RISK ASSESSMENT REPORT

ZINC SULPHATE

CAS-No.: 7733-02-0

EINECS-No.: 231-793-3

GENERAL NOTE

This document contains:

- part I Environment (pages 41)
- part II Human Health (pages 130)

RISK ASSESSMENT

ZINC SULPHATE

CAS-No.: 7733-02-0

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Final report, May 2008

PART 1

Environment

Rapporteur for the risk evaluation of zinc sulphate is the Ministry of Housing, Spatial Planning and the Environment (VROM) in consultation with the Ministry of Social Affairs and Employment (SZW) and the Ministry of Public Health, Welfare and Sport (VWS). Responsible for the risk evaluation and subsequently for the contents of this report is the rapporteur.

The scientific work on this report has been prepared by the Netherlands Organization for Applied Scientific Research (TNO) and the National Institute of Public Health and Environment (RIVM), by order of the rapporteur.

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PREFACE

For zinc metal (CAS No. 7440-66-6), zinc distearate (CAS No. 557-05-1 / 91051-01-3), zinc oxide (CAS No.1314-13-2), zinc chloride (CAS No.7646-85-7), zinc sulphate (CAS No.7733-02-0) and trizinc bis(orthophosphate) (CAS No.7779-90-0) risk assessments were carried out within the framework of EU Existing Chemicals Regulation 793/93. For each compound a separate report has been prepared. It should be noted, however, that the risk assessment on zinc metal contains specific sections (as well in the exposure part as in the effect part) that are relevant for the other zinc compounds as well. For these aspects, the reader is referred to the risk assessment report on zinc.

CONTENTS

0 OVERALL CONCLUSIONS/RESULTS OF THE RISK ASSESSMENT

CAS No. 7733-02-0

EINECS No. 231-793-3

IUPAC Name Zinc Sulphate

( ) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

(X) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

(X) iii*) A conclusion applied to local scenarios in which the local scenario merits conclusion (ii) but where (possibly) due to high regional background concentrations a local risk cannot be excluded.

LOCAL

Conclusion (ii) is drawn for all local scenarios, including secondary poisoning, except those listed below.

Conclusion (iii) or (iii*) is drawn for the specified scenarios, because:

STP

- the PEC_{STP} exceeds the $PNEC_{add}$ for microorganisms for three processing scenarios of zinc sulphate listed in Table 3.4.10 (**conclusion iii**).

Surface water

- the calculated $Clocal_{add}$ in water is greater than the $PNEC_{add}$ in surface water for one processing scenario for zinc sulphate listed in Table 3.4.10 (**conclusion iii**).
- the $Clocal_{add}$ / $PNEC_{add}$ ratio is 1 **(conclusion ii**) for one other processing scenario for zinc sulphate listed in Table 3.4.10, but a potential risk at local scale cannot be excluded due to the possible existence of high regional background concentrations **(conclusion iii***).

Sediment

- the $Clocal_{add}$ in sediment exceeds the $PNEC_{add}$ in sediment for three processing scenarios listed in Table 3.4.10 (**conclusion iii**).
- the $Clocal_{add}$ / $PNEC_{add}$ ratio is 1 for the remaining scenarios for zinc sulphate listed in Table 3.4.10 **(conclusion ii**), but a potential risk at local scale cannot be excluded due to the possible existence of high regional background concentrations **(conclusion iii*).**

Soil

- $PEClocal_{add}$ / $PNEC_{add}$ ratios >1 exist for the terrestrial compartment for three processing scenarios of zinc sulphate listed in Table 3.4.10 **(conclusion iii).**

REGIONAL

The regional risk characterisation is discussed in the RAR on Zinc Metal.

1 GENERAL SUBSTANCE INFORMATION

Identification of the substance

CAS-No.: 7733-02-0
EINECS-No.: 231-793-3
IUPAC name: zinc sulphate
Synonyms: sulphuric acid-zinc salt; zincate; zincomed; white vitriol; zinc vitriol
Molecular formula: $ZnSO_4$
Structural formula: $ZnSO_4$
Molecular weight: 161.4

Purity/impurities, additives

Purity: no data
Impurity: no data
Additives: no data

Physico-chemical properties

In table 1A the physico-chemical properties are summarized.

Table 1A Physico-chemical properties of zinc sulphate

Property	**Result**	**Comment**
Physical state	solid	*
Melting point	600 ºC	*
Boiling point	not applicable	*
Relative density	3.54	*
Vapour pressure	no data	***
Surface tension	no data	***
Water solubility	220 g/l (20ºC)	**
Solubility in other solvents	slowly soluble in alcohol; soluble in MeOH and glycerin	*
Partition coefficient n-octanol/water(log value)	no data	***
Flash point	not applicable	***
Flammability	not flammable	***
Autoflammability temperature	not applicable	***

Property	Result	Comment
Explosive properties	not explosive	***
Oxidizing properties	not oxidizing	***

* More than one apparently independent source. No methods are specified.

** One source.

*** Conclusion based on theoretical and/or structural considerations.

These data are mainly derived from CRC Handbook of Chemistry and Physics (1995), Sax's Dangerous Properties of Industrial Materials (1984), Patty's Industrial Hygiene and Toxicology (1981), Römpp Chemie Lexikon (1995), Ullmann's Encyklopädie der Technischen Chemie (1983), and company information. For an extended description see HEDSET.

Conclusion:
Data on boiling point, vapour pressure, surface tension, and partition coefficient were not provided. In view of the nature of the substance determination of these parameters is considered to be irrelevant. Information on flammability, explosive properties and oxidizing properties is not available. However, on theoretical considerations the compound is concluded to be not flammable, not explosive and not oxidizing. All other required physico-chemical data were submitted. None of these data is based on test results, substantiated with reports. However, the data are considered as sufficiently reliable to fulfil the Annex VIIA requirements.

Classification and labelling (human health, environment and physico-chemical)

Annex 1 of Directive 67/548/EEC contains a list of harmonised classifications and labellings for substances or groups of substances, which are legally binding within the EU.
For zinc sulphate the current Annex 1 classification and labelling (29th ATP, 2004) is as follows:

Classification
Xn; R22
R41
N; R50-53

Labelling
Xn; N
R: 22-41-50/53
S: (2-)22-26-39-46-60-61

The above classification and labelling is for hydrous forms of zinc sulphate (mono-, hexa-, and hepta-hydrate) and for anhydrous zinc sulphate.

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

Zinc sulphate is produced (>1000 t/y) at five known sites in the European Union (see Table 2.1.1)

Table 2.1.1 Production sites of zinc sulphate (>1000 t/y) in the EU (Information from Industry)

Company	**Location**
Floridienne Chimie S.A.	Belgium
Grillo Werke AG	Duisburg, Germany
Pasminco Budel Zink	The Netherlands
Eco-Zinder	Trezzo, Italy
I.C.A. SRL	Arborio, Italy

The total production volume of zinc sulphate in the EU for 1994 is confidential. The same is true for export figures outside the EU. Zinc sulphate was imported from outside the EU by at least one company. The import volume is unknown. Based on production, export and import data the total use volume within the EU is estimated to be circa 28,000 tonnes per year.

2.1.1 Production process

Zinc sulphate is produced starting with zinc bearing material, as for instance zinc ashes, zinc carbonate or other zinc remains. Initially, zinc is leached from these materials with sulphuric acid, followed by various purification processes. Lead sulphate is precipitated and sold as secondary raw material. A reduction of pH to mildly acidic concentrations is affected by addition of zinc oxide, leading to a precipitation predominantly of manganese and iron hydroxide (jarosite sludge). Metallic zinc dust is added, which leads to the precipitation of other heavy metals such as copper, cadmium etc., which is sold as secondary raw material. Pure zinc sulphate is crystallised from the resulting solution (heptahydrate or hexahydrate), or thermally dehydrated to mono-hydrate. In the production process any resulting water is recirculated into the system.

2.2 USE PATTERN

Table 2.2.1 shows the industrial and use categories of zinc sulphate. Zinc sulphate is mainly used for the production of fertilisers and pesticides (60%). The remaining part is used for agriculture pharmaceutical purposes such as feedstuff additives (20%) and the chemical industry (20%).

Minor usages of zinc sulphate are applications in the viscose production as flotation agent in the mining industry, as corrosion inhibitor in the galvanising industry and in water treatment processes (Industry Annex VII A). The main type of use category of zinc sulphate can be characterised as wide dispersive.

Table 2.2.1 Industrial and use categories of zinc sulphate in the EU (Information from Industry)

Industrial category	**EC no.**	**Use category**	**EC no**
Agricultural	1	Fertilisers Feedstuff additive (pharmaceutical)	19 41
Basic chemicals	2	Laboratory chemicals Others	34 55
Agrochemical industry	3	Intermediate for pesticides production	33
Metal extraction, refining and processing industry	8	Flotation agents	23
Others	15/0	Corrosion inhibitors Others: viscose production	14 55

3 ENVIRONMENT

3.1 GENERAL INTRODUCTION

The presence of zinc in the environment due to natural processes (resulting in a natural background concentration of zinc in all environmental compartments, incl. organisms), the chemical processes that will affect the speciation of zinc in the environment, and the fact that zinc is an essential element have implications for the environmental exposure and effect assessment of zinc and thus for the risk characterisation of zinc.

Since the Technical Guidance Document (TGD) does not provide detailed information on how to deal with (essential) elements that have a natural background concentration in the environment, such as zinc, the "added risk approach" (according to Struijs et al., 1997 and Crommentuijn et al., 1997) has been used in this risk assessment report on zinc. In this approach both the "Predicted Environmental Concentration" (PEC) and the "Predicted No Effect Concentration" (PNEC) are determined on the basis of the added amount of zinc, resulting in an "*added* Predicted Environmental Concentration" (PEC_{add}) and "*added* Predicted No Effect Concentration" ($PNEC_{add}$), respectively. It is thus assumed that zinc (and not a specific zinc compound such as zinc sulphate) is the causative factor for toxicity. It is emphasised, however, that the local PEC_{add} values are based on the emissions of zinc due to the production or use of zinc sulphate. The use of the added risk approach (a method that in principle can be used for all naturally occurring substances) implies that only the anthropogenic amount of a substance, i.e. the amount added to the natural background concentration, is considered to be relevant for the effect assessment of that substance. Thus, a possible contribution of the natural background concentration to toxic effects is ignored.

In the present environmental exposure assessment (section 3.2), the use of the added risk approach implies that the local PEC_{add} values have been calculated from zinc emissions due to anthropogenic activities (in this case: zinc emissions due to the production and use of zinc sulphate) Thus, the PEC_{add} is the anthropogenic part of the zinc concentration in the environment. By focusing only on the anthropogenic part of zinc, the problem of the great variety of natural background concentrations of zinc over the different geographic regions is eliminated. Of course it is realised that comparison of the PEC_{add} with measured environmental concentrations must take into account that the latter values comprise the natural background concentration (Cb) and the anthropogenic part.

In the environmental effect assessment (section 3.3), the use of the added risk approach implies that the $PNEC_{add}$ has been derived from toxicity data that are based on the added zinc concentration in the tests. Thus, the $PNEC_{add}$ is the maximum permissible addition to the background concentration. From the background concentration (Cb) and the $PNEC_{add}$, the PNEC can be calculated: $PNEC = Cb + PNEC_{add}$.

Finally, in the environmental risk characterisation (section 3.4), the use of the added risk approach implies the evaluation of the PEC_{add} / $PNEC_{add}$ ratios. In case measured environmental concentrations are used in the risk characterisation, either the background concentration has to be subtracted from the measured environmental concentration (resulting in a "PEC_{add} / $PNEC_{add}$" ratio) or the background concentration has to be added to the $PNEC_{add}$ (resulting in a traditional "PEC / PNEC" ratio).

3.2 EXPOSURE ASSESSMENT

General information about zinc is available in many publications, e.g. the 'Integrated Criteria Document Zinc' (Cleven et al., 1993) and in the 'Environmental Health Criteria for Zinc' (WHO, 1996). In the present series of risk assessment reports on zinc only a summary of the available information is given. In the sections 3.2.2, 3.2.3 and 3.2.4 of the zinc metal RAR, general characteristics are described which are relevant for the release and fate of zinc in the environment. It must be noted that it is very difficult to define the exact form of zinc once emitted by the zinc sulphate industry. Hence, for pragmatically reasons in this document emissions and environmental concentrations are expressed as zinc and not e.g. zinc sulphate, unless otherwise mentioned.

Section 3.2.1 presents the added Predicted Environmental Concentrations ((PE)C_{add}s) for several exposure scenarios. The (PE)C_{add}s are derived from either modelling or measured exposure data. The local exposure assessment for the production and use of zinc sulphate is presented in section 3.2.1.2. This local exposure assessment is focused on the emissions of industrial point sources. A regional exposure assessment is described in section 3.2.5.3 (zinc metal RAR). The regional exposure assessment includes the industrial and diffuse emissions of all current EU priority zinc compounds. In case of diffuse emissions it is not possible to distinguish between emissions from current EU priority zinc compounds and non-EU priority list zinc compounds. The diffuse emissions may thus also comprise emissions from other zinc compounds (Figure 3.2.1) For the local exposure assessment of the other zinc compounds the reader is referred to those separate reports.

A general description about the release and fate of zinc (sections 3.2.2, 3.2.3 and 3.2.4) and the regional exposure assessment (section 3.2.5.3) is only presented in the zinc metal report, but it is applicable to the exposure assessment of all current EU priority zinc compounds.

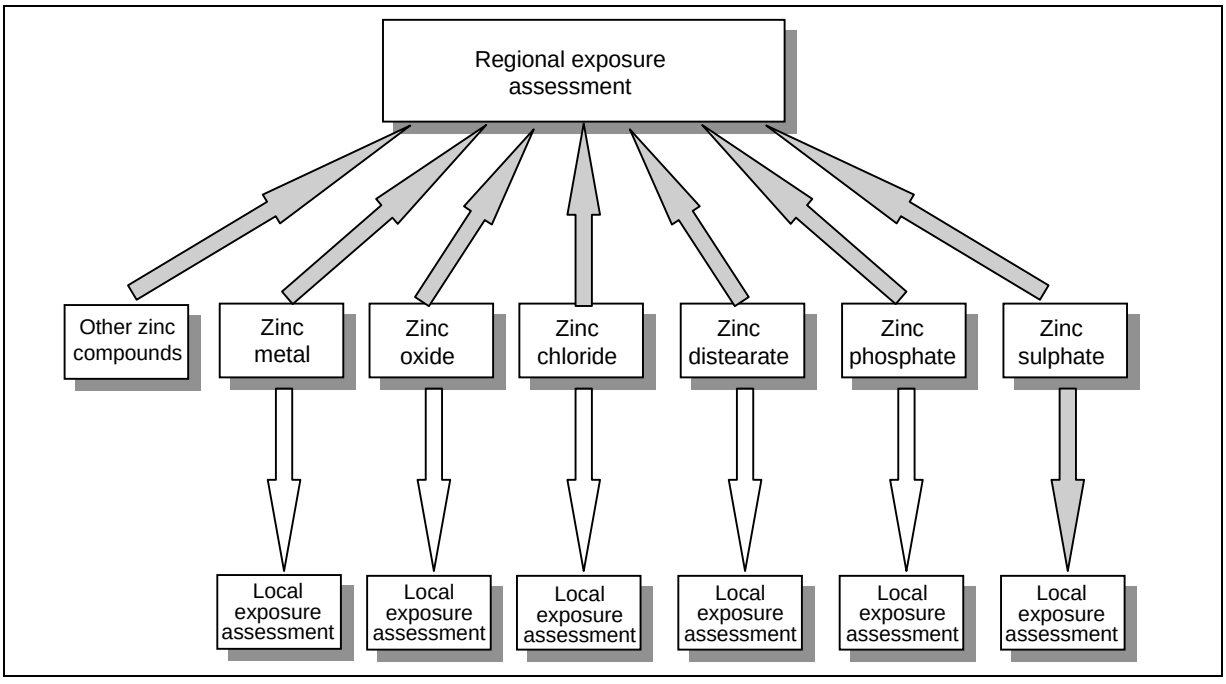

Figure 3.2.1 Theoretical outline for the regional and local exposure assessment for zinc sulphate (and other zinc compounds).

3.2.1 Exposure scenarios

3.2.1.1 General

The objective of this exposure assessment is to determine the emissions, pathways and rates of movement and the transformation of zinc sulphate in order to estimate the predicted environmental concentration ((PE)C) for the different environmental compartments. The EU-Technical Guidance document (TGD, 1996) and the European Union System for the Evaluation of Substances (EUSES 1.0) are used as a guideline to achieve this objective. The entry for estimating the environmental concentrations is, when available, the submitted industrial information, including monitoring data, and/or information gathered from other sources. Deviations from the TGD are mentioned in the text. Otherwise (PE)C values will be calculated according to the TGD. For modelling the behaviour of zinc in the environment the octanol-water partitioning coefficient (Kow) and the water solubility are not appropriate. Measured Kp values are used instead for soil, sediment and suspended matter (TGD (Ap. VIII), 1996). See sections 3.2.2 and 3.2.3 (zinc metal RAR) for more information about the used Kp values. The vapour pressure has been fixed on a low value of 1.10^{-10} Pa and the biotic and abiotic degradation rates have been minimised (TGD (Ap. VIII), 1996).

In the local exposure assessment the agricultural soil concentrations are calculated accounting for accumulation for 10 consecutive years. One should realise that this TGD defined period of 10 years is of lesser relevance to metals than to most organic chemicals. For zinc no steady state will be reached within 10 years. Unless stated otherwise, the input sources to the agricultural soil compartments are the application of sludge and the airborne deposition. For zinc the only removal or output from the agricultural soil compartment is by leaching to deeper soil layers. It is emphasised that other input or output sources, e.g. the use of manure or the crop offtake, are not taken into account for zinc in the local scenarios In the regional exposure assessment steady state agricultural soil concentration are calculated, accounting for the input sources deposition from air, sludge application, corrosion, manure and fertilisers and the output sources leaching to deeper soil layers and offtake via crops. The reason that factors like manure input and removal via crops have been applied in the regional calculations and not in the local modelling is pragmatic: there are reliable, average estimates available for these parameters at a regional level.

The mentioned concentrations ($(PE)C_{add}$) in surface water are mostly expressed as dissolved zinc concentrations. In the exposure scenarios the concentrations effluent water are expressed as total zinc concentrations. Only in the risk characterisation the total effluent concentrations are converted to dissolved effluent concentrations. The concentrations in sediment and soil are initially expressed on a wet weight (wwt) basis. Only when it is explicitly mentioned concentrations are dry weight (dwt) based.

Depending on the information submitted to the rapporteur, the (PE)C calculations start at a different level. The different levels are presented in the flowchart of Figure 3.2.2. A generic scenario is used when no specific industrial emission information is available. In that case the EU (production) tonnage is the starting point for calculating the (PE)C (entry 1). When a regional tonnage or an EU emission is available, which can be possible for the formulating and processing stages, the starting point is subsequently entry 2 or entry 3. With a regional tonnage regional emissions can be derived by multiplying it with the appropriate release fractions (A-Tables, TGD, 1996). An EU emission can be divided by 10 to derive a regional emission. The justification of the use of the 10% rule in the emission estimation is explained in the paragraphs concerning the use categories of zinc sulphate. Also a submitted regional emission can be an entry for the (PE)C calculation (entry 4). With this regional emission a local emission can be derived by multiplying it with the appropriate fraction of main source (B-Tables, TGD, 1996). With a local tonnage (entry 5) also local emissions can be derived by multiplying it with the appropriate release fractions (A-Tables, TGD, 1996). A site specific scenario can be used when local emissions are submitted by the industry (entry 6). The risk characterisation, i.e. the comparison of the PEC with the corresponding PNEC, should be based on the most realistic exposure information. For this, the calculated local PEC values are compared with measured local concentrations, if available (entry 7). In the risk characterisation step a possible correction of the PEC for bioavailability is performed. In the next sections reference is made to Figure 3.2.2 for a better understanding of the procedures followed and entry points of the exposure assessment.

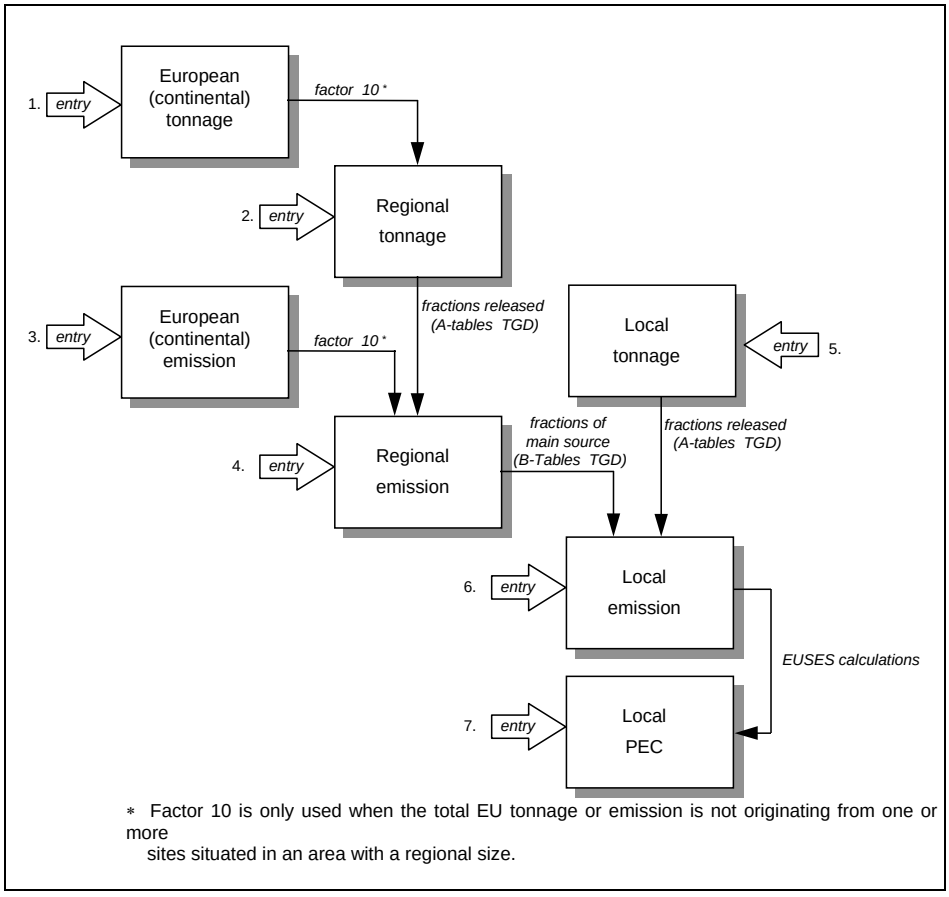

Figure 3.2.2 Flowchart for calculating the PEC, the entry for the calculations is depending on the submitted information.

As stated in section 2.1.1. of the RAR on zinc metal the environmental releases from waste, including mining waste, are not taken into account in the current risk assessment. The Rapporteur recognises that those releases can be significant, but the general instrumentation is currently lacking on how to deal with this type of emissions (mostly landfills).

3.2.1.2 Local exposure assessment

3.2.1.2.1 General

The local environmental exposure assessment of zinc is based on the releases of zinc during the following life cycle stages:

1. Production of zinc sulphate
2. Processing in the agricultural pesticide industry
3. Formulation in the agricultural fertiliser industry
4. Formulation in agricultural feed industry
5. Formulation and processing in chemical industry

For a number of production plants site specific emission scenarios are used for calculating the predicted environmental concentrations ((PE)C_{add}) in the various compartments. This is based on submitted location-specific aquatic, atmospheric and waste emission. Also a generic scenario for production is carried out. The emissions per annum submitted to the rapporteur are corrected for the number of production days according to the TGD (1996). For the zinc sulphate producers it is assumed that they produce 300 days per annum. The submitted aquatic emissions mentioned in this report are assumed to be waste water emissions to a local waste water treatment plant (WWTP) or municipal sewage treatment plant (STP), unless it is otherwise mentioned. For the formulation and processing stages continental EU production tonnages are used for calculating the (PE)C_{add} values in the various compartments according to generic scenarios (entry 1, Figure 3.2.2). Generic scenarios are only used if data are missing from either the industry or other sources in order to carry out a representative local exposure assessment.

It is emphasised that all calculated local C_{add} and PEC_{add} values are expressed as zinc, not as zinc sulphate.

3.2.1.2.2 Production of zinc sulphate

Because of confidentiality reasons, the production tonnages and emissions submitted by three zinc sulphate producing companies (out of 5) in the EU are not presented in this report. For those three production plants a site specific emission scenario is used for calculating the predicted environmental concentrations ((PE)C_{add}) in the various compartments (entry 6,

Figure 3.2.2). Company number three not only produces zinc sulphate, but also zinc metal. Although not explicitly mentioned, the rapporteur assumes that no distinction can be made between the emissions of zinc metal and zinc sulphate at that particular site. Hence, the emissions of the company mentioned in the zinc metal RAR are also applicable to zinc sulphate. For the exposure assessment of this company is referred to the zinc metal RAR. The company number, mentioned in the zinc metal RAR, is not given here for confidentiality reasons. For the remaining two production companies the C_{add} values are based on a realistic worst case scenario. For this scenario the highest calculated emission factor to air and waste water is used, which is based on actually submitted emission data of the other sites. The calculated emission factor to air is $1.14.10^{-5}$ (based on site 1) and for waste water this factor is zero.

Air

For the zinc sulphate producers in the EU the site-specific emission data is used for calculating the C_{add} value in air. One company reported that there is no emission to air. No separate assessment is executed for company number 3, because this company is already described in the zinc metal RAR. From the daily amounts released to air the EUSES model calculates local annual average atmospheric C_{add} values at a distance of 100 meters from a point source. The emission amounts during emission period and the calculated local annual average concentrations of zinc in air are presented in Table 3.2.2. The calculated local C_{add} values in air for site 1 and 2 are **0.078 µg/m^3** and **0 µg/m^3**, respectively. The calculated local C_{add} values in air for the realistic worst case scenario's (company 4-5) are **0.043 µg/m^3** and **0.035 µg/m^3**.

Water and sediment

The zinc sulphate producers number 1 and 2 in the EU submitted to the rapporteur that there are no emissions to water, because all process water is maintained in a closed system (Table 3.2.2). Based on the production process, the emissions to water of company 1 and 2 are assumed to be representative for the remaining companies 4 and 5. The emissions of company 4 and 5 are also assumed to be zero. Therefore for all production sites the local concentrations in water and sediment are not calculated. As stated above no separate assessment is executed for company number 3, because this company is already described in the zinc metal RAR.

Table 3.2.2 Summary of the local production tonnages, emission rates and calculated C_{add} values.

Company no.	Production (t/y)	Emission air (kg Zn/d)	Emission water (kg Zn/d)	C_{add} air (µg/m^3)	Concentr. effl. STP (µg/l)	C_{add} water (µg/l)	C_{add} sediment (mg/kg$_{wwt}$)
1	Conf.	Conf.	0	0.078	0	0	0
2	Conf.	Conf.	0	0	0	0	0
3	Conf.	- 2)	- 2)	- 2)	- 2)	- 2)	- 2)
4	5000 3)	0.189 1)	0 1)	0.0433	0	0	0
5	4000 3)	0.152 1)	0 1)	0.0347	0	0	0

1) No data submitted, emission calculated with release factor of company 1
2) No data submitted. For the exposure assessment of company three is referred to the zinc metal RAR
3) Preliminary estimated tonnage (by lead company), because the producer did not respond

Soil

According to the TGD (1996) both the application of STP sludge on agricultural soil and the deposition from air are taken into account for calculating the zinc levels in the terrestrial compartment. For all production companies the zinc concentration in the terrestrial compartment is only calculated from the emission to air, followed by a distribution and deposition model, because there is no emission to water. For production company 2 the zinc concentration in the terrestrial compartment is zero, because there is no emission to air or water. For production companies 4 and 5 a realistic worst case scenario is used for calculating the local C_{add} value in agricultural soil. The calculated concentrations of zinc in agricultural soils at a local scale are presented in Table 3.2.3. For company 1 the calculated local C_{add} value in agricultural soil is **0.029 mg/kg$_{wwt}$**. The calculated local C_{add} values in agricultural soil for the generic scenario's (company 4-5) are **0.013 mg/kg$_{wwt}$** and **0.016 mg/kg$_{wwt}$**.

Table 3.2.3 Summary of the local emission rates and calculated C_{add} values for agricultural soils

Company no.	Emission Air (kg Zn/d)	Emission waste water (kg Zn/d)	C_{add} agricultural soil (mg/kg$_{wwt}$)
1	Conf.	Conf.	0.029
2	Conf.	Conf.	0
3	- [1)]	- [1)]	- [1)]
4	0.189 [2)]	0 [2)]	0.016
5	0.152 [2)]	0 [2)]	0.013

1) No data submitted. For the exposure assessment of company three is referred to the zinc metal RAR

2) No data submitted, emission calculated with realistic worst case scenario

Waste

Inherent to the production process of zinc sulphate, i.e. by using secondary products or zinc waste as raw material, waste will be generated.. The produced solid waste is mainly sludge, containing mainly lead sulphate, and further sand, stones, zinc ferrite and some zinc sulphate. This sludge is used as raw material in the lead industry. The produced jarosite sludge is dumped, because of the low metal content. Copper and cadmium are cemented with zinc dust. This residue is further processed by the metal industry. The composition of the produced sludge depends on the composition of the used raw material (Industry Annex VII A).

For one company circa 400 t/y of jarosite sludge are deposited as waste in a disposal site. The annual deposition volume of this disposal site is 40,000 tonnes. In the leachate of the deposited sludge amount of 400 t/y, zinc concentrations of circa 1.3 mg/l were measured. The total leachate volume of this entire waste disposal site amounts to about 10,000 cubic meters per year, with a total average zinc concentration of about 0.25 mg/l (apparently dilution occurs from 1.3 to 0.25 mg/l). According to industrial information there is no upward trend in zinc concentrations in the leachate during time. This disposal site maintains an own leachate treatment plant, involving a biological treatment unit, an active carbon adsorption unit and an ion exchange unit. The processed leachate is subsequently discharged to a communal STP.

For the other companies no information about waste emissions are available. See general note on waste in section 3.2.1.1.

3.2.1.2.3 General information on the use categories of zinc sulphate in the EU

Zinc sulphate is mainly used in the agricultural industry for the production of pesticides and the formulation of fertilisers. Zinc sulphate is further used in the agricultural feed industry, and in the chemical industry. The distribution and EU tonnage of these use categories in the EU are presented in Table 3.2.4.

Table 3.2.4 Distribution and EU tonnage for the different use categories of zinc sulphate in the EU for 1994 (Information from the industry).

Use category	fraction	EU tonnage
Agricultural pesticide industry	± 30%	7,760
Agricultural fertiliser industry	± 30%	7,760
Agricultural feed industry	± 20%	4,880
Chemical industry	± 20%	5,400
Total	**100%**	**25,800**

The EU production tonnages were submitted by the zinc sulphate industry. When relevant for the use categories the EU production tonnages are divided by 10 (the so-called 10% rule) to obtain regional tonnages. With the regional tonnages regional emissions are obtained, when the release fractions are applied (A-tables, TGD 1996).

With the regional emission values local daily emission values are calculated by multiplying them with the fraction of main source and with a correction factor for the number of processing days (B-tables, TGD, 1996). See Figure 3.2.2, page 15 , entry 1. The regional tonnage for this life cycle stage is used as input to obtain the fraction of main source. With the local emission values local C_{add} values are calculated for each compartment.

For the water compartment of all formulation and processing stages no information is available about the adsorbed fraction of zinc in waste water belonging to a particular process. Adsorption is the most important removal process in STPs, other removal processes (vaporisation, degradation) are considered not to be relevant for zinc. More information about the suspended and dissolved forms of zinc is presented in section 3.2.2.1 of the zinc metal RAR. For the generic scenarios one rate of removal of zinc in a WWTP or STP will be applied. It is assumed that 74% of the total emission to waste water is directed to sewage sludge (Figure 3.2.3). This percentage is based on measured influent and effluent concentrations of STPs. The average removal of zinc in the examined STPs was about 74% (RIZA, 1996). In absence of specific information it is assumed that this value is also representative for the removal in industrial STP's. The removal rate of 74% is further used for calculating the C_{add} water from the calculated waste water emissions.

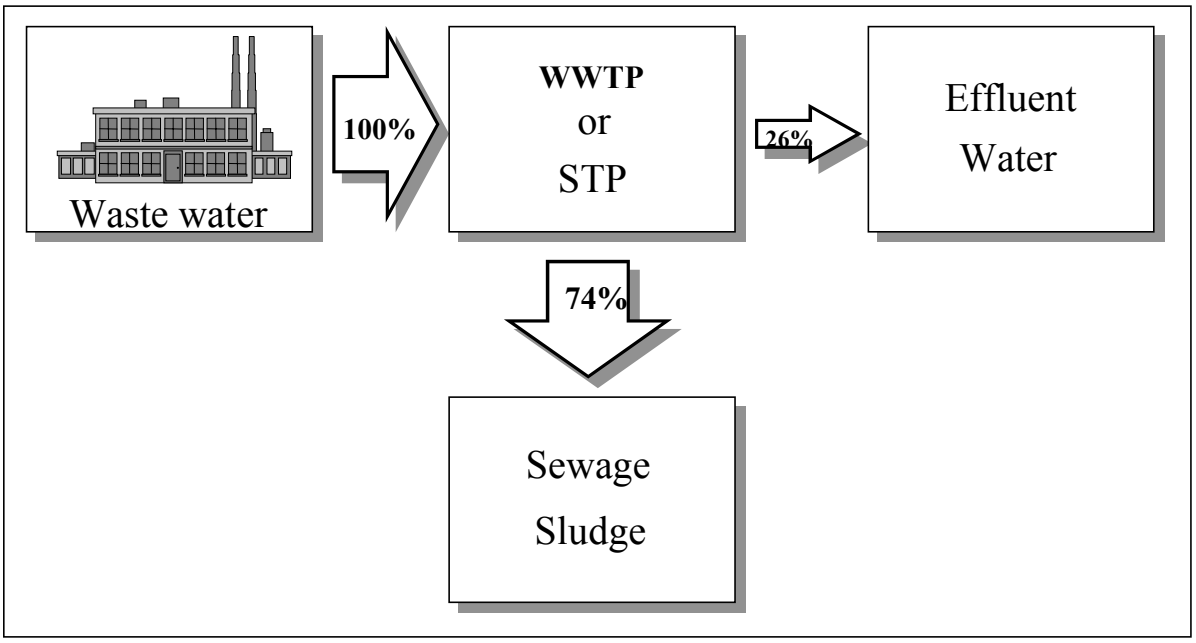

Figure 3.2.3 Distribution estimates of zinc in an STP.

For the generic scenarios a default size for the STP of 2000 m^3/d is used for calculating the C_{add} values in water. The concentration of zinc in the effluent is calculated with the equations:

$$C\,local_{influent} = \frac{EMISSION\,local}{EFFLUENT\,local_{STP}}$$

$Clocal_{influent}$:	concentration in untreated waste water (kg/m^3)
EMISSIONlocal:	local emission rate to waste water (kg/d)
$EFFLUENTlocal_{STP}$:	effluent discharge rate of local STP (m^3/d)

$$Clocal_{effluent} = Clocal_{in\,fluent} \cdot Fstp_{water}$$

$Clocal_{effluent}$:	concentration in effluent water (kg/m^3)
$Clocal_{influent}$:	concentration in untreated waste water (kg/m^3)
$Fstp_{water}$:	fraction of emission directed to water after treatment (-)

Subsequently, from the effluent concentration in the STP the local concentration of the receiving water surface water during the emission episode can be calculated with following equation. Dilution in the receiving surface water and sorption to suspended solids are taken into account.

$$C_{add}\,local_{water} = \frac{C\,local_{effluent}}{(1 + Kp_{susp} * C_{susp}) * D}$$

C_{add} $local_{water}$: local concentration in water during emission episode (kg/m^3)

Kp_{susp}: solids-water partition coefficient of suspended matter. For zinc 110 m^3/kg (see Partition coefficients zinc metal RAR (Stortelder et al., 1989))

$C_{susp:}$ concentration of suspended matter in river water (0.015 kg_{dwt}/m^3, TGD)

D: dilution factor (default = 10)

The local concentrations in sediment (wet weight) during emission episode can be estimated from the local C_{add} values in water, the suspended matter-water partition coefficient and the bulk density of suspended matter. The local concentrations in sediment are calculated according to the following equation:

$$C_{add}local_{sed} = \frac{K_{susp-water}}{RHO_{susp}} * PEC_{add}local_{water}$$

where: $$K_{susp-water} = Fwater_{susp} + Fsolid_{susp} * Kp_{susp} * RHOsolid$$

C_{add} $local_{sed}$: concentration in sediment during emission episode (kg/kg_{wwt})

$K_{susp\text{-}water}$: suspended matter-water partition coefficient (calculated $2.75.10^4$ m^3/m^3)

$RHO_{susp:}$ bulk density of suspended matter (1150 kg_{wwt}/m^3)

$Fwater_{susp}$: fraction of water in suspended matter (0.9)

$Fsolid_{susp}$: fraction of solids in suspended matter (0.1)

Kp_{susp}: solids-water partition coefficient of suspended matter. For zinc 110 m^3/kg (see Partition coefficients zinc metal RAR (Stortelder et al., 1989))

RHOsolid: density of solid phase (2500 kg/m^3)

With the local emission values local C_{add} values are calculated for the compartments air and soil as described earlier in the production section 3.2.1.2.2.

For the soil compartment of the use categories of zinc sulphate both the application of STP sludge on agricultural soil and the deposition from air are taken into account according to the TDG (1996). In the TGD (1996) it is assumed that the total sewage sludge load is applied on agricultural soil. For the sludge part the daily waste water release is the input for calculating the C_{add}. The waste water releases are calculated from the submitted effluent water releases in which it is assumed that zinc is removed in the STP for 74%.

3.2.1.2.4 Processing in agricultural pesticide industry

According to the industry zinc sulphate is only used in the pesticide industry as an intermediate for the production of fungicides such as “Zineb” and “Mancozeb”. Zinc sulphate itself is thus not a primary constituent of pesticides. See also RAR on zinc chloride on this point (choice of IC/UC combination of 3/33). No data were submitted on the releases of zinc sulphate to air and water for processing in agricultural pesticide industry in the EU. Hence, a generic scenario is carried out starting with the EU production tonnages for this life cycle stage (entry 1, Figure 3.2.2). The EU production tonnage is derived from information

submitted by the zinc sulphate industry. It should be noted that for the local exposure assessment direct emissions to agricultural soil via pesticides are beyond the scope of the TGD. Diffuse emissions via this route are accounted for in the regional exposure assessment (see zinc metal document). The scenario used to obtain local C_{add} values is described in section 3.2.1.2.3 (page 19). For this scenario it is assumed that zinc sulphate is not processed at the production sites but elsewhere. The emission factor of 0.007 for the release to water is based on a production volume of >1000 t/y. According to the existing emission scenario document for the assessment of the environmental release of intermediates (IC-3), the 90th percentile of the river flows for 112 locations is 60 m^3/s (TGD, 1996). This river flow is used for calculating a dilution factor of 2592. Table 3.2.5 contains the input data and results of the local exposure assessment for processing of zinc sulphate in the agricultural pesticide industry.

The 10% rule is not used for this scenario, because the industry indicated that a very limited amount of companies in the EU produce zinc-containing pesticides. The rapporteur is aware of the difference with the zinc chloride RAR on the use of the 10% rule for the pesticide scenario.

Table 3.2.5 Input data and results for the local exposure assessment for processing of zinc sulphate in the agricultural pesticide industry.

	processing, generic scenario
Regional tonnage (t/y)	7,760
Industrial category / use category	3/33
Fraction released to air (A-tables TGD, 1996)	0.00001
Fraction released to water (A-tables TGD, 1996)	0.007
Fraction of main source (B-tables TGD, 1996)	0.25
Number of days	300
Calculated local amount released to air (kg/d)	0.0647
Calculated local amount released to waste water (kg/d)	45.3
Size of STP (m^3/d)	2,000
River flow (m^3/d) Emission scenario document IC-3 (TGD, 1996)	5,184,000
Calculated dilution factor	2,593
Results	
Conc. Effluent STP (µg/l)	5,885
C_{add} water (µg/l)	0.856
C_{add} air, 100m (µg/m^3)	$1.48.10^{-2}$
C_{add} sediment (mg/kg_{wwt})	20.5
C_{add} agricultural soil (mg/kg_{wwt})	767

3.2.1.2.5 Formulation in agricultural fertiliser industry

According to the industry zinc sulphate is used as a trace component in fertilisers. Zinc sulphate is predominantly selected due to its water solubility for formulation in liquid fertilisers. Thus, the actual handling of this low-dust material is more or less restricted to

incorporation into a solution matrix. Consequently, in absence of any dust-forming processes, any emission to air may be regarded as negligible (information from industry). The rapporteur agrees with the zero emission to air for this use category. No site-specific data were submitted on the releases of zinc sulphate to water for the formulation in the agricultural fertiliser industry in the EU. Hence, a generic scenario is carried out starting with the EU production tonnages for this life cycle stage (entry 1, Figure 3.2.2). It should be noted that for the local exposure assessment direct emissions to agricultural soil via fertilisers are beyond the scope of the TGD. Diffuse emissions via this route are accounted for in the regional exposure assessment (see zinc metal document). The scenario used to obtain local C_{add} values is described in section 3.2.1.2.3 (page 19). The percentage of zinc sulphate used in fertilisers is about 0.1%. According to that use percentage the tonnage for use of the B-tables (TGD, 1996) was adjusted and therefore also the used fraction of main source was revised from 1 to 0.6. Table 3.2.6 contains the input data and results of the local exposure assessment for processing of zinc sulphate in the agricultural pesticide industry. The emission factor of 0.02 for the release to water is based on a production volume of <1000 t/y.

The 10% rule is used for this scenario, because the industry submitted valid data on the number of processing sites and their distribution in the EU.

Table 3.2.6 Input data and results for the local exposure assessment for formulation of zinc sulphate in the agricultural fertiliser industry.

	Formulation, generic scenario
Regional tonnage (t/y)	776
Industrial category / use category	1/19
Fraction released to air (information from industry)	0
Fraction released to water (A-tables TGD, 1996)	0.02
Use level in end product (%)	0.1
Correction factor for tonnage for use of B-Tables	1,000
Used tonnage for B-Tables (B-tables TGD, 1996)	776,000 (≥50,000)
Fraction of main source (B-tables TGD, 1996)	0.6
Number of days	300
Local amount released to air (kg/d)	0
Local amount released to waste water (kg/d)	31
Size of STP (m^3/d)	2,000
Dilution factor	10
Results	
Conc. effluent STP (µg/l)	4,040
C_{add} water (µg/l)	152
C_{add} air, 100m (µg/m^3)	0
C_{add} sediment (mg/kg_{wwt})	3,640
C_{add} agricultural soil (mg/kg_{wwt})	526

3.2.1.2.6 Formulation in agricultural feed industry

Only for one company site-specific data was submitted on the releases of zinc sulphate to air and water during the formulation in the agricultural feed industry in the EU. The company reported that the process involved is only dry mixing in a containment and that there are no emissions to water. The company also claims emissions to air being zero.

Because no emission data was available for the remaining sites also a generic scenario is carried out starting with the EU production tonnages for this life cycle stage (entry 1, Figure 3.2.2). The EU production tonnage is derived from information submitted by the zinc sulphate industry. Industry is of the opinion that the release factors employed in this scenario exaggerate the release fractions to air and the aquatic environment, particularly since this a dry process. According to expert judgement in general the emission to waste water is probably not zero, because of cleaning operations. The dedicated equipment probably needs some cleaning and therefore the TGD default of 2% is rather high. According to expert judgement a more realistic emission factor to waste water of 0.1% is used for this scenario.

It should be noted that for the local exposure assessment direct emissions to agricultural soil via food or feedstuff additives are beyond the scope of the TGD. Diffuse emissions via this route are accounted for in the regional exposure assessment (see zinc metal document). The scenario used to obtain local C_{add} values is described in section 3.2.1.2.3 (page 19). Table 3.2.7 contains the input data and results of the local exposure assessment for formulation in the agricultural feed industry. It is clear the default emissions from the generic scenario deviate from the zero emissions to air and water from the site-specific scenario.

The 10% rule is used for this scenario, because the industry submitted valid data on the estimated number of processing sites and their distribution in the EU.

Table 3.2.7 Input data and results for the local exposure assessment for formulation of zinc sulphate in the agricultural feed industry

	Formulation, Site specific (one company)	Formulation, Generic scenario
Regional tonnage (t/y)	Not appl.	488
Industrial category / use category	1/41	1/41
Fraction released to air (A-tables TGD, 1996)	Not appl.	0.0025
Fraction released to water (A-tables TGD, 1996)	Not appl.	0.001
Fraction of main source (B-tables TGD, 1996)	Not appl.	1
Number of days	300	300
Local amount released to air (kg/d)	0 (submitted)	4.06 (calculated)
Local amount released to waste water (kg/d)	0 (submitted)	1.63 (calculated)
Size of STP (m^3/d)	2,000	2,000
Dilution factor	10	10
Results		
Conc. effluent STP (µg/l)	0	211

C_{add} water (µg/l)	0	7.98
C_{add} air, 100m (µg/m^3)	0	0.928
C_{add} sediment (mg/kg$_{wwt}$)	0	191
C_{add} agricultural soil (mg/kg$_{wwt}$)	0	27.9

Not appl. Not applicable

3.2.1.2.7 Processing of zinc sulphate in the chemical industry

Zinc sulphate is used in the chemical industry for the manufacturing of lithopones. Lithopone is a brilliant white pigment used in paints, inks, leather, paper, linoleum and face powder. Lithopone is an insoluble mixture of barium sulphate and zinc sulphide that precipitates upon mixing solutions of barium sulphide and zinc sulphate. The precipitate is recovered by filtration, then calcined (roasted) at temperatures above 600 ºC. The use of zinc sulphate for the production of lithopone corresponds to IC3: chemicals used as an intermediate. No site-specific data on the releases of zinc sulphate to air and water were submitted for processing at that site. Therefore a generic scenario is used based on the total EU processing volume. The scenario used to obtain local PEC_{add} values is described in section 3.2.1.2.3. Table 3.2.8 contains the input data and results of the local exposure assessment for processing of zinc sulphate in the chemical industry.

The 10% rule is used for this scenario, because the industry submitted valid data on the estimated number of processing sites and their distribution in the EU.

Table 3.2.8 Input data and results for the local exposure assessment for processing of zinc sulphate in the chemical industry.

	processing, generic scenario
Regional tonnage (t/y)	540
Industrial category / use category	3/33
Fraction released to air (A-tables TGD, 1996)	0
Fraction released to water (A-tables TGD, 1996)	0.02
Fraction of main source	0.4
Number of days	54
Calculated local amount released to air (kg/d)	0
Calculated local amount released to waste water (kg/d)	80
Size of STP (m^3/d)	2,000
Dilution factor	2,593
Results:	
Conc. effluent STP (µg/l)	10,400
C_{add} water (µg/l)	1.51
C_{add} air, 100m (µg/m^3)	0
C_{add} sediment (mg/kg$_{wwt}$)	36.2
C_{add} agricultural soil (mg/kg$_{wwt}$)	1,356

The zinc emissions from the subsequent use of litophone are not further addressed in the zinc sulphate RAR.

3.2.1.2.8 Measured local data in the environment

No measured concentrations available.

3.2.1.2.9 Summary of results for the local exposure assessment

Company	**Conc. effluent STP (total) (µg/l)**	**C_{add} water episode (dissolved) (µg/l)**	**C_{add} sediment episode (mg/kg_{wwt})**	**C_{add} agricultural soil (mg/kg_{wwt})**	**C_{add} air (100m) ($\mu g/m^3$)**
Production companies:					
Company 1	0	0	0	0.029	0.078
Company 2	0	0	0	0	0
Company 3 [1)]	-	-	-	-	-
Company 4	0	0	0	0.016	0.0433
Company 5	0	0	0	0.013	0.0347
Use categories:					
Agricultural pesticide industry: processing	5,885	0.856	20.5	767	$1.48.10^{-2}$
Agricultural fertiliser industry: formulation	4,040	152	3,640	526	0
Agricultural feed industry: formulation (site specific)	0	0	0	0	0
Agricultural feed industry: formulation (generic)	211	7.98	191	27.9	0.928
Chemical industry: processing	10,400	1.51	36.2	1,356	0

1) No data submitted. For the exposure assessment of company 3 is referred to the zinc metal RAR.

3.3 EFFECTS ASSESSMENT

3.3.1 Aquatic and terrestrial compartment

The ecotoxicity of zinc sulphate has been studied extensively in laboratory tests, both with aquatic organisms and terrestrial organisms. The data include many short-term toxicity studies (used to derive acute LC50 and EC50 values for zinc) and many long-term toxicity studies (used to derive chronic NOEC values for zinc). A number of the aquatic toxicity data for zinc sulphate were submitted by Industry ($ZnSO_4$ IUCLID data sheet, *Grillo-version of 7 March 1997*). The further data were retrieved from reviews and updates (literature searches) made by Industry and the rapporteur. For a comprehensive overview of the aquatic and terrestrial toxicity of (soluble) zinc, including zinc sulphate, see the RAR Zinc metal and especially the Annexes of that report; the Annexes include detailed data on the ecotoxicity data bases for (soluble) zinc.

Once emitted into the environment, zinc sulphate, which has a high water solubility, will dissociate into the zinc cation and the sulphate anion. The further speciation of zinc, which includes complexation, precipitation and sorption, depends on the environmental conditions. Therefore, emitted zinc sulphate as well as other emitted zinc species (e.g. zinc chloride) will contribute to the effect of the total amount of zinc in the environment, regardless of the original source or chemical form. For this reason the risk characterisation is based on zinc (regarding zinc as the causative factor for toxicity), not on zinc sulphate as such. Thus, in the local risk characterisation for zinc sulphate, the $PNEC_{add}$ values for zinc (see Table 3.3.1) have been compared with the local PEC_{add} values which are also expressed as zinc, but derived from the local emissions due to the production or use of zinc sulphate. In the regional risk characterisation, which is not for zinc sulphate specifically but for zinc from "all" anthropogenic sources, the $PNEC_{add}$ values for zinc have been compared with PEC_{add} values for zinc, the latter values derived from the sum of the regional emissions due to industrial and non-industrial sources, diffuse sources included (see also earlier in section 3.2 for further explanation). For the regional risk characterisation the reader is referred to the Risk Assessment Report on Zinc metal (RAR Zinc metal).

In the RAR Zinc metal, $PNEC_{add}$ values have been derived for zinc, on the basis of tests with soluble zinc salts (especially zinc sulphate or zinc chloride), using the "added risk approach" (see also earlier in section 3.1 of the present report for an explanation of the added risk approach). These $PNEC_{add}$ values for zinc are listed in Table 3.3.1 and used in the risk characterisation (see section 3.4).

Table 3.3.1 $PNEC_{add}$ values for zinc (from RAR Zinc metal)

Environmental compartment	**$PNEC_{add}$**	**$PNEC_{add}$ value, as Zn**	**Remark**
Freshwater (Hardness ≥ 24 mg/L) (1)	$PNEC_{add, aquatic}$	7.8 µg/l 21 µg /l	Dissolved zinc Total zinc (2)
Freshwater (Hardness <24 mg/L) (1)	$PNEC_{add, aquatic}$ softwater	3.1 µg/l	Dissolved zinc
Freshwater sediment	$PNEC_{add, sediment}$	49 mg/kg dwt 11 mg/kg wwt	Dry weight of sediment (3) Wet weight of sediment (3)
STP effluent	$PNEC_{add, microorganisms}$	52 µg/l	Dissolved zinc
Soil	$PNEC_{add, terrestrial}$	26 mg/kg dwt 23 mg/kg wwt	Dry weight of soil (4) Wet weight of soil (4)

(1) Total hardness (mg/l), as $CaCO_3$.

(2) Total-Zn concentration: calculated from the $PNEC_{add, aquatic}$ of 7.8 µg/l for dissolved zinc, a C_{susp} of 15 mg/l (according to the TGD, 2003) and a Kp_{susp} of 110,000 l/kg.

(3) For the dry to wet weight normalisation of the $PNEC_{add, sediment}$ it is assumed that wet sediment contains 10% solids (density 2500 kg/m^3) and 90% water (density 1000 kg/m^3) by volume, i.e. 22% solids by weight. These properties are set equal to those of suspended matter, thus the $PNEC_{add, suspended\ matter}$ equals the $PNEC_{add, sediment}$ (according to the TGD, 2003).

(4) For the dry to wet weight normalisation of the $PNEC_{add, terrestrial}$ it is assumed that wet soil contains 60% solids (density 2500 kg/m^3) and 20% water (density 1000 kg/m^3) by volume, i.e. 88% solids by weight.

3.3.2 Atmosphere

No data available.

3.3.3 Secondary poisoning

Based on data on bioaccumulation of zinc in animals and on biomagnification (i.e. accumulation and transfer through the food chain), secondary poisoning is considered to be not relevant in the effect assessment of zinc, see further the RAR Zinc Metal.

3.4 RISK CHARACTERISATION

3.4.1 General

The use of the added risk approach implies that in the risk characterisation the added Predicted Environmental Concentrations (PEC_{add}'s) in the various environmental compartments are compared with the corresponding added Predicted No Effect Concentrations ($PNEC_{add}$'s). In section 3.2.1.2 local concentrations are calculated for STP, soil, water, sediment and air. Except for the PEC_{STP}, these local concentrations have to be corrected for the regional background (PEC_{add} regional), according to the TGD equation $PEClocal_{add} = Clocal_{add} + PECregional_{add}$. The regional exposure assessment, including regional monitoring data is described in the RAR on zinc metal. In case measured environmental concentrations are used in the risk characterisation, either the natural background concentration has to be subtracted from the measured environmental concentration (resulting in a "PEC_{add} / $PNEC_{add}$" ratio) or the natural background concentration has to be added to the $PNEC_{add}$ (resulting in a traditional "PEC / PNEC" ratio). Finally, a correction for bioavailability is carried out in the risk characterisation stage. For those scenarios where the uncorrected PEC values would yield a PEC/PNEC ratio above 1, a (possible) bioavailability correction is made for surface water, sediment and soil (see sections 3.3.2.1.1, 3.3.2.2.1 and 3.3.3.1.1 of Zinc Metal RAR). Final conclusions of the risk assessment are based on the corresponding 'corrected' PEC/PNEC ratios.

The reader is referred back to section 3.1 for more background information on the use of the added risk approach.

For air, the average measured concentration in the Netherlands of 0.04 µg/m^3 is chosen as regional background. (The natural background component in the value of 0.04 µg/m^3 is assumed to be negligible). Preference is given to this measured value as it is the result of a valid, representative monitoring programme. Besides, this figure is within the same order of magnitude as the calculated PEC_{add}'s at regional scale (0.006 µg/m^3 for the NL-region and 0.01 for the EU-region). For soil, following the TGD, the PEC regional in natural soil has to be added as background to the local concentration. The calculated value of 0.5 mg/kg wwt is used as regional background in the current risk assessment. For water PEC_{add}'s regional (dissolved) of 6.7 µg/l or 8.8 µg/l could be chosen as background values. These concentrations are derived from the measured average 90th percentile value of 41 µg/l [1](total) for regional waters in the Netherlands in 1997 corrected for, respectively, 3 and 12 µg/l natural background. Preference is given to these measured values as they are the result of valid, representative monitoring programmes. The figure for the Netherlands is supported by data from the large EU-survey (Denzer et al., 1998) in which a average 90-percentile value of

[1] Natural background value of 3 and 12 µg/l are subtracted from this value and, subsequently, the total figures are re-calculated to a dissolved zinc concentration (41-3 = 38 µg/l divided by 4.3 results in 8.8 µg/l; 41-12 = 29 µg/l divided by 4.3 results in 6.7 µg/l)

59.2 µg/l (total) is reported for the EU during the period 1994-1998. (Shortcomings of the Denzer et al. database are discussed in section 3.2.5.3.4 of the zinc metal RAR. Although only considered as 'indicative' in the current risk assessment, the 90P value for total zinc from Denzer et al. does give some overall EU picture that is useful for comparison purposes as described above).For comparison: the calculated $PECregional_{add}$ values (dissolved) amounts to 4.5 µg/l (12.2 µg/l total) for the NL-region and 6.2 µg/l (16.8 µg/l total) for the EU-region. The PECs sediment are calculated from the PEC water ($PEClocal_{add}$ = $Clocal_{add}$ + $PECregional_{add}$) via the equilibrium partitioning method.

For water and sediment, in the current local risk characterisation initially only the $C_{local,\ add}$ values (thus without the regional PEC_{add}) will be compared with the $PNEC_{add}$. At first the local aquatic risk characterisation thus focuses on the contribution of point sources to the potential risks, thereby neglecting the contribution of diffuse sources. If the regional PEC_{add} would have been added for sediment, all local scenarios would have resulted in PEC_{add} / $PNEC_{add}$ ratios larger than 1. This because the regional PEC_{add} already exceeds the $PNEC_{add}$ of 11 mg/kg wwt.. This holds for both calculated and measured sediment concentrations. For this reason for sediment all scenarios with a $Clocal_{add}$ / $PNEC_{add}$ ratio between 0 and 1 a **conclusion iii*** will be drawn, indicating that due to (possibly) high added regional background concentrations a risk for sediment at local scale cannot be excluded. It has to be noted that this conclusion would not be influenced by applying the generic sediment bioavailability correction factor of 0.5.

The situation is somewhat less pronounced for the surface water compartment. With a $PNEC_{add}$ of 7.8 µg/l the regional PEC_{add} / $PNEC_{add}$ would lie between 0.8 (PEC_{add} of 6.7 µg/l) and 1.1 (PEC_{add} of 8.8 µg/l). When using an (arbitrary) average bioavailability correction factor of 0.6[2] these ratios would become, respectively 0.5 and 0.7. As a result of this, it is decided that for $Clocal_{add}$ / $PNEC_{add}$ ratios between 0.5[3] and 1 a **conclusion iii*** will be drawn, indicating that due to (possibly) high (added) regional background concentrations a local risk for water cannot be excluded. For scenarios with a surface water $Clocal_{add}$ / $PNEC_{add}$ ratio < 0.5 the local contribution to the (added) regional background is assumed to be negligible (**conclusion ii**).

For those scenarios in which the involved process type does intrinsically not result in water emissions a **conclusion ii)** is drawn for water and sediment.

It is important to note that the above-mentioned distinction between a (normal) conclusion iii) and a conclusion iii*) is not only made because of transparency, but also because the regional background is due to a variety of zinc compounds (and thus not only the zinc compound specifically addressed in the local risk characterisation).

In section 3.4.2 of the zinc metal RAR a general reflection is given on the uncertainties in the zinc risk assessments.

[2] See Table 3.4.67 in RAR on Zinc Metal. Average of realistic worst case and average BioF for average NL data.

[3] A $Clocal_{add}$ / $PNEC_{add}$ of between 0.5 and 1 should theoretically also be corrected for bioavailability. This would give ratios between 0.3 and 0.6 when using the correction factor of 0.6. Such ratios could just raise the overall PEC_{add} / $PNEC_{add}$ ratio, thus including the regional background, to levels above one.

Table 3.4.9 The local (PE)C_{add} values and (PE)C_{add} / $PNEC_{add}$ ratios used in the local risk characterisation of zinc sulphate. The (PE)C_{add} / $PNEC_{add}$ ratios for water, soil and sediment are based on no correction for bioavailability.

Company	**PEC effluent STP (dissolved)**	**Cadd water (dissolved)**	**Cadd sediment**	**PEC agricultural soil**	**PEC/PNEC STP**	**Cadd/PNEC water**	**Cadd/PNEC sediment**	**PEC/ PNEC agr. soil**
	(µg/l)	**(µg/l)**	**(mg/kgwwt)**	**(mg/kgwwt)**				
Production companies:								
Company 1	0	0	0	0.529	0	0	0	0.02
Company 2	0	0	0	0.5	0	0	0	0.02
Company 3	-	-	-	-	-	-	-	-
Company 4	0	0	0	0.516	0	0	0	0.02
Company 5	0	0	0	0.513	0	0	0	0.02
Use categories:								
Agricultural pesticide industry: processing	1,369	0.856	20.5	768	26	0.11	2	32
Agricultural fertiliser industry: formulation	940	152	3,640	527	18	19	350	22
Agricultural feed industry: formulation (site specific)	0	0	0	0.5	0	0	0	0.02
Agricultural feed industry: formulation (generic)	49.1	7.98	191	28.4	0.94	1	18	1.2
Chemical industry: processing	2,419	1.51	36.2	1,357	47	0.19	3.5	57

3.4.2 Local risk characterisation

The local (PE)C_{add} values and the corresponding (PE)C_{add} / $PNEC_{add}$ ratios are listed in Table 3.4.9. It is emphasised that these C_{add} and PEC_{add} values and the (PE)C_{add} / $PNEC_{add}$ ratios are not corrected for bioavailability (first step in bioavailability decision trees in sections 3.3.2.1.1, 3.3.2.2.1 and 3.3.3.1.1 of RAR zinc metal). The applied bioavailability correction methods are summarised in Appendix 3.4 at the end of this Chapter. Subsequent corrections for the bioavailability of zinc in water, sediment and soil (if allowed) are discussed in the sections below.

Table 3.4.10 finally presents the overall results of the local risk characterisation after the various bioavailability correction steps (if relevant). Bioavailability correction is only carried out in case the uncorrected (PE)C_{add} / $PNEC_{add}$ ratio exceeds one. In addition, no bioavailability correction is done for the PEC STP.

3.4.2.1 Aquatic compartment

3.4.2.1.1 STP effluent

STP effluent

The PECs STP (total) as calculated in paragraph 3.2.1.2 for the various scenarios have been re-calculated to dissolved values. This because the $PNEC_{add}$ of 52 µg/l for microorganisms is expressed as a dissolved zinc concentration.

Production

At the production sites there is no emission to waste water, hence the PEC_{add} values and subsequently the PEC_{add} / $PNEC_{add}$ values for STP effluent are 0, thus <1 (**conclusion ii**)[4].

Use categories

The PEC_{STP} for the processing sites of zinc sulphate exceeds the $PNEC_{add}$ for microorganisms in three scenarios ('agricultural pesticide industry processing', 'agricultural fertiliser industry formulation' and 'chemical industry processing') (**conclusion iii**). All these scenarios are based on generic release estimates. For the remaining two scenarios the PEC / PNEC values for microorganisms are <1 (**conclusion ii**).

[4] For zinc sulphate production site No. 3 (company 3) the risk assessment is included in the RAR Zn Metal, as this company produces both zinc sulphate and zinc metal. Hence, the results for this production site are not discussed in the RAR Zinc sulphate.

3.4.2.1.2 Surface water (incl. sediment)

Production

Surface water.
At the production sites there is no emission to waste water, hence the PEC_{add} values and subsequently the PEC_{add} / $PNEC_{add}$ values for surface water are 0, thus <1 (**conclusion ii**)[4].

Sediment.
At the production sites there is no emission to waste water, hence the PEC_{add} values and subsequently the PEC_{add} / $PNEC_{add}$ values are 0, thus <1 (**conclusion ii**)[4].

Use categories

Surface water
The $Clocal_{add}$ in water for the processing sites of zinc sulphate exceeds the $PNEC_{add}$ for surface water in the generic scenario 'agricultural fertiliser industry formulation'. As relevant data are lacking to perform a correction for bioavailability for surface water (BLM), no additional correction can be carried out for this scenario. This implies that the original surface water risk characterisation ratio from Table 3.4.9 remains unchanged (**conclusion iii**).
The $Clocal_{add}$ / $PNEC_{add}$ ratio for the generic scenario 'agricultural feed industry formulation' is 1, indicating that due to (possibly) high regional background concentrations a potential risk at local scale cannot be excluded **(conclusion iii***), see also the explanation in section 3.4.1.
For the remaining scenarios the $Clocal_{add}$ / $PNEC_{add}$ is < 0.5 **(conclusion ii)**.

Sediment
For sediment the $Clocal_{add}$ / $PNEC_{add}$ is >1 in four scenarios ('agricultural pesticide industry processing' and 'agricultural fertiliser industry formulation', 'agricultural feed industry formulation (generic)' and 'chemical industry processing'). As relevant data are lacking to perform a site-specific correction for bioavailability in sediment (SEM/AVS method), only the generic sediment bioavailability correction factor of 0.5 can be applied these scenarios. This implies that the original sediment $Clocal_{,add}$ from Table 3.4.9 are multiplied with a factor 0.5. After this correction the $Clocal_{add}$ / $PNEC_{add}$ remains above 1 for these scenarios (**conclusion iii**), except for 'agricultural pesticide industry processing' (corrected $Clocal_{add}$ / $PNEC_{add}$ is 1). In this case (i.e. scenario 'agricultural pesticide industry processing') a potential risk at the local scale cannot be excluded due to (possibly) high regional background concentrations (**conclusion iii***),
For the remaining scenario, i.e. 'agricultural feed industry formulation' (site specific) the $Clocal_{add}$ / $PNEC_{add}$ is 0 (no local emission to waste water, thus no local emission to surface water or sediment, resulting in a **conclusion ii)** for this site.

3.4.2.2 Terrestrial compartment

Production

The PEC_{add} soil is below the $PNEC_{add}$ for soil at all production sites of zinc sulphate (**conclusion ii**).

Use categories

The PEC_{add} exceeds the $PNEC_{add}$ in four scenarios ('agricultural pesticide industry processing', 'agricultural fertiliser industry formulation', 'agricultural feed industry formulation (generic)' and 'chemical industry processing'). For these scenarios there are no site-specific data that allow a site-specific bioavailability correction on the basis of soil type characteristics, thus only the generic soil correction factor of 3 (R_{L-F}: ageing aspects) can be applied. This implies that the corrected PEC_{add} / $PNEC_{add}$ values are 3-times lower than the uncorrected values (as the PEC_{add} values are divided by a factor of 3). After this correction the PEC_{add} / $PNEC_{add}$ values remains >1 for three scenarios ('agricultural pesticide industry processing', agricultural fertiliser industry formulation' and ' chemical industry processing'). (**conclusion iii**). The corrected PEC_{add} /$PNEC_{add}$ for scenario 'agricultural feed industry formulation (generic)' is <1 (**conclusion ii**).
The (uncorrected) PEC_{add} / $PNEC_{add}$ ratio is <1 for the site-specific scenario 'agricultural feed industry formulation' **(conclusion ii).**

3.4.2.3 Atmospheric compartment

A quantitative risk characterisation for exposure of organisms to airborne zinc is not possible. This because there are no useful data on the effects of airborne zinc on environmental organisms and thus no PNEC for air could be derived.
The PECs in air will be used for the risk assessment of man indirectly exposed via the environment (see Human Health part of the RAR).

3.4.2.4 Secondary poisoning

Not relevant.

Table 3.4.10 Summary of the uncorrected and corrected local (PE)C_{add} / $PNEC_{add}$ ratios of the local risk characterisation of zinc sulphate.

	Uncorrected				Corrected	
Company	**PEC/PNEC STP**	**Cadd/PNEC water**	**Cadd/PNEC sediment**	**PEC/PNEC agr. soil**	**Cadd/PNEC sediment**	**PEC /PNEC agr. soil**
Production companies:						
Company 1	0	0	0	0.02		
Company 2	0	0	0	0.02		
Company 3	-	-	-	-		
Company 4	0	0	0	0.02		
Company 5	0	0	0	0.02		
Use categories:						
Agricultural pesticide industry: processing	**26**	0.11	2	32	1	**11**
Agricultural fertiliser industry: formulation	**18**	**19**	350	22	**175**	**7.3**
Agricultural feed industry: formulation (site specific)	0	0	0	0.02	0	
Agricultural feed industry: formulation (generic)	0.94	1	18	1.2	**9**	0.40
Chemical industry: processing	**47**	0.19	3.5	57	**1.7**	**19**

3.4.3 Regional risk characterisation

See RAR on zinc metal.

APPENDIX 3.4 BIOAVAILABILITY CORRECTIONS

In the first step of the risk characterisation, the local added Predicted Environmental Concentrations ($PEClocal_{add}s$) in the various environmental compartments are compared with the corresponding added Predicted No Effect Concentrations ($PNEC_{add}s$). In case this yields a PEC_{add} / $PNEC_{add}$ ratio above 1, the risk characterisation includes (if possible) a second step in which a bioavailability correction is made, see the table below for a summary of the bioavailability correction methods applied and see RAR Zinc metal sections 3.3.2.1.1 (water), 3.3.2.2.1 (sediment) and 3.3.3.1.1 (soil) for a comprehensive explanation of the derivation and application of these bioavailability correction methods[5]. In all cases the bioavailability correction is applied to the PEC_{add}, not to the generic $PNEC_{add}$, although for the resulting corrected PEC_{add} / $PNEC_{add}$ ratio it makes no difference whether the correction is applied to the PEC_{add} or to the $PNEC_{add}$.

- For water there is only a site-specific bioavailability correction, i.e. a bioavailability correction is only applied in case there are reliable site-specific data on the abiotic water characteristics that are needed to apply the BLM models. Bioavailability factors are being derived for two scenarios of abiotic conditions. One scenario refers to an average setting and the second one to a 'realistic worst case' setting. The highest bioavailability factor ($BioF_{water}$) is subsequently used in the risk characterisation by multiplying the original $(PE)C_{add}$ with this $BioF_{water}$. If a site has a discharge to seawater, no bioavailability correction is performed, as the BLM models were developed for freshwaters.
- For sediment the bioavailability correction is either site-specific (preference) or generic.
- For soil the bioavailability correction starts with the application of the generic lab-to-field correction factor (R_{L-F}) and if the corrected PEC_{add} / $PNEC_{add}$ ratio still is >1, then a further, site-specific bioavailability correction is applied.

Final conclusions of the risk assessment are based on the corresponding 'corrected' PEC_{add} / $PNEC_{add}$ ratios.

Bioavailability corrections as applied in the EU RARs on zinc and zinc compounds

Compartment	***Added* Predicted Environmental Concentration (PEC_{add})**	
	Bioavailability correction (generic)	**Bioavailability correction (site-specific or region-specific)**
Water	None	Biotic Ligand Models (BLMs) for algae, Daphnia and fish (a)
Sediment	Factor of 2 (b)	Acid Volatile Sulphide (AVS) method (c)
Soil	Factor of 3 (d) (R_{L-F})	Regression lines for invertebrates, plants and microbial processes (e)

(a) Water – BLMs: Based on the relationship between toxicity of zinc and water characteristics, e.g. pH, dissolved organic carbon (DOC) and hardness (see RAR Zinc metal Section 3.3.2.1.1 for further explanation).

(b) The PEC_{add} (or measured concentration) for zinc in sediment is divided by a generic, AVS-related correction factor of 2 to obtain the bioavailable concentration of zinc (note that in the original description of this method in section 3.3.2.2.1 of the RAR Zinc metal it is stated that the PEC_{add} is multiplied with a factor of 0.5). The corrected PEC_{add} is subsequently used in the assessment of the PEC_{add} / $PNEC_{add}$ ratio.

(c) Sediment – AVS method: Based on the inverse relationship between toxicity of zinc and AVS content in sediment (see RAR Zinc metal Section 3.3.2.2.1 for further explanation).

[5] No bioavailability correction is done for the PEC_{STP}

This method is also described as the SEM/AVS-method, as also the toxicity of other metals, i.e. Cd, Cu, Ni, Hg and Pb, referred to as Simultaneously Extracted Metals (SEM) is reduced by AVS.

(d) The PEC_{add} (or measured concentration) for zinc in soil is divided by a generic, ageing-related lab-to-field correction factor (R_{L-F}) of 3 to obtain the bioavailable concentration of zinc. The corrected PEC_{add} is subsequently used in the assessment of the PEC_{add} / $PNEC_{add}$ ratio.

(e) Soil – Regression lines: Based on the relationship between toxicity of zinc and soil characteristics, e.g. pH and cation exchange capacity (CEC) (see RAR Zinc metal Section 3.3.3.1.1 for further explanation).

4 REFERENCES

REFERENCES EXPOSURE ASSESSMENT

The reference list applies to zinc and the five zinc compounds and is presented in the zinc metal RAR.

European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

Existing Substances

European Union Risk Assessment Report

CAS No: 7733-02-0

EINECS No: 231-793-3

zinc sulphate

$ZnSO_4$

2nd Priority List

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European Union Risk Assessment Report

ZINC SULPHATE

Part II – Human Health

CAS No: 7733-02-0

EINECS No: 231-793-3

RISK ASSESSMENT

Cataloguing data can be found at the end of this publication
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ZINC SULPHATE

Part II – Human Health

CAS No: 7733-02-0

EINECS No: 231-793-3

RISK ASSESSMENT

Final Report, 2004

This document has been prepared by the Ministry of Housing, Spatial Planning and the Environment (VROM) in consultation with the Ministry of Social Affairs and Employment (SZW) and the Ministry of Public Health, Welfare and Sport (VWS), on behalf of the European Union.

The scientific work on this report has been prepared by the Netherlands Organisation for Applied Scientific Research (TNO) and the National Institute for Public Health and the Environment (RIVM), by order of the rapporteur.

Contact point:

Chemical Substances Bureau
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The Netherlands

Date of Last Literature Search:	**2003**
Review of report by MS Technical Experts finalised:	**2001**
Final report:	**2004**

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.

The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

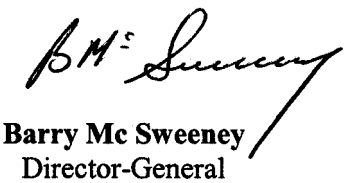
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a Meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) which gives its opinion to the European Commission on the quality of the risk assessment.

If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.

The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992.

This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.



Barry Mc Sweeney
Director-General
DG Joint Research Centre

Catherine Day
Director-General
DG Environment

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1 O.J. No L 084, 05/04/199 p.0001 – 0075

2 O.J. No L 161, 29/06/1994 p. 0003 – 0011

3 Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS No:	7733-02-0
EINECS No:	231-793-3
IUPAC Name:	zinc sulphate

Human health (toxicity)

Workers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

The information available gives no reasons for concern for adverse health effects due to zinc sulphate exposure at the workplace.

Consumers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Human health (physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

CONTENTS

TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS No: 7733-02-0
EINECS No: 231-793-3
IUPAC Name: Zinc sulphate
Synonyms: Sulphuric acid-zinc salt; zincate; zincomed; white vitriol; zinc vitriol
Molecular formula: $ZnSO_4$
Structural formula: $ZnSO_4$
Molecular weight: 161.4

1.2 PURITY/IMPURITIES, ADDITIVES

Purity: no data
Impurity: no data
Additives: no data

1.3 PHYSICO-CHEMICAL PROPERTIES

In **Table 1.1** the physico-chemical properties are summarised.

Table 1.1 Physico-chemical properties of zinc sulphate

Property	Result	Comment
Physical state	solid	*
Melting point	600ºC	*
Boiling point	not applicable	*
Relative density	3.54	*
Vapour pressure	no data	***
Surface tension	no data	***
Water solubility	220 g/l (20ºC)	**
Solubility in other solvents	slowly soluble in alcohol; soluble in MeOH and glycerin	*
Partition coefficient n-octanol/water(log value)	no data	***
Flash point	not applicable	***
Flammability	not flammable	***
Autoflammability temperature	not applicable	***
Explosive properties	not explosive	***
Oxidizing properties	not oxidizing	***

* More than one apparently independent source. No methods are specified
** One source
*** Conclusion based on theoretical and/or structural considerations

These data are mainly derived from CRC Handbook of Chemistry and Physics (1995), Sax's Dangerous Properties of Industrial Materials (1984), Patty's Industrial Hygiene and Toxicology (1981), Römpp Chemie Lexikon (1995), Ullmann's Encyklopädie der Technischen Chemie (1983), and company information. For an extended description see hedset.

Conclusion

Data on boiling point, vapour pressure, surface tension, and partition coefficient were not provided. In view of the nature of the substance determination of these parameters is considered to be irrelevant. Information on flammability, explosive properties and oxidizing properties is not available. However, on theoretical considerations the compound is concluded to be not flammable, not explosive and not oxidizing. All other required physico-chemical data were submitted. None of these data is based on test results, substantiated with reports. However, the data are considered as sufficiently reliable to fulfil the Annex VIIA requirements.

1.4 CLASSIFICATION

Decision of the CMR Working Group:

At the September 2002 meeting, it was agreed to classify zinc sulphate (hydrous forms) for acute oral toxicity and serious eye irritation, thereby replacing the current classification for skin and eye irritation (for the anhydrous form). The anhydrous form is to be added to the new entry for the hydrous forms because the anhydrous form not really exists because of very fast hydration.

Both hydrous and anhydrous forms were included in Annex 1 with the 29th ATP as:

Xn; R22, R41
S(2-)22-26-39-46

R phrases

R22 harmful if swallowed
R41 risk of serious damage to eyes

S phrases

S(2-) keep out of reach of children
S22 do not breathe dust
S26 in case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S39 wear eye / face protection
S46 if swallowed, seek medical advice immediately and show this container or label

2 GENERAL INFORMATION ON EXPOSURE

(will be added later)

3 ENVIRONMENT

(will be added later)

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

Zinc sulphate is made by leaching of zinciferous material with sulphuric acid followed by various hydrometallurgical processes for purification. The resulting, purified zinc sulphate solution is then concentrated and crystallised at room temperature (yielding the heptahydrate) or at 40-50°C (yielding the hexahydrate) (Kirk-Othmer, 1982a). The corresponding monohydrate is generated from these compounds by thermal dehydration. It is marketed as a mono-, hepta-, and hexahydrate. Zinc sulphate is mainly used in the animal feedstuff industry and in the formulation of fertilisers. It is also used in other areas, such as chemical catalysis or the production of pharmaceuticals. It is also the starting material for the manufacture of some zinc chemicals. In Europe, according to industry, zinc sulphate is not used in potential or former uses in textile dying and printing, flotation reagents, electro galvanising, paper bleaching, glue and dental impression material. The use in rayon manufacture has declined to practically zero due to a general decrease in rayon production and technical alterations in the process. Approximately 75% of the production is in the form of the crystalline hexa- and hepta-hydrate. The monohydrate is used predominantly in the animal feedstuff industry, whereas the hexa/heptahydrate is mainly used in the formulation of fertilisers and secondly in other areas (Industry, 1998, 1999a). A particle size distribution for the heptahydrate shows a very coarse product (mean diameter > 500 μ, 99% > 100 μ), while the monohydrate has a broad particle size distribution with a mean diameter of 170 μ, 14% < 10 μ and 6% < 5 μ (Industry, 1999b). A study of dustiness, using the modified Heubach method, that includes a multi-stage impactor to separate different aerosol fractions, shows a total dustiness of 26.7 mg/g for monohydrate and 0.25 for hexahydrate. For monohydrate 92.11% of the generated dust is larger than 8.13 μm and 79.85% larger than 15.8 μm. For hexahydrate 97.02% of the generated dust is larger than 8.13 μm and 85.01% larger than 15.8 μm. For comparison, the total dustiness of zinc oxide is 30 mg/g with 84.53% larger than 8.13 μm and 73.92% larger than 15.8 μm (Deutsche Montan Technologie GmbH, 2000).

Occupational limit values

No occupational limit values have been established for zinc sulphate.

4.1.1.2 Occupational exposure

Exposure to zinc sulphate may occur during the manufacture and the use of products containing zinc sulphate, by the inhalation or dermal route. In the production of fertiliser the crystalline hexa- and heptahydrate are used. This product is delivered in big bags. In the animal feedstuffs industry the finely ground monohydrate is used and is delivered in 25 kg bags (Industry, 1998). The use in animal feedstuff is probably not a source for exposure because one bag of 50 kg zinc sulphate is sufficient for approximately 500 tons of animal feedstuff.

The following data are used for occupational exposure assessment:

- physico-chemical data, physical appearance and vapour pressure,
- data regarding the production process and use pattern of the products and amount of the zinc compound in the product,
- exposure data from the HEDSET,
- measured data for zinc compounds or analogues,
- results from exposure models (EASE-model).

The exposure is assessed using the available information on substance, processes and work tasks. More detailed information on these parameters may lead to a more accurate exposure assessment.

In this part of the assessment, external (potential) exposure is assessed using relevant models and other available methods in accordance with the Technical Guidance Document (TGD) and agreements made at official Meetings of Competent Authorities. Internal dose depends on external exposure and the percentage of the substance that is absorbed (either through the skin or through the respiratory system).

The exposure is assessed without taking account of the possible influence of personal protective equipment (PPE). If the assessment as based on potential exposure indicates that risks are to be expected, the use of personal protective equipment may be one of the methods to decrease actual risks, although other methods (technical and organisational) are to be preferred. This is in fact obligatory following harmonised European legislation.

Knowledge of effectivity of PPE in practical situations is very limited. Furthermore, the effectivity is largely dependent on site-specific aspects of management, procedures and training of workers. A reasonably effective use of proper PPE for skin exposure may reduce the external exposure with 85%. For respiratory protection the efficiency depends largely on the type of protection used. Without specific information, a tentative reduction efficiency of 90% may be assumed, equivalent to the assigned protection factors for supplied-air respirators with a half mask in negative pressure mode (NIOSH, 1987). Better protection devices will lead to higher protection. Imperfect use of the respiratory protection will lower the practical protection factor compared to the assigned factor. These estimations of reduction are not generally applicable "reasonable worst-case" estimations, but indicative values based on very limited data. They will not be used directly in the exposure and risk assessment. Furthermore, the reduction of external exposure does not necessarily reflect the reduction of absorbed dose. It has to be noted, that the use of PPE can result in a relatively increased absorption through the skin (effect of occlusion), even if the skin exposure is decreased. This effect is very substance-specific. Therefore, in risk assessment it is not possible to use default factors for reduction of exposure as a result of the use of PPE.

In some specific situations the model estimates with normal assumptions for input parameters in the assessed exposure scenarios are expected not to lead to a reasonable assessment of exposure. For situations with high risk of direct acute effects, such as manual handling of corrosive substances and hot materials, or possible inhalation exposure of substances with severe acute effects on the respiratory tract, the total level of containment given by all exposure control measures is assumed to be higher than for similar scenarios with other substances. For estimating a single day exposure an extra protection is assumed, reducing exposure with 90%. The extra protection can be reached by a combination of technical and organisational control measures and

personal protective equipment. If the extra protection is reached (mainly) by using personal protective equipment, this is an unwanted situation that should be changed by further technical and organisational control measures.

The estimate of repeated dermal exposure depends on the knowledge of a "maximum non-corrosive concentration". If such a concentration can be estimated, this concentration will be used in estimating repeated dermal exposure. Otherwise the estimate for single day exposure will be used.

The main result of the estimations is the so-called reasonable worst-case estimate. This value intends to estimate the exposure level in a reasonable worst-case situation, i.e. in a situation with exposures in the higher ranges of the full distribution of exposure levels, but below the extremes reached. If a large number of suitable data is available, a 90th percentile is generally used as an estimator of the reasonable worst-case value. If limited data sets are available (e.g. only measurements from one site or only small numbers of measurements or measurements with very little detail on tasks, working conditions, etc.) often the highest measured value is taken or the results of modelling are preferred or a conservative intermediate value is chosen to account for the weaknesses in the different data sets.

From the uses of zinc sulphate the following scenarios for exposure to zinc sulphate will be discussed:

- Scenario 1: Production of zinc sulphate,
- Scenario 2: Production of fertilisers or animal feedstuff,
- Scenario 3: Use of fertilisers.

4.1.1.2.1 Scenario 1: Production of zinc sulphate

The incoming raw material is subjected to the initial leaching process. In a closed stirring vessel containing water and sulphuric acid, zinc containing raw material is deposited by a wheel loader. This process is conducted at ambient temperature. Manganese and iron and other heavy metals are precipitated, leading to a technically pure zinc sulphate solution. The zinc sulphate is crystallised and packed. Another process is the thermal dehydration of a minor portion of the production of the hexahydrate to monohydrate, including packaging. The dustiness of the hexahydrate is much less than the monohydrate: 0.252 mg/g vs. 26.7 mg/g.

Measured data

From one producer, measured data were received. During adding of the raw material from big bags to the solution total dust was below 0.17 mg/m^3 and zinc oxide was detected at 0.021 and 0.029 mg/m^3 (two stationary samples). During drying and packing total zinc was detected in workplace air at 0.44 mg/m^3 (stationary sampling) and 0.59 mg/m^3 (personal sampling). The highest level recalculates into approximately 1.5 mg/m^3 zinc sulphate monohydrate or 2 mg/m^3 zinc sulphate hexahydrate. These data were extended, by measurements in the same facility, to a 22 point data set collected during raw material handling and packaging of zinc monohydrate as a finished product (EBRC, 2001g). Sampling was done both personal and static over a full shift. During handling of raw material (n = 9) the median was 0.1 mg zinc/m^3, and the 90th percentile was 0.2 mg zinc/m^3. In the monohydrate bagging personal and static sampling were combined (n = 11). The median was 0.3 mg zinc/m^3 and the 90th percentile was 0.7 mg zinc/m^3. The median and 90th percentile for personal samples were 0.4 mg zinc/m^3 and 0.9 mg zinc/m^3.

Measured data (for analogous substances)

Since hardly any measured data are available for these processes exposure is estimated using an analogous substance. The exposure to zinc sulphate may mainly take place during packaging of the solid. For bag filling and bag dumping "reasonable worst-case" estimates in the presence of LEV with limited effectiveness were deduced, based on literature on a number of solid powders, of 1.8 and 10 mg/m^3 (respirable and total dust concentrations during bag filling) and 10 mg/m^3 (total dust concentration during bag dumping (Lansink et al., 1996a)). Measurements presented for high-exposure tasks in production of zinc oxide (that has a comparable dustiness to zinc sulphate monohydrate) show typical exposure levels of 1.4-1.5 mg ZnO/m^3 and 90th percentiles of 5.6-5.8 mg ZnO/m^3 (n = 132, two work categories; EBRC, 2001). The work category with the highest number of data (n = 110) had a 90th percentile of 5.6 mg ZnO/m^3. From measurements in the paint industry with calcium carbonate (Lansink et al., 1996b) exposure levels of the hands during different activities were derived. The mean (GM) for collecting raw material was 476 mg/day (range 139-1,090). This activity, involving only the handling of (closed) bags, is assumed to be most similar to handling of filled bags during bagging.

Hughson and Cherrie (2001) studied dermal exposure to zinc in a number of facilities producing zinc compounds. The measurement method was repeated wet wiping of the skin at a number of places considered representative of the skin area. The recovery of the method was found to be around 100%. The study was done in two surveys. In Survey 1, the sampling for hands was done by wet wipes from the back of the hand only. In Survey 2, the palm of the hand was sampled too. Furthermore, in Survey 2, the sample for the chest was placed further from the 'V' of the neck, because this sample was intended to represent exposure underneath clothing. The measured values, expressed as μg zinc/cm^2, were recalculated into mass of zinc by multiplication with the area for which a sample was considered representative (see **Table 4.1**).

In Survey 1, a zinc oxide production plant was studied. In Survey 2, two plants producing zinc dust and zinc oxide and one plant producing zinc oxide only were studied. Hughson and Cherrie (2001) cluster the results in results for tasks with intermittent direct handling and results for tasks with extensive direct handling. This is done for comparison with EASE. In this risk assessment report the results are, however, clustered per job or task name, with all workers performing a task called "packing", "blending", "pelletising" or "classifying" in the group "high-exposure task" and all others in the group "low-exposure task". The division in tasks could only be made for plants B and D in the second survey, since the workers in the plant in the first survey and those in plant A in the second survey only had more general tasks (e.g. "operator"). For plants A and B also a clustering of zinc and zinc oxide workers is made.

Results are summarised in **Table 4.1**.

Table 4.1 Results of the measurement of zinc exposure levels (mg zinc) in plants producing zinc oxide and/or zinc dust (Hughson and Cherrie, 2001)

Result	N	Minimum	Maximum	GM	GSD	Remarks
Survey 1 hands and forearms	15	41.3	587.2	158.6	2.6	zinc oxide plant; for one worker the value for whole body was equal to the value for hands and forearms because of missing samples
Survey 1 whole body	15	57,8	722,1	251,9	2.2	
Survey 2 hands and forearms	10	141	1,005	513	1.8	all workers plant A
	6	232	1,005			zinc oxide workers plant A
	4	141	812			zinc dust workers plant A
Survey 2 whole body	10	160	1,125	637	1.7	all workers plant A
	6	569	1,125			zinc oxide workers plant A
	4	160	822			zinc dust workers plant A
Survey 2 hands and forearms	8	315	2,216	906	2.2	all workers plant B, except a worker with a missing sample for the forearm
	2	315	340			furnace operators plant B
	2	448	2,216			zinc oxide high exposure (packing) plant B
	4	901	1,911			zinc dust high exposure plant B
Survey 2 whole body	7	413	2,682	1,094	2.2	all workers plant B, except two workers with missing samples
	2	413	520			furnace operators plant B
	2	553	2,378			zinc oxide high exposure (packing) plant B
	3	1,118	2,682			Zinc dust high exposure plant B
Survey 2 hands and forearms	11	121	2,157	472	2.8	all workers plant D
	6	121	401			plant D low exposure group
	5	419	2,157			plant D high exposure group
Survey 2 whole body	11	135	2,369	541	2.7	all workers plant D
	6	135	565			plant D low exposure group
	5	439	2,369			plant D high exposure group

In general, the exposure was mostly to hands and forearms. However, some workers had considerable exposure of the head/face, neck and/or chest as well.

Task specific exposures were measured 6 times. The results are mentioned in **Table 4.2**.

Table 4.2 Task specific dermal exposures to zinc measured in zinc powder (oxide and dust) production facilities

Job description	Facility	Dermal exposure (µg zinc/cm²) on hands and forearms
Manual IBC emptying	A	202
Manual IBC emptying	A	319
ZnO packing – 25 kg sacks	B	389
IBC changeover	D	130
ZnO packing – 25 kg sacks	D	49
ZnO packing – 25 kg sacks	D	27

Models

With a negligible vapour pressure at room temperature and the production as a powder, the inhalation exposure for monohydrate is estimated as 2-5 mg/m^3 (dry manipulation with local exhaust ventilation; TGD, 1996). For the less dusty hexahydrate the option low dust technique is probably a better choice: the inhalation exposure is 0-1 mg/m^3.

Dermal exposure during bagging is estimated to be 420 mg/day, assuming intermittent, non-dispersive use and direct handling of the substance, leading to an exposure level of 0.-1 mg/cm^2/day, with the half of two hands exposed (420 cm^2). This activity may occur every working day.

Conclusions

Inhalation exposure

The values of the measured data of personal sampling during packaging of zinc monohydrate will be used for the risk assessment. The 90-percentile of the data will be considered as the full-shift reasonable worst-case value: 2.25 mg $ZnSO_4$/m^3 (0.9 mg Zn/m^3). The median will be taken to represent the typical value: 1 mg $ZnSO_4$/m^3 (0.4 mg Zn/m^3). As a short term value of twice the reasonable worst-case value is used: 4.5 mg $ZnSO_4$/m^3 (2 mg Zn/m^3).

For zinc sulphate hexahydrate no measured data are available that can be used to estimate typical and reasonable worst-case exposure levels. It is expected, based on EASE modelling, that the reasonable worst-case exposure level is 1 mg zinc sulphate hexahydrate/m^3 (0.4 mg zinc/m^3). A typical value cannot be estimated. This value is expected to be relevant for 2-4 hours of these high-exposure activities. Therefore, the reasonable worst-case value for a full shift taken forward to risk characterisation will be half these values.

The following uncertainties have to be considered in the evaluation of the MOS. The measured data on zinc sulphate is extremely limited and does not give sufficient basis for any assessment of exposure. For high-exposure activities (e.g. packaging) the exposure to zinc sulphate monohydrate is expected to be similar as the exposure to zinc oxide in similar activities. This analogy may be flawed if the processes in these activities, or the time spent in these tasks are not sufficiently similar. For hexahydrate, the use of EASE is presently the only option. In this case the result of EASE is used for full-shift estimates. This may lead to an over estimate of exposure if the actual duration of handling is substantially below 8 hours per day. Industry is convinced that the exposure levels in zinc sulphate producing facilities should be substantially below those in zinc oxide and zinc dust production, due to the largely wet process and the better containment

of the process. All these uncertainties indicate that exposure levels are probably overestimated. More data is expected in the near future to estimate the true exposure levels.

Dermal exposure

The results of Hughson and Cherrie (2001) will be used to conclude on reasonable worst-case and typical dermal exposure levels for production of zinc sulphate. Because of the less proper method for sampling in the first survey, the data from the second survey will be used to draw conclusions on dermal exposure. The dustiness of zinc sulphate hexahydrate is substantially lower than that of zinc sulphate monohydrate, that again is comparable to the dustiness of zinc oxide.

The tasks within these factories clearly lead to different exposure levels. Therefore the exposure levels were clustered in levels for "high" and "low" exposure tasks. "High-exposure tasks" are packing, classifying, blending and pelletising. "Low-exposure tasks" are furnace operation, warehouse operation and general operator. For both these clusters of tasks dermal exposure levels will be concluded. Zinc oxide and zinc dust have different particle sizes and different dustiness. The results of the measurements however do not show clear differences between workers in zinc oxide and zinc dust sections of the plants A and B. Therefore, the assessment for dermal exposure in the production of zinc sulphate will be based on the combined results of zinc oxide and zinc dust workers. Six of the eleven "high-exposure group" workers in plants B and D have dermal exposure levels to zinc of hands and forearms between 1,750 and 2,250 mg zinc. A reasonable worst-case value for hands and forearms is therefore estimated as 2,000 mg zinc. Six of the ten "high-exposure group" workers (with full sets of samples) in plants B and D had whole body dermal exposure levels between 1,950 and 2,700 mg zinc. The highest value was found for a worker that had exceptionally high-exposure values for head/face and neck. Discarding this outlier, the highest five whole body dermal exposure values in the high-exposure group are between 1,950 and 2,400 mg zinc. A reasonable worst-case value for whole body dermal exposure is therefore estimated as 2,200 mg zinc. These values are first recalculated into values for zinc oxide (the substance handled in most of the measurements). The values recalculated for zinc oxide are used as values for the full zinc sulphate products as well (i.e. 100 mg of zinc oxide would equal 100 mg of the concerning zinc sulphate). Then these values are calculated back to values in zinc. These recalculations lead to the following exposure levels for zinc sulphate products and zinc.

- hexahydrate, reasonable worst case = 2,700 mg zinc sulphate hexahydrate (2,200/65.38·81.4); this is approximately 810 mg zinc (2,740/221.4·65.38),
- hexahydrate, typical = 1,620 mg zinc sulphate hexahydrate (1,300/65.38·81.4) is approximately 480 mg zinc (1,620/221.4·65.38),
- monohydrate, reasonable worst case = 2,700 mg zinc sulphate monohydrate (2,200/65.38·81.4); this is approximately 1,050 mg zinc (2,740/171.4·65.38),
- monohydrate, typical = 1,620 mg zinc sulphate monohydrate (1,300/65.38·81.4) is approximately 620 mg zinc (1,620/171.4·65.38).

The following uncertainties have to be considered when evaluating the MOS. The data from zinc oxide production are assumed applicable for zinc sulphate production. Because the zinc sulphate production overall is a process where the substance is mostly in a wet phase, contamination of the workplace is expected to be substantially over estimated by this method. The over estimation is even higher for hexahydrate, that is substantially less dusty than zinc oxide. However, the measured data for zinc oxide production and zinc dust production are similar, with similar

technology but a substantially lower dustiness for zinc dust. The effect of dustiness on dermal exposure therefore does not appear to be very large. The measurement method used for the measurements of zinc oxide (repeated sampling at the same spot) may lead to over estimation of dermal exposure levels if the adherence slopes to a maximum that is lower than the adherence calculated by this method. The highest adherence calculated in the study by Hughson and Cherrie (2001) is close to 2 mg/cm^2. This is clearly below the maximum adherence of approximately 10 mg/cm^2 that is concluded in a review (SAIC, 1996), so there is no clear indication that this is a source of bias in this case.

Approximately 75% of the production is in hexa/heptahydrate. It is therefore assumed that exposure to this substance may occur daily, while exposure to the monohydrate is expected to occur at most 100 days/year.

4.1.1.2.2 Scenario 2: Production of fertilisers or animal feedstuff

Several zinc compounds are used in manufacturing fertilisers. Zinc is added as a micro nutrient in the form of zinc sulphate (hexa- or heptahydrate) or zinc phosphate to a mixture of other ingredients. In the animal feedstuff industry the monohydrate of zinc sulphate is used as a micronutrient. Production of fertilisers and animal feedstuff is assumed to be a mostly physical process of mixing of ingredients. Handling of zinc sulphate is assumed to be (weighing and) dumping of the substance from bags into mixtures. Exposure is possible during adding of zinc sulphate and during drying and packaging. Measured exposure levels are very scarce and reported with hardly any details and are below 1 mg/m^3 (expressed as zinc) (Industry, 1998).

Measured data

During initial production of fertiliser the raw material (hexa- or heptahydrate) is added in big bags with hardly any contact with the substance. No measured data are available for this scenario.

Measured data (for analogous substances or situations)

Measured data for zinc oxide or dust during the handling of zinc oxide in the manufacture of rubber, ceramics, ferrites and paint have been gathered by industry. In the tire industry data from 27 plants were compiled to a so-called “median plant”. Exposure of workers is mentioned to be up to 50% of the working time and exposure levels of ZnO are reported to be 0.5 mg/m^3. A “median plant” has also been constructed from 14 plants who have answered questions for the general rubber industry. Exposure is reported for up to 30% of working time with total dust levels of 5.9 mg/m^3 and ZnO concentration of 1.5 mg/m^3. Measured data for zinc (oxide) or dust during the handling of zinc oxide in the manufacture of ceramics, ferrites and paint have been gathered by industry. Data from several industries are presented in **Table 4.3**.

Table 4.3 Exposure to Zn or dust in several industries during the use of ZnO (Industry, 1999c)

Industry	n	Duration	Exposure levels (mg/m^3)*	References and remarks
Frits, enamals and ceramic pigments	212	n.g.	206 values < 0.8	
Ferrites	n.g.	n.g.	< 0.1	no details presented
Ferrites (specific company)	n.g.	n.g.	0.18-0.92	ZnO delivered in big bags exposure levels measured in several parts of the plant
Catalysts	n.g.	8 h	0.1-20.5 (typical)	(at plant operations and bag unloading)
Catalysts (specific company)	108	180-510 min	0.001-6.13 0.16(GM) 6.8 (GSD)	90th percentile calculated from GM and GSD as 1.9 mg/m^3.
Ceramics (one specific company)	n.g.	8 h	1-7 (dust) with 10-14% ZnO	ZnO loaded from bulk transport to bulk storage
Feedstuff additives	n.g.	8 h	< 5	no details presented

* Exposure levels generally expressed as amount of Zn/m3.

Data provided by the paint industry are presented in **Table 4.4**.

Table 4.4 Exposure to total dust in the production of paint (CEPE, 1998)

Set	Situation	n	Duration of sampling (min)	Results (mg/m^3)	Exposure calculated over 8 hours (mg/m^3)
A	Emptying ZnO form big bags into dispensers	3	22-33	2.6-4.9	0.17-0.28
B	Loading powders from 25 kg bags into dispensers	19	< 30	n.g.	0.01-1.5, average 0.29
C	Loading powders from big bags into dispensers	12	< 30	n.g.	0.01-1.34, average 0.27
D	bag disposal	n.g.	n.g.	average 1.04 maximum 2.2	n.g.

n.g. = not given

These results are for total dust. The ZnO content in the dust is unknown.

In a recent study on the exposure to inhalable dust during loading of powders into mixers in 10 different facilities both exposure during loading and full-shift exposure was measured (Marquart et al., 1999). All mixers were equipped with LEV, that was observed to function properly in all but one situations. A variety of powders was loaded (not including zinc sulphate), generally from 25 kg bags, but in some cases also from big bags or drums. Exposure levels of inhalable dust averaged over all loading tasks of one worker ranged from 1.9 to 27.6 mg/m^3. Duration of total loading tasks varied from 20 to 222 minutes and the amount of powder loaded by one worker during the shift from 330 to 11,369 kg. Full-shift exposure levels to inhalable dust measured ranged from 0.8 to 12.1 mg/m^3.

The exposure to zinc sulphate mainly takes place when the compound is added as a solid, during manual weighing and during actual loading of the mixer. The duration of this activity is

estimated to be 2-4 hours per day. For bag filling and bag dumping "reasonable worst-case" estimates in the presence of LEV with unknown effectiveness of 1.8 and 10 mg/m^3 (respirable and total dust concentrations during bag filling) and 10 mg/m^3 (total dust concentration during bag dumping) were deduced from literature (Lansink et al., 1996a).

Dermal exposure data for zinc oxide and zinc dust production are also available (see Scenario 1). The full-shift measured data on zinc powders include exposure due to contamination of surfaces caused by relatively dusty processes, but may be relevant for the handling of the dusty monohydrate in bags. The measured task based levels for emptying intermediate bulk containers (IBCs) are partly relevant for the handling of the hexahydrate in big bags, although they refer to the substantially dustier zinc oxide. These levels are between 130 and 319 µg zinc/cm^2 on hands and forearms (2,030 cm^2), leading to 264-648 mg zinc. This can be recalculated into 329-807 mg zinc oxide. Measurements on calcium carbonate in the paint industry (Lansink et al., 1996b) showed that exposure levels in dumping from bags where higher than in handling of closed bags, manual weighing and discarding of emptied bags. These data show exposure levels of 123-4,214 mg calcium carbonate on hands and forearms for dumping from bags. The measurement method (gloves) may have resulted in overestimation of exposure levels.

Models

The EASE model calculates for inhalation exposure 2-5 mg/m^3 for production of products containing zinc sulphate (dry manipulation with LEV) and a dermal exposure of 0.1-1 mg/cm^2/day (non-dispersive use and intermittent contact). If it is assumed that the palms of both hands are exposed (420 cm^2), the exposure is 42-420 mg/day for production.

Conclusions for production of animal feedstuff

Inhalation exposure

The worst-case situation appears to be the use of monohydrate in the production of animal feedstuff, when zinc sulphate is added by hand from bags to mixers. It is assumed that during this activity LEV is present. Several sets of data are available that are relevant for the production of products containing zinc sulphate. The data presented by various users of zinc oxide are variable in detail. Some appear to relate to "typical exposures" and some other sets are more detailed and also present the full range for the facility measured. The data from the paint industry appear to relate to one facility producing anti-fouling paints and are partly related to ZnO and partly to powders in general. In general, undetailed information is presented by industry sectors, while detailed information is available from single companies. Where detailed data are available, they are not all fully consistent with the summarised industry sector data. It is therefore very difficult to assess the representativeness of the data for the full industry sectors. The data from Marquart et al. (1999) were all measured for other substances than zinc sulphate and included both coarse granular substances and fine powders, often within one measurement. This study shows that there is remarkable variation in exposure levels, at least partly due to differences in powders handled. The study also shows, that exposure is not negligible during other activities than loading (e.g. manual weighing and other handling of powders). Based on the literature survey by Lansink et al. (1996a) and the study by Marquart et al. (1999) it can be concluded that exposure levels can be as high as 20 mg/m^3 during manual handling of large amounts of dusty powders and up to 10 mg/m^3 for an 8-hour shift. Measurements related to another zinc compound (ZnO) are reported as medians over 8 hours of < 0.1-1.5 mg/m^3 and ranges over up to 8 hours of < 0.2-6 mg/m^3 with short-term exposure levels in one facility during loading of

2.5-5 mg/m^3. The model EASE presents 2-5 mg/m^3 as the exposure level for manual handling of powders with LEV. It is concluded that reasonable worst-case exposure levels during loading of zinc sulphate monohydrate from bags (up to 2 hours per day) is comparable to the exposure levels estimated for zinc oxide during dumping in the production of paint and may be up to 5 mg/m^3 (zinc sulphate monohydrate). The reasonable worst-case full-shift exposure level is calculated to be up to 1.25 mg/m^3 (zinc sulphate monohydrate). For a typical value for inhalation exposure during dumping the value of 1 mg/m^3 (zinc sulphate monohydrate) is used, taken from the median values mentioned by industry for ZnO, leading to an estimated full-shift value of 0.25 mg/m^3 (zinc sulphate monohydrate).

The following uncertainties should be considered in the evaluation of the MOS. The exposure estimate is largely based on assumptions regarding similarity of processes and substance with the production of paint and zinc oxide. It is unclear from the available data whether these assumptions are fully valid. Furthermore, the original estimate for zinc oxide in the paint industry is also uncertain, though it is based on measured data. The information with the measured data is however too limited to come to conclusions with a high level of certainty. The duration of handling of the substance is a rough estimate, increasing the uncertainty of the full-shift levels.

Dermal exposure

There are no measured data on dermal exposure for dumping of zinc sulphate from bags or big bags into mixers.

Because zinc sulphate monohydrate has a similar dustiness as zinc oxide, the results for this scenario can be compared to the results for the scenario "production of paint (and some other products) containing zinc oxide" from the risk assessment report on zinc oxide. It is assumed that the exposure to the total powder is similar (i.e. if exposure to zinc oxide is 100 mg/day, exposure to zinc sulphate is also assumed to be 100 mg/day). Two sets of data are available for situations that are somehow analogous to the situation to be assessed. The data by Hughson and Cherrie (2000) are for a similar substance, but for a process that is different from the assessed process. The data on calcium carbonate from Lansink et al. (1996b) are for another substance, but a highly similar process to the one to be assessed. A difference between the studies is, that the data from Hughson and Cherrie are for a full shift, while the data from Lansink et al. are for one batch of paint. The data on calcium carbonate could therefore under estimate full-shift exposure levels. It is not known how many batches of animal feed stuff are made per day using zinc sulphate monohydrate, but a number of batches above two is not expected. On the other hand, calcium carbonate is much more dusty than zinc sulphate monohydrate and this may lead to over estimation of exposure to zinc oxide by the calcium carbonate data. A comparison of dermal exposure in the production of zinc oxide and zinc dust (that is of substantially lower dustiness than zinc oxide) does not show clear differences due to dustiness. The measurement method of Lansink et al. (1996b) – cotton gloves - may have led to an over estimation of the true exposure levels because powder may adhere better to cotton than to bare skin. A comparison on the basis of the measured values shows that the estimates of reasonable worst-case based on Hughson and Cherrie (2000) and Lansink et al. (1996b) are comparable: 2,740 mg zinc oxide for high-exposure tasks in the production of zinc oxide or zinc dust and 3,000 mg calcium carbonate for dumping into mixers. The typical value is lower for dumping of calcium carbonate: 900 mg versus 1,300 mg for high-exposure tasks in the production of zinc oxide or zinc dust. No information is available to show what possible bias in measurement method, process, number of batches or substance characteristics is more influential. Therefore, the most conservative of the two available analogous 90th percentiles is taken forward to the risk characterisation: 3,000 mg

zinc sulphate monohydrate/day (i.e. 1,140 mg zinc/day). Similarly, the most conservative of the two available typical values is taken forward to the risk characterisation: 1,620 mg zinc sulphate monohydrate/day (i.e. 620 mg zinc/day).

The uncertainties that should be considered in the evaluation of the MOS are largely mentioned above. Although the repeated sampling by wet wipes may also over estimate exposure levels (due to the prevention of a possible "sloping effect"), this is not likely to be very important in this case, since the total level of contamination per cm^2 is still clearly below values that were considered to represent the maximum adherence of powders to the skin by SAIC (1996). The value of 3,000 mg is 1.5 mg/cm^2 for a 2,000 cm^2 surface area, while SAIC concludes that the maximum adherence of powders is approximately 10 mg/cm^2, based on literature studies. The process of dumping powders from bags is considered to lead to higher dermal exposure than the filling of bags, due to the higher powder/air interaction in dumping and possible direct contact of the flow of powder with the skin. The reasonable worst-case and typical values may therefore be under estimated by the values taken forward to risk characterisation.

Zinc sulphate (monohydrate) is used only as a trace compound in animal feedstuff. It is therefore assumed that it is used in only a minority of working days when preparing pre-mixes. The duration of exposure is estimated to be up to 8 hours per day, with a frequency of up to 20 days per year.

Conclusions for production of fertilisers

Inhalation exposure

The exposure levels for handling crystalline zinc sulphate (hexahydrate) in the fertiliser industry are expected to be substantially lower than for handling the powder in the animal feed industry, due to the composition of the product and the use of big bags. Based on the limited data on zinc sulphate and on the use of zinc oxide from big bags, combined with the other data on dumping from bags, it is estimated that the reasonable worst-case exposure level in the fertiliser industry is 1.5 mg/m^3 (4 hours), recalculated to 0.8 mg/m^3 over 8 hours. A typical exposure level over 8 hours is expected to be 0.4 mg/m^3 (expert judgement). All the values mentioned above are for zinc sulphate hexahydrate.

The following uncertainties should be considered in the evaluation of the MOS. The estimate is based on a combination of measured data for other substances used in other facilities. This leads to uncertainty. The influence of dustiness is partly accounted for by expert judgement, without substantial information. This increases the uncertainty in the assessment.

Dermal exposure

There are no suitable measured dermal exposure levels for dumping crystalline material of very low dustiness from big bags. The measured task based levels for emptying intermediate bulk containers (IBCs) are considered to be most relevant, although they refer to the substantially dustier zinc oxide. These values (329-807 mg zinc oxide) probably over estimate the dermal exposure due to handling of the zinc sulphate hexahydrate that has a dustiness of only 0.25 mg/g, compared to 30 mg/g for zinc oxide (Armbruster, 2000). On the other hand, the results (expressed as mg zinc/day) for zinc dust (dustiness 1,5 mg/g; Armbruster, 2000) were highly comparable to the results for zinc oxide. To account for the lower dustiness of zinc sulphate hexahydrate, the total dermal exposure to this substance is assumed to be only half of the lowest task based value for zinc oxide: 165 mg zinc sulphate hexahydrate/day. This recalculates to

approximately 100 mg zinc/day. The typical value is estimated by the lower limit of the range of EASE, assuming an exposed area of only 420 cm^2: 42 mg zinc sulphate hexahydrate/day, recalculated in approximately 12 mg zinc/day.

The following uncertainties should be considered in the evaluation of the MOS. The estimate is based on very limited measured data for another, substantially dustier substance used in other facilities but in similar processes. The influence of dustiness is partly accounted for by expert judgement, without substantial information. The actual influence of dustiness on dermal exposure is unknown. This increases the uncertainty in the assessment.

4.1.1.2.3 Scenario 3: Use of fertilisers

Exposure to fertiliser is possible during mixing and loading of the equipment and during application. No data on exposure to fertiliser during use are known, so comparison with a similar process must be made. For comparison exposure to granular pesticides may be used, assuming that equipment and working conditions are similar. Some exposure data for granular pesticides are mentioned in literature. Granular pesticides have a majority of particles larger than 250 μm and only 0.2-2.2% is smaller than 150 μm.

Measured data on zinc compounds

There are no measured data on zinc compounds in the use of fertilisers.

Measured data on possible analogues

No data on exposure to fertilisers are available. An estimation using a less close analogy approach, the handling of granular pesticides, is used. Three studies were available with measured data for granular pesticides. Study 1, application with tractor mounted equipment, showed average respiratory exposures between 0.2 and 4.5 mg/day and maxima were between 0.7 and 6.6 mg/day. The potential dermal exposure (on the boundaries of the body, including the amounts on (protective) clothing) was 800-1,300 mg/day. Actual dermal exposure (on the skin) was estimated to be 50% of the potential dermal exposure (Lloyd and Bell, 1976).

In a second study (application in a greenhouse) respiratory exposure was measured ranging from 0.1-5.1 mg/m^3. No further details were mentioned (Wagner and Hermes, 1987).

In a third study (application by hand) respiratory exposure ranged from 0.3-18 mg formulation/hour (assuming a pulmonary ventilation rate of 10 m^3/day this is equivalent to 0.3-15 mg/m^3) and dermal exposure was 280-5,880 mg/hour (Wolfe et al., 1974).

Models

The EASE model calculates for inhalation exposure 5-50 mg/m^3 (dry manipulation with no LEV present) and a dermal exposure of 5-15 mg/cm^2/day (wide dispersive use and extensive contact. If it is assumed that the palms of both hands are exposed (420 cm^2), the exposure is 2,100-6,300 mg/day for use.

Conclusions

Inhalation exposure

The values calculated for the granular pesticides (though limited) are used in the assessment of exposure because the EASE model seems to give an overestimation, which is probably due to the fact that the estimation is based on powders and not on granules. Application of fertiliser by hand may lead to the highest inhalation and dermal exposure levels. The mentioned data are for the formulation. Assuming a zinc content of 1% (= 4.4% zinc sulphate hexahydrate) in the formulation, the maximum inhalation exposure is 15 (exposure to formulation in mg/m^3)·0.01 (fraction of zinc in formulation)·4.4 (conversion from zinc to zinc sulphate hexahydrate) = 0.66 mg/m^3 zinc sulphate hexahydrate (for one hour of exposure). Time weighted average exposure over 8 hours is calculated to be up to 0.08 mg/m^3, assuming negligible exposure outside of the one-hour exposure period.

Dermal exposure

Reasonable worst-case dermal exposure is 59 mg zinc or 260 mg zinc sulphate hexahydrate per day, assuming one hour of exposure (5,880 mg formulation / hour·1% of zinc or 4.4% of zinc sulphate hexahydrate).

A work task of one hour/day is assumed and frequency of exposure is estimated to be 20-50 days per year.

Table 4.5 Conclusions of the occupational exposure assessment

Scenario	Activity	Frequency (days/year)	Duration (hours/day)	Inhalation exposure zinc sulphate (zinc)				Skin exposure zinc sulphate (zinc)* ‡ (mg/day)	
				Reasonable worst-case (mg/m^3)‡	Method	Typical exposure ($mg/m)^3$‡	Method	Reasonable worst-case	Typical
1a) Production of zinc sulphate monohydrate	short term	50-100	2-4	4.5 (2)	calculated				
	full shift	50-100	8	2.25 (0.9)	measured	1.1 (0.4)	measured	2,700 (1,050)	1,620 (620)
1b) Production of zinc sulphate hexahydrate	short term	100-200	2-4	1.0 (0.4)	EASE				
	full shift	100-200	8	0.5 (0.2)	calculated	n.e.		2,700 (810)	1,620 (480)
2a) Production of animal feedstuff (monohydrate)#	dumping from bags	10-20	1-2	5 (1.9)	analogues/ EASE	1 (0.4)	analogues		
	full shift	10-20	6-8	1.25 (0.5)	calculated	0.25 (0.1)	calculated	3,000 (1,140)	1,620 (620)
2b) Production of fertiliser (hepta/ hexahydrate)#	dumping from big bags	100-200	2-4	1.5 (0.4)	analogues/EASE				
	full shift	100-200	6-8	0.8 (0.2)	calculated	0.4 (0.1)	expert	165 (100)	42 (12)
	short term	100-200	0.25	3.0 (0.7)	expert				
3) Use of fertiliser (hexahydrate)	manual application	20-50	0-1	0.66 (0.2)**	analogy	n.e.		260 (59)**	
	full shift	20-50	8	0.08 (0.02)	calculated	n.e.			

EASE = Calculation with the EASE model
Calculated = 8-hour time weighted average was calculated by assuming exposure at the short term exposure level during the maximum short term exposure duration and negligible exposure during the remainder of the 8-hour working day
Analogy = based on measured data for other substances used in similar exposure situations
n.e. = not estimated
‡ Data without parenthesis are expressed in the mono- or hexa hydrate or both, as mentioned in the first column, the data between parenthesis are expressed as zinc. The recalculation is done by
dividing by the molar weight of the mono- or hexahydrate (171.4, respectively 221.4) and multiplying by the molar weight of zinc (65.38) .
** Based on measured data for granular pesticides (with a majority of particles > 250 µm)
# The monohydrate has a substantially larger percentage of relatively small particles (< 14% < 10 µm), while the hepta- and hexahydrate consists of very large particles (99% > 100 µm)

4.1.1.3 Consumer exposure

According to the HSDB (1998) zinc sulphate is used in fertilizer solutions, as astringent in cosmetics (e.g. skin freshener), in glue, in deodorant anhydrotics, in mouthwashes and in ophthalmic preparations (drops, lotions). Three countries gave information on the consumer products containing zinc sulphate, but without quantitative data or more specific uses. In Sweden zinc sulphate can be found in 3 types of products, namely dispersion adhesives (construction industry), disinfectants, and feedstuff (micronutrients). The percentages of zinc sulphate in these products range from 0 to 10%.

Germany mentioned the use of zinc sulphate in fertilizers. Apart from the uses mentioned already, the US stated that zinc sulphate may be used in vaginal deodorants (0.25-4%) and as herbicide and miticide.

Apparently zinc sulphate is used in several consumer products, but no details on concentration and specific use pattern were given, which makes it difficult to predict consumer exposure. Furthermore, the total daily exposure to zinc can be higher by the use of consumer products containing other zinc compounds. Zinc compounds are also known to be used in dietary supplements, which consumers can buy over the counter.

More specified information was found for zinc compounds used in the product categories paint, cosmetics and drugstore products (VVVF, 1996; Natuur en Milieu, 1984; Annema, 1988; Rundervoort, 1992; KNMP, 1996). The default-values for paint, cosmetics and drugstore products are according to the TGD (1996) or, where no defaults are available, according to the Factsheets "verf" (paint) (Bremmer and van Veen, 2000) and "cosmetica" (cosmetics) (Bremmer et al., 2001). These factsheets are developed in order to refine the CONSEXPO program. The calculations are in accordance with the TGD (1996). For the separate use scenarios, based on the default-values found, the assumption is made that there is no uptake through inhalation when using these products and that the dermal absorption of the zinc compounds from any of the consumer products considered will be 2% for zinc solutions/suspensions and 0.2% for zinc dust/powder (see also Section 4.1.2.2.6).

Remarks

The section below is identical for all six zinc compounds evaluated under EU Regulation 793/93. Specific information is available for five of the six zinc compounds under evaluation (zinc phosphate, zinc distearate, zinc oxide, zinc chloride and zinc sulphate), as well as for some other zinc compounds not under evaluation. The latter information has also been included, because consumers (knowingly or unknowingly) at the same time can be exposed to several zinc-containing products, and irrespective of the original zinc compounds in these products, exposure will ultimately be to Zn^{2+}.

Paint

- Anti-corrosive primer containing 30% zinc phosphate: Assuming a frequency of 0.5 events/year with a dermal exposure of 2.7 g (paintbrush) or 10.8 g (spraying; roughly estimated as 4·paintbrush) primer/event, the maximum exposure will be 1.62 g zinc phosphate/year ≈ 2.25 mg Zn^{2+}/day. With a dermal absorption of 2% the uptake is estimated to be 0.045 mg Zn^{2+} /day,
- Impregnating agent containing 40% zinc naphthenate: Assuming a frequency of 0.5 events/year with a dermal exposure of 2.7 g impregnating agent/event, the exposure will

be 0.54 g zinc naphthenate/year ≈ 0.44 mg Zn^{2+}/day (percentage of zinc in zinc naphthenate is estimated at 30%). With a dermal absorption of 2% the uptake is estimated to be 0.0088 mg Zn^{2+}/day.

Cosmetics

- Eye shadow containing 10% zinc distearate (it mainly concerns glossy, emulsion like eye shadows): By an application of 10 mg/event for 3 times/day, the exposure to eye shadow is 30 mg/day, which contains 3 mg zinc distearate ≈ 0.31 mg Zn^{2+}/day. Assuming a dermal absorption of 2% the uptake is estimated to be 0.0062 mg Zn^{2+} /day,
- Sunscreen containing 10% zinc oxide (refers to a protection factor 20-25!): By an application of 9 g sunscreen/event, 3 events/day during 18 days/year the exposure will be 1,332 mg sunscreen/day, being 107 mg Zn^{2+}/day. Assuming a dermal absorption of 2% the uptake is estimated to be 2.14 mg Zn^{2+}/ day,
- Deodorant containing 10-20% large organic zinc compounds, but apparently no ZnO: The dermal exposure is 3 g or 0.5g/event by using a spray or a roll-on, respectively. In both cases the use is once a day. Maximum dermal exposure to deodorant is 3,000 mg/day ≈ 300 mg zinc compounds/day ≈ 30 mg Zn^{2+}/day (percentage of zinc in these zinc compounds is estimated at 10%). Assuming a dermal absorption of 2% the uptake is estimated to be 0.6 mg Zn^{2+}/day,
- Dandruff shampoo containing 5% zinc compounds such as zinc pyrithione and zinc omadine (5% is estimated based on other active components in dandruff shampoos): By a usage of 12 g shampoo/event for 4 times/week, the dermal exposure to shampoo will be 6,800 mg/day with a content of 340 mg zinc compounds. Assuming that 10% of these compounds consist of zinc and that the dermal absorption is 2%, the uptake via the use of dandruff shampoo will be 0.68 mg Zn^{2+}/day.

Drugstore products

- "Baby care" ointment containing 15% zinc oxide for the irritated skin (intensive ointment) or 5% zinc oxide for protective treatment when changing diapers: The assumption was made that the usage will be 50 g of the intensive ointment/year, leading to a dermal exposure of 7.5 g ZnO/year ≈ 16.5 mg Zn^{2+}/day. Assuming a dermal absorption of 2% the uptake is estimated to be 0.33 mg Zn^{2+}/day,
- Gargle containing 6.88 mg zinc chloride/ml: Assuming a use of 10 g gargle/event (≈ 10 ml/event), 4 times/day for 4 weeks/year, the exposure during these 4 weeks will be 1,120 g gargle/year ≈ 3.1 g gargle/day, which is ≈ 10 mg Zn^{2+}/day. Assuming that almost nothing will be swallowed, there is only buccal uptake via the mucous membranes. As the contact time is very short, the uptake is assumed to be very limited. Hence, with an arbitrary absorption value of 2% the uptake is estimated to be 0.2 mg Zn^{2+}/day,
- Eye drops containing 0.25% zinc sulphate (2.5 mg/ml): The assumption was made that the usage will be 2 eye drops (0.025 ml/drop)/event, 6 times/day during 4 weeks/year, leading to an exposure of 8.4 ml eye drops/year ≈ 23 mg eye drops/day ≈ 0.058 mg zinc sulphate/day ≈ 0.023 mg Zn^{2+}/day. Assuming an absorption of 2% the uptake is estimated to be 0.00046 mg Zn^{2+}/day.
- Zinc oil containing 60% ZnO, which is merely used medically for the treatment of skin disorders: The assumption was made that the usage will be 100 g/year, leading to an

exposure of 60 g ZnO/year ≈ 0.131 g Zn^{2+}/day. Assuming a dermal absorption of 2% the uptake is estimated to be 2.62 mg Zn^{2+}/day.
Remark: it is noted that with skin disorders uptake might be higher than 2%. However, how much more is not known. Besides, it is not expected that the possible higher amount absorbed will disturb the homeostatic balance (see also Section 4.1.2.2.5).

- Dietary supplements containing zinc: Results from a recent report on the food intake of the general population in the Netherlands (Hulshof et al., 1998) indicate that approximately 10% of the population uses dietary supplements, which amongst others can contain zinc. As it is not known how much zinc-containing dietary supplements are used and in what frequency, it is difficult to estimate the exposure to zinc from dietary supplements from this report.
  A dietary survey in the UK showed that < 1-3% of the participants in different age groups took zinc supplements, providing median zinc intakes of 0.3-3.4 mg/day. However, the contribution of this supplemental zinc intake to the population average zinc intakes from food and supplements combined was negligible (EVM, 1999).

Conclusion

The compound specific exposure estimates for the different zinc compounds are taken across to the risk characterisation. However, the total daily exposure to zinc can be higher since several zinc compounds are used in consumer products. Not all of these products are used regularly or at the same time (see above). It is assumed that dandruff shampoo, deodorant, eye shadow, and possibly baby care ointment will be used on a regular basis (more than once a week), resulting in a cumulative uptake of approximately 1.6 mg Zn^{2+}/day. Therefore this value will also be taken across to the risk characterisation, as this is a more realistic calculation of the daily consumer exposure to zinc.

4.1.1.4 Humans exposed via the environment

It should be noted that in this section the zinc cation is discussed, not the salt from which it originates.

4.1.1.4.1 General exposure

The most important exposure to zinc for the general population is by the ingestion of foods. Especially meat and meat products, milk and milk products, bread and starchy foods contribute to the dietary zinc intake. The average dietary intake of zinc by adults in nine European countries was reported to be 9.1-12.3 mg/day. Only for adult males in Germany and Italy a higher daily dietary intake of 14-15 mg/day was reported (Van Dokkum, 1995). These figures are confirmed for the Netherlands in a recent report on the food intake of the general population (Hulshof et al., 1998): the average daily intake of zinc is 9.4 mg with a minimum of 0.6 mg and a maximum of 39 mg. The 95th value is 15.4 mg (P_5 = 4.7, P_{10} = 5.5, median = 9.0, P_{90} = 13.8). The intake figures are based on a random group of 6,250 persons. The differences in zinc intake vary due to source and variety of the food.

An epidemiological study has been carried out by Kreis (1992) in which the health effects of cadmium (and zinc) were investigated in a contaminated area in the southern part of the Netherlands (Kempenland). A population sample aged 30-69, with a residence of at least

15 years in a rural village in Kempenland (NL) was compared with a control population of an unpolluted area. About 75% of the inhabitants of both areas consumed at least half of their vegetables from local gardens. The plasma concentration of zinc did not differ between the exposed (n = 299) and the reference population (n = 295) after adjustment for age and gender. The author concluded that, in contrast to cadmium, zinc exposure probably did not differ between the two villages.

In the section on measured regional data in the environment in the zinc metal risk assessment report, national monitoring data are presented for groundwater, surface water and air. In the following a compilation of these data is given. Via the National Soil Monitoring Network maximum zinc concentrations in upper groundwater of 1.1 mg/l (cattle farms) and 3.1 mg/l (forest locations) have been reported in the Netherlands. Recent zinc concentrations in large surface water in the Netherlands are found to be all below 0.1 mg/l. Recent atmospheric zinc concentrations in the Netherlands are below 0.1 $\mu g/m^3$ (annual averages). Higher concentrations, up to 14 $\mu g/m^3$, were reported for Belgium (older data).

Under normal conditions, drinking water and ambient air are minor sources of zinc intake. Cleven et al. (1993) estimated the intake by drinking water and ambient air to be < 0.01 mg/day and 0.0007 mg/day, respectively. The monitoring data above indicate somewhat higher intakes, but it is to be noted that nowadays in the EU upper groundwater and large surface water are not directly representative for drinking water. In the Netherlands, monitoring of zinc in drinking water is ceased (at water companies) or about to be ceased (at pump stations) (personal communication by RIVM-LWD, 1999).

Conclusion

The recent average dietary intake of zinc is around 10 mg/day. This value is taken across to the risk characterisation. Compared to this intake via food, intake via drinking water and ambient air is considered negligible.

4.1.1.4.2 Local exposure

Estimated local zinc concentrations in water and air around industrial facilities

In surface water a maximum local zinc concentration (PEC_{add}) of 410 µg/l (total zinc) has been estimated for the processing of zinc sulphate. For the production of zinc sulphate there is no emission to water (see Section on local exposure assessment in the environmental part).

Maximum atmospheric zinc concentrations ($PEC_{add}s$) are 0.078 $\mu g/m^3$ and 0.928 $\mu g/m^3$, for production and processing, respectively (see Section on local exposure assessment in the environmental part).

Conclusion

The $PEC_{add}s$ mentioned above are taken across to the risk characterisation.

4.1.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.1.2.1 Introduction

Basic assumptions

Large parts of the hazard section are identical in the risk assessment reports on the six zinc compounds now under review under EU Regulation 793/93. This is because of the basic assumption that the zinc cation (as measure for dissolved zinc species) is the determining factor for systemic toxicity.

It is realised that for zinc (and other metal) compounds it would be important to define the actual or bioavailable concentration which is important for toxicity, both in laboratory animals and in humans. Due to several physico-chemical processes, zinc will exist in different chemical forms, some of which are more bioavailable than others. It is thus realised that the bioavailability is affected by various physico-chemical parameters (ionic behaviour, solubility, pH, alkalinity etc.). Although there is some information on the solubility of the six zinc compounds (they are soluble in water (sulphate, chloride) or in diluted acids (phosphate, distearate and oxide) and elemental zinc is attacked by HCl to yield Zn^{2+} (Windholz et al., 1983)), adequate information is lacking how to quantitatively determine or estimate the bioavailable fraction of all the different zinc compounds in either laboratory animals or humans. Therefore, it is assumed that all zinc compounds (including metallic zinc) are changed (at least in part) to the ionic species (cf. TGD for environmental risk assessment for metals and metal compounds), and all toxicity data (independent of the tested compound) were used and expressed as the zinc cation.

With respect to *local* effects, it is not always possible to use data from all zinc compounds. Hence, for local effects only data from the specific zinc compound were used, or, where there were derogations, data from zinc compounds with more or less the same solubility characteristics.

A problem might arise for the route-to-route extrapolation for inhalation and dermal exposure, since the differences in physico-chemical properties of the zinc compounds can change the toxicokinetics (absorption) and subsequently the toxic effects. Although it is possible to predict the systemic effects after inhalation or dermal exposure from oral toxicity data of the zinc compound itself or other zinc compounds, this is only justifiable after careful consideration of all available data to establish adequate extrapolation factors.

Furthermore it is assumed that the influence of the background intake levels of zinc cations in animal studies will be the same for humans.

Database

A lot of information was provided by industry. Much additional data on zinc and zinc compounds have been published, some of which are referred to in good quality reviews by ATSDR (1994) and Walsh et al. (1994). By using these reviews plus (where relevant) the primary literature, it is felt that in the risk assessment reports most of the essential data to establish possible hazards/risk of zinc for human health have been covered. As not for all studies mentioned in the risk assessment reports the primary literature has been checked, some studies have been described in less detail than others. In the text of the risk assessment reports,

information cited from reviews is marked by a *(r)* after the reference. This information is not included in the hedset.

4.1.2.2 Toxicokinetics, metabolism and distribution

Some data were provided on the toxicokinetics of zinc sulphate. Data on other zinc compounds have also been used, as the basic assumption is made that after intake all zinc compounds (including metallic zinc) are changed (at least in part) to the ionic species and that it is this zinc cation that is the determining factor for the biological activities of the zinc compounds (see Section 4.1.2.1).

4.1.2.2.1 Absorption

Oral

Studies in animals

Furchner and Richmond (1962) added zinc acetate to the diet of Sprague-Dawley rats (9/group) to reach concentrations of Zn of 58 (no zinc acetate added; normal concentration in "control" feed), 117, 175, 293, 410 or 664 mg/kg via the feed, corresponding to ca. 3, 6, 9, 14.5, 20.5 or 33 mg Zn/kg bw. After 28 days the unfasted animals were dosed with 1.2 μCi of $^{65}ZnCl_2$ (ca. 0.15 ng). Whole-body radioactivity was determined at various time points up to 11 days post dosing using a whole-body gamma counter.

In the group which received the non-supplemented diet (i.e. 58 mg Zn/kg feed) ca. 20% of the administered radioactivity was retained at 24 hours post dosing which gradually decreased to about 9% at day 11. The amount of radioactivity retained at 24 h post dosing declined with increasing dietary zinc levels to about 13% for the group with the highest dietary zinc. In this group after 11 days only ca. 2.3% of the administered radioactivity was left. The data indicated that low dietary zinc results in increased zinc retention and that at higher dietary zinc levels absorption of zinc is reduced.

After a pre-exposure period of 7 days, male Wistar rats, kept on a semi-synthetic diet, were dosed orally with 86-130 μg ^{65}Zn as $ZnCl_2$ (n = 15), $ZnCO_3$ (n = 15) or $Zn_5(OH)_8Cl_2 \cdot H_2O$ (n = 20) added to a test meal. It was assumed that during the first 5 days post dosing non-absorbed zinc was excreted via the faeces. Absorption of labelled Zn^{2+} was calculated from *in vivo* whole-body gamma counting results over the period 5-14 days post dosing. The uptake was calculated to be 40, 45 or 48% for $Zn_5(OH)_8Cl_2 \cdot H_2O$, $ZnCl_2$ and $ZnCO_3$, respectively (Galvez-Morros et al., 1992).

Studies in humans

A wide range in absorption (8-80%) is observed in humans, probably due to the amount and type of food eaten (Hunt et al., 1991*(r)*; Reinhold et al., 1991*(r)*; Sandstrom and Sandberg, 1992*(r)*). Persons with adequate nutritional levels of Zn^{2+} absorb approximately 20-30% of all ingested Zn^{2+}. Those who are zinc-deficient absorb greater proportions of administered Zn^{2+} (Johnson et al., 1988*(r)*; Spencer et al., 1985*(r)*), while in persons with excessive zinc intake gastrointestinal uptake can be less (Babcock et al., 1982).

Zn^{2+} absorption in the gastrointestinal tract occurs throughout the entire small intestine with the highest rate in the jejunum and the rate of total absorption appears to be concentration-dependent (Lee et al., 1989*(r)*).

The Zn^{2+} absorption process in the intestines includes both passive diffusion and a carrier-mediated process (Tacnet et al., 1990*(r)*). At low zinc concentrations a cysteine-rich intestinal protein (CRIP) is involved in this process. This protein binds Zn^{2+} entering the intestinal cells from the lumen but this process appears to be saturable. Metallothionein, a metal-binding protein (also rich in cystein), may be involved at higher zinc concentrations (Gunshin et al., 1991*(r)*; Hempe and Cousins, 1992*(r)*; Sturniolo et al., 1991*(r)*). Zinc cations can induce metallothionein production in intestinal mucosa cells (Richards and Cousins, 1975*(r)*).

Payton et al. (1982) determined the intestinal absorption following single oral administration of ^{65}[Zn]-chloride to 6 groups of 5 healthy adult volunteers by comparison of whole body radioactivity counting and faecal excretion data. The individuals fasted overnight prior to dosing. Approximately 55% of the administered ^{65}[Zn]-chloride was absorbed at doses of 18, 45 and 90 µmol (~ 1.2, 2.9 or 5.8 mg) of zinc. The absorption was reduced with increasing dose, indicating that zinc absorption is saturable. At test dose levels of 180, 450 and 900 µmol (~ 11.6, 29 or 58 mg of Zn), only 51, 40 and 25% of the ^{65}Zn was absorbed, respectively. Additional studies in 15 in human volunteers with various intestinal diseases indicated that absorption of Zn occurred mainly in the proximal parts of the intestine.

From this study it appears that in healthy persons with intake levels differing by a factor of 10, uptake levels vary maximally by a factor of two.

The absorption of orally administered ^{65}Zn was studied in 50 patients with taste and smell dysfunction. The study was conducted in three phases. Prior to the start of the study 10 patients were admitted to a metabolic ward and put on a fixed daily diet containing 8-13 mg Zn. In the first phase all patients were studied for 21 days after receiving a single oral dose of 3-18 µCi of ^{65}Zn (~ 0.4 to 1.2 ng zinc) as $ZnCl_2$ after an overnight fast. In the second phase, which started after 21 days and continued for 290 to 440 (mean 336) days, all 50 patients received placebo. To study the effect of additional zinc intake on elimination of previously sequestered radioactivity, in the third phase of the study 14 patients continued on placebo while 36 received $ZnSO_4$ (100 mg Zn^{2+}/day) for 112 to 440 (mean 307) days. Phases two and three were a controlled clinical trial of the effects of zinc on retention of the ^{65}Zn tracer. The results of phase two and three are described in Section 4.1.2.2.4.

Total body retention and activity in plasma and red cells were measured for all patients throughout the study. It was estimated that for the ten in-patients ca. 55% of the administered radioactivity was absorbed while for the whole group of 50 patients the absorption was approximately 60 percent (Aamodt et al., 1982).

Remark: From the study description it is not clear whether the radioactivity was administered as pure radioactive zinc chloride or whether it was diluted with unlabelled zinc chloride. As the authors stated that "patients were given 3 to 18 µCi carrier free ^{65}Zn" for the calculation of the dose of ^{65}Zn in terms of nanogram zinc, it has been assumed that all zinc administered was in fact ^{65}Zn.

The absorption of zinc from soluble zinc acetate, zinc sulphate, zinc aminoate, zinc methionine and insoluble zinc oxide was compared in ten human volunteers who were dosed orally with 50 mg Zn in various forms separated by two weeks intervals. Bioavailability of zinc from the various forms was compared on the basis of plasma zinc levels and area under the plasma curve

analysis. Plasma peak levels were observed after about 2.5 h for all forms, but maximal plasma Zn concentration amounted to 221 and 225 μg/dl for the acetate and the sulphate form while the peak plasma level for Zn from the oxide was only 159 μg/dl. When AUC values for the different zinc forms were compared, it appeared that the bioavailability of zinc oxide was about 60% of the bioavailability of the soluble forms. Information on absolute bioavailability was not obtained (Prasad et al., 1993).

Nève et al. (1991) reported an absorption half-life of 0.4 hour when 45 mg Zn^{2+} as zinc sulphate was administered once in gelatine capsules to 10 healthy young men. Serum concentrations were measured frequently during a total investigation time of 8 hours. A mean maximum concentration of 8.2 μmol Zn^{2+}/l serum was found after 2.3 hours (t_{max}). There is evidence of an enteral recirculation, the first rebound effect appeared after 1.4 hours during the absorption phase before t_{max} was reached, and exhibited mean reabsorption rates of 70% of the dose given. The subsequent ones (max. of 5) appeared at regular intervals of 1.2 hours with a decrease of the quantity reabsorbed.

Factors that influence the gastrointestinal absorption of zinc cations include ligands (for example a decreased Zn^{2+} absorption may occur by intake of plant proteins, such as soy and phytate (Sandstrom and Sandberg, 1992*(r)*), by intake of alcohol (Antonson and Vanderhoff, 1983*(r)*) or use of EDTA (Solomons et al. 1979*(r)*; Spencer et al., 1966*(r)*)), or other trace elements in the diet (Solomons, 1988(*r*)). Also the zinc status of the body, the endogenous zinc secretion into the intestinal lumen via epithelial cells, bile and pancreatic secretion, and the intracellular transport have an influence on the Zn^{2+} absorption in the gastrointestinal tract (Cunnane, 1988*(r)*; Flanagan et al., 1983*(r)*). The mechanism by which zinc is transferred to or across the mucosal surface of the microvilli is not known (Cousins, 1989*(r)*).

Inhalation

Studies in animals

Rates or percentages of absorption of zinc cations after inhalation are not available, but there are some studies on Zn^{2+} retention in the lung.

Pistorius et al. (1976) exposed male and female rats to 15 mg ZnO dust/m^3 (particle size < 1 μm) for 4 hours/day, 5 days/week during 1 day or for 2, 4 or 8 weeks. Animals were killed 24 hours after the last exposure and the zinc content of the lungs, liver, kidneys, tibia and femur was measured. After 1 day of exposure the total zinc content of the lung in males and females was about 46 and 49 μg, respectively. In lung, liver, kidney and bone only minimal differences in tissue zinc content were seen during the experiment. As tissue zinc levels in non-treated animals were not studied, it is not clear whether tissue zinc comes from the experimental or from dietary exposure. However, as the pulmonary zinc level did not rise throughout the study it can be assumed that pulmonary deposition is very low and/or that pulmonary clearance is very high.

After exposure to 4.3 mg (rat), 6.0 mg (rabbit), 11.3 mg (guinea pig) mg ZnO (aerosol)/m^3 for 2-3 hours, the pulmonary retention in rats, rabbits and guinea pigs was 11.5%, 4.7% and 19.8%, respectively. The aerosol had a very small mass median diameter of 0.17 μm (Gordon et al., 1992).

In a time course experiment male Wistar rats (3/group) received a single intratracheal instillation of 0.4 ml ZnO suspension (ZnO particles < 2 μm, but they appeared to form aggregates of 10-20 particles) at a dose of 100 μg Zn^{2+}/rat and the rats were killed 1/3, 1, 2, 3, 5, 7, 14 and 21 days

after administration. In a dose-response experiment 0.4 ml ZnO suspension (ZnO particles < 2 μm, but they appeared to form aggregates of 10-20 particles) was instilled in the lungs of male Wistar rats (3/group) at doses of 20, 50, 100, 200, 500 and 1,000 μg Zn^{2+}/rat. The rats were killed after 2 days. Control animals were included in the experiments.

In the time course experiment a significantly increased lung wet weight 1 day after instillation and remaining throughout the time course was seen. Only a limited portion of Zn could be retrieved in the bronchoalveolar lavage fluid (BALF). No measurable amount of exogenous Zn was observed after 5 days. The half-life of ZnO instilled in the lung was calculated to be 14 hours.

In the dose-response experiment the lung wet weight increased with dose of ZnO 2 days after instillation. The results indicated that the rat lung was able to clear ZnO particles up to a dose of 50 μg Zn^{2+}/rat at least within two days. No measurable accumulation of Zn was observed in the liver and kidneys even at a dose of 1,000 g Zn^{2+}/rat (Hirano et al., 1989).

In a study from Oberdörster et al. (1980) the lung clearance rate of zinc aerosols was determined in male Wistar rats (8/group) 0, 2, 4, 8 and 24 hours after exposure to ZnO aerosol at a concentration of 12.8 mg/m^3 (mean aerodynamic diameter of 1 μm) for 17 hours. The ZnO aerosol was created by pyrolysis of a micronized Zn-acetate aerosol at 500°C. 8 Animals were kept in clean air and served as controls. The lungs and trachea of the animals were removed and their zinc content was determined by flame photometry. In comparison with the controls, the lungs of exposed rats were increased in weight (presumably because of oedema), which increase was significant at 8 hours and even more pronounced at 24 hours. The zinc content in the trachea was not uniform but was above control values except after 24 hours. The zinc content in the lungs decreased monoexponential and was 7% of the initial burden after 24 hours. According to the short half-life of 6.3 hours found in this study for the pulmonary zinc content, a fast dissolution of the particles must occur, as the alveolar clearance of an inert Fe_2O_3 aerosol occurred with a half-life of about 34 hours. It is not clear whether the clearance of Zn from the lungs is affected by the pathological condition of the lungs.

Studies in humans

Elevated zinc concentrations in blood and urine (Hamdi et al., 1969; Trevisan et al., 1982*(r)*) of persons occupationally exposed to zinc oxide fumes suggest that there is some pulmonary absorption, but no quantitative human data are available.

Other

Data were provided on the particle size distribution of zinc aerosol in three different industry sectors, i.e. the galvanising sector (three plants, 4 samples each), the brass casting sector (two plants, 3 and 4 samples respectively) and the zinc oxide production sector (one plant, 10 samples), by using personal cascade impactors with cut-off diameters of 0.52, 0.93, 1.55, 3.5, 6.0 and 21.3 μm, and a final filter diameter of 0.3 μm (Groat et al., 1999). These data served as input for the Multiple Path Particle Deposition Model (MPPDep version V1.11; Freijer et al., 1999) in order to estimate the airway deposition (in head, tracheobronchial and pulmonary region) for workers, by using:

- the human – five lobar lung model,
- a polydisperse particle distribution (i.e. this distribution contains a wide range of particle sizes), by taking the mean size distribution of the 10 samples for zinc oxide production

(MMAD 15.2 μm, GSD 4.0). Using this MMAD and GSD for the total polydisperse distribution is preferred above treating the polydisperse particles on individual impactor stages (with given cut-off diameters) as being monodisperse particles, also because the maximum particle size in the MPPDep model (20 μm) is lower than the largest size fraction of the cascade impactor (21.3 μm),

- both the oral breathing and the oronasal (normal augmenter) mode, but not the nasal breathing mode. The latter is considered to present an underestimate because 1) many people are oronasal or oral breathers, independent of their activities, 2) people with a cold will not normally breath nasally, and 3) with heavy exercise, short-term deep oral breathing will occur, resulting in increased deep pulmonary deposition,
- the possibility of inhalability adjustment for the oronasal augmenter. Inhalability is defined as that fraction of particles in an aerosol that can enter the nose or mouth upon inhalation. It must be noted that inhalability is different from respirability, which term relates to the deposition of particles after making their entrance inside the airways. If "inhalability adjustment" is "off", the calculations start by assuming that the airflow is in line with the direction of the nasal entrance. However, in reality this will not be the case because the airflow has to make turns to enter the nose. This results in losses that are larger with increasing particle size. Ménache et al. (1995) have described the relations between exposure concentration and concentration at the entrance of the airways for laboratory animals and humans,
- a tidal volume and breathing frequency corresponding to the default breathing rate of 10 m^3 for an 8-hour shift (1,100 ml and 20 breaths/min, respectively). This breathing rate is more representative for light exercise activities than for more moderate or heavy exercise activities (EPA, 1997), which can be expected to take place in the zinc industry (see Section 4.1.1.2). Therefore, also a non-default tidal volume and breathing frequency corresponding to a breathing rate of 19 m^3 for an 8-hour shift have been taken (1,700 ml and 23 breaths/min, respectively, based on a breathing volume of 40 l/min for moderate exercise activities (EPA, 1997)). It must be noted that at a minute volume < 35.3 l/min for normal augmenters breathing is only through the nose, while at a minute volume ≥ 35.3 ml/min there is combined nose and mouth breathing. For oral breathers, breathing is always only through the mouth, independent of the minute volume used.

Results of the MPPDep modelling are given in **Table 4.6**. It must be noted that the MPPDep only models deposition, not clearance and retention.

Table 4.6 Deposition fractions for oral breathers and for oronasal augmenters, using a polydisperse particle distribution (MMAD 15.2 μm, GSD 4.0)

	Inhalability	Tidal volume (ml)	Breaths/min	Deposition region			
				Head	Tracheo-bronchial	Pulmonary	Total
Oral	off	1,100 1,700	20 23	0.638 0.676	0.071 0.100	0.139 0.101	0.848 0.877
Oronasal	off	1,100 1,700	20 23	0.927 0.804	0.011 0.064	0.021 0.064	0.960 0.932
Oronasal	on	1,100 1,700	20 23	0.519 0.585	0.011 0.063	0.021 0.064	0.551 0.713

From **Table 4.6** it can be seen that for oral as well as for oronasal breathers the largest part of the deposition takes place in the head region, irrespective of the breathing rate. When inhalability adjustment is "on" head region deposition is somewhat reduced. However, the following is to be noted. As stated above, the corrections for inhalability of particles is based on relationships derived by Ménache et al. (1995). For humans this is based on experiments with only 4 healthy adult volunteers. The experiments are thus too limited to conclude for sure that this correction is valid for all human subjects and all situations (children, elderly, exercise activity, etc). It is therefore fair to estimate the deposition without inhalability adjustment to get an idea of a worst-case situation. The situation with inhalability adjustment "on" will not be taken further into account.

The fate and uptake of deposited particles depends on the clearance mechanisms present in the different parts of the airway. In the head region, most material will be cleared rapidly, either by expulsion (not the case for oral breathers) or by translocation to the gastrointestinal tract (half-time 10 min). A small fraction will be subject to more prolonged retention, which can result in direct local absorption. More or less the same is true for the tracheobronchial region, where the largest part of the deposited material will be cleared to the pharynx (mainly by mucociliary clearance (half-time 100 min)) followed by clearance to the gastrointestinal tract, and only a small fraction will be retained (ICRP, 1994). Higher uptake rates may be assumed for the pulmonary region than for the head and tracheobronchial region.

Once translocated to the gastrointestinal tract, uptake will be in accordance with oral uptake kinetics. Hence, for that part of the material deposited in head and tracheobronchial region that is cleared to the gastrointestinal tract, the oral absorption figures (20% for soluble zinc compounds and 12% for less soluble/insoluble zinc compounds, see Section 4.1.2.2.6) can be taken. However, there are no data available on zinc to estimate the part that is cleared to the gastrointestinal tract and the part that is absorbed locally in the different airway regions. With respect to the latter though, there are some data available for radionuclides. After instillation of small volumes (2-3 µl for rats, 10 µl for hamsters, 0.3 ml for dogs) of solutions or suspensions of radionuclides into each region of the respiratory tract, retention and absorption into blood was measured. For the more soluble chemical forms (a.o. citrate and nitrate) absorption values of 4.8-17.6% in the nasopharynx, 12.5-48% in the tracheobronchial region and up to 100% in the pulmonary region were found. For the more insoluble chemical forms (i.e. oxide) retention and absorption in the nasopharynx and tracheobronchial region was negliglible (ICRP, 1994). There are no data on how the solubility of the different chemical forms of the radionuclides compares to the solubility of the soluble zinc compounds. Although the applicability of the radionuclide figures to the zinc compounds is not quite clear, it is probably a reasonable worst case to take the upper values found (i.e. 20, 50 and 100% in head, trachebronchial and pulmonary region, respectively) for local absorption of soluble zinc compounds. For the less soluble/insoluble zinc compounds it is probably safe to assume negligible absorption for the head and tracheobronchial region and 100% absorption for the pulmonary region. This is supported by the findings in the study by Oberdörster et al. (1980), where the dissolution halftime of 1 µm diameter zinc oxide particles in the deep lung was approximately 6 hours. Given that the clearance to the gastrointestinal tract occurs within a time frame of minutes (10-100 min in head and tracheobronchial region), there will be no significant dissolution in these areas. Besides, most of the particles in these areas will have a diameter >1 µm, thus dissolution halftimes for these larger particles will be longer.

Based on the above information, inhalation absorption was estimated by assuming the following:

	soluble zinc compounds (chloride and sulphate)	less soluble/insoluble zinc compounds (metal, oxide, phosphate, distearate)
fraction absorbed in airway region	20% head 50% tracheobronchial 100% pulmonary	0% head 0% tracheobronchial 100% pulmonary
fraction cleared to g.i. tract, followed by oral uptake kinetics	80% head · 20% 50% tracheobronchial · 20% 0% pulmonary	100% head · 12% 100% tracheobronchial · 12% 0% pulmonary

The result of applying these assumptions to the deposition fractions given in Table 4.6 is given in **Table 4.7**.

Table 4.7 Estimation of inhalation absorption percentage for soluble zinc compounds and for less soluble/insoluble zinc compounds

	Inhalability	Tidal volume (ml)	Breaths/ min	Soluble zinc compounds (chloride and sulphate)	Less soluble/insoluble zinc compounds (metal, oxide, phosphate, distearate)
Oral	off	1,100 1,700	20 23	41.1 40.4	22.4 19.4
Oronasal	off	1,100 1,700	20 23	36.1 39.2	13.4 16.8

Inhalation absorption for the soluble zinc compounds (zinc chloride and zinc sulphate) is at maximum 40%, while for the less soluble/insoluble zinc compounds (zinc metal, zinc oxide, zinc phosphate and zinc distearate) inhalation absorption is at maximum 20%. These figures will be taken forward to the risk characterisation as a reasonable worst case, because these figures are thought to cover existing differences between the different zinc industry sectors with respect to type of exercise activities (and thus breathing rate) and particle size distribution.

Dermal

Studies in animals

Skog and Wahlberg (1964) estimated the percutaneous uptake of ^{65}[Zn]-chloride by the dorsal skin of the guinea pig by monitoring the decline of radioactivity emitted by ^{65}[Zn]-chloride in at least 10 trials for each concentration tested ranging from 0.8 to 4.87 M $ZnCl_2$ (pH 1.8-6.1). It appeared that the loss of radioactivity after 5 hours was less than 1% except for the trials with the lowest pH where it might have been between 1 and 2%. The study gives too little details to be used for risk assessment.

ZnO, zinc omadine, zinc sulphate and zinc undecylenate (131 μCi/mole of $^{65}Zn^{2+}$) were used for topical application on shaved skin on the back of rabbits. Each application consisted of 2.5 mg Zn-compound containing 5 μCi $^{65}Zn^{2+}$. Two animals received one application on four skin areas left of the spine, while the four skin areas on the right side received two applications, the second one 24 hours after the first one. The rabbits were killed 6 and 24 hours after the second application. One rabbit served as control animal.

No significant differences were found in the amount and location of $^{65}Zn^{2+}$ in skin treated with 4 different zinc compounds. High concentrations of $^{65}Zn^{2+}$ were observed in the cortical and cutical zones of the hair shaft, being the highest in the keratogenous zone. Accumulation of $^{65}Zn^{2+}$ in epidermis was very low but heavy in the subdermal muscle layer. Since no different rates of absorption and concentrations of zinc compounds with different oil/water solubility, pH, and molecular weight were seen, it was suggested that the major mode of $^{65}Zn^{2+}$ uptake in skin is by diffusion through the hair follicles due to the heavy localization of $^{65}Zn^{2+}$ primarily in the hair shaft and hair follicles. According to Kapur et al. (1974) this emphasizes that chemical differences in the compounds may not play a very important role in the skin uptake of $^{65}Zn^{2+}$. No data were given on systemic absorption.

The dermal absorption of $^{65}Zn^{2+}$ from $ZnCl_2$ and ZnO was studied by applying the zinc preparations under occlusion on the shaven, but intact skin on the back of male Sprague-Dawley rats (Hallmans and Lidén, 1979). The zinc absorption, being the ration between ^{65}Zn-activity in the carcass, liver and gastrointestinal tract, and the ^{65}Zn-activity in carcass, liver, gastrointestinal tract, skin and bandage, was reported to range from 1.6 to 6.1%. It should be noted that the higher percentages (3.6 to 6.1%) were achieved after application of $ZnCl_2$ in acidic solution (pH = 1). Less acidic solutions with $ZnCl_2$ or with ZnO resulted in a dermal absorption of less than 2%. In this study only the absorption into the body, excluding the skin, was determined. No data were available as to the effect of zinc chloride solutions with pH = 1 on dermal integrity.

Topical application of zinc chloride in an oil vehicle to pregnant Sprague-Dawley rats which were fed a zinc-deficient diet for 24 hours increased the plasma concentration of zinc cations to normal or slightly above normal levels (Keen and Hurley, 1977). The absorbed fraction was not determined so it can be concluded that dermal absorption is possible but no quantification can be given.

Agren et al. (1991) showed that application of zinc oxide dressings (containing 250 μg Zn^{2+}/cm^2) to rats for 48 hours with full-thickness skin excision resulted in a 12% delivery of zinc ions from the dressing to each wound, while application of zinc sulphate dressings (containing 66 μg Zn^{2+}/cm^2) resulted in a 65% delivery of ions to each wound. The data suggest that the application of zinc oxide resulted in sustained delivery of zinc ions causing constant wound-tissue zinc cation levels due to its slow dissociation rate, while the more water soluble zinc sulphate delivers zinc ions more rapidly to the wound fluid with subsequent rapid transferral into the blood.

Studies in humans

There are no quantitative data which indicate that zinc (cations) can be absorbed through the intact skin, but absorption was reported through damaged or burned skin (EHC, 1996).

An increase in serum Zn^{2+} levels was observed in 8 patients suffering from second and third degree burns, who were treated with adhesive zinc tape (ca. 7.5 g ZnO/100 g dry weight). The maximum value (up to 28.3 μmol/litre) was reached within 3-18 days during treatment. It is noted that the absorption through intact skin cannot be assessed based on this study with burn patients (Hallmans, 1977).

The systemic absorption from topical application of 40% zinc oxide ointment (with petrolatum) was investigated by Derry et al. (1983) in healthy subjects and in patients receiving total parenteral nutrition (TPN) for a minimum of 3 days prior to the start of the experiment. TPN is

known to result in zinc deficiency (mean decrease 6.6 μg/dl/week), and the longer the period of TPN without zinc supplementation, the greater the decrease in serum zinc concentration.

Healthy volunteers: in a controlled, cross-over study (on two separate days, one week apart) 6 healthy subjects received a topical application of 100 g of the 40% zinc oxide ointment or 60 g of control ointment (100% white petrolatum base) to the chest, upper legs and lower legs (exposed skin area: not specified; occlusion: not specified) for 3 hours. Each subject fasted for 12 hours before treatment started (only water *ad libitum*). During the study no food or water was consumed. Blood samples were taken after the 12 hour-fast (baseline value), and at 1, 2 and 3 hours after the start of the topical application. Mean serum Zn^{2+} concentrations at these time points were 107.3, 116.1, 105.3 and 112.6 μg/dl for the zinc ointment and 115.2, 103.5, 105.5 and 110.5 for the control ointment, respectively. Normal serum zinc concentrations were considered to be in the range of 68 to 136 μg/dl. An increase in serum zinc over the baseline value was observed in 4/6 subjects. In 3 of them, the rise was most pronounced after 1 hour. In 2/6 no increase was observed throughout the treatment. Overall, there was a mean serum Zn^{2+} increase of 8.8 μg/dl over baseline 1 hour after application. This represented an 8.2% rise in serum zinc, which however was not statistically significant.

Patients: 6 Patients received (under occlusion) a topical application of 15 g of the 40% zinc oxide ointment onto the upper legs (10·15 cm) once daily for 8 consecutive days. Blood samples were taken before treatment (baseline value), at 4, 6 and 8 days (just prior to application), and at day 10. The mean baseline level of the patients (88.6 μg/dl) differed significantly from the mean baseline level of the healthy subjects. The mean zinc concentration in the 3 patients that completed the study remained relatively stable over the 10-day period (78-93 μg/dl).

It can be concluded that topical applications of 40% zinc oxide ointment did not result in a significant increase in serum zinc concentration in healthy human subjects over a 3-hour period nor in TPN-patients over 10 days.

Remark: It is theorised by the authors that after topical application zinc is locally absorbed and stored in the hair follicles where it is relatively unavailable for immediate systemic absorption in subjects with normal serum zinc concentrations. In subjects that are hypozincemic, there is absorption from the storage depot at a rate sufficient to prevent a decline in serum zinc concentration. It is agreed with the authors that the 3-hour sampling time in normal subjects may have been of insufficient length to allow for appreciable systemic absorption from the storage depot.

When ZnO-mediated occlusive dressings (25% w/w; 4·5 cm) were applied to the lower arm of 10 healthy volunteers for 48 hours it appeared that the mean release rate of zinc to normal skin was 5 μg/cm^2/hour. After treatment of 5 other volunteers with the ZnO dressings for 48 hours the zinc content was significantly increased in the epidermis and the accumulated blister fluid (as a model for percutaneous absorption suction blisters were used). It should be noted, however, that the zinc penetration was enhanced during the formation of blisters, indicating that the barrier function was impaired (Agren, 1990).

In another study of Agren (1991) five human volunteers were exposed to different occlusive ZnO dressings (with hydrocolloid vehicle or gum rosin). After 48 hours, suction blisters on treated skin were raised and Zn^{2+} concentration in blister fluid was determined. Furthermore the Zn^{2+} concentration in the stratum corneum (10 successive tape strippings) was determined. The absorbed amount cannot be determined from the data presented but it appeared that the vehicle is an important factor for Zn^{2+} penetration.

In vitro studies

Pirot et al. (1996a) studied the dermal absorption of zinc 2-pyrrolidone 5-carboxylate, ZnO and $ZnSO_4$ (16 mg formulation/cm^2; 0.02–5.62% Zn^{2+}) in different formulations (3 emulsions and 2 ointments) using human abdominal skin. The receptor medium was 0.9% NaCl. After application for 72 hours, the skin was washed and stripped twice. The percutaneous absorption was determined as a percentage of the applied dose found in receptor medium and cutaneous bioavailability. It never exceeded 2%. The percentages for zinc from ointments containing ZnO and $ZnSO_4$ were 0.36% and 0.34%, respectively. The percutaneous absorption of zinc from the emulsion containing zinc 2-pyrrolidone 5-carboxylate was 1.60% of the applied dose. Furthermore the experiment showed a vehicle effect on absorption.

Pirot et al. (1996b) studied the dermal absorption of $ZnSO_4$ and $ZnCl_2$ (20 mg formulation/cm^2) in petrolatum and hydrophilic gels using human breast or abdominal skin. The receptor medium was isotonic saline. After application for 72 hours, the skin was washed and the epidermis was removed from the dermis. The result of the study was that the absorption was low, whatever vehicle was used.

The use of the data generated by Pirot et al. (1996a, 1996b) is limited because in these studies

- the integrity of the membranes was not assessed,
- it is not clear whether or not the skin was occluded,
- cutaneous bioavailability might be underestimated in the first study due to double stripping,
- in the second study, absorption is based on Zn in fresh dermis and receptor fluid, the fresh epidermis is not included.

Industry initiated an *in vitro* testing programme on two representative zinc compounds (zinc oxide and zinc sulphate) for percutaneous absorption (Grötsch, 1999). In this study, a solution of $ZnSO_4$ monohydrate and a suspension of ZnO, each at a concentration of 40 mg/ml in water, were tested for cutaneous penetration and absorption through pig skin *in vitro*. Skin preparations measuring 1 mm in thickness with stratum corneum, stratum germinativum and blood-vessel-containing parts of the dermis were obtained from pigs using a modified dermatome.

In two independent experiments for each compound seven skin preparations were mounted in Teflon flow-through diffusion chambers which were continuously rinsed with physiological receptor fluid (0.9% NaCl in aqua bidest with antibiotics). After an integrity check using the marker substance caffeine, each of the test formulations were applied to six skins at a dose of 1 mg/cm^2 for 8 hours without occlusion, and subsequently washed off with a neutral shampoo. After 0, 2, 4, 6, 8, 16, 24, 40, 48, 64 and 72 hours, the cutaneous permeation was determined by quantifying zinc with atomic absorption spectroscopic analysis (detection limit: 10 ng/ml) in the receptor fluid. The experiment was stopped at 72 hours. Furthermore, zinc was analysed in the skin preparations and the rinsing fluids. In addition, blanks were measured in an unloaded control chamber. Results are summarized in **Table 4.8**.

Table 4.8 Dermal absorption of Zn (% of dose) through pig skin in vitro within 72 hours [a)]

	$ZnSO_4$	ZnO
Receptor fluid	0.3 %	0.03 %
Horny layer	1.3 %	12.3 %
Residual skin	0 %	2.6 %
Potentially absorbed dose	1.6%	14.9%

a) Corrected for background levels of zinc in receptor fluid and skin

Total recoveries of applied zinc in both experiments ranged from 82.0 to 109.6%. The results of analysis of the receptor fluid used and of the blank chambers without topical application of zinc compounds indicated that both the receptor fluid and porcine skin contain an intrinsic level of zinc. The amounts of zinc detected in receptor fluid and different layers of the skin were therefore corrected for background levels.

The authors concluded that dermal penetration of zinc was below 1% based on the cumulative amount recovered from the receptor fluid at 72 hours. However, the amount retained in the skin should be regarded as being absorbed because it may become available at a later stage. Hence, the rapporteur concludes that the dermal absorption of zinc from a solution of zinc sulphate monohydrate and a suspension of zinc oxide in this *in vitro* system may amount to 1.6% and 14.9%, respectively.

4.1.2.2.2 Distribution

Inhalation

No data available.

Dermal

No data available.

Oral

Studies in animals

The highest levels of radioactivity were found in the small intestine followed by the kidney, liver and large intestine six hours after a single oral administration of 0.1 µCi of $^{65}Zn^{2+}$ as zinc chloride to Wistar rats. Smaller amounts were found in the lungs and spleen. 14 Days after the administration, highest levels of radioactivity could be found in the hair, testicles, liver and large intestines (Kossakowski and Grosicki, 1983*(r)*).

Organs with high zinc concentrations (ranging from 20 to 60 mg/kg fresh weight) are liver, gut, kidney, skin, lung, brain, heart and pancreas (Bentley and Grubb, 1991*(r)*; He et al., 1991*(r)*; Llobet et al., 1988). High concentrations of zinc were also detected in the retina and in sperm (Bentley and Grubb, 1991*(r)*).

Studies in humans

After absorption from the gastrointestinal tract, Zn^{2+} is bound in plasma primarily to albumin and then transported to the liver and subsequently throughout the body.

The normal plasma zinc concentration is ca. 1 mg/l, the total zinc content of the human body (70 kg) is in the range of 1.5-2 g (ATSDR, 1994).

Zinc is found in all tissues and tissue fluids and it is a cofactor in over 200 enzyme systems. In humans, the major part of total body zinc is found in muscle and bone, approximately 60% and 30%, respectively (Wastney et al., 1986*(r)*). Under normal conditions, the highest zinc concentrations/kg tissue are found in bone, hair and prostate (Cleven et al., 1993).

The distribution of zinc in humans appears to some degree to be influenced by age. The zinc concentrations increase in the liver, pancreas and prostate and decrease in the uterus and aorta with age. Levels in kidneys and heart peak at approximately 40-50 years of age and then decline. Levels in the aorta decline after 30 years of age (Schroeder et al., 1967*(r)*).

Other routes

The tissue uptake of $^{65}Zn^{2+}$ (as zinc chloride) was determined in adult male Wistar rats after intraperitoneal injection of 15 μCi $^{65}Zn^{2+}$. The liver displayed the greatest uptake for zinc ions, followed by the kidney, pancreas, spleen, ileum, lung, heart, bone, testis, blood cells, muscle and brain. Additional data on Zn^{2+} uptake by the brain indicate that the blood brain barrier is minimally permeable to zinc cations (Pullen et al., 1990*(r)*).

Eight hours following intravenous administration of ^{65}[Zn]-chloride to rabbits, tissue levels were highest in the liver, intestine and kidney with levels being ≥ 10%/g in tissue (Lorber et al., 1970*(r)*).

4.1.2.2.3 Metabolism

Zinc is mostly bound to organic ligands rather than free in solution as a cation (Gordon et al., 1981). Zinc is found in diffusible and nondiffusible forms in the blood and about 66% of the diffusible form of zinc in the plasma is freely exchangeable and loosely bound to albumin (Cousins, 1985*(r)*). A small amount of the nondiffusible form of zinc is tightly bound to α_2-macroglobulin in the plasma and is not freely exchangeable with other zinc ligands. Zinc is incorporated into and dissociated from α_2-macroglobulin only in the liver (Henkin, 1974*(r)*).

4.1.2.2.4 Excretion

Inhalation

No data available.

Dermal

No data available.

Oral

Studies in animals

After a single oral dose of 86–130 µg of ^{65}Zn as $ZnCl_2$, $ZnCO_3$ or $Zn_5(OH)_8Cl_2 \cdot H_2O$, male rats eliminated ^{65}Zn from the body with a rate of about 1.7% of the absorbed dose during day 5 to 14 post dosing as determined from stool, urinary and *in vivo* whole-body gamma counting results. In male rats who received 25 mg $ZnCO_3$/kg feed or 100 mg $Zn_5(OH)_8Cl_2 \cdot H_2O$/kg feed for 14 days, the radioactivity from a subcutaneous dose of 37 kBq of $^{65}ZnCl_2$ disappeared from the body with a rate of approximately 1% during the period 5 to 14 days post dosing (Galvez-Morros et al., 1992).

Studies in humans

In humans the fecal zinc consists of unabsorbed dietary zinc and endogenous zinc from bile, pancreatic juice and other secretions. About 70-80% of the ingested amount of zinc is excreted via feces (5 to 10 mg/day depending upon the dietary zinc concentration) (Spencer et al., 1976*(r)*; Venugopal and Lucky, 1978; Reinhold et al., 1991*(r)*; Wastney et al., 1986*(r)*). In humans about 10% of the zinc amount consumed is lost via urine (appr. 200 to 600 µg zinc/day). The urinary zinc excretion appears to be sensitive to alterations in the zinc status (Babcock et al., 1982; Aamodt et al., 1982; see below).

Minor routes of zinc excretion are saliva, hair loss, mothermilk, and sweat. In tropical climates about 2-3 mg Zn^{2+}/day may be lost in sweat (Venugopal and Lucky, 1978; Rivlin, 1983*(r)*; Prasad et al., 1963*(r)*; Rossowka and Nakamoto, 1992*(r)*; Henkin et al., 1975*(r)*).

In humans with no excessive intake of zinc, the body burden halftime of absorbed radio-labelled zinc has been observed to range from 162 to 500 days. After parenteral administration of $^{65}Zn^{2+}$, halftimes ranged from 100 to 500 days (Elinder, 1986).

Payton et al. (1982) determined body retention of Zn at 7-10 days after oral administration of 92 µmol of ^{65}Zn (as $ZnCl_2$) to 16 healthy adult human volunteers. It could be demonstrated that about 10% of the initially absorbed amount of Zn was excreted during the first 10 days post dosing. In thirty other volunteers dosed with 18 to 900 µmoles of ^{65}Zn the following elimination data for the 10 to 60 days post dosing period were obtained:

Dose group (µmoles; (mg))	Excretion rate (% of remaining Zn per day)	Biological half-live (days)
18 (1.2)	0.44	157
45 (2.9)	0.62	111
90 (5.8)	0.37	186
180 (11.6)	0.49	141
450 (29)	0.37	186
900 (58)	0.74 [a)]	93

a) Significantly different from the 18 µmoles group

The excretion rates for the 18 to 450 µmoles dose groups were not different, but after the 900 µmole dose elimination was significantly increased.

The effects of additional oral zinc on excretion of orally administered ^{65}Zn were studied in 50 patients with taste and smell disfunction. The study was conducted in three phases. In the first phase all patients were studied for 21 days after receiving a single oral dose of 3-18 μCi of ^{65}Zn (~ 0.4 to 1.2 ng zinc) as $ZnCl_2$ after an overnight fast. In the second phase, which started after 21 days and continued for 290 to 440 (mean 336) days, all 50 patients received placebo. To study the effect of additional zinc intake on elimination of previously sequestered radioactivity, in the third phase of the study 14 patients continued on placebo while 36 received $ZnSO_4$ (100 mg Zn^{2+}/day) for 112 to 440 (mean 307) days. Phases two and three were a controlled clinical trial of the effects of zinc on retention of the ^{65}Zn tracer. The results from the first phase of the study are described in Section 4.1.2.2.1.

Total body retention and activity in plasma and red cells were measured for all patients throughout the study. About one-third of the absorbed radioactivity was eliminated from the body with a half-life of ca. 19 days, while after about 100 days post dosing the remainder of the absorbed dose was eliminated with a biological half-life of 380 days (i.e. phase two of the study). During the third phase patients receiving $ZnSO_4$ showed an accelerated loss of total body ^{65}Zn ($T_{1/2}$ ca. 230 days) which was significantly different ($P > 0.001$) from half-life values during placebo treatment. Accelerated loss of ^{65}Zn from the thigh was apparent immediately while that from the liver began after a mean delay of 107 days. There was no apparent effect of zinc on loss of mean ^{65}Zn activity from red blood cells (Aamodt et al., 1982).

Remark: from the study description it is not clear whether the radioactivity was administered as pure radioactive zinc chloride or whether it was diluted with unlabelled zinc chloride. As the authors stated that "patients were given 3 to 18 μCi carrier free ^{65}Zn" for the calculation of the dose of ^{65}Zn in terms of nanogram zinc, it has been assumed that all zinc administered was in fact ^{65}Zn.

In ten patients from the Aamodt et al. 1982 study (see above) kinetics of ^{65}Zn were studied in more detail by Babcock et al. (1982). These patients received a fixed diet containing 8–13 mg Zn per day for 4 to 7 days before and after the single ^{65}Zn dose, followed by 290-440 (mean 336) days of non-restricted diet, followed by an intake of an additional 100 mg/day of non-radioactive zinc ion (as ZnSO4) over the next 112-440 days (mean 307). The overall kinetic parameters of these 10 patients did not differ from those of the other patients (Aamodt et al., 1982).

The authors further submitted retention-time curve data for whole body, plasma, red blood cells, liver and thigh to a multi-compartment kinetic model. From this model analysis it could be demonstrated that the increase in elimination of Zn during the third phase of the study by Aamodt et al. (1982) can be ascribed entirely to the change in two model parameters: reduction in absorption in the gastrointestinal tract (5-fold: from 43% absorption in the beginning of the study to 9% during the period in which patients were dosed with $ZnSO_4$) and to an increase in the urinary elimination rate (about 2-fold upon administration of $ZnSO_4$ during phase three of the study). Michaelis-Menten type saturation mechanisms were adequate to explain the observed parameter changes. These changes also accounted for the observed mean plasma zinc mass increase of only 37% above pre-load levels in face of an 11-fold increase in zinc intake (viz. from ca. 10 mg/day to ca. 110 mg/d) (Babcock et al., 1982).

Remark: from this model study it was estimated that the total body Zn contents of these 10 patients at the beginning of the study was 1.4 g. Babcock et al. (1982) indicated that normally the body contents of zinc is in the range of 2.1 to 2.5 g. This may indicate that the patients studied by Babcock et al. (1982) and possibly by Aamodt et al. (1982) were somewhat deficient in total body Zn.

4.1.2.2.5 Homeostasis

Within certain limits, mammals can maintain the total body zinc and the physiologically required levels of zinc in the various tissues constant, both at low and high dietary zinc intakes. The sites of regulation of zinc metabolism are: absorption of Zn^{2+} from the gastrointestinal tract, excretion of zinc in urine, exchange of zinc with erythrocytes, release of zinc from tissue, and secretion of zinc into the gastrointestinal tract. Regulation of gastrointestinal absorption and gastrointestinal secretion probably contributes the most to zinc homeostasis. In spite of the mechanism for whole body zinc homeostasis a regular exogenous supply of zinc is necessary to sustain the physiological requirements because of the limited exchange of zinc between tissues (Cleven et al., 1993).

It is hypothesized by Hempe and Cousins (1992*(r)*) that Zn^{2+} entering the luminal cells is associated with CRIP, a diffusible intracellular zinc carrier, and that a small amount is bound to metallothionein; however, as the luminal Zn^{2+} concentration increases, the proportion of cytosolic Zn^{2+} associated with CRIP is decreased and zinc binding to metallothionein increased. CRIP binds 40% of radiolabelled Zn^{2+} entering the intestinal cells of rats when zinc concentration is low; but only 14% when the concentration is high (Hempe and Cousins, 1992*(r)*).

Zinc is initially concentrated in the liver after ingestion, and is subsequently distributed throughout the body. When plasma zinc levels are high, liver metallothionein synthesis is stimulated, which facilitates the retention of zinc by hepatocytes (Richards and Cousins, 1975*(r)*).

4.1.2.2.6 Conclusion on toxicokinetics, metabolism and distribution

Some data were provided on the toxicokinetics of zinc sulphate. Data on other zinc compounds have also been used, as the basic assumption is made that after intake all zinc compounds (including metallic zinc) are changed (at least in part) to the ionic species and that it is this zinc cation that is the determining factor for the biological activities of the zinc compounds.

Within certain limits, the total body zinc as well as the physiologically required levels of zinc in the various tissues can be maintained, both at low and high dietary zinc intake. Regulation of gastrointestinal absorption and gastrointestinal secretion probably contributes the most to zinc homeostasis. In spite of this a regular exogenous supply of zinc is necessary to sustain the physiological requirements because of the limited exchange of zinc between tissues.

The Zn^{2+} absorption process in the intestines includes both passive diffusion and a carrier-mediated process. The absorption can be influenced by several factors such as ligands in the diet and the zinc status.

Persons with adequate nutritional levels absorb 20-30% and animals 40-50%. However, persons that are Zn-deficient absorb more, while persons with excessive Zn intake absorb less. For risk assessment, for the more soluble zinc compounds (chloride, sulphate) the lower bound of the absorption range at adequate nutritional levels is taken (i.e. 20%). For zinc oxide it has been shown that bioavailability is about 60% of that for soluble zinc salts, corresponding to 12-18%. For zinc metal, zinc phosphate and zinc distearate no bioavailability data were present. As these forms have limited solubility in diluted acids (stomach) comparable to zinc oxide, for the less soluble zinc compounds (oxide, phosphate, distearate, metal) an oral absorption value of 12% will be taken for risk assessment.

In situations of exposure excess (e.g. in case of high dermal or inhalation exposure at the workplace) the oral uptake of zinc compounds will probably be less than the values taken for risk assessment (20% and 12%). However, as this reduction in uptake is not quantifiable, also for excess exposure situations the same oral absorption values will be applied. Some justification for this approach can be found in the observation that for intake levels differing by a factor of 10, uptake levels vary maximally by a factor of two.

Quantitative data on the absorption of zinc following inhalation exposure (especially relevant in occupational settings) are not available. Some animal data suggest that pulmonary absorption is possible. In animal studies on zinc oxide retention in the lungs half-life values of 14 and 6.3 hours were reported for dissolution. As the absorption of inhaled zinc depends on the particle size and the deposition of these particles, data were provided on the particle size distribution of zinc aerosol in three different industry sectors. When analysing the particle size distribution data with a multiple path particle deposition (MPPDep) model, it appeared that for zinc aerosols the largest part of the deposition takes place in the head region and much less in the tracheobronchial and pulmonary region. Although most of the material deposited in the head and tracheobronchial region is rapidly translocated to the gastrointestinal tract, a part will also be absorbed locally. Based on data for local absorption of radionuclides in the different airway regions, it is assumed that local absorption for the soluble zinc compounds will amount to 20, 50 and 100% of the material deposited in head, tracheobronchial and pulmonary region, respectively. For the less soluble/insoluble zinc compounds negligible absorption is assumed for head and tracheobronchial region and 100% absorption for the pulmonary region. The remaining part of the material deposited in the different airway regions will be cleared to the gastrointestinal tract where it will follow oral uptake kinetics, hence the oral absorption figures can be applied. Applying the above mentioned assumptions to the deposition fractions as determined by the MPPDep model, inhalation absorption for the soluble zinc compounds (zinc chloride and zinc sulphate) is at maximum 40%, while for the less soluble/insoluble zinc compounds (zinc metal, zinc oxide, zinc phosphate and zinc distearate) inhalation absorption is at maximum 20%. These figures will be taken forward to the risk characterisation as a reasonable worst case, because these figures are thought to cover existing differences between the different zinc industry sectors with respect to type of exercise activities (and thus breathing rate) and particle size distribution.

Adequate quantitative data on the absorption of zinc following dermal exposure (relevant in both occupational and consumer settings) are not available. The human data presented are not considered valid, mainly since either wounded skin was investigated, or suction blisters were raised, impairing the intactness of the skin. Dermal absorption through the intact skin seems to be small (< 2%), based on the results of the *in vivo* animals studies as well as the *in vitro* studies, but unfortunately shortcomings were noted in all *in vivo* studies and none of these studies can be used quantitatively. As for the *in vitro* studies, it is clear that the % in receptor medium generally gives an underestimation of the % systemically available in *in vivo* studies. Therefore, the amount detected in the skin should be included as being absorbed by default. This "potentially absorbed dose" more closely resembles the dose becoming systemically available *in vivo*.

Zinc bound to or in the skin may become systemically available at a later stage. This can be concluded from results in TPN patients, in which an expected decrease in serum zinc levels with time was counteracted by dermal absorption of zinc to result in steady serum zinc levels. Unfortunately, only 3 of the 6 patients completed the 10-day study period. There are no adequate human data available to evaluate the release of zinc from normal skin following single or repeated dermal exposure, as either blood was sampled for a too short period of time (3 hours; Derry et al., 1983) or the skin was damaged (Agren, 1990, 1991; Hallmans, 1977). Therefore, it can be

concluded that following single or repeated dermal exposure zinc can be taken up by the skin, whereas the relevance of this skin depot cannot be judged based on the available data. For example, it is not studied how a large artificial zinc depot in the skin will affect the uptake or homeostasis of other essential ions (e.g. Cu). However, the total database available indicates that skin-bound zinc may not become systemically available in a way that it results in high peak levels of zinc in serum, but rather in a more gradual way. Given the efficient homeostatic mechanisms of mammals to maintain the total body zinc and the physiologically required levels of zinc in the various tissues constant, the anticipated slow release of zinc from the skin is not expected to disturb the homeostatic zinc balance of the body. By expert judgement, based on the aforementioned considerations, the default for dermal absorption of solutions or suspensions of zinc or zinc compounds is therefore chosen to be 2%. Based on the physical appearance, for dust exposure to zinc or zinc compounds a 10-fold lower default value of 0.2% is chosen in the risk assessment.

Zinc is distributed to all tissues and tissue fluids and it is a cofactor in over 200 enzyme systems.

Zinc is primarily excreted via feces, but can also be excreted via urine, saliva, hair loss, sweat and mothermilk.

4.1.2.3 Acute toxicity

4.1.2.3.1 Studies in animals

Studies with zinc sulphate have been carried out in rats and mice by oral and intraperitoneal routes. In rats also a study by dermal route is available. The studies are summarised in **Table 4.9**.

Table 4.9 Acute toxicity

Acute toxicity	Species	Protocol	Results	Reference
Oral	mouse	other	LD_{50} = 926 mg $ZnSO_4 \cdot 2H_2O$/ kg bw	Domingo et al. (1988) #
	mouse	other	LD_{50} = 1,891 mg $ZnSO_4$*/kg bw	Courtois et al. (1978)
	rat	other	LD_{50} = 2,280 mg $ZnSO_4 \cdot 7H_2O$/kg bw (estimated value)	Lorke (1983)
	rat	other	LD_{50} = 2,949 mg $ZnSO_4$*/kg bw	Courtois et al. (1978)
	rat	other	LD_{50} = 920 mg $ZnSO_4$*/kg bw	Litton Bionetics (1974)
	rat	other	LD_{50} = 1,710 mg $ZnSO_4 \cdot 2H_2O$/kg bw	Domingo et al. (1988) #
	rat	OECD 423	LD_{50} > 2,500 mg $ZnSO_4 \cdot 6H_2O$/kg bw	Sanders (2001a)
	rat	OECD 423	LD_{50} = 1,000-2,000 mg $ZnSO_4 \cdot 7H_2O$/kg bw	Sanders (2001b)
Dermal	rat	OECD 402	LD_{50} >2,000 mg $ZnSO_4 \cdot 7H_2O$/kg bw	Van Huygevoort (1999c)
Intraperitoneal	mouse mouse	other other	LD_{50} = 316 mg $ZnSO_4 \cdot 2H_2O$/kg bw LD_{50} = 267 mg $ZnSO_4$*/kg bw	Domingo et al. (1988) # Courtois et al. (1978)
	rat rat	other other	LD_{50} = 200 mg $ZnSO_4 \cdot 2H_2O$/kg bw LD_{50} = 258 mg $ZnSO_4$*/kg bw	Domingo et al. (1988) # Courtois et al. (1978)

* type of zinc sulphate used not specified

\# in the tests by Domingo et al. (1988) the dihydrate is stated to have been used. However, this type of zinc sulphate does not exist

Domingo et al. (1988) calculated the LD_{50} values in male Sprague Dawley rats and male Swiss mice after oral and intraperitoneal administration of zinc sulphate. The authors stated that zinc sulphate dihydrate obtained from Merck (Darmstadt) was given, although this form does not exist. After a preliminary screening with small groups of 3 animals of each species, ten animals in each group were used and observed for 14 days. Death occurred within the first 48 hours. Toxicity signs included miosis, conjunctivitis, decreased food and water consumption and haemorrhages and haematomas in the tail. These changes decreased with time. Oral LD_{50} values for mice and rats were 926 mg/kg bw and 1,710 mg/kg bw, respectively. The LD_{50} values after intraperitoneal administration for mice and rats were 316 mg/kg bw and 200 mg/kg bw, respectively. It must be noted that in this study also other zinc compounds have been tested for acute toxicity and that in the description of the clinical signs no distinction was made for the different zinc compounds.

In the study of Courtois et al. (1978) the acute toxicity of zinc sulphate (type not specified) was determined in male Wistar rats and male Swiss mice both after oral and intraperitoneal administration. Oral LD_{50} values for mice and rats were 1,891 mg/kg bw and 2,949 mg/kg bw, respectively. The LD_{50} values after intraperitoneal administration for mice and rats were 267 mg/kg bw and 258 mg/kg bw, respectively. Toxicity signs included decreased mobility, piloerection, dyspnoeic, and diarrhoea. No further details were given.

Lorke (1983) estimated the oral LD_{50} value for zinc sulphate ($ZnSO_4 \cdot 7H_2O$) in male rats, using 11 animals per group. The LD_{50} value was estimated at 2,280 mg/kg bw. But no further details and no description of toxicity signs were given.

In the study of Litton Bionetics (1974) zinc sulphate (type not specified, only "Zinc sulphate, Rayon") was suspended in 0.85% saline and administered to male Sprague Dawley rats by intubation. Dose levels were 50, 100, 500, 1,000 and 3,000 mg/kg (5 animals per dose). Animals were observed for ten days. Toxicity signs were reddened stomach and intestinal mucosa. The LD_{50} value was determined as 920 mg/kg bw zinc sulphate.

In a recent study according to OECD 423 (acute toxic class protocol) and in compliance with GLP, no deaths and no clinical signs of intoxication were observed after dosing six rats (3/sex) with 2,000 mg/kg bw sulphate hexahydrate (technical) (Sanders, 2001a). According to OECD 423 this result leads to an expected LD_{50} of > 2,500 mg/kg bw.

In a similar study (Sanders, 2001b) with zinc sulphate heptahydrate (pharmaceutical grade), two out of six rats died at an oral dose of 2,000 mg/kg bw (0/3 in first sex tested, 2/3 in second sex tested). Clinical signs were hunched posture, lethargy, ataxia, pilo-erection, splayed or tiptoe gait, decreased respiration rate, laboured respiration, ptosis, emaciation, red/bron staining round eyes and diarrhoea. Necropsy in animals that died revealed haemorrhagic lungs, dark liver and kidneys, white/green coloured and thickened gastric mucosa and haemorrhagic small intestine. At 200 mg/kg bw no death or clinical signs were observed.). According to OECD 423 the results for zinc sulphate heptahydrate (pharmaceutical grade) lead to an expected LD_{50} between 1,000 and 2,000 mg/kg bw.

Because in two of the six oral studies the type of zinc sulphate tested was not specified and in a third study a non-existing form of zinc sulphate was stated to have been used, all available rat LD_{50} values were recalculated (see **Table 4.10** below) to LD_{50}-values for the three main types of zinc sulphate currently marketed (i.e. the mono-, hexa- and heptahydrate).

Table 4.10 Recalculation of rat LD50 values

LD_{50} (mg/kg bw)	Reported for	Source	LD_{50} (mg/kg bw) recalculated for		
			mono	hexa	hepta
1,710	dihydrate	Domingo et al. (1988)	1,554	2,334	2,490
2,280	heptahydrate	Lorke (1983)	1,423	2,137	2,280
2,949	unspecified	Courtois et al. (1978)	1,840 [1)] 2,949 [2)]	2,764 [1)] 4,429 [2)]	2,949 [1)] 4,725 [2)]
920	unspecified	Litton Bionetics (1974)	574 [1)] 920 [2)]	862 [1)] 1,382 [2)]	920 [1)] 1,474 [2)]
> 2,500	hexahydrate	Sanders (2001a)	> 1,665	> 2,500	> 2,667
1,000 < LD_{50} < 2,000	heptahydrate	Sanders (2001b)	624 < LD_{50} < 1,248	937 < LD_{50} < 1,875	1,000 < LD_{50} < 2,000

1) Assuming that the heptahydrate was tested (worst case)
2) Assuming that the monohydrate was tested

The available data are not completely conclusive as to the true value of the oral LD_{50} for the various forms. The older acute oral toxicity studies are difficult to interpret because of gaps in the information, especially with regards to the form that was tested. The two most recent studies by Sanders are contradictory in the sense that one would expect the hexahydrate to be more toxic than, or at least to be equally toxic to the heptahydrate. However, the results of these studies seem to point in the opposite direction.

Based on recalculation of the data it must be assumed that the monohydrate should be classified as harmful via the oral route. As there is good evidence that the heptahydrate is harmful as well, it seems logic to assume that the hexahydrate should also be classified as harmful, despite the result obtained in the study by Sanders (2001a).

Zinc sulphate is not harmful or toxic via the dermal route. In the study of Van Huygevoort (1999c) zinc sulphate heptahydrate was administered to the skin of five Wistar rats of each sex at 2,000 mg/kg bw for 24 hours. Animals were observed for 15 days. Clinical signs of toxicity consisted of erythema (grade 1 and 2, of maximum grade 4), scales and/or scabs (scale 1 and 2, of maximum scale 3) in the treated skin area between observation days 2-8.

No data were available on acute inhalation toxicity of zinc sulphate.

Additional single dose studies

Male Syrian hamsters were exposed via inhalation to zinc sulphate ($ZnSO_4 \cdot 7H_2O$) aerosols in doses of 1.3 to 34.2 mg /m^3 (1.1-7.3 mg Zn^{2+}) for 4 hours. The activity median aerodynamic diameter (AMAD) and geometric standard deviation (GSD) of the aerosols were 0.59 µm and 1.46, respectively. The rate of phagocytosis of insoluble particles by pulmonary macrophages was determined *in situ* by introduction of insoluble gold colloid in the respiratory tract under anesthesia. From a dose of 5.2 up to 34.2 mg $ZnSO_4 \cdot 7H_2O/m^3$ macrophage endocytosis of colloidal gold was significantly reduced 1 h after exposure compared with that in unexposed control animals. After 24 hours the rate of phagocytosis was still depressed, whereas after 48 hours it had returned to normal values. An increase in macrophage cell number was seen at low concentrations followed by depressions in macrophage numbers at high concentrations. No effects were observed at 1.3 mg/m^3 (0.2 mg Zn^{2+}) (Skornik and Brain, 1983).

In anesthetised dogs the pulmonary mechanics were not significantly changed, after inhalation exposure to submicron aerosols of $ZnSO_4$ (geometric standard deviation 2.1) up to 17.3 mg/m^3 (7 mg Zn^{2+}) for 7.5 minutes. Also an exposure of 4 hours to 4.1 to 8.8 mg/m^3 $ZnSO_4$ to anesthetised dogs showed no effect on breathing mechanics, haemodynamics, and arterial blood gases (Sackner et al., 1981).

4.1.2.3.2 Studies in humans

A 15-year-old girl with no history of dyspepsia ingested zinc sulphate tablets of 220 mg twice daily (440 mg $ZnSO_4$/day ≈ 2.6 mg Zn^{2+}/kg bw/day) for treatment of acne. After each capsule the girl experienced epigastric discomfort. After 1 week she showed gastrointestinal haemorrhages accompanied by anemia. No other medicines were used (Moore, 1978).

Brandao-Neto et al. (1990) reported an inhibitory effect of zinc on adrenal cortisol secretion in humans. In 26 healthy individuals of both sexes aged 20-27 years, an infusion was inserted into an antecubital vein at 7.00 am after a 12-hour fast. Basal blood samples were collected at 7.30 and 8.00 am. The experimental group (n = 12) received single doses of 25, 37.5 and 50 mg of zinc (diluted in 20 ml deionised water, ≈ 0.5 mg Zn^{2+}/kg bw), p.o. at 8.00 am. Controls (n = 14) received 20 ml of physiological saline. Serial blood samples were collected over a period of 240 minutes after basal samples. An acute inhibitory effect of zinc on cortisol secretion was detected. After 120 minutes of exposure mean differences in cortisol level were 2.76 µg/dl cortisol in controls whereas in the experimental group this difference was 8.09 µg/dl cortisol.

4.1.2.3.3 Conclusion on acute toxicity

The amount of data available is considered sufficient to fulfil the Annex VIIA requirements for acute toxicity. Based on the acute oral data, zinc sulphate (anhydrous as well as hydrous forms) is harmful via the oral route and therefore needs to be classified as harmful if swallowed (R22) according to EC criteria (Council Directive 67/548/EEC). Zinc sulphate is not acutely toxic via the dermal route and therefore needs not to be classified for acute dermal toxicity. Investigations into the acute inhalation toxicity of zinc sulphate were limited to pulmonary effects.

4.1.2.4 Irritation

Skin

In a well performed primary skin irritation/corrosion study, conducted according to Directive 92/69/EEC B.4 and OECD guideline 404, three male New Zealand White rabbits were exposed to 0.5 g of moistened zinc sulphate ($ZnSO_4 \cdot 7\ H_2O$), applied onto clipped skin for 4 hours using a semi-occlusive dressing. Observations were made 1, 24, 48 and 72 hours after exposure. No symptoms of systemic toxicity and no skin irritation/corrosion were observed and no mortality occurred (Van Huygevoort, 1999d).

Zinc sulphate (unspecified) was tested at a concentration of 0.5 ml (1% in deionised water, pH 5.7) applied daily on the dorsal skin (5 cm^2) for 5 consecutive days. In open patch tests ¼ rabbits, 2/6 mice and 1/8 guinea pigs had slight irritancy (score +; scoring from slight (+) to severe (+++)). In an occlusive patch test 1/4 rabbits had slight irritancy. Erythema but no ulceration or scaling was observed (Lansdown, 1991).

Respiratory tract

There are no data on irritating effects after inhalation exposure. In both inhalation studies mentioned before (Skornik and Brain, 1983; Sackner et al., 1981) no details were given whether zinc sulphate was irritating to the respiratory organs.

Eye

In a well performed eye irritation/corrosion study, conducted according to Directive 92/69/EEC B.5 and OECD guideline 405, three male New Zealand White rabbits were treated by instillation of approximately 98.1 mg of zinc sulphate ($ZnSO_4 \cdot 7\ H_2O$) into the conjuctival sac of one eye. The other eye remained untreated and served as control. The eyes (unrinsed) were examined at 1, 24, 48 and 72 hours and 7, 14 and 21 days after instillation.

No symptoms of systemic toxicity were observed and no mortality occurred. Corneal injury was seen as slight dulling of the normal lustre (opacity grade 0) and/or epithelial damage (10% of the corneal area) in two animals. This injury had resolved within 24 hours in one animal and within 72 hours in the other animal. Irritation of the conjuctivae was seen as redness (mean scores over 24-72 hours 2, 2.7 and 2.7), chemosis (mean scores 2, 2.7 and 3.7) and discharge. Yellow/white spots were observed in the tissue of the lower eyelid, nictitating membrane and/or sclera in all animals from day 7 until termination. These spots were described as signs of necrosis and consisted of encapsulated material of unknown origin which caused protrusions at termination of the study. Reduced elasticity of the eyelids was noted in one animal, 72 hours and 7 days after instillation (Van Huygevoort, 1999e). Based on the degree and persistence of the corneal injury, zinc sulphate is considered to cause severe ocular irritation.

Conclusion on irritation

The data available fulfil the base set requirements for irritation testing of zinc sulphate to skin and eyes. Based on the findings in a well performed study according to EU and OECD guidelines, zinc sulphate is considered not irritating/corrosive to the skin and, therefore, does not have to be classified/labelled.

Zinc sulphate is considered to induce severe ocular irritation. According to EU criteria, zinc sulphate should be labelled as R41 (risk of serious damage to eyes).

4.1.2.5 Corrosivity

The substance is not corrosive to the skin, but can induce serious damage to the eyes (see Section 4.1.2.4).

4.1.2.6 Sensitisation

Zinc sulphate ($ZnSO_4 \cdot 7\ H_2O$) was tested in a mouse local lymphnode assay (Ikarashi et al., 1992), according to the validated test strategy developed by Kimber et al. (1989 and 1990). After gentle dermal abrasion, 25 µl of a 5% zinc sulphate solution in 20% ethanol was applied for three consecutive days at the dorsal side of both ears of 3 Balb/c mice. On the fourth day the animals were sacrificed and the ear-draining lymph nodes were collected. Lymph node lymphocyte proliferation was determined by tritiated thymidin incorporation. The results were compared to those of vehicle treated controls. Zinc sulphate did not induce proliferative activity,

whereas for potassium bichromate, nickel sulphate and cobalt chloride (known dermal sensitisers) positive results were obtained.

Remark: according to OECD Guideline 406 (adopted on 17th July 1992) this test is suitable for the detection of moderate to strong sensitisers. When a negative result is found in this test further testing may be mandatory.

Therefore, the skin sensitising potential of zinc sulphate ($ZnSO_4 \cdot 7\ H_2O$) was also investigated in guinea pigs. A well performed maximisation test, conducted according to Directive 96/54/EC B.6 and OECD guideline 406, was carried out in female Dunkin Hartley guinea pigs. Based on the results of a preliminary study, in the main study 10 experimental animals were intradermally injected with a 0.1% concentration and epidermally exposed to a 50% concentration. Five control animals were similarly treated, but with vehicle (water) alone. Approximately 24 hours before the epidermal induction exposure all animals were treated with 10% SDS. Two weeks after the epidermal application all animals were challenged with a 50% test substance concentration and the vehicle. A second challenge followed one week after the first.

In response to the 50% test substance concentration, in some experimental animals and controls skin reactions of grade 1 were observed 48 hours after the first (5/10 and 2/5, respectively) and the second challenge (4/10 and 2/5, respectively). As the skin reactions were comparable among the experimental and control animals, and as there was poor consistency of the skin reactions among individual experimental animals after the first and second challenge, the observed skin reactions can be considered to be non-specific signs of irritation. Hence, it can be concluded that zinc sulphate did not induce hypersensitivity in experimental animals (Van Huygevoort, 1999f).

Conclusion on sensitisation

The data submitted fulfil the base-set requirements for skin sensitisation testing. Zinc sulphate is not considered a skin sensitiser and does not need to be classified/labelled. No data are available on the potential for respiratory sensitisation.

4.1.2.7 Repeated dose toxicity

4.1.2.7.1 Studies in animals

Several data were provided on the repeated dose toxicity of zinc sulphate. Data on other zinc compounds have also been used, as the basic assumption is made that after intake all zinc compounds (including metallic zinc) are changed (at least in part) to the ionic species and that it is this zinc cation that is the determining factor for the biological activities of the zinc compounds (see Section 4.1.2.1).

The section is divided in two subsections. Under “Relevant studies for risk assessment” more or less guideline repeated dose studies were evaluated that allowed the establishment of a N(L)OAEL. The subsection “Additional studies” comprises studies with animals other than standard laboratory animals, special investigations into specific parameters, limitedly reported studies etc.

Relevant studies for risk assessment

See **Table 4.11**.

Table 4.11 Repeated dose toxicity

Repeated-dose toxicity	Species	Protocol	Results	mg Zn^{2+}/ kg bw	Reference
Oral	mouse	other, but comparable with guideline study: 300 to 30,000 mg $ZnSO_4 \cdot 7\ H_2O$ /kg feed daily via diet for 13 weeks	NOAEL 3,000 mg/kg feed At 30,000 mg/kg feed: heamatological and biochemical effects were observed. Gross pathology and histopathology showed changes in kidney, thyroid, gastrointestinal tract and pancreas.	NOAEL: 104 LOAEL: 1,107	Maita et al. (1981)
	rat	other, but comparable with guideline study: 300 to 30,000 mg $ZnSO_4 \cdot 7\ H_2O$/kg feed daily via diet for 13 weeks	NOAEL 3,000 mg/kg feed At 30,000 mg/kg feed: hematologic effects and pancreatic damage.	NOAEL: 53.5 LOAEL: 564	Maita et al. (1981)
	rat	According to OECD 408: up to 1% Zn-mono glycerolate via diet (~ 31.52 to 758.73 mg/kg bw) for 13 weeks	NOAEL 31.52 mg/kg bw At 0.2% (≈ 127.52 mg/kg bw): effects on pancreas, spleen and clinical chemical parameters	NOAEL: 13.26 LOAEL: 53.65	Edwards and Buckley (1995)

Oral exposure

- Zinc sulphate

ICR mice (12/sex/group) were given daily doses of 300, 3,000 or 30,000 mg $ZnSO_4 \cdot 7\ H_2O$/kg feed (equivalent to 42.7/46.4, 458/479 and 4,927/4,878 mg/kg bw for males/females, respectively) during 13 weeks. A control group was included. At the highest dose level 4 males and 1 female were found dead or killed in extremis. Histological findings of these animals revealed impairment of the urinary tract and regressive changes in the exocrine gland of the pancreas. Only the high dose animals showed moderately lower haematocrit (males: from 42% in controls to 29% in high dose animals; females: from 44% in controls to 31% in high dose animals) and haemoglobin concentrations (males and females: 14 to 10 g/dl). The leucocyte counts of high dose males were moderately decreased (lymphocytes 70 to 60%; monocytes 5.3 to 4.9%). Total protein, glucose and cholesterol were reduced and alkaline phosphatase and urea nitrogen were increased in high dose animals. High dose females showed reduced ALAT and increased calcium levels, ASAT was increased in high dose males. Absolute and relative (in parentheses) thyroid weights of males were increased from 3.3 mg (0.007%) in control animals to 4.2 mg (0.0011%) in the highest dose group. Kidney weights of females were also increased from 0.42 g (0.93%) in controls to 0.53 g (1.62%) at the highest dose. Gross pathology and histopathology showed changes in kidneys, thyroids, pancreas (degeneration/necrosis of acinar cells, clarification of nucleoli), gastrointestinal tract, and spleen. No effects were found on the reproductive organs (i.e. ovaries, testes, accessory sex organs). The NOAEL in this study is 458

and 479 mg $ZnSO_4 \cdot 7\ H_2O$/kg bw for males and females, respectively (≈ 104 mg Zn^{2+}/kg bw) (Maita et al., 1981).

Wistar rats (12/sex/group) were given daily doses of 300, 3,000 or 30,000 mg $ZnSO_4 \cdot 7\ H_2O$/kg feed (equivalent to 23.2/24.5, 234/243, and 2,514/2,486 mg/kg bw for males/females, respectively) during 13 weeks. A control group was included. At the highest dose level a moderate reduction in leucocyte counts was seen in both sexes (males: from $7.3 \cdot 10^3$/mm^3 in controls to $4.7 \cdot 10^3$/mm^3 in high dose animals; females: from $4.5 \cdot 10^3$/mm^3 in controls to $3.3 \cdot 10^3$/mm^3 in high dose animals). Compared to controls, males also showed slightly decreased haematocrit (42 to 40%), decreased total protein (5.2 to 4.4 g/dl) and cholesterol values (96 to 62 mg/dl). Absolute and relative (in parentheses) liver weights were decreased in the high dose males (from 16.1 g (3.55%) in controls to 11.9 g (3.20%) at the highest dose). Absolute kidney weights were decreased in high dose males (2.29 g vs. 2.93 g in controls). Histopathology showed pancreatic damage (degeneration, necrosis of acinar cells, clarification of centroacinar cells and interstitial fibrosis) in high dose animals. No effects were found on the reproductive organs (i.e. ovaries, testes, accessory sex organs). The NOAEL is 234 and 243 mg $ZnSO_4 \cdot 7\ H_2O$/kg bw for males and females, respectively (≈ 53.5 mg Zn^{2+}/kg bw) (Maita et al., 1981).

- Zinc monoglycerolate

Groups of 20 male and 20 female Sprague-Dawley rats were fed zinc monoglycerolate at dietary levels of 0, 0.05 or 0.2% (equal to 0, 31.52 or 127.52 mg/kg for males and 0, 35.78 or 145.91 mg/kg bw for females, respectively) for a period of 13 weeks in a study performed according to OECD 408. A similar group was fed 1% (equal to 719 and 805 mg/kg bw/day for males and females, respectively) of zinc monoglycerolate up to day 58 of the study when a deterioration in their clinical condition (poor physical health and reduced food intake) necessitated reducing the dietary level to 0.5% (equal to 632 and 759 mg/kg bw/day for males and females, respectively). However, as no improvement occurred these rats were killed on humane grounds on day 64 of the study. These rats developed hypocupremia manifested as a hypochromic microcytic regenerative type anaemia (low haemoglobin and haematocrit, decreased MCV and MCH, and increased MCHC, red blood cell and reticulocyte count). Enlargement of the mesenteric lymph nodes and slight pitting of the surface of the kidneys were noted. Severe pancreatic degeneration and pathological changes in the spleen, kidneys, incisors, eyes and bones were observed. The testes of all males showed hypoplasia of the seminiferous tubules to a varying degree and in addition the prostate and seminal vesicles showed hypoplasia. In all but one female the uterus was hypoplastic.

All other rats survived to the end of the 13-week treatment. At a dietary level of 0.2% increases in plasma ALAT, alkaline phosphatase and creatine kinase were observed in males and in plasma creatine kinase in females. Total plasma cholesterol was reduced in both males and females. The changes were statistically significant but small in absolute terms. No changes in haematological parameters were seen at 0.05 and 0.2%. A dose related reduction in the quantity of abdominal fat was noted in male rats at 0.05 and 0.2%. Enlargement of the mesenteric lymph nodes was apparent in 6 out of 20 rats fed 0.2% and in one male fed 0.05%. Microscopic examination showed a reduction in the number of trabeculae in the metaphysis of the tibia of 5 male and 3 female rats fed 0.2%, 4 males and 1 female had a similar reduction in the metaphysis of the femur. Pancreatic cell necrosis was seen in both sexes at 0.2% and a slight, but statistically not significant increase could be noted at 0.05% (3 males and 1 female). This pancreatic cell necrosis was seen also in 1 control male. A reduction in the number of pigmentated macrophages in the red pulp of the spleen was observed in both sexes at 0.2% and a marginal reduction was

also seen in males at 0.05%. In the animals given 0.05 and 0.2% no effects were found on the reproductive organs.

Since the pancreatic cell necrosis, being without statistical significance at 0.05%, was also apparent in 1 control male and because the reduction in pigmented macrophages in the spleen was only marginal at 0.05% without any haematological changes the dose level of 0.05%, is considered as a NOAEL. This dose level is equal to 31.52 or 35.78 mg zinc monoglycerolate/kg bw for males and females, respectively, so the NOAEL in this study is 31.52 mg/kg bw (≈ 13.26 mg Zn^{2+}/kg bw) (Edwards and Buckley, 1995).

Inhalation exposure

No proper inhalation toxicity data are available.

Dermal exposure

No dermal toxicity data are available.

Additional studies

Oral exposure

- Zinc sulphate

A group of 150 C3H mice was given daily doses of 0.5 g $ZnSO_4$ (unspecified)/l drinking (≈ 100 mg $ZnSO_4$/kg bw/day; ≈ 22.6 mg Zn^{2+}/kg bw in case heptahydrate was used) water for 1 year. A 2-month post observation period and a control group were included. At monthly intervals 5 control and 5 test animals were investigated for plasma zinc, glucose and insulin, and for zinc in skin, liver and spleen. Histology, histochemistry and microscopy were performed on adrenals and pancreas, and on adenohypophysis only microscopy. The animals remained healthy throughout the study. Hypertrophy of the adrenal glands (cellular enlargement) and hypertrophy and vacuolisation of pancreatic islets and fasciculata cells in adrenal cortex from month 3 onwards. Changes indicating hyperactivity in the anterior pituitary were noted, such as increased cell size of all cell types in the pituitary. All the other parameters remained the same during the study. The study was undertaken to further investigate the effects of supplemental zinc on endocrine glands and correlate these effects with any change in body zinc levels produced (Aughey et al., 1977).

Mink (3/sex/group) were given diets supplemented with 0, 500, 1,000 or 1,500 mg/kg feed zinc sulphate for 144 days. Zinc concentrations in liver, pancreas and kidney increased with increasing zinc content in the diet. No histological lesions were found in these organs (Aulerich et al., 1991*(r)*).

- Zinc chloride

Wistar rats (2 months, 16 males and 14 females) were given 0.12 mg Zn^{2+}/ml drinking water (equivalent to 12 mg Zn^{2+}/kg bw; 25 mg $ZnCl_2$/kg bw) for 4 consecutive weeks. A control group was included. The body weights of exposed males and food and water intakes of both exposed sexes decreased. A statistically significant decrease in Hb level (85% of control value) and erythrocyte count was reported in the peripheral blood of treated rats. An increased leucocyte count, due to increased lymphocyte numbers was noted in treated males. No inhibition of erythropoiesis in the bone marrow was found. No more details were given in this limited study

performed to investigate the effect of simultaneous oral administration of zinc and vanadium and therefore it cannot be used for risk assessment (Zaporowska and Wasilewski, 1992).

- Zinc oxide

Special studies were conducted to examine the morphological and histoenzymatic changes of the brain. Twelve Wistar rats were given daily doses of 100 mg ZnO (ca. 600 mg ZnO/kg bw ≈ 480 mg Zn^{2+}/kg bw) intragastrically for 10 consecutive days. A control group was included. After 10 days the rats were sacrificed and the brains were examined for morphological and histoenzymatic changes.

Morphological changes included degenerative changes of neurocytes, accompanied with moderate proliferation of the oligodendroglia and glial proliferation in the white matter. Furthermore endothelial oedema was observed in the small arterial and capillary walls. Histoenzymatic changes included decreased activities of ACP (acid phosphatase), ATPase (adenosinetriphosphatase), AChE (acetylcholine esterase), and BChE (butyrylthiocholine esterase). The activities of TPPase (thiamine pyrophosphatase) and NSE (non specific esterase) were increased. No details on quantitative aspects of enzymatic changes were given. No change was seen in the alkaline phosphatase. The authors indicated that observed morphological and histoenzymatic changes were unspecific, indistinctive and most likely reversible (Kozik et al., 1980). Examination of the neurosecretory function of the hypothalamus and the hypophysis in these animals showed an increased neurosecretion in cells of the supraoptic and paraventricular nucleus of the hypothalamus along with a declined neurosecretion in the hypophysis and an enhanced release of antidiuretic hormone in the neurohypophysis (Kozik et al., 1981). It is not clear whether these observations represent an adverse effect of zinc on the brain or whether they are secondary to changes somewhere else in the body.

Four groups of ferrets (3-5/group) were given 0, 500, 1,500 or 3,000 mg zinc oxide/kg feed (equivalent to be 0, 81.3, 243.8 or 487.5 mg ZnO/kg bw, respectively. At the highest dose level (487.5 mg ZnO/kg bw) all animals (3) were killed in extremis within 13 days. Macroscopic examination showed pale mucous membranes, dark coloured fluid in the stomach, blood in the intestines, orange coloured liver and enlarged kidneys showing diffuse necrosis, haemorrhages in the intestine and a severe macrocytic hypochromic anaemia. Histology showed nephrosis and extramedullary haematopoesis in the spleen. At the mid dose level of 243.8 mg ZnO/kg bw the animals (4) were killed on day 7, 14 and 21 (1/2 in extremis) showing poor condition. Macroscopy showed pale livers with fatty infiltration and enlarged kidneys. Histology was comparable with the highest dose group. The haemogram showed macrocytic hypochromic anaemia, increased reticulocytes and leucocytosis.

At the lowest dose level (81.3 mg ZnO/kg bw) the animals (3) were killed on day 48, 138 and 191, respectively. No clinical signs of toxicity or pathological changes were seen, apart from an extramedullary heamatopoesis in the spleen (Straube et al., 1980).

Ellis et al. (1984) conducted a 14-day and a 49-day feeding study in 3 different breeds of sheep that were receiving feed containing 31 mg Zn^{2+}/kg feed. The sheep received additional amounts of Zn^{2+} (from ZnO) at dose levels of 261 and 731 (14 day study) or 731 and 1,431 mg Zn^{2+}/kg feed (49-day study). No effects were seen after 261 mg Zn^{2+}/kg feed. In all other groups pancreatic lesions were seen.

Administration of 240 mg Zinc (as ZnO)/kg bw for 3 times/week during 4 weeks to 42 castrated sheep resulted in an increased incidence of pancreatic lesions (Smith and Embling, 1993*(r)*).

Inhalation exposure

- Zinc oxide

Male Hartley guinea pigs were exposed to 0, 2.3, 5.9 or 12.1 mg/m^3 of ZnO (as ultrafine particles with an average diameter of 0.05 μm) 3 hours a day for 1, 2 or 3 consecutive nose only exposures. Three animals from each group were examined after each exposure period, were sacrificed and lung tissues were microscopically examined, and the pulmonary lavage fluid was also examined.

Exposure to 12.1 mg/m^3 increased the number of nucleated cells in lavage fluid. Exposures to 5.9 and 12.1 mg ZnO/m^3 were associated with increased protein, neutrophils, and activities beta-glucuronidase, acid phosphatase, alkaline phosphatase, lactate dehydrogenase, and angiotensin-converting enzyme. The increases were dose dependent and were detectable after the second exposure, and generally increased after the third exposure. Significant morphologic damage characterized by centriacinar inflammation in the lung was seen at 5.9 and 12.1 mg/m^3. Minimal changes in neutrophils and activities of lactate dehydrogenase and alkaline phosphatase were seen in the pulmonary fluid at the lowest dose level of 2.3 mg/m^3 after 3 exposures but no morphologic changes were observed at this dose level. Based on these results 2.3 mg ZnO/m^3 is considered as a marginal LOAEL in this study (Conner et al., 1988).

Male Hartley guinea pigs were exposed to 6 mg/m^3 of ultrafine ZnO (average diameter of 0.05 μm) for 3 hours a day for 1 to 5 days by nose-only exposure. A control group was included. After each exposure 3 animals were sacrificed and lung tissues were microscopically examined. After first, second and third exposure 3 additional animals were sacrificed and their pulmonary lavage fluid was examined. ZnO-exposure increased the total cell count, neutrophils, protein and the enzyme activities of angiotensin converting enzymes, Acid phosphatase, alkaline phosphatase, and β-glucoronidase. Furthermore a dose related centriacinar inflammation was seen after second exposure (Conner et al., 1986).

Male Hartley guinea pigs were exposed to 0, 2.7 or 7 mg ultrafine (0.05 μm in diameter) ZnO/m^3 3 hours a day for 5 days. Lung function measurements were performed every day after exposure in 5-8 animals. After the last exposure the animals were sacrificed. At the highest exposure level a gradual decrease in total lung capacity (18%) and vital capacity (22%) was seen during the exposure period. At day 4 the carbon monoxide diffusing capacity dropped to below 30% of the control level. Wet-lung weights were increased with 29%, indicating the presence of edema. Exposures up to 2.7 mg ZnO/m^3 did not alter any parameters measured (Lam et al., 1988).

Male Hartley guinea pigs (73) were exposed (nose only) 3 hours a day for 6 days to 5 mg ZnO/m^3 (0.05 μm in diameter). A group of 53 animals served as control group. Lung function tests (in 38 animals) were performed and the respiratory tract of the animals was morphologically examined 1, 24, 48 and 72 hours after the last exposure. Furthermore epithelial permeability (5 animals at 1 and 24 hours) and DNA synthesis in epithelial cells (5 animals at 24, 48 and 72 hours) were determined.

Vital and functional residual capacity, alveolar volume and carbon monoxide diffusing capacity were all decreased and did not return to normal values 72 hours after the last exposure. Lung weights were elevated due to inflammation, still present at 72 hours after last exposure (Lam et al., 1985).

240 Female Wistar rats (80/group) were exposed by inhalation to 15 mg ZnO/m^3 for 1 hour, 4 hours or 8 hours a day for 5 days a week. 20 Animals/group were sacrificed after 14, 28, 56, and 84 days and their lungs were examined for zinc content.

It appeared that the highest daily exposure time resulted in the highest dry lung weights, independent of the duration of the experiment, while the zinc content remained almost constant. The absolute and relative (relative to dried weights of lung tissue) zinc content in the lungs was influenced by the duration of the experiment. After 84 days exposure the zinc content was significantly higher compared to 14 days exposure, independent of the duration of the daily exposure (Dinslage-Schlünz and Rosmanith, 1976).

4.1.2.7.2 Studies in humans

All relevant oral human data concerning metallic zinc and zinc compounds are reported in this section.

Dietary levels were not measured in all of the studies reported here. According to a Total Diet Study performed by the US Food and Drug Administration (FDA) over the period 1982 to 1986, adult males (25-35 years of age) consumed an average of 16.4 mg Zn^{2+}/day. Adult females (25-30 years of age) consumed an average of 9.72 mg Zn^{2+}/day (Pennington, 1989).

Zinc sulphate

In a double blind cross-over trial 47 healthy volunteers (26 females and 21 men) ingested zinc sulphate capsules containing 220 mg zinc sulphate, three times a day with each meal (resulting in a total daily dose of 150 mg Zn^{2+} i.e. ≈ 2.1 and 2.5 mg Zn^{2+}/kg bw /day for males and females, respectively) for six weeks. Plasma zinc and copper levels, plasma cholesterol, plasma low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, serum ceruloplasmin and erythrocyte superoxide dismutase (ESOD) were determined. In 84% of the women and 18% of the men symptoms were reported which included headaches, nausea, vomiting, loss of appetite and abdominal cramps. The study authors reported that the gastric discomfort went together with lower body weights and taking the capsules with small meals (breakfast or morning tea) or no food. Plasma zinc levels rose significantly in both men and women (36% and 57%, respectively). Plasma copper levels did not change significantly. Total plasma cholesterol and HDL were unchanged in both sexes. In females the LDL cholesterol decreased significantly from 2.38 to 2.17 mmol/l. In females a decrease was also found in serum ceruloplasmin (13% reduction) and in ESOD (ca.20% reduction) (Samman and Roberts, 1987, 1988).

Hooper et al. (1980) examined the effect of oral zinc administration on human lipoprotein values. Twelve healthy adult men were given oral doses of 440 mg zinc sulphate/day (≈ 2.3 mg Zn^{2+}/kg bw/day in the form of two zinc sulphate capsules containing 220 mg zinc sulphate (80 mg elemental zinc per capsule resulting in a total daily dose of 160 mg Zn^{2+}), each capsule to be eaten with a main meal for 35 days. Fasting lipid levels were determined on a weekly basis and continued two weeks after zinc supplementation stopped, with a final determination at 16 weeks after start of the experiment. HDL cholesterol levels were decreased by 25% at the 7^{th} week, but had returned to baseline levels at 16 weeks. Total serum cholesterol, triglyceride and LDL cholesterol levels were not changed.

Remark: there is a discrepancy between the dosimetric data in the Samman and Roberts study (1987/1988) as compared to the Hooper et al. study (1980). In the first study, a daily dose of 660 mg zinc sulphate was declared to be equivalent to a dose of 150 mg Zn^{2+} per day, while in

the second study a daily dose of 440 mg zinc sulphate was stated to have resulted in a daily dose of 160 mg Zn^{2+}. This discrepancy can only be explained by assuming that in the Samman and Roberts study zinc sulphate was administered in the form of the heptahydrate, while in the Hooper et al. study the monohydrate has been used. As this is not clearly stated in either of the two studies, the dosimetric data which are presented here are the same as those given in the respective publications.

Chandra (1984) examined the effect of zinc on immune response and serum lipoproteins. Zinc sulphate was administered twice daily to 11 adult men for a total (extra) intake of 300 mg elemental zinc/day (≈ 4.3 mg Zn^{2+}/kg bw/day). Dietary zinc intake amounted to ca. 11 mg/person/day. None of the subjects showed evidence of any untoward side effects. There was a significant increase in serum zinc levels and reduction in lymphocyte stimulating response to PHA after 4 and 6 weeks of treatment. A slight increase in LDL was observed together with a significant reduced level of HDL cholesterol.

In two studies the side effects of zinc administration as a medication in the treatment chronic leg ulcers was investigated:

- in a double-blind trial, 13 humans received 200 mg zinc sulphate (± 135 mg Zn^{2+}) three times a day for 18 weeks, while 14 humans received a placebo. No signs of nephrotoxicity associated with the zinc treatment were reported, but the study was not sufficiently documented to fully appreciate the relevance of its result (Hallbook and Lanner, 1972),
- in a study of Greaves and Skillen (1970) no indications for haematotoxicity, hepatotoxicity or nephrotoxicity, as determined by several clinical biochemical and haematological parameters, were seen in 18 humans after administration of 220 mg zinc sulphate (± 150 mg Zn^{2+}) 3 times a day for 16-26 weeks.

Zinc gluconate

In a 12-week double blind study Black et al. (1988) administered zinc gluconate tablets to 2 groups of healthy male volunteers for 12 weeks at doses equivalent to 50 or 75 mg zinc/kg bw/day (≈ 0.71 and 1.1 mg Zn^{2+}/kg bw/day). A control group received a placebo tablet. No changes in serum cholesterol, triglyceride, and LDL and very-low-density-lipoprotein (VLDL) cholesterol levels were observed.

In a 10-week single-blind oral study by Yadrick et al. (1989) 9 healthy female volunteers were given 50 mg Zn^{2+} (as zinc gluconate)/day (≈ 0.83 mg Zn^{2+}/kg bw/day) and 9 other healthy female volunteers were given 50 mg Zn^{2+} (as zinc gluconate)/day plus 50 mg Fe^{2+} (as ferrous sulphate monohydrate) in two daily doses via their diet to investigate the effect of zinc supplementation on iron, copper and zinc status. The subjects (assumed mean body weight of 60 kg) served as their own controls. In both groups the erythrocyte superoxide dismutase (ESOD) activity was significantly reduced with 47% after 10 weeks. In the zinc supplemented group, after 10 weeks significant decreases in haematocrit (by 4%) and serum ferritin levels (with 23%) were seen, whereas the haemoglobin levels were unchanged. In the zinc + iron supplemented group, serum ferritin levels were significantly increased (by 25%), whereas the haematocrit and haemoglobin levels were unchanged. The ceruloplasmin concentration, another indicator for copper status besides ESOD, was not altered in both groups, but the serum zinc concentration was significantly increased. The NOAEL in this study is less than 0.83 mg Zn^{2+}/kg bw.

A significant decrease of 15% in ESOD activity was reported by Fischer et al. (1984) who administered 50 mg Zn^{2+} (as zinc gluconate)/day (≈ 0.71 mg Zn^{2+} /kg bw) divided in two daily

doses to 13 healthy young men (assumed mean body weight of 70 kg) for 6 weeks in a double blind study design. The other two indices of copper status, i.e. ceruloplasmin activity and plasma copper levels were not changed compared to the controls at 2, 4 or 6 weeks, but the serum zinc levels were significantly increased from 2 weeks of supplementation onwards. Serum zinc showed a significant inverse correlation with ESOD activity at 6 weeks.

The study of Yadrick et al. (1989) as well as the study of Fischer et al. (1984) showed several limitations such as:

- the short duration of the studies and the small number of subjects,
- the absence of a placebo-controlled group in the Yadrick study. However, all subjects served as their own controls,
- the lack of information on the dietary levels of zinc (and iron and copper); the diets were not controlled,
- the absence of physical or medical examination.

Over the course of the past several years, industry has been sponsoring a series of human volunteer studies in conjunction with the Grand Forks Human Nutrition Research Center of the US Department of Agriculture. These studies, recently completed, have been evaluating impacts of moderate zinc deficiency and moderate zinc excess as a function of intake levels for mineral nutrients such as copper. This because extremely high amounts of zinc have been shown to interfere with the uptake and metabolism of copper, and it was questioned if moderately high intakes of zinc would also be antagonistic to copper metabolism. The studies are anticipated to demonstrate the fashion in which subtle biochemical alterations associated with zinc deficiency and excess will vary as a function of copper status, and to evaluate exposure biomarkers with potential applications for monitoring zinc status. The results of two of these studies are now available for public circulation (see studies by Davis et al. and Milne et al. below).

In a controlled metabolic-unit study by Davis et al. (2000), various indicators of zinc status were measured in 25 healthy postmenopausal women (mean age 64.9 years) to evaluate the usefulness of these indicators as a marker for the functional assessment of zinc status in humans. The subjects were kept under close supervision for 200 days, divided into two 90-day dietary periods, each preceded by a 10-day equilibration period. The subjects received a daily diet with a total energy content of 8.4 MJ (or 2,000 kcal). In the equilibration periods the subjects received a diet containing 2 mg copper/day and 9 mg zinc/day. For the 90-day dietary periods the subjects were randomly divided into two groups, one group (n = 12) was fed a low copper diet (1 mg Cu/day) and the other group (n = 13) a high copper diet (3 mg Cu/day). In the first 90-day dietary period both groups received no zinc supplement (low zinc; 3 mg Zn/day), while in the second 90-day dietary period both groups received a zinc supplement of 50 mg per day (high zinc; 53 mg Zn/day). Zinc was supplemented as zinc gluconate and copper as cupric sulphate. Blood samples were taken (after overnight fasting for 12 hours) during each of the equilibration periods and one to twice monthly during the dietary periods, and analysed for various zinc-status indicators.

Zinc concentrations in erythrocytes and erythrocyte membranes, plasma and erythrocyte membrane alkaline phosphatase activities, and erythrocyte membrane 5'nucleotidase activity did not change statistically significantly with the different dietary treatments.

Zinc supplementation significantly increased plasma zinc concentrations and activities of mononuclear 5'nucleotidase and extracellular superoxide dismutase ($P < 0.0001$). For all three indicators the effect of zinc supplementation was dependent on the copper intake although this

was not statistically significant for plasma zinc. In case of mononuclear 5'nucleotidase activity, the difference caused by zinc supplementation was apparent when subjects were fed high dietary copper (92% change) but not when they were fed low dietary copper (5% change). The effects for plasma zinc and for extracellular superoxide dismutase activity were more apparent when subjects were fed low dietary copper (35 vs. 22% and 21 vs. 8% change, respectively). Independent of copper intake, zinc supplementation caused relatively small increases in free thyroxine (7-8%) and triiodothyronine (7-9%) concentrations, platelet zinc concentrations (10-13%) and bone specific alkaline phosphatase activity (18%) ($0.002 < P < 0.08$). The levels of the affected indicators were elevated from the equilibration values at all dietary treatments, with the exception of extracellular superoxide dismutase activity at low copper/low zinc, mononuclear 5'nucleotidase activity at low copper/low zinc, low copper/high zinc and high copper/low zinc, and thyroxine and triiodothyronine concentrations at all dietary treatments. Plasma zinc concentrations were within the normal range for healthy adults (10.7-18.4 μmol/L) throughout the low zinc period, but during zinc supplementation 8 out of 23 subjects had plasma zinc concentrations > 18.4 μmol/L.

Decreased activities upon zinc supplementation were found for plasma 5'nucleotidase activity ($P < 0.0001$), thyroid stimulating hormone concentrations ($P < 0.07$) and erythrocyte superoxide dismutase activity (ESOD; not statistically significant). For these three indicators the decrease was somewhat more apparent when fed high dietary copper (28 vs. 29%, 5 vs. 9%, and 3 vs. 5%, respectively). However, for plasma 5'nucleotidase and ESOD the levels at high dietary copper were higher than at low dietary copper (only at high copper/low zinc the levels were elevated from equilibration values). For thyroid stimulating hormone the levels were depressed from equilibration values at all dietary treatments. Limited data suggested that zinc supplementation in combination with low dietary copper depresses amyloid precursor protein expression in platelets (Davis et al., 2000).

Remark: Data from two volunteers fed low copper diets were not included: they had to be supplemented with dietary copper because of significant changes in their electrocardiograms.

In the same dietary experiment as described by Davis et al. (2000; see above), also other parameters (i.e. copper status and iron status indicators) were investigated to study the effect of moderately excessive and deficient intakes of zinc on copper metabolism and utilization in humans fed low and luxuriant amounts of copper (Milne et al., 2001). For that purpose, urine and faeces were collected during the last 78 days of each 90-day dietary period and copper and zinc were determined (in faeces in 6-day composite samples). Once weekly blood was sampled (after overnight fasting for 12 hours), and blood samples were analysed for various copper-status and iron-status indicators.

Women fed low copper were in negative copper balance. Zinc intake (low or high) did not alter this. Women fed high copper were put into negative copper balance by low zinc. Upon transition to high zinc, women fed high copper came into positive copper balance, which apparently was the result of a lower amount of dietary copper lost in the faeces; urinary copper was not affected.

The zinc balance reflected dietary zinc intake (more positive with increased zinc intake) and was not significantly affected by copper intake.

Copper status indicators were variably affected by dietary treatment. The concentrations of serum ceruloplasmin (enzymatically determined), HDL and VLDL cholesterol, triglycerides and red blood cell zinc did not change statistically significantly with the different dietary treatments.

Independent of zinc intake, plasma copper concentrations were significantly lower on low dietary copper than on high dietary copper ($P < 0.07$). Although plasma copper concentrations were depressed from equilibration values at all dietary treatments, the depression was less for high than for low dietary copper ($P < 0.03$).

Independent of copper intake, zinc supplementation caused increases in the concentrations of serum ceruloplasmin (immunochemically determined; 4-8%, $P < 0.05$) and plasma zinc (19-32%, $P < 0.0001$) and in platelet cytochrome c oxidase activity (on a platelet number basis; 19-27%, $P < 0.0007$), and decreases in the concentrations of red blood cell copper (8-16%, $P < 0.0008$) and whole blood glutathione (8-12%, $P < 0.009$) and in the activities of specific ceruloplasmin (defined as the ratio between enzymatic and immunoreactive ceruloplasmin; 8-11%, $P < 0.0003$) and erythrocyte glutathione peroxidase (11-15%, $P < 0.002$). The levels of these indicators were elevated from equilibration values at all dietary treatments, with the exception of serum immunoreactive ceruloplasmin concentration (reduced at all dietary treatments), platelet cytochrome c oxidase activity (reduced at high copper/low zinc), specific ceruloplasmin activity and whole blood glutathione concentration (essentially at equilibration values at low copper/high zinc and high copper/high zinc), and red blood cell copper concentration (essentially at equilibration value at low copper/low zinc and reduced at low copper/high zinc).

Zinc supplementation significantly decreased ESOD activity (5-7%, $P < 0.03$) as well as the concentrations of total cholesterol (3-4%, $P < 0.005$) and LDL cholesterol (2-6%, $P < 0.003$), but not by much. The effect on ESOD was dependent on copper intake ($P < 0.0001$): compared to equilibration values, ESOD activity decreased on low copper but increased on high copper. Total cholesterol and LDL cholesterol concentrations were significantly higher on low dietary copper than on high dietary copper ($P < 0.02$ and $P < 0.03$, respectively). This suggests a dependency on copper intake, but it should be noted that women fed low copper had higher equilibration values for both indicators than women fed high copper.

The authors state that measured indicators of iron status (serum iron, haemoglobin, haematocrit and percent transferrin saturation) were unaffected by dietary treatment (no data presented), with the exception of haemoglobin, which was lower on high zinc than on low zinc in both the low and high copper groups. The drop in haemoglobin occurred especially during the last month of zinc supplementation, possibly due to the frequent blood sampling.

Remark: Data from another two volunteers (one on a low copper diet and one on a high copper diet) were not included, because they were using an adhesive containing extremely high amounts of zinc for their false teeth.

Remarks on the Grand Forks study, reported by Davis et al. (2000) and Milne et al. (2001):

1. From personal communication with the authors it appears that for ESOD activity the initial equilibration values varied markedly between individuals, and that for women who were assigned to the low copper group ESOD activity was substantially higher than for those assigned to the high copper group. This implicates that for this indicator, the assignment of the subjects to the two groups was suboptimal, which might also be the case for other indicators.

2. The frequent blood sampling (an average of no more than 235 ml per month was drawn) might have compromised the physiology of the subjects (as was suggested for haemoglobin).

3. The subjects served as their own controls: values upon both treatments (i.e. low and high zinc administration) were compared with values upon first equilibration. However, as the second treatment is not independent of the first treatment, the study design is not optimal.

In the human studies described above, the effects of high or moderately high dietary zinc on several indicators known to be associated with copper status have been investigated. These indicators included plasma zinc and copper concentrations, cholesterol and lipoprotein cholesterol concentrations, and several enzym activities (e.g. ESOD and ceruloplasmin). Effects of zinc on the latter are thought to precede changes in plasma and tissue levels of the elements, given the primary role of zinc as a component of different enzymes. In humans supplemented with zinc, plasma zinc concentration was elevated, while plasma copper concentration was not affected. In the earlier studies by Samman and Roberts (1987/1988), Yadrick et al. (1989) and Fischer et al. (1984) reductions in ESOD activity were found upon zinc supplementation. This was thought to be associated with copper deficiency, as was the reduction in ceruloplasmin activity found by Samman and Roberts (1987/1988). In the more recent and more sophisticated studies by Davis et al. (2000) and Milne et al. (2001), however, only very small reductions in ESOD activity were observed that did not correlate with changes in copper balance. The clinical significance of this ESOD reduction can be doubted, because the findings in these studies on more specific copper deprivation signs (decreased serum ceruloplasmin and platelet cytochrome c oxidase) indicate that sub-optimal intake of zinc was more effective than a moderately high intake of zinc in inducing changes associated with a decreased copper status in postmenopausal women. It might also be that the small decrease in ESOD activity with high zinc intake was not caused by an interference with copper metabolism, but was more reflective of reduced oxidative stress given the serum glutathione and erythrocyte glutathione peroxidase findings. However, one can only conclude from the Grand Forks studies (Davis et al., 2000; Milne et al., 2001) that very subtle changes were induced by the different dietary treatments.

From various studies (e.g. Fischer et al., 1990; Barnett and King, 1995; Verhagen et al., 1996 and Puscas et al., 1999) it can be concluded that ESOD activities in healthy human volunteers may show a coefficient of variation of at least 10 to 20%. Although it is impossible to compare the absolute ESOD activities as reported by these authors to those from the Grand Forks studies, due to methodological differences, the relative changes in activities as reported by Davis et al. (2000) and Milne et al. (2001) can be compared to the coefficient of variation of ESOD activity, showing that the changes found in the Grand Forks studies are within the range of natural variation. In addition, Fischer et al. (1990) have demonstrated that in a large group of male and female human volunteers of different ages, ceruloplasmin and serum copper levels were highly correlated, but that no correlation between serum copper concentration and ESOD could be established. ESOD activity was independent of sex, age, pre-post menopausal status, estrogen use (including that in post-menopausal women), smoking or drinking habits, or level of physical exercise.

The general function of ESOD, also within red blood cells, is to catalyze the dismutation of superoxide anion radicals to hydrogen peroxide and oxygen, thus preventing damage of cell constituents and structures by this radical intermediate generated during the oxygen transport function. Concentrations of superoxide anion radicals are in the order of 0.01–0.001 nmol/l under non-pathological conditions. Hydrogen peroxide, on the other hand, is destroyed by catalase being present in high amounts within erythrocytes resulting in concentrations between 1 and 100 nmol/l. According to our knowledge there are only few measured data available showing a direct relationship between changes of intracellular concentrations of free radicals and tissue damage.

Assuming that there is a considerable reduction of the ESOD activity then higher concentration of superoxide radical anions should occur in red blood cells which may lead to destructive effects. Such effects should be detectable, e.g. by changes in haematological parameters (e.g. increased hemolysis, decreased number of erythrocytes, increase in reticulocytes). However, such findings have not been observed in any study. In the Grand Forks studies (Milne et al., 2001) hematocrit, serum iron, and transferrin saturation were unaffected by a dose of 50 mg Zn^{2+}/day leading to a 3-7% reduction of ESOD activity. Yadrick et al. (1989) reported a 47% decrease of ESOD activity after giving 50 mg Zn^{2+}/day over 10 weeks. However, this decrease of ESOD is accompanied by a small decrease in hematocit value.

The subtle changes in clinical-biochemical parameters, as reported in the Grand Forks studies, are hardly indicative for zinc-induced perturbations of the copper homeostasis. These biochemical changes do not lead to detectable deterioration of red blood cell functioning. Therefore, these changes are also of marginal biological significance, if any. Hence, it is concluded that in women supplemented with zinc, a dose of 50 mg Zn^{2+}/day is a NOAEL.

4.1.2.7.3 Conclusion on repeated dose toxicity

Several data were provided on the repeated dose toxicity of zinc sulphate. Data on other zinc compounds have also been used, based on the assumption that after intake the biological activities of the zinc compounds are determined by the zinc cation.

Studies in animals

No repeated dose toxicity studies after dermal exposure are available in animals.

After inhalation exposure mainly studies of short duration (3-6 days) are available. In a 3-day inhalation study with guinea pigs a concentration of 2.3 mg ultrafine ZnO/m^3 (3 hours/day) was a marginal LOAEL, showing changes in neutrophils and activities of lactate dehydrogenase and alkaline phosphatase in the pulmonary fluid. At higher concentrations increased protein concentration, neutrophils, and enzyme activities in lung lavage fluids were seen, together with significant centriacinar inflammation of the pulmonary tissue. A dose of 2.7 mg ultrafine ZnO/m^3 (3 hours/day for 5 days) did not alter the lung function parameters in guinea pigs but at 7 mg ultrafine ZnO/m^3 (3 hours/day for 5 days) or at 5 mg ultrafine ZnO/m^3 (3 hours/day for 6 days) a gradual decrease in total lung capacity, vital capacity and reduction of the carbon monoxide diffusing capacity were seen in combination with inflammatory changes and edema. The relevance of the findings in studies with ultra-fine zinc oxide fumes is unclear with respect to commercial grade zinc oxide, as the latter is of much larger particle size and can have different toxicological characteristics.

In two oral 13-week studies with zinc sulphate (one with rats and one with mice) and an oral 13-week study with zinc monoglycerolate in rats, the lowest oral NOAEL was found in the study with zinc monoglycerolate. This overall NOAEL is 31.52 mg zinc monoglycerolate/kg bw (≈ 13.26 mg Zn^{2+}/kg bw). At higher doses the most important effects the rats developed were hypocupremia, and significant changes in the pancreas (focal acinar degeneration and necrosis) and the spleen (decreased number of pigmented macrophages). It should be noted that in the studies with zinc sulphate mice and rats could be maintained up to 13 weeks on a diet containing 30,000 mg $ZnSO_4 \cdot 7\ H_2O$/kg feed (equivalent to 6,794 mg Zn^{2+}/kg feed), while in the 13-week study with zinc monoglycerolate with rats 1.0% zinc monoglycerolate in the diet (equivalent to

4,420 mg Zn^{2+}/kg feed) was so detrimental that animals had to be killed on humane grounds after 9 weeks.

Studies in humans

Upon supplementing men and women with 150 mg Zn^{2+}/day (as zinc sulphate capsules), women appeared to be more sensitive than men to the effects of high zinc intake: clinical signs such as headache, nausea and gastric discomfort were more frequent among women, and women but not men had decreased activities of serum ceruloplasmin and ESOD. In some earlier oral studies in which humans were supplemented with moderately high amounts of zinc (50 mg Zn^{2+}/day), a reduction in ESOD activity was also observed and again women appeared to be more sensitive to this effect. Hence, a reduction in ESOD was thought to be a sensitive indicator of copper status. However, in more recent and more sophisticated studies using the same dose level, ESOD was only marginally reduced (without a correlation with changes in copper balance), while findings on more specific copper deprivation signs (decreased serum ceruloplasmin and platelet cytochrome c oxidase) indicated that a sub-optimal intake of zinc was more effective than a moderately high intake of zinc in inducing changes associated with a decreased copper status in postmenopausal women. Given this, and degree of the observed ESOD reduction in comparison to the natural variability in its activity, the zinc-induced decrease in ESOD activity is considered to have marginal biological significance, if any, also because it may not have been caused by an interference with copper metabolism.

Overall, it is concluded from studies in which humans were supplemented with zinc (as zinc gluconate), that women are more sensitive to the effects of high zinc intake and that a dose of 50 mg Zn^{2+}/day is a NOAEL. At the LOAEL of 150 mg Zn^{2+}/day, clinical signs and indications for disturbance of copper homeostasis have been observed. The human oral NOAEL of 50 mg Zn^{2+}/day (0.83 mg/kg bw/day) will be taken across to the risk characterisation.

4.1.2.8 Mutagenicity

Several *in vitro* and *in vivo* studies were provided on the genotoxicity of zinc sulphate. Data on other zinc compounds have also been used, as the basic assumption is made that after intake all zinc compounds (including metallic zinc) are changed (at least in part) to the ionic species and that it is this zinc cation that is the determining factor for the biological activities of the zinc compounds (see Section 4.1.2.1).

The tests that are considered useful for the assessment of the genotoxicity of Zn^{2+} are summarised in **Table 4.12**.

Table 4.12 Genotoxicity data

Genetic toxicity	Species	Protocol	Results	Form	Reference
***In vitro* studies**					
Bacterial test (gene mutation)	S. typhimurium (4 strains)	Ames test; 1,000–5,000 µg/plate	negative	oxide	Crebelli et al. (1985) *
Bacterial test (gene mutation)	S. typhimurium (3 strains)	Ames test	negative	oxide	Litton Bionetics (1976) *
Bacterial test (gene mutation)	S. typhimurium (5 strains)	Ames test: with and without m.a.; 5 doses, up to 3,600 µg/plate	negative	sulphate	Gocke et al. (1981)
Bacterial test (gene mutation)	S. typhimurium (1 strain)	other: without m.a.; up to 3,000 nM/plate	negative	sulphate	Marzin and Vo Phi (1985) *
Bacterial test (gene mutation)	S. typhimurium (4 strains)	unknown	negative	chloride	Kada et al. (1980)*(r)*
Bacterial test (gene mutation)	S. typhimurium	Ames test: with and without m.a.	negative	distearate	Litton bionetics (1977)*(r)*
Bacterial test (gene mutation)	S. typhimurium (4 strains)	according to OECD guideline No. 471; 50-5,000 µg/plate; no toxicity up to 5,000 µg/plate	negative	mono-glycerolate	Jones and Gant (1994) **
Bacterial reverse mutation test	E. coli (strain WP2s (λ))	other: induction of λ prophage (adaptation of McCarroll et al. 1981); conc. 3,200 µmol/l; m.a. unknown	ambiguous (two-fold increase of λ prophage induction)	chloride	Rossman et al. (1984)
Eukaryotic assay (gene mutation)	S. cerevisiae (1 strain)	other: without m.a.; single concentration (0.1 mol/l) screening assay	weakly positive (no details given)	sulphate	Singh (1983) *
Eukaryotic assay (gene mutation)	S. cerevisiae (1 strain)	unknown: m.a. unknown; 1,000 and 5,000 ppm	negative	sulphate	Siebert et al. (1970) *
Eukaryotic assay (gene mutation)	S. cerevisiae	unknown	negative	distearate	Litton Bionetics (1977)*(r)*
Eukaryotic assay (gene mutation)	mouse lymphoma cells	unknown: with and without m.a.	positive	oxide	Cameron (1991)(*r*)
Eukaryotic assay (gene mutation)	mouse lymphoma cells	according to OECD guideline No. 476; without m.a. 1-15 µg/ml (toxic at 15 µg/ml) with m.a. 1-30 µg/ml (toxic at 30 µg/ml)	positive: without m.a. from 10 µg/ml with m.a. from 15 µg/ml	mono-glycerolate	Adams and Kirkpatrick (1994) **
Eukaryotic assay (gene mutation)	mouse lymphoma cells	unknown: without m.a.	negative	chloride	Amacher and Paillet (1980)*(r)*
Cytogenetic assay (SCE's)	Syrian hamster embryo cells	unknown; m.a. unknown	ambiguous	oxide	Suzuki (1987) *
Cytogenetic assay	human embryonic lung cells:WI-38	unknown: without m.a.; 0.1, 1.0 and 10 µg/plate	negative	sulphate	Litton Bionetics (1974) *

Table 4.12 continued overleaf

Table 4.12 continued Genotoxicity data

Genetic toxicity	Species	Protocol	Results	Form	Reference
***In vitro* studies** (continued)					
Cytogenetic assay (chromosomal aberrations)	human lymphocytes	other: m.a. unknown; 0, 30 and 300 µM (3mM toxic)	ambiguous	chloride	Deknudt and Deminatti (1978) *
Cytogenetic assay (chromosomal aberrations)	human lymphocytes	according to OECD guideline No. 473; cytotoxicity at 40 µg/ml (MI 51%), con. tested: without m.a. 5–20µg/ml, with m.a. 10–40 µg/ml	positive in the presence of m.a. at 30 and 40 µg/ml	mono-glycerolate	Akhurst and Kitching (1994) **
Cytogenetic assay (chromosomal aberrations)	human lymphocytes	other: without m.a.; 0, 20, and 200 µg/culture (2,000 µg toxic)	negative	chloride	Deknudt (1982) *
Unscheduled DNA synthesis	Syrian hamster embryo cells	unknown: without m.a.; 0.3, 1, 3, 10 and 30 µg/ml	positive ≥ 1 µg/ml	oxide	Suzuki (1987) *
Cell transformation assay	Syrian hamster embryo cells	unknown: without m.a.; 0, 1, 3 µg ZnO/ ml	positive 1 and 3 µg/ml	oxide	Suzuki (1987) *
Cell transformation assay	Syrian hamster embryo cells	unknown; up to 20 µg/ml	negative	chloride	Di Paolo and Casto (1979)*(r)*
Cell transformation assay	Syrian hamster embryo cells	unknown; 0-0.34 mM	equivocal	chloride	Casto et al. (1979)
Cell transformation assay	Syrian hamster embryo cells	unknown; 0-0.2 mM	equivocal	sulphate	Casto et al. (1979)
***In vivo* studies**					
Cytogenetic assay (chromosomal aberrations)	mouse	other: 0.5% zinc in calcium-deficient (0.03% Ca) or standard diet (1.1% Ca) for 30 days	slightly positive in case of calcium deficient diet in the survivors (0.5% Zn with poor Ca-diet resulted in 50% mortality after 30 days)	chloride	Deknudt (1982) *
Cytogenetic assay (chromosomal aberrations)	mouse	other; single i.p. injections of 0, 7.5, 10 or 15 mg $ZnCl_2$/kg bw and repeated i.p. injections every other day of 2 and 3 mg $ZnCl_2$/kg bw for 8, 16 or 24 days.	single dose study: positive; repeated-dose study: positive	chloride	Gupta et al. (1991)
Cytogenetic assay (chromosomal aberrations)	rat	other: 5 months inhalation of 0.1 to 0.5 mg/m^3	only slight increases of chromosomal aberrations were seen; primarily hyperdiploid cells were seen	oxide	Voroshilin et al. (1978) *
Cytogenetic assay (chromosomal aberrations)	rat	other: 2.75, 27.5 or 275 mg/kg bw by gavage once or daily for 5 consecutive days	negative	sulphate	Litton Bionetics (1974)

Table 4.12 continued overleaf

Table 4.12 continued Genotoxicity data

Genetic toxicity	Species	Protocol	Results	Form	Reference
***In vivo* studies** (continued)					
Micronucleus	mouse	other: i.p. 28.8, 57.5 or 86.3 mg/kg bw at 0 and 24 hours	negative	sulphate	Gocke et al. (1981)
Micronucleus	rat	other: resembling OECD guideline No. 474; 0.05%, 0.2%, and 1% in purified diet over a 13-week period	negative	mono-glycerolate	Windebank et al. (1995) **
Host-Mediated Assay	mouse	other: 2.75, 27.5 or 275 mg/kg bw by gavage once or daily for 5 consecutive days	weakly positive	sulphate	Litton Bionetics (1974)
Dominant lethal assay	rat	other: 2.75, 27.5 or 275 mg/kg bw by gavage once or daily for 5 consecutive days	negative	sulphate	Litton Bionetics (1974)
Drosophila SLRL test	drosophila melanogaster	other; 5 mM (in 5% saccharose) adult feeding method	negative	sulphate	Gocke et al. (1981)
Drosophila dominant lethal and SLRL test	drosophila melanogaster	unknown; 0.247 mg/ml adult feeding	negative	chloride	Carpenter and Ray (1969) *

m.a.: metabolic activation

* Although study or study documentation showed limitations (see hedset), the study is considered useful for the evaluation of the genotoxicity of zinc

** Studies on zinc monoglycerolate, submitted within the framework of the EEC Council Regulation

4.1.2.8.1 *In vitro* studies

Exposure to zinc compounds did not increase the mutation frequencies in the bacterial test systems (Gocke et al., 1981; Crebelli et al., 1985; Marzin and Vo Phi, 1985; Kada et al., 1980*(r)*; Litton Bionetics, 1976*(r)*; Jones and Gant, 1994), except for one ambiguous result with zinc chloride reported by Rossman et al. (1984).

A weakly positive and two negative results were found in eukaryotic test systems using the yeast S. cerevisiae (Singh, 1983; Siebert et al., 1970, Litton Bionetics, 1977).

A negative result (Deknudt, 1982) and a positive result (Akhurst and Kitching, 1994) were found for chromosomal aberrations in human lymphocytes. A negative (Amacher and Paillet, 1980*(r)*) and two positive results (Cameron, 1991(*r*); Adams and Kirkpatrick, 1994) were reported in mouse lymphoma assays (gene mutations).

A negative (zinc chloride) as well as a positive (zinc oxide) result in a cell transformation assay using Syrian hamster embryo cells was reported by Di Paolo and Casto (1979*(r)*) and Suzuki (1987), respectively. Equivocal results in this assay were reported for zinc chloride and zinc sulphate, producing enhancement of cell transformation in 3/6 and 3/7 trials, respectively (Casto et al., 1979). Suzuki (1987) reported a positive UDS test and an ambiguous result with zinc oxide in an SCE test.

4.1.2.8.2 *In vivo* studies

Two reliable negative micronucleus tests were reported in mice (Gocke et al., 1981) and rats (Windebank et al., 1995).

Zinc chloride induced chromosomal aberrations in mouse bone marrow in case of an extreme calcium deficient diet. In this study C57Bl mice received during one month a normal (with 1.1% Ca) or poor calcium diet (0.03% Ca) in combination with 0.5% of zinc. After this month 50% of the animals given the poor calcium diet in combination with 0.5% zinc died. No information was given about the mortality in the other groups. Ten survivors of each group were sacrificed another month later and their bone marrow cells were studied on chromosome aberrations. In each group 500 metaphases were studied. Total cells damaged were 9 in controls with normal Ca, 10 in controls with low Ca, 14 in Zn-exposed with normal Ca, and 25 in Zn-exposed with low Ca diet (Deknudt, 1982).

Mice (5 per group) were given intraperitoneal injections of 7.5, 10 or 15 mg zinc chloride/kg bw/day. After treatment of the animals with colchicine bone marrow preparations were collected at 24 h post dosing and 60 metaphases were studied per animal. At all doses an increase (dose-related) in chromosomal aberrations in bone marrow cells was observed as compared to the controls. Next to this, mice (5/group) were i.p. injected for 4, 8 or 12 times with 2 or 3 mg zinc chloride/kg bw every other day and the observed incidence of chromosomal aberrations was compared to the control group of the single dose study. Again an increase in incidence was found (after 4 injections only at the highest dose, at 8 and 12 injections at both doses), but the control group used is not entirely appropriate. The cauda epididymis of the animals in the single dose study were minced and sperm cells were examined. An increase in sperm head abnormalities was found, but further study details and criteria for interpretation were not provided (Gupta et al., 1991). The increase in chromosomal aberrations observed in the single dose study is considered reliable.

No chromosomal aberrations were induced when rats were given 2.75, 27.5 or 175 mg/kg bw zinc (as zinc sulphate) by gavage once or daily for 5 consecutive days (Litton Bionetics, 1974). Only a slight increase in chromosomal aberrations in rat bone marrow was reported by Voroshilin et al. (1978) after exposure to zinc oxide by inhalation. Female rats were subjected to continuous inhalation of a zinc oxide aerosol in concentrations of 0.5 and 0.1 mg/m^3 for 5 months. 200 Metaphases were studied and the total amount of cells damaged were 1.0% in controls, 4.5% in rats exposed to 0.1 mg/m^3, and 6.5% in rats exposed to 0.5 mg/m^3.

Zinc sulphate tested negative in a drosophila SLRL test (Gocke et al., 1981) and a dominant lethal assay in rats (Litton Bionetics, 1974). A drosophila dominant lethal and SLRL test with zinc chloride (Carpenter and Ray, 1969) was also negative.

A host-mediated assay with zinc sulphate appeared to be weakly positive (Litton Bionetics, 1974).

4.1.2.8.3 Conclusion on mutagenicity

Several data were provided on the genotoxicity of zinc sulphate. Data on other zinc compounds have also been used, based on the assumption that after intake the biological activities of the zinc compounds are determined by the zinc cation.

The available data indicate that the genotoxicity results vary widely. Conflicting results have been found, even in the same test systems. Overall, the results of the *in vitro* tests indicate that zinc has genotoxic potential *in vitro* based on positive results in mammalian test systems for gene mutations and chromosomal aberrations and on the positive *in vitro* UDS test.

In *vivo,* increases in chromosomal aberrations were found in calcium-deficient mice exposed via the diet as well as in mice with normal calcium status when dosed intraperitoneally. In mice also negative results were obtained and even at higher intraperitoneal dose levels. Rats tested negative for chromosomal aberrations after oral dosing, either via gavage or via the diet. The positive result for chromosomal aberrations *in vitro* is considered overruled by negative *in vivo* tests for this endpoint.

The positive sperm head abnormality test is considered sufficiently counter-balanced by two negative SLRL tests as well as two negative dominant lethal tests. Moreover, this sperm test is not adequately reported and without details on scoring criteria, interpretation of the observations is rather subjective. In addition, sperm head abnormalities are indicative rather than proof for genotoxicity.

Based on the available data there is insufficient ground to classify zinc as genotoxic. It should be noted that the potential to induce gene mutations was not adequately tested *in vivo*. However, there is no clear evidence from the available data that zinc is genotoxic *in vivo* and without a clear indication for carcinogenicity (see below) guidance for further testing with respect to target tissue is not available.

4.1.2.9 Carcinogenicity

No adequate long-term carcinogenicity studies are available. All the information regarding the carcinogenic properties of zinc or zinc compounds is included in this section.

4.1.2.9.1 Studies in animals

Testicular teratomas were reported in early studies in poultry, birds and rats following repeated intratesticular injection of different zinc compounds, such as $ZnCl_2$ and $ZnSO_4$. No tumourogenic effects have been found when zinc was administered by intramuscular or subcutaneous injection (Léonard et al., 1986).

In a limited older study the tumour incidences in Chester Beatty mice were studied after administration of 1,000 and 5,000 ppm zinc sulphate ($ZnSO_4 \cdot 7\ H_2O$) in drinking water (equal to 4.4 and 22 g/l water; calculated to be 200 or 1,000 mg Zn^{2+}/kg bw) for 45-53 weeks. A control group was included, however concurrent controls were used after a number of animals died after an intercurrent disease (ectromelia). The starting number of animals per group was not given. Only 22-28 mice/group survived at the end of the exposure period. Observations were limited to 'thorough examinations once each week and more cursorily examinations each day', body weight measurements and at the end 'a thorough post-mortem examination' with a histological examination for lesions that were possibly neoplastic. Results were only given for incidence and types of tumours. The incidences of hepatoma, malignant lymphoma, and lung adenoma and the evidence of hyperplasia in the fore-stomach epithelium were not different between exposed and control mice. No other tumours developed (Walters and Roe, 1965).

Although no direct carcinogenic actions of dietary zinc deficiency or supplementation are known, the growth rate or frequency of transplanted and chemically induced tumours is influenced by the zinc content in the diet. Both promoting and inhibiting actions have been reported depending on the experimental conditions. Experiments with rodents suggest that cancer growth is retarded by zinc deficiency and may be promoted by large amounts of zinc intake. These effects may be explained by the fact that zinc is needed in DNA synthesis and cell replication (Deknudt and Gerber, 1979; Léonard et al., 1986).

4.1.2.9.2 Studies in humans

A cohort study of 4,802 refinery workers in nine electrolytic zinc and copper refining plants (i.e. one zinc, one copper + zinc and seven copper refineries), who had been employed between 1946 and 1975, reported slightly reduced mortality in the 1,247 workers who had been exposed to "zinc" alone (978) or in combination with "copper" (269). Employees were incorporated in the study when they had worked in the electrolytic department for at least one year. Age-adjusted Standardized Mortality Ratio's were calculated on the basis of comparison with the mortality rates for the entire US population for the year 1970. Of the 1,247 workers who were exposed to "zinc" (either alone or in combination with "copper"), 88 died before the end of the follow-up. For 12 of these, the cause of death could not be retrieved. 143 workers were lost to follow-up entirely. Cancer rates were only analysed for the entire cohort of refinery workers (i.e. all 4,802 participants). An association between cancer mortality and employment in zinc and/or copper refinery was not found. However, the study does not permit to draw a conclusion about any association between cancer mortality and zinc exposure, because cancer mortality for "zinc"-workers was not analysed separately from cancer mortality for "copper"-workers (Logue et al., 1982).

Neuberger and Hollowell (1982) studied an excess in lung cancer mortality associated with residence in an old-lead/zinc mining and smelting area in the US. The age- and sex-adjusted mortality rates were compared to state and national rates. The analysis determined that lung cancer mortality was elevated in the region. Quantification of inhabitant's exposure to zinc was not part of the study. The authors mentioned several possible causes for the increased lung cancer rates such as smoking habits, occupational exposure (e.g. in mining and associated activities) and residence. Ore contaminants were arsenic, cadmium, iron, sulphur, germanium and radioactivity. Tuberculosis and silicosis were commonly seen among the region's inhabitants. From this study any conclusion on a possible association between exposure to environmental levels of lead or zinc and the increased lung cancer rate cannot be drawn.

Leitzmann et al. (2003) examined the association between supplemental zinc intake (level and duration) and prostate cancer among 46,974 US men participating in the Health Professionals Follow-Up Study. During 14 years of follow-up (from 1986 through 2000), 2,901 new cases of prostate cancer were ascertained, of which 434 cases were diagnosed as advanced cancer. Approximately 25% of the study population used zinc supplements (24% in amounts ≤ 100 mg/day, 1% in amounts > 100 mg/day). Supplemental zinc intake at doses of up to 100 mg/day was not associated with prostate cancer risk. However, compared with non users, users with an excessively high supplemental zinc intake (> 100 mg/day) had a relative risk of advanced prostate cancer of 2.29 (95% CI 1.06 to 4.95). Increasing the duration of supplemental zinc use was unrelated to the risk of total prostate cancer. However, for chronic users (> 10 years) the relative risk of advanced prostate cancer was 2.37 (95% CI 1.42 to 3.95). According to the authors residual confounding by supplemental calcium intake or some unmeasured correlate of zinc supplement use cannot be ruled out. They also indicate that strong

evidence to support a specific mechanism for the association is lacking at present, and that further exploration for the possible role of chronic zinc oversupply in prostate carcinogenesis is needed.

4.1.2.9.3 Conclusion on carcinogenicity

The available data are limited. Zinc deficiency or supplementation may influence carcinogenesis, since promoting and inhibiting actions have been reported. However, there is no clear experimental or epidemiological evidence for a direct carcinogenic action of zinc or its compounds.

4.1.2.10 Toxicity for reproduction

Several data were provided on the reproductive toxicity of zinc sulphate. Data on other zinc compounds have also been used, as the basic assumption is made that after intake all zinc compounds (including metallic zinc) are changed (at least in part) to the ionic species and that it is this zinc cation that is the determining factor for the biological activities of the zinc compounds (see Section 4.1.2.1).

Zinc is necessary for normal growth and development (e.g. gene expression, metabolism of vitamins including folate, retinol) and therefore it is not surprisingly that a zinc deficiency can cause foetal damage as reported in animals (Walsh et al., 1994; ATSDR, 1994). Both human and animal data show that zinc deficiency will also lead to delayed sexual maturation and to impairment of reproductive capacity (WHO, 1996).

4.1.2.10.1 Studies in animals

Fertility

For zinc no 1- or 2-generation studies are available. However, one study is available in which some attention was paid to the effects of zinc on male fertility (Samanta and Pal, 1986), while in another study (Pal and Pal, 1987) effects on female fertility were studied. In addition, three repeated dose toxicity studies are available in which mice and rats were exposed for 13 weeks to dietary zinc. In these three studies the effects of zinc on gonads and accessory sex organs were studied.

18 Male Charles-Foster rats were exposed via diet to 4,000 mg Zn^{2+} (as anhydrous zinc sulphate)/kg feed (about 200 mg Zn^{2+}/kg bw/day) for 30-32 days before mating. 15 Males served as controls. The males were mated individually with female rats of proven fertility and sacrificed the day after mating. There was a statistically significant difference between the number of control females that conceived (15/15) and the treated females (11/18). Zinc treatment resulted in significantly lower numbers of live birth. Increased zinc concentrations were found in the testes (not in the other reproductive organs examined) and sperm of treated males. The motility of the sperm was reduced, but the viability was unaffected (Samanta and Pal, 1986).

When 12 female Charles-Foster rats received via diet 4,000 mg Zn^{2+} (as anhydrous $ZnSO_4$)/kg feed (corresponding to 200 mg Zn^{2+}/kg bw/day) from day 1 until day 18 post coitum, only 5 females conceived versus 12 in the control group. The numbers of implantation sites per pregnant female and per mated female were both lower in the treated group. After administration

of the same dose from day 21-26 prior to mating until sacrifice (day 18 post coitum), 14 out of 15 mated treated females conceived versus 10 out of 11 mated control females. No differences were seen between the groups in the numbers of implantation sites per mated or per pregnant female. According to the study authors the reduced fertility in the post-coitus-only-exposed group was the result of a disturbance of the implantation process. The pre- and postcoitus-exposed animals had the opportunity to adapt to high zinc intake, thus being able to avoid the effect. However, no further studies were done to substantiate this (Pal and Pal, 1987).

In mice and rats, zinc sulphate heptahydrate in dietary concentrations up to 30,000 mg/kg feed did not produce adverse effects on either male or female sex organs after 13 weeks of exposure. This dietary level was equal to ca. 1,100 mg or 565 mg Zn^{2+}/kg bw/day for mice and rats, respectively (Maita et al., 1981; see also Section 4.1.2.7.1).

In another study, male and female rats were exposed to zinc monoglycerolate up to 1% in the diet, equal to ca. 335 mg Zn^{2+}/kg bw/day for 58 days, after which the concentration in the feed was decreased for one week to 0.5%, equal to ca. 300 mg Zn^{2+}/kg bw/day. Subsequently, the animals had to be killed at day 64 because of poor health and compromised food consumption (note also the non-linearity in the Zn^{2+}- doses). The testes of all these males showed hypoplasia of the seminiferous tubules to a varying degree and in addition the prostate and seminal vesicles showed hypoplasia. In all but one female the uterus was hypoplastic. All other rats exposed to 0.05 or 0.2% (ca. 13 or 60 mg Zn^{2+}/kg bw/day, respectively) survived to the end of the 13 weeks treatment, without showing detrimental effects on sex organs (Edwards and Buckley, 1995; see also Section 4.1.2.7.1).

Developmental toxicity

Several developmental toxicity studies with zinc sulphate and zinc oxide are available. Four studies with zinc sulphate were performed at the Food and Drugs Research Labs, Inc. (1973, 1974) and were of a design comparable to the OECD 414 guideline. These studies are mentioned in **Table 4.13** and summarised in more detail below. However, in the reports it was not specified which form of zinc sulphate was used. For this reason the NOAELs in these studies are converted to two NOAELs for Zn^{2+}, one on the assumption that the anhydrate was used and one on the assumption that the heptahydrate was used.

Table 4.13 Developmental toxicity data

Developmental toxicity	Species	Protocol	Result	mg Zn^{2+}/ kg bw	Reference
Oral	mouse	females received daily doses of 0, 0.3, 1.4, 6.5 and 30 mg $ZnSO_4$ (unspecified)/kg bw during days 6-15 of gestation.	NOAEL 30 mg/kg bw (highest dose level tested): no discernible effects were seen on nidation, or maternal or foetal survival. No difference in number of abnormalities found in foetuses.	NOAEL: anhydr: 12 hepta: 6.8	Food and Drugs Research Labs., Inc. (1973) *
	rat	females received daily doses of 0, 0.4, 2.0, 9.1 and 42.5 mg $ZnSO_4$ (unspecified)/kg bw during days 6-15 of gestation.	NOAEL 42.5 mg/kg bw (highest dose level tested): no discernible effects were seen on nidation, or maternal or foetal survival. No difference in number of abnormalities found in foetuses.	NOAEL: anhydr: 17 hepta: 9.6	Food and Drugs Research Labs., Inc. (1973) *

Table 4.13 continued overleaf

Table 4.13 continued Developmental toxicity data

Developmental toxicity	Species	Protocol	Result	mg Zn^{2+}/ kg bw	Reference
	hamster	females received daily doses of 0, 0.9, 4.1, 19, and 88 mg $ZnSO_4$ (unspecified)/kg bw during days 6-15 of gestation.	NOAEL 88 mg/kg bw (highest dose level tested): no discernible effects were seen on nidation, or maternal or foetal survival. No difference in number of abnormalities found in foetuses.	NOAEL: anhydr: 35.2 hepta: 19.9	Food and Drugs Research Labs., Inc. (1973) *
	rabbit	females received daily doses of 0, 0.6, 2.8, 13 and 60 mg $ZnSO_4$ (unspecified)/kg bw during days 6-18 of gestation.	NOAEL 60 mg/kg bw: no discernible effects were seen on nidation, or maternal or foetal survival. No difference in number of abnormalities found in foetuses.	NOAEL: anhydr: 24 hepta: 13.6	Food and Drugs Research Labs., Inc. (1974) *

* Valid study, with restrictions. $ZnSO_4$ form is unspecified. The NOAEL, expressed as Zn cation, has been calculation for both anhydrate- and heptahydrate forms.

Oral exposure

- Zinc sulphate

Female CD-1 mice (25-30 animals/group) received daily doses of 0.3, 1.4, 6.5 and 30 mg unspecified $ZnSO_4$/kg bw by gavage during days 6-15 of gestation. A control group was included. All animals were observed daily for appearance and behaviour with particular attention to food consumption and body weight. Body weights were recorded on day 0, 6, 11, 15 and 17 of gestation. The females were sacrificed at day 17. The urogenital tract of each animal was examined in detail. Between 21 and 23 females were pregnant at term in all groups. No clearly discernible effects on maternal survival, body weight gains, number of corpora lutea, implantations and resorptions were observed. The number of live litters, live and dead foetuses, the foetus weights and sex ratio were not affected by treatment. No difference in number or kind of abnormalities (in either soft or skeletal tissues) was found between exposed and control groups. It can be concluded that the administration of up to 30 mg/kg bw of unspecified zinc sulphate (≈ 12 mg or 6.8 mg Zn^{2+}/kg bw, for anhydrate and heptahydrate, respectively) had no adverse effects on adult mice and their foetuses (Food and Drug Research Labs., Inc., 1973).

Female Wistar rats (25-28 animals/group) received daily doses 0.4, 2.0, 9.1 and 42.5 mg unspecified $ZnSO_4$/kg bw by gavage during days 6-15 of gestation. A control group was included. All animals were observed daily for appearance and behaviour with particular attention to food consumption and body weight. Body weights were recorded on day 0, 6, 11, 15 and 20 of gestation. The females were sacrificed at day 20. The urogenital tract of each animal was examined in detail. At term 25 females were pregnant in all groups. No clearly discernible effects on maternal survival, body weight gains, number of corpora lutea, implantations and resorptions were observed. The number of live litters, live and dead foetuses, the foetus weights and sex ratio were not affected by treatment. No difference in number or kind of abnormalities (in either soft or skeletal tissues) was found between exposed and control groups. It can be concluded that the administration of up to 42.5 mg/kg bw of unspecified zinc sulphate (≈ 17 mg or 9.6 mg Zn^{2+}/kg bw, for anhydrate and heptahydrate, respectively) had no adverse effects on adult rats and their foetuses (Food and Drug Research Labs., Inc., 1973).

Female hamsters (23-25 animals/group; outbred strain of golden hamster) received daily doses of 0.9, 4.1, 19 and 88 mg unspecified $ZnSO_4$/kg bw by gavage during days 6-10 of gestation. A

control group was included. All animals were observed daily for appearance and behaviour with particular attention to food consumption and body weight. Body weights were recorded on day 0, 8, 10 and 14 of gestation. The females were sacrificed at day 14. The urogenital tract of each animal was examined in detail. Between 21 and 24 females were pregnant at term in all groups. No clearly discernible effects on maternal survival, body weight gains, number of corpora lutea, implantations and resorptions were observed. The number of live litters, live and dead foetuses, the foetus weights and sex ratio were not affected by treatment. No difference in number or kind of abnormalities (in either soft or skeletal tissues) was found between exposed and control groups. It can be concluded that the administration of up to 88 mg/kg bw of unspecified zinc sulphate (≈ 35.2 mg or 19.9 mg Zn^{2+}/kg bw, for anhydrate and heptahydrate, respectively) had no adverse effects on adult hamsters and their foetuses (Food and Drug Research Labs., Inc., 1973).

Female Dutch rabbits (14-19 animals/group) received daily doses of 0.6, 2.8, 13 and 60 mg unspecified $ZnSO_4$/kg bw by gavage during days 6-18 of gestation. A control group was included. All animals were observed daily for appearance and behaviour with particular attention to food consumption and body weight. Body weights were recorded on day 0, 6, 12, 18 and 29 of gestation. The urogenital tract of each animal was examined in detail. The females were sacrificed at day 29. Between 10 and 12 females were pregnant at term in all groups. No clearly discernible effects on maternal survival, body weight gains, number of corpora lutea, implantations and resorptions were observed. The number of live litters, live and dead foetuses, the foetus weights and sex ratio were not affected by treatment. No difference in number or kind of abnormalities (in either soft or skeletal tissues) was found between exposed and control groups. It can be concluded that the administration of up to 60 mg/kg bw of unspecified zinc sulphate (≈ 24 mg or 13.6 mg Zn^{2+}/kg bw, for anhydrate and heptahydrate, respectively) had no adverse effects on adult rabbits and their foetuses (Food and Drug Research Labs., Inc., 1974).

Female rats (13) received low protein (10%) diets containing 30 mg Zn^{2+} supplemented with 150 mg Zn^{2+}/kg feed (7.5 mg Zn^{2+}/kg bw) as 2% $ZnSO_4$ solution during days 1-18 of pregnancy. A control group (12 females) was included and received the same diet as the exposure group but without additional zinc. No further study details were given, but it was stated that two resorptions of a total number of 101 implantation sites were found in 2 (1 in each female) of the 12 control females. In 8 (at least 1 resorption each) of the 13 experimental females 11 resorptions out of 116 implantations sites were found. This difference was reported to be statistically significant (Kumar, 1976).

Remark: The low protein diet may have affected the physiology of the animals resulting in an increased sensitivity for zinc. As this cannot be further assessed, and because virtually no study details are available, the study is not taken into account.

12 Female Charles-Foster rats received via diet 4,000 mg Zn^{2+} (as anhydrous $ZnSO_4$)/kg feed (corresponding to 200 mg Zn^{2+}/kg bw) from day 1 until day 18 post coitum and 15 animals received the same diet from day 21-26 prior to mating until sacrifice (day 18 post coitum). Control groups consisted of 12 and 11 animals, respectively. No stillbirths or malformed fetuses were recorded and there were no significant differences in the number of resorptions or the mean placental and fetal weights between the treated females and controls irrespective of the exposure regime (Pal and Pal, 1987).

Campbell and Mills (1979) examined the reproductive performance of Cheviot sheep (6/group) which received 30, 150 and 750 mg $ZnSO_4$ (unspecified)/kg feed during pregnancy until parturition. A control group was included. High-dose sheep showed decreased food

consumption, food utilisation and reduced body weight gains. Blood copper levels, plasma ceruloplasmin and amine oxidase were statistically significantly decreased and plasma zinc levels were greatly increased. The reproductive performance was severely impaired at the highest dose level: Most of the lambs were non-viable, and showed high zinc levels in the livers (this was also seen in the mid-dose) and low copper concentrations. These lambs also showed discontinuous growth of long bones, which was not observed in the lower dose groups. Copper supplementation (2.5 and 10 mg) at the high dose level prevented the development of copper deficiency, but not the other effects such as lamb viability and food consumption/utilisation.

- Zinc oxide

In rats, the administration of 0.4% of Zn^{2+} as ZnO (corresponding to 200 mg Zn^{2+}/kg bw/day) via diet for 21 days prior to mating until day 15 of gestation resulted in resorption of all foetuses. Administration of 0.4% dietary Zn^{2+} from day 0 to day 15, 16, 18 or day 20 of gestation, but not prior to mating, resulted in decreased live fetal body weights and in 4-29% fetal resorptions. When the concentration of Zn^{2+} in the feed was reduced to 0.2% (corresponding to 100 mg Zn^{2+}/kg bw /day), starting 21 days prior to mating until day 15 of gestation no resorptions or effects on fetal body weights were observed. Treatment with dietary zinc did not result in external malformations, irrespective of dose level or treatment regimen. A dose-related significant increase in liver total zinc and liver zinc concentration and a significant decrease in the liver copper concentration was found in foetuses and mothers on all zinc regiments. No other information was given with respect to the health status of the mother animals. Although some of the animals were exposed from day 21 before mating up to study termination, no data were provided on possible consequences for female fertility. The study is too limited to derive an NOAEL for developmental toxicity (Schlicker and Cox, 1968).

Groups of Sprague-Dawley rats (10/group) were fed diets containing 2,000 or 5,000 mg ZnO/kg feed (calculated to be 150 or 375 mg ZnO/kg bw [≈ 120 or 300 mg Zn^{2+}/kg bw/day]) from day 0 of gestation to day 14 of lactation, then mothers and remaining pups were killed. The control animals received a basal diet containing 9 mg Zn^{2+}/kg feed.

Maternal weight, daily food intake, duration of gestation and the number of viable young/litter were not affected. No external malformations were seen.

Two females at 5,000 mg/kg feed had all stillborn litters containing oedematous pups. At 2,000 mg/kg feed 4 stillborn pups (not oedematous) were observed. Dry liver weights of pups (newborn and 14 days old) were decreased at 5,000 mg/kg feed. A dose-related increase in zinc content and a dose-related decrease in iron content were observed. The livers of newborns of zinc-treated dams, however, contained significantly more iron than the controls. This was not observed in the 14-day old pups. The copper levels in the liver were significantly lower only in the newborns of the 5,000 mg/kg level. After 14 days the copper concentrations were significantly lower in all treated pups (Ketcheson et al., 1969).

Bleavins et al. (1983) exposed groups of mink (11 females and 3 males/group) to basal diet (containing 20.2 mg Zn^{2+}/kg diet and 3.1 mg Zn^{2+}/kg diet) or to the diet supplemented with 1,000 mg ZnO/kg diet. No maternal effects were seen. All females on the basal diet produced offspring, 8/11 females of the Zn-supplemented diet group had young. None of the animals (males, females and kits) were sacrificed, so they were only macroscopically examined. The kits were kept on the basal and supplemented diets. The body weight of male kits on the supplemented diet was significantly lower at 12 weeks of age. 8-week old kits on the supplemented diet showed a significant decrease of the Ht-value, the other blood parameters were comparable to the kits on basal diet. The decreased T-cell mitotic response observed in the

Zn-supplemented kits was reversible when the kits were placed on basal diet. Kits (3-4 weeks old) of females fed the Zn-supplemented diet showed effects consistent with copper deficiency, such as grey fur around eyes, ears, jaws and genitals together with hair loss and dermatosis in these areas.

Inhalation exposure

No inhalation toxicity data are available.

Dermal exposure

No dermal toxicity data are available.

Other routes

- Zinc chloride

Chang (1976) reported a study in which single i.p. injections of 12.5, 20.5 or 25 mg $ZnCl_2$/kg bw (6, 9.8 or 12 mg Zn^{2+}) to CF-1 albino mice (7-15/group) on day 8, 9, 10 or 11 of gestation caused a significant dose-related increased incidence of skeletal anomalies without soft tissue anomalies. Toxic effects on mothers and foetuses where the greatest when $ZnCl_2$ was administered at 20.5 mg/kg bw on day 10 of pregnancy. When $ZnCl_2$ was given at 12.5 mg/kg bw on day 11 of gestation no effects on mothers or foetuses were observed. Because no more information was given, these results cannot be used for risk assessment.

4.1.2.10.2 Studies in humans

The majority of human studies are dealing with the association between low indices of maternal zinc status and the negative effects on pregnancy including neural tube defects in babies (Walsh et al., 1994).

Mukherjee et al. (1984) found a highly significant increase in pregnancy complications, including foetal distress and maternal infections, among women with low plasma zinc during the latter half of pregnancy. An association of low plasma zinc levels in early pregnancy and greater likelihood of delivery of a low birth weight infant was observed by Neggers et al. (1990(*r*)). The earlier findings of Meadows et al. (1981(*r*)) reporting an association between low maternal leukocyte and muscle zinc at term and low birth weight and of Cambell-Brown et al. (1985(*r*)) reporting an association between low zinc intakes in Hindu women and low birth weight.

There are no data available indicating that an excess of zinc can impair human pregnancy outcome.

Mahomed et al. (1989) performed a study in pregnant women to examine whether zinc supplementation during pregnancy improves maternal and foetal outcome. Pregnant women were randomly assigned to receive a zinc supplementation or placebo in a double blind trial. 494 Women (246 given zinc supplementation, 248 given placebo) were followed till the end of pregnancy. The zinc supplementation was administered in capsules containing 20 mg Zn^{2+} as zinc sulphate (0.3 mg Zn^{2+}/kg bw/day) once a day during two trimesters. There were no significant differences between the two groups with respect to complications of pregnancy (weight, weight gains, maternal bleeding and hypertension), complications of labour and delivery, gestational age, Apgar scores, neonatal abnormalities and birth.

Two human studies with other zinc compounds than the ones selected showed no effects on the newborns of mothers consuming 0.3 mg Zn^{2+} (as zinc citrate)/kg bw/day (Simmer et al., 1991(*r*)) or 0.06 mg Zn^{2+} (as zinc aspartate)/kg bw/day (Kynast and Saling, 1986) during the last two trimesters of pregnancy.

4.1.2.10.3 Conclusion on toxicity for reproduction

Several data were provided on the reproductive toxicity of zinc sulphate. Data on other zinc compounds have also been used, based on the assumption that after intake the biological activities of the zinc compounds are determined by the zinc cation.

For fertility no 1- or 2-generation or other applicable guideline studies are available.

When male rats were dosed with approximately about 200 mg Zn^{2+}/kg bw via the food for 30-32 days before mating, a statistically significant reduction in male reproductive performance was observed. This effect was attributed to a reduction in sperm motility. In females receiving 200 mg Zn^{2+}/kg bw, reduced conception was observed when they were dosed after mating, but not when they were dosed before and during pregnancy. It is not known whether the reduced sperm motility in males and the contradictory effects on conception in females are a direct effect of zinc on the sperm cells, embryos or uterine function, or whether they are the result of disturbances in other physiological functions. From a study by Schlicker and Cox (1968), it is known that this dose level (and even levels of 100 mg additional Zn^{2+}/kg bw/day) may result in impaired copper balance in females.

In repeated dose toxicity studies with zinc sulphate heptahydrate, no effects on the reproductive organs were seen at dose levels up to ca. 1,100 mg and 565 mg Zn^{2+}/kg bw/day for mice and rats, respectively. In a repeated dose toxicity study with zinc monoglycerolate hypoplasia of several sex organs was observed at doses of ca. 300 mg Zn^{2+}/kg bw/day, but not at 13 or 60 mg Zn^{2+}/kg bw/day. As these effects were only seen at dose levels which produced very severe general toxicity, it is impossible to conclude that these adverse effects are directly related to zinc. It should be noted that these studies are not designed to detect effects on sperm cell motility.

Developmental toxicity studies, according to a study design similar to OECD 414, with mice, rats, hamsters and rabbits were described with unspecified zinc sulphate. These studies do not permit the derivation of a proper NOAEL because neither reproductive nor developmental or maternal effects were observed, not even at the highest dose tested. When it is assumed (worst-case) that the heptahydrate was administered from the study with hamsters it can be calculated that the NOAEL for both maternal effects and effects on the offspring is at least 19.9 mg Zn^{2+}/kg bw/day. In other (non-guideline) studies, higher dose levels (up to 200 Zn^{2+}/kg bw/day) have been reported to result in resorptions and retarded foetal growth, but not in external malformations. No resorptions and growth retardation were seen at 100 mg Zn^{2+}/kg bw/day but as the study was too limited, this dose level cannot be taken as an NOAEL for developmental toxicity, either. Besides, at both 100 and 200 mg Zn^{2+}/kg bw/day changes in maternal and fetal copper status were observed. In absence of better information a NOAEL of > 19.9 mg Zn^{2+}/kg bw/day for developmental toxicity in animals is adopted.

In studies with pregnant women receiving additional 0.3 mg Zn^{2+}/kg bw/day (as zinc sulphate or citrate) during the last 6 months of pregnancy, no reproductive or developmental effects were observed. Clear evidence of zinc toxicity in human pregnancy has not been reported but this may be due to the fact that very high exposures to zinc in human pregnancy are unusual. In contrast,

zinc deficiency during pregnancy can cause a variety of adverse effects on the foetus or may result in reduced fertility or delayed sexual maturation in animals as well as in humans (Walsh et al., 1994; ATSDR, 1994; WHO, 1996).

Hence, with respect to effects on reproduction, zinc deficiency is known to result in impairment of fertility and of foetal development. In humans additional zinc up to 0.3 mg Zn^{2+}/kg bw/day during pregnancy did not result in adverse effects. Available data in animals on zinc excess indicate that adverse effects on fertility and foetal development may occur at dose levels of 200 mg Zn^{2+}/kg bw/day, in conjunction with other effects such as perturbation of parental and foetal copper homeostasis. In humans a small disturbance (if any) of normal physiology, presumably indicative for copper deficiency, has been demonstrated at zinc excess of 50 and 150 mg Zn^{2+}/day (0.83 and 2.5 mg Zn^{2+}/kg bw/day, respectively), while 150 mg Zn^{2+}/day (2.5 mg Zn^{2+}/kg bw/day) resulted in clinical signs. As the margin between the dose at which in humans clinical signs are manifest and the dose at which in animals reproductive effects have been reported is so high (i.e. 80), it is considered unlikely that in humans reproductive effects will occur at exposure levels at which clinical signs are not manifest. Therefore, neither fertility nor developmental toxicity are considered end points of concern for humans. Based on the available information there is no reason to classify metallic zinc nor any of the zinc compounds considered for reproductive toxicity.

4.1.2.11 Interaction with other chemicals

Zinc can interact with other trace elements, such as cadmium, iron, calcium and especially copper, resulting in toxicity which is usually due to depletion of these elements, leading to nutritional deficiencies. Metallothionein is involved in the interaction between zinc and other metals such as copper.

Both copper and zinc appear to bind to the same metallothionein protein, but copper has a higher affinity for it than zinc and displaces the zinc that is attached to the metallothionein (Ogiso et al., 1979*(r)*; Wapnir and Balkman, 1991*(r)*). A number of factors influence the effect of dietary zinc on copper metabolism, including the amount of copper and zinc in the diet, the zinc-to-copper ratio, age of the individual, and the duration of exposure to high zinc levels (Johnson and Flagg, 1986*(r)*).

Prasad et al. (1978*(r)*) and Porter et al. (1977*(r)*) reported that chronic, elevated intake of zinc of 100 mg or more per day induced copper deficiency in humans. Yadrick et al. (1989) and Fischer et al. (1984) observed an altered copper balance in humans at doses of 50 mg zinc/day. However, in more recent studies in which the copper status was closely monitored (Davis et al., 2000; Milne et al., 2001) the daily oral intake of 50 mg Zn^{2+} appeared to enhance rather than impair copper retention in humans.

Normally the influence of iron on zinc absorption may not be significant. Under unusual conditions, however, if large iron supplements are ingested in the absence of food, it is likely that iron could impair the zinc absorption. This is supported by a number of clinical studies (Solomons, 1988*(r)*).

Yadrick et al. (1989) studied the effect of 50 mg daily doses of supplemental zinc or 50 mg zinc together with 50 mg iron during 10 weeks in women. The results suggested that supplemental zinc at a level of 50 mg/day impaired both the iron and copper status. Simultaneous iron supplementation protected the iron status. However, in more recent studies in which the iron

status was closely monitored (Davis et al., 2000; Milne et al., 2001) the daily oral intake of of 50 mg Zn^{2+} did not affect indicators of iron status in humans.

Exposure to cadmium may cause changes in the distribution of zinc, with accumulation of zinc in the liver and kidney. This accumulation may result in a deficiency in other organs. Harford and Sarkar (1991*(r)*) stated that simultaneous administration of cadmium and zinc results in induction of metallothionein in an additive manner.

A high zinc intake is also associated with decreased intestinal calcium absorption, leading to decreased calcium status in the body (Yamaguchi et al., 1983*(r)*; Spencer et al., 1992*(r)*).

Conclusion on interaction with other chemicals

Zinc can interact with other trace elements, especially copper, resulting in toxicity which is usually due to depletion of these elements, leading to nutritional deficiencies. In some older studies, it has been suggested that supplemental zinc at a level of 50 mg/day impaired both the iron and copper status, but these effects were not observed in more recent interaction studies. At least part of the interaction between zinc and other metals such as copper may be related to the effect of zinc on metallothionein.

4.1.2.12 Biological function and recommended levels

Zinc is an essential element for humans and animals and it is required for the optimum function of over 200 enzymes. These enzymes include those required for normal acid, protein, and membrane metabolism, as well as cell growth and division. Zinc also plays a role in the regulation of DNA and RNA synthesis (Vallee and Auld, 1990*(r)*; South and Summers, 1990*(r)*; Berg, 1990*(r)*). Zinc is also a required element for the optimum activity of growth hormone and the normal exocrine and endocrine function of the pancreas (Lee et al., 1990*(r)*).

A zinc deficiency in the diet has been associated with loss of appetite, decreased sense of smell and taste, impaired immune function, poor wound healing and dermatitis. It can also lead to retarded growth and hypogonadism with impaired reproductive capacity. An increased incidence of congenital malformations in infants has also been associated with a zinc deficiency in the mothers (Cotran et al., 1989*(r)*; Elinder, 1986; Sandstead, 1981*(r)*).

The symptoms of zinc deficiency in children may be different from that of adults. In chronic zinc deficiency, anorexia, diarrhoea, irritability, and short stature may be predominant in children while in adults taste and smell malfunction, hypogonadism, and poor wound healing may appear as early signs. The main symptoms observed during an experimental zinc deficiency in male volunteers were loss of body weight and testicular hypofunction (Prasad, 1983).

The following daily zinc levels are recommended by NAS/NRC (1989*(r)*):

Infants (0-1 year)		5 mg/day
Children (1-10 years)		10 mg/day
Males (11-51^{+} years)		15 mg/day
Females (11-51^{+} years)		12 mg/day
Pregnant women		15 mg/day
During lactation	(first 6 months)	19 mg/day
	(next 6 months)	16 mg/day

Other authorities such as the EU (1993) or the Voedingsraad (1992) recommended somewhat lower daily levels of 9-10 mg/day and 7-9 mg/day for males and females, respectively.

Conclusion on biological function and recommended levels

Zinc is an essential element required for the function of a large number of enzymes. It plays a role in DNA and RNA synthesis and many other processes in the body. A zinc deficiency in the diet can lead to notable health effects. Recommended daily zinc levels range from 5 mg/day for infants to 19 mg/day for women during lactation.

4.1.3 Risk characterisation

4.1.3.1 General aspects

The human population may be exposed to zinc sulphate at the workplace, from uses of consumer products and indirectly via the environment (see Sections 4.1.1.2, 4.1.1.3, 4.1.1.4).

Large parts of the hazard section are identical in the risk assessment reports on the six zinc compounds now under review under EU Regulation 793/93. This is because of the basic assumption that the zinc cation (as measure for dissolved zinc species) is the determining factor for systemic toxicity.

It is realised that for zinc (and other metal) compounds it would be important to define the actual or bioavailable concentration which is important for toxicity, both in laboratory animals and in humans. Due to several physico-chemical processes, zinc will exist in different chemical forms, some of which are more bioavailable than others. It is thus realised that the bioavailability is affected by various physico-chemical parameters (ionic behaviour, solubility, pH, alkalinity etc.). Although there is some information on the solubility of the six zinc compounds, adequate information is lacking how to quantitatively determine or estimate the bioavailable fraction of all the different zinc compounds in either laboratory animals or humans. Therefore, it is assumed that all zinc compounds (including metallic zinc) are changed (at least in part) to the ionic species, and all toxicity data (independent of the tested compound) were used and expressed as the zinc cation.

With respect to local effects, it is not always possible to use data from all zinc compounds. Hence, for local effects only data from the specific zinc compound were used, or, where there were derogations, data from zinc compounds with more or less the same solubility characteristics.

A problem might arise for the route-to-route extrapolation for inhalation and dermal exposure, since the differences in physico-chemical properties of the zinc compounds can change the toxicokinetics (absorption) and subsequently the toxic effects. Although it is possible to predict the systemic effects after inhalation or dermal exposure from oral toxicity data of the zinc compound itself or other zinc compounds, this is only justifiable after careful consideration of all available data to establish adequate extrapolation factors.

Furthermore it is assumed that the influence of the background intake levels of zinc cations in animal studies will be the same for humans.

Some data were provided on the toxicokinetics of zinc sulphate. Data on other zinc compounds have also been used, as the basic assumption is made that after intake all zinc compounds (including metallic zinc) are changed (at least in part) to the ionic species and that it is this zinc cation that is the determining factor for the biological activities of the zinc compounds.

Within certain limits, the total body zinc as well as the physiologically required levels of zinc in the various tissues can be maintained, both at low and high dietary zinc intake. Regulation of gastrointestinal absorption and gastrointestinal secretion probably contributes the most to zinc homeostasis. In spite of this a regular exogenous supply of zinc is necessary to sustain the physiological requirements because of the limited exchange of zinc between tissues.

The Zn^{2+} absorption process in the intestines includes both passive diffusion and a carrier-mediated process. The absorption can be influenced by several factors such as ligands in the diet and the zinc status.

Persons with adequate nutritional levels absorb 20-30% and animals 40-50%. However, persons that are Zn-deficient absorb more, while persons with excessive Zn intake absorb less. For risk assessment, for the more soluble zinc compounds (chloride, sulphate) the lower bound of the absorption range at adequate nutritional levels is taken (i.e. 20%). For zinc oxide it has been shown that bioavailability is about 60% of that for soluble zinc salts, corresponding to 12-18%. For zinc metal, zinc phosphate and zinc distearate no bioavailability data were present. As these forms have limited solubility in diluted acids (stomach) comparable to zinc oxide, for the less soluble zinc compounds (oxide, phosphate, distearate, metal) an oral absorption value of 12% will be taken for risk assessment. In situations of exposure excess (e.g. in case of high dermal or inhalation exposure at the workplace) the oral uptake of zinc compounds will probably be less than the values taken for risk assessment (20% and 12%). However, as this reduction in uptake is not quantifiable, also for excess exposure situations the same oral absorption values will be applied. Some justification for this approach can be found in the observation that for intake levels differing by a factor of 10, uptake levels vary maximally by a factor of two.

Quantitative data on the absorption of zinc following inhalation exposure (especially relevant in occupational settings) are not available. Some animal data suggest that pulmonary absorption is possible. In animal studies on zinc oxide retention in the lungs half-life values of 14 and 6.3 hours were reported for dissolution. As the absorption of inhaled zinc depends on the particle size and the deposition of these particles, data were provided on the particle size distribution of zinc aerosol in three different industry sectors. When analysing the particle size distribution data with a multiple path particle deposition (MPPDep) model, it appeared that for zinc aerosols the largest part of the deposition takes place in the head region and much less in the tracheobronchial and pulmonary region. Although most of the material deposited in the head and tracheobronchial region is rapidly translocated to the gastrointestinal tract, a part will also be absorbed locally. Based on data for local absorption of radionuclides in the different airway regions, it is assumed that local absorption for the soluble zinc compounds will amount to 20, 50 and 100% of the material deposited in head, tracheobronchial and pulmonary region, respectively. For the less soluble/insoluble zinc compounds negligible absorption is assumed for head and tracheobronchial region and 100% absorption for the pulmonary region. The remaining part of the material deposited in the different airway regions will be cleared to the gastrointestinal tract where it will follow oral uptake kinetics, hence the oral absorption figures can be applied. Applying the above-mentioned assumptions to the deposition fractions as determined by the MPPDep model, inhalation absorption for the soluble zinc compounds (zinc chloride and zinc sulphate) is at maximum 40%, while for the less soluble/insoluble zinc compounds (zinc metal, zinc oxide, zinc phosphate and zinc distearate) inhalation absorption is at maximum 20%. These figures will be taken forward to the risk characterisation as a reasonable worst case, because these figures are thought to cover existing differences between the different zinc industry sectors with respect to type of exercise activities (and thus breathing rate) and particle size distribution.

Adequate quantitative data on the absorption of zinc following dermal exposure (relevant in both occupational and consumer settings) are not available. The human data presented are not considered valid, mainly since either wounded skin was investigated, or suction blisters were raised, impairing the intactness of the skin. Dermal absorption through the intact skin seems to be small (< 2%), based on the results of the *in vivo* animals studies as well as the *in vitro* studies, but unfortunately shortcomings were noted in all *in vivo* studies and none of these studies can be

used quantitatively. As for the *in vitro* studies, it is clear that the % in receptor medium generally gives an underestimation of the % systemically available in *in vivo* studies. Therefore, the amount detected in the skin should be included as being absorbed by default. This 'potentially absorbed dose' more closely resembles the dose becoming systemically available *in vivo*.

Zinc bound to or in the skin may become systemically available at a later stage. This can be concluded from results in TPN patients, in which an expected decrease in serum zinc levels with time was counteracted by dermal absorption of zinc to result in steady serum zinc levels. Unfortunately, only 3 of the 6 patients completed the 10-day study period. There are no adequate human data available to evaluate the release of zinc from normal skin following single or repeated dermal exposure, as either blood was sampled for a too short period of time (3 hours; Derry et al., 1983) or the skin was damaged (Agren, 1990, 1991; Hallmans, 1977). Therefore, it can be concluded that following single or repeated dermal exposure zinc can be taken up by the skin, whereas the relevance of this skin depot cannot be judged based on the available data. For example, it is not studied how a large artificial zinc depot in the skin will affect the uptake or homeostasis of other essential ions (e.g. Cu). However, the total database available indicates that skin-bound zinc may not become systemically available in a way that it results in high peak levels of zinc in serum, but rather in a more gradual way. Given the efficient homeostatic mechanisms of mammals to maintain the total body zinc and the physiologically required levels of zinc in the various tissues constant, the anticipated slow release of zinc from the skin is not expected to disturb the homeostatic zinc balance of the body. By expert judgement, based on the aforementioned considerations, the default for dermal absorption of solutions or suspensions of zinc or zinc compounds is therefore chosen to be 2%. Based on the physical appearance, for dust exposure to zinc or zinc compounds a 10-fold lower default value of 0.2% is chosen in the risk assessment.

Zinc is distributed to all tissues and tissue fluids and it is a cofactor in over 200 enzyme systems.

Zinc is primarily excreted via feces, but can also be excreted via urine, saliva, hair loss, sweat and mothermilk.

Available data indicate that zinc sulphate (anhydrous as well as hydrous forms) is harmful after acute oral exposure. Dermally, zinc sulphate is not acutely toxic.

Zinc sulphate is not skin irritating and is no skin sensitiser, but can induce ocular corrosion.

Several data were provided on the repeated dose toxicity of zinc sulphate. Data on other zinc compounds have also been used, based on the assumption that after intake the biological activities of the zinc compounds are determined by the zinc cation.

No repeated dose toxicity studies after dermal exposure are available in animals.

After inhalation exposure mainly studies of short duration (3-6 days) are available. In a 3-day inhalation study with guinea pigs a concentration of 2.3 mg ultrafine ZnO/m^3 (3 hours/day) was a marginal LOAEL, showing changes in neutrophils and activities of lactate dehydrogenase and alkaline phosphatase in the pulmonary fluid. At higher concentrations increased protein concentration, neutrophils, and enzyme activities in lung lavage fluids were seen, together with significant centriacinar inflammation of the pulmonary tissue. A dose of 2.7 mg ultrafine ZnO/m^3 (3 hours/day for 5 days) did not alter the lung function parameters in guinea pigs but at 7 mg ultrafine ZnO/m^3 (3 hours/day for 5 days) or at 5 mg ultrafine ZnO/m^3 (3 hours/day for 6 days) a gradual decrease in total lung capacity, vital capacity and reduction of the carbon monoxide diffusing capacity were seen in combination with inflammatory changes and edema.

The relevance of the findings in studies with ultra-fine zinc oxide fumes is unclear with respect to commercial grade zinc oxide, as the latter is of much larger particle size and can have different toxicological characteristics.

In two oral 13-week studies with zinc sulphate (one with rats and one with mice) and an oral 13-week study with zinc monoglycerolate in rats, the lowest oral NOAEL was found in the study with zinc monoglycerolate. This overall NOAEL is 31.52 mg zinc monoglycerolate/kg bw (≈ 13.26 mg Zn^{2+}/kg bw). At higher doses the most important effects the rats developed were hypocupremia, and significant changes in the pancreas (focal acinar degeneration and necrosis) and the spleen (decreased number of pigmented macrophages). It should be noted that in the studies with zinc sulphate mice and rats could be maintained up to 13 weeks on a diet containing 30,000 mg $ZnSO_4 \cdot 7\ H_2O$/kg feed (equivalent to 6,794 mg Zn^{2+}/kg feed), while in the 13-week study with zinc monoglycerolate with rats 1.0% zinc monoglycerolate in the diet (equivalent to 4,420 mg Zn^{2+}/kg feed) was so detrimental that animals had to be killed on humane grounds after 9 weeks.

Upon supplementing men and women with 150 mg Zn^{2+}/day (as zinc sulphate capsules), women appeared to be more sensitive than men to the effects of high zinc intake: clinical signs such as headache, nausea and gastric discomfort were more frequent among women, and women but not men had decreased activities of serum ceruloplasmin and erythrocyte superoxide dismutase (ESOD). In some earlier oral studies in which humans were supplemented with moderately high amounts of zinc (50 mg Zn^{2+}/day), a reduction in ESOD activity was also observed and again women appeared to be more sensitive to this effect. Hence, a reduction in ESOD was thought to be a sensitive indicator of copper status. However, in more recent and more sophisticated studies using the same dose level, ESOD was only marginally reduced (without a correlation with changes in copper balance), while findings on more specific copper deprivation signs (decreased serum ceruloplasmin and platelet cytochrome c oxidase) indicated that a sub-optimal intake of zinc was more effective than a moderately high intake of zinc in inducing changes associated with a decreased copper status in postmenopausal women. Given this, and degree of the observed ESOD reduction in comparison to the natural variability in its activity, the zinc-induced decrease in ESOD activity is considered to have marginal biological significance, if any, also because it may not have been caused by an interference with copper metabolism.

Overall, it is concluded from studies in which humans were supplemented with zinc (as zinc gluconate), that women are more sensitive to the effects of high zinc intake and that a dose of 50 mg Zn^{2+}/day is a NOAEL. At the LOAEL of 150 mg Zn^{2+}/day, clinical signs and indications for disturbance of copper homeostasis have been observed. The human oral NOAEL of 50 mg Zn^{2+}/day (0.83 mg/kg bw/day) will be taken across to the risk characterisation.

Several data were provided on the genotoxicity of zinc sulphate. Data on other zinc compounds have also been used, based on the assumption that after intake the biological activities of the zinc compounds are determined by the zinc cation. The available data indicate that the genotoxicity results vary widely. Conflicting results have been found, even in the same test systems. Overall, the results of the *in vitro* tests indicate that zinc has genotoxic potential *in vitro* based on positive results in mammalian test systems for gene mutations and chromosomal aberrations and on the positive *in vitro* UDS test. The positive result for chromosomal aberrations *in vitro* is considered overruled by negative *in vivo* tests for this endpoint. The positive sperm head abnormality test is considered sufficiently counter-balanced by two negative SLRL tests as well as two negative dominant lethal tests.

Based on the available data there is insufficient ground to classify zinc as genotoxic. It should be noted that the potential to induce gene mutations was not adequately tested *in vivo*. However, there is no clear evidence from the available data that zinc is genotoxic *in vivo* and without a clear indication for carcinogenicity (see below) guidance for further testing with respect to target tissue is not available.

The limited data available indicate that zinc deficiency or supplementation may influence carcinogenesis, since promoting and inhibiting actions have been reported. However, there is no clear experimental or epidemiological evidence for a direct carcinogenic action of zinc or its compounds.

Several data were provided on the reproductive toxicity of zinc sulphate. Data on other zinc compounds have also been used, based on the assumption that after intake the biological activities of the zinc compounds are determined by the zinc cation.

For fertility no 1- or 2-generation or other applicable guideline studies are available.

When male rats were dosed with approximately about 200 mg Zn^{2+}/kg bw via the food for 30-32 days before mating, a statistically significant reduction in male reproductive performance was observed. This effect was attributed to a reduction in sperm motility. In females receiving 200 mg Zn^{2+}/kg bw, reduced conception was observed when they were dosed after mating, but not when they were dosed before and during pregnancy. It is not known whether the reduced sperm motility in males and the contradictory effects on conception in females are a direct effect of zinc on the sperm cells, embryos or uterine function, or whether they are the result of disturbances in other physiological functions. From a study by Schlicker and Cox (1968), it is known that this dose level (and even levels of 100 mg additional Zn^{2+}/kg bw/day) may result in impaired copper balance in females.

In repeated dose toxicity studies with zinc sulphate heptahydrate, no effects on the reproductive organs were seen at dose levels up to ca. 1,100 mg and 565 mg Zn^{2+}/kg bw/day for mice and rats, respectively. In a repeated dose toxicity study with zinc monoglycerolate hypoplasia of several sex organs was observed at doses of ca. 300 mg Zn^{2+}/kg bw/day, but not at 13 or 60 mg Zn^{2+}/kg bw/day. As these effects were only seen at dose levels which produced very severe general toxicity, it is impossible to conclude that these adverse effects are directly related to zinc. It should be noted that these studies are not designed to detect effects on sperm cell motility.

Developmental toxicity studies, according to a study design similar to OECD 414, with mice, rats, hamsters and rabbits were described with unspecified zinc sulphate. These studies do not permit the derivation of a proper NOAEL because neither reproductive nor developmental or maternal effects were observed, not even at the highest dose tested. When it is assumed (worst-case) that the heptahydrate was administered from the study with hamsters it can be calculated that the NOAEL for both maternal effects and effects on the offspring is at least 19.9 mg Zn^{2+}/kg bw/day. In other (non-guideline) studies, higher dose levels (up to 200 Zn^{2+}/kg bw/day) have been reported to result in resorptions and retarded foetal growth, but not in external malformations. No resorptions and growth retardation were seen at 100 mg Zn^{2+}/kg bw/day but as the study was too limited, this dose level cannot be taken as an NOAEL for developmental toxicity, either. Besides, at both 100 and 200 mg Zn^{2+}/kg bw/day changes in maternal and fetal copper status were observed. In absence of better information a NOAEL of > 19.9 mg Zn^{2+}/kg bw/day for developmental toxicity in animals is adopted.

In studies with pregnant women receiving additional 0.3 mg Zn^{2+}/kg bw/day (as zinc sulphate or citrate) during the last 6 months of pregnancy, no reproductive or developmental effects were observed. Clear evidence of zinc toxicity in human pregnancy has not been reported but this may be due to the fact that very high exposures to zinc in human pregnancy are unusual. In contrast, zinc deficiency during pregnancy can cause a variety of adverse effects on the foetus or may result in reduced fertility or delayed sexual maturation in animals as well as in humans (Walsh et al., 1994; ATSDR, 1994; WHO, 1996).

Hence, with respect to effects on reproduction, zinc deficiency is known to result in impairment of fertility and of foetal development. In humans additional zinc up to 0.3 mg Zn^{2+}/kg bw/day during pregnancy did not result in adverse effects. Available data in animals on zinc excess indicate that adverse effects on fertility and foetal development may occur at dose levels of 200 mg Zn^{2+}/kg bw/day, in conjunction with other effects such as perturbation of parental and foetal copper homeostasis. In humans a small disturbance (if any) of normal physiology, presumably indicative for copper deficiency, has been demonstrated at zinc excess of 50 and 150 mg Zn^{2+}/day (0.83 and 2.5 mg Zn^{2+}/kg bw/day, respectively), while 150 mg Zn^{2+}/day (2.5 mg Zn^{2+}/kg bw/day) resulted in clinical signs. As the margin between the dose at which in humans clinical signs are manifest and the dose at which in animals reproductive effects have been reported is so high (viz. 80), it is considered unlikely that in humans reproductive effects will occur at exposure levels at which clinical signs are not manifest. Therefore, neither fertility nor developmental toxicity are considered end-points of concern for humans.

Zinc can interact with other trace elements, especially copper, resulting in toxicity which is usually due to depletion of these elements, leading to nutritional deficiencies. In some older studies, it has been suggested that supplemental zinc at a level of 50 mg/day impaired both the iron and copper status, but these effects were not observed in more recent interaction studies. At least part of the interaction between zinc and other metals such as copper may be related to the effect of zinc on metallothionein.

Zinc is an essential element required for the function of a large number of enzymes. It plays a role in DNA and RNA synthesis and many other processes in the body. A zinc deficiency in the diet can lead to notable health effects. Recommended daily zinc levels range from 5 mg/day for infants to 19 mg/day for women during lactation.

For the risk characterisation, an overall oral NOAEL of 50 mg Zn^{2+}/day (0.83 mg/kg bw/day) is set on the human volunteer study by Grand Forks (Davis et al. 2000; Milne et al., 2001). Given that this study was with women (the most sensitive population in zinc supplementation studies), and that in women clinical signs begin to appear only at a dose three times this NOAEL, a minimal MOS of 1 is considered sufficient when comparing the human NOAEL with the exposure levels for workers/consumers/general population.

Note: In the absence of useful dermal and inhalation toxicity studies, in the risk characterisation no distinction is made for systemic exposure to zinc via oral, dermal or inhalation exposure. For inhalation exposure this seems reasonable, given that the majority of the inhaled zinc is cleared via the gastro-intestinal tract. It is not entirely clear whether this route-to-route extrapolation, using the oral NOAEL as starting point, is also justified for dermal exposure. This because it is not certain whether the effects of zinc on copper homeostasis at higher doses are only the result of a local interference of zinc with the regulation of copper absorption or that also systemic factors are involved. For a worst-case approach it will be assumed that it is possible to evaluate the systemic effects after dermal exposure to zinc based on the oral NOAEL.

Previously, other organisations have evaluated the toxicity data of zinc, also taking into account that zinc is an essential element. In these evaluations the information generated in the Grand Forks study has not been considered, because this study is of more recent date. For sake of completeness the opinions of these organisations are given below.

In 1982, the WHO set a provisional maximum tolerable daily intake for zinc at 0.3-1.0 mg/kg bw (basis not quite clear). Later on, several scientific committees have based their recommendation for a maximum daily intake (EU, 1993; Gezondheidsraad, 1998) or oral reference dose (US EPA, 1992) on the study in humans by Yadrick et al. (1989). This study was also taken into account by WHO in 1996. Because the dose of 50 mg Zn^{2+} was additional to the amount of zinc that was already in the normal diet (approximately 10 mg Zn^{2+}/day), the US EPA (1992) recalculated the LOAEL to be approximately 60 mg/day (1 mg/kg bw/day). By using an uncertainty factor of 3 (based on a minimal LOAEL from a moderate duration study of the most sensitive humans and consideration of a substance that is an essential dietary nutrient) they set an oral reference dose of 0.3 mg/kg bw/day for zinc and zinc compounds. The EU (1993) stated that as "short-term intakes of about 50 mg zinc daily interfered with the metabolism of both iron and copper (Yadrick et al., 1989) ..... it would be unwise to exceed a daily zinc intake of 30 mg in adults". The Dutch Health Council (Gezondheidsraad, 1998) followed this recommendation. The WHO (1996) stated that "interactions with other nutrients influencing their absorption and utilization have been detected biochemically at total zinc intakes as low as 60 mg/day when zinc was given in the form of a supplement to a diet that, it is reasonable to assume, already provided 10 mg of zinc/day". In order "to ensure that very few individuals in a population have an intake of zinc of 60 mg or higher, the Expert Consultation recommended that the adult population mean intake should not exceed 45 mg if a 20% variation in intake is assumed" (WHO, 1996).

4.1.3.2 Workers

Assuming that oral exposure is prevented by personal hygienic measures, the risk characterisation for workers is limited to the dermal and inhalation routes of exposure.

4.1.3.2.1 Acute toxicity

Based on the results from the acute dermal toxicity study with zinc sulphate heptahydrate there is no concern with respect to acute toxicity after dermal exposure: **conclusion (ii)**.

No acute inhalation studies, performed according to the guidelines, with zinc sulphate are available. However, in view of the results from the acute inhalation studies with zinc sulphate in hamsters and dogs, it is concluded that there is no concern with respect to acute toxicity after inhalation exposure: **conclusion (ii)**.

4.1.3.2.2 Irritation and corrosivity

Skin

Based on the findings in a well performed skin irritation study it is concluded that zinc sulphate is not irritating or corrosive to the skin and therefore is of no concern for workers with regard to acute skin irritation and corrosivity: **conclusion (ii)**.

Eyes

Based on a well performed eye irritation/corrosion study zinc sulphate is considered to induce severe ocular irritation. Therefore, it is concluded that zinc sulphate is of concern for workers with regard to eye irritation. However, if the required protection (based on current R36/38 Annex 1 classification) is strictly adhered to, **conclusion (ii)** is justifiable. It is noted that based on the available data classification with R41 is warranted (see Section 4.1.2.4).

Respiratory tract

In the available inhalation studies in hamsters and dogs, no details were given whether zinc sulphate was irritating to the respiratory tract. In hamsters respiratory exposure for 4 hours to 5.2-34.2 mg $ZnSO_4.7H_2O/m^3$ caused effects on lung macrophages, which were absent at 1.3 mg/m^3. The observed decrease in phagocytosis by lung macrophages is however considered a functional response to the inhalation exposure to aerosols. These effects are not seen as adverse nor indicative of respiratory irritation. Furthermore, respiratory exposure for 4 hours to 4.1-8.8 mg $ZnSO_4/m^3$ in dogs showed no effects on e.g. breathing mechanics.

Finally, it is noted that finely dispersed solutions of $ZnSO_4$ generated under laboratory conditions are not representative of occupational circumstances. The particle size distribution of zinc sulphate monohydrate and hexa/heptahydrate (see Section 4.1.1.2) will significantly reduce the potential for inhalation exposure. Therefore, it is concluded that zinc sulphate (both monohydrate and hexa/heptahydrate) is of no concern for workers with regard to acute respiratory irritation: **conclusion (ii)**.

4.1.3.2.3 Sensitisation

In a well performed maximisation test and in a mouse local lymph node assay, zinc sulphate heptahydrate was not sensitising. Therefore, it is concluded that zinc sulphate is of no concern for workers with respect to sensitisation: **conclusion (ii)**.

4.1.3.2.4 Repeated dose toxicity

Because there are no dermal and respiratory repeated dose toxicity studies available, risk characterisation for local skin and respiratory effects after repeated exposure to zinc sulphate cannot be described and it is unknown whether local or systemic effects of $ZnSO_4$ are critical. Risk characterisation is limited to the systemic effects of the Zn^{2+}-ion.

The NOAEL of 50 mg Zn^{2+}/day derived from a 10-week oral study with human volunteers is used as a starting point for the risk characterisation for repeated dose toxicity. This NOAEL of 50 mg Zn^{2+}/day results in an internal NOAEL of 10 mg Zn^{2+}/day by correction for oral absorption (20%; worst case, because of the homeostasis the relative absorption will be smaller by excess of Zn^{2+}-intake (see Section 4.1.2.1.6)). The occupational health risk due to the $ZnSO_4$ exposure is determined, comparing the internal NOAEL of 10 mg Zn^{2+}/day with the internal occupational exposure.

The dermal and respiratory exposure levels of $ZnSO_4$ for the occupational scenarios (see Section 4.1.1.2 and Table 4.5) are estimated. The reasonable worst-case exposure levels are used as a starting point in determining the internal exposure level due to occupational exposure, by correction for dermal and inhalation absorption, respectively. For zinc sulphate, 40% respiratory

absorption is assumed (see Section 4.1.2.2). For dermal absorption 0.2% is taken into account for exposure to dust (all scenarios).

The MOSs between the internal NOAEL and the internal occupational exposure estimates are mentioned in **Table 4.14**. The MOSs are evaluated by comparison with the minimal MOS. Since the NOAEL that is used as a starting point is derived from a study with human volunteers, a minimal MOS of 1 is considered appropriate (see Section 4.1.3.1). There is concern when the calculated MOS is significantly lower than the minimal MOS.

Table 4.14 Occupational risk assessment of zinc sulphate for repeated dose toxicity after dermal and inhalation exposure (systemic effects)

Scenario / subscenario #	Risk characterisation for dermal and inhalation exposure			
	Estimated external dermal exposure in mg Zn^{2+}/day; between brackets internal exposure in mg Zn^{2+}/day a)	MOS b)	Estimated external inhalation exposure in mg Zn^{2+}/m^3; between brackets internal exposure in mg Zn^{2+}/day c)	MOS b)
1a) The production of zinc sulphate monohydrate	1,050 (2.1)	4.8	0.9 (3.6)	2.8
1b) The production of zinc sulphate hexahydrate	810 (1.6)	6.3	0.2 (0.8)	13
2a) Production of animal feedstuff	1140 (2.3)	4.3	0.5 (2)	5
2b) Production of fertilisers	100 (0.2)	50	0.2 (0.8)	13
3) Use of fertiliser	59 (0.1)	100	0.02 (0.08)	125

The risk assessment is only based on full-shift exposure levels. It is noted that possible higher risks resulting from daily performance of activities associated with higher short-term exposures is not accounted for

a) Estimated internal dermal exposure to Zn^{2+} used for calculating the risk, assuming a dermal absorption of 0.2%.

b) MOS values based on comparison of the internal NOAEL of 10 mg Zn^{2+}/day with the internal exposure

c) Estimated internal inhalation exposure to Zn^{2+} used for calculating the risk, assuming a respiratory absorption of 40% and a respiratory volume of 10 m^3 for a worker/day

Given the calculated MOS values for dermal and inhalation exposure as mentioned in Table 4.14, it is concluded that, based upon the present information, no health risks are expected for dermal or inhalation exposure in any occupational scenario: **conclusion (ii)**.

The risk characterisation for systemic effects is made with several assumptions:

- the internal values are calculated with worst-case assumptions for percentages absorption,
- it is assumed that other factors influencing route-specificity are not of importance. In case of Zn^{2+}, metabolism does not play a role, which favours this assumption,
- the human study was not performed with $ZnSO_4$, so it is assumed that the effects are due to Zn^{2+},
- the background intake of zinc in the experimental situation (human) and in workers are comparable,
- the background intake via food is considered to be comparable in the different EU-countries,
- physiological role of Zn^{2+} is comparable between species.

The NOAEL was derived from the human volunteer study, in which a restricted amount of parameters was used. As the toxicity study with zinc monoglycerolate with rats showed more specific adverse effects (pancreas), the results from this toxicity study can be used for comparison. However, a study with zinc sulphate itself is available and should be used for comparison. Starting with the NOAEL of 234 mg $ZnSO_4 \cdot 7H_2O$/kg bw/d (corresponding with 131 mg $ZnSO_4$/kg bw/d and 53.5 mg Zn^{2+}/kg bw/d) from a 13-week study with rats, results in an internal NOAEL of 21 mg Zn^{2+}/kg bw/d or 1,484 mg Zn^{2+}/day for a 70-kg worker (see Appendix A). The calculated MOSs range from 645-14,840 and 412-18,550 for dermal and inhalation exposure, respectively. After comparing these values with the minimal MOS of 360 (see Appendix A), it is concluded that risk characterisation based on the human study is adequate to protect also against adverse effects as observed in animal studies.

Combined exposure

The assessment of the risk after combined exposure (i.e., the risk due to the internal exposure resulting from both the dermal and the inhalation exposure) can only be made with the assumption that both dermal and inhalation exposure contribute to the internal exposure every working day. The total internal occupational exposure 0.18-5.7 mg Zn^{2+}/day (see Table 4.14) compared to the internal NOAEL of 10 mg Zn^{2+}/day results in MOS values of 1.8-56. Based on these calculations, it is concluded that combined exposure to zinc sulphate is of no concern for workers: **conclusion (ii)**.

4.1.3.2.5 Mutagenicity

Given the results from the mutagenicity studies, it is concluded that zinc sulphate is of no concern for workers with regard to mutagenicity: **conclusion (ii)**.

4.1.3.2.6 Carcinogenicity

There are no adequate carcinogenicity studies available. However, because at the moment, there is no experimental or epidemiological evidence for carcinogenicity after exposure to zinc or zinc compounds, there is no reason to require a carcinogenicity study: **conclusion (ii)**.

4.1.3.2.7 Toxicity for reproduction

There are no indications that Zn^{2+} caused adverse effects on fertility based on the results of the oral repeated dose toxicity study in rats with zinc monoglycerolate **conclusion (ii)**. Furthermore, there are no indications that Zn^{2+} is of concern for developmental effects based on the results of developmental toxicity studies in different species (mice, rats, hamsters and rabbits) and several studies in which pregnant women were exposed to soluble zinc compounds: **conclusion (ii)**.

4.1.3.2.8 Occupational Exposure Limits

At the moment, occupational limit values for zinc sulphate have not been established.

4.1.3.3 Consumers

Table 4.15 Consumer exposure estimates

	Internal exposure (compound specific)	Internal exposure (not compound specific)
Zinc metal	negligible	
Zinc oxide	2.5 mg Zn^{2+}/day (5.1 including medically used zinc oil)	
Zinc chloride	0.2 mg Zn^{2+}/day	
Zinc sulphate	0.00046 mg Zn^{2+}/day	
Zinc phosphate	0.045 mg Zn^{2+}/day	
Zinc distearate	0.0062 mg Zn^{2+}/day	
Personal care products used regularly		1.6 mg Zn^{2+}/day

Only data on the use of zinc sulphate in eye drops are available. For this use, a consumer exposure of 0.00046 mg zinc/day was calculated.

4.1.3.3.1 Acute toxicity/Irritation/Corrosivity/Sensitisation

Given the data available, it is concluded that zinc sulphate is of no concern for consumers with respect to acute toxicity, skin and respiratory tract irritation, corrosivity and skin sensitisation: **conclusion (ii)**. Given the low concentration in eye drops, the minimal amount that consumers will use and the resulting low exposure, it is also expected that eye irritation is of no concern for consumers: **conclusion (ii)**.

4.1.3.3.2 Repeated dose toxicity

Starting point for the risk characterisation for systemic effects is the human oral NOAEL of 50 mg zinc/day. Assuming 20% absorption, this NOAEL corresponds to an internal dose of 10 mg zinc/day. The MOS between this (internal) NOAEL and the internal exposure by the use of eye drops (0.00046 mg/day) is > 20,000.

However, consumer products containing zinc sulphate are probably not used regularly. Besides, consumers can also be exposed to other zinc compounds in consumer products, some of which may be used on a regular basis (more than once a week). The use of regularly used products (dandruff shampoo, deodorant, eye shadow, and possibly baby care ointment) results in a cumulative (internal) exposure of approximately 1.6 mg zinc/day (see Section 4.1.1.3 and Table 4.15). Comparing the (internal) NOAEL with this more realistic exposure, a MOS of 6.25 can be calculated.

These MOSs are considered sufficient (see Section 4.1.3.1), and it can be concluded that there is no concern for consumers (**conclusion (ii))**, neither for zinc sulphate nor for regularly used zinc compounds taken together.

4.1.3.3.3 Mutagenicity/Carcinogenicity/Toxicity for reproduction

Given the results from the mutagenicity studies, it is concluded that zinc sulphate is of no concern for consumers with regard to mutagenicity: **conclusion (ii)**.

As there is no experimental or epidemiological evidence for carcinogenicity, there is no concern for carcinogenicity: **conclusion (ii)**.

Given the data available, it is concluded that zinc sulphate is of no concern for reproductive toxicity: **conclusion (ii)**.

4.1.3.4 Humans exposed via the environment

4.1.3.4.1 Repeated dose toxicity

General exposure

For zinc, the ingestion of foods appears to be the most important exposure route for the general population, compared to which the intake by drinking water and ambient air is negligible. Recently, the average dietary intake of zinc is reported to be around 10 mg/day with a minimum of 0.6 mg and a maximum 39 mg. Both the reported average intake and the maximum intake are well below the human oral NOAEL of 50 mg/day and also well below the upper limit of safe intake as recommended by WHO (45 mg/day; 1996).

Hence, it can be concluded that there is no concern for the general population exposed indirectly to zinc via the environment: **conclusion (ii)**.

Local exposure

Starting point for the risk characterisation for systemic effects are the local PEC_{add}s in air and water as presented in Section 4.1.1.4.2 and the human oral NOAEL of 50 mg zinc/day. Assuming 20% absorption, this NOAEL corresponds to an internal dose of 10 mg zinc/day. The local PEC_{add}s in air and water are converted to internal doses by correction for inhalatory and oral absorption (40% and 20%, respectively), and by assuming a breathing volume of 20 m^3/day and a drinking water consumption of 2 l/day (see **Table 4.16**).

Table 4.16 Internal exposure levels via water and air at local scale

	PEC_{add}-water (in µg/l)	Internal exposure (in mg zinc/day)	PEC_{add}-air (in µg/m^3)	Internal exposure (in mg zinc/day)
Production	-	-	0.078	0.00062
Processing	410	0.16	0.928	0.0074

Comparing the (internal) NOAEL with the internal exposures, MOSs are in the range 62.5-16,129. These MOSs are considered sufficient (see Section 4.1.3.1), and it can be concluded that there is no concern for human health: **conclusion (ii)**. Moreover, it must be noted that the internal exposure via water is an overestimate. It is based on untreated surface water, which nowadays in the EU is not directly representative for drinking water.

4.1.3.4.2 Mutagenicity/Carcinogenicity/Toxicity for reproduction

General and local exposure

Given the results from the mutagenicity studies, it is concluded that zinc sulphate is of no concern with regard to mutagenicity for the general population exposed indirectly to zinc via the environment: **conclusion (ii)**.

As there is no experimental or epidemiological evidence for carcinogenicity, there is no concer for carcinogenicity: **conclusion (ii)**.

Given the data available, it is concluded that zinc sulphate is of no concern for reproductive toxicity: **conclusion (ii)**.

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

n.a.

4.2.2 Effects assessment: Hazard identification

4.2.2.1 Explosivity

Test data on explosive properties are not available. However, on theoretical considerations the substance is concluded not to be explosive.

4.2.2.2 Flammability

Test data on flammable properties are not available. However, on theoretical considerations the substance is concluded not to be flammable.

4.2.2.3 Oxidising potential

Test data on oxidising properties are not available. However, on theoretical considerations the substance is concluded not to be oxidising.

4.2.3 Risk characterisation

Given the physico-chemical data, zinc sulphate is considered not to form a risk with respect to explosive, flammable and oxidising properties: **conclusion (ii)**.

5 RESULTS

5.1 ENVIRONMENT

n.a.

5.2 HUMAN HEALTH

5.2.1 Human health (toxicity)

5.2.1.1 Workers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

The information available gives no reasons for concern for adverse health effects due to zinc sulphate exposure at the workplace.

Table 5.1 Overview of conclusions with respect to occupational risk characterisation

End point	Conclusions valid for the occupational scenarios									
	Scenario 1a		Scenario 1b		Scenario 2a		Scenario 2b		Scenario 3	
	MOS	conclusion	MOS	conclusion	MOS	conclusion	MOS	conclusion	MOS	conclusion
Acute toxicity										
- dermal	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii
- inhalation	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii
Irritation and corrosivity, single exposure										
- dermal	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii
- inhalation	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii
- eyes	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii
Sensitisation										
- dermal	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii
- inhalation	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii
Repeated dose toxicity, systemic effects										
- dermal	4.8	ii	6.3	ii	4.3	ii	50	ii	100	ii
- inhalation	2.8	ii	13	ii	5	ii	13	ii	125	ii
- combined	1.8	ii	4.2	ii	2.3	ii	10	ii	56	ii
Mutagenicity	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii
Carcinogenicity	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii

Table 5.1 continued overleaf

Table 5.1 continued Overview of conclusions with respect to occupational risk characterisation

End point	Conclusions valid for the occupational scenarios									
	Scenario 1a		Scenario 1b		Scenario 2a		Scenario 2b		Scenario 3	
	MOS	conclusion	MOS	conclusion	MOS	conclusion	MOS	conclusion	MOS	conclusion
Reproductive toxicity										
Fertility	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii
Developmental effects										
- dermal	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii
- inhalation	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii
- combined	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii	n.a.	ii

n.a not applicable

5.2.1.2 Consumers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

5.2.1.3 Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

5.2.2 Human health (physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

6 REFERENCES

The reference list applies to zinc and the five zinc compounds.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
BOD	Biochemical Oxygen Demand
bw	body weight / *Bw*, *bw*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CEPE	European Committee for Paints and Inks
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 90 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
foc	Organic carbon factor (compartment depending)
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives

JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OC	Organic Carbon content
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic

PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex IV of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
ThOD	Theoritical Oxygen Demand
UC	Use Category
UDS	Unscheduled DNA Synthesis

UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
VOC	Volatile Organic Compound
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organization
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

Appendix A Internal NOAEL and minimal MOS calculation based on the NOAEL from the repeated dose study in the rat

Toxicological starting-point for the calculation of the internal NOAEL for systemic effects of Zn^{2+} due to $ZnSO_4$ exposure is the NOAEL of 234 mg $ZnSO_4 \cdot 7H_2O$/kg bw/day (corresponding with 131 mg $ZnSO_4$/kg bw/day and 53 mg Zn^{2+}/kg bw/d) from the 13-week study with rats. For oral absorption a value of 40% is used for the rat study (worst case estimations) (see Section 4.1.2.1.6), resulting in an internal NOAEL of 21 mg Zn^{2+}/kg bw/d or 1,484 mg Zn^{2+}/day for a 70 kg worker.

The risk characterisation for systemic effects is made with several assumptions:

- the internal NOAEL is calculated with worst case assumptions for oral absorption,
- it is assumed that other factors influencing route specificity are not of importance. In case of Zn^{2+}, metabolism does not play a role, which favours this assumption,
- the background intake and requirement of zinc in the experimental situation (rats) and in workers are assumed to be comparable,
- the physiological role of zinc is comparable between rat and man.

Dermal and inhalation exposure

Given the estimated frequency of exposure (100-200 d/year), chronic exposure is assumed for risk characterisation.

The assessment factors applicable for the calculation of the minimal MOS are mentioned in **Table A1**.

Table A.1 Assessment factors applied for the calculation of the minimal MOS

Aspect	Assessment factors applied on oral NOAEL
Interspecies differences	4 · 3 a)
Intraspecies differences	3
Differences between experimental conditions and exposure pattern of the worker	10
Type of critical effect	1
Dose-response curve	1
Confidence of the database	1 b)
Overall	360

a) Extrapolation based on differences in caloric demands, together with a factor 3 for remaining uncertainties
b) Database exists of the available toxicological data with zinc and zinc compounds

The minimal MOS amounts to 360 when the 13-week oral toxicity study in rats with $ZnSO_4 \cdot 7H_2O$ is taken as a starting point for the risk characterisation for repeated dose toxicity.

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Zinc sulphate, Volume 46

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Environment and quality of life series

The report provides the comprehensive risk assessment of human health part of the substance zinc sulphate. It has been prepared by the Netherlands in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans and the environment, laid down in Commission Regulation (EC) No. 1488/94.

The evaluation considers the emissions and the resulting exposure to the environment and the human populations in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined. For human health the scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The human health risk assessment for zinc sulphate concludes that there is no concern for workers, consumers and humans exposed via the environment.

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