and 7,5g yeast extract (Difco) in 1 liter of hot water. After filtration PPYSm was autoclaved for 20 min at 120°C. Just prior to use, PPYSm was supplemented with 2% of sterilized inorgnic salts solution containing 250 mg $ZnCl_2$ (Merck) and 5 g $CaCl_2$ (Merck) per liter of deionized water.
Test endpoint	Population growth
Test conditions	Two test methods were compared: flask method and microplate method. Flask tests were conducted in 500 ml Fernbach flasks containing 100 ml of medium. Cellular density was determined by counting with an electronic particles Coulter Counter. For the microplate technique some 2-mL aliquots of TP cultures were treated with tested substances in sterile tubes. Then the treated samples were mixed thoroughly and dispensed (200 µL/well) into a 96-well microplate. The absorbance of each well was spectrophotometrically measured against PPYSm as blank with a Biotech EL 340 microplate reader equipped with a 540-nm test wavelenght. A computer, directly connected to the spectrophotometer, allowed automatic calcultion of the optical density (OD). The microplates were incubated for 36 h at a temperature of 28 °C. OD measurements were repeated every 2h.
Comments	❑ Test concentrations were not measured. No evidence was given that the concentrations were maintained during the test. ❑ No hardness and alkalinity of the test medium are reported. ❑ No pH of the test medium is reported. ❑ Effect levels are calculated by linear regression analysis. No 95% confidence limits were reported. ❑ No concentration range is reported ❑ Acute toxicity of Sb^{3+} inhibitory concentration (Relative Doubling Time) ▪ Microplate technique IC_{50} (36h): 6 mg/L. ▪ Flask technique IC_{50} (36h):16 mg/L.
Reliability	This study is being classified as reliable. However, important information is missing concerning the hardness and pH of the dilution medium.

Data Reliability Evaluation Sheet

Title	Effects of organic and inorganic substances on the cell proliferation of *L-929 Fibroblasts* and *Tetrahymena pyriformis GL* protozoa used for toxicological bioassays
Author	Sauvant M.P., Pépin D., Grolière C.A., Bohatier J. (1995)
Test substance	$SbCl_3$
Test species	❑ *L-929 murine fibroblasts,* Origin: Laboratory culture, ECACC n° 85011425. Tests were initiated with cells which were in the exponential growth phase. *Tetrahymena pyriformis GL;* Origin: the ciliated protozoa *T. pyriformis*, strain GL was cultured in the laboratory. Tests were initiated with cells which were in the exponential growth phase.
Test medium	❑ *L-929 Fibroblasts:* Eagle's Minimum Essential Medium (MEM) supplemented with 5% fetal calf serum, 1% L-glutamine, 1% non-essential amino acids; 1% vitamins and 0,1 g gentamicine/l ❑ *Tetrahymena pyriformis GL:* an autoclaved proteose peptone/yeast extract medium enriched with inorganic salts (PPYS).
Test endpoint	Population growth
Test conditions	❑ *L-929 Fibroblasts:* 10-mL cultures in exponential growth phase were treated with sterile solutions of chemical substances and incubated at 37°C in a 5%-CO_2 humidified atmosphere. After a 24-hr exposure the viability was evaluated by Trypan Blue Dye exclusion and the viable cell proliferation (CPR) was evaluated by counting achieved in Malassez hematocytometer under contrast phase microscope. Tests were terminated after 24 h. ❑ *Tetrahymena pyriformis GL:* Chemical substances were added to the medium of exponential 100-ml growth culture (10^4 TP/ml) in a constant 1%-volume. The viability and mobility were monitored by examination with a photonic microscope (Nikon). After fixation of the 1-mL cell suspension with 1 mL 4% fomaldehyde in Isoton buffer, the cell density of the culture was determined after 3, 6 an 9 h incubation period, using an electronic particles Coulter Counter ZM.

Comments	❑ Test concentrations were not measured. No evidence was given that the concentrations were maintained during the test.
	❑ No hardness and alkalinity of the test medium are reported.
	❑ No pH of the test medium is reported.
	❑ Effect levels are calculated by linear regression analysis. No 95% confidence limits were reported.
	❑ No concentration range is reported.
	❑ Acute toxicity of Sb^{3+} inhibitory concentration
	  ❑ *L-929 Fibroblasts:*
	  ▪ IC_{50} (24h): 22 mg/L.
	  ❑ *Tetrahymena pyriformis GL:*
	  ▪ IC_{50} (3h): 60 mg/L.
	  ▪ IC_{50} (6h): 38 mg/L.
	  ▪ IC_{50} (9h): 20 mg/L.

Reliability	This study is being classified as reliable. However, important information is missing concerning the hardness and pH of the dilution medium.

Data Reliability Evaluation Sheet

Title	Toxicity of less common metals to fishes
Author	Tarzwell C.M. and Henderson C. (1960)
Test substance	$SbCl_3$ and Sb_2O_3
Test species	Pimephales promelas *(fathead minnows)*
Test medium	Tests were carried out in hard (hardness: 400 mg/L. as $CaCO_3$, total alkalinity: 360 mg/L as $CaCO_3$ and pH 8.2) and soft waters (hardness: 20 mg/L. as $CaCO_3$, total alkalinity: 18 mg/L. as $CaCO_3$ and pH 7.4).
Test conditions	not described
Comments	❑ No temperature of the test medium is reported ❑ Test concentrations were not measured. No evidence was given that the concentrations were maintained during the test. ❑ No 95% confidence limits were calculated. ❑ No concentration range is reported. ❑ Only exploratory tests were made. ❑ Acute toxicity (mg Sb^{3+}/L.): ▪ LC_{50} (96h) for $SbCl_3$ = 9 mg/L. (soft water) and 17 mg/L. (hard water) ▪ LC_{50} (96h) for Sb_2O_3 > 80 mg/L. for both soft and hard water.
Reliability	This study is being classified as not reliable since background information is missing on the methodology used. Furthermore the exposure concentrations of antimony trioxide exceeded the solubility.

Data Reliability Evaluation Sheet

Title	In-depth studies on health and environmental impacts of selected water pollutants (Table of data available from Charles E. Stephan)
Author	U.S. Environmental Protection Agency (1978) (also referred to in USEPA (1980 en 1988).
Test substance	Sb_2O_3
Test species	*Selenastrum capricornutum* (currently renamed *Raphidocelis subcapitata*)
Test medium	Unknown
Test endpoint	Inhibition of chlorophyll (i) Reduction in cell number (r)
Test conditions	Unknown
Comments	❑ Acute toxicity (mg Sb^{3+}/L.) ▪ EC_{50}i (96h): 0.610 mg/L. ▪ EC_{50}r (96h): 0.630 mg/L.
Reliability	This study is being classified as not reliable due to the complete lack of information on test methodology, test medium and used statistics.

Data Reliability Evaluation Sheet

Title	Aquatic toxicity testing using the nematode, *Caenorhabditis elegans*
Author	Williams P.L. and Desenbery D.B.(1990).
Test substance	$SbCl_3$
Species	*Caenorhabditis elegans.* Origin: Laboratory culture, var. Bristol (strain N2). Young adult nematodes (3 to 4 days old) were used.
Test medium	Nutrient-free medium consisting of deionized, distilled H_2O with 1.23 g of NaCl and 0.968 g of KCl (K-medium). Food source *E. coli* strain OP50 was added.
Test endpoint	Mortality
Test conditions	The dilutions of the metal were directly made into K-Medium, with the final dilutions in K-medium containing the OP50. Tests were conducted in multiwell plates (1 ml of test solution). 25 replicates for each concentration. Tests were incubated at 20 °C and scored at each 24 h interval. Complete duration of the test was 96 h.
Comments	❑ No hardness and alkalinity data reported. ❑ No pH range of the test solution reported. ❑ Test concentrations were not measured. No evidence was given that the concentrations were maintained during the test. ❑ No 95% confidence limits were calculated because only lower limits for LC_{50} values could be reported due to solubility limitations. ❑ The concentrations used were within the water solubility range for the metallic salt. ❑ No concentration-effect relations are reported ❑ A 96-h lethality rate of 3% in the parallel control cultures is reported ❑ Acute toxicity (mg Sb^{3+}/L.) ▪ LC_{50} (96h) > 20 mg/L.
Reliability	This study is being classified as not reliable since the reported effect data are unbounded. In addition important information is missing concerning the hardness and pH of the dilution medium.

ANNEX C: Classification categories and criteria in the "Commission Directive 93/21/EEC adapting to technical progress for the 18th time Council Directive 67/548/EEC "

Acute toxicity

Class: Acute I	
Acute toxicity:	
96h-LC_{50} (for fish)	≤ 1 mg/L or
48h-EC_{50} (for *Daphnia*)	≤ 1 mg/L or
72 h-IC_{50} (for algae)	≤ 1 mg/L
Risk phrases: R50-R53: Very toxic to aquatic organisms and may cause long-term adverse effects to the aquatic environment and the substance is not readily degradable or the log Pow ≥ 3 (unless the experimental determined BCF is ≥ 100	
Class: Acute II	
Acute toxicity:	
96h-LC_{50} (for fish)	>1 - ≤ 10 mg/L or
48h-EC_{50} (for *Daphnia*)	>1 - ≤ 10 mg/L or
72 h-IC_{50} (for algae)	>1 - ≤ 10 mg/L
Risk phrases: R51-R53: Toxic to aquatic organisms and may cause long-term adverse effects to the aquatic environment and the substance is not readily degradable or the log Pow ≥ 3 (unless the experimental determined BCF is ≥ 100	
Class: Acute III	
Acute toxicity:	
96h-LC_{50} (for fish)	>10 - ≤ 100 mg/L or
48h-EC_{50} (for *Daphnia*)	>10 - ≤ 100 mg/L or
72 h-IC_{50} (for algae)	>10 - ≤ 100 mg/L
Risk phrases: R52-R53: Harmfull to aquatic organisms and may cause long-term adverse effects to the aquatic environment and the substance is not readily degradable.	

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