GreenScreen® Chemical Assessment

Zinc Oxide (1314-13-2)

Method Version: GreenScreen® Version 1.4

Assessment Details:

Assessment Type:	Certified
Assessment Prepared By:	WAP Sustainability Consulting, LLC (Eric Rosenblum Ph.D. DABT Donald K. Ward, NSF International)
Assessment Prepared For:	Tile Council of North America (TCNA)
Date Assessment Completed:	January 31st, 2021
Assessment Expiration Date:	N/A
Assessor Type:	Licensed GreenScreen® Profiler

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GREENSCREEN BENCHMARK™ SUMMARY:

This chemical assessment report includes a GreenScreen Benchmark™ score and results for Zinc Oxide (1314-13-2) only. No marketing claims can be made without licensing through Clean Production Action.

GreenScreen Benchmark Score: BM-1

Zinc Oxide (1314-13-2) was assigned a GreenScreen® Benchmark Score of 1 ("Avoid—Chemical of High Concern") based on Very High persistence combined with High Group II* Human Toxicity (repeat dose systemic) or Very High persistence combined with Very High Ecotoxicity (chronic aquatic toxicity). This corresponds to GreenScreen® benchmark classification 1b ("vPT = very High P + [very High T (Ecotoxicity or Group II Human) or High T (Group I or II* Human)]") in CPA 2018. Data gaps (DG) also exist for carcinogenicity, single dose neurotoxicity, and bioaccumulation. As outlined in CPA (2018) Section 13.2 (Step 8 – Conduct a Data Gap Analysis to assign a final Benchmark score), zinc oxide meets requirements for a GreenScreen® Benchmark Score of 1 despite the hazard data gaps. In a worst-case scenario, if zinc oxide were assigned a High score for any of the data gaps it would continue to be categorized as a Benchmark 1 Chemical.

HAZARD CLASSIFICATION SUMMARY

Table 1. GreenScreen Hazard Summary Table:

GreenScreen Hazard Summary Table for Zinc Oxide – BM-1																			
Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
Carcinogenicity	Genotoxicity/Mutagenicity	Reproductive Toxicity	Developmental Toxicity	Endocrine Activity	Acute Toxicity	Systemic Toxicity		Neurotoxicity		Skin Sensitization*	Respiratory Sensitization*	Skin Irritation	Eye Irritation	Acute Aquatic Toxicity	Chronic Aquatic Toxicity	Persistence	Bioaccumulation	Reactivity	Flammability
						single	repeat*	single	repeat*	*	*								
DG	*M*	*M*	L	M	L	M	H	DG	*M*	L	H	L	L	vH	vH	vH	DG	L	L

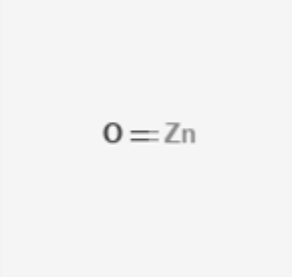
Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in italics reflect lower confidence in the hazard classification while hazard levels in BOLD font reflect higher confidence in the hazard classification. Group II Human Health endpoints differ from Group II* Human Health endpoints in that Group II Human Health endpoints have four hazard scores (i.e., vH, H, M and L) instead of three (i.e., H, M and L), and are based on single exposures instead of repeated exposures. Group II* Human Health endpoints are indicated by an * after the name of the hazard endpoint or after "repeat" for repeated exposure sub-endpoints.

SCOPE OF ASSESSMENT

Zinc Oxide (1314-13-2):

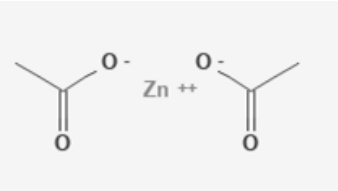
Also Called: Lassar Paste; Oxozinc; Zinc White; Amalox; Permanent white; Chinese white; Snow white; Emanay zinc oxide; Felling zinc oxide; Flowers of zinc (PubChem 2019)

Chemical Structure:

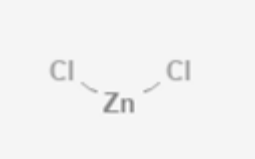


Suitable analogs or moieties of chemicals used in this assessment (CASRN(s)):

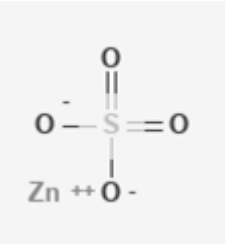
Chemical Structure(s) of suitable analog(s) and/or moieties:



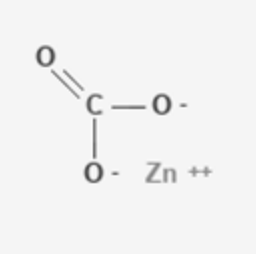
Zinc acetate
(CAS# 557-34-6)



Zinc chloride
(CAS# 7646-85-7)



Zinc sulfate
(CAS# 7733-02-0)



Zinc carbonate
(CAS# 3486-35-9)

For Inorganic Chemicals and relevant particulate organics (if not relevant, list NA)

Define Properties:

1. Particle size (e.g., silica of respirable size): N/A
2. Structure (e.g., amorphous vs. crystalline) : N/A
3. Mobility (e.g., water solubility, volatility) : N/A
4. Bioavailability: N/A

Identify potential applications/functional uses of the chemical:

1. Used to treat diaper dermatitis and minor skin irritations
2. Used to protect against sunburn and other skin damage
3. Used as an antimicrobial agent in food-contact polymers
4. Used as a color additive in drugs and cosmetics
5. Used as a preservative in agrochemicals (PubChem 2019)

Table 2. Environmental Transformation Products Summary

Life Cycle Stage	Transformation Pathway	Environmental Transformation Product	CAS #	Feasible (Yes or No)	Relevant (Yes or No)	GreenScreen List Translator Score or GreenScreen Benchmark Score
		None				

HAZARD CLASSIFICATION SUMMARY

GROUP I HUMAN HEALTH EFFECTS (GROUP I HUMAN)

Carcinogenicity (C): DG

Zinc oxide was assigned a hazard classification level of Data Gap for carcinogenicity based on (1) inadequate information to assess the carcinogenic potential of zinc, (2) because studies of humans occupationally-exposed to zinc are inadequate or inconclusive, (3) adequate animal bioassays of the possible carcinogenicity of zinc are not available and (4) results of genotoxic tests of zinc have been equivocal.

Lists

- Authoritative:
  - US EPA - IRIS Carcinogens (1986) Group D - Not classifiable as to human carcinogenicity
  - US EPA - IRIS Carcinogens (1999) Data are inadequate for an assessment of human carcinogenic potential
  - US EPA - IRIS Carcinogens (2005) Inadequate information to assess carcinogenic potential
- Screening: None

Measured Data

ECHA 2019

A study of one-year duration was conducted to evaluate the carcinogenic potential of the zinc sulphate in mice. Chester Beatty stock mice (newly-born litters) were used. They were housed in the metal cages (4-6 per cage). The doses of zinc sulphate were 22 g/L (5,000 ppm zinc) and 4.4 g/L (1,000 ppm zinc) in drinking water along with a control group fed a basal diet and normal drinking water. The animals were examined thoroughly once a wk throughout the experiment and daily when fed. Weighing was done once every 2 wk. Deaths of animals occurred during the first 8 wk of experiment due to an epizootic of ectromelia. The survivors were vaccinated with sheep lymph and animals showing a negative or accelerated response were sacrificed. New group of weanling mice (4 -5 wk old) were added to supplement the control group. All the surviving animals were sacrificed after 1 year of treatment and examined for gross pathology. Histopathological examination was done for suspected neoplastic lesions. Stomachs were examined for tumors and other changes in the forestomach and glandular epithelium. No differences in carcinogenic effects were observed between treatment and control groups under the test conditions. Under the test conditions, zinc sulphate was found to be non-carcinogenic in mice.

There are a range of epidemiological studies that investigated the association between zinc exposure either through occupational activities or food supplementation and increased cancer risks. While no associations were found between occupational zinc exposure and excess cancer risk, the main association that has been made in this context is related to dietary/supplemental

zinc and prostate cancer risk. In contrast to established clinical and experimental evidence that prostate cancer is associated with a decrease in the zinc uptake, numerous epidemiology studies and reports of the effect of dietary and supplemental zinc on the incidence of prostate cancer have provided divergent, inconsistent and inconclusive results which range from adverse effects of zinc, protective effects of zinc and no effect of zinc on the risk of prostate cancer. Clinical and experimental studies have established that zinc levels are decreased in prostate cancer and support a role of zinc as a tumor suppressor agent. In a recent critical assessment of epidemiology studies regarding dietary/supplemental zinc and prostate cancer risk, Costello et al., concluded that epidemiological studies have not provided an established relationship for any effect or lack thereof of dietary/supplemental zinc on the risk of prostate cancer. Proclamations of an association of dietary/supplemental zinc and increased prostate cancer are based on inconclusive and uncorroborated reports (Costello et al.,2007).

US EPA 2005

Under the U.S. EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), there is inadequate information to assess carcinogenic potential of zinc, because studies of humans occupationally-exposed to zinc are inadequate or inconclusive, adequate animal bioassays of the possible carcinogenicity of zinc are not available, and results of genotoxic tests of zinc have been equivocal.

Either zinc deficiency or excessively high levels of zinc may enhance susceptibility to carcinogenesis, whereas supplementation with low to moderate levels of zinc may offer protection (Mathur, 1979; Woo et al., 1988). For example, zinc deficiency enhanced carcinomas of the esophagus induced by MBN (Fong et al., 1978) but retarded the development of oral cancer induced by 4-NQO (Wallenius et al., 1979). Thus, zinc's modifying effect on carcinogenesis may depend on the dose of zinc as well as the carcinogen being affected.

Aughey et al. (1977) investigated the effects of supplemental zinc on endocrine glands in groups of 75 male and 75 female C3H mice by administering 0 or 0.5 g/L zinc (as zinc sulfate) in the drinking water for up to 14 months. The authors reported that the body weight in the control group ranged from 21 to 30 g, and the mean weight of the zinc-fed mice was approximately 1 g higher. Using the midpoint of the body weight range (0.022 to 0.031 kg), a water intake of 0.0069 L/day was calculated (U.S. EPA, 1988), resulting in average daily drinking water doses of 0 or 135 mg Zn/kg-day. At 1-month intervals, five mice in each of the treated and control groups were killed. After 6 months of exposure to zinc, there were no significant changes in plasma insulin or glucose levels as compared to controls. Histological alterations were observed in the pancreas, pituitary gland, and adrenal gland of zinc-exposed mice. The histological changes in the mice were first observed after 3 months of exposure to zinc. In the zinc-supplemented mice, the pancreatic islets were enlarged and had a vacuolated appearance. The β-cells of the pancreatic islets were larger with enlarged mitochondria and prominent Golgi apparatus. The severity of the pancreatic lesions appeared to increase with increasing exposure durations. Pituitary alterations consisted of changes in the adrenocorticotrophic hormone-producing cells that indicated increased synthesis and secretion, including increased number and size of granules and more prominent rough endoplasmic reticulum and Golgi apparatus. Hypertrophy of the adrenal zona fasciculata and increased adrenal cortical lipid and cholesterol deposition were also observed. No tumors were reported in the pancreas, pituitary gland, or adrenal gland of zinc-exposed mice; data on other organs were not reported. A study by Halme (1961) reported potential increases in zinc-induced tumors in a multi-generation study in rats, but was not sufficiently descriptive to allow for a complete evaluation of the study.

No suitable human or animal studies were identified which examined the carcinogenicity of zinc following chronic inhalation exposure.

ATSDR 2005

Female Porton strain mice (98–100/group) exposed to 121.7 mg zinc/m^3 of a zinc oxide/hexachloroethane smoke mixture (which produces zinc chloride), 1 hour/day, 5 days/week, for 20 weeks had a statistically significant increase in the incidence of alveologenic carcinoma (30 versus 8% in control) thirteen months after the end of exposure (Marrs et al. 1988). No increased tumor incidences were seen in mice exposed to 1, 1.3, or 12.8 mg zinc/m3. Guinea pigs and rats were also tested with similar dose levels, and no significant carcinogenic response was observed. A number of factors limit the usefulness of this study, including the presence of several compounds in the smoke that may have carcinogenic potential, the use of only female animals, and the short duration of the exposure (20 weeks).

The carcinogenicity of zinc in experimental animals following oral exposure was evaluated by Walters and Roe (1965). The incidence of tumors was not increased in mice exposed to 951 mg zinc/kg/day as zinc sulfate in drinking water for 1 year compared to controls. However, important details regarding the study protocol were lacking including the age and sex of the mice, the number of mice at the beginning of the study, the purity of the test material, and a complete list of the organs and tissues examined at necropsy. The control mice developed intercurrent disease (ectromelia), which resulted in a number of deaths; supplementary control mice were added to the study, but they were not concurrent controls. The number of animals in treated and control groups surviving at 1 year (study termination) was small (22– 28 mice/group). The exposure period (1 year) was less than the standard bioassay period (18–24 months). There were no data in the study (e.g., survival or body weight data) to indicate that a maximum tolerated dose was achieved. These limitations reduce the sensitivity of the study by Walters and Roe (1965) to detect a carcinogenic response.

Halme (1961) exposed tumor-resistant and tumor-susceptible strains of mice to zinc in drinking water. In a 5-generation study, groups of tumor-resistant mice (strain not specified) received 0, 10, 20, 50, 100, or 200 mg zinc/L as zinc chloride in the drinking water. The spontaneous tumor frequency for this strain of mice was 0.0004%. The tumor frequencies were reported as: F0=0.8%; F1=3.5%; F1 and F2=7.6%; and F3 and F4=25.7%. The majority of the tumors were seen in the 10- and 20-mg zinc dose groups. No individual or group tumor incidence data were reported, and a discussion of statistical analysis was not included. In the tumor-susceptible mice, strains C3H and A/Sn received 10–29 mg zinc/L in their drinking water for 2 years; 33/76 tumors were observed in the C3H strain (31 in females) and 24/74 tumors were observed in the A/Sn strain (20 in females). Most of the tumors were reported to be adenocarcinomas, but the tissues in which they occurred were not reported. The numbers of specific tumor types were not reported. The overall tumor frequencies (43.4% for C3H and 32.4% for A/Sn) were higher than the spontaneous frequency (15% for each strain), but statistical analyses were not reported.

Estimated Data - None

Mutagenicity/Genotoxicity (M): M

Zinc oxide was assigned a hazard classification level of Moderate for mutagenicity based on available data indicating that the genotoxicity results vary widely. Specifically, 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. Equivocal results have also been reported from in vivo tests for mutagenicity. The hazard score of Moderate is based on equivocal findings (both positive and negative results) and therefore is reported as low confidence.

Lists

- Authoritative: None
- Screening: None

Measured Data

ECHA 2019

Several in vitro studies and one in vivo study are available on the genotoxicity of zinc oxide. Data on other zinc compounds have also to be taken into account, 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. The genotoxicity of soluble and slightly soluble zinc compounds have been extensively investigated in a wide range of in vitro and in vivo studies. The in vitro investigations included non-mammalian and mammalian test systems covering the endpoints of gene mutation, chromosomal aberrations, sister chromatide exchange, unscheduled DNA synthesis (UDS), as well as cell transformation. Available in vivo genotoxicity assays included the micronucleus test, sister chromatide exchange (SCE) and chromosomal aberration test and the dominant lethal mutation assay in mouse or rat as well as investigations for sex-linked recessive lethal mutation in drosophila melanogaster.

The investigated zinc compounds did not increase the mutation frequencies in the majority of bacterial or mammalian cell culture systems. For example, zinc chloride, zinc sulphate, zinc bis(dihydrogen phosphate), zinc oxide or zinc monoglycerolate were consistently negative in the Ames test. While zinc chloride was also negative for gene mutations in the mouse lymphoma assays, there was some evidence that zinc oxide, zinc acetate or zinc monoglycerolate induced in the absence of metabolic activation the formation of mutation colonies. Several reviewers noted, however, that these mutations were observed at cytotoxic concentrations and that the analysis did not distinguish between big and small colonies which could be caused by gene mutation or chromosomal aberrations (Thompson et al.,1989, WHO, 2001; EU RAR, 2004; MAK, 2009).

Conflicting information was further found when zinc compounds were examined for their potential to induce chromosomal aberrations or sister chromatide exchange in mammalian cell systems or when evaluated in the cell transformation assay. Positive as well as negative results were obtained in these cell systems with either soluble or slightly soluble zinc compounds. In those studies where chromosomal aberrations or sister chromatide exchange has been observed, these were generally considered to be weak and occurred only at high, often cytotoxic concentrations. Moreover, these positive in vitro findings have also to be seen in context of the impact that changes in zinc levels can have on cell system processes that are controlled by a strict metal homeostasis. A change of this metal homeostasis due to increased zinc levels, may lead to a binding of zinc to amino acids like

cystein and therefore to an inhibition of certain enzymes. This can lead to interactions with the energy metabolism, signal transmission and apoptotic processes which can lead to the observed clastogenic or aneugenic effects in in vitro systems (EU RAR, 2004; MAK, 2009).

In addition to above mentioned in vitro investigations, various soluble and slightly soluble zinc compounds have also been studied in a range of in vivo studies including the micronucleus test, SCE and chromosomal aberration test or dominant lethal mutation assay in mice or rats as well as in the Drosophila Melanogaster SLRL test. The zinc compounds were consistently negative in the micronucleus and in the assay with Drosophila Melanogaster. Zinc sulphate was further negative in a dominant lethal assay in rats.

Equivocal and sometimes contradicting results were found for the induction of chromosomal aberrations which have been studied in bone marrow cells harvest from animals exposed to zinc compounds zinc chloride, and zinc oxide. Negative findings for chromosome aberrations have been produced after intraperitoneal injection of zinc chloride into mice (Vilkina et al., 1978) or when rats were given zinc sulphate by gavage once or daily for 5 consecutive days (Litton Bionetics, 1974). In contrast, increased aberrations have been reported in rats after inhalation exposure to zinc oxide (Voroshilin et al., 1978), in rats after oral exposure to zinc chloride and in mice after multiple intraperitoneal injections of zinc chloride (Gupta et al., 1991). Moreover, increased chromosomal aberrations were found in calcium-deficient mice when fed zinc (in form of zinc chloride) via the diet (Deknudt, 1982).These equivocal finding likely a reflection of inter-study differences in routes, levels, and duration of zinc exposure, the nature of lesions scored (gaps compared to more accepted structural alterations) and great variability in the technical rigour of individual studies (WHO, 2001). The German MAK committee reviewed the existing in vivo evidence and concluded that particularly those studies indicating clastogenic effects involved a lot of methodological uncertainties which do not allow overruling those in vivo studies which did not provide any evidence for chromosomal aberrations in vivo. Moreover, the Dutch rapporteur of EU risk assessment of zinc compounds under the EU existing substance legislation considered the positive in vitro findings for chromosomal aberration and SCE assays to be overruled by the overall weight of evidence of negative in vivo tests for this endpoint (EU RAR, 2004).

The only identified publicly available genotoxicity study in humans related to the identification of chromosomal aberrations in lymphocytes of 24 workers in a zinc smelting plant (Bauchinger et al.,1976). This study was, however, not suitable to draw any conclusions to the association of these effects with zinc exposure, as the workers displayed also increased blood levels of lead and cadmium, and the clastogenic effects were predominantly attributed to cadmium exposure. There were no further reports in the accessible literature on genotoxic effects of zinc compounds in human populations.

Nano-ZnO did not increase the mutation frequencies in bacterial cell culture systems: it was consistently negative in the Ames test. There was some evidence that nano-ZnO induced the formation of mutation colonies. However, significantly increased mutation frequencies were always linked to acute cytotoxicity. For this reason and the limited significance of the test system for particles, the test results should more likely be judged as equivocal/questionable in L5178Y/TK cells under the conditions and restrictions of this assay, implying a possible false positive result.

Nano-ZnO data have shown that DNA damage was detected in the comet assay in the absence of a metabolic activation system after exposure to uncoated ZnO nanoparticles; there was evidence that these effects were mediated by generation of oxygen species and oxidative stress. Other studies have also shown induction of DNA damage but these occurred only at high, often cytotoxic concentrations. Conflicting information was found when nano-ZnO was examined for its potential to induce chromosomal aberrations or micronucleus induction. A chromosome aberration test according to OECD Guideline 473 was performed with V79 cells using Z-COTE® HP1, Z-COTE®, and micronised zinc oxide. No increase in structural chromosome aberrations and no aneugenic activity were detected. In contrast, other data revealed clastogenic activity also below the threshold for cytotoxicity.

Systemic clastogenic and aneugenic effects were investigated in a sub-acute nose-only inhalation toxicity study in male and female Wistar rats using the bone marrow micronucleus assay (according to OECD Guideline 474). No systemic chromosome mutagenic activity was detected after inhalation exposure toZ-COTE® HP1 or uncoated ZnO nanoparticles or micronised ZnO at dose levels inducing local effects (see section repeated dose toxicity (Bellmann, 2011)) in the lung.

Z-COTE HP1 tested in the in vivo micronucleus test in bone marrow cells of mouse after single intraperitoneal injection (according to OECD Guideline 474) does not induce any chromosome damaging (clastogenic) effect, and there were no indications of any impairment of chromosome distribution in the course of mitosis (aneugenic activity) in bone marrow cells in vivo.

In mice administered via intragastric gavage both ZnO nanoparticles and ZnO microparticles, it was shown that both ZnO nanoparticles and ZnO microparticles were not considered to be clastogenic in the in vivo micronucleus assay.

DNA strand breaks and oxidative DNA damage were analysed ex vivo in BAL cells of Wistar rats using the hOGG1-modified Comet assay. DNA damage was not observed 24h after last exposure, but occurred after 14 days of recovery. The available data on genotoxicity in vitro partly supported evidence for local genotoxic effects. However, it is questionable, whether BAL cells are a suitable surrogate for epithelial cells in the lung, which are the relevant cells for tumour development. No oxidative DNA damage was detected in epithelial cells of the lung parenchyma using immunohistochemical methods indicating lack of local genotoxicity in relevant cells mediated by Reactive oxygen species (ROS). Although an increase in pro-inflammatory cytokines (coated ZnO) was observed, there is no evidence of substance specific genotoxic potential.

EU RAR 2004

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.

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/m3 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/m3 , 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).

Estimated Data - None

Reproductive Toxicity (R): M

Zinc oxide was assigned a hazard classification level of Moderate for reproductive toxicity based on a statistically significant reduction in male reproductive performance that was observed when male rats were dosed with approximately about 200 mg Zn2+/kg bw via food for 30-32 days before mating. This effect was attributed to a reduction in sperm motility. In females receiving 200 mg Zn2+/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. In addition, exposures to zinc have been associated with decreased number of implantation sites and increased number of resorptions. Due to the uncertain mechanism behind the impaired sperm motility and effect on conception in females, along with mixed positive and negative effects reported in numerous studies, the hazard score of Moderate is assigned a low level of confidence.

Lists

- Authoritative: None
- Screening:
  - GHS – Japan Toxic to reproduction - Category 2 [H361]

Measured Data

ECHA 2019

A study was conducted to determine the effects of dietary zinc supplementation on male fertility in Charles-Foster rats. 4,000 ppm zinc as zinc sulphate was fed to 18 test males in diet for 30 -32 d. 15 control males were fed normal diet for the same duration. All animals mated with individual normal females once between Day 30 and 32. After mating, males were sacrificed for sperm characterization and zinc concentration analysis in different reproductive organs. Mated females were allowed to have full term gestation. Mating by treated males caused significant lowering of incidence of conception and number of live births per mated female. However, no stillbirth or malformed litter was observed. Motility of the sperm was significantly reduced in the treated rats but viability was unaffected. Zinc content was significantly increased only in the testis and sperm of the treated rats. The results indicate that dietary zinc supplementation at 4,000 ppm reduced male fertility in rats under the conditions of the study.

Maita et al. (1981) reported that mice and rats fed with zinc sulphate 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. 1100 mg or 565 mg Zn/kg bw/day for mice and rats, respectively.

Edwards and Buckley (1995) showed that rats exposed to 13 or 60 mg Zn/kg bw/day in the diet over a period of 90 days did not show any detrimental effects on sex organs. In the exposure group of 335 mg Zn/kg bw/day, all males showed hypoplasia in testes and seminiferous tubules in males hypoplastic uterus in females, but these findings are not considered reliable as the animals of this

high dose group were generally of poor health conditions and killed for humane reasons prior to study termination.

In addition to those key reproductive toxicity studies(Khan et al.,2001, 2003, 2007; Samanta et al, 1986), some additional studies indicating high oral doses of zinc (i.e., exposures greater than 25 mg day/kg bw/day) to impair fertility as indicated by a decreased number of implantations sites and increased number of resorptions are of note:

A study was carried out to determine the effect of zinc supplementation on the number of implantation sites and resorptions in pregnant rats. The control group consisting of 12 pregnant females was maintained on 10 % vegetable protein diet (containing 30 ppm zinc) from Day 1 through Day 18 of pregnancy. The experimental group consisting of 13 animals was also maintained on the same diet, but received additionally 150 ppm zinc as a 2% zinc sulphate solution administered daily orally. All the animals were sacrificed on Day 18 of pregnancy, and their uteri examined for implantation sites and resorptions. Of a total number of 101 implantation sites in the 12 control animals there were two resorptions, one in each of two animals. In marked contrast, in the 13 zinc supplemented animals, there were 11 resorptions out of 116 implantations. Eight of the animals had at least one resorption each. This difference was statistically significant. The result indicates that oral administration of moderately high levels of zinc (150 ppm) may be associated with harmful effects in the course of pregnancy of rat (Kumar et al., 1976). The low protein diet may have affected the physiology of the animals resulting in an increased sensitivity for zinc. As this hypothesis cannot be further assessed and also considering the limited available study information, this study is only of limited validity for the assessment of effects of zinc exposure on fertility (EU RAR, 2004).

Another study aimed at determining the effect of post-coitum, and pre- and post-coitum dietary zinc supplementation on the conception in the Charles-Foster rat. In the post-coitum study (test 1), two groups of 15 pregnant rats were fed 0 and 4,000 ppm zinc as zinc sulphate in diet (i.e., approximately 200 mg Zn/kg bw/day) from day 1 through day 18 of pregnancy. In the pre- and post-coitum study (test 2), two groups of 15 female rats were treated with same doses for 21 days pre-mating period, maximum 5 days of mating period and 18 days of post-coitum period. All the females were sacrificed on Day 18 of gestation and uterus content and fetuses were examined. In test 1, significant decrease in the incidences of conception and number of implantation sites per mated female was observed in the treatment group with respect to the control group. However, the difference in implantation sites when considered per pregnant female was not significant. In test 2, no significant difference in incidences of conception and implantation sites was observed in the control and treatment groups. In both the tests, there was no treatment-related change in the fetal and placental weights, stillbirths and malformed fetuses were absent and the number of resorption sites was negligible. Based on these results, dietary zinc supplementation at 4,000 ppm did not affect the fetal growth in pregnant rats. This dose, however, altered the normal conception when started after coitus but showed no effect when initiated sufficient time before coitus (Pal et al., 1987).

The available information suggests that high oral doses of zinc (i. e., exposure levels greater than 20 mg Zn/kg bw/day) may adversely affect spermatogenesis and result in impaired fertility indicated by decreased number of implantation sites and increased number of resorptions (US EPA, 2005). However, these effects were only observed in the presence of maternal toxicity as seen in the one or two generation studies conducted by Khan et al.(2001, 2003, 2007) or, in case of the study

conducted by Kumar et al. (1976), when other study non-zinc relevant study specificities could have impacted the study outcome.

Overview of experimental key studies on fertility:

Test Substance: Zinc Chloride

Method 1: One-generation study in rats administered zinc chloride at doses of 0, 3.6, 7.2, 14.4 mg Zn/kg bw/d in water over one generation by gavage. Exposure started 77 days prior to mating

- Results:
  - As of 3.6 mg Zn/kg bw/day:
    - P - Mortality↑; body weight gain↓; fertility indext↓; thymus atrophy
    - F1 - litter size (non significant)↓; number of surviving pubs (non significant)↓;
  - As of 7.2 mg Zn/kg bw/day:
    - P – hemosidosis of spleen; lymphocyte deficiency
    - F1 - number of surviving pubs↓; BW gain (PND 21)↓
- Remarks: 2 (reliable with restrictions) key study
- Reference: Khan et al., 2001

Method 2: One-generation study in mice administered zinc chloride at doses of 0, 0.75, 1.5 and 3, mg Zn/kg bw/d (male)respectively, 0. 1.5, 3 and 6, mg Zn/kg bw/d (female) in water with 1.5mL HNO_3/l over one generation by gavage. Exposure started 49 days prior to mating

- Results:
  - 0.75 resp. 1.5 mg Zn/kg bw/day:
    - P- Mortality↑; body weight gain↓; abs./rel. Liver/thymus/ spleen weight↓; fertility indext↓; number pregnancies↓
    - F1- litter size (non significant)↓; number of surviving pubs (non significant)↓;
  - 1.5 resp. 3 mg Zn/kg bw/day:
    - P- body weight gain↓; F1– 14day survival index↓;
  - 3 resp. 6 mg Zn/kg bw/day:
    - F1– only 1 birth; 9 still births.
- Remarks: 2 (reliable with restrictions) key study
- Reference: Khan et al., 2003

Method 3: Two-generation study in rats administered zinc chloride at doses of 7.5, 15and 30 mg zinc chloride/kg bw/d (3.6, 7.2 and 14.4 mg Zn/kg bw/day) in water over two successive generations via the oral route. Application procedure not specified but likely oral gavage. Exposure started 77 days prior to mating.

- Results:
  - As of 3.6 mg Zn/kg bw/day:

        - P - Mortality↑; body weight gain↓; abs/rel liver/kidney weight↓; lesions in GI tract, inflammation in prostate
        - F1 - Mortality↑; body weight gain↓; abs/rel brain/prostate/spleen weight↓;
        - F2 – no effects
    - 7.2 mg Zn/kg bw/day:
        - P – abs./rel. brain/seminal vesicle weight↓;F1 - abs/rel liver/adrenal/seminal vesicle weight↓
        - F2 – no effects
    - 14.4 mg Zn/kg bw/day:
        - P – abs./rel. Spleen/uterus weight↓;
        - F1 - body weight gain (PND21)↓; abs/rel kidney weight↓; litter size and #surviving pubs until PND4↓;
        - F2 – body weight gain (PND21)↓; abs/rel kidney weight↓; litter size and number surviving pubs until PND4↓;
    - Maternal toxicity at any dose level. The NOAEL for fertility and development toxicity is about 15 mg ZnCl2/kg bw/d, this corresponds to 7.2 mg zinc/kg bw/day. No NOAEL for systemic toxicity could be derived.
- Remarks: 2 (reliable with restrictions) key study
- Reference: Khan et al., 2007

Test Substance: Zinc Sulphate

Method 1: Charles foster rats fed with a diet containing 4000ppm Zn (in form of zinc sulphate); exposure equals 200 mg Zn/kg bw exposure started 30-32 days prior to mating.

- Results:
    - 200 mg Zn/kg bw/day
        - P – Zn-concentration in testis and sperm↑; sperm mobility↓; number of pregnancies↓
        - F1 – number of live births↓As of 7.2 mg Zn/kg bw/day:
- Remarks: 2 (reliable with restrictions) key study
- Reference: Samanta et al., 1986

A double-blind trial was conducted in 56 pregnant women at risk of delivering a small for gestational-age baby to determine the effects of dietary zinc supplementation during the last 15-25 weeks of pregnancy following administration of 22.5 mg zinc/day. No adverse reproductive effects were observed (Simmer et al.,1991).

Pregnant women who received 0.3 mg zinc/kgbw/day as zinc sulphate capsules during the last two trimesters did not exhibit any changes in maternal body weight gain, blood pressure, postpartum haemorrhage or infection, inidicating no adverse reproductive effects (Mahomed et al.,1989).

ATSDR 2005

Following an initial exposure of rats, mice, and guinea pigs to concentrations as high as 119.3 or 121.7 mg zinc/m^3 as zinc chloride smoke (which also contained other compounds) for 1 hour/day,

5 days/week, for 20 weeks; histological evaluation revealed no adverse effects on the mammary glands, ovaries, fallopian tubes, or uteri were observed at 18 months (Marrs et al. 1988).

No measurable effect on gestational length or litter size was observed when female mink ingested a timeweighted average dose of 20.8 mg zinc/kg/day as zinc sulfate (Bleavins et al. 1983).

Exposure to up to 8 mg zinc/kg/day every other day for 14 days showed no effects on the levels of abnormal sperm in Wistar rats (Piao et al. 2003).

Preimplantation loss increased in rats fed diets containing 200 mg zinc/kg/day as zinc sulfate on gestational days 0–18 (Pal and Pal 1987). When the rats received 200 mg zinc/kg/day 21 days prior to mating, no effects on implantation or other adverse reproductive effects were observed (Pal and Pal 1987).

Exposure of up to 372 mg zinc/kg/day in mice prior to and throughout pregnancy did not result in changes in reproductive index (Lastra et al. 1997).

Animal studies suggest that exposure to very high levels of dietary zinc is associated with reduced fetal weight, alopecia, decreased hematocrit, and copper deficiency in offspring. For example, second generation mice

US EPA 2005

The reproductive and developmental toxicity of zinc has been investigated in several animal studies. Studies in rats provide evidence that high oral doses of zinc (>25 mg/kg-day) adversely affect spermatogenesis (Saxena et al., 1989; Evenson et al., 1993) and result in impaired fertility (decreased number of implantation sites and increased number of resorptions) in exposed females (Sutton and Nelson, 1937; Schlicker and Cox, 1968; Kumar, 1976; Pal and Pal, 1987).

In two separate experiments, Saxena et al. (1989) exposed an unspecified number of adult male Sprague-Dawley rats to 0 or 500 ppm of supplemental zinc (zinc form not specified) in the diet for 3 or 6 weeks. Using averages of the weekly body weight and food intake data provided, the supplemental zinc intake is calculated to have been 20 mg/kg-day for the 3-week experiment and 28 mg/kg-day for the 6-week experiment. In general, there were no adverse effects on food intake or body weight gain in the rats fed the high zinc diet for 3 or 6 weeks. The study authors noted an increase in swelling of the cervical and pectoral girdle lymph nodes and lameness of the forelimbs in the zinc-exposed animals, and that the degree of swelling increased with exposure duration; however, no data were provided to assess the statistical significance of this effect. General loss of hair and roughness of fur with subcutaneous hematomas were also noted in the rats exposed for 6 weeks. With the exception of a statistically significant increase in caput epididymis weight in the rats exposed for 3 weeks, there were no significant alterations in relative weights of reproductive tissues (testes, caput epididymis, cauda epididymis, seminal vesicles, prostate). Zinc intake significantly affected enzyme activities in tissues of the male reproductive system. Significant decreases in lactic dehydrogenase were observed in the testes, caput epididymis, cauda epididymis (6 weeks only), seminal vesicles, and prostate (6 weeks only) after 3 or 6 weeks of exposure. Increases in arylsulfatase activity were observed in the seminal vesicles after 3 or 6 weeks of exposure and in the cauda and caput epididymis after 6 weeks of exposure. Leucyl aminopeptidase activity was significantly increased in the testes, caput epididymis (3 weeks only), cauda epididymis, seminal vesicles (3 weeks only), and prostate gland after 3 or 6 weeks of exposure. Histological examination

of the gonads of rats consuming increased levels of zinc for 3 weeks revealed meiotic arrest at the primary spermatocyte stage, degenerating secondary spermatocytes, fluid accumulation within the seminiferous tubules, and reduced epithelial cell height in the epididymis. After 6 weeks of exposure, histological examination of the testes revealed additional evidence of arrested spermatogenesis. The germinal epithelium contained only spermatogonia, one layer of primary spermatocytes, and a few pyknotic secondary spermatocytes; no mature spermatozoa were present in the cauda epididymis. Necrotic nuclei were observed among Sertoli cells, Leydig cells, and in the epithelia of prostatic follicles and seminal vesicles. Fertility tests were not carried out in this study.

Evenson et al. (1993) fed groups of 10 male Sprague-Dawley rats a diet containing deficient, adequate or excessive amounts of zinc (4, 12, or 500 mg total Zn/kg food) for 8 weeks; using the average of the initial and terminal body weight data provided in this paper and an allometric equation for food intake (U.S. EPA, 1988), the average dosages of zinc are estimated to be 0.4, 1, or 49 mg total Zn/kg-day. Body weight gain was directly related to the zinc dose, but there was no effect on the relative testicular weight. Flow cytometric data revealed that excess zinc caused abnormalities in the chromosome structure of sperm. The authors suggested that excess zinc, represented by the highest dose group, destabilizes disulfide bonds and complexes with protamine (a basic protein in the sperm) molecules, leading to a destabilization of sperm chromatin quaternary structure and greater susceptibility to DNA denaturation. No fertility tests were carried out in this study.

Sutton and Nelson (1937) maintained groups of young female (n=3) and male (n=2) rats on basal diets supplemented with 0, 0.10, 0.50, or 1.0% zinc as zinc carbonate for 10-39 weeks. Using reference values for body weight (0.124 kg) and food intake (14 g) (U.S. EPA, 1988), supplemental zinc intake is estimated as 0, 113, 565, or 1130 mg/kg-day. Growth, reproduction, and development were reported to be normal for the 0.10% group over several generations. Adverse reproductive effects were observed in the 0.50% group; there were several stillbirths in the first pregnancy, after which there were no live young born. Rats in this group ceased to become pregnant after 5 months, although their body weights appeared normal. Reproduction and development were reported to have returned to normal in this group after excess zinc was withheld from the diet. No data were presented in support of this statement, so the timeframe of recovery is not known. Most of the animals on the 1.0% zinc diet failed to grow normally and some died within 4 weeks; no reproduction occurred in this dose group Since both males and females were treated with zinc, but no histopathological examination of the gonads was performed, it is not possible to determine the immediate cause of reproductive failure at higher dose levels.

Pal and Pal (1987) added 4000 ppm of zinc as zinc sulfate to the diet of 12 Charles-Foster female rats for 18 days beginning immediately after coitus. Using the reference values for food intake and body weight (U.S. EPA, 1988), supplemental zinc intake is estimated at 450 mg/kgday. The incidence of conception in the treated group was significantly reduced compared to controls (5/12 vs. 12/12). In those animals that did conceive, the number of implantation sites per pregnant female was not significantly altered. Zinc treatment had no effect on the number of resorption sites and there were no stillbirths or malformations among the offspring of treated rats. In a separate experiment in which female rats were fed 4000 ppm supplemental zinc for 3 weeks prior to mating, the incidence of conception and fetal outcome were not adversely affected by treatment.

In a series of four studies conducted by Schlicker and Cox (1968), groups of 10-20 female Sprague-Dawley rats were fed a control diet or a diet containing supplemental zinc oxide prior to mating

and/or during gestation. The exposure protocols for the four studies were as follows: (1) 10 rats fed 0 or 0.4% dietary zinc on gestational days 0 through 15 or 16, (2) 20 rats fed 0 or 0.4% supplemental zinc on gestational days 0 through 18 or 20, (3) 20 rats fed 0 or 0.4% supplemental zinc for 21 days prior to mating through delivery, and (4) 10 rats fed 0 or 0.2% supplemental zinc for 21 days prior to mating through gestational day 15. Using initial body weight data provided and an allometric equation for food intake (U.S. EPA, 1988), excess zinc intake by dams is estimated as 0, 200, or 400 mg/kg-day for the 0, 0.2, and 0.4% dietary concentrations, respectively. Dams were sacrificed on the final day of exposure, and the fetuses removed for examination. A 4-29% fetal resorption rate was observed in the dams exposed to 0.4% zinc beginning on gestational day 0 (studies 1 and 2). In rats exposed to 0.4% zinc prior to mating and during gestation, there was a 100% resorption of the fetuses. Significant decreases in body weight were observed in the fetuses of rats exposed to 0.4% zinc on gestational days 0-15 16, 18, or 20, but not in the 0.2% group exposed prior to mating and during gestational days 0-15. No external malformations were observed in the 0.4% group exposed during gestation or in the 0.2% group exposed prior to and during gestation.

In a single-generation study of reproductive performance, Khan et al. (2001) exposed groups (n=5-7) of male and female Sprague-Dawley rats to 0, 3.6, 7.2, 14.4, or 28.8 mg Zn/kg day, as zinc chloride, by gavage. Animals were exposed 7 days per week for 77 days prior to cohabitation and throughout the 21-day cohabitation period; females were also exposed during each of the 21-day gestation and lactation periods. Evaluated reproductive parameters included fertility, viability index, weaning index, litter size, and pup body weight. No significant changes were seen in body weights of the exposed rats prior to birth, but postpartum dam body weights for the mid- and high-dose groups were significantly decreased, relative to controls. The fertility indices in all dose groups were significantly lower than in the control group, though no dose related trends were noted. At the highest two dose levels, the number of live pups per litter, but not total pups per litter, was significantly decreased, as was live pup weight at postnatal day 21, though not at days 4, 7, or 14. No other changes in reproductive parameters were noted, and no effects on serum clinical chemistry endpoints were reported.

Kumar (1976) compared the effect of different levels of dietary zinc on pregnancy in an unspecified strain of rats. Beginning on day 1 of pregnancy, 12 control rats were fed a basal diet containing 30 ppm of zinc (3.39 mg/kg-day), and 13 rats were fed the basal diet plus 150 ppm supplemental zinc (as zinc sulfate, ~20 mg/kg-day total zinc). The dams were sacrificed on gestational day 18. No alterations in the number of implantation sites were found, but a statistically significant increase in the number of resorptions (9.5%) was observed in the zincsupplemented group. Kinnamon (1963) fed groups of five Sprague-Dawley female rats a diet containing 0 or 0.5% supplementary zinc as zinc carbonate for 5 weeks prior to mating with untreated males and for the first 2 weeks of gestation. At the end of the 7-week period, the rats were injected with radiolabelled zinc chloride, then housed in metabolism cages for 4 days prior to sacrifice. Using the body weight data provided and an allometric equation for food intake (U.S. EPA, 1988), supplemental zinc doses of 0 or 500 mg/kg-day were calculated. No significant differences in number of fetuses per litter, wet weight of the litter, or average weight per fetus were observed.

Estimated Data - None

Developmental Toxicity incl. Developmental Neurotoxicity (D): L

Zinc oxide was assigned a hazard classification level of Low for developmental toxicity based on weight of evidence which considers the predominance of negative developmental effects reported for most developmental endpoints. While alopecia and achromotrichia have been observed in the offspring of mice and mink exposed to high doses of zinc during gestation and lactation, it is unclear that these results are due to zinc exposures or associated with decreased copper deficiency. Based on the numerous studies reporting negative developmental effects following zinc exposure, the Low hazard score is assigned a high level of confidence.

Lists

- Authoritative: None
- Screening: None

Measured Data

ECHA 2019

Female hamsters (23-25 animals/group; outbred strain of golden hamster) received daily doses of 0.9, 4.1, 19 and 88 mg unspecified ZnSO4/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. Under the conditions of the test, administration of up to 88 mg/kg bw of unspecified zinc sulphate (ca. 35.2 mg or 19.9 mg Zn2+/kg bw, for anhydrate and heptahydrate, respectively) had no adverse effects on adult hamsters and their foetuses.

Female CD-1 mice (25-30 animals/group) received daily doses of 0.3, 1.4, 6.5 and 30 mg unspecified ZnSO4/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. 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. Under the conditions of the test, administration of up to 30 mg/kg bw of unspecified zinc sulphate (ca.12 mg or 6.8 mg Zn2+/kg bw, for anhydrate and heptahydrate, respectively) had no adverse effects on adult mice and their foetuses.

Female Dutch rabbits (14-19 animals/group) received daily doses of 0.6, 2.8, 13 and 60 mg unspecified ZnSO4/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. Under the conditions of the test, administration of up to 60 mg/kg bw of unspecified zinc sulphate (ca. 24 mg or 13.6 mg Zn2+/kg bw, for anhydrate and heptahydrate, respectively) had no adverse effects on adult rabbits and their foetuses.

The developmental toxicity of zinc compounds can be assessed on the basis of prenatal toxicity studies that have been conducted with soluble zinc sulphate and zinc chloride and slightly soluble zinc carbonate in rats, mice, hamsters or rabbits. Moreover, a total of three (one or two generation) reproductive toxicity studies conducted by Khan et al.(2001, 2003, 2007) provide further information on potential teratogenic effects of zinc compounds. No prenatal toxicity was observed with either zinc sulphate, zinc chloride or zinc carbonate at exposure levels up to 50 mg Zn/kg bw/day by oral gavage or 200 mg Zn/kg bw/day if the zinc was dosed via the diet. Established NOAELs in these studies were typically at highest dose tested and systemically tolerated by the dams. Developmental effects such as decrease in body or organ weights were, however, observed in F1 and/or F2 generations in the one or two generation reproductive toxicity studies conducted by Khan et al. (2001, 2003, 2007). These studies are not considered suitable for the assessment of teratogenic effects for hazard classification or risk assessment purposes since they were always observed in the presence of maternal toxicity.

Overview of experimental key studies on developmental toxicity:

Test Substance: Zinc Sulphate

Species: Mouse CD-1

- Route: Oral
- Method: Females received daily doses of 0, 0.3, 1.4, 6.5 and 30 mg $ZnSO_4$(unspecified)/kg bw by oral gavage during days 6-15 of gestation.
- Result: No discernible effects were seen on or maternal or foetal survival. No difference in number of abnormalities found in foetuses.
    - NOAEL:
        - 30 mg/kg bw/day equalling
        - 12mg Zn/kg bw/d (anhydrate);
        - 6.8mg Zn/kg bw/d (heptahydrate)
- Remark: 2 (reliable with restrictions) Key study
- Reference: Food and Drugs Research Labs., Inc, 1973*

Species: Rat Wistar

- Route: Oral

- Method: Females received daily doses of 0, 0.4, 2.0, 9.1 and 42.5 mg $ZnSO_4$(unspecified)/kg bw by oral gavage during days 6-15 of gestation
- Result: No discernible effects were seen on or maternal or foetal survival. No difference in number of abnormalities found in foetuses.
    - NOAEL:
        - 42.5 mg/kg bw/day equalling
        - 17mg Zn/kg bw/d (anhydrate);
        - 9.6 mg Zn/kg bw/d (heptahydrate)
- Remark: 2 (reliable with restrictions) Key study
- Reference: Food and Drugs Research Labs., Inc, 1973*

Species: Rat Charles Foster

- Route: Oral
- Method: Females received daily doses of 0, and 200 mg Zn/kg bw (in form of $ZnSO_4$) in diet during days 1-18 of gestation
- Result: No discernible effects were seen on or maternal or foetal survival. A reduced number of implantations observed. No difference in number of abnormalities found in foetuses.
    - NOAEL:
        - 200 mg/kg bw/day
- Remark: 2 (reliable with restrictions) Key study
- Reference: EU RAR, 2004

Species: Hamster

- Route: Oral
- Method: Females received daily doses of 0, 0.9, 4.1, 19, and 88 mg $ZnSO_4$ (unspecified)/kg bw by oral gavage during days 6-10 of gestation.
- Result: No discernible effects were seen on or maternal or foetal survival. No difference in number of abnormalities found in foetuses.
    - NOAEL:
        - 88 mg/kg bw/day
        - equalling
        - 32.5mg Zn/kg bw/d (anhydrate);
        - 19.9mg Zn/kg bw/d (heptahydrate);
- Remark: 2 (reliable with restrictions) Key study
- Reference: Food and Drugs Research Labs., Inc, 1973*

Species: Rabbit Dutch

- Route: Oral
- Method: 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.

- Result: No discernible effects were seen on or maternal or foetal survival. No difference in number of abnormalities found in foetuses.
    - NOAEL:
        - 60 mg/kg bw/day
        - equalling
        - 24mg Zn/kg bw/d (anhydrate);
        - 13.6mg Zn/kg bw/d (heptahydrate)
- Remark: 2 (reliable with restrictions) Key study
- Reference: Food and Drugs Research Labs., Inc, 1974*

Species: Rat Sprague Dawley

- Route: Oral
- Method: Females received daily doses of 0, 2.5, and 50 mg Zn/kg bw (in form of $ZnCO_3$) in diet during days 1-20 of gestation.
- Result: No discernible effects were seen on or maternal or foetal survival. No difference in number of abnormalities found in foetuses.
    - NOAEL:
        - 50 mg/kg bw/day
- Remark: 2 (reliable with restrictions) Key study
- Reference: Uriu-Hare, 1989

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

A study was conducted on pregnant women to determine the effects of nutrients during pregnancy on maternal and fetal outcome. Four hundred fifty women were observed during pregnancy and postpartum. Forty-three variables including 12 laboratory indices of maternal nutrient status were assessed. Maternal plasma zinc levels were inversely correlated with fetal weight. Blood examinations revealed a significant association between the total occurrence of fetomaternal complications or fetal distress, and lowest quartile zinc/albumin and highest quartile folate. Under the study conditions, plasma zinc was determined to be a discriminator for fetomaternal complications only in women in the lowest quartile for plasma zinc (Mukherjee et al., 1984).

A double-blind trial was conducted on pregnant women to determine the effects zinc supplementation during pregnancy on maternal and fetal outcome. 494 women booking before 20-week of gestation in a hospital were prescribed either 66 mg zinc sulphate (equivalent to 20 mg elemental zinc, 0.3 mg zinc/kgbw/day) capsules or placebo for once daily use, starting from day of booking till delivery. Various adverse outcomes were tested, including maternal bleeding, hypertension, complications of labor and delivery, gestational age, Apgar scores, and neonatal abnormalities. The main outcome measure was birth weight. There were no differences between the mothers and neonates of the zinc supplemented and placebo group. Under the test conditions,

zinc supplementation during pregnancy did not affect maternal or fetal outcome (Mahomed et al.,1989).

In summary, in studies with women receiving zinc supplementation during pregnancies at levels of approximately ≤ 0.3 mg Zn/kg bw/day, no reproductive or developmental effects were observed (WHO, 2001; SCF, 2003). Evidence of zinc toxicity during 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 is necessary for normal growth and development (e.g., gene expression, vitamin metabolism) and therefore it is not surprising that zinc deficiency during pregnancy can cause a variety of adverse effects to the foetus or may result in reduced fertility or delayed sexual maturation in animals as well as in humans (EU RAR, 2004; WHO, 2001).

A recent prenatal developmental study in rats performed specifically on nano-ZnO, demonstrates that maternal toxicity was evident by increase of lung weights and lung inflammations at 7.5 mg/m^3. But there were no effects on reproductive parameters (conception rate, corpora lutea, implantation sites, pre- and postimplantation loss, resorptions, and dead fetuses), no increase in external and soft tissue malformations and variations resulting in a NOAEC developmental toxicity of 7.5 mg/m^3.

EPA 2005

Several studies have examined the developmental toxicity of zinc. Studies by Schlicker and Cox (1968) and Ketcheson et al. (1969) have found decreases in body weights in the offspring of rats exposed to high doses of zinc in the diet. Additionally, alopecia and achromotrichia have been observed in the offspring of mice and mink exposed to high doses of zinc during gestation and lactation (Bleavins et al., 1983; Mulhern et al., 1986).

Ketcheson et al. (1969) fed groups of 10 pregnant female Sprague-Dawley rats a basal diet containing 9 ppm of zinc or 0.2% or 0.5% supplemental zinc as zinc oxide, throughout gestation and lactation day 14. Using an estimated body weight of 0.300 kg and reported food intake data, estimated maternal supplemental zinc doses are 120 and 280 mg/kg-day during gestation in the 0.2 and 0.5% groups, respectively, and 150 and 400 mg/kg-day during lactation. No significant alterations in maternal body weight or food intake were observed in the zinc supplemented groups relative to controls. No significant alterations in duration of gestation or the number of viable pups per litter were observed. Significant alterations in newborn and 14- day-old pup body weights were observed; the alterations consisted of an increase in the 0.2% group and a decrease in the 0.5% group. The increase in pup body weight at the 0.2% dietary level suggests that the basal diet did not provide a sufficient amount of zinc to support pregnancy and lactation. No external malformations were reported.

Uriu-Hare et al. (1989) fed groups of eight-nine Sprague-Dawley rats diet containing low, adequate (control group), or high amounts of zinc (4.5, 24.5, or 500 ppm total zinc) during gestational days 1-20. Using estimates of body weight (0.285 kg) and food intake (17 g/day) data presented in graphs, the total dietary intake of zinc is estimated to have been 0.27, 1.45, or 30 mg/kg-day. No adverse effects on maternal body weight gain, hematocrit levels, or the incidences of resorptions, malformations, fetal body weight, or fetal length were observed in the high zinc group, as compared to the adequate zinc group. Adverse effects, including decreases in maternal body weight and increases in resorptions, malformations, and fetal growth were observed in the low-zinc group only.

Mulhern et al. (1986) fed an unspecified number of female weanling C57BL/6J mice a diet containing 50 (normal) or 2000 (high) ppm of zinc as zinc carbonate and, at age 6 weeks, mated them with unexposed males. Each dam and her offspring were assigned to one of 10 groups receiving 50 or 2000 ppm total zinc during gestation, lactation, and postweaning until age 8 weeks. Decreases in hematocrit and body weight were observed in the F1 mice exposed to 2000 ppm zinc during gestation, lactation, and postweaning. The study authors noted that decreases in body weight gain were observed in other groups; however, the magnitude and statistical significance were not reported. Alopecia was observed in all groups of F1 mice exposed to 2000 ppm during lactation, regardless of gestational exposure. The mice began to lose hair between 2 and 4 weeks of age, and exhibited severe alopecia at 5 weeks. Exposure to 2000 ppm during lactation and/or post weaning resulted in achromotrichia, which the authors suggest may result from the effects of zinc-induced copper deficiency.

Bleavins et al. (1983) fed groups of adult mink (11 females and 3 males) a basal diet containing 20.2 ppm of zinc or the basal diet supplemented with 500 ppm of zinc as zinc sulfate heptahydrate. After 2 months the animals were mated during an 18-day period; since no clinical signs of zinc toxicity or copper deficiency were noted for the 500-ppm group, 3 days before the end of the mating period, the high dose of zinc was increased to 1000 ppm. Using the reference body weight and an allometric equation for food intake (U.S. EPA, 1988), the intake of zinc is calculated to have been 56 mg/kg-day. Fewer dams (8/11) on the high-zinc diet produced offspring than those on the control diet (11/11); however, gestational length, litter size, birth weights and kit mortality to weaning were not affected. Zinc had no effect on body, liver, spleen or kidney weights, or on hematological parameters (leukocyte, erythrocyte, Hb, hematocrit) in adults. Clinical signs associated with copper deficiency (alopecia, anemia, achromotrichia) were also not observed in adults. However, 3- to 4-week-old kits exhibited achromotrichia around the eyes, ears, jaws, and genitals, with a concomitant loss of hair and dermatosis in these areas. Subsequently, achromotrichia and alopecia spread over much of the body. At 8 weeks, treated kits had lower hematocrit and lower lymphocyte counts, but higher numbers of band neutrophils. At 8 weeks, treated kits exhibited signs of immunosuppression (significantly lowered thymidine incorporation by lymphocytes after stimulation by concanavalin A). Treated male kits had lower body weights than controls at 12 weeks. After weaning, the kits were placed on the basal diet, and within several weeks they recovered.

ATSDR 2005

Zinc is necessary for normal fetal growth and development. Fetal damage may result from zinc deficiency. Only one report in the literature suggested adverse developmental effects in humans due to exposure to excessive levels of zinc (Kumar 1976). Four women were given zinc supplements of 0.6 mg zinc/kg/day as zinc sulfate during the third trimester of pregnancy. Three of the women had premature deliveries, and one delivered a stillborn infant. However, the significance of these results cannot be determined because very few details were given regarding the study protocol, reproductive histories, and the nutritional status of the women. Other human studies have found no developmental effects in the newborns of mothers consuming 0.3 mg zinc/kg/day as zinc sulfate (Mahomed et al. 1989) or zinc citrate (Simmer et al. 1991) or 0.06 mg zinc/kg/day as zinc aspartate (Kynast and Saling 1986) during the last two trimesters. There has been a suggestion that increased serum zinc levels in pregnant women may be associated with an increase in neural tube defects (McMichael et al. 1994), but others have failed to confirm this association (Hambidge et al. 1993).

Animal studies suggest that exposure to very high levels of dietary zinc is associated with reduced fetal weight, alopecia, decreased hematocrit, and copper deficiency in offspring. For example, second generation mice exposed to zinc carbonate during gestation and lactation (260 mg/kg/day in the maternal diet), and then continued on that diet for 8 weeks, had reduced body weight, alopecia, and signs of copper deficiency (e.g., lowered hematocrit and occasional achromotrichia [loss of hair color]) (Mulhern et al. 1986). It is likely that the alopecia resulted from zinc-induced copper deficiency, which is known to cause alopecia in monkeys (Obeck 1978).

Estimated Data - None

Endocrine Activity (E): M

Zinc oxide was assigned a hazard classification level of Moderate for endocrine activity based on histological alterations observed in the pancreas, pituitary gland, and adrenal gland of zinc-exposed mice. The reported endocrine activity is not associated with an adverse effect such as carcinogenicity, reproductive toxicity, developmental toxicity and/or systemic toxicity (repeated dose, typically, thyroid). Therefore, no modification of the score from moderate to high is needed. The hazard score of Moderate is based on well-reported study data using a high-quality analog and therefore is reported as high confidence.

Lists

- Authoritative: None
- Screening: None

Measured Data

ATSDR 2005

Only one human exposure study has evaluated endocrine effects of oral zinc exposure. Davis et al. (2000; Milne et al. 2001) reported a slight (<10%) decrease in serum T4 levels in postmenopausal women exposed to 0.68 mg zinc/kg/day as zinc gluconate; the difference did not attain statistical significance, and no changes in free T3 or thyroid stimulating hormone (TSH) levels were reported. Piao et al. (2003) exposed groups of Wistar rats to 0, 4, or 8 mg zinc/kg as zinc acetate every other day for a 14-day period. Levels of T3 were decreased in both groups of exposed rats, relative to controls, but levels of T4 and TSH were not significantly altered. Zinc exposure resulted in increased levels of serum cortisol, which was significant from controls at the 8 mg zinc/kg exposure level.

US EPA 2005

Aughey et al. (1977) investigated the effects of supplemental zinc on endocrine glands in groups of 75 male and 75 female C3H mice by administering 0 or 0.5 g/L zinc (as zinc sulfate) in the drinking water for up to 14 months. The authors reported that the body weight in the control group ranged from 21 to 30 g, and the mean weight of the zinc-fed mice was approximately 1 g higher. Using the midpoint of the body weight range (0.022 to 0.031 kg), a water intake of 0.0069 L/day was calculated (U.S. EPA, 1988), resulting in average daily drinking water doses of 0 or 135 mg Zn/kg-day. At 1-month intervals, five mice in each of the treated and control groups were killed. After 6 months of exposure to zinc, there were no significant changes in plasma insulin or glucose levels as

compared to controls. Histological alterations were observed in the pancreas, pituitary gland, and adrenal gland of zinc-exposed mice. The histological changes in the mice were first observed after 3 months of exposure to zinc. In the zinc-supplemented mice, the pancreatic islets were enlarged and had a vacuolated appearance. The β-cells of the pancreatic islets were larger with enlarged mitochondria and prominent Golgi apparatus. The severity of the pancreatic lesions appeared to increase with increasing exposure durations. Pituitary alterations consisted of changes in the adrenocorticotrophic hormone-producing cells that indicated increased synthesis and secretion, including increased number and size of granules and more prominent rough endoplasmic reticulum and Golgi apparatus. Hypertrophy of the adrenal zona fasciculata and increased adrenal cortical lipid and cholesterol deposition were also observed. No tumors were reported in the pancreas, pituitary gland, or adrenal gland of zinc-exposed mice; data on other organs were not reported.

Estimated Data- None

GROUP II AND II* HUMAN HEALTH EFFECTS (GROUP II AND II* HUMAN)

Note: Group II and Group II* endpoints are distinguished in the v1.4 Benchmark system (the asterisk indicates repeated exposure). For Systemic Toxicity and Neurotoxicity, Group II and II* are considered sub-endpoints. See GreenScreen Guidance v1.4, Annex 2 for more details.

Acute Mammalian Toxicity (AT): L

Zinc oxide was assigned a hazard classification level of Low for acute mammalian toxicity based on reported oral LD50s that are >2000 mg/kg and an inhalation LC50 reported that is >5 mg/L. The hazard score of Moderate is based on study data and therefore reported as high confidence.

Lists

- Authoritative: None
- Screening: None

Measured Data

ECHA 2019

Acute oral toxicity: key studies carried out according to OECD guideline no 401 or 423 indicating for both micro- and nanomaterial zinc oxide LD50 > 2000 mg/kg bw

Acute inhalation toxicity: key study carried out according to OECD guideline no 403 indicating for micro zinc oxide LC50 > 5.7 mg/L/4hrs.

Acute dermal toxicity: key study carried out according to OECD guideline no 402 indicating for nano zinc oxide LD50 >2000 mg/kg bw.

The study was conducted to determine the acute dermal toxicity of nanoscaled ZnO according to the OECD Guideline 402 in compliance with GLP. 2000 mg/kg bw of the test substance were semiocclusively administered for 24 h as pasty formulation in corn oil to the shaved and defaffed back of 5 female and 5 male rats. After the end of the exposure period, the test substance paste was recovered as effectively as possible using water and the animals were observed for 14 d. During the present study no mortality occurred and there were no indications of systemic toxicity, no effects regarding the body weight and neither clinical signs nor pathological findings observed. The LD50 of the test substance is therefore estimated to be > 2000 mg/kg bw

Tests performed specifically on nano-ZnO demonstrate alsovery low acute oral toxicity (i.e. LD50 values consistently exceeding 2,000 mg/kg bw). The only available inhalation data on nano-ZnO indicates an LC50 value of > 1.79 mg/L. However, only this one single dose of 1.79 mg/L was tested which wasthe maximum attainable exposure concentration for achieving respirable particle size.Data on nano-ZnO confirms low acute dermal toxicity with LD50>2000 mg/kg bw.

In conclusion, for nano-ZnO no nano-specific acute toxicity could be identified. Zn^{2+}ion determines the toxicity of ZnO and read across between various forms of ZnO (micro-scale, nano, coated or not) is fully supported.

Estimated Data- None

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-single): M

Zinc oxide was assigned a hazard classification level of Moderate for single dose systemic toxicity/organ effects based on study data that associate the compound with metal fume fever in humans. Specifically, symptoms of metal fume fever associated with inhalation of zinc generally appear a few hours after exposure and are reversible 1–4 days following cessation of exposure. Transient effects following single exposures are classified under GHS as category 3 hazards and equivalent to a GreenScreen moderate hazard score. In addition, an oral study in rats reported incidences of microscopic lesions in liver, pancreas, heart and stomach which were higher at lower doses compared to higher dose following oral exposures. The hazard score of Moderate is based on human study data and supported by effects reported in animal exposures and therefore reported as high confidence.

Lists

- Authoritative: None
- Screening: None

Measured Data

ECHA 2019

In an acute toxicity test Wistar rats (5/sex) were given a single dose of 5 g ZnO/kg bw (in water) by gavage and observed for 14 days. No signs of toxicity were observed.

In an acute inhalation toxicity study, 10 male and 10 female animals per group were exposed to zinc oxide aerosol (head and nose only) for 4 h. Aerosol concentration was 5.7 mg/l and the particle size distribution had a mass median aerodynamic diameter of 4mm ± 2.9 (GSD). Only one concentration and a control group were tested. All animals survived up to day 14 post exposure. Apart from a dusty fur on the head the day after the exposure, no effects were seen. Body weights developed normally. At pathological examination all organs were normal

The study was conducted to determine the acute dermal toxicity of nanoscaled ZnO according to the OECD Guideline 402 in compliance with GLP. 2000 mg/kg bw of the test substance were semiocclusively administered for 24 h as pasty formulation in corn oil to the shaved and defaffed back of 5 female and 5 male rats. After the end of the exposure period, the test substance paste was recovered as effectively as possible using water and the animals were observed for 14 d. During the present study no mortality occurred and there were no indications of systemic toxicity, no effects regarding the body weight and neither clinical signs nor pathological findings observed.

Of significance for humans from an acute toxicity standpoint is the occurrence of metal fume fever following exposure to ultrafine particles of special grades of zinc oxide in context of very specific operations such as cutting or welding of galvanised steel. Metal fume fever is exclusively associated with freshly formed ultrafine particulate zinc oxide (<0.1 µm). As these ultrafine particles (nanoparticles) rapidly agglomerate to bigger particles, which are normally encountered at production and processing sites, at these sites there is no indication for metal fume fever. According to the response from 11 zinc companies to a questionnaire, there have been no observations of zinc metal fume fever over the last decade and in recent occupational practice (EU RAR, 2004a-f). However in light of responsible care and since no studies are available that allow the establishment

of a NOAEL for metal fume fever with a reasonable degree of certainty, a LOAEL (5 mg ZnO/ m^3) for 2 hours (showed the typical metal fume fever symptoms beginning 4 to 8 hours after exposure and disappearing within 24 hours) can be used for metal fume fever based on the study by Gordon et al.(1992).

An acute oral toxicity study was performed with ZnO nanoparticles using Sprague Dawley rats according to OECD guideline 423. A group of 10 fasted animals (five males and five females) were administered with 5, 50, 300, 1,000 and 2,000 mg/kg bw of ZnO nanoparticles suspended in distilled water once through oral gavage. The animals were observed for mortality/morbidity, clinical signs of toxicity, weekly body weight and weekly food consumption during the experimental period. The study focused on the effects of the test substance on biochemical and hematological parameters which were analyzed on Day 14 of administration. At the end of 14 days the animals were sacrificed and subjected to gross pathological examination along with histopathology or organs. An inverse dose-dependent increase was noted in AST, ALT serum levels when compared with control. Calcium levels in the serum were also significantly high from control. However, there are no significant differences in rest of the parameters analyzed. There were also no statistically significant changes in the hematologic parameters. No gross pathological lesions were observed in any of the treatment groups. Histopathological evaluation showed incidences of microscopic lesions in liver, pancreas, heart and stomach which were higher at lower doses compared to higher dose. Although an LD50 value was not reported in the study report, based on the study description and considering that no mortality occurred at any of the tested dose levels, the LD50 value can be established at >2,000 mg/kg bw. Overall the authors concluded that the high toxicity at low doses which were evident in the present study may be due to the fewer number of nanoparticles in that mass, which may result in less agglomeration thereby making them penetrate into the cells.

<u>ATSDR 2005</u>

The effects of inhalation exposure to zinc and zinc compounds vary somewhat with the chemical form of the zinc compound, but the majority of the effects seen will occur within the respiratory tract. Following inhalation of zinc oxide, and to a lesser extent zinc metal and many other zinc compounds, the most commonly reported effect is the development of “metal fume fever.” Metal fume fever is characterized by chest pain, cough, dyspnea, reduced lung volumes, nausea, chills, malaise, and leukocytosis. Symptoms generally appear a few hours after exposure and are reversible 1–4 days following cessation of exposure. Exposure levels associated with the development of metal fume fever have not been identified, though are generally in the range of 77–600 mg zinc/ m^3. Acute experimental exposures of humans to lower concentrations of zinc oxide (14 mg/m3 for 8 hours or 45 mg zinc/ m^3 for 20 minutes) and occupational exposures to low concentrations of zinc (8–12 mg zinc/ m^3 for 1–3 hours and 0.034 mg zinc/m3 for 6–8 hours) did not produce symptoms of metal fume fever.

Metal fume fever, a well-documented acute disease induced by inhalation of metal oxides, especially zinc, impairs pulmonary function but does not usually progress to chronic lung disease. Symptoms generally appear within a few hours after acute exposure, usually with dryness of the throat and coughing. The most prominent respiratory effects of metal fume fever are substernal chest pain, cough, and dyspnea. The impairment of pulmonary function is characterized by reduced lung volumes and a decreased diffusing capacity of carbon monoxide. Leukocytosis persisting for approximately 12 hours after the fever dissipates is also a common manifestation of metal fume

fever. In general, the symptoms of metal fume fever resolve within 1–4 days after cessation of exposure and do not lead to long-term respiratory effects. Inhalation of "ultrafine" zinc oxide particles may also result in metal fume fever, as well as histologic damage and inflammation of the lung periphery.

Exposure levels leading to the development of metal fume fever have been characterized. Minimal changes in forced expiratory flow were observed 1 hour after a 15–30-minute exposure to 77 mg zinc/m3 as zinc oxide, while at higher levels (300–600 mg/ m^3, from 10 minutes to 3 hours), shortness of breath, nasal passage irritation, cough, substernal chest pain, persistent rales of the lung base, and a decreased vital capacity have been reported. Exposure to lower levels of zinc oxide, either for acute (14 mg zinc/ m^3 for 8 hours or 45 mg zinc/ m^3 for 20 minutes) or chronic (8–12 mg zinc/ m^3 for 1–3 hours and 0.034 mg zinc/ m^3 for 6–8 hours) duration did not result in the symptoms of metal fume fever. However, analysis by bronchoalveolar lavage of volunteers exposed to zinc oxide for up to 2 hours (mean concentration 16.4 mg zinc/ m^3) revealed an increase in levels of the cytokines TNF, IL-6 and IL-8, and increases in the number of polymorphonuclear leukocytes and lymphocytes in the BAL fluid. Thus, it appears that while the precursor events for the development of metal fume fever begin to occur even at very low zinc concentrations, the condition itself does not appear to fully manifest until exposure levels reach much higher (>75 mg/ m^3) levels. Similar effects, including decreased ventilation, an inflammatory response, and changes in cytokine levels, have also been seen in animal studies of zinc oxide inhalation.

Acute experimental exposures to lower concentrations of zinc oxide (14 mg/ m^3 for 8 hours or 45 mg zinc/ m^3 for 20 minutes) and occupational exposures to similar concentrations (8–12 mg zinc/ m^3 for 1– 3 hours and 0.034 mg zinc/ m^3 for 6–8 hours) did not produce symptoms of metal fume fever (Drinker et al. 1927b; Hammond 1944; Marquart et al. 1989). In a single-blind experiment, exposure of subjects to 3.9 mg zinc/ m^3 as zinc oxide resulted in sore throat and chest tightness but no impairment of pulmonary function (Gordon et al. 1992). It is speculated that subjects in other studies may have been less susceptible because of the development of tolerance to zinc (Gordon et al. 1992).

Kuschner et al. (1995) exposed a group of 14 volunteers to a single exposure of varying levels of zinc oxide fume (mean concentration 16.4±12.5 mg/ m^3) for 15–120 minutes (mean duration 45±28 minutes) and evaluated the response by bronchoalveolar lavage (BAL). Significant increases were reported in the number of polymorphonuclear leukocytes and lymphocytes in the BAL fluid, but not in the number of macrophages or in lymphocyte subpopulations; aside from a decrease in FEV1, no changes were reported in pulmonary function tests.

In a follow-up study by the same group (Kuschner et al. 1997), single-exposed volunteers showed an increase in levels of the cytokines TNF, IL-6, and IL-8 as a result of zinc oxide inhalation. Recurrent episodes of cough and dyspnea were reported in a former mild smoker 3 years after beginning work in a metal foundry where exposure to zinc oxide presumably occurred (Ameille et al. 1992). This case was distinguishable from metal fume fever because of the lack of tolerance to zinc (as shown by the late emergence of symptoms).

In a group of 14 welders acutely exposed to 77–153 mg zinc/m^3 as zinc oxide, significant correlations between the concentration of airborne zinc and the proportion of activated T cells, T helper cells, T inducer cells, T suppressor cells, and activated killer T cells were observed 20 hours

after exposure (Blanc et al. 1991). In addition, significant increases in levels of polymorphonuclear leukocytes, macrophages, and all types of lymphocytes were observed in the bronchoalveolar lavage fluid 20 hours after exposure. Increased levels of lymphocytes, with a predominance of CD8 cells, in the bronchoalveolar lavage fluid were reported in a case study of a smelter exposed to unspecified levels of zinc fumes (Ameille et al. 1992).

US EPA 2005

In a multispecies study, Gordon et al. (1992) exposed an unspecified number of male Hartley guinea pigs, Fischer 344 rats, and New Zealand rabbits to freshly generated zinc oxide particles. The guinea pigs and rats received nose-only exposure to 0, 2.5, or 5.0 mg/m^3 zinc oxide for 3 hours; the rabbits received nose-only exposure to 0 or 5.0 mg/m^3 zinc oxide for 2 hours. Animals were sacrificed 0, 4, or 24 hours following cessation of exposure. The lungs were lavaged, and the lavage fluid and recovered cells were examined for evidence of inflammation. Significant increases in lavage fluid parameters (lactate dehydrogenase, β-glucuronidase, and protein content) were observed 24 hours after the guinea pigs and rats were exposed to 2.5 or 5.0 mg/m^3. No significant alterations in lavage parameters were observed in the rabbits. The ability of alveolar macrophages to phagocytize particles was assessed in guinea pigs and rabbits. In the guinea pigs exposed to 5.0 mg/m^3, there was a significant reduction in phagocytic capacity (percentage of viable macrophages engulfing four or more particles), but no effect on phagocytic index (percentage of macrophages engulfing particles). Phagocytic ability was not adversely affected in the rabbits. The authors suggested that the reason rabbits were less affected was a lower retention of the inhaled zinc particles (4.7% in rabbits, compared to 11.5% in rats and 19.8% in guinea pigs), resulting in a lower dose per unit tissue mass.

Lam et al. (1988) exposed groups of seven-eight male Hartley guinea pigs to 2.7 or 7 mg/m^3 (average concentrations) freshly formed ultrafine zinc oxide aerosols (count median diameter of 0.05 :m; geometric standard deviation of 2.0) for 3 hours/day for 5 days. Two groups of eight guinea pigs were exposed to furnace gases for 3 hours on one of two days; the two groups were combined and served as the control group. No significant alterations in tidal volume, functional residual capacity, residual volume, respiratory frequency, airway resistance, or compliance were observed. Gradual decreases in total lung capacity (significant after day 4), vital capacity (significant after day 2), and single-breath diffusing capacity for carbon monoxide (significant after day 4), relative to controls, were observed in the 7 mg/m^3 group, but not in the 2.7 mg/m^3 group. Significant increases in relative and absolute lung weights were also observed in the 7 mg/m^3 group.

Lam et al. (1988) also assessed the effect of a single high peak of zinc oxide on lung function. In the first of the two experiments, eight male Hartley guinea pigs were exposed to 4.0 mg/m^3 zinc oxide for 3 hours on day 1; on day 2, the animals were exposed to 34 mg/m^3 for the first hour and to 4.0 mg/m^3 for the remaining 2 hours. Significant decreases in total lung capacity and vital capacity were observed on days 2, 3, 4, and 5; apparent alveolar volume was decreased on day 3. Relative lung weights were decreased on days 2-5. In general, the decrements in lung function parameters and lung weight changes peaked at day 3. Increase in respiratory resistance and decrease in respiratory compliance were observed on days 1 and 2. Increases in absolute and relative lung weights were observed on days 2-5. In the second experiment, eight male Hartley guinea pigs were exposed to 6 mg/m^3 (average concentration) 3 hours/day for 5 days; the animals were exposed to 25 mg/m^3 during the first hour of exposure on day 1. Several lung function parameters were significantly

altered, including decreases in vital capacity and total lung capacity on days 1-5, decreases in functional residual capacity and residual volume on days 2-5, a decrease in apparent alveolar volume on day 3, and increases in single-breath diffusing capacity for carbon monoxide on days 1-5. A gradual, but statistically significant increase in respiratory resistance and decrease in respiratory compliance was observed on days 1-5. Increases in absolute and relative lung weights were observed on days 2-5.

Amdur et al. (1982) exposed groups of 23 male Hartley guinea pigs to 0.91 mg/m^3 freshly-generated zinc oxide for 1 hour. A significant decrease in respiratory compliance was observed immediately after exposure and 1 hour postexposure. No alterations in respiratory frequency, tidal volume, or minute volume were observed. Similar results were observed in another study by this group in which seven guinea pigs were exposed to 0.90 mg/m^3 zinc oxide for 1 hour. This study showed that compliance continued to decrease between the first and second postexposure hours

Estimated Data- None

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-repeat) (Group II*): H

Zinc oxide was assigned a hazard classification level of High for repeated dose systemic toxicity/organ effects based on study data showing a significant reduction in erythrocyte superoxide dismutase activity, hematocrit, and serum ferritin, compared to pretreatment levels, in female subjects who received supplements (as capsules) of 50 mg zinc/day as zinc gluconate for 10 weeks. The human oral NOAEL of 50 mg Zn2+/day is equivalent to 0.83 mg/kg bw/day) has been used in the EU risk assessment, the ATSDR MRL development and the US EPA RfD for zinc. It is based on well-reported human studies. In addition, an NOAEC of 0.5 mg/m^3 (0.0005 mg/L) based on degeneration/regeneration of the olfactory tract in nasal cavity supports the High classification. The hazard score of High is based on numerous quality studies using high quality analogs and therefore is reported as high confidence.

Lists

- Authoritative: None
- Screening:
  - GHS Japan Category 1 – Specific target organs/systemic toxicity following repeated exposure.

Measured Data

ECHA 2019

In a subacute inhalation study the toxicity profile of ZnO after inhalation exposure, was performed in Wistar rats nose-only exposed to dynamic atmosphere of ZnO for 6 hours per day on 5 consecutive days per week for 4 weeks (28-day study). The target concentrations were 0.5, 1.5, 3.0 and 4.5 mg/m³. Inhalation exposure of 4.5 mg/m3 ZnO caused alopecia in ear region of female animals and impaired the body weight development in males. In bronchoalveolar lavage fluid, neutrophils and other cytological and biochemical parameters were changes significantly in animals exposed to 1.5 mg/m³ and higher. At 3.0 and 4.5 significantly increased absolute and relative lung

weight was found. Histological examination revealed degeneration/regeneration of the olfactory tract in nasal cavity. In accordance to findings in lavage fluid and the increased lung weight, histology of the lung reveals multifocal alveolar histiocytosis which were associated with single or few inflammatory cells. Based on the above-mentioned findings, the NOAEC was 0.5 mg/m3 under the current study condition.

-In a 90-day repeated dose inhalation toxicity study, male Wistar rats were exposed (nose only) at 0.3, 1.5 and 4.5 mg/m3 with coated nanoscaled ZnO (Z-COTE HP1) and at 4.5 mg/m^3 with non-coated microscaled ZnO. Fresh air treated animals served as concurrent control. Test substance related findings or losses of animals did not occur. Effects indicating systemic toxicity were not observed. The LOAEL was 4.5 mg/m^3 and the NOAEL 1.5 mg/m^3. Adverse effects were restricted to the high dose group: very slight bronchiolo-alveolar hyperplasia and increased incidences in very slight to slight infiltration of interstitial mononuclear cells in the lung, increased activity of lactate dehydrogenase in BAL and the increased numbers of lymphocytes in the BAL. The reversibility of all effects has been demonstrated. Effects on the olfactory epithelium were detected only in the reference group exposed to 4.5 mg/m^3 microscaled ZnO particles. In conclusion repeated dose toxicity inhalation studies showed local irritation of nasal cavity and respiratory tract, local lung inflammation and all effects were reversible within 28d after 90 d exposure.

In the only study available on repeated dose toxicity in the skin, the dose levels tested (75, 180 and 360 mg/kg bw) on Sprague-Dawley rats through dermal route did not show toxicity. On repeated application, nano zinc oxide at low doses caused collegen decrease compared with their high dose and control (LOAEL= 75 mg/kg bw/day). However, these effects were reversible in a period of 14 days. In general, no systemic effects were observed.

ATSDR 2005

Treatment-related changes in hematological parameters have been observed in humans and animals after intermediate or chronic exposure to zinc or zinc-containing compounds. Long-term administration (1– 8 years) of zinc supplements has caused anemia in humans (Broun et al. 1990; Gyorffy and Chan 1992; Hale et al. 1988; Hoffman et al. 1988; Patterson et al. 1985; Porter et al. 1977; Prasad et al. 1978; Ramadurai et al. 1993; Salzman et al. 2002; Stroud 1991; Summerfield et al. 1992). Exposure of one patient to 2 mg zinc/kg/day as zinc sulfate for 10 months resulted in anemia (Hoffman et al. 1988). A significant reduction in erythrocyte superoxide dismutase activity (47% decrease), hematocrit, and serum ferritin, compared to pretreatment levels, occurred in female subjects who received supplements (as capsules) of 50 mg zinc/day as zinc gluconate for 10 weeks (Yadrick et al. 1989); this study was selected as the basis for the intermediate-duration oral MRL. A 15% decrease in erythrocyte superoxide dismutase activity was reported in male volunteers receiving 50 mg zinc/day as zinc gluconate for 6 weeks (Fischer et al. 1984). A more recent study by Davis et al. (2000; Milne et al. 2001) reported increases in bone specific alkaline phosphatase levels (~25%) and extracellular superoxide dismutase (~15%), while significant decreases were seen in mononuclear white cell 5'-nucleotidase (~30%) and plasma 5'-nucleotidase activity (~36%) following exposure of postmenopausal women to a combined (dietary+supplemental) 53 mg zinc/day as zinc glycine chelate. Healthy men given 200 mg zinc/day as elemental zinc for 6 weeks showed a reduction in lymphocyte stimulation response to phytohemagglutinin as well as chemotaxis and phagocytosis of bacteria by polymorphonuclear leukocytes (Chandra et al. 1984); however, no changes in lymphocyte cell number or in the

proportion of lymphocyte populations were noted. Exposure of male volunteers to 0.48 mg zinc/kg/day, as zinc glycine chelate, had no effect on markers of coagulation (Bonham et al. 2003b) relative to unexposed subjects. While the changes in hematological end points following long-term zinc exposure in humans are noteworthy, they were subclinical in nature, and therefore, are generally considered to be non-adverse.

US EPA 2005

Maita et al. (1981) exposed groups of 12 male and 12 female Wistar rats and ICR mice to 0, 300, 3000, or 30,000 ppm zinc sulfate (hydration state not reported) in the diet for 13 weeks. The study authors estimated zinc sulfate intakes of male rats to be 23.2, 234, and 2514 mg/kgday (5.3, 53, and 572 mg supplemental Zn/kg-day). In the case of females, the authors estimated the doses as 24.5, 243, and 2486 mg ZnSO4/kg-day (5.6, 55, and 565 mg supplemental Zn/kg day). For male mice the estimated doses were 42.7, 458, and 4927 mg ZnSO4/kg-day (9.7, 104, and 1119 mg supplemental Zn/kg-day) and 46.4, 479, and 4878 mg ZnSO4/kg-day (10.5, 109, and 1109 mg supplemental Zn/kg-day) for female mice. Zinc intakes from the control diet were not estimated. In rats, no adverse clinical signs or increases in mortality were observed (Maita et al., 1981). Body weight gain was decreased in the high-dose male rats, as was food and water intake. Several statistically significant alterations in hematology and serum clinical chemistry parameters were observed in the high-dose rats; these included decreases in hematocrit and Hb levels in males, decreases in leukocyte levels in males and females, decreases in serum total protein, cholesterol, and calcium levels in males, and decreases in serum calcium levels in females. Significant decreases in absolute and relative liver and spleen weights were observed in the high-dose male rats; decreases in absolute weight were also observed in a number of other organs in the high-dose males which were probably related to the decreased body weight. No other consistent alterations in organ weights were observed. Histopathological lesions were limited to the pancreas of high-dose rats; however, significant increases in the incidence of degeneration and necrosis of acinar cells, decreased number of acinar cells, clarification of centroacinar cells and “ductule-like” metaplasia of acinar cells, and interstitial fibrosis were observed. Incidences of these lesions were not reported. In mice, an increase in mortality was observed in the high-dose group (5/24 mice died); impairment of the urinary tract and regressive changes (decreased number of acinar cells) in the pancreas were observed in the animals dying early (Maita et al., 1981). Decreases in body weight gain were also observed in both sexes of high-dose mice. In the low- and mid-dose male mice, there were significant increases in Hb and erythrocyte levels. Significant decreases in hematocrit, Hb, and erythrocyte levels were observed in the high-dose male and female mice; a significant decrease in hematocrit level was also observed in the mid-dose male mice. Total leukocyte levels were also decreased in the high-dose male mice. Several statistically significant alterations in serum clinical chemistry parameters were observed in the high-dose mice, including slight-to-moderate decreases in total protein, glucose, and cholesterol and moderate-to marked increases in alkaline phosphatase and urea nitrogen. Decreases in total protein and increases in alkaline phosphatase and urea nitrogen were also observed in the mid-dose male mice, although the study authors stated that the values were within acceptable historical limits. Histological alterations were observed in the pancreas, gastrointestinal tract, and kidneys of high-dose mice; incidences were not reported. Pancreatic alterations included an increased number of acinar cells, many displaying necrosis, swollen nuclei, and/or ductule-like metaplasia. Slight-to-moderate ulcerative lesions in the boundary

of the forestomach, inflammation of the mucous membranes of the "upper intestine" with proliferation of epithelial cells, and edema at the lamina propria were observed.

In a study by L'Abbe and Fischer (1984a), groups of 10 weanling male Wistar rats were fed a basal diet supplemented with 15, 30, 60, 120, or 240 ppm zinc as anhydrous zinc sulfate for 6 weeks; the 30 ppm group served as the control group. Using a reference body weight of 0.217 kg and food intake of 0.020 kg/day (U.S. EPA, 1988), daily doses of 1.4, 2.8, 5.5, 11, and 22 mg supplemental Zn/kg-day were estimated. Although a linear relationship between zinc intake and serum ceruloplasmin levels was not established, the number of animals with abnormal ceruloplasmin levels increased with increasing doses. Abnormal ceruloplasmin levels were observed in 0, 0, 11, 30, and 100% of the animals in the 15, 30, 60, 120, and 240 ppm groups, respectively. The study authors estimated that the ED50 for low ceruloplasmin levels was approximately 125 ppm. Dose-related decreases in liver Cu, Zn-superoxide dismutase and heart cytochrome c oxidase activities were observed at dietary zinc levels greater than 30 ppm, reaching statistical significance in the 120 and 240 ppm groups. Heart Cu, Zn-superoxide dismutase and liver cytochrome c oxidase activities were not affected.

In a second study, L'Abbe and Fischer (1984b) fed groups of 10 weanling male Wistar rats diets containing normal (30 mg Zn/kg diet) or supplemented (240 mg Zn/kg diet) zinc (as zinc sulfate) and normal (6 mg Cu/kg diet) or deficient (0.6 mg Cu/kg diet) copper for up to 6 weeks. Groups of rats were sacrificed at 2, 4, and 6 weeks. Blood, heart, and liver samples were collected for analysis. No significant differences in body weight or food consumption were noted among treated groups. Similarly, no differences were seen in Hb levels. Serum and heart copper levels were significantly decreased in rats fed either zinc-supplemented or copper deficient diets. In both the high zinc and copper-deficient groups, activity levels of serum ceruloplasmin, liver and heart Cu, Zn-superoxide dismutase, and liver and heart cytochrome c oxidase were significantly reduced relative to control animals by 2 weeks of exposure and remained reduced throughout the study.

Zaporowska and Wasilewski (1992) exposed groups of 13 male and 16 female Wistar rats to 0 or 0.12 mg Zn/mL as zinc chloride in the drinking water for 4 weeks. The study authors estimated the daily drinking water dose to be 11.66 mg Zn/kg-day in males and 12.75 mg Zn/kg day for females. Although significant decreases in food and water intake were observed, body weight gain was not significantly different from controls. Significant alterations were observed in several hematological endpoints including decreases in erythrocyte and Hb levels, increases in total and differential (neutrophils and lymphocytes) leukocyte levels, and increases in the percentage of reticulocytes and polychromatophilic erythrocytes.

Bentley and Grubb (1991) fed groups of seven-eight male New Zealand white rabbits diets containing 0, 1000, or 5000 :g supplemental zinc/g as zinc carbonate (0, 34, 170 mg supplemental Zn/kg-day using an estimated time-weighted-average body weight of 2.5 kg and an allometric equation for food intake [U.S. EPA, 1988]) for 8 (1000 :g/g group) or 22 weeks (5000 :g/g group); the basal diet contained 105.5 :g Zn/g. No adverse alterations in body weight gain were observed. A significant decrease in Hb levels were observed in the 5000 :g/g group. Significant decreases in serum copper and increases in serum and tissue (liver, kidney, brain, testis, pancreas, thymus, skin, bone, and hair) zinc levels were also observed in the 5000 :g/g group. No effects were reported at other dose levels.

de Oliveira et al. (2001) exposed groups of 9 or 12 male and female Swiss mice to 0 or 1% hydrated zinc acetate (0 or 793 mg Zn/kg-day), assuming reference body weight and drinking water consumption values from U.S. EPA (1988), beginning in the first month of life and lasting for 60 days. Animals were evaluated using a shock avoidance behavioral test at the end of their 60-day exposure period. The animals were placed in a two-compartment chamber where one compartment was dark and the other lighted. When placed in the lighted compartment, the mice (who prefer the dark) moved into the dark compartment where they received an electric shock upon contact with the dark room floor. On the next day when the animals were placed in the lighted compartment, the time before they moved into the dark compartment increased significantly from the time on the first day, signifying that they had learned from the adverse day zero experience. There was no significant difference in the time before dark room entry between the control and zinc-exposed animals on test day 1. Entry into the dark chamber did not result in shock treatment on test day 1. The control and zinc-exposed animals continued to be tested on days 7, 14, 21, and 28. No shock was given on any of these test days. The initial period in the lit room before entering the dark room decreased over time for both the control and the zinc-exposed groups. However, the decrease over time was greater in the zinc-exposed group signifying a more rapid extinction of the learned avoidance response. The time spent in the lighted chamber before entry into the dark room was significantly lower (about half of that for the controls) for the zinc-exposed animals on day 28. Accordingly, postnatal zinc exposure appeared to have a negative effect on the retention of a learned behavioral response.

Llobet et al. (1988) examined the effects of subchronic oral administration of zinc in Sprague-Dawley rats. Forty female rats were exposed to 0, 160, 320, and 640 mg/kg-day zinc acetate dihydrate in the drinking water (0, 48, 95, and 191 mg Zn/kg-day) for 12 weeks. Sugar was added to all drinking water of all groups to reduce unpalatability. Food and water were provided ad libitum. Food and water consumption, volume of urine, and weight of excreted feces were measured daily and body weights were measured weekly. After 12 weeks of treatment, blood samples were collected and analyzed for hematocrit, Hb, glucose, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase, urea, and creatinine concentrations. The brain, heart, lungs, spleen, liver, and kidneys were weighed, analyzed for zinc concentration, and (all but the brain) examined histologically. Zinc concentrations were also determined for bone, abdominal muscle, and blood. Clinical signs noted were apathy and two deaths in the 640 mg/kg-day group. Statistically significant decreases in water intake and urine output were observed in the 640 mg/kg-day group; a decrease in urine output was also observed in the 320 mg/kg-day group for 3 of the 6 two-week measurement periods. No alterations in body weight gain or organ weights were observed. Increases in blood urea and creatinine levels in the 640 mg/kg-day group were the only significant alterations in hematological or serum clinical chemistry parameters. Zinc concentrations were significantly increased in the liver, kidneys, heart, bone, and blood of rats in the 320 and 640 mg/kg-day groups. The study authors noted that the "most severe histological alterations were observed in kidneys," but it is unclear, from the limited reporting of the histological results, if lesions were observed in other tissues. The described renal lesions included flattened epithelial cells in the Bowman's capsule, desquamation of the proximal convoluted tubules, and pyknotic nuclei in the 640 mg/kg-day group.

Straube et al. (1980) examined the effects of excess dietary zinc in ferrets. Adult ferrets (six males, nine females), weighing 500-700 g, were divided into four groups and fed a basal diet of canned

dog food (that contained 27 ppm zinc and 3.3 ppm copper) plus 0 (five animals), 500 ppm (three animals), 1500 ppm (four animals), or 3000 ppm (three animals) supplemental zinc as zinc oxide. Doses of 0, 142, 425, and 850 mg supplemental Zn/kg-day, respectively, are estimated using the midpoint of the range of initial body weights and the amount of food given to each animal (170 g per day, assumed to be consumed completely each day). Animals in the 1500 and 3000 ppm groups showed signs of severe toxicity and were sacrificed or died within the first 3 weeks. Animals in the 500 ppm group were sacrificed on days 48, 138, and 191, and the controls were sacrificed on days 27, 48, 138, 147, and 197. The following parameters were used to assess toxicity: hematology (Hb, packed cell volume, erythrocyte, leukocyte, and reticulocyte levels), serum clinical chemistry (urea nitrogen, bilirubin, ceruloplasmin oxidase activity, and blood glucose), and histopathology (kidney, liver, pancreas, lung, heart, stomach, intestine, spleen, bone marrow, and brain). Severe decreases in food intake (80%) and body weight loss (12-50%) were observed in the 1500 and 3000 ppm groups. Additional effects observed in the 1500- and 3000-ppm groups included: macrocytic hypochromic anemia, increased reticulocyte count, diffuse nephrosis, and the presence of protein, glucose, blood, and bilirubin in the urine. The 500 ppm group showed no clinical signs of toxicity. Increases in tissue zinc levels, decreases in copper levels, and decreased ceruloplasmin oxidase activity were observed at all three dietary concentrations.

Aughey et al. (1977) investigated the effects of supplemental zinc on endocrine glands in groups of 75 male and 75 female C3H mice by administering 0 or 0.5 g/L zinc (as zinc sulfate) in the drinking water for up to 14 months. The authors reported that the body weight in the control group ranged from 21 to 30 g, and the mean weight of the zinc-fed mice was approximately 1 g higher. Using the midpoint of the body weight range (0.022 to 0.031 kg), a water intake of 0.0069 L/day was calculated (U.S. EPA, 1988), resulting in average daily drinking water doses of 0 or 135 mg Zn/kg-day. At 1-month intervals, five mice in each of the treated and control groups were killed. After 6 months of exposure to zinc, there were no significant changes in plasma insulin or glucose levels as compared to controls. Histological alterations were observed in the pancreas, pituitary gland, and adrenal gland of zinc-exposed mice. The histological changes in the mice were first observed after 3 months of exposure to zinc. In the zinc-supplemented mice, the pancreatic islets were enlarged and had a vacuolated appearance. The $-cells of the pancreatic islets were larger with enlarged mitochondria and prominent Golgi apparatus. The severity of the pancreatic lesions appeared to increase with increasing exposure durations. Pituitary alterations consisted of changes in the adrenocorticotrophic hormone-producing cells that indicated increased synthesis and secretion, including increased number and size of granules and more prominent rough endoplasmic reticulum and Golgi apparatus. Hypertrophy of the adrenal zona fasciculata and increased adrenal cortical lipid and cholesterol deposition were also observed. No tumors were reported in the pancreas, pituitary gland, or adrenal gland of zinc-exposed mice; data on other organs were not reported.

In a 1-year study, an unspecified number of newborn Chester Beatty stock mice (sex not reported) were administered 0, 1000, or 5000 ppm zinc (approximately 0, 170, or 850 mg/kg/day) as zinc sulfate in drinking water (Walters and Roe, 1965). A separate group of mice received zinc oleate in the diet at an initial dose of 5000 ppm supplemental zinc; this dose was reduced to 2500 ppm after 3 months and to 1250 ppm after an additional 3 months because of mortality due to anemia. An epidemic of the ectromelia virus caused the deaths of several mice during the first 8 weeks; consequently, additional control and test-diet groups were established. There was no difference in

body weight gain between control and treated groups, except for the dietary zinc group which became anemic. Survival was not reported in treated compared with control groups. An apparent increase in the incidence of hepatomas was observed in treated mice surviving for 45 weeks or longer relative to controls (original and replacement mice were pooled). The hepatoma incidences in the control, low-dose drinking water, high-dose drinking water, and test-diet groups were 3/24 (12.5%), 3/28 (10.7%), 3/22 (13.6%), and 7/23 (30.4%), respectively. Incidences of malignant lymphoma in the control, low dose drinking water, high- dose drinking water, and test-diet groups were 3/24 (12.5%), 4/28 (14.3%), 2/22 (9%), and 2/23 (8.7%), respectively. Incidences of lung adenoma in the control, low-dose drinking water, high-dose drinking water, and test-diet groups were 10/24 (41.7%), 9/28 (32.1%), 5/22 (22.7%), and 9/23 (39.1%), respectively. None of these were significantly elevated in a statistical analysis of these data performed by the EPA.

Halme (1961) exposed tumor-resistant and tumor-susceptible strains of mice to zinc in drinking water. In a 3-year, 5-generation study, zinc chloride was added to the water of tumorresistant mice (strain not specified); the groups received 0, 10, 20, 50, 100, or 200 mg Zn/L. The spontaneous tumor frequency for this strain of mice was 0.0004%. The tumor frequencies in the generations were reported as: F0=0.8%, F1=3.5%, F1 and F2=7.6%, and F3 and F4=25.7%. Most of the tumors occurred in the 10- and 20-mg Zinc dose groups. No statistical analyses and no individual or group tumor incidence data were reported. In the tumor-susceptible mice, strains C3H and A/Sn received 10-29 mg Zn/L in their drinking water for 2 years; 33/76 C3H strain mice developed tumors (31 in females) and 24/74 A/Sn strain mice developed tumors (20 in females). Most of the tumors were reported to be adenocarcinomas, but the tissues in which they occurred were not reported. The numbers of specific tumor types were not reported. The overall tumor frequencies (43.4% for C3H and 32.4% for A/Sn, both sexes combined) were higher than the spontaneous frequency (15% for each strain), although no statistical analyses were reported.

EU RAR

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 Zn2+/day is a NOAEL. At the LOAEL of 150 mg Zn2+/day, clinical signs and indications for disturbance of copper homeostasis have been observed. The human oral NOAEL of 50 mg Zn2+/day (0.83 mg/kg bw/day) will be taken across to the risk characterization.

Estimated Data- None

Neurotoxicity (N-single): DG

Zinc Oxide was assigned a hazard classification level of Data Gap for single dose neurotoxicity based on insufficient available data. While nonspecific signs and symptoms of neurotoxicity (light-headedness, dizziness, headache, and lethargy) have been reported in humans following acute oral exposure to zinc, it is not known whether these observations represent direct effects of the compound on the nervous system. In addition, it is unclear if these effects are reversible or not. No data was located for analogs to address this data gap.

Lists

- Authoritative: None

- Screening: None

Measured Data

ATSDR 2005

Zinc appears to be necessary for normal brain function (Sandstead et al. 1983), but excess zinc is toxic. A 16-year-old boy who ingested ≈86 mg zinc/kg/day of metallic zinc over a 2-day period in an attempt to promote wound healing, developed signs and symptoms of lethargy, light-headedness, staggering, and difficulty in writing clearly (Murphy 1970). Lethargy was also observed in a 2-year-old child who ingested a zinc chloride solution (≈1,000 mg zinc/kg) (Potter 1981). It is not known whether these observations represent direct effects on the nervous system.

Estimated Data- None

Neurotoxicity (N-repeated) (Group II*): M

Zinc Oxide was assigned a hazard classification level of Moderate for repeated dose neurotoxicity based on gradual decrease in learning extinction in mice following exposures to 0 .5 mg zinc/kg/day as zinc acetate for 28 days. This concentration range is less than the 10 mg/kg/day threshold associated with a GHS category 1 classification for repeat exposure. The hazard classification of High is based on a poorly-reported study that is less than the 90-day exposure duration typically associated with repeat exposure studies and therefore reported as low confidence.

Lists

- Authoritative: None
- Screening: None

Measured Data

ATSDR 2005

Mice exposed postnatally to 0.5 mg zinc/kg/day as zinc acetate for 28 days showed no changes in memory formation but showed a gradual decrease in learning extinction throughout the study (de Oliveira et al. 2001).

EU RAR 2004

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 Zn2+/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 (Butyrylthiocholineesterase). The activities of TTPase (thiamine pyrophophatase) 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, undistinctive 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.

Estimated Data- None

Skin Sensitization (SnS): L

Zinc oxide was assigned a hazard classification level of Low for skin sensitization based on the weight of evidence that does not indicate that zinc oxide is a very potent sensitising agent in animals, if any. In addition, the results of human patch tests do not indicate that zinc oxide acts as a sensitizing agent in humans. The hazard score of Low is based on numerous high-quality studies including human data and therefore is reported as high confidence.

Lists

- Authoritative: None
- Screening: None

Measured Data

ECHA 2019

The skin sensitizing potential of zinc oxide (purity 99.69%) was investigated in female Dunkin Hartley guinea pigs in two well-performed maximization tests, conducted according to Directive 96/54/EC B.6 and OECD guideline 406. Based on the results of a preliminary study, in the main studies experimental animals (10 in each test) were intradermally injected with a 20% concentration and epidermally exposed to a 50% concentration (i. e. the highest practically feasible concentration). Control animals (5 in each test) 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. In the first study, in response to the 50% test substance concentration skin reactions of grade 1 were observed in 4/10 experimental animals 24 hours after the challenge (40% sensitization rate), while no skin reactions were evident in the controls. In contrast, in the second study no skin reactions were evident in the experimental animals (0% sensitization rate), while a skin reaction grade 1 was seen in one control animal. The skin reaction observed in one control animal is probably a sign of non-specific irritation (Van Huygevoort, 1999b1, 1999b2).

In a third well-performed maximization test, conducted according to the same guidelines and with the same experimental design, another analytical grade zinc oxide was tested (Zincweiß Pharma A; purity 99.9%). The only difference with the studies described above was the intradermal induction concentration, which was 2% as for Zincweiß Pharma A this was considered the highest concentration that could reproducibly be injected. In this test no skin reactions were evident in both experimental and control animals, hence a 0% sensitization rate for Zincweiß Pharma A. White

staining of the treated skin by the test substance was observed in some animals 24 and 48 hours after challenge (Van Huygevoort, 1999a).

Human data: In a human patch test performed with 100 selected leg-ulcer patients, 11/100 patients gave an allergic reaction with zinc ointment (60% ZnO and 40% sesame oil). However, 14/81 patients gave a positive response when treated with sesame oil alone. This study does not give any indication for a skin sensitizing potential of zinc oxide in humans (Malten and Kuiper, 1974).

The effect of zinc oxide on contact allergy to colophony was investigated. With 14 patients with earlier history of moderate patch test reactions to colophony (a patch test) with 10% ZnO (2.3 mg Zinc/cm²) with and without colophony was performed. No positive response was observed in the 14 patients when only a 10% solution of zinc oxide was used. The addition of zinc oxide to colophony decreased the allergic reaction induced by colophony (Söderberg et al., 1990).

Repeat human insult patch tests performed specifically on nano-ZnO demonstrate that coated and uncoated ZnO (Zano 10 Plus and Zano 10, respectively) tested as 25% oily dispersion did not produce any skin irritation or skin sensitization in human volunteers under the conditions of this test (Tatsene 2007).

EU RAR 2004

The data submitted fulfil the base-set requirements for skin sensitization testing. While some studies with guinea pigs produced conflicting results, the weight of evidence does not indicate that zinc oxide is a very potent sensitizing agent in animals, if any. In addition, the results of human patch tests do not indicate that zinc oxide acts as a sensitizing agent in humans, either. Zinc oxide does not have to be classified/labelled for skin sensitization. This is supported by the fact that zinc compounds, especially zinc oxide and zinc distearate, have been used for over decades in a variety of pharmaceutical and cosmetic products (some of them even dermatological preparations against skin irritation) without any such reported effects.

Estimated Data- None

Respiratory Sensitization (SnR): H

Zinc oxide was assigned a hazard classification level of High for respiratory sensitization based on its classification as a sensitizer-induced asthmagen by the AOEC. GreenScreen® criteria classify chemicals as a Moderate to High hazard for respiratory sensitization when a chemical is classified as a sensitizer-induced asthmagen by the AOEC (CPA 2012a). Zinc oxide is conservatively assigned a High. The High hazard score is based on listing under an Authoritative B list with no supporting data and therefore is reported as low confidence.

Lists

- Authoritative:
  - AOEC – Asthmagens Asthmagen (Rs) – sensitizer-induced
  - AOEC – Asthmagens Suspected asthmagen –® - but does not meet AOEC criteria)
- Screening: None

Measured Data- None

Estimated Data- None

Skin Irritation/Corrosivity (IrS): L

Zinc oxide was assigned a hazard classification level of Low for skin irritation/corrosivity based on negative results reported in numerous animal studies. These negative results are reported in high-quality studies and therefore the hazard score of Low is reported as high confidence.

Lists

- Authoritative: None
- Screening: None

Measured Data

ECHA 2019

A study was conducted to evaluate the skin irritation potential of zinc oxide in New Zealand White rabbits by both open and occlusive patch methods. In open patch test, 0.5 mL of 20 % w/v zinc oxide suspension in 0.1 % Tween 80 or vehicle was applied to clipped sites (5 cm^2) of groups of four animals for 5 d. Skin sites were observed during and after 5 d exposure period. Animals were killed 24 h after the fifth daily treatment. In occlusive patch test, groups of four animals were treated similarly to open patch test, but the test suspension or vehicle was applied to the skin using occlusive dressing for 3 d. After 3 d, dressings were removed and skin sites evaluated for irritancy. Two rabbits were killed at this stage. The remaining animals were redressed with freshly impregnated gauzes and then re-examined and killed after a further 2 d. In both tests, representative samples of each test and control skin site of killed animals were analyzed histologically and stained with morin dye to study epidermal keratin binding of zinc. No obvious reactions were observed in both control and treatment groups in any of the performed tests after 5 d exposure. No evidence of damage was observed upon histological examination. No morin fluorescence was observed in skin sections. Based on these results, 20 % w/v zinc oxide was found to be non-irritating to rabbit skin.

A study was conducted to evaluate the skin irritation potential of zinc oxide in Dunkin-Hartley white guinea-pigs. 20 % w/v zinc oxide suspension in 0.1 % Tween 80 or vehicle was applied to shaved sites (5 cm2) of groups of eight animals for 5 consecutive days. Skin sites were observed during and after 5 d exposure period. 24 h after the fifth daily treatment, animals were killed and representative samples of each test and control skin sites were analyzed histologically and stained with morin dye to study epidermal keratin binding of zinc. No obvious reactions were observed in both control and test groups after 5 d exposure. No evidence of damage was observed upon histological examination. No morin fluorescence was observed in skin sections. Based on these results, 20 % w/v zinc oxide was found to be non-irritating to guinea-pig skin.

A study was conducted to evaluate the skin irritation potential of zinc oxide in TO (outbred) strain mice. 0.5 mL of 20 % w/v zinc oxide suspension in 0.1 % Tween 80 or vehicle was applied to clipped sites (5 cm2) of groups of six animals for 5 consecutive days. Skin sites were observed during and after 5 d exposure period. 24 h after the fifth daily treatment, animals were killed and representative samples of each test and control skin sites were analyzed histologically and stained with morin dye to study epidermal keratin binding of zinc. No obvious reactions were observed in both control and

treatment groups after 5 d exposure. No evidence of damage was observed upon histological examination. No morin fluorescence was observed in skin sections. Based on these results, 20 % w/v zinc oxide was found to be non-irritating to mouse skin.

In a skin irritation test, no dermal reactions were noted following the application of 500 mg ZnO/animal during 24 hours under occlusion to the ears of 2 New-Zealand white rabbits. The observation period was for 7 days.

Two EpiDerm tissues each were treated for 3 minutes and 1 hour, respectively, with the test item. Water was used as negative control and 8 N KOH as positive control. At the end of the exposure period the cell viabilities of the treated tissues were measured using MTT test and calculated as percent relative to the negative control. Based on the results of the present study Z-COTE HP1 was classified as non-corrosive to skin while the positive control was classified as corrosive to skin as expected.

Estimated Data- None

Eye Irritation/Corrosivity (IrE): L

Zinc oxide was assigned a hazard classification level of Low for eye irritation/corrosivity based on numerous studies in which the positive eye irritation responses do not meet the criteria outlined within the GHS methodology for classification as an eye irritant. While one animal showed erythema that persisted for 7-days, all other studies report reversible effects within a 7-day period. Therefore, the hazard score is based on weight of evidence which show the mild irritation does not fulfil the GHS criteria for a mild eye irritant. The hazard score of Moderate is based on well-reported studies and is therefore reported with high confidence.

Lists

- Authoritative: None
- Screening: None

Measured Data

EU RAR 2004

In an eye irritation study in 2 NZW rabbits (Löser, 1977) 50 mg ZnO/animal caused erythema (mean scores over 24-72 hours: 3 and 2) and edema (mean scores over 24-72 hours: 1.3 and 0.3) up to 48 hours after treatment. In the first rabbit erythema persisted for 7 days. No effects were seen on the iris and cornea. Zinc oxide is borderline positive for irritation to the rabbit eye in this study.

In another eye irritation study using 2 NZW rabbits 50 mg ZnO/animal (Thijssen, 1978) caused slight erythema (mean scores over 24-72 hours: 0.7 and 0.7) of the conjunctiva that lasted for 2 days. No effect on the iris or cornea was seen in the 7-day observation period. Zinc oxide is not irritating to the rabbit eye in this study.

In a well-performed eye irritation/corrosion study, performed according to Directive 92/69/EEC B.5 and OECD guideline 405, three male New Zealand White rabbits were treated by instillation of approximately 64 mg of zinc oxide (a volume of about 0.1 ml) into the Conjunctival sac of one eye. The other eye remained untreated and served as control. After 24 hours, both eyes of two animals were rinsed with water. The eyes were examined at 1, 24, 48 and 72 hours after instillation. No

symptoms of systemic toxicity were observed and no mortality occurred. Slight iridial irritation (grade 1) was observed in one animal, at 1 hour only. Slight irritation of the conjunctivae (grade 1-2) was seen as redness (mean scores over 24-72 hours 0.7, 1 and 1), which had completely resolved at 72 hours in all animals. Chemosis (grade 2) and discharge (grade 1) were also observed in all animals, but at 1 hour only. No corneal opacity or epithelial damage was observed in any of the animals (Van Huygevoort, 1999a).

Estimated Data- None

ECOTOXICITY (ECOTOX)

Acute Aquatic Toxicity (AA): vH

Zinc oxide was assigned a hazard classification level of Very High for acute aquatic toxicity based on LC50 results reported for all three trophic levels that are <1 mg/L. The hazard score of Very High is based on numerous studies in a variety of organisms and therefore is reported as high confidence.

Lists

- Authoritative:
  - EU - GHS (H-Statements) H400 - Very toxic to aquatic life
- Screening:
  - GHS – Japan Hazardous to the aquatic environment (acute) - Category 1 [H400]

Measured Data

ECHA 2019

Short-term toxicity to fish: Key data (lowest LC50 values) are:

- for Oncorrhynchus Mykiss: 0.169 mg Zn/l (single value) at neutral/high pH and low hardness
- for Pimephales promelas (single values) : 0.780 mg Zn/l at low pH (high hardness) and 0.330 mg Zn/l at neutral/high pH, high hardness
- for Pimephales promelas: LC50 0.780 mg Zn/l (at low pH); 0.33mg Zn/l at neutral/high pH
- for the nano values, only one species was examined (Danio rerio) for which the lowest LC50 was 1.79 mg Zn/l or 1.44 mg Zn/l hence far above the ones reported above for bulk form.

Short term toxicity for aquatic invertabrates: Key data (lowest EC50 values) are:

- for Ceriodaphnia dubia: 0.413 mg Zn/l (single value) at low pH and low hardness
- for Ceriodaphnia dubia: >0.53 mg Zn/l (single value) at low pH and high hardness
- for Ceriodaphnia dubia: 0.147 mg Zn/l (geomean value) at neutral/high pH and low hardness
- for Ceriodaphnia dubia: 0.228 mg Zn/l (geomean value) at neutral/high pH and high hardness

Acute exposure (48h) of D. magna to ZnO MNPs (modified OECD test guideline 202) caused a concentration-dependent increase on mortality (LC50 1.55 mg l-1). D. magna exposed to sublethal concentrations of ZnO MNPs had a lower feeding rate compared to bulk ZnO and soluble zinc. This did not appear to be related to physical impairment or obstruction of the organism's feeding apparatus. Bioimaging using Coherent Anti-stokes Raman Scattering (CARS) and optical light microscopy confirmed that D. magna ingested large quantities of both MNPs, but in the case of CeO2 MNPs the high uptake did not affect survival.

Acute toxicity to freshwater algae: lowest IC50 0.136 mg Zn/l (Selenastrum capricornutum; single value) (neutral/high pH). In conclusion, the dissolution of ZnO nanostructures in our medium was found to occur within hours and to be essentially independent of either nanoparticle morphology or particle concentrations. The lack of a particle concentration and morphology dependence of ZnO nanoparticle dissolution was attributed to the aggregation effect, which inherently alters the surface area of nanoparticles in solution. The complete suppression of growth in T. pseudonana could be explained on the basis of Zn dissolution since the lowest concentration of ZnO used (10mgL−1) released sufficient Zn ions to be acutely toxic to these cells.

Estimated Data- None

Chronic Aquatic Toxicity (CA): vH

Zinc oxide was assigned a hazard classification level of Very High for chronic aquatic toxicity based on LC50 results reported for all three trophic levels that are <0.1 mg/L. The hazard score of Very High is based on numerous studies in a variety of organisms and therefore is reported as high confidence.

Lists

- Authoritative:
    - H410 - Very toxic to aquatic life with long lasting effects
- Screening: None

Measured Data

ECHA 2019

Freshwater fish Data on 7 species available. Species NOECs range between 0.044 and 0.530 mg Zn/l (dissolved concentrations). Marine fish: Data on 1 species available. NOEC = 0.025 mg Zn/l (dissolved concentrations) Species NOECs have been put into the respective species sensitivity distributions. For ZnO nano form, only one study is of good quality but only compares ZnO bulk and zinc ion at one dose for control and the concentrations are nominal. Additionally, it gives unbound NOECs. It concludes though that ZnO bulk and ionic form of zinc are more toxic at equal dose. No value is retained for long-term toxicity in fish.

Freshwater: invertebrates Data on 13 species available. Species NOECs range between 0.014 and 0.400 mg Zn/l (dissolved concentrations). Marine invertebrates Data on 26 species available. Species NOECs range between 0.0056 and 0.9 mg Zn/l (dissolved concentrations) Species NOECs have been put into their respective species sensitivity distributions.

Chronic toxictiy to freshwater algae: lowest NOEC 0.019 mg Zn/l (Pseudokircherniella subcapitata =Selenastrum capricornutum; geomean of 27 data) chronic toxicity to marine algae: 12 species available which NOECs range between 0.0078 and 0.67 mg/l (dissolved concentrations)

Estimated Data- None

ENVIRONMENTAL FATE (FATE)

Persistence (P): vH

Zinc oxide was assigned a hazard classification level of Very High for persistence based on being an inorganic material and is not expected to be biodegradable. GreenScreen® criteria classify chemicals as a Very High hazard for persistence when substances are recalcitrant (CPA 2012a). The hazard score of Very High is based on physical/chemical properties of the substance and is therefore reported as high confidence.

Lists

- Authoritative: None
- Screening: None
  - EC - CEPA DSL Persistent

Measured Data- None

Estimated Data- None

Bioaccumulation (B): DG

Zinc oxide was assigned a hazard classification level of Data Gap for bioaccumulation. While some sources indicate that zinc bioconcentrates moderately in aquatic organisms; bioconcentration is higher in crustaceans and bivalve species than in fish, the data was not sufficient to allow for a hazard classification.

Lists

- Authoritative: None
- Screening: None

Measured Data

ECHA 2019

Zinc is an essential element which is actively regulated by organisms, so bioconcentration/bioaccumulation is not considered relevant for all inorganic zinc substances. As a rule, the ranges of BCF values observed have no relation to toxicity. They are the result of these active regulation mechanisms that keep the internal zinc concentration of the organisms within an optimal range.

McGeer et al (2003) extensively reviewed the evidence on bioconcentration and bioaccumulation of zinc as a function of exposure concentration in a number of taxonomic groups (algae, molluscs, arthropods, annelids, salmonid fish, cyprinid fish, and other fish). The data clearly illustrated that internal zinc content is well regulated. All eight species taxonomic groups investigated exhibited very slight increases in whole body concentration over a dramatic increase in exposure concentration. In fact, most species did not show significant increases in zinc accumulation when exposure levels increased, even when exposure concentrations reached those that would be predicted to cause chronic effects. This suggests that adverse effects related to Zn exposure are independent of whole-body accumulation. Due to the general lack of increased whole body and

tissue concentrations at higher exposure levels, the zinc BCF data showed an inverse relationship to exposure concentrations. In all cases, the relationship of BCF to exposure was significant and negative. The slopes of the BCF/BAF – exposure relations were: algae: -1.0, insects: -0.79, arthropods: -0.73, molluscs: -0.83, salmonids: -0.92, Centrarchids: -0.80, Killifish: -0.84, other fish: -0.87. Overall, species mean slope was -0.85 +/- 0.03 (McGeer et al 2003). The physiological basis for the inverse relationship of BCF to zinc exposure concentration arises from Zn uptake and control mechanisms. At low environmental zinc levels, organisms are able to sequester and retain Zn in tissues for essential functions. When Zn exposure is more elevated, aquatic organisms are able to control uptake. There is clear evidence that many species actively regulate their body Zn concentrations, including crustaceae, oligochaetes, mussels, gastropods, fish, amphipods, chironomids by different mechanisms (McGeer et al 2003).

Hao 2013

In this study, bioaccumulation and sub-acute toxicity of water-borne nano-ZnO in the test fish, juvenvile carp (Cyprinus Carpio) were evaluated. To clarify the contribution of particle size and free Zn ion to NPs toxicity, its bulk counterparts (bulk-ZnO) and the released Zn(2+) were also tested. The results showed that after a 30-day exposure, 50mg/L of nano-ZnO and bulk-ZnO could be significantly accumulated and distributed in various tissues of fish, but nano-ZnO exhibited more hyper-bioaccumulation than bulk-ZnO. Liver and gill might be the target tissues with exposure to nano-ZnO, instead, the target tissue for bulk-ZnO might be intestine. Also, 50mg/L of nano-ZnO caused more severe histopathological changes than the same concentration of bulk-ZnO, which was in accordance with the induction of higher levels of intracellular oxidative stress. The effects of dissolved Zn ions were assessed and the ion toxicity was negligible herein. The results of this study indicated that the observed toxicities of nano-ZnO were not likely a result solely of particle dissolution and identified as a function of particle toxicity and the possibility for a size dependence. The main toxic mechanism of nano-ZnO was possibly by increasing cellular oxidative stress response.

ATSDR 2005

Zinc bioconcentrates moderately in aquatic organisms; bioconcentration is higher in crustaceans and bivalve species than in fish. Zinc does not concentrate in plants, and it does not biomagnify through terrestrial food chains.

In some fish, it has been observed that the level of zinc found in their bodies did not directly relate to the exposure concentrations. A recent study shows that bioaccumulation of zinc in fish is inversely related to the aqueous exposure (McGeer et al. 2003). This evidence suggests that fish placed in environments with lower zinc concentrations can sequester zinc in their bodies.

Estimated Data- None

PHYSICAL HAZARDS (PHYSICAL)

Reactivity (Rx): L

Zinc oxide was assigned a hazard classification level of Low for reactivity based on NFPA Reactivity Hazard classifications from multiple sources, including Chemtrade (Chemtrade, 2018). The NFPA rates a score of 0 for reactivity since the material by themselves are normally stable, even under fire conditions. The hazard score of Low is based on primary reporting sources and is supported by measured data and is therefore reported as high confidence

Lists

- Authoritative: None
- Screening: None

Measured Data

Chemtrade 2018

Reactivity: Violent reaction may occur if chlorinated rubber and zinc oxide are heated to 215°C. Exothermic reaction will occur when mixed with flax oil with the possibility of ignition. Violent reaction may occur if magnesium powder or aluminum powder is heated with zinc oxide with the possibility of ignition.

Storage Conditions: Store in a dry, cool and well-ventilated place. Keep container closed when not in use. Extremely high or low temperatures and incompatible materials.

Incompatible Materials: Strong acids. Strong bases.

Fisher Scientific 2015

The NFPA rates a score of 0 for reactivity since the material by themselves are normally stable, even under fire conditions.

Estimated Data- None

Flammability (F): L

Zinc oxide was assigned a hazard classification level of Low for flammability based on reporting as not flammable by multiple sources, including Chemtrade (Chemtrade, 2018). The NFPA rates a score of 0 for flammability since the material will not burn under typical fire conditions, including intrinsically noncombustible materials such as concrete, stone, and sand. The hazard score of Low is based on both the HMIS and NFPA rating system and on primary reporting sources which is supported by measured data and is therefore reported as high confidence.

Lists

- Authoritative: None
- Screening: None

Measured Data

Chemtrade 2018

Melting Point: 1975 °C (3587 °F)

Explosion Hazard: Product is not explosive.

Fisher Scientific 2015

The NFPA and HMIS rates a score of 0 for flammability.

Estimated Data- None

REFERENCES

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APPENDIX A: HAZARD CLASSIFICATION ACRONYMS

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

APPENDIX B – PHAROS OUTPUT FOR ZINC OXIDE (1314-13-2)

Hazard Lists

ENDPOINT	HAZARD LEVEL	GS SCORE	LIST NAME	HAZARD DESCRIPTION
Carcinogenicity	H-L	LT-UNK	US EPA - IRIS Carcinogens	(1986) Group D - Not classifiable as to human carcinogenicity *
	H-L	NoGS	US EPA - IRIS Carcinogens	(1999) Data are inadequate for an assessment of human carcinogenic potential *
	H-L	NoGS	US EPA - IRIS Carcinogens	(2005) Inadequate information to assess carcinogenic potential *
Reproductive Toxicity	M	LT-UNK	GHS - Japan	Toxic to reproduction - Category 2 [H361]
Endocrine Activity	H-M	LT-P1	TEDX - Potential Endocrine Disruptors	Potential Endocrine Disruptor
Acute Mammalian Toxicity	pC	NoGS	US EPA - OPP - Registered Pesticides	FIFRA Registered Pesticide
Respiratory Sensitization	H-M	LT-UNK	AOEC - Asthmagens	Asthmagen (Rs) - sensitizer-induced
	M	NoGS	AOEC - Asthmagens	Suspected asthmagen (R) - but does not meet AOEC criteria) *
Acute Aquatic Toxicity	vH	LT-UNK	EU - GHS (H-Statements)	H400 - Very toxic to aquatic life
	vH	LT-UNK	GHS - Japan	Hazardous to the aquatic environment (acute) - Category 1 [H400]
	vH	LT-UNK	GHS - Malaysia	H400 - Very toxic to aquatic life
Terrestrial Ecotoxicity	M	NoGS	GHS - New Zealand	9.3C - Harmful to terrestrial vertebrates
Persistence	vH-H	LT-UNK	EC - CEPA DSL	Persistent
T & P and/or B [(Chronic Aquatic Toxicity and Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	U	LT-P1	EU - GHS (H-Statements)	H410 - Very toxic to aquatic life with long lasting effects
	U	LT-P1	GHS - Australia	H410 - Very toxic to aquatic life with long lasting effects
	U	LT-UNK	GHS - New Zealand	9.1A (algal) - Very ecotoxic in the aquatic environment
	U	LT-UNK	GHS - New Zealand	9.1A (crustacean) - Very ecotoxic in the aquatic environment
	U	LT-UNK	GHS - New Zealand	9.1A (fish) - Very ecotoxic in the aquatic environment
Human and/or Aquatic toxicity and/or Persistence and/or Bioaccumulation	U	LT-P1	German FEA - Substances Hazardous to Waters	Class 2 - Hazard to Waters
T & P and/or B [(Chronic Aquatic Toxicity and sometimes Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	H	LT-UNK	GHS - Malaysia	H410 - Very toxic to aquatic life with long lasting effects
	U	LT-P1	GHS - Japan	Hazardous to the aquatic environment (chronic) - Category 1 [H410]
Systemic Toxicity/Organ Effects [Single Exposure] and/or Neurotoxicity [Single Exposure]	vH	LT-UNK	GHS - Japan	Specific target organs/systemic toxicity following single exposure - Category 1 [H370]
Acute aquatic toxicity; Chronic aquatic toxicity	U	LT-UNK	EC - CEPA DSL	Inherently Toxic in the Environment (iTE)