ECHA Scientific report

for the evaluation of limit values for copper and its inorganic compounds at the workplace

Prepared by the European Chemicals Agency

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List of abbreviations

Abbreviation	Definition
AGS	Ausschuss für Gefahrstoffe (German Committee on Hazardous Substances)
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANSES	Agence Nationale de SEcurité Sanitaire de l'alimentation, de l'environnement et du travail (French Agency for Food, Environmental and Occupational Health & Safety)
ASA	Alveolar surface area
AST	Aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry (USA)
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage Fluid
BGV	Biological Guidance Value
BLV	Biological Limit Value
bw	Body weight
CAD	Chemical Agents Directive 98/24/EC
CAS RN	CAS Registry Number (unique identifier providing an unambiguous means to distinguish chemical substances or molecular structures when there are many possible systematic, generic, proprietary or otherwise trivial names)
CAT	Catalase
CI	Confidence Interval
CLP	Regulation EC No 1272/2008 on the Classification, Labelling and Packaging of substances and mixtures (CLP Regulation)
CMD/ CMRD	Carcinogens and Mutagens Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work. The amendment of the CMD, Directive 2022/431/EU also brought reprotoxic substances within the scope of the directive, changing the original title on the protection of workers from the risks related to exposure to carcinogens or mutagens at work to the protection of workers from the risks related to exposure to carcinogens, mutagens or reprotoxic substances at work (CMRD).
DFG	Deutsche Forschungsgemeinschaft (German Research Foundation)
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EU	European Union
FEF	Forced Expiratory Flow
FEV	Forced Expiratory Volume
FSH	Follicle-stimulating hormone
FVC	Forced Vital Capacity
GESTIS Substance	GEfahrSToffInformationsSystem (German information system for the safe

Abbreviation	Definition
Database	handling of hazardous substances and other chemical substances at work) Substance Database
GGT	gamma-glutamyl transferase
GLP	Good Laboratory Practice
GSH	Glutathione
HEC	Human equivalent concentration
IARC	International Agency for Research on Cancer (World Health Organization)
IOELV(s)	Indicative Occupational Exposure Limit Value(s)
i.p.	Intraperitoneal
LD50	Median lethal dose
LDH	Lactate dehydrogenase
LH	Luteinizing hormone
LOQ	Limit of quantification
MDA	Malondialdehyde
MMAD	Mass Median Aerodynamic Diameter
MoA	Mode of action
MPPD	Multiple-Path Particle Dosimetry Model
NIOSH	National Institute for Occupational Safety and Health (USA)
NP	Nanoparticle
NTP	National Toxicology Program (USA)
OECD	Organisation for Economic Co-operation and Development
OECD TG	OECD Test Guidelines for the testing of chemicals
OEL(s)	Occupational exposure limit(s)
OSHA	Occupational Safety and Health Administration (USA)
PBPK	Physiologically Based Pharmacokinetic
PBTK	Physiologically Based Toxicokinetic
PM	Particulate Matter
RAC	Risk Assessment Committee
REACH	Regulation (EC) No 1907/2006 of the European Union concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals
SCOEL	Scientific Committee on Occupational Exposure Limits (former committee of the European Commission)
SLA	Service Level Agreement
SOD	Superoxide dismutase
STEL	Short term exposure limit
TWA	Time-Weighted-Average
WPC	Working Party on Chemicals

Procedure and outcome

I. European Commission request

The European Commission is responsible for preparing the proposals for amendment of Directive 98/24/EC on the protection of the health and safety of workers from the risks related to exposure to chemical agent work (CAD).

Therefore, the Commission requested ECHA on 29 January 2026, in accordance with the Service Level Agreement (SLA) (Ares(2022)711149), to evaluate the following substance: Copper (EC number 231-159-6) and its inorganic compounds, in accordance with the CAD.

In response to the Commission's request, ECHA has prepared a scientific report concerning occupational limit values for copper (EC number 231-159-6) and its inorganic compounds:

- ECHA launched a call for evidence between 01 April 2026 and 01 July 2026
- ECHA launched a consultation between 12 October 2026 and 14 December 2026.

RAC will developed its opinion on occupational limit values (OELs) based on ECHA's scientific report, which will be attached as an Annex to the adopted RAC opinion.

All information is available on the OEL activity list (click on <Details> at the end of the row).

II. Literature search & data collection

This report is based on international assessments such as (ATSDR, 2024, DFG, 2014, SCOEL, 2014). The literature search was performed using the scientific literature databases: PubMed[2] and Web of Science[3] by Clarivate Analytics with publication data mainly searched beyond the year 2000. Searches covered the years: 1975 – 2026.

Search strings were developed and targeted literature searches performed on the basis of the Commission's request with a focus on occupational exposure, general human exposure and occurrence data, and a general search on all the toxicological endpoints considered.

All references are listed at the end of the report. Databases used were last accessed: August 2026.

III. ECHA evaluation and recommendation

The tables below present the outcome of the scientific evaluation to derive limit values for copper and its inorganic compounds.

Table 1: Outcome of the scientific evaluation

Derived Limit Values	Value
OEL as 8-hour TWA	0.01 mg Cu/m^3 (respirable fraction) 0.1 mg Cu/m^3 (inhalable fraction)
STEL	None proposed
BLV	None proposed
BGV	60 µg Cu/L (urine)

Notations	Value
None proposed	

[2] https://pubmed.ncbi.nlm.nih.gov/
[3] https://www.webofscience.com/wos/woscc/basic-search

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1. Chemical agent identification and physico-chemical properties

1.1 Copper

The chemical identifiers and main physico-chemical properties of copper are listed in Table 2 and Table 3.

Table 2: Chemical Identification

Identifier	
IUPAC Name	copper
Synonyms	-
EC/ List No	231-159-6
CAS RN	7440-50-8
Molecular formula	Cu
Molecular weight	63.546

Table 3: Physico-chemical properties[4]

Property	
Physical state (20°C / 1013 hPa)	solid
Boiling point	Not applicable
Melting point (°C)	1059-1069
Density	8.78
Vapour pressure	Not applicable
Water solubility	Insoluble

1.2 Inorganic copper compounds

In total, there are 45 inorganic copper compounds registered under REACH. Of these substances, 11 are only used during the manufacture and recycling of copper and its compounds. These 11 substances are all of complex and variable composition and contain other constituents than only copper compounds. They are therefore not very useful in terms of evaluating the specific properties of copper and its compounds and are not considered in the present evaluation. Copper and 4 out of the 34 remaining copper compounds, which are registered according to the REACH legislation, cover the use of more than 99.7% of the total estimated tonnage of the inorganic copper compounds at the time of writing, with copper alone accounting for 97.5% of the total tonnage. Therefore, only copper and these 4 inorganic copper compounds are described in detail (in terms of properties, hazards, uses etc.) in this report.

The substance identification and physico-chemical properties of the 4 inorganic copper compounds are described in Table 4 and Table 5.

The 46 registered under REACH compounds (i.e., copper and inorganic copper compounds) are listed in Appendix 1 of this report.

[4] Values obtained from registration data published on ECHA Chemicals Database (ECHA CHEM).

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Table 4: Substance identification

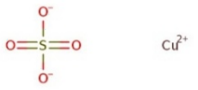
Substance name	EC No	CAS RN	Synonyms	Molecular formula	Chemical structure	Molecular weight
Copper oxide	215-269-1	1317-38-0	-	CuO		79.545
Dicopper oxide	215-270-7	1317-39-1	-	Cu_2O		143.091
Copper chloride	231-842-9	7758-89-6	-	Cl_2Cu		134.446
Copper sulphate	231-847-6	7758-98-7	-	CuO_4S		159.602

Table 5: Physico-chemical properties[6]

Substance name	EC No	Physical state (20°C / 1013 hPa)	Density (g/cm^3)	Melting point (°C)	Boiling point (°C)	Vapour pressure (kPa)	Water Solubility
Copper oxide	215-269-1	solid	6.31	1326	Not applicable	Not applicable	0.23 g/l (at pH 5.1)
Dicopper oxide	215-270-7	solid	5.87	399,85	Not applicable	Not applicable	$6.39x10^{-4}$g/l (pH 6.5 – 6.6)
Copper chloride	231-842-9	solid	4.14	423	1490	Not applicable	47 mg/l
Copper sulphate	231-847-6	solid	3.6	Decomposes without melting at 560°C	Decomposes without boiling at 560°C	Not applicable	22g/100 g H_2O at 25°C

2. EU harmonised classification and labelling - CLP (EC) 1272/2008

The harmonised classification for copper and selected inorganic compounds is presented in Table 6.

Table 6: EU classification: Summary of existing classifications

Index No	International chemical ID	EC number	CAS RN	Annex VI of CLP hazard class and category	Hazard statement code
029-026-00-0	Copper	231-159-6	7440-50-8	Aq. Acute 1 Aq. Chronic 1	H400 H410
029-016-00-6	Copper oxide	215-269-1	1317-38-0	Aq. Acute 1 Aq. Chronic 1	H400 H410
029-002-00-X	Dicopper oxide	215-270-7	1317-39-1	Acute Tox. 4 Acute Tox. 4 Eye Dam. 1 Aq. Acute 1	H332 H302 H318 H400

[6] Values obtained from registration data published on ECHA Chemicals Database (ECHA CHEM).

Index No	International chemical ID	EC number	CAS RN	Annex VI of CLP hazard class and category	Hazard statement code
				Aq. Chronic 1	H410
029-001-00-4	Copper chloride	231-842-9	7758-89-6	Acute Tox. 4 Aq. Acute 1 Aq. Chronic 1	H302 H400 H410
029-004-00-0	Copper sulphate	231-847-6	7758-98-7	Acute Tox. 4 Skin Irrit. 2 Eye Irrit. 2 Aq. Acute 1 Aq. Chronic 1	H302 H315 H319 H400 H410

3. Chemical Agent and Scope of Legislation – Regulated uses in the EU

Table 7: Scope of legislation

Legislation	Applicable	Comment
Directive 98/24/EC (CAD)	Not listed	/
Directive 2004/37/EU (CMRD)	Not listed	/
Regulation (EC) No 1907/2006 (REACH) – Annex XIV (Authorisation)	Not listed	/
Regulation (EC) No 1907/2006 (REACH) – Annex XVII (Restriction)	Not listed	/
Regulation (EU) 528/2012 – Biocidal Products	Authorised	Copper, granulated was approved on 1st January 2017.
Regulation (EC) 1107/2009 - Plant Protection Product	Authorised	Copper compounds (copper hydroxide, copper oxychloride and tribasic copper sulphate). In the context of the peer review process of plant protection products (PPPs) in 2008, EFSA established an acceptable daily intake (ADI) of 0.15 mg Cu/kg bw per day (corresponding to 10 mg/day for a 70-kg adult). Dietary exposure assessments were performed in 2015 by EFSA's Panel on Nutrition, Novel Foods and Food Allergens (NDA) and in 2018 under the framework of the review of the existing maximum residue levels (MRLs) for copper compounds according to Article 12 of Regulation (EC) No 396/2005. The levels of copper in foods of animal origin for human consumption resulting from the use of copper-containing PPPs are regulated according to Annex III A Reg. 396/2005 and Reg. (EC) No 149/2008.
Directives 2001/83/EC – Human Medicinal Products	Not listed	/
Directives 2004/28/EC – Veterinary Medicinal Products	Not listed	Several copper compounds are authorised to be used as nutritional additives in feed for all species. Based on the authorisation, the introduction of copper into the food chain is regulated by setting authorised maximum content of total copper in complete animal feed [e.g., Regulation (EU) 2018/1039].

3.1 REACH Registrations

Table 8: REACH Registrations and cumulative tonnage

Substance(s)		Cumulative tonnage (tonnes/annum) [8]
Name	**EC number**	
Copper	231-159-6	1 000 000 – 10 000 000; 236 active registrants (of which 0 are for intermediate use)
Copper oxide	215-269-1	10 000 – 100 000; 64 active registrants (of which 2 are for intermediate use)
Dicopper oxide	215-270-7	1 000 – 10 000; 14 active registrants (of which 0 are for intermediate use)
Copper chloride	231-842-9	100 – 1 000; 12 active registrants (of which 1 are for intermediate use)
Copper sulphate	231-847-6	10 000 – 100 000; 60 active registrants (of which 0 are for intermediate use)

4. Existing Occupational Exposure Limit values

4.1 Occupational Exposure Limits (OEL)

Several EU Member States and non-EU countries have established OEL values for copper and its inorganic compounds. Some countries have additionally established short-term limit values (STEL) (Table 9).

The list should not be considered as exhaustive.

Table 9: Existing Occupational Exposure Limits (OELs)

Country	TWA (8 hrs)		STEL (15 min)		Remarks / Notations
	ppm	mg/m³	ppm	mg/m³	
Copper and its compounds, inorganic (as copper)					
EU					
Austria		1 (1) 1 (2)		4	(1) Inhalable fraction. (2) Inhalable aerosol. Copper, dusts and mists
Belgium		1			Copper, dusts and mists
Denmark		1		2	Copper, dusts and mists
Germany (AGS)		0.045 (1) 0.2 (2)		0.36 (1) 0.8 (2)	(1) Respirable fraction (2) Inhalable fraction
Germany (DFG)		0.01		0.02	Respirable fraction. Same for copper, dusts, mists
Finland		0.02			Respirable fraction. Copper sulphate,

[8] ECHA Chemicals Database (ECHA CHEM), accessed August 2026

Country	TWA (8 hrs)		STEL (15 min)		Remarks / Notations
	ppm	mg/m³	ppm	mg/m³	
					copper oxide, dicopper oxide
France		1		2	Copper, dusts and mists
Hungary		0.1 1 (2)		0.2 4 (2)	(1) Copper and its inorganic compounds (2) Copper, dusts and mists
Ireland		1			Copper, dusts and mists
Latvia		0.5		1	Inhalable fraction
The Netherlands		0.1			Inhalable fraction. Also for copper, dusts and mists
Romania		0.5		1.5	Copper, dusts and mists
Poland		0.2			
Spain		0.01			Respirable fraction. Also for copper, dusts and mists
Sweden		0.01			Respirable fraction. Also for copper, dusts and mists
Non-EU					
Australia		1			Copper, dusts and mists
China		1			Copper, dusts and mists
Canada-Ontario		1			Copper, dusts and mists
Canada-Québec		1			Copper, dusts and mists
New Zealand		0.01			Respirable fraction. Dermal sensitiser. Also for copper, dusts and mists
Singapore		1			Copper, dusts and mists
Switzerland		0.1		0.2	Inhalable fraction
South Africa		0.4 (1) 2 (2)			(1) Copper oxide (2) Copper, dusts and mists
South Africa mining		0.2 (1) 1 (2)		2 (2)	(1) Copper oxide (2) Copper, dusts and mists
South Korea		1		2	Copper, dusts and mists
United Kingdom		1		2	Copper, dusts and mists
USA (NIOSH)		0.1			Copper oxide
USA (OSHA)		0.1			Copper oxide

Notes: AGS: Committee on Hazardous Substances; DFG: Deutsche Forschungsgemeinschaft; TWA: Time-Weighted-Average; STEL: Short term exposure limit; NIOSH: National Institute for Occupational Safety and Health; OSHA: Occupational Safety and Health Administration.

Source: GESTIS Substance Database, last accessed August 2026 / not exhaustive.

4.2 Biological Limit Values (BLV) and Biological Guidance Values (BGV)

No BLV or BGV were identified for copper and its inorganic compounds.

5. Occurrence, uses and occupational exposure

5.1 Occurrence

Copper is one of the few native metals, meaning metals that occur naturally in a directly usable, unalloyed metallic form. It occurs in the Earth's crust at an average abundance of about 50 parts per million. It is primarily mined from large-scale, low-grade deposits (often less than 1% copper), with Chile, Peru, Congo, and China leading global production (Data, 2026). Copper readily bonds with other elements, so it is often extracted alongside other commercially valuable metals like gold, silver, lead, and zinc.

Copper occurs in a variety of minerals, depending on geological conditions (Davenport, 2001):

- Native Copper: rarely found in pure metallic form, sometimes in massive irregular masses or veins (historically in Michigan's Keweenaw Peninsula).
- Sulphide Ores: The most common source; includes chalcopyrite ($CuFeS_2$), bornite (Cu_5FeS_4), and chalcocite (Cu_2S).
- Oxide & Carbonate Ores: Found closer to the surface; includes minerals like malachite (green), azurite (blue), and cuprite.

Copper mineralisation is linked to historical volcanic and sedimentary processes:

- Porphyry Copper Deposits: formed by cooling magmas; these account for the majority of the world's copper resources (around 60%).
- Sedimentary Copper: formed in ancient river and marine basins (e.g., in Central Africa and parts of China) independently of volcanoes or magmatic activity (accounts for roughly 23% of the world's copper).
- Volcanogenic Massive Sulphide (VMS) Deposits: formed by underwater volcanic vents, they represent a significant source of the world's copper.

5.2 Production and uses

The EU classifies copper as a Strategic Raw Material under the Critical Raw Materials Act (CRMA) to ensure supply chain security for its green transition (JRC, 2026). It is a soft, malleable, and ductile metal with very high thermal and electrical conductivity. The demand for copper is driven by the growth of the global middle class and an increasingly electrified world. Copper is used in everything from power grids and vehicles to defence and industry. Due to its crucial role in decarbonisation technologies, copper demand is expected to grow by 35% in the EU by 2050. While Poland is the top primary copper producer and Germany leads in refining, the bloc relies heavily on external imports and robust recycling to meet demand.

The copper industry in Europe includes copper mines, smelters, refiners, recycling facilities and fabricators of semi-finished products from copper and copper alloys, such as tubes, wire rods and bars. Copper is an important contributor to the European economy, and the copper industry employs approximately 50 000 people in Europe. Many more individuals are employed indirectly.

5.2.1 Primary Copper – mining/extraction

The EU accounts for about 3.6% of global primary production but nearly 10% of refined production, with Poland and Sweden as the top miners and Germany acting as the largest refiner (ICSG, 2025).

Major copper extraction operations in the EU:

- Poland is home to KGHM Polska Miedź, a major global producer operating three massive

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underground mines in the Lubin area (Lubin, Rudna, and Polkowice-Sieroszowice). The region holds some of the largest ore deposits in Europe.
- Sweden is home to Boliden which operates Aitik, one of Europe's largest and most productive open-pit copper mines, located near Gällivare. The Swedish government also approved Boliden's extraction concession for the large Nautanen copper deposit in Norrbotten.

Other locations where copper mining occurs include Finland, Portugal and Spain**.**

Production from primary sources starts with the extraction of copper-bearing ores from open-pit or underground mines. The ores typically contain between 0.25 and 1% of copper, which are then enriched (via milling and froth flotation) into copper concentrates with a copper content between 20 and 30%. It should be noted that there are different names for the copper at each distinct stage of manufacturing, and in this stage the copper starts as copper ore and is transformed into copper concentrate (ICSG, 2026). Subsequently, two different production routes exist (pyrometallurgical and hydrometallurgical), depending on the characteristics of the raw material-sulphide or oxide ores.

5.2.2 Secondary copper – recycling/recovery from copper containing (used) material

Production from secondary sources is fed by copper scrap that originates either from semi-finished or finished products manufacturing waste ("new scrap"), or from copper-containing products reaching the end of their life ("old scrap"). After initial treatment, which usually includes sorting and shredding, the copper scrap enters the pyrometallurgical production process at different stages. In the EU, the copper industry has developed advanced technologies and made considerable investments to be able to process a wide range of copper scrap, including complex, low-grade residues and electronic scrap characterised by variable copper content and a broad concentration range of other metals.

5.2.3 Pyrometallurgical (Smelting & Refining)

Used for sulphide ores (and recycling/secondary copper). There are a number of different intermediate products of copper metallurgy. Black copper (60-85% copper) is produced by smelting oxidised copper ores or impure scrap, usually in a blast furnace. Copper matte (50-70% copper) is produced by smelting copper concentrate and then further oxidised to remove iron and sulphur, converting it into blister copper (98-99% copper). Anode copper (99% copper) is then produced from blister copper by reduction with natural gas or wood to remove oxygen and other impurities in an anode furnace. Finally, the anode copper is purified through electrorefining, during which an electric current moves copper from the anodes onto stainless steel blanks, producing ultra-pure sheets to produce cathode copper (99.99% copper), which are the standard for global metal trading.

5.2.4 Hydrometallurgical (Leaching & Solvent extraction and electrowinning)

Often used for oxide ores. Copper is dissolved from crushed ore using a liquid solution (lixiviant), most commonly dilute sulphuric acid, extracting the copper into a liquid (Pregnant Leach Solution; PLS). The PLS then undergoes solvent extraction (where the extractant selectively bonds with the copper ions, pulling them from the aqueous phase into the organic phase). This is followed by electrowinning (by passing a direct electric current between an inert anode and a metal cathode, positively charged metal ions migrate to the cathode and deposit as a pure solid metal) to produce pure cathode copper (99.99% copper).

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5.2.5 REACH Registration of "intermediates" during copper manufacture

Table 10: Substances registered during the manufacture of copper*

EC Number	Name
918-168-7	Anode, copper
266-967-8	Matte, copper
918-452-0	Black copper, copper smelting
273-752-2	Electrolytes, copper-manufg., spent
701-554-2	Copper and silver nitrates and nitric acid from electrochemical refining of silver anodes
273-925-2	Lead, dross, copper-rich
273-836-9	Speiss, copper
266-964-1	Cement copper
919-583-6	Cupro, copper processing
273-744-9	Scale (coating), copper
951-951-1	Sodium copper sulphate, sodium zinc chloride, zinc sulphate, recovery products from copper and zinc dusts

*These are in order of estimated registration tonnage, from highest to lowest

5.2.6 Semi-fabrication

Pure copper cathodes are melted down and alloyed (or formed) into raw commercial materials:

- Wire Rod: The primary starting material for electrical wiring, made by continuously casting and rolling molten cathodes into long coils.
- Billets, Cakes, and Slabs: Cast shapes of copper (or copper alloys like brass and bronze) that are ready to be rolled, extruded, or drawn into final products.
  Strip, Sheet, and Tube: Intermediate rolled or extruded shapes used in construction, radiators, and electronics.

5.2.7 Component manufacturing

Semi-fabricated copper is shaped into commercial goods:

- Wire and Cable: Drawn wire rod shielded with insulation for telecommunications and power transmission.
- Standoffs and Fittings: Machined or stamped parts for plumbing, automotive radiators, and electrical components.
- Foil: Ultra-thin rolled copper used to produce printed circuit boards (PCBs)

5.2.8 Uses

99.7% of the REACH registered tonnage for copper and copper compounds (not including copper manufacturing) is accounted for by the following five substances which along with their main uses (indicated by X) are presented in Table 11.

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Table 11: Copper and copper substances accounting for most of the REACH registered tonnage and their main uses

EC number	Name	Conductive/ plating agent	Pigment/ dye	Fertiliser	Catalyst	Other uses
231-159-6	Copper	X				
231-847-6	Copper sulphate	X	X	X		Intermediate, lab reagent
215-269-1	Copper oxide	X	X	X	X	Intermediate, lubricant, lab reagent
215-270-7	Dicopper oxide	X	X	X	X	Intermediate, thermite welding
231-842-9	Copper chloride		X	X	X	Intermediate, lab reagent

5.2.8.1 Copper

Copper itself, at more than 5 million tonnes/year, accounts for about 97.5% of the total registration tonnage. In the European Union, copper is classified as a strategically vital raw material. Given its widespread use in decarbonisation technologies, global copper demand is expected to increase in the run up to 2050 (European Copper Institute-Copper Alliance, 2023) . According to a 2022 study by KU Leuven commissioned by Eurométaux (Leuven, 2022), the demand for refined copper in Europe is expected to grow by 1% annually, over the period from 2020 to 2050, under a scenario of keeping the production of decarbonisation technologies in Europe (solar panels, wind turbines, electric vehicles, …) at current or increasing levels.

The primary uses of copper are split between four core sectors (ICSG, 2025):

- building construction (wiring, plumbing);
- infrastructure (power grids, telecom);
- equipment manufacturing (appliances, industrial machinery);
- transportation.

5.2.8.1.1 Building construction (wiring, plumbing)

Copper is vital in building construction for its exceptional electrical and thermal conductivity, high corrosion resistance, and longevity. It is primarily utilized in electrical wiring, plumbing systems, and architectural elements like cladding. Copper's high conductivity makes it the industry standard for safe and energy-efficient power transmission, reducing thermal stress and electrical loss within buildings. Copper (and brass) are the materials of choice for plumbing, taps, valves, and fittings. Copper tubes also help protect water systems from potentially lethal bacteria such as *Legionella*. In addition, copper is used in building facades, canopies, doors, window frames, roofs and fire sprinkler systems.

5.2.8.1.2 Infrastructure (power grids, telecom)

Due to its unparalleled electrical and thermal conductivity, it is the essential metal used in generation windings, high-voltage transmission cables, transformers, and electrical substations to minimize energy loss during transfer. Renewable energy systems (like wind turbines and solar farms) require four to six times more copper per installed megawatt than conventional thermal or nuclear plants because their generation is spread over much larger land areas, increasing the demand for copper.

Copper also plays a key role in worldwide information and communications technologies. Copper and copper alloy products are used in domestic subscriber lines, wide and local area networks, mobile phones, and personal computers. By using copper for circuitry in silicon chips,

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microprocessors are able to operate at higher speeds, using less energy. Copper heat sinks help remove heat from transistors and keep computer processors operating at peak efficiency. Copper is also used extensively in other electronic equipment in the form of wires, transformers, connectors, and switches. The rapid growth of data centres is also driving increasing demand for copper.

5.2.8.1.3 Equipment manufacturing (appliances, industrial machinery)

Due to their durability, machinability, and ability to be cast with high precision and tolerances, copper alloys are ideal for making products such as gears, bearings, and turbine blades.

Copper's superior heat transfer capabilities and ability to withstand extreme environments make it an ideal choice for heat exchange equipment, pressure vessels, and vats. The corrosion-resistant properties of copper and copper alloys (such as brass, bronze, and copper-nickel) make them especially suitable for use in marine and other demanding environments. Vessels, tanks, and piping exposed to seawater, propellers, oil platforms, and coastal power stations all depend on copper's corrosion resistance for protection.

5.2.8.1.4 Transportation

Copper-nickel alloys are used on the hulls of boats and ships to reduce marine biofouling, thereby reducing drag and improving fuel consumption. Automobiles and trucks rely on copper motors, wiring, radiators, connectors, brakes, and bearings. Today, the average internal combustion engine contains about 22.5 kg (50 lbs.) of copper, while luxury cars on average contain around 1 500 copper wires totalling about 1.6 km (1 mile) in length.

Electric vehicles (EVs) contain approximately four times more copper than conventional cars. It is used in batteries, windings, and copper rotors used in electric motors, wiring, busbars, and charging infrastructure. To reduce carbon emissions, the use of EVs will continue to rise, and this increase will result in greater demand for copper.

Copper is also used extensively in new-generation airplanes and trains. New high-speed trains can use anywhere from 2 to 4 tonnes of copper, significantly higher than the 1 to 2 tonnes used in traditional electric trains.

5.2.8.2 Copper compounds

5.2.8.2.1 Conductive/ plating agent

Copper compounds (oxides, copper sulphate and copper chloride) are predominantly used in industrial plating as anode materials and replenishment agents (Britannica, 2026). They are dissolved into electrolytes to continuously maintain the copper ion concentration in baths (without requiring solid copper anodes) for printed circuit boards (PCBs), semiconductors, and electroforming. In some specialized electrochemical or electroless processes, copper oxides can be reduced to generate thin, uniform, nanostructured copper coatings or to apply copper to non-conductive surfaces like glass or polymers. Copper chloride is frequently used in etching, pickling, or activating bath formulations. It helps clean and slightly roughen a base metal surface at a microscopic level, maximizing the adhesion (mechanical grip) of subsequent copper or multi-layer coatings.

5.2.8.2.2 Pigments

Copper oxides are highly versatile inorganic colorants primarily used in ceramics, glass, and specialty coatings. The final colour depends on the oxide type-Copper(II) Oxide (CuO) is black, and Copper(I) Oxide is red or yellow-as well as the kiln atmosphere and glaze chemistry, producing blue, red, and green, and sometimes grey, pink, or black glazes. Copper oxides and copper chloride can also be used in fireworks and pyrotechnics. Copper sulphate is primarily used in pigment production as a chemical precursor rather than a direct pigment itself. It is a crucial ingredient for synthesizing inorganic pigments like cobalt blue and manganese blue, as well as manufacturing specialised colorants for glass and ceramic.

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5.2.8.2.3 Fertilisers

Copper sulphate is a highly bioavailable micronutrient fertiliser used to correct copper deficiencies in crops, promoting essential functions like photosynthesis and lignin synthesis. It typically contains around 25% elemental copper in a water-soluble, pentahydrate form. It is also widely used as a preventative fungicide and algaecide.

Copper oxides are also used as micronutrient fertilisers to supply essential copper but because they are slowly soluble, they provide a long-lasting release of copper and are heavily utilised in agriculture, organic farming, and nano-agrochemical formulations. In addition to correcting copper deficiencies, copper oxides act as sanitising agents that help protect high-value crops (such as citrus, grapes, and vineyards) from fungal outbreaks and diseases. Modern agriculture is increasingly utilizing Copper Oxide Nanoparticles (CuO NPs).

5.2.8.2.4 Catalysts

Copper compounds are highly versatile and cost-effective catalysts. They cycle efficiently between oxidation states to drive essential industrial processes and synthetic organic transformations.

In environmental remediation, copper oxide nanocatalysts drive the low-temperature oxidation of carbon monoxide (CO) into CO_2 (often used in gas sensors and off-gas purification). Copper oxides are widely used in heterogeneous catalysis for the selective oxidation of alcohols to aldehydes or ketones, off-gas purification (removing VOCs, CO, and No_x) and N_2O decomposition. They are also fundamental in low-temperature water-gas shift reaction

Copper chloride is the standard industrial catalyst for producing chlorine gas from hydrogen chloride and oxygen at 400–450°C in the Deacon Process. It also facilitates the synthesis of chlorinated hydrocarbons (such as vinyl chloride, dichloromethane, trichloroethylene, and precursors to pharmaceuticals). In laboratory and fine-chemical manufacturing, it is heavily used for the Sandmeyer reaction (substituting amine groups with halides) and the aerobic oxidation of phenols. In vitamin and monomer production, it drives specialty catalytic steps in industrial vitamin synthesis and the direct synthesis of chlorosilanes used in silicone manufacturing.

5.2.8.2.5 Other uses

The listed copper compounds are used as chemical intermediates in the synthesis of other copper-based compounds (including specialised catalysts and pigments). Their primary role is to supply reactive copper ions Cu^{2+} required to build more complex chemical formulations.

The listed copper compounds are also versatile inorganic laboratory reagents. They can be used as oxidising agent for elemental analysis, catalysts, precursors to generate pure copper salts, and sources of copper ions in analytical chemistry

In addition, there are some specific uses of certain compounds. Copper oxide is used in lubrication as a nanoscale additive to base oils and greases. When suspended as nanoparticles (typically 0.1% to 1.0% by weight) in lubricants, it significantly reduces the coefficient of friction and wear rates. Additionally, it improves the lubricant's thermal conductivity and viscosity index. Dicopper oxide is used in copper thermite (or exothermic) welding to create high-integrity, electrically conductive joints. It acts as a powerful oxidiser that, when combined with aluminium powder, produces molten elemental copper.

5.3 Occupational exposure

According to REACH data, there are activities during copper manufacturing and use that lead to higher exposures (up to 1 mg/m^3) such as smelting, copper powder or flake manufacture, handling of raw material and scrap, the production of articles, maintenance etc. Many of these activities involve melting and the inhalation of fumes or manual handling (especially of powders or flakes) by workers where the activities are not enclosed but use a mixture of risk management measures such as local exhaust ventilation (LEV), or general ventilation with respiratory protective equipment (RPE) to reduce exposure. There are also activities, such as melting and

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casting of alloys, etching etc., that are more enclosed or automated (requiring less worker intervention) that have much lower worker exposures (~0.01 mg/m³).

The exposure data is based on a Voluntary Risk Assessment (VRA) for copper managed by European Copper Institute (European Copper Institute (ECI), 2008). The Scientific Committee on Health and Environmental Risks (SCHER) generally agrees with the approach and conclusions. It describes the VRA as following basically the Technical Guidance Document (TGD). For occupational exposure, data for twelve industrial processes from several industrial sites, from copper smelting to production of copper chemicals and other copper products have been compiled according to the approach from 2008 (with the latest exposure data from 2022). Inhalation exposure assessment was based on measured personal exposure data and also included acute inhalation exposure (short-term peak values) as a separate assessment. Some exposure data was corrected by correction factors for differences in samplers used for data collection, with the corrections justified. In the absence of dermal exposure data for copper (since copper does not cause skin irritation), data on zinc (zinc oxide) was used by the "analogous substance" principle for workers. The use of zinc data was justified by a similar industrial process of handling, dustiness and particle size with the most copper compounds and inadequacy of the Estimation and Assessment of Substance Exposure (EASE) model in exposure assessment for hot metal processes.

There are relatively few occupational exposure studies for copper (and its compounds) compared to some other metals. Unlike metals, such as arsenic, beryllium, lead, and mercury, copper has not been generally associated with appreciable occupational health concerns and has not received significant research attention in this context. Historically, health outcomes that have been associated with occupational copper exposures include respiratory irritation, nausea, and metal fume fever.

Poland et al. (2026) conducted a review to examine the toxicological impact of copper inhalation, from its toxicological mode of action to respiratory effects in animal models and humans, in the context of adversity, to identify relevant points of departure (PoDs) for establishing an OEL (Poland et al., 2026). Exposure information compiled across five European companies involved in copper manufacturing (including recycling) indicated typical personal 8-h TWA of respirable copper exposures ranging from 2.96 µg/m^3 (screening area) to 84.89 µg/m^3 (furnace area), with inhalable copper concentrations ranging from 10.60 µg/m^3 to 457.0 µg/m^3 in different work areas.

Kelvin et al. (2023) present a method to characterise airborne dust (inhalable and respirable) from a European copper smelter, performed using a combination of chemical analysis and quantitative mineralogy ((using Quantitative Evaluation of Materials by Scanning Electron Microscopy (QEMSCAN)); a relatively novel application to industrial hygiene. The data confirms that exposure to specific copper phases differs throughout an operation and is unique to the activities that are performed at each location, reflecting the progressive refinement during the smelting process, with the highest copper concentrations reported during batch preparation for both the inhalable (460 µg Cu/m³) and respirable fractions (40 µg Cu/m³). The idea is that such data will help determine if specific species of copper are dominant in health-related particle size fractions and how they differ amongst various activities that are conducted at an operation. This can aid in evaluating potential health implications of repeated inhalation exposure to copper minerals and thereby provide important information towards setting better OEL values for copper, and similarly for other metals.

Haase et al. (2022) conducted a study to assess the effect of exposure to copper-containing dust on lung function and inflammatory endpoints among workers of a German copper plant. The inhalable copper dust exposure level during the study period was approximately 0.120 mg/m^3 (based on n=12 measurements in 2018), similar to earlier years. The results suggest the absence of the detectable effects of copper-containing dust exposure on lung function or chronic inflammation within the investigated cohort.

Haase et al. (2021) conducted a health surveillance study among workers employed at a copper smelter between 1972 and 2018. The primary study objective was to determine whether differences in lung function could be detected between the copper-containing smelter dust-

exposed study population and an internal reference group (consisting of workers in the precious metal and lead facilities located at the same site). The secondary objective was to evaluate if lung function depended on tertiles of cumulative copper dust exposure among exposed study subjects. The study findings indicate that cumulative inhalable copper dust exposure averaging 4.61 mg/m^3-years over an exposure duration of ~22 years is not associated with adverse effects on lung function.

5.3.1 Routes of exposure

The primary routes of exposure for workers is by inhalation (of fumes and dusts), by accidental ingestion (by hand to mouth exposure in case of insufficient personal hygiene and housekeeping) and by dermal absorption through direct contact with copper dusts, powders, or copper-containing solutions.

5.4 General population exposure

Copper is an essential trace mineral vital to the human body. An adult body contains roughly 50 to 120 mg copper (National Institutes of Health, 2022), primarily stored in the liver, bones, and muscles. It is actively involved in several of the body's most fundamental biological processes such as energy production, iron absorption, connective tissue and bones, nervous system and antioxidant defence**.**

5.4.1 Occurrence

5.4.1.1 Diet

The European Food Safety Authority (EFSA) has established Adequate Intakes (AIs) for copper. For adults, the recommended daily AI is 1.6 mg/day for men and 1.3 mg/day for women (EFSA, 2015). The minimum and maximum limits of copper authorised in infant and follow-on formula and processed cereal-based foods and baby food are set by EC Regulation 609/20139 and EC delegated EC Regulation 2016/12710. The regulatory limits for infant formula and follow-on formula set copper content to a minimum of 60 lg/100 kcal and a maximum of 100 lg/100 kcal, whilst for processed cereal-based foods and baby food, it sets copper content to a maximum of 40 lg/100 kcal. Directive 2002/46/EC11 and EC Regulation 1925/200612 provide the forms of copper that are authorised for use in food supplements and addition to foods, respectively. These legislations establish that maximum amounts of vitamins and minerals added to foods and to food supplements shall be set, including for copper. To date, provisions regarding these maximum amounts are not harmonised among the EU Member States.

The human body cannot manufacture copper, therefore it must be obtained via the diet. Excellent food sources include:

- Oysters and shellfish;
- Organ meats (such as liver);
- Nuts and seeds;
- Whole grains and beans;
- Dark chocolate and cocoa.

As a natural element in the Earth's crust, copper exists in most of the world's surface water and groundwater, although the actual concentration of copper in natural waters varies geographically. Drinking water also contributes to dietary copper (Sadhra et al., 2007) but across the EU, the contribution is variable. It can be impacted by the conveyance mechanisms, which can be copper piping. Copper concentrations in bottled mineral water have also been reported.

5.4.1.2 Supplementation

Supplementation is generally not recommended for healthy adults who consume a well-balanced diet which includes a wide range of foods. However, supplementation under the care of a physician may be necessary for premature infants or those with low birth weights, infants fed unfortified formula or cow's milk during the first year of life, and malnourished young children.

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Physicians may consider copper supplementation for:

- illnesses that reduce digestion (e.g., children with frequent diarrhoea or infections; alcoholics);
- insufficient food consumption (e.g., the elderly, the infirm, those with eating disorders or on diets);
- patients taking medications that block the body's use of copper;
- anaemia patients who are treated with iron supplements;
- anyone taking zinc supplements;
- those with osteoporosis.

Copper supplements can help prevent copper deficiency and are available at vitamin and herb stores and grocery stores and online retailers. Different forms of copper supplementation have different absorption rates.

5.4.1.3 Tobacco smoke

Tobacco plants naturally absorb heavy metals like copper from the soil. As a result, copper is found in tobacco leaves, filters, and cigarette smoke. The average values found of copper were in the range of 0.49, 4.22 and 12.8 µg/cigarette for the post-burning filter, ashes, and tobacco, respectively (Lisboa et al., 2019). It was observed that filters had low absorption after a simulated firing, which suggests that significant amounts of copper (and other metals), carried by smoke, can reach the airways of smokers.

5.4.1.4 Vehicular emissions via brake pad wear

While copper is not a primary byproduct of fuel combustion, it is highly prevalent in vehicular emissions via brake pad wear. Copper is extensively used in many brake pad formulations (particularly non-asbestos, organic and hybrid-ceramic pads) because it helps dissipate heat and improves stopping power. Brake pads may contain up to ~15% copper (Denier van der Gon et al., 2007), which makes brake wear from vehicles potentially an important source of atmospheric (particulate) copper concentrations, and may be responsible for 50–75% of the total copper emissions to air for most of Western Europe.

6. Monitoring exposure

6.1 Monitoring methods (external exposure)

Several validated methods are available for measuring copper and copper compounds in air. Several validated methods are available for measuring copper (compounds) in air. The principle of the methods is as follows: air sampling is performed by passing air actively through a filter. The retained copper (compound) is then extracted for analysis by different digestion methods followed by analysis via inducted couple plasma or atomic absorption.

Table 12 presents some of the available methods and limits of quantification (LOQ) for measurement of copper in air. It is possible to measure copper compounds in air, at below the µg/m^3 or ppb range.

Table 12: Overview of validated sampling and analytical methods for air monitoring of copper and copper compounds at the workplace

Method	Analytical technique	LOQ Sampling volume Time	Similar methods/ comments
(DFG, 2012)	ICP-OES	27.1 µg/m3 (420 L) 2 hours	
(IRSST, 2010)	ICP -MS	0.0005 mg/m³ (180 L) 2 hours	

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Method	Analytical technique	LOQ Sampling volume Time	Similar methods/ comments
(IRSST, 2023)	ICP -MS	0.0009 mg/m³ (180 L) 2 hours	
(NIOSH, 2014a)	ICP -AES (microwave digestion with nitric acid)	0.07 µg/m^3 for a 1000 L Sample	Not inhalable sampler Inhalable/ Respirable sampler could be used instead
(NIOSH, 2014b)	ICP -AES (microwave digestion with conc. nitric acid)	0.07 µg/m^3 for a 1000 L sample	Not inhalable sampler Inhalable/ Respirable sampler could be used instead
(NIOSH, 2015)	Analysis by ICP-AES (Internal capsule instead of filter)	0.009 µg/ m^3 for a 1000 L sample	Not inhalable sampler
(OSHA, 2005)	ICP-MS	0.3 µg/m^3 for a 480 L sample	Not inhalable sampler Inhalable/ Respirable sampler could be used instead

6.2 Biomonitoring of exposure (internal exposure)

The detection of copper is possible in blood, serum, plasma and urine (DFG, 2018). There is also limited data of copper concentration in hair.

Urine is the most common, non-invasive sample used in human biomonitoring studies. In general, urinary measurements of copper are used for the diagnosis of diseases affecting copper homeostasis and the liver, in which copper levels are raised. In healthy individuals, the concentrations of copper in urine are much lower (50–60 times) than those in blood (ATSDR, 2024)(ATSDR, 2024).

Copper is an essential trace element. The copper concentration in the body is finely regulated via the liver. Thus, under normal physiological conditions, intestinal absorption and biliary excretion effectively regulate systemic copper concentrations and prevent excessive accumulation (See section 7 for more details on copper toxicokinetic). It is assumed that homeostatic regulation takes place over a wide intake range of 0.8 to 5.5 mg/day.

6.2.1 Background levels for general population, i.e., non-occupationally exposed

The HBM4EU Rapid Response Mechanism performed a study upon request of the European Commission's Directorate-General for Health and Food Safety (DG SANTE) in October 2018 to support the regulatory renewal of copper compounds used in pesticides (HBM4EU, 2018). The study pools 35 different data collections across 13 European countries (with significant heterogeneity between datasets including different sampling methods, age distributions, and an overrepresentation of women, including pregnant women). HBM4EU concluded that the data were very similar across Europe (due to the homeostatic regulation) and that copper concentrations in the general population were generally within established clinical reference ranges .[9]

For urinary copper, the reported normal range was approximately 10–60 µg/L, and almost all general-population datasets had 95th percentile concentrations within this range. In contrast, occupationally exposed workers showed somewhat higher urinary copper concentrations than the general population, although the available occupational data were limited and exposure-

[9] Detailed values can be consulted at https://www.hbm4eu.eu/wp-content/uploads/HBM4EU_4.3_Rapid-response-Mechanism_Response-Document_Copper-compounds.pdf

specific information was insufficient to establish a clear exposure–response relationship. Blood serum copper concentrations are more difficult to interpret because they are influenced by physiological factors, particularly pregnancy and use of intrauterine contraceptives.

(DFG, 2018), summarises the data available in several populations. In addition, Canada and the United States have also collected data on background exposures from their respective programmes of general population biomonitoring ((CDC, 2026) and (Health Canada, 2026)).

A summary of the data available from these sources is available in Table 13 and Table 14.

Table 13: Copper concentrations (µg copper/L) in blood serum and plasma from studies in the general population

Country	Population	Assay material	n	Mean value (µg Cu/L)	P95	Reference
Greece	All	serum	506	1150.46 ± 235.6 (1)		(Kouremenou-Dona et al., 2006)
	Male		414	1129.5 ± 219.5 (1)		
	Female		92	1267.9 ± 271.1 (1)		
Germany	All	blood	3968	970	1300	(Krause et al., 1996)
	Male		1939	890	1100	
	Female		2029	1010	1500	
Germany	All	plasma	69	16.5 ± 4.3 µmol/ 1050 ± 0.48 (3)		(Rükgauer et al., 1997)
Southern Spain	All	plasma	340	1370 ± 480		(Sánchez et al., 2010)
	Male		167	1310 ± 470		
	Female		173	1420 ± 400		
Spain	All	serum		1100 ± 320		(Terrés-Martos et al., 1997)
	Male			1110 ± 2510		
	Female			1090 ± 360		
Canada (2009-20011)	All	blood	6070	900 (2)	1200	(Health Canada, 2026)
	Male		2940	840 (2)	1000	
	Female		3130	970 (2)	1300	
US (2015-2016)	All	serum	2436	1150 (2)	1690	(CDC, 2026) NHANES Survey 2015-2016
	Male		1201	1040 (2)	1420	
	Female		1235	1250 (2)	1900	

(1) X ± SD=mean ± Standard deviation
(2) GM: Geometric mean
(3) Calculated

Table 14: Copper concentrations in urine (µg copper/L; unless otherwise stated) from studies in the general population

Country	Population	n	Mean value (range)	P95	Reference
Belgium	All	28	0.31 µmol/g creatinine 19.7 µg/g creatinine (3)		(Peretz et al., 1989)
Belgium	All	9	(11.5–34 µg/day)		(Cornelis et al., 1975)
Italy	All	50	23 ± 6.9 (1) (4.2–75)		(Minoia et al., 1990)

Country	Population	n	Mean value (range)	P95	Reference
Czech Republic	All (4)		21.9 µg/g creatinine (0.4–331 µg/g creatinine)	94.1 µg/g creatinine	(Benes et al., 2002)
Tenerife/Spain	All	97	22.68 ±0.99 (5.81–47.42)		(Rodríguez and Díaz, 1995)
	Male	46	23.34 ± 1.63 (5.81–47.42)		
	Female	51	22.02 ± 1.16 (7.21–36.78)		
Germany (west/north)		87	9 (4–30)	13	(Heitland and Köster, 2006)
Spain	All	434	26.6 µg/g creatinine (2) 39.0 (2)		(Schuhmacher et al., 1994)
German		4002	11.64	22.9	(Krause et al., 1996)
		1955	10.48	20.4	
		2047	12.75	20.4	
Canada (2009-2001)	All	6311	11	28	(Health Canada, 2026) Survey 2009-2011
	Male	3036	10	27	
	Female	3275	12	28	

(1) X± SD = mean ± Standard deviation
(2) GM: Geometric mean
(3) Calculated
(4) Significant differences between male and female

Despite the available data on background exposure, it is not proposed to establish a biological guidance value as due to the homeostatic regulation, no significant increase of exposure in urine or blood can be expected in the occupationally exposed population

6.2.2 Correlations between internal and external exposure

After occupational inhalation exposure to 0.64–1.05 mg copper/m^3, the plasma copper concentrations in exposed workers did not differ significantly from those of non-exposed control persons (108 ± 4 µg/dL versus 99 ± 3 µg/dL) (Finelli, 1981). From this, it can be concluded that no dose-dependently increased internal copper concentrations are to be expected up to an inhalation exposure of about 1 mg copper/m^3 (SCOEL, 2014).

Regarding copper in urine, few publications report data on both air and urine concentrations and no correlation has been found.

It is assumed that due to the homeostatic regulation of copper, no increased concentrations in blood or urinary copper levels are expected for exposures in the range of the air limit value.

Thus, no BLV can be established.

6.2.3 Biomonitoring analytical methods

Several analytical methods are available for the determination of the biomarkers described at the beginning of section 6.2. Table 15 provides an overview of the methods available to measure copper in blood and urine.

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Table 15: Analytical methods for copper in blood and urine

Method	Specimen	Analytical technique	LOQ /Range
NHANES 2015-2016 Laboratory Methods	Serum	ICP-DRC-MS	25 µg/L
(Heitland and Köster, 2006)	Urine	ICP MS	0.41 µg/L

7. Health Effects

7.1 Toxicokinetics (ADME-absorption, distribution, metabolism and excretion)

7.1.1 Human data

7.1.1.1 Absorption

Copper is an essential trace element that is absorbed primarily via the gastrointestinal tract following oral exposure. Oral absorption occurs mainly in the stomach and proximal small intestine and is tightly regulated by efficient homeostatic mechanisms that maintain copper balance within a narrow physiological range (Turnlund et al., 2005, Wapnir, 1998). Consequently, the fraction of ingested copper that is absorbed is influenced by dietary intake, age, nutritional status and physiological demand.

Stable isotope studies in healthy adult volunteers have demonstrated that intestinal copper absorption is variable, with reported absorption efficiencies generally ranging from approximately 12 to 71%, depending on dietary copper intake and copper status (Turnlund et al., 1998). Fractional absorption decreases as dietary copper intake increases, reflecting efficient homeostatic regulation of copper uptake (Turnlund et al., 2005, Turnlund et al., 1998).

Following intestinal absorption, copper enters the portal circulation where it is transported primarily bound to albumin and transcuprein to the liver, the principal organ regulating copper homeostasis. Hepatic copper is incorporated into ceruloplasmin before redistribution to extrahepatic tissues, whereas excess copper is excreted into bile. Urinary excretion accounts for only a minor proportion of copper elimination (Wapnir, 1998).

At the workplace, the main route of uptake of metallic copper is via the airways. Quantitative information on copper pulmonary absorption following inhalation of dust, fumes and smoke is limited. Available evidence indicates that respiratory absorption of copper depends on the physicochemical characteristics of the inhaled copper compound, including particle size and solubility, but reliable quantitative estimates are lacking (SCOEL, 2014). Similarly, dermal absorption of copper is considered negligible under normal exposure conditions. Experimental studies indicate that metallic copper and copper salts are poorly absorbed through intact skin, although dermal uptake may increase when the skin barrier is compromised or penetration-enhancing substances are present (SCOEL, 2014, WHO, 1998).

Overall, oral exposure represents the toxicokinetically relevant route for systemic copper uptake in humans. Under normal physiological conditions, intestinal absorption and biliary excretion effectively regulate systemic copper concentrations and prevent excessive accumulation. However, these homeostatic mechanisms may be impaired in individuals with inherited disorders of copper metabolism, such as Wilson disease or Menkes disease, resulting in altered copper disposition and an increased susceptibility to copper toxicity (SCOEL, 2014, WHO, 1998).

7.1.1.2 Distribution

Following gastrointestinal absorption, copper is transported via the portal circulation to the liver, the principal organ responsible for maintaining systemic copper homeostasis. In plasma, newly absorbed copper is initially bound predominantly to albumin and the α2-globulin transcuprein, which facilitate hepatic uptake. Within hepatocytes, copper is incorporated into intracellular

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proteins or exported into the circulation as ceruloplasmin, the major copper-carrying protein in blood (Linder and Hazegh-Azam, 1996, Wapnir, 1998).

Approximately 90–95% of circulating copper is bound to ceruloplasmin, while the remaining fraction is associated with albumin, transcuprein and low-molecular-weight amino acids. Ceruloplasmin-bound copper is relatively stable and contributes primarily to physiological copper transport rather than immediate tissue uptake, whereas the non-ceruloplasmin-bound fraction represents the metabolically active pool available for uptake by peripheral tissues (Linder and Hazegh-Azam, 1996, Wapnir, 1998).

Copper is widely distributed throughout the body, with the highest concentrations occurring in the liver, brain, heart and kidneys. Lower concentrations are present in skeletal muscle and bone; however, because of their larger mass, these tissues collectively contain a substantial proportion of total body copper. Intracellular copper is tightly regulated through binding to metallothionein, copper chaperone proteins and cuproenzymes, thereby minimizing the concentration of free copper ions and limiting oxidative damage (Linder and Hazegh-Azam, 1996, WHO, 1998). Under normal physiological conditions, whole-body copper homeostasis is maintained by coordinated regulation of intestinal absorption, hepatic storage and biliary excretion rather than by redistribution between tissues (Wapnir, 1998).

Copper readily crosses the human placenta to meet foetal developmental requirements, with active regulation ensuring adequate foetal copper supply throughout pregnancy. Copper is also secreted into breast milk, providing an important source of the element during early infancy (WHO, 1998). Individuals with inherited disorders affecting copper transport, including Wilson disease and Menkes disease, exhibit markedly altered tissue distribution due to defects in ATP-dependent copper transport, resulting in either excessive hepatic and extrahepatic accumulation or impaired tissue delivery of copper (Bull et al., 1993, WHO, 1998).

Available occupational studies indicate that blood copper concentrations are of limited value as biomarkers of exposure because circulating copper is under strict homeostatic control. SCOEL reported that workers exposed to airborne copper concentrations of approximately 0.64–1.05 mg Cu/m³ showed plasma copper concentrations (108 ± 4 µg/dL) that were not significantly different from those of unexposed controls (99 ± 3 µg/dL) (Finelli, 1981). Similarly, workers exposed to 0.001–0.082 mg Cu/m³ had only marginally higher serum copper concentrations than controls (104.5 ± 15.1 versus 99.7 ± 12.1 µg/dL), despite measurable occupational exposure (Kossowska et al., 2010, Kossowska et al.). In contrast, hair copper concentrations were substantially increased in exposed workers, suggesting that hair may reflect longer-term exposure, although considerable inter-individual variability limits its usefulness as a quantitative biomarker (Finelli, 1981, Kempson et al., 2007, Rodrigues et al., 2008). The available evidence indicates that systemic copper distribution is tightly regulated in humans, and that blood copper concentrations generally remain within the normal physiological range even following occupational exposure levels that produce local respiratory effects (SCOEL, 2014).

7.1.1.3 Metabolism

Absorbed copper is subject to tightly regulated intracellular transport, storage and incorporation into biologically active proteins. Following uptake by the liver, copper is distributed to specific intracellular compartments by copper chaperone proteins, which deliver the metal to target enzymes while preventing the accumulation of free copper ions (Linder and Hazegh-Azam, 1996, Wapnir, 1998).

Copper is incorporated into numerous cuproenzymes involved in essential physiological processes, including oxidative phosphorylation, antioxidant defence and iron metabolism. It is also incorporated into ceruloplasmin, the principal copper-carrying protein in plasma, which is synthesized in the liver and released into the circulation (Linder and Hazegh-Azam, 1996, WHO, 1998). Excess intracellular copper is bound to metallothionein or exported via the ATP7B copper transporter for biliary excretion, thereby maintaining copper homeostasis (Bull et al., 1993, WHO, 1998).

Inherited defects in copper transport demonstrate the importance of these regulatory mechanisms. Mutations in the *ATP7B* gene cause Wilson disease, resulting in impaired biliary

copper excretion and tissue accumulation, whereas mutations in *ATP7A* lead to Menkes disease, characterised by impaired intestinal copper transport and systemic copper deficiency (Bull et al., 1993, Vulpe et al., 1993, WHO, 1998).

Overall, copper metabolism is characterised by efficient intracellular trafficking and incorporation into essential proteins rather than chemical biotransformation. These homeostatic processes ensure an adequate supply of copper for physiological functions while preventing excessive intracellular accumulation under normal exposure conditions (ATSDR, 2024, SCOEL, 2014).

7.1.1.4 Excretion

Copper excretion is also tightly regulated to maintain systemic homeostasis, with the liver playing a central role in controlling body copper concentrations. Following hepatic uptake, excess copper is excreted into bile and eliminated in the faeces (Wapnir, 1998, WHO, 1998). Biliary excretion is the predominant mechanism regulating copper elimination in humans and represents the principal homeostatic process controlling systemic copper concentrations under both physiological and increased exposure conditions (ATSDR, 2024, SCOEL, 2014).

Urinary excretion accounts for only a small proportion of total copper elimination in healthy individuals because circulating copper is efficiently conserved through homeostatic mechanisms (Linder and Hazegh-Azam, 1996, WHO, 1998). Small amounts of copper are also eliminated through sweat, hair, nails and desquamated skin cells; however, these pathways contribute negligibly to overall copper excretion (WHO, 1998).

Human balance studies have demonstrated that copper excretion increases with increasing dietary copper intake, primarily through enhanced biliary secretion rather than increased urinary elimination, thereby maintaining relatively constant body copper stores over a wide range of exposures (Turnlund et al., 2005, Turnlund et al., 1998). Conversely, reduced biliary excretion, as occurs in individuals with Wilson disease due to mutations in the *ATP7B* gene, results in progressive hepatic copper accumulation and subsequent deposition in extrahepatic tissues (Bull et al., 1993, WHO, 1998).

7.1.2 Animal data

7.1.2.1 Absorption

The absorption of orally administered copper in animals depends, e.g., on the administered dose. The mechanisms regulating absorption of copper are comparable to those observed in humans (SCOEL, 2014).

In a bioequivalence study bioavailability of five inorganic copper compounds, i.e., copper hydroxide, copper oxychloride, Bordeaux mixture (an aqueous solution of calcium hydroxide and 1-2% copper sulphate), tribasic copper sulphate and copper oxide, were compared to the bioavailability of copper sulphate pentahydrate in rats. The study demonstrated that following oral administration of 20 mg copper/kg bw to rats, the absorption of these copper compounds was similar to copper sulphate (EFSA et al., 2018).

Copper is rapidly absorbed following oral administration, with peak plasma concentrations (C_{max}) observed 1.5 hours after a single gavage dose of 79.5 mg copper/kg in rats administered as copper gluconate in water (Garcia-Martinez et al., 2021). Age-dependent differences in copper absorption have been reported in rats. Copper absorption was linear and non-saturable in suckling (16 days old) and weanling (21–22 days old) rats, whereas it was saturable in adolescent rats (6 weeks old). Intestinal copper retention was also higher in suckling rats than in weanling or adolescent rats (Varada et al., 1993).

Other metals can affect copper absorption. High zinc and cadmium content in the diet may decrease copper absorption in rats (ATSDR, 2024).

No quantitative animal data are available to characterize absorption rate following inhalation exposure of copper (ATSDR, 2024, SCOEL, 2014).

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Evidence from animal studies suggests that copper may penetrate dermal barriers when co-administered with penetration-enhancing vehicles, including salicylic acid and phenylbutazone (ATSDR, 2024).

7.1.2.2 Distribution

Several studies have shown that after repeated oral exposure to inorganic copper compounds, copper concentration increases in various tissues in rats including liver, kidney, testis and brain (Ali et al., 2023, Arafa et al., 2019, Fuentealba et al., 2000, Haywood, 1980, Haywood and Loughran, 1985, Hébert et al., 1993, Kumar et al., 2019, Kumar et al., 2016b, Kumar et al., 2016a, Kumar et al., 2015, Patwa and Flora, 2020, Yu et al., 2023, Yu et al., 2021a).

The bioequivalence study ((EFSA, 2018, as cited in (EFSA et al., 2023)) showed that the distribution rate of five inorganic copper compounds was similar to that of copper sulphate after oral ingestion in rats (see above, Section 7.1.2.1.). Liver copper concentration increased significantly with T_{max} at 12 hours after oral administration of 20 mg copper/ kg bw.

A study investigating respiratory toxicity in rats reported significantly elevated copper concentrations in the liver and plasma, indicating systemic distribution following inhalation exposure (Romeu-Moreno et al., 1994). Non-significant increases in copper levels were also observed in the kidneys and lungs after daily 1-hour inhalation exposure to aerosolized copper sulphate for up to 10 days (Romeu-Moreno et al., 1994).

In an OECD TG 412 inhalation study (Poland et al., 2022), Sprague-Dawley rats were exposed to dicopper oxide aerosols in whole-body chambers at copper concentrations of 0, 0.17, 0.35, 0.7, or 1.7 mg/m³ for 4 weeks. Copper concentrations were measured in the lungs, bronchoalveolar lavage fluid (BALF), liver, and brain. Additional assessments were conducted after 1–3 weeks of exposure and following a 13-week recovery period in control and high-exposure groups. Lung copper concentrations remained below the detection limit (2.5 µg/g tissue) in nearly all animals exposed to ≤0.7 mg/m³. In contrast, exposure to 1.7 mg/m³ resulted in measurable increases after 1–4 weeks, with concentrations ranging from 4.23–5.09 µg/g in males and 4.44–6.87 µg/g in females. Following the 13-week recovery period, copper levels in both the lungs and BALF were below the respective detection limits. In BALF, animals exposed to 1.7 mg/m³ showed the highest copper concentrations after 1 week (400 ng/mL in males and 458 ng/mL in females), with lower levels observed after 3 weeks (204 and 243 ng/mL, respectively). After 4 weeks, BALF copper concentrations remained detectable only in the 1.7 mg/m³ group (232 ng/mL in males and 347 ng/mL in females), whereas concentrations at lower exposure levels were below the detection limit of 100 ng/mL (Poland et al., 2022). In the liver, slight increases in copper concentration were observed only in animals exposed to 1.7 mg/m³. After 4 weeks, liver concentrations reached 7.58 µg/g in males and 7.64 µg/g in females, compared with 6.60 and 6.64 µg/g in controls, respectively. These differences were no longer statistically significant after the 13-week recovery period. Peak liver concentrations occurred after 1 week of exposure, indicating no evidence of hepatic copper accumulation. No treatment-related increases in brain copper concentrations were observed after 1–4 weeks of exposure.

Intraperitoneal and intravenous administration of radioisotopes of copper (^{67}Cu or ^{64}Cu) to non-pregnant and lactating rats showed that approximately 60% of the copper in lactating animals was directly taken up by the mammary gland and was also present in the milk (Donley et al., 2002).

No data is available on distribution of copper after dermal exposure in animals.

7.1.2.3 Metabolism

Water-soluble inorganic copper compounds, such as copper sulphate and copper chloride, can be hydrolysed in animals similarly to humans to release copper ions. Copper can exist in two oxidation states, i.e., cuprous (Cu^{1+}) or cupric (Cu^{2+}) ions. Water-insoluble inorganic copper compounds, such as copper oxide, can also release copper ions in the acidic environment of stomach. Regarding inorganic copper compounds, the potential toxicity is driven by the copper ions and not by the accompanying anions (such as sulphate and chloride). Similarly to humans, copper ions can be bound to various proteins maintaining the copper homeostasis in animals,

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such as ceruloplasmin and metallothionein (ATSDR, 2024).

7.1.2.4 Excretion

The mechanisms regulating excretion of copper in animals are comparable to those observed in humans (SCOEL, 2014). Biliary excretion of copper in faeces is the main pathway while urinary excretion is less important (ATSDR, 2024).

The bioequivalence study (EFSA et al., 2018) showed that the excretion rate of five inorganic copper compounds was similar to that of copper sulphate after oral ingestion in rats (see Section 7.1.2.1.). The main elimination route was the excretion via the bile.

Levenson and Janghorbani (1994), ((as cited in (ATSDR, 2024)), estimated copper half-lives in several rat tissues to characterize tissue-specific copper elimination kinetics. By restricting dietary copper intake, the authors evaluated the balance between copper excretion and physiological conservation mechanisms. Copper turnover was modelled using two kinetic components: a rapidly turning over component 1 with a relatively constant half-life regardless of dietary copper status, and a slower component 2, whose half-life increased markedly under copper restriction. Although the contribution of each component could not be resolved for all tissues, estimates were obtained for several organs. The calculated half-lives (component 1/component 2) were 3.9/21 days for the liver, 5.4/35 days for the kidney, and 23/662 days for the heart. In contrast, copper turnover in the brain appeared to follow a single-phase process with an estimated half-life of 457 days.

No studies are available to characterize the rate and extent of excretion in animals after inhalation or dermal exposure to copper (ATSDR, 2024). In a study examining the effects after intratracheal instillation of copper sulphate in rats ((Hirano et al., 1990), as cited in (ATSDR, 2024)), copper was rapidly cleared from the lung with a half-life of 7.5 hours.

7.1.3 Toxicokinetic modelling

Physiologically based pharmacokinetic (PBPK) models have been developed to describe copper kinetics in humans using data from stable isotope studies.

The first human compartmental model was developed by Scott and Turnlund (1994), who used plasma, urinary and faecal data from healthy adult males receiving oral and intravenous doses of the stable isotope ^{65}Cu, under different dietary copper intakes. The model comprised seven compartments representing ceruloplasmin-bound and non-ceruloplasmin plasma copper, two liver compartments, other tissues, urine and faeces, and was designed to describe copper absorption, tissue distribution and excretion under normal physiological conditions. The model adequately reproduced the observed plasma copper kinetics and demonstrated the importance of hepatic uptake and biliary excretion in maintaining copper homeostasis.

Harvey et al. (2005) subsequently developed a more mechanistic compartmental model incorporating eight compartments representing plasma, liver, gastrointestinal tract, other tissues and faeces. Model parameters were calibrated using stable isotope tracer studies in healthy volunteers and successfully predicted plasma copper concentrations following oral and intravenous administration. The model estimated that approximately 74% of absorbed copper undergoes first-pass extraction by the liver, with most circulating copper bound to ceruloplasmin. Although these models provide valuable insight into human copper homeostasis and support the interpretation of stable isotope studies, they have not been widely applied in environmental or occupational human health risk assessment because of the complexity of copper homeostasis and the limited validation of model predictions under conditions of excessive exposure.

Overall, the PBPK models developed by (Scott and Turnlund, 1994) and (Harvey et al., 2005) provide a mechanistic description of human copper toxicokinetics and highlight the central role of the liver in maintaining copper homeostasis through hepatic uptake and biliary excretion. Although these models improve the understanding of copper kinetics under physiological conditions, their application in human health risk assessment is limited because they were developed using stable isotope studies in healthy individuals and have not been extensively

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validated for excessive environmental or occupational exposures.

HBM4EU (2018) adapted the INTEGRA ((integrated exposure and internal-dose modelling platform, developed originally through a Cefic Long-Range Initiative project with researchers at the Centre for Research and Technology Hellas (CERTH) and collaborators)), physiologically based toxicokinetic (PBTK) model to describe the absorption, distribution, metabolism and excretion of copper in humans. The HBM4EU copper-adapted PBTK model showed that copper is strongly regulated by homeostatic mechanisms, particularly through intestinal absorption and biliary excretion. The model reproduced observed copper concentrations under normal and controlled dietary exposure, but the available data were insufficient to adequately validate its application to occupational exposure. Overall, modelling indicated that blood and urinary copper concentrations do not increase proportionally with external exposure and therefore cannot be used as straightforward quantitative measures of occupational copper exposure.

7.1.4 Summary

Copper is an essential trace element whose toxicokinetics are tightly regulated in humans to maintain systemic homeostasis. Following oral exposure, copper is absorbed primarily in the stomach and proximal small intestine, with absorption efficiency varying according to dietary intake, nutritional status and physiological demand. Intestinal uptake is subject to efficient homeostatic control, whereby absorption decreases as dietary copper intake increases, thereby limiting excessive systemic accumulation. In contrast, quantitative data on the absorption of inhaled copper in humans are limited. Available evidence in humans indicates that respiratory absorption depends on the physicochemical properties of the inhaled particles, including their size and solubility, although no reliable quantitative estimates are available.

After absorption, copper is transported via the portal circulation to the liver, the principal organ responsible for copper homeostasis. In the circulation, most copper is bound to ceruloplasmin, while smaller amounts are associated with albumin and other plasma proteins. Copper is subsequently distributed throughout the body, with the highest concentrations occurring in the liver, brain, heart and kidneys. At the cellular level, copper is tightly regulated through binding to specific chaperone proteins, metallothionein and copper-dependent enzymes, minimizing the presence of free copper ions and preventing oxidative damage.

Unlike organic chemicals, copper does not undergo metabolic transformation. Instead, it is incorporated into numerous cuproenzymes involved in essential physiological processes, including energy production, antioxidant defence, connective tissue formation and iron metabolism. Intracellular copper transport and storage are regulated by specialised copper transport proteins, ensuring adequate copper supply while preventing toxicity.

Excretion of copper occurs predominantly via biliary secretion into the faeces, which constitutes the major route of elimination under normal physiological conditions. Urinary excretion contributes only minimally to total copper elimination. Together, the coordinated regulation of intestinal absorption, hepatic distribution, intracellular trafficking and biliary excretion maintains copper homeostasis over a wide range of dietary and environmental exposures. Consequently, systemic copper concentrations generally remain stable in healthy individuals, although these homeostatic mechanisms may be impaired in inherited disorders of copper metabolism or following excessive exposure.

PBPK/PBTK models developed from stable-isotope studies have provided a mechanistic understanding of human copper kinetics, highlighting the central role of hepatic uptake and biliary excretion in maintaining copper homeostasis. Two existing models successfully described copper absorption, distribution and excretion under physiological conditions, but their applicability to excessive environmental or occupational exposure remained limited. HBM4EU subsequently adapted an existing PBTK model to incorporate copper-specific homeostatic regulation and demonstrated that the model could reproduce observed copper concentrations under normal and controlled dietary exposure. However, the available data were insufficient to adequately validate the model for occupational exposure, and the modelling indicated that blood and urinary copper concentrations do not increase proportionally with external exposure and

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therefore cannot be regarded as straightforward quantitative measures of occupational copper exposure.

7.2 Acute toxicity

7.2.1 Human data

Acute intake of copper following oral exposure can cause reversible gastrointestinal symptoms in humans, such as nausea, epigastric pain, vomiting, or diarrhoea (Health Canada, 2019). Controlled human exposure studies have demonstrated gastrointestinal effects following acute oral exposure to copper sulphate in drinking water (Araya et al., 2001, Pizarro et al., 2001, Pizarro et al., 1999), with healthy volunteers presenting nausea after receiving a 10 mg Cu/L solution (Araya et al., 2003b, Gotteland et al., 2001). Acute gastrointestinal effects, including nausea, vomiting and abdominal pain, have been reported in infants consuming drinking water containing approximately 3.7 mg Cu/L (Spitalny et al., 1984). Supporting evidence for the gastrointestinal effects of acute oral ingestion of copper in humans comes from numerous case reports and community health investigations as described in (ATSDR, 2024).

In a case report of severe copper sulphate poisoning from dermal absorption, a 53-year-old male worker developed intravascular haemolysis with methemoglobinemia, acute kidney injury and had an asymptomatic cerebral ischemic stroke, and melena due to multiple shallow gastric ulcers, although he recovered after intense haemodialysis and blood transfusions (Park et al., 2018).

In a suicidal attempt case, a man of 65 presented with severe hepatotoxicity as well as caustic oesophagitis mainly coming from intake of Bordeaux mixture, a widely used copper-based fungicide that contains 1-2.5% copper sulphate (with pH neutralized via hydrated lime) (Carvalho et al., 2021). In other studies, no changes in hepatic enzyme levels were observed after acute-duration (Pizarro et al., 2001, Pizarro et al., 1999) or intermediate-duration oral exposure to copper sulphate (Araya et al., 2003a, Araya et al., 2004).

Storage of food in unlined copper containers has been associated with an increased risk of childhood cirrhosis in genetically or otherwise susceptible children (Tanner, 1998). In addition, copper may leach from domestic copper plumbing, particularly in areas supplied with soft or acidic water, resulting in elevated copper concentrations in drinking water that may exceed 100 mg/L after prolonged stagnation (Olivares et al., 1998, WHO, 1998).

Neurological impairment and hyperglycaemia have been described in another case report of a young girl after a massive oral ingestion of copper sulphate (approximately 50 grams), which only partially remitted at discharge. She also presented other symptoms of acute copper poisoning such as deteriorating respiratory function, acute gastroenteritis, haemolytic anaemia and hepatorenal failure (Zhu et al., 2026).

For inhalation exposure, the principal acute effects are local irritation of the upper and lower respiratory tract. Occupational exposure to airborne copper dusts and fumes may cause transient irritation of the nose, throat and airways, accompanied by coughing and chest discomfort. Exposure to freshly generated copper oxide fumes during welding or smelting may also produce metal fume fever, a self-limiting syndrome characterised by fever, chills, malaise, headache and myalgia that typically resolves within 24–48 hours after exposure ceases, as reported by a few studies (Armstrong et al., 1983, Gleason, 1968). However, a later review of seven published reports of copper-induced metal fume fever concluded that the reported cases lacked sufficient evidence to establish a causal association because of limitations including inadequate exposure assessment (and co-exposure with other potentially causative metals), atypical clinical presentations and inconsistencies in the occupational settings in which the effects were reported (Borak et al., 2000). Similarly, a cross-sectional study of brass workers reported acute symptoms consistent with metal fume fever; however, the workers were also exposed to zinc, and exposure concentrations were not reported (Jayawardana, 2004). Since zinc oxide is a well-established cause of metal fume fever, the contribution of copper to the observed effects could

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not be determined. Finally, copper (in addition to other metals, namely zinc oxide, magnesium or cadmium) has been identified in ultrafine particles leading to metal fume fever among welders (Schraufnagel, 2020).

A single case report described a 2-year-old girl who unintentionally inhaled metallic copper dust and developed severe respiratory toxicity. Respiratory failure occurred within a few hours of exposure and progressed to acute respiratory distress syndrome after three days. The patient also developed haemolytic anaemia, hepatic failure, oliguric renal failure and evidence of acute tubular injury. Copper-positive macrophages were identified in BALF, indicating pulmonary deposition of inhaled copper particles (Donoso et al., 2007).

7.2.2 Animal data

7.2.2.1 Acute oral toxicity

Copper sulphate, dicopper oxide and copper chloride have entries in Annex VI of the CLP regulation as Acute Tox 4 (oral) substances (H302) (see Table 6). Several oral acute toxicity studies following standard test guidelines are summarised below:

Table 16: Summary of studies on acute oral toxicity of copper and inorganic copper compounds

Method, route	Species, strain, sex, number/group	Test material, doses	Results	References
Standard acute method	Rat, Sprague-Dawley, 5 males and 5 females per dose, except 215 mg/kg bw and 316 mg/kg bw 10 males and 10 females per dose	Copper chloride, 147, 215, 261, 316, 383, 464 mg/kg bw	LD50 ca. 336 mg/kg bw	Study report 1978 as cited in (ECHA, 2026)
Acute oral toxicity (OECD TG 401)	Rat, Sprague-Dawley, 5 males and 5 females per dose	Dicopper oxide, 200, 431, 928, 2000 mg/kg bw	LD50 male 1625 mg/kg bw; LD50 female ≥928, ≤2000 mg/kg bw; combined 1340 mg/kg bw	Study report 1984 as cited in (ECHA, 2026)
Acute oral toxicity, fixed dose procedure (OECD TG 401)	Rat, Sprague-Dawley, 5 males and 5 females per dose	Copper sulphate pentahydrate, 447, 562, 708 and 893 mg/kg bw	LD50 (male and female combined) 481 mg/kg bw	Study report 1994 as cited in (ECHA, 2026)
Acute toxic class method (OECD TG 423)	Rat, Sprague-Dawley, 3 males and 3 females at low dose, 3 females at high dose	Coated copper flakes, 200 and 2000 mg/kg bw	LD50 300-500 mg/kg bw	Study report 2001 as cited in (ECHA, 2026)
Acute toxic class method (OECD TG 423)	Rat, Sprague-Dawley, 2 groups with 3 males	Copper oxide, 2000 mg/kg bw	LD50 >2500 mg/kg bw	Study report 2002 as cited in (ECHA, 2026)
Up and down method	Mouse, Swiss Albino, sex unclear, 2 animals per dose	Copper oxide nanoparticles (NPs) 250, 400, 600 mg/kg bw; copper sulphate 100 mg/kg bw	LD50 copper oxide NPs: 400 mg/kg bw; Copper	(Kadammattil et al., 2018)

Method, route	Species, strain, sex, number/group	Test material, doses	Results	References
			sulphate 100 mg/kg bw	
Acute oral toxicity, fixed dose procedure (OECD TG 420)	Rat, Sprague-Dawley, males, number of animals unclear	Copper NPs sized 30, 50 or 50 nm, copper micro sized particles, copper chlorides, up to 5000 mg/kg bw	LD50 30nm Cu NPs: 1022mg/kg bw; 50 nm Cu NPs: 1750mg/kg bw; 80 nm Cu NPs: 2075 mg/kg bw; Cu micro: >5000 mg/kg bw; Cu chloride: 359.6 mg Cu/kg bw	(Tang et al., 2018)

7.2.2.2 Acute dermal toxicity

Copper and copper compounds do not cause acute toxicity via the dermal route. Several dermal acute toxicity studies following standard test guidelines are summarised below:

Table 17: Summary of studies on acute dermal toxicity of copper and inorganic copper compounds

Method, route	Species, strain, sex, number/group	Test material, doses, duration of exposure	Results	References
Acute dermal toxicity, fixed dose procedure (OECD TG 402)	Rat, Sprague-Dawley, 5 males and 5 females per dose	Dicopper oxide pentahydrate, 2000 mg/kg bw, 24 hours	LD50 >2000 mg/kg bw	Study report 1988 as cited in (ECHA, 2026)
Acute dermal toxicity, fixed dose procedure (OECD TG 402)	Rat, Sprague-Dawley, 5 males and 5 females per dose	Coated copper flakes, 2000 mg/kg bw, 24 hours	LD50 >2000 mg/kg bw	Study report 2001 as cited in (ECHA, 2026)
Acute dermal toxicity, fixed dose procedure (OECD TG 402)	Rat, Sprague-Dawley, 5 males and 5 females per dose	Copper oxide, 2000 mg/kg bw, 24 hours	LD50 >2000 mg/kg bw	Study report 2002 as cited in (ECHA, 2026)
Acute dermal toxicity, fixed dose procedure (OECD TG 402)	Rat, Sprague-Dawley, 5 males and 5 females per dose	Copper sulphate pentahydrate, 2000 mg/kg bw, 24 hours	LD50 >2000 mg/kg bw	Study report 2002 as cited in (ECHA, 2026)
Acute dermal toxicity, fixed dose	Rat, Sprague-Dawley, 5 females per dose, 5 males at top dose only	Copper chloride, 500, 1000, 1500, 2000 mg/kg bw	LD50 males >2000	Study report 2003 as cited in (ECHA,

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Method, route	Species, strain, sex, number/group	Test material, doses, duration of exposure	Results	References
procedure (OECD TG 402)			mg/kg bw; LD50 females 1224 mg/kg bw	2026)

7.2.2.3 Acute inhalation toxicity

Copper and copper compounds are not known as acutely toxic via the inhalation route. Several inhalation acute toxicity studies following standard test guidelines are summarised below:

Table 18: Summary of studies on acute inhalation toxicity of copper and inorganic copper compounds

Method, route	Species, strain, sex, number/group	Test material, doses, duration of exposure	Results	References
Acute inhalation toxicity (OECD TG 403)	Rat, Sprague-Dawley, 5 males and 5 females per dose	Dicopper oxide, 5.78 mg/L air (nose only), 4 hours	LC50 ca. 5 mg/L air	Study report 1985 as cited in (ECHA, 2026)
Acute inhalation toxicity (OECD TG 403)	Rat, Sprague-Dawley, 5 males and 5 females per dose	Dicopper oxide, 3.45, 4.43, 5.09 mg/L air (nose only), 4 hours	LC50 ca. 5.36 mg/L air	Study report 1985 as cited in (ECHA, 2026)
Acute inhalation toxicity (OECD TG 403)	Rat, Wistar, 5 males and 5 females per dose	Dicopper oxide, nominal conc. 30 mg/L air (nose only), 4 hours	LC50 >30 mg/L air	Study report 1988 as cited in (ECHA, 2026)
Acute inhalation toxicity (OECD TG 403)	Rat, Sprague-Dawley, 5 males and 5 females per dose	Dicopper oxide, 5.075 mg/L air (nose only), 4 hours	LC50 >5 mg/L air	Study report 1990 as cited in (ECHA, 2026)
Acute inhalation toxicity (OECD TG 403)	Rat, Sprague-Dawley, 5 males and 5 females per dose	Dicopper oxide, 1.28, 2.37, 5.25 mg/L air (nose only), 4 hours	LC50 male 2.92 mg/L air; LC50 female 3.69 mg/L air; LC50 combined 3.34 mg/L air	Study report 1999 as cited in (ECHA, 2026)
Acute toxic class method (OECD TG 436)	Rat, Sprague-Dawley, 3 males and 3 females per dose	Coated copper flakes, 1.24 or 5.11 mg/L air (nose only), 4 hours	LC50 >5.11 mg/L air	Study report 2011 as cited in (ECHA, 2026)

7.2.3 Summary

In humans, gastrointestinal irritation is the main adverse effect following acute oral exposure to copper, and it is generally reversible. Acute effects, including mortality, have also been reported in animal studies after oral administration. Acute inhalation toxicity of copper is dominated by local respiratory irritation and, at higher exposures to copper fumes, there is some evidence of

metal fume fever, while evidence of acute systemic toxicity from inhalation in occupational settings is limited. No acute effects have been reported via the dermal route.

7.3 Specific target organ toxicity/Repeated dose

Copper and its inorganic compounds do not have a harmonised classification for specific target organ toxicity after repeated dose exposure. Copper chloride (EC 215-704-5) has been self-classified as STOT SE 3 (H335, may cause respiratory irritation).

7.3.1 Human data

7.3.1.1 Respiratory effects

Suciu et al. (1981) examined about 100 workers chronically exposed to 111-464 mg Cu/m^3 as copper dust. The study involved 100, 97, 75, and 97 workers in 1970, 1971, 1972, and 1973, respectively, most of whom were exposed for 6-9 years. At the higher concentration levels, the authors reported an increased incidence in respiratory effects, as well as gastrointestinal complaints, neurotoxic symptoms, cardiovascular and peripheral vascular disorders, hepatomegaly and impotence. No control group was included in this study, so the findings are difficult to interpret.

Finelli (1981) reported the occurrence of bronchitis (as well as mild anaemia and hepatomegaly) among workers exposed to copper dust at concentrations of 0.64-1.05 mg/m^3. However, the workers were also exposed to iron, lead and cadmium, making it difficult to attribute the observed effects specifically to copper.

More recent epidemiological studies of respiratory effects in humans exposed to airborne copper are summarized in Table 19, below.

In a study of automotive workers in Iran, those occupationally exposed to copper-containing welding fumes reported respiratory symptoms such as cough, sputum production and wheezing (Saadiani et al., 2023). The mean airborne copper concentration in the welding unit was 0.107 mg Cu/m^3, and exposure was also associated with a reduction in forced expiratory volume in one second (FEV_1).

Two Egyptian studies examined respiratory health among employees at secondary copper smelters, where workers were also exposed to arsenic, lead and cadmium (Fouad and Ramadan, 2022, Mourad and El-Sherif, 2022). Smelter workers reported respiratory complaints, including cough, expectoration and nasal irritation, more frequently than administrative workers, and showed poorer spirometric measures. In (Fouad and Ramadan, 2022), radiographic abnormalities were reported in 36% of smelter workers compared with 4% of administrative workers, with reticular infiltrates being the predominant finding. Neither study measured or reported airborne copper concentrations, and the potential effects of co-exposure to other metals were not assessed. However, serum copper concentrations were higher in smelter workers than in the reference groups, suggesting greater internal copper exposure in the earlier group.

Three epidemiological studies, based on the PIAMA prospective birth cohort in the Netherlands, examined respiratory effects of copper exposure in particulate matter in ambient air.

In the first one, including 661 subjects evaluated before and after implementation of traffic reduction policies in a before-after controlled intervention study, Boogaard et al. (2013) reported that decreased copper concentrations in particulate matter were associated with improved forced vital capacity (FVC).

In the two subsequent studies, (Yu et al., 2021b) and (Gehring et al., 2015) showed inconsistent relationships between spirometry measures and copper concentration in particulate matter (PM). Gehring et al. (2015) investigated long-term exposure to elemental components of ambient $PM_{2.5}$ and PM_{10}, in relation to respiratory and allergic outcomes in 3 702 children at 8 and 11–12 years of age. Mean copper concentrations at the birth address were 3.1 ng/m^3 for $PM_{2.5}$ and 12.8 ng/m^3 for PM_{10}. Copper exposure was associated with lower FEV_1 for $PM_{2.5}$ and lower

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FEF_{25-75} for PM_{10}, while no association was observed with FVC; higher PM_{10} copper was also associated with increased allergic sensitisation. Copper was one of several correlated PM constituents, making it difficult to determine whether the associations were attributable specifically to copper. Yu et al. (2021b) included 706 adolescents and examined associations between modelled exposure to elemental components of ambient PM at ages 13–16 years and lung function at age 16. Mean copper concentrations were approximately 2.6 ng/m³ in $PM_{2.5}$ and 11 ng/m³ in PM_{10}. Higher copper exposure was not associated with FEV_1 or FVC, providing no evidence of an adverse effect of copper on lung function during adolescence. The main limitations were exposure estimation using land-use regression rather than personal measurements and the inability to fully separate the effects of correlated particulate constituents.

Two occupational health studies explored the potential long-term effects of inhaling copper dust at a German copper smelting facility (Haase et al., 2021, Haase et al., 2022), and found that chronic exposure to an average copper concentration of 4.61 mg/m³ (inhalable) over a mean exposure duration of 22 years did not adversely affect respiratory function. More specifically:

Haase et al. (2021) conducted a medical surveillance study of copper smelter workers for a period of 22 years utilising spirometry data. The exposure data derived from historical airborne monitoring data allowed the classification of cumulative inhalable Cu exposure into low (<=3.25mg Cu/m^3-years), medium (>3.25 to 5.5mg Cu/ m^3-years), and high (>5.5mg Cu/m^3-years) tiers. Spirometry analyses were routinely performed with at least two examination dates between ages 25 and 70 years, with the first and last examinations at least 5 years apart. After adjusting for normalized rates of respiratory decline with age, no accelerated decline in forced expiratory volume in 1 second (FEV1) was observed in never-smokers between the exposure and referent groups that could be attributed to exposure to copper dust, in any of the cumulative exposure tertiles (Table 19). Regarding respirable Cu, this would result in a cumulative mean concentration of 1.06 mg Cu/m^3 (range 0.32–1.8 mg respirable Cu/m^3-years). The study did not identify a clinically meaningful adverse effect of cumulative copper exposure on FVC. The study has limitations in relation to exposure assessment, since it assumed that all exposed workers had the same annual copper exposure, based on facility-wide measurements, and it also considered that the exposure levels measured in 1982-1984 were the same in the 1972-1981 period (for which there was no historical workplace measurement data). The absence of an observed association may, in part, reflect a healthy worker effect, whereby selection of a healthier working population could have attenuated the exposure–outcome association.

In the cross-sectional study of (Haase et al., 2022), 104 currently exposed workers were compared with 70 reference subjects from the same copper smelter plant as in the previous study using spirometry, plethysmography and inflammatory biomarkers to detect subclinical effects of copper exposure. No significant effect of chronic exposure on lung function or inflammation was observed at an exposure level of 0.21 mg Cu/m³ (inhalable fraction).

Table 19: Summary of epidemiological studies on respiratory effects of copper and inorganic copper compounds

Description	Copper exposure levels	Outcomes and Results	Study
Cross-sectional study of male automobile-industry workers in Iran. N= 1 152 welders (exposed) and 1 152 administrative workers (controls). Mean ages: 37.5 ± 5.09 years in welders and 38.5 ±	Occupational inhalation exposure to welding fumes. Annual workplace measurements were performed using the NIOSH 7 300 method. The average concentration of copper in the welding unit was	Pulmonary function was assessed by periodic spirometry and respiratory symptoms by questionnaire. Cough, sputum and wheezing were more prevalent among welders (P <0.001). FVC, FEV_1, FEV_1/FVC, FEF_{25-75} and PEF were all significantly lower in welders than controls (P <0.001). Cu concentration in welding fumes was significantly associated with FEV_1	(Saadiani et al., 2023)

Description	Copper exposure levels	Outcomes and Results	Study
7.18 years in controls.	0.107 mg Cu/m³.	(β=−0.133, 95% CI: −12.234 to −1.204) and FEF_{25-75} (β= −0.162, 95% CI: −20.981 to −3.730) after adjustment for age, BMI, work experience, smoking, and exposure to Fe, Pb and Cu fumes.	
Cross-sectional study of 75 exposed male smelter workers and 75 administrative employees (controls) in Egypt. Mean ages 43.19 years (exposed) and 44.05 years (controls).	Serum copper and arsenic were measured by atomic absorption spectrophotometry. Mean serum copper 191.41 µg/dL in exposed workers and 137.30 µg/dL in controls. Airborne copper concentrations not reported.	Higher prevalence of of nasal irritation, rhinitis, sinusitis, cough, expectoration, wheeze, dyspnea in copper-smelter workers. 36% of exposed workers had radiological infiltrates on chest X-ray vs 4% of controls. Most infiltrates were reticular. Exposed workers had reduced ventilatory parameters (FVC, FEV_1, FVC/FEV_1 and FEF). 58.7% of exposed workers had restrictive impairment and 40% had obstructive impairment.	(Fouad and Ramadan, 2022)
Cross-sectional Egyptian workers including 65 male copper-smelter workers and 41 matched administrative controls.	Mean serum copper was 175.2 µg Cu/dL in exposed and 93.44 µg Cu/dL in controls	Higher prevalence of exertional dyspnoea, cough and expectoration was higher among copper-smelter workers than matched administrative workers.	(Mourad and El-Sherif, 2022)
Before and after intervention study in a cohort in the Netherlands. Residents were assessed in 2008 and 2010, before and after implementation of traffic-reduction measures. N=661, aged ≥4 years,	Ambient outdoor air pollution (including particulate copper) measured in 12 study locations (fixed monitoring stations). Mean ambient Cu concentration decreased by 27.2 ng/m³ between 2008 and 2010.	In adjusted analyses, each IQR decrease in Cu was associated with approximately 1% increases in FVC. (Cu was highly correlated with soot, NO_2, NOx and Fe.) No association of Cu was observed with exhaled NO.	(Boogaard et al., 2013)
Prospective birth cohort study (PIAMA) in the Netherlands. N=3 702 children; followed from childhood, with lung-function assessment at approximately 8 and 11–12 years.	Long-term residential exposure to ambient PM copper, estimated using land-use regression models based on monitoring campaigns. Copper was measured in $PM_{2.5}$ and PM_{10} using X-ray fluorescence. At birth addresses, mean Cu concentrations were 3.1 ng/m³ in $PM_{2.5}$	Higher Cu in PM_{10} at the current address was associated with: • asthma symptoms (OR 1.06, 95% CI 1.00–1.12 per IQR) • lower FEF_{25-75} (−2.3%, 95% CI −4.3 to −0.3%). Cu in $PM_{2.5}$ at the current address was associated with: • lower FEV_1 (−1.1%, 95% CI −2.1 to −0.1%). Copper in PM_{10} at the birth address was associated with allergic sensitization (RR 1.07, 95% CI 1.01–1.14). No association was observed with FVC. High correlations between some PM constituents and PM mass make independent	(Gehring et al., 2015)

Description	Copper exposure levels	Outcomes and Results	Study
	(range 1.1–10.6 ng/m³) and 12.8 ng/m³ in PM_{10} (range 6.6–52.3 ng/m³). Copper was mainly associated with the coarse fraction of PM_{10} and was considered a marker of non-tailpipe traffic emissions, (particularly brake/tire wear).	attribution to copper difficult	
Prospective birth cohort study, follow-up of PIAMA participants in the Netherlands. N=706 adolescents (47.3% male) with lung-function measurements at approximately 16 years of age.	Long-term residential exposure to ambient PM elemental components and ultrafine particles (UFP) during approximately 13–16 years, estimated using land-use regression. Copper was assessed in $PM_{2.5}$ and PM_{10}. Mean current-residence concentrations were approximately 2.6 ng Cu/m³ in $PM_{2.5}$ and 11 ng Cu/m³ in PM_{10}.	Long-term residential exposure to $PM_{2.5}$ Cu was associated with lower FEV_1 at age 16 (−2.10%, 95% CI −3.40 to −0.79% per IQR increase of 1.4 ng/m³) PM_{10} Cu exposure was not significantly associated with FEV_1 (−0.38%, 95% CI −1.26 to 0.50% per IQR increase of 3.2 ng/m³). Neither $PM_{2.5}$ nor PM_{10} Cu was significantly associated with FVC. Cu and Fe were highly correlated (r = 0.83–0.96), both represented non-tailpipe traffic emissions	(Yu et al., 2021b)
Historical occupational health-surveillance cohort study of workers employed at a German copper smelter from 1972–2018, including 479 copper-smelter workers and 172 referent workers (from the precious-metal/lead facility)	Occupational inhalation exposure to copper-containing dust reconstructed from historical workplace airborne measurements. Mean cumulative inhalable copper exposure was approximately 4.61 mg/m³-years, with an average exposure duration of 22 years.	Lung function, primarily longitudinal changes in FEV_1 and other spirometry parameters. Among never-smokers, FEV_1 declined by approximately 29 mL/year, consistent with expected age-related decline. No evidence of an adverse association between cumulative inhalable copper exposure and lung-function decline, including among workers with higher cumulative exposure.	(Haase et al., 2021)
Cross-sectional occupational study of currently employed workers at the same	Occupational long-term inhalation exposure to copper-containing dust/aerosols,	Lung function, FeNO, systemic inflammatory markers and airway inflammation were assessed. Lung-function values in both groups were	(Haase et al., 2022)

Description	Copper exposure levels	Outcomes and Results	Study
German copper smelter: 104 copper-exposed workers and 70 referent workers employed in precious-metal/lead facilities. A smaller subgroup underwent induced-sputum analysis (25 copper exposed and 11 referent workers).	assessed using workplace exposure data. Reported Cu inhalable concentrations approximately 0.12 mg/m³. Serum copper was measured by atomic absorption spectrometry.	generally within healthy reference ranges, with no clinically relevant adverse differences between exposed and referent workers. Sputum findings did not indicate clinically meaningful chronic pulmonary inflammation. Some inflammatory blood markers were higher among workers not using respiratory protection, but smoking had a stronger influence on these markers.	

Notes: BMI=Body Mass Index; FEF=Forced Expiratory Flow; FEV_1=Forced Expiratory Volume in the first second; FVC=Forced Vital Capacity; IQR=Interquartile Range; PEF=Peak Expiratory Flow; PM=Particulate Matter.

7.3.1.2 Neurological effects

A large prospective cohort study in the United States (>10 000 participants) reported an association between higher dietary copper intake and incident dementia among participants with diets high in saturated fat. Specifically, each 1 mg/day increase in copper intake was associated with a higher risk of incident dementia (HR 1.49, 95% CI 1.04–1.95), whereas no such association was observed among participants whose diets were not high in saturated fat (Wei et al., 2022). Higher copper intake was also associated with a decline in word fluency scores over the 20-year follow-up in both dietary groups. Dietary copper intake was assessed at enrolment (1987–1989) and again in 1993–1995 using an interviewer-administered, validated food frequency questionnaire, while cognitive function was assessed at three time points beginning in 1996–1998. Strengths of the study included the large sample size, prospective design and long follow-up; limitations included the inability to account for copper intake from drinking water or other local sources and the limited number of cognitive assessments.

A meta-analysis of 56 studies investigating copper biomarkers in serum (Squitti et al., 2021) showed that serum copper excess was associated with a three to fourfold increase in the likelihood of having Alzheimer's Dementia (OR: 3.30, 95%CI: 1.86 to 5.85), with very high study heterogeneity (I2=95.9%; p <0.001). The meta-analysis included 2 929 patients with Alzheimer's dementia and 3 547 healthy controls for the serum/plasma analyses. Additionally, carriers of the *ATP7B* AG haplotype were significantly more frequent in the AD group, suggesting that people with this genetic subtype seem especially prone to copper imbalance. These findings show an association with altered copper status in the patients' group but do not demonstrate that external copper exposure causes Alzheimer's disease.

An earlier systematic review by (Loef and Walach, 2012) suggested that high dietary copper intake may be associated with accelerated cognitive decline and risk of Alzheimer's disease, particularly in the context of a diet high in saturated and trans fats. However, this conclusion was largely based on findings from a single large prospective cohort study by (Morris et al., 2006), which included 3 718 older adults and reported a substantially faster rate of cognitive decline among participants with high copper and saturated-fat intakes. The authors of the systematic review noted that the available prospective evidence was limited and that further studies were needed to clarify the relationship between dietary copper, dietary fat and cognitive decline.

Several cross-sectional studies have studied cognitive function in relation to copper exposure. A small study of 245 women attending a menopause clinic in Japan reported an association between higher estimated dietary copper intake (estimated using a brief, self-administered diet

history questionnaire) and greater severity of subjective forgetfulness among women aged ≥55 years, but not among those aged 40–54 years (Odai et al., 2020). In contrast, a cross-sectional analysis of 2 483 adults aged ≥60 years participating in NHANES (2011–2014) reported nonlinear associations between total dietary copper intake and cognitive function. Higher copper intake was positively associated with cognitive test scores below study-specific inflection points, whereas no significant associations were observed at higher intake levels, including among participants with copper intake exceeding the recommended dietary intake (Wang et al., 2021). Using the same NHANES 2011–2014 cohort, (Jia et al., 2025) similarly examined dietary copper intake and cognitive function among 2 420 adults aged ≥60 years. In contrast to (Wang et al., 2021), who reported nonlinear associations with weaker or nonsignificant associations at higher copper intakes, (Jia et al., 2025) found that higher copper intake was generally associated with better cognitive performance. However, as both studies used the same NHANES cycles and overlapping populations, the findings should not be considered independent evidence.

The European Food Safety Authority (EFSA) characterised the evidence for a causal relationship of dietary copper intake and neurological effects in humans as 'speculative and inconclusive' (EFSA et al., 2023).

7.3.1.3 Other chronic effects

7.3.1.3.1 Hepatic

Wilson's disease, Indian childhood cirrhosis (ICC), and idiopathic copper toxicosis (ICT) are chronic disorders of copper homeostasis characterized by excessive accumulation of copper in the liver and associated with hepatic effects, as described by (ATSDR, 2024).

Hepatomegaly was reported in workers occupationally exposed to copper dust during grinding and sieving (Suciu et al., 1981). Estimated copper exposure concentrations decreased from 464 to 111 mg Cu/m³ over the 3-year observation period; however, the prevalence of hepatomegaly increased during this period despite the reduction in exposure. Consequently, the relationship between copper exposure and hepatomegaly is difficult to interpret from this study alone. Additionally, the study did not provide an unexposed comparison group nor reported information on how the outcome was measured.

Several observational studies report associations between higher blood/serum copper and non-alcoholic fatty liver disease. In NHANES 2011-2014 (n=3 211), the highest serum-copper quartile was associated with approximately 97% higher odds of non-alcoholic fatty liver disease, versus the lowest quartile (Chen et al., 2021). A later NHANES analysis (n=1 377) similarly found higher serum copper associated with Non-Alcoholic Fatty Liver Disease (NAFLD) (OR 2.07) and advanced fibrosis (OR 2.40) (Li et al., 2024). Reverse causation is a major concern because liver dysfunction itself can alter copper metabolism and distribution.

A few controlled human oral exposure studies on copper evaluated hepatic clinical chemistry parameters, including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH), and found no significant changes in serum enzyme activities following copper exposure of up to 0.138 mg Cu/kg/day as copper sulphate in drinking water for up to 2 months (Pizarro et al., 2001, Pizarro et al., 1999, Araya et al., 2003a), in the diet for 6 weeks (O'Connor et al., 2003), or in capsule form for 6 months (Rojas-Sobarzo et al., 2013). Similarly, administration of 0.15 mg Cu/kg/day as copper gluconate in capsules for 12 weeks to seven adults did not significantly alter serum enzyme activities (Pratt et al., 1985). In infants exposed to 0.319 mg Cu/kg/day as copper sulphate in drinking water for 9 months, no changes in total bilirubin or serum ALT, AST, or GGT activities were observed (Olivares et al., 1998). The studies were relatively small and are limited by the use of clinical chemistry endpoints that may not detect subclinical or structural hepatic effects.

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7.3.1.3.2 Cardiovascular

A meta-analysis of 16 studies including 41 322 participants found that higher serum copper was associated with myocardial infarction (OR 1.31, 95% CI 1.17–1.46), total stroke (OR 1.49, 1.22–1.82), and cardiovascular mortality (OR 1.60, 95% CI: 1.39–1.86) (Muñoz-Bravo et al., 2023).

A separate meta-analysis restricted to longitudinal studies, including 47 813 participants, similarly reported associations of copper with stroke, cardiovascular mortality, and coronary-artery-disease mortality, although it did not find a statistically significant association with acute myocardial infarction (Zhao et al., 2024).

A Mendelian-randomization study-which uses genetic variants associated with blood copper as proxies for lifelong differences in copper levels-reported an inverse association between genetically predicted copper and coronary artery disease (OR 0.92, 95% CI 0.86–0.99) and systolic blood pressure. The authors' multivariable analysis suggested that systolic blood pressure could mediate the association of copper with coronary artery disease rather than altered lipoprotein metabolism (Jäger et al., 2022).

Another meta-analysis of 22 studies of cross-sectional and case-control studies found no significant difference in serum copper levels between people with hypertension and controls, although some subgroups showed different results (Li et al., 2019).

7.3.2 Animal data

7.3.2.1 Studies by oral route

Most of the available oral repeated dose studies were performed with copper sulphate or copper sulphate pentahydrate (Table 20).

In an NTP study (Hébert et al., 1993, NTP, 1993), toxicity of copper sulphate was investigated in rats and mice after oral exposure for 2 weeks (diet or drinking water) or 13 weeks (diet).

In the 13-week oral NTP study, male and female rats (F344/N), 10 animals/ sex/ dose, received 0, 500, 1 000, 2 000, 4 000 and 8 000 ppm copper sulphate via feed (*ad libitum*) for 92 days:

- Except for one female in the 1 000 ppm group that was accidentally euthanized, all animals survived to study termination. Reduced terminal body weights and body weight gains were observed in males at ≥500 ppm and in females at 8 000 ppm. The greatest decrease occurred in high-dose males. Feed consumption was generally comparable to controls up to 4 000 ppm but was slightly reduced at 8 000 ppm in both sexes. No treatment-related clinical signs were observed. Mean copper sulphate intake was 32, 64, 129, 259, and 551 mg/kg/day in males and 34, 68, 135, 267, and 528 mg/kg/day in females.
- Significant changes in absolute organ weights were restricted to the 8 000 ppm group. Males exhibited reduced brain, heart, kidney, liver, lung, and thymus weights, while females showed only reduced kidney weights. Relative organ weights were generally unchanged or increased at 4 000 and 8 000 ppm, consistent with lower body weights.
- Copper sulphate produced dose-related haematological alterations in both sexes throughout the study indicating sustained, dose-dependent disruption of erythropoiesis and haematological homeostasis. Dose-related clinical chemistry changes were observed primarily at 4 000 and 8 000 ppm and were indicative of a clear dose-dependent hepatic toxicity of copper sulphate (see Table 20). Transmission electron microscopy demonstrated degenerative changes in periportal hepatocytes, including increased and enlarged secondary lysosomes containing crystalline and electron-dense material. Similar changes occurred in the kidney, where proximal tubular epithelial cells exhibited

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increased protein droplets, particularly in males. Large crystalline inclusions were observed only in male kidneys.

- Bile salt concentrations showed time- and sex-dependent changes. Total protein concentrations were decreased throughout the study in high-dose animals and, to a lesser extent, in intermediate-dose groups. Albumin concentrations were also reduced, particularly in the three highest dose groups (see Table 20).
- Urea nitrogen concentrations were increased in a dose-related manner, particularly at the two highest dose levels. Creatinine was unaffected except for a transient increase in high-dose females. Urinalysis revealed significant increases in urinary aspartate aminotransferase (AST) activity at 4 000 and 8 000 ppm in both sexes, with occasional increases at lower doses. Urinary N-acetyl-β-D-glucosaminidase activity was increased in the 4 000 and 8 000 ppm groups, indicating renal tubular effects. Increased urinary glucose excretion was observed in males at ≥2 000 ppm, while urinary protein excretion was decreased at Day 19 but increased by Day 90 in high-dose males. No treatment-related effects on urinary glucose or protein excretion were observed in females.
- Tissue analysis demonstrated dose-dependent copper accumulation in the liver and kidneys, accompanied by increased zinc concentrations. Copper concentrations were elevated in all treated groups in the liver and kidney and at ≥2 000 ppm in plasma and testes. Plasma calcium was reduced at 4 000 and 8 000 ppm, while magnesium concentrations were increased in selected tissues and dose groups.
- Treatment-related gross lesions were confined to the forestomach and consisted of enlargement of the limiting ridge in animals receiving ≥2 000 ppm, with the highest incidence at 4 000 and 8 000 ppm. Histologically, these gross findings corresponded to dose-dependent squamous mucosal hyperplasia and hyperkeratosis at the limiting ridge, occasionally accompanied by inflammation and oedema. No ulceration or lesions elsewhere in the squamous mucosa were identified. Treatment-related liver lesions consisted of dose-dependent chronic active inflammation, characterized by periportal mononuclear inflammatory infiltrates and associated hepatocellular necrosis. These changes occurred predominantly at 4 000 and 8 000 ppm.
- Renal histopathology revealed increased cytoplasmic protein droplets in male and female rats at ≥2 000 ppm, with greater severity in males. Occasional protein droplets were also present within tubular lumina, particularly in males. In males, protein droplets were larger and more numerous than in females and stained positively for protein but not for iron, pre-autophagosomal structure , or lipofuscin. Immunohistochemistry for α2u-globulin was inconclusive and did not demonstrate qualitative differences between treated and control animals. Additional renal findings included minimal tubular karyomegaly in high-dose animals and tubular epithelial degeneration in three females at 8 000 ppm.
- Copper staining confirmed deposition in the liver and kidneys of animals receiving 4 000 or 8 000 ppm. Hepatic staining was concentrated in periportal to midzonal hepatocytes, while renal staining was restricted to cortical tubular epithelium and associated protein droplets. No copper deposition was detected in the heart or spleen at any dose level. Perl's iron staining revealed reduced splenic hemosiderin deposition in treated animals, most notably at 8 000 ppm, with less pronounced reductions at 2 000 and 4 000 ppm.
- No treatment-related effects were observed on sperm morphology or vaginal cytology in animals exposed to up to 4 000 ppm copper sulphate. These evaluations were performed only at 0, 500, 2 000 and 4 000 ppm.

In the 13-week oral NTP study (Hébert et al., 1993, NTP, 1993), in addition to rats, also male and female mice ($B6C3F_1$), 10 animals/ sex/ dose, received 0, 1 000, 2 000, 4 000, 8 000 and 16 000 ppm copper sulphate via feed (*ad libitum*) for 92 days:

- No treatment-related mortality occurred during the study. A dose-related reduction in body weight gain was observed, with earlier onset and greater severity at higher dose levels. Final mean body weights and body weight gains were slightly reduced in males at

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4 000 ppm and significantly reduced in males and females at 8 000 and 16 000 ppm compared with controls. Mean daily feed consumption was comparable to or greater than control values across all treated groups. No treatment-related clinical signs were observed. Mean compound intake was 173, 382, 736, 1 563, and 3 201 mg/kg/day in males and 205, 494, 1 048, 2 106, and 5 157 mg/kg/day in females.

- At 16 000 ppm, male mice showed significantly reduced absolute heart and kidney weights, while females exhibited significantly reduced absolute thymus and kidney weights. Absolute liver weights decreased in a dose-dependent manner in both sexes, reaching statistical significance at 8 000 and 16 000 ppm in males and females, and at 4 000 ppm in males. Relative organ weights were generally increased in treated animals, with many changes significant at higher dose levels. These organ weight changes were considered secondary to reduced terminal body weights in the high-dose groups.
- Treatment-related gross lesions were limited to the forestomach of seven males and four females at 16 000 ppm. Lesions consisted of focal white discoloration of the squamous mucosa at the limiting ridge adjacent to the glandular stomach. Histopathological examination revealed minimal to mild squamous cell hyperplasia and hyperkeratosis at the limiting ridge in mice exposed to ≥4 000 ppm copper sulphate. No associated inflammation, erosion, or ulceration was observed, and no increase in these lesions was noted elsewhere in the forestomach.
- Liver from males and females at 16 000 ppm stained weakly positive for copper, with only a few hepatocytes showing positive staining. No copper staining was detected in the liver at lower dose levels (only male livers were examined). Copper was not detected in the kidneys of any animals.
- No treatment-related effects on sperm morphology or vaginal cytology were observed in animals exposed to up to 8 000 ppm compared with controls. These evaluations were performed only at 0, 1 000, 2 000 and 8 000 ppm.

In the 2-week oral NTP study (Hébert et al., 1993, NTP, 1993), male and female rats (F344/N), 5 animals/ sex/ dose, received 0, 1 000, 2 000, 4 000, 8 000 or 16 000 ppm copper sulphate via feed (*ad libitum*) for 15 days. The study included tissue metal level analysis in male rats at 0, 1 000 or 16 000 ppm:

- No mortality occurred during the exposure period. At the two highest dose levels, mean final body weight and body weight gain were significantly reduced compared with controls. In the highest dose group (16 000 ppm), feed consumption was also decreased. The reduced feed intake during the first week was likely attributable, at least partly, to poor diet palatability. Thinness was the only clinical sign observed and was restricted to the 8 000 and 16 000 ppm groups. Mean compound intake was 92, 180, 363, 777, and 1 275 mg/kg bw/day in males and 89, 174, 367, 769, and 1 121 mg/kg bw/day in females.
- Absolute organ weights were generally lower in the two highest dose groups than in controls. Female rats in the 4 000 ppm group had significantly reduced absolute liver weights compared with controls. Reduced absolute liver weights were also observed in males and females at the two highest dose levels. These changes were likely secondary to reduced feed consumption and lower body weight gain.
- Analysis of tissue metal concentrations (three males per dose group) showed that liver and kidney copper concentrations in males receiving 16 000 ppm copper sulphate were approximately 130-fold and 25-fold higher, respectively, than in controls. Liver zinc concentrations were also increased in males at this dose level. No treatment-related effects were observed on manganese, calcium, or magnesium concentrations in the liver, kidneys, testes, or plasma.
- Hyperplasia with hyperkeratosis of the squamous mucosa on the limiting ridge separating the forestomach from the glandular stomach was observed in all rats at dose levels of

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2 000 ppm and above. Minimal to mild mononuclear inflammatory cell infiltrates were present in the livers of males at the two highest dose levels and in females at the highest dose level (16 000 ppm). Bone marrow depletion of haematopoietic cells was observed at 8 000 ppm (minimal severity) and 16 000 ppm (mild to moderate severity) in both sexes. In addition, reduced cortical and trabecular bone density was noted in many animals at 16 000 ppm. Minimal to mild reductions in splenic erythroid haematopoiesis were also observed in males and females at this dose level.

- At the three highest dose levels, renal cortical tubular cytoplasmic alterations were observed in both sexes. These changes were characterised by increased numbers and sizes of eosinophilic protein droplets within the cytoplasm and tubular lumen.

In the 2-week oral NTP study (Hébert et al., 1993, NTP, 1993), male and female mice ($B6C3F_1$), 5 animals/ sex/ dose, received 0, 1 000, 2 000, 4 000, 8 000 or 16 000 ppm copper sulphate via feed (*ad libitum*) for 15 days:

- No mortality occurred during the exposure period. In the two highest dose groups, mean terminal body weight and body weight gain were lower in both males and females compared with controls, although terminal body weights remained within 6% of control values. A similar effect on body weight was observed in females at 2 000 ppm. At 16 000 ppm, reduced feed consumption was also noted and was most pronounced during the first week of exposure, likely contributing to the slight reduction in terminal body weight. Thinness was the only clinical sign reported, occurring in one male and two females at 8 000 ppm and in one female at 16 000 ppm. Mean compound intake was 168, 362, 773, 1 154, and 2 817 mg/kg bw/day in males and 210, 408, 849, 1 563, and 3 068 mg/kg bw/day in females.
- Compared with controls, mice exposed to 16 000 ppm showed significantly increased relative brain and liver weights in both sexes. In males, significant increases in relative brain weight were also observed at 8 000 ppm and in relative liver weight at 4 000 and 8 000 ppm. As these changes were not accompanied by relevant pathological findings, they were considered incidental and unrelated to treatment.
- No treatment-related gross lesions were observed in either sex. Histopathological findings were confined to the forestomach and consisted of minimal squamous mucosal hyperplasia with hyperkeratosis at the limiting ridge. These lesions were present in all animals at 8 000 and 16 000 ppm and in 3 of 5 males and 2 of 5 females at 4 000 ppm.

In the 2-week oral NTP study (Hébert et al., 1993, NTP, 1993), male and female rats (F344/N), 5 animals/ sex/ dose, received 0, 300, 1 000, 3 000, 10 000 or 30 000 ppm copper sulphate via drinking water (*ad libitum*) for 15 days:

- Exposure to copper sulphate resulted in clear dose-dependent toxicity. One female rat in the 3 000 ppm group died and all animals in the 10 000 and 30 000 ppm groups either died or were euthanized in a moribund condition before study termination. By Day 8, mean body weights of males and females at ≥3 000 ppm were significantly lower than those of controls. Due to complete mortality, terminal body weight assessment in the 10 000 and 30 000 ppm groups was not possible. However, animals exposed to 3 000 ppm showed pronounced growth retardation, with terminal body weights 48% lower in males and 46% lower in females compared with controls.
- Clinical signs of toxicity were observed in the three highest dose groups and included ruffled fur, emaciation, abnormal posture, hypoactivity, and nasal discharge. These effects were associated with markedly reduced water consumption, which was attributed to the poor palatability of the test solutions. Mean daily water intake in the 3 000 ppm group was less than 30% of control values and less than 6% of controls in the 10 000 and 30 000 ppm groups. Based on water consumption, estimated copper sulphate intake was 41, 113, 175, 140, and 379 mg/kg bw/day in males and 39, 102, 121, 120, and 279 mg/kg bw/day in females.

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- Treatment-related effects on organ weights were limited to the highest surviving dose group (3 000 ppm). Absolute organ weights were significantly reduced in both sexes, while relative organ weights were generally increased. The report did not specify the affected organs. These changes were considered secondary to the substantially reduced terminal body weights in animals exposed to 3 000 ppm copper sulphate.
- No treatment-related macroscopic lesions were observed in either sex. Histopathological findings were restricted to the kidneys of males at 300 and 1 000 ppm, where minimal cytoplasmic alterations in proximal convoluted tubule epithelial cells were characterized by increased size and number of protein droplets. No comparable findings were reported in females.

In the 2-week oral NTP study (Hébert et al., 1993, NTP, 1993), male and female mice ($B6C3F_1$), 5 animals/ sex/ dose, received 0, 300, 1 000, 3 000, 10 000 or 30 000 ppm copper sulphate via drinking water (*ad libitum*) for 15 days:

- One male and three female mice in the 3 000 ppm group, as well as all mice in the 10 000 and 30 000 ppm groups, died before study termination. Mean body weights on day 8 were lower than controls in both sexes at 3 000, 10 000, and 30 000 ppm. As all animals in the two highest dose groups died before the end of the study, final body weights and body weight changes could not be assessed for these groups. At study completion, significantly reduced final body weights and weight gains were observed at 3 000 ppm, i.e., males weighed 22% less and females 34% less than their respective controls.
- Clinical signs of toxicity were observed at the three highest dose levels and included ruffled fur, emaciation, abnormal posture, hypoactivity, dyspnoea, tremors, and prostration. These effects were attributed primarily to markedly reduced water consumption, which was less than one-third of control values. The reduced water intake was considered secondary to the poor palatability of the drinking water solutions. Based on water consumption measurements, estimated compound intake was 41, 95, 226, 524, and 1 442 mg/kg/day in males and 58, 140, 245, 683, and 1 296 mg/kg/day in females.
- Treatment-related changes in organ weights were largely confined to the highest surviving dose group (3 000 ppm). In this group, absolute thymus weights were significantly reduced in both sexes. Females also showed significantly lower absolute brain, kidney, liver, and lung weights at 3 000 ppm, while absolute brain and lung weights were reduced at 1 000 ppm. These changes were considered secondary to dehydration, reduced water consumption, and lower terminal body weights rather than direct effects of copper sulphate.
- Atrophy and cellular depletion were observed in various tissues in the three highest dose groups. However, these findings were considered secondary to reduced water intake and impaired body weight gain and were not regarded as direct toxic effects of copper sulphate.

Other available non-guideline oral repeated dose studies on copper sulphate have mainly focussed on organ toxicity (Ali et al., 2023, Arafa et al., 2019, de Vries et al., 1986, Draper et al., 2023, Haywood, 1980, Haywood and Comerford, 1980, Kumar et al., 2016b, Kumar et al., 2016a, Liu et al., 2020a, Liu et al., 2020b, Patwa and Flora, 2020).

In a 15-week oral dietary study, rats (strain, sex and number of animals per dose group not specified) were exposed to 2 000 mg copper per kg as copper sulphate for 15 weeks (Haywood, 1980, Haywood and Comerford, 1980).

- Administration of high dose of copper sulphate resulted in copper accumulation in the liver and kidneys. Early findings in histological examination indicated cellular disturbances that progressed to marked cellular disruption in the liver and kidney. However, similar regenerative and healing responses observed in the final phase suggest adaptive mechanisms to prolonged excessive copper exposure in both organs (Haywood, 1980).

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- Copper sulphate exposure resulted in clear, time-dependent effects on copper homeostasis and liver function. Blood and plasma copper concentrations increased during the first week, returned to baseline levels, and subsequently peaked at week 9. A corresponding gradual increase in alanine aminotransferase (ALT) activity, reaching its highest level at week 9, indicated a hepatic response to copper exposure. In contrast, alkaline phosphatase (ALP) and bilirubin concentrations remained unaffected. No changes were observed in erythrocyte copper concentrations, and no evidence of haemolysis was reported (Haywood and Comerford, 1980).

In an 11-month study, female Sprague-Dawley rats (8 animals/group) received 0.125% copper sulphate in drinking water *ad libitum* from weaning, corresponding to an estimated copper intake of approximately 12 mg per day (de Vries et al., 1986).

- Chronic copper exposure increased copper concentrations in the liver and parietal cortex without affecting body or liver weights. While striatal dopamine levels remained unchanged, a significant 25% reduction in 3,4-dihydroxyphenylacetic acid (DOPAC) was observed, indicating altered dopamine metabolism. Copper exposure was also associated with increased dopamine D2 receptor affinity, a trend toward reduced receptor numbers, and inhibition of ligand binding in radioligand assays. Collectively, these findings demonstrate that long-term copper exposure can modify central dopaminergic neurotransmission despite the absence of overt systemic toxicity (de Vries et al., 1986).

In a 90-day oral gavage study, male Wistar rats (18 animals per dose group) received copper sulphate at 0, 100, or 200 mg/kg bw/ day, with six animals per group examined after 30, 60, and 90 days (Kumar et al., 2016b, Kumar et al., 2016a, Kumar et al., 2015).

- Marked copper accumulation occurred in the liver, kidney, and brain ($p < 0.01$) being highest in the liver, followed by the kidney and brain. The concentrations increased up to 29-fold in the liver compared with control.
- No mortality was observed. However, copper exposure produced clear treatment-related effects. Body weight was significantly reduced at nearly all time points, while haematological and biochemical findings indicated systemic toxicity, including decreased haemoglobin and increased bilirubin and transaminase levels. Neurobehavioral performance was consistently impaired, as demonstrated by reduced grip strength, rotarod performance, and Y-maze attention scores, with the greatest effects at 200 mg/kg bw/day. These findings provide evidence of dose-related systemic and neurobehavioural toxicity following repeated copper exposure (Kumar et al., 2015).
- The liver was the primary target organ, exhibiting severe fatty degeneration, centrilobular necrosis, and inflammatory changes, while the brain showed neuronal damage and demyelination, and the kidney displayed tubular degeneration, congestion, and fibrosis after prolonged exposure. Histopathological severity was consistently greatest in the liver and correlated with tissue copper concentrations. No treatment-related histopathological changes were observed in control animals (Kumar et al., 2016b).
- Clear evidence of oxidative stress was also detected, characterized by increased malondialdehyde levels and decreased glutathione and total antioxidant capacity. These effects were most pronounced in the liver, followed by the brain and kidney, and consistently correlated with tissue free copper concentrations across all time points (Kumar et al., 2016b, Kumar et al., 2016a).

In a 16-week oral gavage study in male Sprague-Dawley rats (the number of animals per group not specified), daily exposure to copper sulphate pentahydrate (10 or 20 mg/kg bw) produced evidence of neurotoxicity (Kumar et al., 2019).

- No mortality was observed. Copper-treated animals showed increased brain copper accumulation, reduced serum ceruloplasmin activity, and dose-related neurobehavioural impairments. The observed neurobehavioural effects included decreased passive avoidance, locomotor activity, and novel object exploration, alongside increased immobility. These functional changes were accompanied by marked indicators of oxidative stress in the brain, including elevated lipid peroxidation, nitrite and 8-

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hydroxydeoxyguanosine (8-OHdG) levels, reduced glutathione, and decreased antioxidant enzyme activity. The effects were observed at both dose levels and were generally more pronounced at 20 mg/kg bw, supporting a treatment-related adverse effect of copper exposure on neurological function and brain oxidative status (Kumar et al., 2019).

In a 90-day oral gavage study in male Wistar rats (10 animals/group), administration of copper sulphate pentahydrate at 200 mg/kg bw/day resulted in marked copper accumulation, particularly in the liver (41-fold) and intestine (36-fold), with additional increases in the kidneys (3-fold) and testes (2-fold) (Arafa et al., 2019).

- The copper overload was associated with clear adverse effects, including hypertension, evidenced by significantly elevated systolic blood pressure and serum angiotensin II levels alongside reduced serum nitric oxide. Moreover, pronounced testicular toxicity was observed, characterized by decreased relative testis weight, reduced testosterone and luteinizing hormone (LH) concentrations, diminished testicular zinc levels, increased oxidative stress, and downregulation of key steroidogenic genes. These findings demonstrate that prolonged exposure to high levels of copper can lead to systemic toxicity and significant impairment of male reproductive function (Arafa et al., 2019).

In a 21- and 42-day oral gavage study, ICR mice (60 animals per sex per dose group) received 0, 4, 8, or 16 mg Cu^{2+} /kg/ day as copper sulphate (Liu et al., 2021a, Liu et al., 2021b, Liu et al., 2020a, Liu et al., 2020b).

- Histopathological evaluation revealed clear dose- and time-dependent liver toxicity. Hepatocyte vacuolar degeneration was observed at 8 mg/kg after 21 days, while the 16 mg/kg group showed granular and vacuolar degeneration together with hepatocellular necrosis. By Day 42, hepatocellular degeneration was evident at doses above 4 mg/kg, and necrotic hepatocytes with inflammatory cell infiltration occurred at doses above 8 mg/kg. These findings were supported by increased serum liver injury biomarkers (ALP, AST, ALT, GGT, albumin, and total bilirubin). Mechanistic analyses further demonstrated activation of the MAPK and NF-κB signalling pathways, accompanied by upregulation of pro-inflammatory cytokines (IL-2, IL-1β, and IL-8) and suppression of the anti-inflammatory mediator TGF-β. Collectively, the results provide evidence that repeated copper exposure induces progressive hepatotoxicity and hepatic inflammation in mice (Liu et al., 2020a).
- Although reductions in body weight gain were observed from 4 mg/kg onwards, these changes were not statistically significant. A significant increase in liver copper concentrations was detected at 16 mg/kg on day 42 ($p < 0.01$). Histopathological examination revealed progressively greater hepatic injury with increasing dose and duration, including granular and vacuolar degeneration of hepatocytes. At doses ≥8 mg/kg, severe degenerative changes, including karyorrhexis, karyolysis, hypochromasia, and hepatocellular necrosis, were observed. These findings were accompanied by evidence of oxidative stress in the liver, demonstrating a consistent relationship between copper accumulation and adverse hepatic effects (Liu et al., 2020b).
- Liver lesions were evident from 4 mg/kg, including hepatocyte granular and vacuolar degeneration. At doses of 8 mg/kg and above, additional findings such as disrupted hepatic cord architecture and hepatocellular necrosis were observed. By day 42, all major liver lesions were present at ≥4 mg/kg, with severity increasing consistently with dose, providing strong evidence of a progressive and dose-dependent adverse effect of copper sulphate on the liver (Liu et al., 2021a, Liu et al., 2021b).

In a 16-week oral gavage study, male Sprague-Dawley rats (18 animals per group) received copper sulphate at 0 or 20 mg/kg bw/day (Patwa and Flora, 2020).

- Treatment had no effect on body or liver weight. However, it resulted in increased hepatic copper accumulation and elevated levels of the copper-transport protein ceruloplasmin. In addition, serum ALT and low-density lipoprotein (LDL) concentrations were increased, indicating potential liver injury and altered lipid metabolism. Evidence of oxidative stress

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was observed through elevated hepatic reactive oxygen species (ROS) levels. Histopathological examination revealed marked liver damage, including hepatocyte necrosis, distortion of the sinusoidal spaces and central vein architecture, as well as collagen deposition, consistent with hepatic fibrosis (Patwa and Flora, 2020).

In a 16-week oral gavage study, male Sprague-Dawley rats were administered copper sulphate at 20 mg/kg/day (24 exposed animals) or vehicle control (18 animals) (Patwa et al., 2022).

- Chronic copper exposure induced significant neurobehavioural impairments, characterized by reduced spontaneous locomotor activity and motor coordination, alongside increased resting behaviour, immobility, and anxiety. These effects were accompanied by marked oxidative stress in the brain, evidenced by elevated ROS, lipid peroxidation, and nitrite levels, as well as depletion of key antioxidant enzymes (superoxide dismutase and catalase). Furthermore, copper exposure triggered a pronounced inflammatory response, with increased levels of tumour necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), highlighting the potential of copper to drive neurotoxicity through interconnected oxidative stress and inflammatory pathways (Patwa et al., 2022).

In a 4-month oral drinking water study, Sprague-Dawley rats (sex not specified) exposed to 250 ppm copper sulphate pentahydrate (equivalent to 100 ppm copper; 4-6 animals per dose group) exhibited clear evidence of cardiac toxicity (Ali et al., 2023).

- Treatment was associated with increased oxidative stress and histopathological alterations in the heart, including myocyte damage and necrosis, mast cell infiltration, and increased collagen deposition. Although the increase in cardiac copper concentration did not reach statistical significance, the overall findings indicate biologically relevant adverse effects on cardiac tissue following chronic copper exposure (Ali et al., 2023).

In a 28-day oral gavage study, male Sprague-Dawley rats (8 animals per group) were administered copper sulphate pentahydrate at 0 and 161.5 mg/kg bw/day (Draper et al., 2023).

- Histopathological examination of pulmonary tissue revealed clear treatment-related adverse effects, including thickening of inter- and intra-alveolar spaces, epithelial stratification, smooth muscle disruption, early fibrotic changes, and bronchiolar epithelial desquamation. Transmission electron microscopy further demonstrated marked ultrastructural damage, characterized by nucleolar indistinctness, nuclear membrane detachment, chromatin condensation, and increased collagen and elastin fibre deposition. Collectively, these findings provide evidence of pulmonary toxicity following repeated oral exposure to copper sulphate pentahydrate (Draper et al., 2023).

In a 3- to 10-week oral study, mice (strain, sex and number of animals per dose group not specified) exposed to 50, 100, 200 or 300 ppm copper sulphate in drinking water exhibited clear dose- and duration-dependent effects on immune function (Pocino et al., 1991).

- Exposure reduced the proliferative response to concanavalin A at 100 ppm after 8 weeks and at 200 ppm after 3 or 8 weeks. While low-dose short-term exposure (50 to 100 ppm for 3 weeks) initially increased the proliferative response to lipopolysaccharide (LPS), prolonged exposure resulted in inhibition. Delayed-type hypersensitivity responses were similarly affected, with no effects observed at 100 ppm after 3 weeks, but significant suppression following longer exposure and at 300 ppm after 5 to 10 weeks. These findings indicate that sustained copper sulphate exposure can adversely affect multiple immune endpoints in mice (Pocino et al., 1991).

Additional oral repeated dose exposure studies on inorganic copper compounds include studies with copper chloride and copper carbonate, or studies in which the used copper compound has not been specified.

In a combined repeated-dose and reproductive/developmental toxicity study, Sprague-Dawley rats (12 animals/ sex/ dose) were exposed by gavage to copper monochloride at doses of 0, 1.3, 5, 20, or 80 mg/kg bw/day, corresponding to 0, 0.83, 3.2, 12.8, and 51.3 mg Cu/kg bw/day, respectively (Chung et al., 2009).

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- The study demonstrated clear treatment-related toxicity with increasing dose levels. Mortality occurred in females at 80 mg/kg bw/day (3 of 12 died), while no deaths were observed in males. Clinical signs increased in a dose-dependent manner. At 80 mg/kg bw/day, males showed pronounced haematological alterations indicative of anaemia and inflammatory responses, accompanied by changes in serum biochemistry and reduced organ weights. Histopathological evaluation revealed a marked, dose-related increase in squamous cell hyperplasia of the stomach in both sexes, reaching statistical significance from ≥20 mg/kg bw/day in males and ≥5 mg/kg bw/day in females. Additional treatment-related findings included increased bone marrow and splenic haematopoiesis at the highest dose. Based on these adverse effects, the systemic NOAEL was established at 5 mg/kg bw/day for males and 1.3 mg/kg bw/day for females (Chung et al., 2009). Reproductive and developmental toxicity findings are described in Section 7.8.

In a 60-day oral dietary study, Fischer rats (sex and the number of animals per group not specified) were exposed to 150-600 ppm copper chloride from weaning (Sugawara et al., 1995).

- Increasing dietary copper levels were associated with elevated serum AST and ALT activities, increased biliary copper excretion, and higher copper accumulation in the liver and kidneys. At the highest dose (600 ppm), approximately 25% of cytosolic liver copper was bound to metallothionein, indicating an adaptive response to increased copper burden. These findings demonstrate a consistent relationship between dietary copper exposure and systemic copper handling and tissue accumulation (Sugawara et al., 1995).

In a 12-week oral gavage study, male rats (30 animals per group; strain not specified) were exposed to tribasic copper chloride daily at doses equivalent to 0, 20, 40, 80, or 160 mg Cu/kg bw/day (Yu et al., 2023).

- Treatment resulted in clear, dose-related evidence of copper accumulation, neurotoxicity, and impaired cognitive performance. Serum copper concentrations increased significantly at ≥80 mg Cu/kg bw, while brain copper levels showed a dose-dependent rise, reaching more than tenfold the control values at 160 mg Cu/kg bw. Cognitive deficits observed in the Morris water maze included reduced platform crossings, prolonged escape latency, impaired target quadrant performance, and the emergence of an inefficient "edge-search" strategy at higher doses. These functional impairments were supported by marked neuropathological changes in the cortex and hippocampus, including neuronal degeneration, oedema, vascular alterations, disorganized hippocampal architecture, and a fivefold reduction in Nissl bodies at 160 mg Cu/kg bw. Ultrastructural examination further revealed mitochondrial damage and altered mitophagy, demonstrating a consistent dose-related pattern of copper-induced neurotoxicity (Yu et al., 2023).

In a 24-week oral dietary study in male Sprague-Dawley rats (24 animals per group) administered tribasic copper chloride at 15 (control), 30, 60, or 120 mg Cu/kg bw/day, clear dose-related evidence of hepatic copper accumulation and liver toxicity was observed (Yu et al., 2021a).

- Liver copper concentrations increased with dose and were significantly elevated at 120 mg/kg bw/day. High-dose animals showed impaired antioxidant defence, increased oxidative stress, and significant histopathological alterations, including hepatocyte degeneration, disruption of hepatic architecture, and ultrastructural features consistent with apoptosis. Molecular analyses further demonstrated dysregulation of mitophagy and dose-dependent activation of pro-apoptotic pathways. Collectively, these findings indicate that prolonged exposure to elevated dietary copper can compromise liver integrity and function, with the most pronounced adverse effects occurring at 120 mg/kg bw/day in male rats (Yu et al., 2021a).

In a 20-week rat study (Wistar and spontaneously hypersensitive rats, sex not specified, 10 animals per dose), dietary exposure to 100 mg Cu/kg feed (as cupric carbonate) resulted in a clear increase in systolic blood pressure compared with 18 mg Cu/kg feed, with the effect being particularly pronounced in Wistar rats (Liu and Medeiros, 1986).

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- Elevated haemoglobin levels were also observed at the higher copper concentration, while body weight, urine output, feed intake, and water consumption remained unaffected. These findings indicate that increased dietary copper intake can influence cardiovascular parameters without affecting general growth or consumption patterns (Liu and Medeiros, 1986).

In a 16-week (young rats) or 18-week (adult rats) non-GLP dietary study in male and female Fischer rats (the number of animals per dose not specified), administration of 1 500 ppm copper (copper compound not specified) in feed resulted in marked hepatic copper accumulation and clear evidence of liver toxicity. Control animals received diet containing <10 ppm copper (Fuentealba et al., 2000).

- Treated animals exhibited elevated serum liver enzyme activities ((ALT, GGT, and sorbitol dehydrogenase (SDH)), morphological liver damage, and histopathological lesions, including multifocal hepatitis and widespread single-cell necrosis. Notably, rats exposed from birth accumulated higher hepatic copper concentrations and developed more severe liver effects than adult animals, indicating increased susceptibility in younger rats (Fuentealba et al., 2000).

Haywood and Loughran (1985) demonstrated a clear adaptive response to dietary copper exposure in male rats (strain and number of animals per dose not specified) exposed 3 000, 4 000, 5 000 or 6 000 mg copper (copper compound not specified) per kg for 15 weeks, or 3 000 mg copper per kg for 52 weeks. In addition, one group of male rats which earlier received 3 000 mg copper per kg were exposed to 6 000 mg copper per kg.

- Initial copper supplementation led to hepatic copper accumulation and liver damage. However, animals receiving up to 5 000 mg copper per kg diet showed a subsequent decline in liver copper concentrations, liver regeneration, and resumed growth, indicating the development of tolerance. A similar adaptation was observed following 52 weeks of exposure to 3 000 mg copper per kg diet. In contrast, rats exposed to 6 000 mg copper per kg diet failed to recover and developed hepatic copper overload. Notably, rats previously exposed to elevated copper levels were less susceptible to adverse effects than non-pre-exposed animals, suggesting that prior exposure enhanced tolerance to copper-induced hepatotoxicity.

Table 20: Summary of studies on repeated dose toxicity (oral route) with inorganic copper compounds

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
Copper sulphate or copper sulphate pentahydrate; copper(II)sulphate				
13-week study Oral (feed)	Rat, F344/N Male and female 10 animals per sex per dose	0, 500, 100, 2 000, 4 000 or 8 000 ppm via the feed 92 days, 7 days per week Males: 0, 32, 64, 129, 259 and 551 mg/kg/day Females: 0, 34, 68, 135, 267 and 528 mg/kg/day	No mortality • Only one female at 1 000 ppm accidentally killed Terminal body weight and body weight gains ↓ • ≥ 500 ppm in males • At 8 000 ppm in females Feed consumption ↓ • At 8 000 ppm in males and females (only slightly) No treatment-related clinical signs Organ weights	(Hébert et al., 1993, NTP, 1993)

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• 8 000 ppm: absolute brain, heart, kidney, liver, lung and thymus weights ↓ in males and absolute kidney weight ↓ in females • Generally, relative organ weights were similar or increased at 4 000 and 8 000 ppm Haematology Haematocrit and haemoglobin • Day 5: Hematocrit and hemoglobin ↑ in males and females at 8 000 ppm • Day 21: Hematocrit and hemoglobin ↓ in males (4 000 and 8 000 ppm) and in females (2 000, 4 000 and 8 000 ppm) • Day 92: Hematocrit and hemoglobin ↓ in males (4 000 and 8 000 ppm) and in females (8 000 ppm) Erythrocytes • Day 5: Erythrocytes ↑ in males (4 000 and 8 000 ppm) and in females (8 000 ppm) • Day 92: Erythrocytes ↑ only in males (8 000 ppm) • Day 5: Reticulocytes ↓ in males and females (4 000 and 8 000 ppm Reticulocytes • Day 21: Reticulocytes ↑ in males and females (4 000 and 8 000 ppm) • Day 92: Reticulocytes ↑ only in males (8 000 ppm) Mean cell volume • Day 5: mean cell volume ↓ in males (4 000 and 8 000 ppm) and in females (8 000 ppm) and mean cell hemoglobin ↓ in males (4 000 and 8 000 ppm) • Days 21 and 92: Mean cell volume and mean cell hemoglobin ↓ in males and females	

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			(2 000, 4 000 and 8 000 ppm) Platelets • Days 5 and 21: Platelets ↑ in males and females (2 000, 4 000 and 8 000 ppm). Also, at 1 000 ppm in males. • Day 92: Platelets ↑ in males and females (4 000 and 8 000 ppm). Statistically significant only in males Leukocytes • Days 5, 21 and 92: Leukocytes ↑ in males and females (4 000 and 8 000 ppm) Lymphocytes • Day 5: Lymphocytes ↑ in males (8 000 ppm) • Day 21: Lymphocytes ↑ in males (2 000 and 4 000 ppm) • Day 92: Lymphocytes ↑ in females (8 000 ppm) Serum chemistry Alanine aminotransferase (ALT) • Days 5, 21 and 92: ALT ↑ at 4 000 and 8 000 ppm in males and females, increase observed also at 1 000 and 2 000 ppm in males on day 92 Alkaline phosphatase (ALP) • Days 5 and 21: ALP ↓ at 4 000 and 8 000 ppm in males and females (except in males at 4 000 ppm on day 21) Sorbitol dehydrogenase (SDH) • Days 21 and 92: SDH ↑ in males at 4 000 and 8 000 ppm and in females at 8 000 ppm on Days 21 and 92 5'-nucleotidase • Day 5 and 21: 5'-nucleotidase ↓ at 8 000 in males on Day 5 and in females on Days 5 and 21. 5'-nucleotidase ↑ in males at 4 000 and 8 000 ppm on Day 92. Bile salts	

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• Day 5: Bile salts ↑in males at 2 000, 4 000 and 8 000 ppm and bile salts ↓ in females at all dose levels 5. • Day 21: Bile salts ↑ in males at 4 000 and 8 000 ppm. No significant effects on bile salts in females. • Day 92: Bile salts ↑in males at 8 000 ppm and in females at 2 000 and 4 000 ppm. Total protein • Days 5, 21 and 92: Total protein ↓in males at 8 000 ppm and females at 4 000 and 8 000 ppm. Total protein decreased also in males at 4 000 ppm and in females at 2 000 ppm on Days 5 and 21. Albumin • Days 5 and 21: Albumin ↓ in males and females at 2 000, 4 000 and 8 000 ppm • Day 92: Albumin ↓ in males at 8 000 ppm and in females at 4 000 and 8 000 ppm Urea nitrogen • Days 5 and 21: Urea nitrogen ↑ in males at 2 000, 4 000 and 8 000 ppm and in females at 8 000 ppm • Day 92: Urea nitrogen ↑ in males at 8 000 ppm and in females at 1 000, 2 000, 4 000 and 8 000 ppm Creatinine • Day 92: Creatinine ↑in females at 8 000 ppm. No other effects on creatinine observed in males or females Urinalysis Urinary aspartate aminotransferase (AST) • Days 19 and 90: Urinary AST ↑ in males and females at 8 000 ppm N-acetyl-β-D-glucosaminidase	

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• Day 90: N-acetyl-β-D-glucosaminidase ↑ in males and females at 8 000 ppm Glucose output • Day 19: Glucose output ↑ in males at 2 000 ppm • Day 90: Glucose output ↑ in males at 4 000 and 8 000 ppm • No effects in females Protein output • Day 19: Protein output ↓ in males at 8 000 ppm • Day 90: Protein output ↑ in males at 4 000 and 8 000 ppm No effects in females Copper, zinc, magnesium and calcium in the liver, kidney, testis and plasma in male rats • Copper ↑ in kidney and liver in all treated groups. It was accompanied by an accumulation of zinc to these tissues (2 000, 4 000 and 8 000 ppm). • Copper ↑ in plasma and testis (2 000, 4 000 and 8 000 ppm) • Calcium ↓ in plasma (4 000 and 8 000 ppm) • Magnesium ↑ in kidney and plasma (2 000 ppm, in plasma also at 8 000 ppm) Gross pathology • ≥ 2 000 ppm: gross lesions in the forestomach in males and females • The limiting ridge that forms the junction of the forestomach squamous mucosa with the glandular gastric mucosa appeared enlarged in all rats at 4 000 and 8 000 ppm, and in 9/10 males and in 7/10 females at 2 000 ppm Histopathology • ≥ 2 000 ppm (forestomach): minimal to moderate hyperplasia	

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			of the squamous mucosa at the site of the limiting ridge in the forestomach in males and females. Also, hyperkeratosis was a component of hyperplasia. Moderate hyperplasia was often associated with increased number of inflammatory cells and/ or oedema in the lamina propria of the limiting ridge. • ≥ 4 000 ppm (kidney): Cytoplasmic alteration in males and females characterised with increased size and number of cytoplasmic protein droplets. Less severe in females. Degeneration of the renal tubule epithelium was observed in 3/10 females at 8 000 ppm and minimal nuclear enlargement (karyomegaly) in renal tubule cells in males and females at 8 000 ppm. • ≥ 4 000 ppm (liver): Chronic-active inflammation in males and females. Also, in one male at 2 000 ppm Ultrastructural changes in the liver and kidney • Degenerative changes in the cytoplasm of hepatocytes in the periportal area consisting of increased number of secondary lysosomes • The number and size of electron-dense protein droplets in the cytoplasm of the proximal convoluted tubule epithelium ↑ in males and females. In males, many of the droplets had irregular crystalline shapes. Presence of iron (hemosiderin) in the spleen • 8 000 ppm: only few iron-positive granules in	

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			the cytoplasm of macrophages in the red pulp in males and females • 2 000 and 4 000 ppm: The reduction in iron-positive material in spleens was much less prominent compared to high dose group. The minimal decrease was evident when compared to the normal amount of iron-positive granules in the spleen of control rats. Sperm morphology and vaginal cytology • No significant findings observed	
13-week study Oral (feed)	Mouse, B6C3F1 Male and female 10 animals per sex per dose	0, 1 000, 2 000, 4 000, 8 000 or 16 000 ppm via the feed (*ad libitum*) 92 days, 7 days per week Males: 0, 173, 382, 736, 1 563 and 3 201 mg/kg/day Females: 0, 205, 494, 1 048, 2 106 and 5 157 mg/kg/day	No mortality Terminal body weight gain↓ • ≥ 8 000 ppm in males and females Feed consumption • Comparable to or exceeded control values in all treated groups in males and females No treatment-related clinical signs Organ weights • 16 000 ppm: absolute heart and kidney weights ↓ in males and absolute thymus and kidney weights ↓ in females • ≥ 8 000 ppm: absolute liver weight ↓ in males (also at 4 000 ppm) and females • Generally relative organ weights ↑ in males and females in all dose groups Gross lesions • 16 000 ppm: Forestomach lesions in males (7/10) and females (4/10) characterised as a focal white discoloration of the squamous mucosa in the area of the limiting ridge where it forms a junction	(Hébert et al., 1993, NTP, 1993)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			with the glandular gastric mucosa Histopathology • ≥ 4 000 ppm: minimal to mild squamous cell hyperplasia with hyperkeratosis of the forestomach mucosa at the site of the limiting ridge • No evidence of inflammation or erosion/ ulceration in the forestomach • No increased hyperplasia or hyperkeratosis in other portions of the forestomach mucosa Copper in the liver and kidney • Liver: extremely minimal positive staining only at 16 000 ppm in males and females • Kidney: no staining Sperm morphology and vaginal cytology • No significant findings observed	
2-week study Oral (feed)	Rat, F344/N Male and female 5 animals per sex per dose	0, 1 000, 2 000, 4 000, 8 000 or 16 000 ppm via feed (*ad libitum*), 15 days Males: 0, 92, 180, 363, 777 and 1 275 mg/kg/day Females: 0, 89, 174, 367, 769 and 1 121 mg/kg/day	No mortality Body weight and weight gain ↓ • ≥ 8 000 ppm Feed consumption↓ • 16 000 ppm Clinical signs • ≥ 8 000 ppm: thinness Organ weights↓ • ≥ 8 000 ppm: absolute organ weights in general lower than in controls (not specified), but included at least liver in males and females • 4 000 ppm: decreased absolute liver weight in females Histopathology • ≥ 2 000 ppm: hyperplasia with hyperkeratosis of the squamous mucosa in all males and females • ≥ 4 000 ppm: cytoplasmic alteration in the cytoplasm and lumen of the renal cortical	(Hébert et al., 1993, NTP, 1993)

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			tubules in the kidneys of male and female rats. Characterised by an increased number and size of eosinophilic-staining protein droplets. • ≥ 8 000 ppm: inflammation of the liver (minimal to mild mononuclear inflammatory cell infiltrate) in males (in females only at the highest dose level) and depletion of hematopoietic cells in bone marrow in males and females • 16 000 ppm: bone mass (cortex and trabecular density) ↓ in many animals and erythroid hematopoiesis (minimal to mild) in the spleens of males and females Copper concentration in the liver and kidneys↑ • 16 000 ppm: 130 times higher in the liver and 25 times higher in the kidneys of males compared to controls • only males examined	
2-week study Oral (feed)	Mouse, B6C3F1 Male and female 5 animals per sex dose	0, 1 000, 2 000, 4 000, 8 000 or 16 000 ppm via feed (*ad libitum*) 15 days Males: 168, 362, 773, 1 154 and 2 817 mg/kg/day Females: 0, 210, 408, 849, 1 563 and 3 068 mg/kg/day	No mortality Body weight ↓ • ≥ 8 000 ppm in males and females (also at 2 000 ppm) • Only slight decrease as remained within 6% of control values Body weight gain ↓ • ≥ 8 000 ppm in males and females Feed consumption ↓ • 16 000 ppm Clinical signs • 16 000 ppm: thinness 1/5 females • 8 000 ppm: thinness 1/5 males and 2/5 females Organ weights • ≥ 4 000 ppm: relative liver weight ↑ in males • ≥ 8 000 ppm: relative brain weight ↑in males	(Hébert et al., 1993, NTP, 1993)

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• 16 000 ppm: relative brain and liver weights ↑ in females No treatment-related gross lesions Histopathology ≥ 4 000 ppm: minimal squamous mucosal hyperplasia in the forestomach with hyperkeratosis at the limiting ridge	
2-week study Oral (drinking water)	Rat, F344/N Male and female 5 animals per sex per dose	0, 300, 1 000, 3 000, 10 000 or 30 000 ppm via drinking water (*ad libitum*) 15 days Males: 0, 41, 113, 175, 140 and 379 mg/kg/day Females: 0, 39, 102, 121, 120 and 279 mg/kg/day	Mortality • 3 000 ppm: 1/5 female died • ≥ 10 000 ppm: all males and females died Body weight ↓ • ≥ 3 000 ppm • 3 000 ppm: 48 % (males) and 46% (females) lower than in controls • no terminal body weights at higher dose levels due to mortality Clinical signs • ≥ 3 000 ppm: ruffled fur, emaciation, abnormal posture, hypoactivity, nasal discharge Water consumption ↓ • ≥ 3 000 ppm • 3 000 ppm: <30% of control • ≥ 10 000 ppm: <6% of control Organ weights (3 000 ppm, the highest surviving dose group) • absolute organ weights↓ • relative organ weights↑ • affected organs not specified Gross lesions • no treatment-related effects Histopathology • 300 and 1 000 ppm: protein droplets within proximal convoluted tubule epithelial cells ↑ only in male rats	(Hébert et al., 1993, NTP, 1993)
2-week study Oral (drinking water)	Mouse, B6C3F1 Male and female	0, 300, 1 000, 3 000, 10 000 or 30 000 ppm via drinking water (*ad libitum*)	Mortality • 3 000 ppm: 1/5 male and 3/5 females died • ≥ 10 000 ppm: all males and females died Body weight ↓	(Hébert et al., 1993, NTP, 1993)

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
	5 animals per sex per dose	15 days Males: 0, 41, 95, 226, 524 and 1 442 mg/kg/day Females: 0, 58, 140, 245, 683 and 1 296 mg/kg/day	• ≥ 3 000 ppm • 3 000 ppm: 22% (males) and 34% (females) lower than in controls • no terminal body weights at higher dose levels due to mortality Clinical signs • ≥ 3 000 ppm: ruffled fur, emaciation, abnormal posture, hypoactivity, dyspnea, tremors and prostration Water consumption↓ • ≥ 3 000 ppm: <33% of control Organ weights • Males (3 000 ppm): absolute weight of thymus↓ • Females (3 000 ppm): absolute weight of thymus, brain, kidney, liver and lung↓ • Females (1 000 ppm): absolute weights of brain and lung↓ Gross lesions • no treatment-related effects Histopathology • ≥ 3 000 ppm: atrophy and cellular depletion in various tissues (not specified) • considered secondary to dehydration and reduced terminal body weights	
11-month study Oral (drinking water) Non-TG	Rat, Sprague-Dawley Female 8 animals per dose group	0 and 0.125% Copper sulphate in drinking water (*ad libitum*) from weaning for 11 months 0.125% copper sulphate in drinking water corresponds to copper intake of about 12 mg/day based on water intake of approximately 25 ml/day	Concentration of copper in liver and parietal cortex ↑ No effects on body or liver weights Catecholamine and metabolite concentrations in the corpus striatum • No effects on dopamine • 3,4-dihydroxyphenylacetic acid ↓ (25% decrease) in the copper-treated group Dopamine receptors • The affinity of D-2 receptor ↑ whereas a trend for the number of receptors to decrease in the copper-treated group Copper inhibited ligand binding	(de Vries et al., 1986)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			in a radioligand binding assay	
90-day study Oral (gavage) Non-TG	Rat, Wistar Male 18 animals per dose 6 males per dose group were sacrificed at the end of 30, 60 and 90 days	0, 100 and 200 mg/kg bw/day for 30, 60 or 90 days Copper sulphate pentahydrate	No mortality Body weight • Significantly lower in both copper-exposed groups and at all time points, except low dose group at 30 days Blood analysis • Haemoglobin ↓, and serum bilirubin and transaminases ↑ in both copper-exposed groups Behaviour • The maximum peak force in grip strength, latency to fall in rotarod and percentage attention score in Y-maze ↓at all examined time points • Most prominent effect at 200 mg/kg bw/d Copper concentrations in liver, kidney and brain • significantly higher in the copper-treated animals compared to control animals at both dose levels and all examined time points ($p<0.01$) • Copper concentration highest in the liver (29-fold), followed by kidney (3-fold) and brain (1.5-fold)	(Kumar et al., 2015)
90-day study Oral (gavage) Non-TG	Rat, Wistar Male 18 animals per dose 6 males per dose group were sacrificed at the end of 30, 60 and 90 days	0, 100 and 200 mg/kg bw/day for 30, 60 or 90 days Copper sulphate	No mortality Copper concentrations • Higher in copper-exposed rats compared to controls • Highest in the liver, followed by kidney and brain Oxidative stress • Higher in copper-exposed rats compared to controls • Highest in the liver, followed by brain and kidney Histopathology in copper-treated animals • Liver: massive fatty change and centrilobular necrosis, and also hepatitis characterised by mononuclear cells	(Kumar et al., 2016b)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			infiltration mostly macrophages and lymphocytes around central veins and in portal areas • Brain: cell swelling, neuronal vacuolation, demyelination, neuronophagia, and satellitosis • Kidney: mild congestion in the glomeruli, focal congestion, and vacuolar degeneration of tubular cells. Also, inflammation with fibrosis at 90 days. • More severe histopathological changes in the in the liver compared to brain and kidney at all time points. • Histopathological changes correlated with the copper concentration in the liver, brain and kidneys. No histopathological changes in the control animals.	
90-day study Oral (gavage) Non-TG	Rat, Wistar Male 18 animals per dose 6 males per dose group were sacrificed at the end of 30, 60 and 90 days	0, 100 and 200 mg/kg bw/day for 30, 60 or 90 days Copper sulphate	Copper concentrations in the liver, kidney and brain • Higher in copper-exposed rats compared to controls • Highest in the liver, followed by kidney and brain Oxidative stress in copper exposed animals • MDA ↑ • GSH and total antioxidant capacity ↓ • Effects most prominent in liver, followed by brain and kidney at all time points • Observed effects correlated with tissue free copper concentration	(Kumar et al., 2016a)
16-week study Oral (gavage) Non-TG	Rat, Sprague-Dawley Male Number of animals per dose group not specified	0, 10 and 20 mg/kg bw, once per day for 16 weeks Copper sulphate pentahydrate	No mortality Body weight • Lower in copper-exposed group (not statistically significant) Copper concentration in brain ↑ • In both copper exposed groups	(Kumar et al., 2019)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• Most prominent at 20 mg/kg bw Serum ceruloplasmin activity ↓ • In both copper exposed groups • Statistically significant at 20 mg/kg bw Neurobehavioural parameters in copper exposed rats • Passive avoidance ↓ • Immobility ↑ • Locomotor activity ↓ • Novel object exploration time ↓ Oxidative stress in brain ↑ in copper exposed rats • Lipid peroxidation and nitrite level ↑ • 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels ↑ • Reduced GSH ↓ • Activity of antioxidant enzymes (SOD and CAT) ↓	
90-day study Oral (gavage) Non-TG	Rat, Wistar albino Male, 10 animals per dose	0 and 200 mg/kg bw/day for 90 days Copper sulphate pentahydrate	Copper overload • Liver (41-fold higher) • Intestine (36-fold higher) • Kidneys (3-fold higher) • Testes (2-fold higher) Hypertension • Significant elevation in systolic blood pressure and serum angiotensin II levels • Reduction in serum nitric oxide level Testicular dysfunction • Relative weights of testes ↓ • Testosterone and LH ↓ • Testicular Zn ↓ • Oxidative stress ↑ • Down-regulation of steroidogenic genes in testis	(Arafa et al., 2019)
21- and 42-day study Oral (gavage) Non-TG	Mouse, ICR Male and female 60 animals per sex per dose	0, 4, 8 or 16 mg/kg Cu^{2+} as copper sulphate, once per day for 21 or 42 days Animals killed and hepatic tissues collected on Days 21 and 42	Histopathology • 8 mg/kg group at 21 days: hepatocyte vacuolar degeneration • 16 mg/kg group at 21 days: hepatocyte granular and vacuolar degeneration, and necrotic hepatocytes • > 4 mg/kg group at 42 days: hepatocyte granular and vacuolar degeneration	(Liu et al., 2020a)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• > 8 mg/kg group at 42 days: necrotic hepatocytes and inflammatory cells Copper exposure increased serum activities of ALP, AST, ALT and GGT, and increased also albumin and total bilirubin Copper exposure activated the mitogen-activated protein kinases and nuclear factor-kappa B signalling pathways High copper uptake up-regulated expression of some pro-inflammatory mediators such as interleukin-2, interleukin-1β and interleukin-8, and down-regulated expression of transforming growth factor beta (an anti-inflammatory mediator).	
21- and 42-day study Oral (gavage) Non-TG	Mouse, ICR Male and female 60 animals per sex per dose	0, 4, 8 or 16 mg/kg Cu^{2+} as copper sulphate, once per day for 21 or 42 days Animals killed and liver samples collected on days 21 and 42.	Body weight gain ↓ • ≥ 4 mg/kg (not statistically significant) Copper concentration in the liver on Day 42↑ • 16 mg/kg ($p < 0.01$) Histopathological lesions in the liver ↑ • The number hepatic cells with granular and vacuolar degeneration increased dose- and time-dependent manner • ≥ 8 mg/kg: karyorrhexis, karyolysis and hypochromatosis in the necrotic hepatocytes Oxidative stress in the liver	(Liu et al., 2020b)
21- and 42-day study Oral (gavage) Non-TG	Mouse, ICR Male and female 60 animals per sex per dose	0, 4, 8 or 16 mg/kg Cu^{2+} as copper sulphate, once per day for 21 or 42 days Animals killed and liver samples collected on days 21 and 42.	Histopathological lesions in the liver on Day 21 • ≥ 4 mg/kg: hepatocyte granular and vacuolar degeneration • ≥ 8 mg/kg: disorder of hepatic cord arrangement and necrotic hepatocytes • Dose-dependent increase in severity Histopathological lesions in the liver on Day 42 • ≥ 4 mg/kg: hepatocyte granular and vacuolar degeneration, disorder of hepatic cord arrangement, necrotic hepatocytes	(Liu et al., 2021a, Liu et al., 2021b)

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• Dose-dependent increase in severity	
16-week study Oral (gavage) Non-TG	Rat, Sprague-Dawley Male 18 animals per dose	0 and 20 mg copper sulphate/kg, once per day for 16 weeks	No effect on body weight and liver weight In copper treated rats • Copper concentration in the liver ↑ and copper carrying protein (ceruloplasmin) ↑ • No effects on AST and cholesterol levels • ALT and LDL levels ↑ • ROS and nitrosative stress (nitrite levels) ↑ in the liver • No effects on GSH • Antioxidant enzyme activity (superoxide dismutase and catalase) ↓ • Pro-inflammatory markers (IL-1β and IL-6) ↑and anti-inflammatory marker (IL-4) ↓ • No effect on TNF-α expression • Apoptosis (Cleaved Caspase-3 level) ↑ • Serum immunoglobulins IgE, IgM, IgA and IgG ↑ • Histopathological lesions in the liver: A marked necrosis of hepatocytes, distorted sinusoidal space, central vain distortion and collagen deposition	(Patwa and Flora, 2020)
16-week study Oral (gavage) Non-TG	Rat, Sprague-Dawley Male 18 animals in the control group 24 animals in copper-exposed group	0 and 20 mg coppers sulphate/kg once per day for 16 weeks	Copper exposure • neurobehavioral abnormalities including spontaneous locomotor activity and time on rotating rod ↓, and resting time, immobility time and anxiety ↑ • Oxidative stress (ROS, lipid peroxidation and nitrite level in the brain) ↑ • Antioxidant enzymes (SOD and CAT) ↓ • Inflammatory markers (tumor necrosis factor-α and interleukin-1β) ↑	(Patwa et al., 2022)
4-month study Oral	Rat, Sprague-Dawley	0 and 250 ppm, corresponds to 100 ppm of Cu for 4 months	Oxidative stress ↑ Histopathology of heart	(Ali et al., 2023)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
(drinking water) Non-TG	Sex not specified 4 - 6 rats per dose	Copper sulphate pentahydrate	• Myocyte damage, necrosis, infiltration of mast cells increased deposition of collagen Copper concentration in heart ↑ (not statistically significant)	
28-day study Oral (gavage) Non-TG	Rat, Sprague-Dawley Male 8 animal per dose	0 and 161.5 mg/kg bw/day, once per day for 28 days Copper sulphate pentahydrate	Histopathology of pulmonary tissue • Thickened inter- and intra-alveolar spaces, stratified epithelium, disrupted smooth muscle, early fibrosis and desquamation of the epithelia of the bronchioles Ultrastructural changes of pulmonary tissue (transmission electron microscopy) • Indistinct nucleolus, nuclear membrane detachment, chromatin condensation, increase in collagen and elastin fibres	(Draper et al., 2023)
3 to 10-week study Oral (drinking water) Non-TG	Mouse Strain and sex not specified Number of animals per dose group not specified	50, 100, 200 or 300 ppm in the drinking water for 3 to 8 weeks Copper sulphate	Inhibition of proliferative response Concanavalin A • At 100 ppm for 8 weeks • At 200 ppm for 3 or 8 weeks Proliferative response to LPS • Increased at 50 or 100 ppm for 3 weeks • Inhibited following prolonged exposure Production of autoantibodies against bromelain-treated mouse erythrocytes Delayed-type hypersensitivity response • No effects at 100 ppm for 3 weeks • Depressed at 100 ppm after a prolonged exposure • Inhibition at 300 ppm exposure for 5 or 10 weeks	(Pocino et al., 1991)
Copper monochloride				
Combined repeated dose and reproductive /developmental toxicity study	Rat, Sprague-Dawley Male and female	0, 1.3, 5, 20 and 80 mg/kg bw/day Males (exposure): 30 days beginning 14 days before	Mortality • 3 females died at 80 mg/kg bw/day • No mortality among males Clinical signs	(Chung et al., 2009)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
Oral (gavage) TG not specified	12 animals per sex per dose	mating Females (exposure): 14 days before mating, throughout the mating and gestation period to day 3 of lactation Copper monochloride	• Dose-dependent increase in the incidence of clinical signs Haematology (statistically significant effects) • In males at 80 mg/kg bw/d: RBC, HGB, HCT, MCV and MCH ↓ and WBC, PLT and neutrophils ↑ • In females at 80 mg/kg bw/d: MCH ↓ and PLT ↑ Serum biochemistry (statistically significant effects) • In males at 80 mg/kg bw/d: total protein and total bilirubin ↓, and A/G ↑ • In males at 1.3 mg/kg bw/d: total bilirubin ↑ • In males at 1.3 and 5 mg/kg bw/d: potassium ↓ • No statistically significant effects in females Organ weights (statistically significant effects) • In males: absolute weight of the salivary gland (20 and 80 mg/kg bw/d) ↓, seminal vesicles (20 mg/kg bw/d) ↓ and thyroid gland (1.3 and 20 mg/kg bw/d) ↓ • No statistically significant effects in females Histopathology (males) • The incidence of squamous cell hyperplasia of the stomach ↑: 1/12, 2/12, 8/12 and 11/12 at 1.3, 5, 20 and 80 mg/kg bw/d, respectively • Haematopoiesis of the femur ↑ (8/12) and extramedullary haematopoiesis of the spleen (3/12) at 80 mg/kg bw/d • Lymphoid cell infiltration of the kidney in 2/12 in the control group and 1/12 at 80 mg/kg bw/d • Tubular basophilia of the kidney in 4/12 in the control group and 4/12 at 80 mg/kg bw/d	

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• The incidence of squamous cell hyperplasia of the stomach in the ≥ 20 mg/kg groups and bone marrow hyperplasia of the femur in the 80 mg/kg group were significantly higher than those in the vehicle control group. Histopathology (females) • The incidence of squamous cell hyperplasia of the stomach ↑: 2/12, 5/12, 6/12 and 9/12 at 1.3, 5, 20 and 80 mg/kg bw/d, respectively • Extramedullary haematopoiesis of the spleen (1/12) at 5 mg/kg bw/d • Lymphoid cell infiltration of the kidney in 1/12 in control group and in 4/12 at 80 mg/kg bw/d • Tubular basophilia of the kidney in 3/12 in control group and 6/12 at 80 mg/kg bw/d • Congestion of the kidney (3/12) at 80 mg/kg bw/d • The incidence of squamous cell hyperplasia of the stomach in the ≥ 5 mg/kg groups was significantly higher than those in the vehicle control group NOAEL (systemic toxicity) • 5 mg/kg bw/d for males • 1.3 mg/kg bw/d for females Reproductive/developmental toxicity findings described in Section 7.8.	
60-day study Oral (feed) Non-TG	Rat, Fischer Sex not specified Number of animals per group not specified	150 – 600 ppm in the diet for 60 days from weaning Copper chloride; Not specified whether copper(I) or	Serum activities of AST and ALT ↑ with the increase of copper concentration in the diet Biliary excretion of copper was related to copper level in the diet Liver and kidney copper concentrations ↑ • Dose-dependent	(Sugawara et al., 1995)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
		copper(II) chloride	• 25% of cytosolic copper was bound to metallothionein in the liver at 600 ppm	
Tribasic copper chloride				
12-week study Oral (gavage) Non-TG	Rat Strain not specified Male 30 animals per dose	0, 34.5, 69, 138 and 276 mg tribasic copper chloride /kg bw Corresponds to 0, 20, 40, 80 and 160 mg Cu/kg bw Once per day for 12 weeks Examinations after 0, 6 and 12 weeks of exposure	Body weight • Decreasing trend at 80 and 160 mg Cu/kg bw • At 3 weeks, weight of rats ↓ at 160 mg/kg bw ($p<0.05$) Copper content in serum and brain at 12 weeks • Serum copper ↑ at 80 and 160 mg/kg bw ($p<0.05$) • Brain copper ↑ (dose-dependent) • Brain copper was at least 10-fold higher at 160 mg/kg bw than in the control group ($p<0.05$) Examination of cognitive function (Morris water maze) • Total distance travelled ↑ at 80 and 160 mg/kg bw ($p<0.05$) • The number of platform crossings ↓ at 160 mg/kg bw/d at 12 weeks ($p<0.001$) • Swimming speed not affected • Significantly longer escape latency at 160 mg/kg bw/d on Days 3 and 4 at 12 weeks ($p<0.05$) • Proportion of movements in the target quadrant ↓ at 80 mg/kg bw/d and exhibited a typical 'edge search' strategy at 160 mg/kg bw/d Histopathology of cortex and hippocampus at 12 weeks • 80 mg/kg bw/d: Cortical neurons showed pyknotic hyperstaining, hyperemia and dilatation of blood vessels. Many neurons with obvious pyknosis and deep staining in the hippocampus, accompanied by loose oedema and vascular dilatation in the interstitium.	(Yu et al., 2023)

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• 160 mg/kg bw/d: Cortical neurons exhibited a disordered arrangement, neuronal oedema, and dilated blood vessels. Dead neurons in the cerebral cortex, increased microglia and the glial cells exhibiting a typical 'satellite phenomenon'. Whole hippocampal structure was abnormal and the arrangement of neurons in the hippocampal area was disorganised with significant pyknosis and vacuoles. Nissi bodies in the hippocampus ↓ being 5 times less than in control animals ($p<0.001$) Ultrastructural changes in the hippocampus • Mitophagy in copper exposed rats • 80 and 160 mg/kg bw/d: mitochondrial vacuolation, extension, swelling, and the ridges decreased of disappeared • 40 and 80 mg/kg bw/d: the number of mitophagosomes ↑ ($p<0.05$) • 160 mg/kg bw/d: mitophagosomes depleted	
24-week study Oral (feed) Non-TG	Rat, Sprague-Dawley Male 24 animals per dose group	15 (control), 30, 60 and 120 mg Cu/kg for 24 weeks Tribasic copper chloride Liver tissue examined at 24 weeks	Copper concentration in liver and serum • Dose-dependent ↑ • Statistically significant increase in the liver at 120 mg/kg bw/d ($P<0.05$) Liver enzymes • 30 and 60 mg/kg bw/d: AST and ALT ↓ • 120 mg/kg bw/d: ALP ↑ ($p<0.05$) Antioxidant enzyme activity and lipid peroxidation • Activity of T-SOD, CAT and GSH-Px ↓ (dose-dependent), statistically significant at 120 mg/kg bw/d ($p<0.05$) Histopathology of liver tissue	(Yu et al., 2021a)

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• Increased dietary copper decreased the number and proportion of normal hepatocytes and reduced cell volume • 60 and 120 mg/kg bw/d: Swollen nuclei and vacuoles in the cytoplasm of hepatocytes. The number of cells with hyperchromatic nuclei and concentrated cytoplasm ↑. The hepatic cords were damaged, disordered or absent. Ultrastructural changes in the liver • 120 mg/kg bw/d: chromatic condensation and nuclear fragmentation of hepatocytes (characteristics of apoptosis) • The number of mitophagosomes increased dose-dependently and peaked at 60 mg/kg bw/d, but decreased at 120 mg/kg bw/d indicating inhibition of mitophagy process in the high dose group. Mitophagy in the liver • 60 mg/kg bw/d: the relative mRNA expression of mitophagy-related genes ↑ ($p<0.05$) • 120 mg/kg bw/d: the expression of mitophagy-related genes ↓ ($p<0.001$, compare to mid dose) Apoptotic markers in the liver • The relative mRNA levels of Bax, Bax/Bcl-2, Cleaved Caspase-3, Cytc and p53 ↑ (dose-dependent) except that at 120 mg/kg bw/d the gene expression of Bcl-2 was decreased ($p<0.05$).	
Copper carbonate/cupric carbonate				
20-week study Oral (feed)	Rat, Wistar and spontaneously hypersensiti	18 and 100 mg Cu as cupric carbonate/kg feed	Blood pressure • Rats fed with the higher copper content diet showed increased systolic blood pressure	(Liu and Medeiros, 1986)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
Non-TG	ve rats Sex not specified 10 rats dose		compared to rats received lower amount of copper. • Increase more pronounced in Wistar rats Hemoglobin ↑ at 100 mg Cu/kg feed No differences in body weights, urine output, feed and water consumption	
Copper compound not specified				
15- and 52-week study Oral (feed) Non-TG	Rat Strain not specified Male Number of animals per group not specified	3 000, 4 000, 5 000 and 6 000 mg Cu/kg in the diet for 15 weeks 3 000 mg Cu/kg in the diet for 52 weeks 6 000 mg Cu/kg in the diet for 3 weeks to animals earlier received 3 000 mg copper/kg in the diet or control diet (10-20 mg copper/kg) Copper compound not specified	Liver • First, accumulation of copper in the liver and liver damage in rats receiving copper supplemented diet • Then, liver copper concentration declined, regeneration occurred, growth resumed in animals received up to 5000 mg copper/kg in the diet indicating tolerance. • Similar adaptation was observed in rat receiving 3 000 mg copper/kg in the diet for 52 weeks • Rats receiving 6 000 mg copper/kg in the diet did not recover and showed hepatic overload of copper • Rats pre-exposed to copper were less susceptible compared to animals without pre-exposure	(Haywood and Loughran, 1985)
16- and 18-week study Oral (feed) Non-TG	Rat, Fischer Male and female Number of animals per sex per dose not specified	Control diet (< 10 ppm copper) and 1 500 ppm copper in the diet for 18 weeks (adult rats) or 16 weeks (young rats from birth) Copper compound not specified	Rats receiving copper-loaded diet • Copper concentration in the liver ↑ • Liver injury: morphological changes and increased activity of liver enzymes (ALT, GGT and SDH) in the serum • Histopathological changes in the liver: multifocal hepatitis and widespread single-cell necrosis Young rats accumulated more hepatic copper and had more severe liver effects compared to adult rats	(Fuentealba et al., 2000)

Notes: A/G: albumin/globulin; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate

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aminotransferase; CAT: catalase; GGT: gamma-glutamyl transferase; GSH: glutathione; GSH-Px: glutathione peroxidase; HCT: hematocrit; HGB: hemoglobin; LDL: low-density lipoprotein; MCH: Mean Corpuscular Hemoglobin; LH: Luteinizing hormone; LPS: Lipopolysaccharide; MCV: Mean Corpuscular Volume; MDA: malondialdehyde; PLT: platelet; RBC: Red blood cell; ROS: Reactive oxygen species; SDH: Sorbitol dehydrogenase; T-SOD: total superoxide dismutase; WBC: White blood cell.

7.3.2.2 Studies by inhalation route

A limited number of repeated dose inhalation studies are available on inorganic copper compounds.

A recent publication (Poland et al., 2022) describes a short-term 28-day inhalation study (OECD TG 412) study on dicopper oxide in rats. (DFG, 2014) and (SCOEL, 2014) refer to the same study with reference to ICA (2010). The study included also 14-days range finding studies on dicopper oxide and copper sulphate pentahydrate (Poland et al., 2022).

In range finding studies via inhalation (Poland et al., 2022), male and female rats (Crl:CD(SD)), 5 animals per sex for each dose, were exposed to 0, 0.2, 0.8 and 10 mg dicopper oxide/m^3 or 0, 0.7, 2.8, 7.0 and 35 mg copper sulphate pentahydrate/m^3 for 2 weeks (6 hours per day, 5 days per week, whole-body, aerosol). These concentrations correspond to 0, 0.18, 0.71, 1.78 and 8.9 mg Cu/m^3.

- Neither mortality nor treatment-related clinicals signs were reported in the range finding studies. Exposure to the highest concentration of copper sulphate pentahydrate resulted in statistically significantly lower mean body weights in males on day 4 and 11. Also, a significantly reduced weight gain during day 0 to day 4 and day 4 to day 11 was observed in males. In females, slight but statistically significantly lower mean body weight gain was observed from day 0 to day 4 at the highest tested concentration with copper sulphate pentahydrate. The observed effects on mean body weights and body weight gain correlated with reduced mean food consumption in males and females. Dicopper oxide did not cause similar effects at the highest concentration, although the copper content was the same. The lowest dicopper oxide concentration reduced body weight gain during day 7 to day 11.
- Copper sulphate pentahydrate caused enlargement of mediastinal lymph nodes at 2 highest concentrations (7.0 and 35 mg/m^3) in some animals. Enlarged tracheobronchial lymph nodes were observed in some animals at the highest concentration of dicopper oxide (10 mg/m^3).

In the 28-day study (OECD TG 412) via inhalation (Poland et al., 2022), male and female rats (Crl:CD(SD)) were exposed to 0, 0.2, 0.4, 0.8 and 2.0 mg dicopper oxide/m^3 corresponding to 0, 0.18, 0.35, 0.70 and 1.75 mg Cu/m^3 for 4 weeks (6 hours per day, 5 days per week, whole-body, aerosol) in the core study. In the control and highest dose groups, 20 animals/ sex/ group were used. The other groups had 10 animals/ sex/ dose. Half of males and females at the control and highest dose group were subject to recovery period of 13 weeks to investigate reversibility of effects observed at Day 28 of exposure. Furthermore, the study included satellite studies in which the toxicological response was investigated at 0 and 2.0 mg dicopper oxide/m^3 on Days 7, 14 and 21.

- No mortality or treatment-related clinical signs were observed in the main study. Treatment had no effect on mean body weight or food consumption, except for a transient, statistically significant reduction in body weight gain in males exposed to 2.0 mg/m^3 during Days 0-4 and 4-11. The decrease in body weight gain was less than 10% and was associated with slightly reduced food consumption during Days 0-4. No comparable effects were observed in the satellite studies (1-, 2-, or 3-week exposures).
- Haematology was assessed only in the main study. Dicopper oxide increased mean neutrophil counts in both sexes at 28 days, with statistically significant increases at 0.4 and 0.8 mg/m^3. No other treatment-related haematological effects were reported.
- Bronchoalveolar lavage fluid (BALF) was collected at the end of exposure from all groups and after the 13-week recovery period from control and 2.0 mg/m^3 groups. Treatment-

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related changes in BALF cytology were observed in both sexes after 4 weeks. Total cell counts were significantly increased at 0.8 mg/m³ in males and at 0.8 and 2.0 mg/m³ in females, reflecting inflammatory cell influx. Changes were characterized by increased proportion of neutrophils and decreased proportion of macrophages, with statistically significant effects at all exposure concentrations and a clear dose-response relationship. The strongest responses occurred at 2.0 mg/m³, where neutrophils represented 73.3% and 69.6% of BALF cells in males and females, respectively. Notably, increases in neutrophils were already evident at 0.2 mg/m³. A small but statistically significant increase in lymphocytes was observed in females at 0.2 and 0.4 mg/m³. However, in the absence of a dose-response relationship, this was not considered toxicologically relevant.

- In satellite studies (0 and 2.0 mg/m³), BALF analyses showed significantly increased neutrophil counts at all exposure durations (1-3 weeks) in both sexes. BALF neutrophils and macrophages peaked after 2-3 weeks and subsequently declined despite continued exposure at 4 weeks. The authors suggested that it is an indication of an acute inflammatory response and an adaptation or resolution. However, the number of neutrophils was still statistically significantly higher compared to control at 4 weeks
- BALF total protein and lactate dehydrogenase (LDH) concentrations were also increased in a dose-dependent manner, indicating pulmonary toxicity. Total protein was significantly elevated at ≥0.4 mg/m³ in both sexes, while LDH was significantly increased at ≥0.4 mg/m³ in males and ≥0.8 mg/m³ in females. Although total protein concentrations remained significantly elevated throughout exposure (from week 1 to week 4), they declined progressively over time. The authors consider that it suggests an adaptive or resolving response. In animals exposed to 2.0 mg/m³, total protein decreased by approximately 40% in males and 34% in females between weeks 1 and 4, with the greatest reduction occurring between weeks 1 and 2. LDH concentrations showed a similar temporal pattern, decreasing by 27.2% in males and 20.3% in females between weeks 1 and 4. All BALF parameters normalized following recovery.
- Enlargement of lung-associated lymph nodes (bronchial and/ or mediastinal) was observed only in males exposed to 0.8 mg/m³ and in both sexes exposed to 2.0 mg/m³ for 1 week. At the end of exposure, bronchial lymph node weights were significantly increased in both sexes at 0.8 and 2.0 mg/m³, while mediastinal lymph node weights were significantly increased only in high-dose males. These changes resolved during recovery. Histopathology revealed lymphoid hyperplasia of bronchial lymph nodes in most animals exposed to ≥0.4 mg/m³, while mediastinal lymph node hyperplasia occurred only sporadically. In satellite studies, lymphoid hyperplasia was present at all exposure durations but did not increase in incidence or severity over time. All findings resolved after recovery.
- Histopathological examination of the lungs identified diffuse alveolar histiocytosis characterized by accumulations of foamy macrophages, particularly around terminal bronchioles and alveolar ducts. The lesion occurred in both sexes at all exposure concentrations and increased in severity with dose, ranging from minimal at 0.2 mg/m³ to moderate at 2.0 mg/m³. In satellite animals, the lesion was evident after 1 week and increased only slightly in severity with prolonged exposure.
- Acute neutrophilic inflammation in the lung was observed in all animals exposed to ≥0.4 mg/m³ and only minimally at 0.2 mg/m³. Severity increased in a dose-related manner from minimal to mild. Satellite studies showed that the response developed rapidly, being present after 1 week and remaining stable thereafter. Perivascular inflammatory cell cuffing was observed in males at ≥0.8 mg/m³ and females at ≥0.4 mg/m³. Despite inflammatory changes, the alveolar epithelium remained morphologically normal. Neutrophilic inflammation had completely resolved by the end of the recovery period. Minimal residual alveolar histiocytosis remained in some animals.
- Assessment of fibrotic potential using Masson's Trichrome staining showed only minimal increases in collagen deposition in the lungs of high-dose animals. Morphometric analysis

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confirmed small, non-significant increases in lung collagen content. Overall, there was no evidence of pulmonary fibrosis after 28 days of exposure.

- In the nasal cavity, minimal to mild lymphocytic inflammation was observed only in males exposed to 2.0 mg/m³. Minimal olfactory epithelial degeneration was observed in one male each at 0.8 and 2.0 mg/m³. Both lesions resolved during recovery. No treatment-related histopathological changes were observed in the brain, liver, or kidneys.
- Mean lung weights increased in a concentration-dependent manner in both sexes at ≥0.4 mg/m³ at the end of exposure. Lung weights returned to control levels in females after recovery, while males showed largely resolved changes. Similar increases were observed in the satellite studies, where lung weights plateaued after the first week of exposure rather than increasing progressively. Concentration-dependent increases in lung wet and dry weights were observed from 0.4 mg/m³ onwards. However, no significant increases in lung wet/dry ratios were detected, indicating the absence of pulmonary oedema. Findings were consistent with particle deposition and inflammatory cell infiltration rather than fluid accumulation. Following recovery, lung wet and dry weights remained modestly elevated (10-13%) in the high-dose group but were substantially lower than at the end of exposure, indicating progressive recovery. The authors consider time-course changes in lung weights, wet/dry ratios, BALF parameters, and histopathology consistent with an early inflammatory response followed by stabilization and adaptation during continued high-dose exposure.
- Copper concentrations were measured in the lungs, BALF, liver, and brain. Lung copper concentrations remained below the limit of quantification (LOQ) at 0.2 and 0.4 mg/m³ but increased at 2.0 mg/m³ in both sexes. Satellite studies showed that lung copper concentrations were elevated after 1 week but remained relatively stable thereafter. Following recovery, lung copper concentrations returned below the LOQ in females, while variable concentrations persisted in males. The authors considered these male findings likely attributable to contamination or analytical variability, as elevated values were also present in recovery control males. BALF copper concentrations exceeded the LOQ only at 0.8 and 2.0 mg/m³, with the highest levels observed in the 2.0 mg/m³ groups (232 ng/mL in males and 347 ng/mL in females). No treatment-related increase in brain copper concentrations was detected. Small increases in liver copper content were observed at 2.0 mg/m³ but were minimal and had resolved by the end of the recovery period.

Additional repeated dose inhalation studies on inorganic copper compounds include studies by (Drummond et al., 1986, Ginoian, 1976, Johansson et al., 1983, Johansson et al., 1984). These studies are also summarised in (SCOEL, 2014) and (DFG, 2014).

Ginoian (1976) evaluated the effects of inhalation exposure to copper(II) oxide aerosol in rats (strain and the number of animals per concentration not specified) exposed for 90 to 100 days. Two exposure groups were used: 0.01 to 0.1 mg/m³ and 1 mg/m³ copper(II) oxide, corresponding to 0.008 to 0.08 and 0.8 mg Cu/m³, respectively.

- As only the publication abstract is available, study details are limited. Increased serum protein levels were reported in the lower-exposure group, whereas elevated haemoglobin concentrations and erythrocyte counts were observed in the higher-exposure group. No additional information on study methods and results was provided.

Johansson et al. (1983), (Johansson et al., 1984) investigated the effects of inhalation exposure to 0.6 mg Cu/m³ (as copper(II) chloride; MMAD 0.5-1 µm) in male rabbits (n = 8) exposed for 4-6 weeks (6 h per day, 5 days per week).

- No adverse effects on respiratory function were observed. Exposure resulted in a 1.5-fold increase in type II alveolar cell density and evidence of increased lamellar inclusions in macrophages and membrane damage. However, these findings had resolved within three days after cessation of exposure, at which time no histopathological lung alterations or macrophage dysfunction were detected.

Drummond et al. (1986) investigated the respiratory effects of copper(II) sulphate aerosols (MMAD 0.54 µm) in CD-1 mice (22 males per dose and 18-24 females per dose) following

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repeated 3-hour inhalation exposures at copper concentrations of 0.12 mg/m³ for 5 days or 0.13 mg/m³ for 10 days. Pulmonary host defence was assessed by concurrent exposure to *Streptococcus zooepidemicus*, with endpoints including alveolar macrophage bactericidal activity, mortality, and respiratory tract histopathology.

- No increase in mortality was observed after 5 days of exposure to 0.12 mg Cu/m³ followed by inhalation of 10 colony-forming units (CFU) of streptococci per animal. In contrast, 10 days of exposure to 0.13 mg Cu/m³, combined with the same bacterial challenge, resulted in a significant 28% increase in mortality in both sexes compared with controls, suggesting a time-dependent impairment of pulmonary immune function.
- Histopathological evaluation was inconclusive due to poor baseline respiratory epithelial integrity in control animals, characterised by necrosis, ciliary loss, and desquamation. Alveolar macrophage bactericidal activity was assessed using 35S-labelled *Klebsiella pneumoniae*, with viable and non-viable bacteria quantified in lung homogenates 4 hours after exposure. Exposure to 0.12 mg Cu/m³ for 5 days (22 males, 24 females) produced a small but significant reduction in bactericidal activity in females (94% of control values). Following 10 days of exposure to 0.13 mg Cu/m³ (22 males, 18 females), bactericidal activity was significantly reduced in both males and females, reaching 95% and 85% of control values, respectively.

Table 21: Summary of studies on repeated dose toxicity (inhalation route) with inorganic copper compounds

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
Copper sulphate or copper sulphate pentahydrate; copper(II)sulphate				
14-day dose range finding study Inhalation (whole-body) Non-TG	Rat, Crl:CD(SD) 5 animals per sex per dose	0, 0.7, 2.8, 7.0 and 35 mg/m³ Corresponds to 0, 0.18, 0.71, 1.78 and 8.9 mg Cu^{2+}/m³ 6 h per day, 5 days per week for 2 weeks MMAD 1.9 – 2.7 µm (GSD 2.31 – 3.10) Copper sulphate pentahydrate	No mortality No test substance related clinical findings Body weight and body weight gain • 35 mg/m³ (males): mean body weight lower on Day 4 (10.3% lower) and Day 11 (13.8%) compared to control. Also, significant reduction in weight gain between Day 0-4 and 4-11 • 35 mg/m³ (females): no effect on mean body weight. A slight but significantly ($p<0.01$) lower body weight gain between Days 0-4 compared to control. Food consumption • 35 mg/m³: mean food consumption ↓ Organ weights • ≥ 7.0 mg/m³: absolute and relative lung weight ↑ in males and females ($p<0.01$) Gross pathology	(Poland et al., 2022)

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• ≥ 7.0 mg/m^3: Enlargement of mediastinal lymph nodes in some of the animals (not specified further) Histopathology • ≥ 7.0 mg/m^3: Acute neutrophilic inflammation in the lung with accumulation of neutrophils within alveolar spaces • ≥ 2.8 mg/m^3: Alveolar histiocytosis in males and females. This was characterised with accumulation of macrophages which appeared to be activated due to a microvacuolated (foamy) cytoplasm. Identical macrophages also in enlarged mediastinal lymph nodes. • ≥ 2.8 mg/m^3: Hyperplasia of the Type II pneumocytes lining interalveolar septae, i.e., bronchioalveolar hyperplasia, in males and females. In females only at the 2 highest concentrations. • ≥ 7.0 mg/m^3: degeneration of the olfactory epithelium	
5- and 10-day study Inhalation Non-TG	Mouse, CD-1 Male and female 22 males per dose 18-24 females per dose	0 and 0.12 mg Cu^{2+}/mg for 5 days (3 hours/day) or 0 and 0.13 mg Cu^{2+}/mg for 10 days (3 hours/day) Copper sulphate	Mortality after bacterial challenge • no mortality after 5 days exposure • 28% increase in mortality in males and females after 10 days exposure Alveolar macrophage bactericidal activity • small but significant decrease in females (94% of control) after 5 days exposure • small but significant decrease in males (95% of control) and females (85% of control) after 10 days exposure	(Drummond et al., 1986)
Copper oxide				
7-day study Intranasal instillation	Mice, C57BL/6 Male	0, 0.1, 0.2, 0.4 and 1.0 mg/kg on days 1, 3 and 5	Physicochemical characterization of nanoparticles • Primary size 43.96±12.36 nm	(Kim et al., 2024)

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
Non-TG	6 animals per dose group	2 days after the last instillation, mice were euthanized Copper oxide nanoparticles (<50 nm)	BALF analysis • 0.1 mg/kg: inflammatory cell counts and cytokine levels ↑ (not statistically significant) • ≥ 0.2 mg/kg: neutrophils, macrophages, lymphocytes, IL-1β, IL-6 and TNF-α ↑ Histopathology • Inflammatory cell infiltration in bronchial and alveolar tissue • Mucus production ↑ Inflammation-related proteins ↑	
Dicopper oxide; copper(II) oxide				
28-day study Inhalation (whole-body) OECD TG 412	Rat, Crl:CD(SD) Male and female 20 animals per sex per dose for control and highest concentration 10 animals per sex per dose for other concentrations	<u>Main study</u> 0, 0.2, 0.4, 0.8 or 2 mg/m^3 in the main study Corresponds to 0, 0.18, 0.35, 0.70 or 1.76 mg/m^3 Cu^{2+} 6 h per day, 5 days per week for 4 weeks 10 animals per sex per dose MMAD 1.7 – 1.8 µm (GSD 1.67 – 1.79) Dicopper oxide <u>Recovery groups</u> 0 and 2 mg/m^3 At the end of the exposure period of 28 days, animals were retained for a 13-week recovery 10 animals per sex per dose <u>Satellite groups</u>	<u>Main study</u> No mortality No test substance related clinical findings Body weight and weight gain • No effect on body weight • 2 mg/m^3: A transient lower body weight gain in males between Days 0-4 and 4-11 Food consumption • 2 mg/m^3: slightly lower food consumption among males between Days 0-4 Haematology • Neutrophils ↑ • Increase in neutrophils was observed at all dose levels in males and females but was significant ($p<0.05$) only at 0.4 and 0.8 mg/m^3 in males and 0.8 and 2.0 mg/m^3 in females ($p<0.01$) • No other exposure-related haematological changes BALF analysis • ≥ 0.2 mg/m^3: Effects on the lavage fluid cytology in males and females • Total cells ↑: significant in males at 2.0 mg/m^3 and in females at 0.8 and 2.0 mg/m^3 • The increase in cell number associated with	(Poland et al., 2022)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
		0 and 2.0 mg/m^3 6 h per day, 5 days per week for 1, 2 or 3 weeks. 5 animals per sex per dose	higher proportion of neutrophils and lower proportion of lung macrophages being maximal at 2.0 mg/m^3, i.e., 73.3% (males) and 69.6% (females) of neutrophils • 0.2 and 0.4 mg/mg^3: lymphocytes ↑ in females, small but statistically significant increase without dose-response • ≥ 0.4 mg/m^3: Total protein and LDH ↑ in males and females. LDH increase in females ≥ 0.8 mg/m^3. • 13-week recovery: BALF parameters returned to control levels Organ weights • Lung weight (wet and dry) ↑ which was statistically significant in males and females at > 0.4 mg/m^3 • No significant increase in the lung wet/dry ratio, i.e., lack of pulmonary oedema • ≥ 0.8 mg/m^3: bronchial lymph node weight ↑ in males and females • 2.0 mg/m^3: mediastinal lymph node weight ↑ only in males • No effects in other organs • After 13-weeks recovery: In females, lung weights returned to control values whereas in males the absolute lung weights were lower than in controls ($p<0.05$), but relative lung weight was marginally above the control values. The lung wet/dry weights were slightly higher at 2.0 mg/mg^3 (10-13%), statistically significant only in males. Weights of bronchial and mediastinal lymph nodes returned to the control levels. Gross pathology	

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• 0.8 mg/m^3: Enlargement of the lung associated lymph nodes (bronchial or mediastinal) in 2/10 males Histopathology • ≥ 0.4 mg/m^3: Lymphoid hyperplasia of the bronchial lymph nodes in majority of males and females • Hyperplasia of the mediastinal lymph nodes in 1/10 male and 1/10 female at 0.4 mg/m^3 and 1/10 female at 2.0 mg/m^3 • ≥ 0.2 mg/m^3: Alveolar histiocytosis characterised by intra-alveolar accumulation of macrophages with microvacuolated (foamy) cytoplasm in males and females • ≥ 0.4 mg/mg^3: acute inflammation in the lung (accumulation of neutrophils) in males and females. At 0.2 mg/m^3, minimal severity neutrophilic inflammation was observed in 1 male and 1 female. • 2.0 mg/mg^3: slight increase in collagen • 2.0 mg/m^3: minimal to mild inflammation (submucosal infiltrates of lymphocytes) in the upper respiratory tract (nasal cavity) only in males. These infiltrates clearly distinct from normal nasal-associated lymphoid tissue. • Minimal degeneration of the olfactory epithelium in 1 male at 0.8 and 2.0 mg/m^3 • No histological changes in in the brain, kidney and liver • After 13-weeks recovery: Bronchial and mediastinal lymph nodes appeared normal. The acute neutrophilic inflammation resolved	

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			fully in the high exposure group. Alveolar histiocytosis was observed in several control and 2.0 mg/m^3 animals. Full reversal of the collagen content. Findings in the nasal cavities were not apparent. Organ and BALF copper content • Lung copper concentration clearly above the LOQ at 2.0 mg/m^3 in males and females. At 0.8 mg/m^3, copper content was at LOQ in 1/5 males and marginally above LOQ in 3/5 females. • BALF copper concentration consistently above the LOQ ≥0.8 mg/m^3 in males and females • Brain copper content not increased • Liver copper content slightly increased in males and females at 2.0 mg/m^3 • After 13-week recovery time, lung copper concentration returned to control level (<LOQ) in high dose female group, but not in the male group. BALF copper concentration <LOQ. <u>Satellite studies</u> (1, 2 and 3 weeks) No mortality No test substance related clinical findings Body weight and weight gain • No effects Food consumption • No effects BALF analysis • Macrophages and neutrophils ↑ at 2.0 mg/m^3, peaks in weeks 2/3. After this a reduction in absolute cell	

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			numbers. However, the number of neutrophils is still statistically significantly higher compared to control at 28 days at 2.0 mg/m^3 in males and females. • Total protein and LDH ↑ at 2.0 mg/m^3. The highest concentration is measured at week 1 but is statistically significantly higher at every time (1-4 weeks) when compared to control values in males and females. Organ weights • Relative lung weight ↑ in males and females ($p<0.01$) at 2.0 mg/m^3, plateaued between 1-3 weeks • Lung wet/dry ratio was statistically lower only at week 2 in males and females. Histopathology • Mild neutrophilic inflammation after 1-week exposure and was similar in severity and incidence across all 3 exposure periods Organ copper content • Lung: clearly above the LOQ at 2.0 mg/m^3 in males and females at weeks 1, 2 and 3. In females, a trend towards a slight reduction with exposure time was observed.	
14-day dose range finding study Inhalation (whole-body) Non-TG	Rat, Crl:CD(SD) Male and female 5 animals per sex per dose	0, 0.2, 0.8, 2.0 and 10 mg/m^3 Corresponds to 0, 0.18, 0.71, 1.78 and 8.9 mg/m^3 Cu^{2+} 6 h per day, 5 days per week for 2 weeks MMAD 2.3 – 3.1 µm (GSD 2.94	No mortality No test substance related clinical findings Body weight and weight gain • No effects on mean body weight • 0.2 mg/m^3: significant reduction in body weight gain between Days 7-11 Organ weights • ≥ 2.0 mg/m^3: absolute lung weight ↑ in males	(Poland et al., 2022)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
		– 3.74) Dicopper oxide	(at 10 mg/m^3 only) and females ($p<0.01$) Gross pathology • 10.0 mg/m^3: Enlargement of tracheobronchial lymph nodes in some of the animals (not specified further) Histopathology • 10.0 mg/m^3: Acute neutrophilic inflammation in the lung with accumulation of neutrophils within alveolar spaces • ≥ 2.0 mg/m^3: Alveolar histiocytosis in males and females. This was characterised with accumulation of macrophages which appeared to be activated due to a microvacuolated (foamy) cytoplasm. Identical macrophages also in enlarged tracheobronchial lymph nodes. • ≥ 2.0 mg/m^3: degeneration of the olfactory epithelium	
90 to 100-day study Inhalation Non-TG	Rat Strain not specified Number of animals per concentration not specified	0.01 to 0.1 or 1 mg/m^3 Corresponds to 0.008 to 0.08 or 0.8 mg Cu^{2+}/m^3 90 to 100 days	Low-exposure group • serum protein levels ↑ High-exposure group • haemoglobin levels and erythrocyte counts ↑ No further study details provided	(Ginoian, 1976)
Copper chloride, copper(II)chloride				
4 to 6-week study Inhalation Non-TG	Rabbit Strain not specified Male 8 animals per dose	0.6 mg Cu^{2+}/m^3 MMAD = 0.5 – 1 µm 4 to 6 weeks, 5 days/week, 6 hours/day	Respiratory function was not affected Exposure was associated with a 1.5-fold increase in type II alveolar cell density Indications of increased lamellar inclusions in macrophages and membrane damage. These effects were not observed three days after exposure ended, when neither histopathological lung changes nor macrophage dysfunction were detected.	(Johansson et al., 1983, Johansson et al., 1984)

Notes: BALF: Bronchoalveolar lavage Fluid; LDH: Lactate dehydrogenase; LOQ: Limit of Quantitation; MMAD: Mass Median Aerodynamic Diameter; GSD: Geometric Standard Deviation.

7.3.3 Summary

Earlier occupational studies in humans reported respiratory and other systemic effects among workers exposed to copper dust, but the absence of control groups and frequent co-exposure to other metals limit interpretation. More recent studies of welding and secondary copper smelting have reported respiratory symptoms, impaired lung function, and radiographic abnormalities, although attribution to copper is complicated by exposure to other metals and, in some cases, the absence of quantitative copper exposure data. Studies of ambient particulate matter have produced mixed findings, with some reporting associations between copper-containing particles and reduced lung function or allergic sensitisation, while others found no association with lung function. In contrast, two studies of long-term occupational exposure at a German copper smelter found no evidence of clinically meaningful adverse effects on lung function or accelerated respiratory decline, although limitations in exposure assessment and the potential for a healthy worker effect should be considered. Substantial uncertainty remains on the relationship between inhaled copper exposure and chronic respiratory effects due to co-exposures, exposure-assessment limitations, and differences in study populations and designs.

In humans, the evidence for copper in relation to Alzheimer's disease or cognitive decline is limited and unclear. Many of the studies are cross-sectional and no occupational studies have been identified. There is insufficient evidence to conclude that either high or low copper status independently causes cardiovascular disease, despite some meta-analyses reporting increased serum copper in patients with myocardial infarction and ischaemic stroke compared to controls, the available data does not show a consistent dose-response relationship between copper exposure/status and chronic cardiovascular disease, and differences between exposure metrics and the potential for confounding and reverse causation remain important uncertainties.

A limited number of animal studies have investigated the toxicity of inorganic copper compounds via inhalation. In a 28-day inhalation study (OECD TG 412) in rats, dicopper oxide induced acute neutrophilic lung inflammation, which resolved during a 13-week recovery period but not by the end of the 4-week exposure. However, no subchronic or chronic inhalation studies are available to determine whether prolonged exposure may cause irreversible pulmonary effects or systemic toxicity.

Short-term (2-week) and subchronic (13-week) oral exposure to copper sulphate produced local gastrointestinal effects in rats and mice, including gross lesions and minimal to mild squamous cell hyperplasia with hyperkeratosis of the forestomach. Rats were more sensitive than mice. Comparable findings were reported in a combined repeated-dose and reproduction/developmental toxicity screening study with copper monochloride in rats. In non-guideline oral studies, additional target organs of inorganic copper compounds included the liver, brain, kidneys, and heart, with evidence of histopathological changes and oxidative stress. Neurobehavioural alterations have also been reported in rats.

7.4 Irritancy and corrosivity

Copper sulphate has an entry in Annex VI of the CLP regulation as a Skin Irrit. 2 and Eye Irrit. 2 substance, and dicopper oxide has an entry as Eye Dam. 1 (see Table 6).

7.4.1 Human data

Airborne copper particles are respiratory irritants in humans. Occupational exposure to 111–464 mg/m^3 metallic copper dust caused symptoms of irritation of the respiratory tract (Suciu et al., 1981). Respiratory tract and eye irritation have been reported in occupational studies of workers exposed to copper dust or copper oxide; however, exposure concentrations were not quantified (Askergren and Mellgren, 1975, Jayawardana, 2004).

Eye irritation has been described in workers occupationally exposed to copper dust. Finelli (1981) reported cases of conjunctivitis in workers exposed to copper dust concentrations ranging from

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0.64 to 1.05 mg Cu/m³; however, concurrent exposure to iron, lead and cadmium limits the attribution of these effects to copper alone. In contrast, (Gleason, 1968) found no evidence of lower respiratory tract irritation in workers exposed to copper dust concentrations of 0.12–0.36 mg Cu/m³.

Two occupational cross-sectional studies at secondary copper smelters in Egypt evaluated respiratory effects of copper in workers who were simultaneously exposed to arsenic, lead, and cadmium (Fouad and Ramadan, 2022, Mourad and El-Sherif, 2022). These studies compared smelter workers with administrative workers from the same plants. In the first study, 75 male smelter workers had a mean blood copper concentration of 191.41 µg Cu/dL, compared with 137.30 µg Cu/dL among controls. In the second study, 65 male smelter workers had a mean blood copper concentration of 175.2 µg Cu/dL, compared with 93.44 µg Cu/dL among controls. Neither study accounted for potential co-exposures. Compared to administrative workers without metal dust exposure, copper smelter workers exhibited in both studies, higher prevalence of symptoms of respiratory irritation (nasal irritation, rhinitis, sinusitis, cough, expectoration and exertional dyspnoea) (Fouad and Ramadan, 2022, Mourad and El-Sherif, 2022). In addition, automotive workers in Iran (n=1 152) occupationally exposed to copper-containing welding fumes (average air concentration in welding unit: 0.107 mg Cu/m^3) reported a higher prevalence of respiratory symptoms compared to administrative staff (n= 1 152), including cough, sputum production and wheezing (Saadiani et al., 2023).

A prospective birth cohort study in the Netherlands (the PIAMA study, including 3 702 participants) did not find associations of ambient air copper in particulate matter (annual average concentrations) with asthma symptoms or rhinitis in school children aged 8 and 12 (Gehring et al., 2015).

Dermal exposure to copper salts may cause skin irritation, including erythema and pruritus, while direct ocular contact has been associated with conjunctivitis and, in severe cases, corneal ulceration, corneal opacity and adhesion of the eyelids to the ocular surface (WHO, 2002).

7.4.2 Animal data

7.4.2.1 Skin effects

Copper chloride has been reported to cause irritation of the skin of test animals. Other copper compounds tested in standard test guideline studies did not show skin irritation.

Table 22: Summary of studies on dermal irritation of copper and inorganic copper compounds

Method	Species, strain, sex, number/group	Test material, duration of exposure	Results	References
Acute dermal irritation/corrosion (OECD TG 404)	Rabbit, New Zealand White, 10 animals (sex not specified)	Dicopper oxide, 4 hours	Not irritating	Study report 1988 as cited in (ECHA, 2026)
Acute dermal irritation/corrosion (OECD TG 404)	Rabbit, New Zealand White, 3 males	Copper sulphate, pentahydrate, 4 hours	Not irritating	Study report 1994 as cited in (ECHA, 2026)
Acute dermal irritation/corrosion (OECD TG 404)	Rabbit, New Zealand White, 2 males and 1 female	Coated copper flakes, 4 hours	Not irritating	Study report 2001 as cited in (ECHA, 2026)
Acute dermal irritation/corrosion (no test guideline)	Rabbit, Vienna White, 2 animals (sex not specified)	Copper chloride, 1, 5, 15 minutes and 20 hours	Irritating	Study report 2002 as cited in (ECHA, 2026)

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Method	Species, strain, sex, number/group	Test material, duration of exposure	Results	References
Acute dermal irritation/corrosion (OECD TG 404)	Rabbit, New Zealand White, 3 males	Copper oxide, 4 hours	Not irritating	Study report 2002 as cited in (ECHA, 2026)

7.4.2.2 Eye effects

Various degrees of eye irritation have been reported in standard test guideline studies in rabbits, as reported in the table below.

Table 23: Summary of studies on eye irritation of copper and inorganic copper compounds

Method	Species, strain, sex, number/group	Test material, duration of exposure	Results	References
Acute eye irritation/corrosion (no test guideline)	Rabbit, Vienna White, 2 animals (sex not specified)	Copper chloride, duration not specified	Irreversible effects on the eyes	Study report 1978 as cited in (ECHA, 2026)
Acute eye irritation/corrosion (OECD TG 405)	Rabbit, New Zealand White, 3 animals (sex not specified)	Dicopper oxide, single treatment (no rinsing)	Irritating	Study report 1984 as cited in (ECHA, 2026)
Acute eye irritation/corrosion (OECD TG 405)	Rabbit, New Zealand White, 6 females	Dicopper oxide, single treatment (no rinsing)	Slightly irritating	Study report 1988 as cited in (ECHA, 2026)
Acute eye irritation/corrosion (OECD TG 405)	Rabbit, New Zealand White, 3 males	Copper sulphate, pentahydrate, single treatment (no rinsing)	Highly irritating	Study report 1994 as cited in (ECHA, 2026)
Acute eye irritation/corrosion (OECD TG 405)	Rabbit, New Zealand White, 6 males, 3 females	Dicopper oxide, 3 male/female: single treatment (no rinsing); 3 males 30 seconds	Slightly irritating	Study report 1994 as cited in (ECHA, 2026)
Acute eye irritation/corrosion (OECD TG 405)	Rabbit, New Zealand White, 3 females	Coated copper flakes, single treatment (no rinsing)	Slightly irritating	Study report 2001 as cited in (ECHA, 2026)
Acute eye irritation/corrosion (OECD TG 405)	Rabbit, New Zealand White, 3 males	Copper oxide, 72 hours	Mild irritation	Study report 2002 as cited in (ECHA, 2026)

7.4.3 Summary

Overall, the evidence in humans indicates that copper exposure may cause respiratory and ocular irritation, particularly following occupational exposure to copper dust, fumes, or salts. However, interpretation is limited by exposure-assessment limitations and concurrent exposure to other metals, making it difficult to attribute observed effects specifically to copper. Evidence from lower-exposure occupational and ambient settings is more limited and generally does not show consistent respiratory effects. Animal studies show eye irritation.

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7.5 Sensitisation

7.5.1 Human data

Copper and copper sulphate have been reported to induce allergic contact dermatitis; however, the available evidence indicates that confirmed cases of copper-specific skin sensitisation are rare. Positive patch test reactions have been observed in patients with contact dermatitis and in workers occupationally exposed to copper dust or fumes following application of copper sulphate at concentrations of up to 5%. Nevertheless, only a small number of cases have demonstrated clear sensitisation to copper, and these reactions have generally occurred at relatively high test concentrations (Walton, 1983a, Walton, 1983b). Furthermore, as noted in SCOEL (2014) many of the reported positive reactions were non-specific or attributable to cross-reactivity in individuals with nickel allergy, while in some cases nickel contamination of the copper test material could not be excluded.

A single case of possible occupational respiratory sensitisation to copper was reported by (Cirla, 1985), in which a worker from the galvanic industry experienced an approximately 30% decline in FEV_1 following bronchial provocation with copper sulphate at 1 mg/m³. However, the finding was based on a single case within a study primarily investigating nickel-induced occupational asthma.

In the PIAMA birth cohort in the Netherlands, increased risk of allergic sensitization was positively associated with modelled estimates of copper in airborne PM_{10} at both children's birth and current home address, and also asthma symptoms were positively associated with copper in PM_{10} at the current address (OR: 1.06, 95% CI: 1.00 to 1.12), whereas no association of copper in PM_{10} was found with incident asthma or hay fever (Gehring et al., 2015).

7.5.2 Animal data

7.5.2.1 Respiratory sensitisation

No studies were found.

7.5.2.2 Skin sensitisation

None of the copper compounds tested in standard skin sensitisation tests have shown indications of sensitisation, as summarised in the table below.

Table 24: Summary of studies on skin sensitisation of copper and inorganic copper compounds

Method, route	Species, strain, sex, number/group	Test material, doses, duration of exposure	Results	References
Guinea pig maximisation test (OECD TG 406)	Guinea pig, Pirbright-Hartley, males/females, in total 20 animals in the treatment group	Dicopper oxide; Day 0: intradermal induction 0.25% in Na-carboxy methyl cellulose (CMC); Day 7 topical induction 50% in vaseline; Day 21 topical challenge 50% in vaseline. Scoring 24 h and 48 h after challenge.	Not sensitising	Study report 1993, as cited in (ECHA, 2026)
Freund's complete adjuvant test (OECD TG 406)	Guinea pig, Hartley, males/females, in total 20 animals in the treatment group	Copper sulphate, pentahydrate; Day 1: intradermal induction 0.1% in water; Day 8 topical induction 10% in water; Day 22 topical challenge 50% in water. Scoring 24 h and 48 h after	Not sensitising	Study report 1994, as cited in (ECHA, 2026)

Method, route	Species, strain, sex, number/group	Test material, doses, duration of exposure	Results	References
		challenge.		
Guinea pig maximisation test (OECD TG 406)	Guinea pig, Dunkin-Hartley, 10 males	Coated copper flakes; Day 0: intradermal induction 0.1% w/w in arachis oil BP; Day 7: topical induction 50% w/w; Day 21 topical challenge 50% and 25% w/w. Scoring 24 h and 48 h after challenge.	Not sensitising	Study report 2001, as cited in (ECHA, 2026)
Guinea pig maximisation test (OECD TG 406)	Guinea pig, Dunkin-Hartley, 10 males	Copper oxide; Day 0: intradermal induction 0.1% w/w in arachis oil BP; Day 7: topical induction 75% w/w; Day 21 topical challenge 10% and 5% w/w. Scoring 24 h and 48 h after removal of the occlusive dressing.	24h: 4/10 animals with discrete or patchy erythema after 10% challenge and 2/10 showed signs of erythema after 5% challenge. 48h: no signs of erythema. Not sensitising	Study report 2002, as cited in (ECHA, 2026)
Guinea pig maximisation test (OECD TG 406)	Guinea pig, Dunkin-Hartley, 10 females	Copper chloride; Day 1: intradermal induction 1% in PEG 300; Day 8 topical induction 10% in PEG 300; Day 22 topical challenge 0.5% in PEG 300. Scoring 24 h and 48 h after challenge.	24h: Discrete/patchy erythema in 2/10 animals. 48h: No findings. Not sensitising.	Study report 2010, as cited in (ECHA, 2026)

7.5.3 Summary

Based on the limited human data available, there is not sufficient evidence that copper is a respiratory or skin sensitiser. None of the copper compounds tested *in vivo* showed sensitising effects.

7.6 Genotoxicity

Copper or its inorganic compounds have no entry in Annex VI of the CLP regulation for the hazard class germ cell mutagenicity. In 2018, a RAC opinion concluded that for granulated copper (EC 231-159-6; CAS 7440-50-8) no proposal for classification for germ cell mutagenicity could be made due to the absence of relevant data (RAC, 2018).

7.6.1 Human data

De Olivera et al. (2012) reported significantly higher levels of DNA damage in peripheral blood leukocytes of male workers (n=11) employed at a copper smelting plant, with employment durations ranging from 0.2 to 25 years, compared with unexposed controls (n=11), using the Comet assay. However, the extent of DNA damage was not associated with blood copper concentrations nor with duration of exposure, suggesting that the observed genotoxic effects could not be directly attributed to internal copper exposure.

Shubber et al. (1998) evaluated cytogenetic endpoints in women who had used copper-containing intrauterine devices (IUDs) for 1-4 years. The study included 40 women using IUDs and 22 control women matched for age and socioeconomic status. The IUD users had significantly higher plasma copper concentrations than controls (1.25 ± 0.14 µg/mL vs 0.89 ± 0.04 µg/mL) and showed increased frequencies of chromosomal aberrations and sister chromatid

exchanges in peripheral blood lymphocytes. However, the small sample size of the study and its cross-sectional design preclude establishing a causal relationship between the elevated plasma copper concentrations and the observed cytogenetic changes.

In a controlled double-blind repeated crossover study, in which the same participants received copper supplementation and placebo during different periods, (O'Connor et al., 2003) administered copper sulphate supplements to 22 healthy adults (12 men and 12 women, all non-smokers) in alternating 6 week-periods at doses of up to 0.067 mg Cu/kg body weight/day for women and 0.067 mg Cu/kg body weight/day for men. Copper was administered as copper sulphate at 3 mg Cu/day and as copper amino-acid chelates at 3 and 6 mg Cu/day. Assessment of peripheral blood leukocytes (assessed using a modified alkaline Comet assay) showed no increase in DNA damage compared with baseline or control values, indicating no evidence of genotoxic effects at the administered doses of copper.

Meramat et al. (2017) conducted a cross-sectional study to investigate pulmonary function, oxidative stress and DNA damage in 30 male workers employed in a copper processing industry and 30 age- and socioeconomic status-matched controls. Occupational exposure was assessed based on employment in the copper processing facility; however, quantitative airborne copper concentrations were not reported, limiting characterization of the exposure. Pulmonary function tests showed significantly lower FVC, FEV_1, peak expiratory flow rate (PEFR) and maximum voluntary ventilation (MVV) in exposed workers compared with controls. Biomarkers of oxidative stress indicated significantly higher serum malondialdehyde (MDA) concentrations and lower total antioxidant capacity (FRAP) and erythrocyte glutathione (GSH) levels in exposed workers. In addition, DNA damage in blood cells, assessed using the Comet assay, was significantly greater among exposed workers, and reductions in pulmonary function were correlated with increased oxidative stress. The authors concluded that occupational exposure to copper particulates may impair lung function and induce oxidative stress and DNA damage. However, interpretation of the findings is limited by the small sample size, the cross-sectional study design, the absence of quantitative exposure measurements, and the potential influence of co-exposure to other workplace contaminants (e.g., other metal dusts, welding dusts, silica, sulphur compounds or particulate matter).

No studies were located regarding genotoxicity in humans after dermal exposure to copper or its inorganic compounds.

7.6.2 Animal data

Copper and its inorganic compounds induced mutagenic and genotoxic effects in most of the studies in experimental animals summarised in Table 25. In studies via intraperitoneal (i.p.) or via oral exposure, copper sulphate caused the formation of chromosomal aberrations in the mouse, chicken and rabbit, and of micronuclei in mouse and chicken. This chemical also showed DNA damage after oral exposure in several studies in mice, rats and drosophila and was positive in the γ-H2AX assay in mice after i.p. or oral exposure. Copper sulphate also induced lethal mutations in drosophila, sperm abnormalities in mice and sister chromatid exchanges in rabbit. Copper chloride caused DNA damage in rats after oral administration.

Table 25: Summary of *in vivo* genotoxicity studies of inorganic copper compounds

Study endpoints/ method	Species, test system	Dose levels, route of administration	Results	References
Copper sulphate				
Cytogenetic assays				
Chromosomal aberrations	Mouse, inbred Swiss Tissue: bone marrow	Acute: 5, 10, 20mg/kg bw **Intraperitoneal (i.p.)** and **subcutaneous**	**Positive** (acute and chronic treatments)	(Bhunya and Pati, 1987)

Study endpoints/ method	Species, test system	Dose levels, route of administration	Results	References
		(s.c.), recovery times times (6, 24 and 48 h) Chronic: **i.p.**, 4 mg/kg daily x5 days (total dose 20 mg/kg) sacrifice 24h after last injection.		
Chromosomal aberrations	Mouse, White Swiss Tissue: bone marrow cells	1.1–6.6 mg/kg bw **i.p.** injection	**Positive**	Agarwal et al. 1990, as cited in (ATSDR, 2024)
Chromosomal aberrations	Chicken, White Leghorn Tissue: bone marrow cells	Acute: 5-7.5-10 mg/kg bw, **i.p.** and oral, sampling at 6, 24 and 48 h after treatment Sub-acute: **i.p.**, 2 mg/kg daily x5 days (total dose 10 mg/kg) sacrifice 24h after last injection.	**Positive** (acute and sub-acute treatments)	Bhunya and Jena 1996, as cited in (ATSDR, 2024)
Chromosomal aberrations	Mouse, White Swiss Tissue: spermatocytes	2.06, 4.13, 8.25 mg/kg bw **i.p.** x1 and **i.p.** x3	**Positive** (both single and repeated treatments)	Fahmy 2000, as cited in (ATSDR, 2024)
Chromosomal aberrations	Rabbit, New Zealand Tissue: blood cells	Dose not reported oral exposure	**Positive**	Georgieva et al. 2013, as cited in (ATSDR, 2024)
Micronuclei	Mouse, inbred Swiss Tissue: bone marrow	5, 10, 20mg/kg bw **i.p.** x2 daily, sacrifice 6h after second injection.	**Positive**	Bhunya and Pati 1987, as cited in (ATSDR, 2024)
Micronuclei	Mouse, CBA Tissue: bone marrow cells	Doses not reported **i.p.** injection	Negative	Tinwell and Ashby 1990, as cited in (ATSDR, 2024)
Micronuclei	Mouse, CD1 Tissue: blood	447 mg/kg bw as copper sulphate pentahydrate Oral/gavage	Negative	Riley, 1994, as cited in (ECHA, 2026)
Micronuclei	Chicken, White Leghorn Tissue: bone marrow erythrocytes	5-7.5-10 mg/kg bw, **i.p.** x2 daily, sacrifice 6h after second injection. 10 mg/kg bw, p.o.	**Positive** (both routes)	Bhunya and Jena 1996, as cited in (ATSDR, 2024)
Micronuclei	Chicken, White Leghorn Tissue: peripheral erythrocytes	5-7.5-10 mg/kg bw, **i.p.** x2 daily, sacrifice 6h after second injection. 10 mg/kg bw, p.o.	**Positive** (only i.p. route)	Bhunya and Jena 1996, as cited in (ATSDR, 2024)
Micronuclei	Mouse, White Swiss Tissue: marrow cells	2.06, 4.13, 8.25 mg/kg bw **i.p.** x1, sacrifice 24h after	**Positive**	Fahmy 2000, as cited in (ATSDR, 2024)
Micronuclei	Mouse, CF1 Tissue: blood	8.25 mg Cu/kg bw, x 6 days Oral/gavage	**Positive**	Prá et al. 2008, as cited in ATSDR, 2024

Study endpoints/ method	Species, test system	Dose levels, route of administration	Results	References
Micronuclei	Mouse, Swiss Albino Tissue: bone marrow cells	Dose not reported **oral**	**Positive**	Kadammattil et al. 2018, as cited in (ATSDR, 2024)
DNA damage assays				
Comet assay	Mouse, Swiss Albino Tissue: blood leukocytes	1.25, 2.5, 5, 7.5, 10 and 12.5 mg/kg bw copper sulphate **oral gavage**	**Positive**	Saleha Banu et al. 2004, as cited in (ATSDR, 2024)
Comet assay	Mouse, Swiss Webster Tissue: blood cells	8.50 Cu mg/kg bw **oral gavage**	**Positive**	Franke et al. 2006, as cited in (ATSDR, 2024)
Comet assay	Mouse, CF1 Tissue: blood	8.25 mg Cu/kg bw, x 6 days, sacrifice one day after last dose. **oral gavage**	**Positive**	Prá et al. 2008, as cited in (ATSDR, 2024)
Comet assay	Drosophila melanogaster	50–200 µM, **Oral (diet)**	**Positive**	Shukla et al. 2011, as cited in (ATSDR, 2024)
DNA strand breaks	Rat, male albino (species not reported) Tissue: liver	39.8 or 119 mg Cu/kg/day for 7 days **oral**	**Positive**	Alhusaini et al., 2018b, as cited in (ATSDR, 2024)
DNA fragmentation	Mice, C57BL/6 Tissue: kidney	50, 100, 200 mg/kg/day for 28 days **oral**	**Positive**	(Dai et al., 2020)
γ-H2AX	Mouse, ICR Tissue: testis and spleen	10, 20 or 40 mg/kg,by **oral gavage** X 21- and 42-days.	**Positive**	Guo et al. 2021, as cited in (ATSDR, 2024)
***In vivo* mutagenicity**				
Lethal mutation rate	Drosophila melanogaster	0.1%, **injection**	**Positive**	Law, 1938, as cited in (ATSDR, 2024)
Unscheduled DNA synthesis (UDS)				
UDS	Rat, Wistar male Tissue: liver	632.5 and 2000 mg/kg bw as copper sulphate pentahydrate **oral gavage**	Negative	Ward, 1994, as cited in (ECHA, 2026)
Sister chromatid exchanges				
Sister chromatid exchanges	Rabbit, New Zealand Tissue: blood cells	Dose not reported **oral**	**Positive**	Georgieva et al. 2013, as cited in (ATSDR, 2024)
Sperm abnormalities				
Sperm abnormalities	Mouse, inbred Swiss Tissue: bone marrow	1, 2, 4 mg/kg bw, **i.p.** x5 daily, sacrifice 35 days after first injection.	**Positive**	Bhunya and Pati 1987, as cited in (ATSDR, 2024)
Sperm abnormalities	Mouse, Swiss Albino Tissue: bone marrow cells	Dose not reported **oral**	**Positive**	Kadammattil et al. 2018, as cited in (ATSDR, 2024)
Sperm abnormalities	Mouse, White Swiss Tissue: marrow cells	2.06, 4.13, 8.25 mg/kg bw **i.p.** x3 daily, sacrifice 35 days after first injection.	**Positive**	Fahmy 2000, as cited in (ATSDR, 2024)

Study endpoints/ method	Species, test system	Dose levels, route of administration	Results	References
Copper chloride				
DNA damage assays				
DNA strand breaks	Rat, Wistar Tissue: intestinal	5, 15, 30 and 40 mg copper chloride/kg bw **Oral** (single treatment)	**Positive**	Husain et al. 2021, as cited in (ATSDR, 2024)

Note: Positive outcomes are always presented in bold.

7.6.3 *In vitro* data

In vitro tests investigating the mutagenic and genotoxic activity of copper and its inorganic compounds showed rather inconsistent results. The studies are summarised in Table 26. Copper was positive in the comet assay in three rodent cell lines and one bovine cell line, while it was negative in a human cell line. Copper chloride was positive in one out of four assays in prokaryotic organisms, while it induced DNA damage in two out of five tests in human or mouse cell lines. DNA damage and sister chromatid exchanges were observed in Chinese hamster cells treated with copper nitrate. Copper sulphate was negative for reverse mutation in three out of four tests in bacteria, and in one test in *Saccharomyces cerevisiae*. This chemical however caused DNA strand breaks, DNA damage, unscheduled DNA synthesis and oxidative DNA damage in rodent, mammalian or human cell lines.

Table 26: Summary of *in vitro* genotoxicity studies of copper and inorganic copper compounds

Study endpoints, method	Species, test system	Concentration /dose levels	Results		References
			With metabolic activation	Without metabolic activation	
Copper					
Eucaryotic organisms					
Comet assay	Human $CD4^+$ T lymphocytes	0.05-5 mM (318 mg/L) for 48h	No data	Negative	(Caicedo et al., 2008)
Comet assay	CHO cells	5.67–7.42 mg/L	No data	**Positive**	(Grillo et al., 2010)
Comet assay	Mouse primary lymphocytes	10–200 µM	No data	**Positive**	Jing et al. 2016, as cited in (ATSDR, 2024)
Comet assay	Bovine cumulus cells	0, 120, 240, and 360 µg/dL Cu	No data	**Positive**	(Anchordoquy et al., 2017)
Micronuclei	Rat splenocytes	1-100 µM, for 12h	No data	**Positive**	(Mandil et al., 2020)
Copper chloride					
Prokaryotic organisms					
DNA damage (rec- assay)	*Bacillus subtilis*	Concentration not reported	No data	Negative	Nishioka 1975, as cited in (ATSDR, 2024)
DNA damage (rec- assay)	*Bacillus subtilis*	Concentration not reported	No data	Negative	Kanematsu et al., 1980, as cited in (ANSES, 2017)
Errors in DNA synthesis	Avian myeloblasts virus, DNA polymerase	Concentration not reported	No data	**Positive**	Sirover and Loeb 1976, as cited in (ATSDR, 2024)

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Study endpoints, method	Species, test system	Concentration /dose levels	Results		References
			With metabolic activation	Without metabolic activation	
Reverse mutation	*S. typhimurium* TA100	Concentration not reported	No data	Negative	Tso and Fung 1981, as cited in (ATSDR, 2024)
Reverse mutation	*S. typhimurium* TA98, TA102, TA1535, TA1537	160 ppm and 200 ppm	Negative	Negative	Wong 1988, as cited in (ATSDR, 2024)
Eucaryotic organisms					
DNA synthesis	CHO cells	Concentration not reported	No data	**Positive**	Garrett and Lewtas 1983, as cited in (ATSDR, 2024)
DNA fragmentation	Human blood cells	up to 40 mM (2.5 g/L) for 30 minutes	No data	Negative	Prasad et al. 2006, as cited in (ATSDR, 2024)
DNA strand breaks	Human lymphocytes	0.01 to 2.5 mM, for 1 h at 37 °C.	No data	**Positive**	(Husain and Mahmood, 2019)
DNA strand breaks	Human lymphocytes	0.5 mM, for 1 h at 37 °C.	No data	**Positive**	(Husain and Mahmood, 2020)
DNA strand breaks	FE1 Mouse pulmonary alveolar epithelial cells	0.5-50 µg/mL	No data	Negative	Boyadzhiev et al. 2022, as cited in (ATSDR, 2024)
Copper nitrate					
Eucaryotic organisms					
DNA strand breaks	Chinese hamster V79 cells	Concentration not reported	No data	**Positive**	Sideris et al. 1988, as cited in (ATSDR, 2024)
Sister chromatid exchange	Chinese hamster V79 cells	10-5 M for 24h	No data	**Positive**	Sideris et al. 1988, as cited in (ATSDR, 2024)
Copper sulphate					
Prokaryotic organisms					
Reverse mutation	*Escherichia coli*	Concentration not reported	No data	**Positive**	Demerec et al. 1951, as cited in (ATSDR, 2024)
Reverse mutation	*S. typhimurium* TA 98, 100, 1535, 1537, 1538, and *E. coli WP2*	Up to 5 000 µg/plate	Negative	Negative	Moriya et al., 1983, as cited in (ANSES, 2017)
Reverse mutation	*S. typhimurium* TA 102	10, 30, 100, 300, 100 and nM/plate	No data	Negative	Marzin and Phi 1985, as cited in (ANSES, 2017)
Reverse mutation	*S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 and TA 102	Up to 1000 µg/plate	Negative	Negative	Ballantyne, 1994, as cited in (ECHA, 2026)
Eucaryotic organisms					
Reverse mutation	*Saccharomyces cerevisiae*		No data	Negative	Singh, 1983, as cited in

Study endpoints, method	Species, test system	Concentration /dose levels	Results		References
			With metabolic activation	Without metabolic activation	
					(ATSDR, 2024)
Recombination	*Saccharomyces cerevisiae*	Concentration not reported	No data	Negative	Sora et al. 1986, as cited in (ATSDR, 2024)
DNA strand breaks	Rat hepatocytes	Concentration not reported	No data	**Positive**	Sina et al. 1983, as cited in (ATSDR, 2024)
DNA strand breaks	Human HeLa S3 cells	0.5-3 mM for 2h and 0.05-0.4 mM for 24h	No data	**Positive**	(Schwerdtle et al., 2007)
DNA fragmentation	HEK293 (human embryonic kidney)	0.4 mM	No data	**Positive**	(Dai et al., 2020)
Unscheduled DNA synthesis	Rat hepatocytes	7.9–78.5 µM	**Positive**	**Positive**	Denizeau and Marion 1989, as cited in (ATSDR, 2024)
Oxidative DNA damage	Human HeLa S3 cells	0.5-3 mM for 2h, and 0.05-0.4 mM for 24h	No data	**Positive**	Schwerdtle et al. 2007, as cited in (ATSDR, 2024)
Oxidative DNA damage	Porcine oocytes	15, 25, 50, or 100 mg/mL for 44h	No data	**Positive**	Chen et al. 2021, as cited in (ATSDR, 2024)

Note: Positive outcomes are always presented in bold.

7.6.4 Summary

Overall, the evidence for genotoxic effects of copper in humans is limited and inconsistent. Some studies have reported increased DNA damage or cytogenetic abnormalities, whereas a controlled crossover study of copper supplementation found no evidence of increased DNA damage. Interpretation of the positive occupational findings is constrained by small sample sizes, cross-sectional designs, limited exposure characterization, and potential co-exposure to other workplace contaminants. No evidence was identified regarding genotoxicity following dermal exposure to copper or its inorganic compounds.

In animals, most of the available studies were positive and showed the formation of chromosomal aberrations, micronuclei or DNA damage in mice, rats or chicken exposed to copper sulphate and copper nitrate via i.p. or oral route. Copper sulphate also induced lethal mutations in drosophila, and sperm abnormalities, sister chromatid exchanges and phosphorylation of H2AX in rodents.

In bacteria and in mammalian cells, the mutagenic and genotoxic activity of copper and its inorganic compounds was inconsistent. Some positive and negative results were obtained for DNA damage or reverse mutations with copper, copper chloride and copper nitrate. Copper sulphate was negative in most reverse mutation tests in bacteria and *Saccharomyces cerevisiae*, while it caused DNA damage in several rodent, mammalian or human cell lines.

Overall, the data suggest that copper sulphate may induce genotoxic effects *in vivo* and *in vitro*.

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7.7 Carcinogenicity

The IARC has not evaluated the carcinogenicity of copper. Neither the NTP nor the EPA has evaluated the carcinogenicity of copper (IRIS 1988; NTP 2021). In 2018, a RAC opinion concluded that for granulated copper (EC 231-159-6; CAS 7440-50-8), no proposal for classification for carcinogenicity can be made due to the absence of relevant data (RAC, 2018).

7.7.1 Human data

Some epidemiological studies reported increased incidences for overall cancer and lung cancer, as well as increased mortality due to lung and stomach cancer among workers employed in copper production and smelting industries (copper oxide). Due to the lack of exposure characterisation, the fact that some of the studies had measured exposure to copper in airborne particulate matter or indoor dust after the outcome had occurred, and the presence of uncontrolled confounding factors (smoking, co-exposure to arsenic, silica and iron, and elevated individual copper serum levels as a consequence of several diseases including cancer), these studies, as described in ATSDR (2024), are not adequate to derive a causal relationship between inhalation exposure to copper compounds and cancer in humans (Chen et al., 1995, Chen et al., 1993, Enterline et al., 1995, Viren and Silvers, 1994, Welch et al., 1982).

Suciu et al. (1981) reported the occurrence of 7 pituitary adenomas in 75 to 100 workers examined several times between 1970 and 1973, who were exposed between 111 and around 464 mg Cu/m^3 as copper dust. Due to the insufficient diagnosis and description of these tumours, it is not possible to draw a firm conclusion regarding the carcinogenic potency of copper dust. Moreover, the patients were suffering from obesity, hypertension, impotence and osteoporosis at the same time.

No studies on the carcinogenic action of copper in humans via the oral route were identified.

7.7.2 Animal data

The few animal carcinogenicity investigations on copper or its inorganic compounds were not performed according to OECD test guidelines. The available studies mostly assessed the inhibitory effects of copper compounds on the carcinogenesis of known carcinogens.

Table 27: Summary of animal carcinogenicity studies of inorganic copper compounds

Species, strain, sex, number/ group	Doses, route, duration of exposure	Results	Remarks	References
Copper sulphate				
Rat				
Sprague-Dawley rats, M/F, 23-26 animals/ group	530 and 1 600 ppm (27 and 80 mg Cu/kg bw/day) **Oral (diet)** 42-44 weeks	No report of evidence of neoplasms in any treatment group.	This study used only two dose levels.	Harrison et al, 1954, as cited in (ECHA, 2026)
Sprague-Dawley rats, M, 50-58 animals/ group	1 and 800 ppm (0.05, 40 mg Cu/kg/bw/day) **Oral (diet)** 42-44 weeks	No evidence of formation of copper related neoplasms.	This study investigated the inhibitory effects of copper on the carcinogenesis of acetylaminofluorene and	Carlton et al, 1973, as cited in (ECHA, 2026)

Species, strain, sex, number/ group	Doses, route, duration of exposure	Results	Remarks	References
			dimethylnitrosamine.	
Mouse				
Mouse C57BL/6J, female 12 animals/group	198 mg/l (app. 10 mgCu/kg/bw/day) **Oral (drinking water)** 46 weeks	No evidence of formation of copper-related tumours in lung or ovaries.	This study used only one dose level.	Burki & Okita, 1969, as cited in (ECHA, 2026)
Copper acetate				
Rat				
Wistar rats, M, n=150 for 12 groups	130 mg Cu/kg/day as copper acetate. **Oral (diet)** 24 weeks	No increase in incidence of liver tumours.	This study investigated the inhibitory effects of copper on the carcinogenesis of DL-ethionine. It used only a single dose level of copper, tested small groups of animals, exposed animals for less than a lifetime, and examined the tumour formation only in liver.	Kamamoto et al. (1973), as cited in (ATSDR, 2024)
Copper compound (unspecified)				
Rat				
Sprague-Dawley rats, M, n=10/group	9 mg Cu/kg/day as unspecified copper compound **Oral (diet)** 16 weeks	No increase in incidence of large intestine.	This study assessed the effect of dietary copper on intestine tumour induced by the carcinogen 1,2-dimethylhydrazine. It used only a single dose level of copper compound, tested small groups of animals, exposed animals for less than a lifetime, and examined only large intestine for tumour formation.	Greene et al. (1987), as cited in (ATSDR, 2024)

7.7.3 Summary

Epidemiological studies have reported associations between occupational copper exposure and certain cancers in workers, primarily in the copper smelting and production industry, but limitations including inadequate exposure characterization, uncontrolled confounding, co-exposure to other carcinogens, and insufficient tumour documentation preclude establishing a causal relationship.

In animals, no proper carcinogenicity study was performed on copper or its inorganic compounds. The limited data available via oral route in rats and mice showed no evidence to

support copper sulphate or copper acetate as potential carcinogens.

7.8 Reproductive toxicity

7.8.1 Human data

7.8.1.1 Fertility

In an occupational health study, sexual impotence was reported in 16% of workers (75–100 workers examined) chronically exposed to copper dust (declining over time from 464 to 111 mg Cu/m^3) during grinding and sieving operations, particularly in persons with obesity and hypertension (Suciu et al., 1981). The study did not evaluate changes in the prevalence of impotence with declining exposure concentrations.

Samir et al. (2024) assessed 40 male workers exposed in a secondary copper smelter and 40 non-exposed workers (from other sectors of the same factory not occupationally exposed to the copper smelting process) with ages ranging from 20 to 55 years for both groups from the same factory in a comparative cross-sectional occupational study. Exposure was not assessed by air monitoring; instead, serum copper was measured. Mean serum copper was 1 409.4 ± 611.2 µg/L in exposed workers compared with 808.0 ± 322.2 µg/L in controls. Exposed workers had significantly higher follicle-stimulating hormone (FSH) (15.17 vs. 7.25 mIU/mL), luteinizing hormone (LH) (8.97 vs. 6.59 mIU/mL) and malondialdehyde (MDA) (117.12 vs. 33.52 mmol/mL), and lower testosterone (309.92 vs. 416.61 ng/dL); erectile dysfunction was reported in 45% of exposed workers versus 12.5% of the controls. Within exposed workers, copper was positively correlated with FSH and MDA and negatively correlated with testosterone. This was an occupational study involving potential inhalation exposure, but the actual airborne copper concentration was not reported. A major limitation of the study is that workers were simultaneously exposed to arsenic and lead, with substantially higher levels of these contaminants also observed in the exposed group, making it difficult to attribute the reproductive findings specifically to copper. The small sample size, cross-sectional design, lack of quantitative inhalation exposure data, and use of hormonal/biochemical endpoints rather than demonstrated impaired fertility further limit causal interpretation on the role of copper in male fertility.

Grieger et al. (2019) evaluated maternal copper status and fertility in 1 060 nulliparous women from the Adelaide SCOPE prospective cohort. Maternal plasma copper was measured at 15±1 weeks of gestation, and the fertility outcomes time to pregnancy and subfertility (>12 months to conceive) were assessed. Copper concentrations were categorized as exposure tertiles in <25.97, 25.97–34.78 and >34.78 µmol/L. No significant association was observed between copper concentrations and time to pregnancy or subfertility. Copper was measured after conception rather than before pregnancy and time to pregnancy was retrospectively reported.

7.8.1.2 7.8.1.2 Developmental toxicity

Li et al. (2018) conducted a prospective cohort study within the Ma'anshan Birth Cohort in China to investigate the association between umbilical cord serum copper concentrations and neonatal birth outcomes. The study included 2 689 mother–singleton infant pairs with available umbilical cord serum samples and detailed birth records. The median umbilical cord serum copper concentration was 298.2 µg/L (range: 123.1–699.6 µg/L). Copper concentrations were categorized into exposure tertiles, and associations with gestational age and birth outcomes were evaluated after adjustment for maternal age, pre-pregnancy body mass index, education, parity, smoking, alcohol consumption, pregnancy complications and other potential confounders. Compared with infants in the middle copper tertile, those in the lowest tertile had a significantly increased risk of preterm birth (adjusted OR ≈5.1, 95% CI: 2.7–9.3) and early-term birth (adjusted OR ≈1.4), whereas infants in the highest tertile had an increased risk of late- or post-term birth (adjusted OR ≈1.5, 95% CI: 1.1–2.0). Umbilical cord copper concentrations were positively associated with gestational duration, suggesting that both low and high foetal copper status may be associated with adverse birth outcomes. However, interpretation of the findings

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is limited by the measurement of copper at delivery rather than earlier in pregnancy and the potential for residual confounding (e.g., maternal nutritional status).

A population-based nested case–control study including 1 599 stillbirths and 9 594 live-born controls in Harris County, Texas, examined associations between maternal exposure to metal constituents of ambient $PM_{2.5}$ and stillbirth risk (Rammah et al., 2019). Exposure was estimated from chemically speciated $PM_{2.5}$ measurements from stationary monitoring sites, rather than individual measurements. No statistically significant association was observed between modelled $PM_{2.5}$-bound copper exposure and stillbirth risk for each subject's pregnancy. The median modelled copper concentration in $PM_{2.5}$ was 7.06 ng Cu/m^3. For copper, the adjusted OR for stillbirth per IQR increase was 0.93 (95% CI: 0.83 to 1.06). A main limitation of the study was the potential for exposure misclassification due to the use of a single monitoring station and the lack of individual exposure measurements.

Chen et al. (2023a) conducted a cross-sectional study of 102 mother–infant pairs in an e-waste recycling area in China to examine associations between prenatal exposure to heavy metals and birth outcomes. Copper concentrations were measured in human milk collected four weeks postpartum, with a mean copper concentration of 46.64 ± 17.73 µg/L (range 13.07–98.07 µg/L), well below the World Health Organisation (WHO) recommended range of 180–310 µg/L. After adjustment for maternal characteristics and co-exposure to other metals, no significant associations were observed between copper concentrations and gestational age, birth weight or birth length. The study was limited by its small sample size, the use of postpartum human milk as a surrogate of prenatal exposure and the limited copper exposure contrast reducing the study's power to detect an exposure–response relationship.

The prospective birth cohort study within the Jiangsu Birth Cohort in China by (Lv et al., 2024) included 1 675 parent–offspring trios. Urinary concentrations of 25 metals were measured in both parents during early pregnancy, and children were followed to one year of age for birth defects. Copper was detected in >95% of samples, with median values of 13.47 and 17.70 µg/L in paternal and maternal samples, respectively. A one-unit increase in log-transformed urinary copper was associated with an increased risk of birth defects in both fathers (adjusted RR 1.69, 95% CI: 1.27–2.25) and mothers (RR 1.48, 95% CI: 1.10–1.99), after adjustment for relevant parental and socioeconomic factors and mutual adjustment for the spouse's exposure. Joint high paternal and maternal copper exposure was also associated with an increased risk (adjusted RR 1.94, 95% CI: 1.16–3.24) compared with low exposure in both parents. However, the study assessed multiple metals simultaneously, so residual confounding and co-exposure are important considerations. In addition, urinary copper reflects relatively recent exposure and only one single urine sample was measured which may not represent exposure during the relevant developmental window and could have introduced exposure misclassification; and the number of individual birth-defect cases was relatively small which limited further analysis for specific defect subtypes.

Hernandez-Castro et al. (2024) used the prospective Project viva birth cohort in the US to investigate first-trimester metal exposure and hypertensive disorders of pregnancy. Copper and other metals were measured in red blood cells at a median gestational age of 10.1 weeks in 1 386 women; 52 (3.8%) developed preeclampsia and 94 (6.8%) developed gestational hypertension. The geometric mean erythrocyte copper concentration was 565 µg/L (median 564 µg/L; range 86.3–3,970 µg/L). A doubling of first-trimester erythrocyte copper concentration was associated with lower odds of preeclampsia (OR 0.22, 95% CI: 0.08–0.60). The association remained important in mixture analyses, with lower concentrations of the essential-metal mixture (primarily driven by copper) associated with higher odds of preeclampsia. However, the relatively small number of preeclampsia cases limits statistical power, and erythrocyte copper provides an indirect measure of overall copper exposure. During pregnancy, copper metabolism changes substantially, which can further complicate interpretation of blood copper concentrations as an exposure measure. Residual confounding, particularly by diet and nutritional status, cannot be excluded.

Monangi et al. (2024) carried out a multi country meta-analysis using data from 18 international pregnancy cohorts, comprising 11 160 pregnancies overall, of which 10 449 singleton live births had maternal plasma or serum copper measurements and gestational age data available. The

cohorts included population- or hospital-based studies, case-control samples enriched for preterm births, and two intervention cohorts. Copper was measured in maternal plasma or serum, with a mean concentration of 1.92 ± 0.43 µg/mL overall. In the meta-analysis of 10 017 pregnancies with samples collected before 28 weeks of gestation, higher maternal copper was associated with increased preterm birth (pooled OR 1.30, 95% CI: 1.08–1.57 per 1 µg/mL increase), which was stronger when copper was measured before week 16 of pregnancy compared with later sampling, and with shorter gestational duration (1.64 days shorter, 95% CI: 0.56–2.73). Associations were generally consistent across cohorts, and low-to-moderate heterogeneity was observed (I^2 = 32.8% for preterm birth and 43.9% for gestational duration). The association was somewhat stronger when samples were collected before 16 weeks of gestation. However, copper was strongly correlated with inflammatory markers and infections in the Malawi cohort, and adjustment for these factors attenuated the observed associations. Important limitations included differences in study design and gestational-age assessment, variation in timing of copper sampling, incomplete information on environmental and socioeconomic factors, inability to distinguish spontaneous from iatrogenic preterm birth, and the absence of ceruloplasmin measurements. The authors noted the possibility of reverse causation, since copper may partly act as a marker of inflammation or other processes such as HIV or malaria (reflecting acute-phase responses and infections) associated with preterm birth, rather than being a direct causal factor.

7.8.2 Animal data

7.8.2.1 Reproductive toxicity/ Fertility

Reproductive effects of copper compounds have been studied in animals after oral administration. No inhalation studies were found.

In an oral (gavage) combined repeated-dose and reproductive/developmental toxicity study (similar to OECD TG 422), Sprague-Dawley rats (12 animals/ sex/ dose) were exposed to copper monochloride at doses of 0, 1.3, 5, 20, or 80 mg/kg bw/day, corresponding to 0, 0.83, 3.2, 12.8, and 51.3 mg Cu/kg bw/day, respectively (Chung et al., 2009).

- No treatment-related effects were observed on the copulation index, fertility index, sperm parameters (count, motility, and morphology), or serum testosterone levels at any tested dose.
- No treatment-related effects were observed on the precoital interval, copulation index, fertility index, delivery index, gestation length, corpora lutea count, implantation sites, stillbirths, live births, sex ratio, viability index, or offspring body weight.
- Treatment-related dose-dependent general toxicity was observed. 25% of females died at the highest dose level. No mortality was observed in males. The systemic NOAEL was established at 5 mg/kg bw/day for males and 1.3 mg/kg bw/day for females. For details see section 7.3.2.1.

A two-generation reproductive toxicity test (OECD TG 416) was conducted with copper sulphate pentahydrate in Sprague-Dawley rats (30 animal/sex/dose) ((Study report 2005 as reported in (ECHA, 2026)). The test material was administered in the diet to parental generation (P1) males and females at concentrations of 0, 100, 500, 1 000 and 1 500 ppm for pre-mating periods of at least 70 days and continued for females until two weeks of lactation. For details on doses, see Table 28. At weaning, a group of 30 first generation (F1) pups/sex/group were selected and administered the same dose of test material in the diet as their respective P1 parental generation, with the same pre-mating period of at least 70 days before co-housing to produce the second generation (F2) litter.

- No treatment-related effects were observed on precoital interval length, mating, fertility, gestation length, number of implantation sites, or implantation efficiency at any dose level. No histopathological findings were reported,

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- No treatment-related effects were observed on sperm motility, morphology, epididymal sperm or testicular spermatid numbers in male P1 and F1 males at any dose level.
- No treatment-related effects were observed on the mean percent days in oestrus, dioestrus, or proestrus, ovarian follicle counts or mean cycle length were observed among P1 and F1 females.
- No treatment-related effects were observed on weight of testes, epididymides, right cauda epididymis, seminal vesicles, prostate, ovaries, uterus, thyroid gland.
- A NOAEL of 1 500 ppm (top dose) was reported for reproductive toxicity in all groups.

Additional non-guideline studies on the effects of copper exposure on male fertility have focussed mainly on the effects on testes and sperm in mice (Guo et al., 2021, Kheirandish et al., 2014, Sakhaee et al., 2014, Sakhaee et al., 2016a, Sakhaee et al., 2016b) and rats (Babaei and Abshenas, 2013, Sakhaee et al., 2012). These studies describe adverse effects of copper compounds (mainly copper sulphate) on sperm quality and histopathological changes in testes.

In an oral (gavage) study (Kheirandish et al., 2014), male NMRI mice (15 animals/ dose) were treated with copper sulphate (0 or 200 mg/kg) once a day for 56 days.

- Histopathological examination of the control group revealed normal seminiferous tubules, spermatogenesis, and Sertoli cells. In the copper-treated group, the seminiferous tubules were shrunken and exhibited moderate to severe degeneration of the germinal epithelium.
- Compared with the control group, the mean seminiferous tubule diameter, Sertoli cell nuclear diameter, and germinal epithelium height were significantly reduced in the Cu-treated groups ($P < 0.05$). A significant decrease in Sertoli cell nuclear diameter was also observed on day 56 compared with days 14 and 28.
- Copper treatment significantly reduced both the meiotic index and spermatogenesis compared with the control group ($P < 0.05$).

In an oral study (Sakhaee et al., 2014), investigated sperm quality and effects on testes in male NMRI mice (12 animals per dose) after treatment (gavage) with 0 and 200 mg/kg bw/day copper sulphate for 4 and 6 weeks.

- Copper treatment decreased statistically significantly motility and viability of epididymal sperm ($P < 0.05$) in comparison with control.
- The testes of the control group exhibited normal seminiferous tubules containing all stages of germ cell development, from spermatogonia to spermatozoa. In contrast, the copper-treated group showed varying degrees of seminiferous tubular degeneration. Some tubules were shrunken and exhibited epithelial vacuolation and cellular sloughing, while others lacked spermatids and spermatozoa and were lined primarily by Sertoli cells, spermatogonia, and primary spermatocytes.
- Copper treatment increased also liver enzymes and copper concentration in the liver compared with control ($P < 0.05$) as described in Table 28.

Sakhaee et al. (2016a) investigated the effects of 0 and 100 mg/kg copper sulphate administered by gavage (every other day) on sperm and testes in male NMRI mice (12 animals per dose) after 28- and 42-day treatment. Similar study design is described in (Sakhaee et al., 2016b) with an exception that 6 male NMRI mice per dose group were used and investigations performed only after a 42-day treatment.

- Sperm concentration, motility, and viability in the copper-treated group decreased significantly compared with those in the control group at weeks 4 and 6 ($P < 0.05$). In the control group, testicular sections exhibited normal seminiferous tubule morphology, with germ cells arranged in well-organized concentric layers. In the copper-treated group, seminiferous tubules showed varying degrees of degeneration, characterized primarily by depletion and vacuolation of the seminiferous epithelium. These alterations were more pronounced at 6 weeks.

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In a study by (Guo et al., 2021), ICR male mice (60 mice per dose) were administered 0, 10, 20 or 40 mg/kg copper sulphate by gavage. After 21 and 42 days of treatment, mice were sacrificed and effects on testes and sperm quality were investigated.

- Copper sulphate impaired spermatogenesis, as evidenced by reduced sperm concentration and motility and an increased sperm malformation rate. Quantitative analysis demonstrated a statistically significant increase in sperm malformation rates in all three copper-treated groups on Days 21 and 42 compared with the control group. Sperm motility was also significantly reduced in all treatment groups at both time points. In addition, sperm concentration was significantly decreased in the 20 and 40 mg/kg groups on Day 42, relative to the control group.
- Relative testis weight was significantly reduced following exposure to 20 and 40 mg/kg copper sulphate for 42 days. Histopathological examination revealed time- and dose-dependent alterations. These changes included significant reductions in spermatogonia and spermatocytes, as well as vacuolar degeneration and necrosis of spermatogenic cells. Furthermore, testicular copper concentrations were significantly elevated in the 20 and 40 mg/kg treatment groups after 42 days compared with the control group.
- Copper sulphate promoted ROS accumulation, impaired antioxidant defence mechanisms and induced DNA damage and apoptosis, while suppressing multiple DNA repair pathways, in the testes (see Table 28).

In an oral (gavage) study in male Wistar rats (Sakhaee et al., 2012), effects of copper sulphate (0, 100 and 200 mg/kg bw/d; 20 animals per group) after 56 days of exposure on sperm quality and testis.

- Sperm concentration, motility, and viability were significantly reduced in copper-treated groups compared with the control group throughout the experimental period.
- Copper treatment-induced testicular lesions included tubular shrinkage and collapse, reduced numbers of germ cells, and decreased epithelial height. In addition, the number of seminiferous tubules containing spermatozoa was reduced in the copper-treated groups.

A study by (Babaei and Abshenas, 2013) investigated effects of copper exposure on epididymal sperm quality in rats. Male Sprague-Dawley rats (12 animals per dose) received 0 or 200 mg/kg copper sulphate by gavage, once a day for 56 days.

- A non-significant reduction in testicular weight was observed after 28 days of copper exposure. After 56 days of copper exposure, testicular weight was significantly lower than in the control group. Copper exposure for 56 days significantly reduced spermatozoa count compared with controls.
- Copper exposure significantly reduced the percentage of live spermatozoa after 28 days, with an even greater reduction observed after 56 days.
- Compared with the control group, mean total sperm motility decreased significantly after 28 days of copper treatment. The effect was substantially greater following long-term exposure (56 days).

Seven et al. (2020) determined the effects of copper on testes and sperm quality. Male Wistar rats (6 animals per dose) received 0 and 128 mg/kg copper sulphate pentahydrate by gavage for 21 days.

- Compared with the control group, copper administration significantly reduced sperm motility and concentration, while significantly increasing the proportion of abnormal spermatozoa and abnormal spermatogenesis.
- In testicular tissue, copper treatment significantly increased oxidative stress and significantly reduced antioxidant defence mechanisms, and induced apoptosis.
- Testicular tissues from the control exhibited normal histoarchitecture, with intact seminiferous tubules, normal germinal epithelium, and the presence of spermatozoa,

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spermatocytes, and spermatids. In contrast, copper-treated rats showed marked histopathological alterations, including loss, disorganization, and vacuolation of the germinal epithelium, interstitial oedema, proliferation of interstitial cells, and the presence of immature spermatogenic cells within the lumen of some seminiferous tubules.

In an oral rat study (Parlak Ak et al., 2021), male Wistar rats (6 animals per dose) received 0 and 500 mg/kg bw copper sulphate pentahydrate by gavage (once per day) for 21 days.

- No significant differences were observed among the groups in the weights of the right and left testes, right and left epididymides, seminal vesicles, or the right cauda. However, sperm motility and epididymal sperm concentration were significantly lower in the copper-treated group than in the control group. The rats receiving excess copper also exhibited a significantly higher rate of abnormal sperm compared with the control group.
- Testicular sections from the control group exhibited normal, well-defined seminiferous tubules with intact germinal epithelium and orderly progression of spermatogenesis. The lumens were filled with mature spermatozoa, and the seminiferous tubules were separated by compact interstitial tissue. In contrast, the copper-treated group showed marked histopathological alterations, including seminiferous tubular shrinkage, atrophy, and structural irregularities, with widened intertubular spaces. Degeneration of the germinal epithelium, vacuolization, and substantial loss of germ cells (spermatogonia, spermatocytes, and spermatids) were observed. Spermatogenesis was disrupted, and immature spermatogenic cells were present within the lumens of some seminiferous tubules. Interstitial oedema and vascular congestion were also evident. Also, rats receiving excessive copper demonstrated significantly lower Johnsen Testicular Biopsy Score values than the control group (p <0.05).
- Copper-treatment induced oxidative stress and reduced antioxidant defence mechanisms. Copper induced apoptosis as shown by strong caspase-3 immunoreactivity in spermatogonia and spermatocytes.

In an oral study (Liu et al., 2016), male Wistar rats (10 animals per dose) were administered daily 0, 100, 200 and 400 mg/kg bw copper sulphate by gavage for 30 days.

- Sperm counts decreased dose-dependently throughout the experiment in all copper-treated groups and were significantly lower than in control group. Conversely, sperm malformation rates increased over time following copper treatment. Malformation rates were significantly higher at 200 and 400 mg/kg bw than in control.
- Serum testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) concentrations decreased in copper-treated groups throughout the experimental period. LH and FSH levels in the low-dose group were significantly lower than those in the control group.
- Throughout the experiment, copper exposure was associated with increased oxidative stress and decreased activity of antioxidant enzymes.

Sarawi et al. (2022) investigated the effects of copper sulphate (0 and 100 mg/kg) on male reproductive system after daily gavage for 7 days in male Wistar rats (8 animals per dose).

- Compared with controls, copper-treated rats showed significantly reduced serum LH, FSH, and testosterone levels.
- Copper administration induced marked degeneration of the seminiferous tubules, disruption of the spermatogenic lineage, and complete absence of mature spermatozoa.
- Copper sulphate significantly reduced the expression of all steroidogenic genes and increased oxidative stress, reduced antioxidant defence mechanisms and induced apoptosis in testes.

In an oral rat study (Abdel-Baky, 2019), male Albino rats (6 animals per dose) were administered 0, 100 and 200 mg/kg bw copper sulphate by daily gavage for 2 weeks.

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- Treatment with copper sulphate resulted in a significant reduction in the serum concentrations of total testosterone, FSH, LH and prolactin compared with the corresponding control group.
- Rats administered copper sulphate exhibited significantly elevated levels of renal function markers (serum concentrations of urea, uric acid and creatine) and liver enzymes (ALP, AST and ALT) compared with controls. The increases were more pronounced at the higher dose, indicating a dose-dependent effect.

Limited number of studies have investigated the effects of inorganic copper compounds on female fertility.

Chen et al. (2023b) investigated effects of copper exposure on ovarian folliculogenesis in rats. Female Wistar rats (10 animals per dose) were administered 0, 6, 12 and 25 mg Cu^{2+}/kg by gavage as copper sulphate pentahydrate, once a day for 35 days, until sexual maturity.

- The observed effects suggest that copper exposure can impair ovarian development. Copper exposure significantly decreased the proportion of preantral follicles and increased the proportion of atretic follicles. The proportion of antral follicles increased in the 6 mg/kg group but decreased at 12 and 25 mg/kg. In addition, the proportion of corpora lutea was significantly elevated in the 12 and 25 mg/kg groups.
- Transmission electron microscopy showed that in the control group, granulosa cells exhibited normal morphology, with regular cellular arrangement, homogeneous chromatin distribution, and intact intercellular junctions, and no pathological changes were observed. In contrast, the 12 mg/kg group showed swelling of the rough endoplasmic reticulum, chromatin condensation, disruption of intercellular junctions, and the presence of apoptotic bodies. In the 25 mg/kg group, both necrotic cells and apoptotic bodies were detected. The apoptotic rate of ovarian granulosa cells decreased in the 6 mg/kg group but increased significantly in the 12 and 25 mg/kg groups compared with controls. Collectively, these findings indicate that copper exposure impairs ovarian development, at least in part, through the induction of granulosa cell apoptosis.
- The expression of apoptosis-related mRNAs and proteins in the ovaries of copper-exposed rats suggests that low-dose copper exposure (6 mg/kg) suppresses apoptosis, whereas higher doses (12 and 25 mg/kg) promote apoptotic activity.

An additional study on female reproductive system includes an oral study in mice. Babaei et al. (2012) investigated the effects of 0, 100 and 200 mg/kg copper sulphate on ovaries after daily gavage for 14 and 35 days.

- After 14 days, treatment with 100 mg/kg copper sulphate resulted in a significant reduction in the number of antral follicles compared with the control group, while no significant changes were observed in other follicular classes.
- In contrast, treatment with 200 mg/kg copper sulphate markedly reduced the numbers of all follicular classes, including primordial, primary, growing, secondary, and antral follicles, as well as corpora lutea, compared with controls. Histomorphometric analysis further demonstrated that the 200 mg/kg dose significantly decreased the mean numbers of all follicle classes and corpora lutea on Day 14 and/or Day 35.

Table 28: Summary of studies on reproductive toxicity of inorganic copper compounds

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
Copper monochloride				
Combined	Rat,	0, 1.3, 5, 20	No effects on:	(Chung et al.,

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
repeated dose and reproductive/ developmental toxicity study Oral (gavage) TG not specified	Sprague-Dawley Male and female 12 animals per sex per dose	and 80 mg/kg bw/day Males (exposure): 30 days beginning 14 days before mating Females (exposure): 14 days before mating, throughout the mating and gestation period to day 3 of lactation Copper monochloride	• the copulation index, fertility index, sperm parameters (count, motility, and morphology), or serum testosterone levels • the precoital interval, delivery index, gestation length, corpora lutea count, implantation sites, stillbirths, live births, sex ratio, viability index, or offspring body weight	2009)
Copper sulphate/ copper sulphate pentahydrate				
Two-generation reproductive toxicity (OECD TG 416) Oral (diet)	Rat, Sprague-Dawley Male and female 30 animals per sex per dose	Concentrations in feed: 100, 500, 1 000, 1 500 ppm, corresponding to: 1.53, 7.7, 15.2 and 23.6 mg Cu/kg/day P1 males during premating; 1.92, 9.6, 19.1 and 29.5 mg Cu/kg/day P1 females during premating; 1.67, 8.6, 17.0 and 26.2 mg Cu/kg/day P1 females during gestation; 3.39, 17.7, 33.8 and 55.7 mg Cu/kg/day P1 females during the first 2 weeks of lactation;	No effects on: • the copulation index, fertility index, sperm parameters (count, motility, and morphology) • the precoital interval, gestation length, number of implantation sites, or implantation efficiency	Study report 2005, as cited in (ECHA, 2026)

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
		2.25, 11.5, 23.5 and 36.1 mg Cu/kg/day F1 males during premating; 2.65, 13.3, 26.7 and 43.8 mg Cu/kg/day F1 females during premating; 1.69, 8.5, 17.1 and 26.5 mg Cu/kg/day F1 females during gestation; 3.27, 17.6, 35.2 and 55.4 mg Cu/kg/day F1 females during the first 2 weeks of lactation.		
Study on male fertility Oral (gavage) Non-TG	Mouse, NMRI Male 15 animals per dose	0 and 200 mg/kg 56 days Copper sulphate	Histopathology of testes • Shrunken seminiferous tubules and moderate to severe degeneration of the germinal epithelium in the copper-treated animals • Controls animals had normal seminiferous tubules, spermatogenesis, and Sertoli cells Morphometry in copper- treated animals • The mean seminiferous tubule diameter, Sertoli cell nuclear diameter, and germinal epithelium height ↓ • the meiotic index and spermatogenesis ↓	(Kheirandish et al., 2014)
Study on male fertility Oral (gavage) Non-TG	Mouse, NMRI Male 12 animals per dose	0 and 200 mg/kg bw/d 4 and 6 weeks Copper sulphate	Copper treatment had effects on liver enzymes, sperm parameters and testes histopathology Liver enzymes at 6 weeks • AST and ALT ↑ Copper concentration • Serum and liver ↑	(Sakhaee et al., 2014)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			Sperm parameters • Motility and viability of epididymal sperm ↓ Histopathology of testes • varying degrees of seminiferous tubular degeneration • Some tubules were shrunken and exhibited epithelial vacuolation and cellular sloughing, while others lacked spermatids and spermatozoa and were lined primarily by Sertoli cells, spermatogonia, and primary spermatocytes. • Control testes normal	
Study on male fertility Oral (gavage) Non-TG	Mouse, NMRI 12 animals per dose (2016a) 6 animals per dose (2016b)	0 and 100 mg/kg 28 and 42 days, (2016a) 42 days (2016b) Exposure every other day Copper sulphate	Copper exposure had effects on sperm and histopathology of testes Sperm parameters • Sperm concentration, motility, and viability ↓ Histopathology • seminiferous tubules showed varying degrees of degeneration, characterized primarily by depletion and vacuolation of the seminiferous epithelium • Control testes normal	(Sakhaee et al., 2016a, Sakhaee et al., 2016b)
Study on male fertility Oral (gavage) Non-TG	Mouse, ICR Male 60 mice per dose	0, 10, 20 or 40 mg/kg Daily for 21 and 42 days Copper sulphate	Copper exposure had effects on sperm parameters and testes Sperm parameters • Sperm motility ↓ and sperm malformation rate ↑ at all dose levels on Day 21 and 42 p < 0.05 or 0.01 • Sperm concentration ↓ at 20 and 40 mg/kg on Day 42 p <0.05 or 0.01 Weight and histopathology of testes • Relative weight ↓ on Day 21 and 42 P < 0.05 • Spermatogonia and spermatocytes ↓ • Vacuolar degeneration and necrosis of spermatogenic cells ↑	(Guo et al., 2021)

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• Copper concentration in testes ↑ at 20 and 40 mg/kg on Day 42 P <0.05 or 0.01 Oxidative stress in testes • ROS ↑ at all dose levels on Day 21 and 42 P <0.05 or 0.01 • SOD activity ↓ at 20 and 40 mg/kg on Day 21 and at all dose levels on Day 42 P <0.05 or 0.01 • Catalase activity ↓ at 40 mg/kg on Day 21 and at all dose levels on Day 42 P <0.05 or 0.01 • GSH-Px activity ↓ at 20 and 40 mg/kg on Day 21 and 42 P <0.05 or 0.01 • GSH levels ↓ at 40 mg/kg on Day 21 and at all dose levels on Day 42 P <0.05 or 0.01 Apoptosis in testes • Apoptotic cells ↑ at all dose levels on Day 21 and 42 P <0.01 • Anti-apoptotic protein Bcl-2 and its mRNA ↓ P <0.05 or 0.01 • Pro-apoptotic proteins: Bax, cleaved caspase-3, -8, -9 and 12, and cleaved PARP and their mRNA ↑ P <0.05 or 0.01 DNA damage in testes • γ-H2AX levels ↑ at 20 and 40 mg/kg on Day 21 and at all dose levels on Day 42 P <0.01 DNA repair in testes • Expression of MMR genes: MLH1 and MSH6 proteins ↓. No effect on MSH1 protein expression. • Expression of genes in the BER pathway (*Ogg1*, *Ape1* and *Lig3*) ↓. No effect on XRCC1 expression. • Expression of proteins in NER pathway (XPA, XPB,	

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			XPF, XPG, ERCC1, and RAD23B) ↓. No effect on XPC expression. • Expression of proteins in the homologous recombination (HR) pathway (BRCA1, BRCA2, RAD50, RAD51, FANCD2, and PALB2) ↓ • Expression of proteins in the non-homologous end joining (NHEJ) pathway (Ku70, Ku80, XRCC4, LIG4 and DNA-PK) ↓	
Study on male fertility Oral (gavage) Non-TG	Rat, Wistar Male 20 animals per dose	0, 100 and 200 mg/kg bw/d Daily for 56 days Copper sulphate	Copper exposure had effects on sperm parameters and testis histopathology Sperm parameters • Sperm concentration, motility and viability ↓ at all dose levels P <0.05 Histopathology in the testes (at all dose levels) • Tubular shrinkage and collapse ↑ • Number of germ cells and epithelial height ↓ Number of seminiferous tubules containing spermatozoa ↓	(Sakhaee et al., 2012)
Study on male fertility Oral (gavage) Non-TG	Rat, Sprague-Dawley Male 12 animals per dose	0 and 200 mg/kg Daily for 56 days Copper sulphate	Copper exposure had effects on testicular weight and sperm parameters Testes • Weight ↓. Non-significant at 28 days, significant at 56 days (p =0.002) Sperm parameters • Spermatozoa ↓ P =0.001 (56 days) • Percentage of live spermatozoa ↓ (28 and 56 days) P <0.001) • Mean total sperm motility ↓ (28 and 56 days) P <0.001)	(Babaei and Abshenas, 2013)
Study on male fertility Oral (gavage) Non-TG	Rat, Wistar Male 6 animals per dose	0 and 128 mg/kg Daily for 21 days Copper sulphate pentahydrate	Copper exposure had effects on sperm parameters and caused oxidative stress and apoptosis in testes Sperm parameters • Sperm motility ↓ P <0.01	(Seven et al., 2020)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• Sperm concentration ↓ P <0.05 • Abnormal spermatozoa ↑ P <0.01 • Abnormal spermatogenesis as Johnsen's score ↓ P <0.05 Oxidative stress in testes • MDA ↑ P <0.001 • GSH and activities of SOD and catalase ↓ P <0.001 Histopathological changes in testes • loss, disorganization, and vacuolation of the germinal epithelium, interstitial oedema, proliferation of interstitial cells, and the presence of immature spermatogenic cells within the lumen of some seminiferous tubules ↑ • no histopathological changes in controls Apoptosis in testes • Active caspase-3 positive cells ↑	
Study on male fertility Oral (gavage) Non-TG	Rat, Wistar Male 6 animals per dose	0 and 500 mg/kg bw Daily for 56 days Copper sulphate penthydrate	Copper treatment increased oxidative stress and affected sperm parameter and caused histopathological changes in testes Reproductive organs • No effects on the weights of testes, epididymides, seminal vesicles or the right cauda Oxidative stress • MDA ↑ (p<0.001) • GSH levels and activity of SOD ↓ (p<0.001) Sperm parameters • Sperm motility ↓ (p<0.01) • Epididymal sperm concentration ↓ (p<0.05) • Rate of abnormal sperm ↑ (p<0.01) Histopathology	(Parlak Ak et al., 2021)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
			• Seminiferous tubular shrinkage, atrophy, and structural irregularities, with widened intertubular spaces ↑ • Degeneration of the germinal epithelium, vacuolization, and substantial loss of germ cells (spermatogonia, spermatocytes, and spermatids) were observed ↑ • Spermatogenesis was disrupted, and immature spermatogenic cells were present within the lumens of some seminiferous tubules. Interstitial oedema and vascular congestion were also evident • Control testes histologically normal Apoptosis • Cleaved Caspase-3 immunoreactivity ↑ in spermatogonia and spermatocytes	
Study on male fertility Oral (gavage) Non-TG	Rat, Wistar Male 10 animals per dose	0, 100, 200 and 400 mg/kg bw Daily for 30 days Copper sulphate	Copper treatment affected sperm parameters and had effects on reproductive hormones and increased oxidative stress Sperm parameters • Sperm counts ↓at all dose levels ($p<0.5$ or 0.01) • Sperm malformations ↑ at 2 highest dose levels Reproductive hormones • Serum testosterone, FSH and LH ↓ at all dose levels ($p<0.05$ or 0.01) Oxidative stress • Activity of GSH-Px and SOD ↓ at all dose levels ($p<0.05$ or 0.01) • Concentration of MDA ↑ ($p<0.05$ or 0.01)	(Liu et al., 2016)
Study on male fertility Oral (gavage) Non-TG	Rat, Wistar Male 8 animals per dose	0 and 100 mg/kg Daily for 7 days Copper	Copper exposure reduced the levels of reproductive hormones, expression of steroidogenic genes, caused oxidative stress and apoptosis Reproductive hormones	(Sarawi et al., 2022)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
		sulphate	• Testosterone, LH and FSH ↓ ($p<0.001$) Expression of steroidogenic genes: • StAR, 3β-HSD, CYP17A1 and 17β-HSD protein levels ↓ ($p<0.001$) • AR protein ↓ ($p<0.001$) Oxidative stress • MDA and NO levels ↑ ($p<0.001$) • GSH, SOD and CAT↓ ($p<0.001$) • Nrf2, HO-1 and TNF-α protein ↓ in testes ($p<0.001$) • NF-kB p65 and iNOS mRNA ↑ in testes ($p<0.001$) Apoptosis • Testicular Bax and cleaved caspase-3 expression ↑ ($p<0.001$) • Bcl-2 and PCNA ↓ ($p<0.001$) Histopathology • Degeneration of the seminiferous tubules, disruption of the spermatogenic lineage, and complete absence of mature spermatozoa • Control rats had normal testes histology	
Study on male fertility Oral (gavage) Non-TG	Rat, Albino Male 6 animals per dose	0, 100 and 200 mg/kg bw Daily for 2 weeks Copper sulphate	Copper exposure had effects on reproductive hormones and renal function Reproductive hormones • Serum concentration of total testosterone, FSH, LH and prolactin ↓ Renal function • Serum concentration of urea, uric acid and creatinine ↑ Liver • ALP, AST and ALT enzyme activity ↑	(Abdel-Baky, 2019)
Study on female fertility Oral (gavage) Non-TG	Rat, Wistar Female 10 animals per dose	0, 6, 12 and 25 mg Cu^{2+}/kg Daily for 35 days Copper sulphate	Copper had adverse effects on ovaries and caused apoptosis Ovaries • Ovarian coefficients ↓ at 2 highest dose levels $p<0.05$) • Proportion of preantral follicles ↓	(Chen et al., 2023a)

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
		pentahydrate	• Proportion of atretic follicles ↑ • Proportion of antral follicles ↑ at the lowest dose level and ↓ at 2 highest dose levels ($p<0.05$) • Proportion of corpora lutea ↑ at 2 highest dose level ($p<0.05$) Apoptosis • 12 mg/kg: swelling of the rough endoplasmic reticulum, chromatin condensation, disruption of intercellular junctions, and the presence of apoptotic bodies • 25 mg/kg: both necrotic cells and apoptotic bodies were detected • 6 mg/kg: Apoptotic rate ↓ • 12 and 25 mg/kg: Apoptotic rate ↑ ($p<0.05$) • The expression of apoptosis-related mRNAs (*Casp3* and *Casp8*) and proteins (cleaved caspase-3, cleaved caspase-8, BCL2, BAX and FAS) suggest that low dose copper (6 mg/kg) suppresses apoptosis and higher doses (12 and 25 mg/kg) promote apoptotic activity	
Study on female fertility Oral (gavage) Non-TG	Mouse, NMRI Female 6 animals in control group 12 animals per copper dose	0, 100 and 200 mg/kg Daily for 14 and 35 days Copper sulphate	Copper exposure had adverse effects on ovaries Ovaries • Number of antral follicles ↓ at 100 mg/kg P =0.043 • Numbers of all follicular classes, including primordial, primary, growing, secondary, and antral follicles, as well as corpora lutea ↓ at 200 mg/kg Serum • Copper concentration ↑ at 200 mg/kg	(Babaei et al., 2012)

Notes: ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BER:

Base excision repair; FSH: Follicle-stimulating hormone; GSH: glytathione; GSH-Px: glutathione peroxidase; LH: Luteinizing hormone; MDA: Malondialdehyde; MMR: DNA mismatch repair; NER: Nucleotide Excision Repair; ROS: Reactive oxygen species; SOD: superoxide dismutasehyde.

7.8.2.2 Developmental toxicity

A limited number of studies investigating the effects of inorganic copper compounds on developmental toxicity is available. No inhalation studies were found.

In a pre-natal developmental toxicity study (OECD TG 414), pregnant New Zealand White rabbits were exposed via the oral route to copper hydroxide at doses of 6, 9, and 18 mg Cu/kg bw/day during gestation days 7-28 ((Study report 2003, as cited in (ECHA, 2026)).

- Severe maternal toxicity effects, including deaths were reported at the highest dose, and additional signs of maternal toxicity (diarrhoea, body weight loss, reduced food consumption) were also observed in the mid-dose group.
- No effects on the number of foetuses, numbers of early and late embryonic deaths, or on sternebral ossification were observed.
- Developmental effects observed in foetuses at the highest dose included lower mean foetal weight, delayed ossification of skull and pelvic bones, increased incidences of hemivertebra, and supernumerary ribs. In the robust study summary, a reference is also made to "*an increased incidence of a common skeletal variant in foetuses at 9 mg/kg bw/day*".
- The lowest dose of 6 mg Cu/kg bw/day was in the robust study summary ((Study report 2003, as cited in (ECHA, 2026)) considered as the NOAEL for maternal and developmental effects.

A two-generation reproductive toxicity test (OECD TG 416) was conducted with copper sulphate pentahydrate in Sprague-Dawley rats (30 animal/sex/dose) ((Study report 2005 as cited in (ECHA, 2026)). The test material was administered in the diet to parental generation (P1) males and females at concentrations of 0, 100, 500, 1 000 and 1 500 ppm for pre-mating periods of at least 70 days and continued for females until two weeks of lactation. For details, see section 7.8.2.1.

- No treatment-related effects were reported on number of pups born alive on Day 4, 7, 14, or 21, sex ratio, and survival indices during the lactation period in F1 and F2 litters.
- No treatment-related effects on body weights and clinical observations in F1 and F2 litters during lactation or on age at preputial separation in F1 males and vaginal opening in F1 females.
- Decreased spleen weights were reported in F1 and F2 male and female weanlings (and in P1 adult females) of the high-dose (1 500 ppm) group. These findings were considered adverse, but in the robust study summary ((Study report 2005 as reported in (ECHA, 2026)) it is suggested that this may have been a transient physiological alteration. No microscopical examination of the spleens was conducted.
- 1 000 ppm was considered as the NOAEL.

In an oral study similar to OECD TG 422 (Chung et al., 2009), some developmental effects of monochloride copper were observed. The study design is described under Section 7.8.2.1, above.

- The incidence of pups with gross lesions, specifically icterus, was significantly higher in the 80 mg/kg group than in the vehicle control group. In addition, the number of runt pups was significantly increased at 80 mg/kg compared with controls. These effects were observed at dose level causing marked toxicity in maternal animals, i.e., 25% of females died. No mortality was observed in males. The systemic NOAEL was established at 5 mg/kg bw/day for males and 1.3 mg/kg bw/day for females. For details see section 7.3.2.1.

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In a study by (Kadammattil et al., 2018), pregnant female Swiss Albino mice received by the oral route, 0 or 10 mg/kg bw/d copper sulphate once per day for 7 days; gestational day (GD) 7 to GD 12. Animals were sacrificed on GD 18.

- No differences in the number of implantations, viability of embryos, resorption, mean body weight of embryos and frequency of anomalies between copper sulphate and control groups.

Haddad et al. (1991) and (Kasama and Tanaka, 1988) studied effects of copper exposure in neonatal mice. Mice were exposed to copper either by direct or indirect administration. In the direct exposure group, neonatal male mice (C3H/HeN, the number of animals not specified) received copper chloride (5 µg copper per 1 g bw) by intraperitoneal injection at 7 and 10 days of age and were subjected to analysis at 13 days of age. Control neonatal males received physiological saline. In the indirect exposure group, female mice (C3H/HeN, the number of animals not specified) were divided to 3 groups. Group 1 females were exposed to copper via drinking water (6 ppm copper as copper sulphate) from GD 13 to delivery (late gestational period) and during the lactational period (5 ppm copper in the drinking water). Group 2 females received copper during the late gestational period (similar to group 1) and received tap water during the lactational period. Group 3 females served as controls and received only tap water.

- Direct administration of copper: No significant differences in neonatal body weight were observed between the copper and control groups at 7, 10, or 13 days of age, nor were there differences in tissue wet weights at 13 days. Thus, two intraperitoneal copper injections did not affect neonatal growth.
- Indirect administration of copper: Maternal exposure to 6 ppm copper during late gestation did not affect foetal growth. Similarly, no significant differences were observed in tissue wet weights. However, offspring in the group 1 exhibited reduced postnatal growth relative to the other two groups, as reflected by lower body weights and tissue wet weights. These findings indicate that maternal exposure to 5 ppm copper during lactation adversely affected neonatal growth. Consistent with this observation, protein concentrations in the liver and kidney were also reduced in these animals. Changes in copper concentrations were tissue specific. Maternal copper administration increased foetal cerebral copper levels, and elevated concentrations persisted in neonatal cerebrum for at least 13 days after birth, regardless of maternal drinking water during lactation. In contrast, hepatic copper concentrations were increased only in the group 1, indicating that copper exposure during lactation contributed to copper accumulation in the liver.

Table 29: Summary of studies on developmental toxicity of inorganic copper compounds

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
Copper monochloride				
Combined repeated dose and reproductive/ developmental toxicity study Oral (gavage) TG not specified	Rat, Sprague-Dawley Male and female 12 animals per sex per dose	0, 1.3, 5, 20 and 80 mg/kg bw/day Males (exposure): 30 days beginning 14 days before mating Females (exposure):	At 80 mg/kg bw/d: • the incidence of pups with gross lesions, specifically icterus ↑ the number of runt pups ↑	(Chung et al., 2009)

Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
		14 days before mating, throughout the mating and gestation period to day 3 of lactation Copper monochloride		
Neonatal developmental toxicity study Intraperitoneal injection Non-TG	Mouse, C3H/HeN Male (neonatal) Number of animals not specified	0 and 5 µg of copper per 1 g bw Single exposure at 7 or 10 days of age Copper chloride	Neonatal body weight • Copper had no effects Oxidative stress • Activity of SOD and cytochrome oxidase ↑ in the cerebrum and lived of copper exposed mice Organ copper concentration • Copper accumulated transiently in the cerebrum, liver and kidney at Day 3 but returned to control level by Day 23	(Kasama and Tanaka, 1988)
Copper sulphate				
Two-generation reproductive toxicity (OECD TG 416) Oral (diet)	Rat, Sprague-Dawley, Male and female 30 animals per sex per dose	Concentrations in feed: 100, 500, 1 000, 1 500 ppm, corresponding to: 1.53, 7.7, 15.2 and 23.6 mg Cu/kg/day in P1 males during premating; 1.92, 9.6, 19.1 and 29.5 mg Cu/kg/day P1 females during premating; 1.67, 8.6, 17.0 and 26.2	Decreased spleen weights reported in F1 and F2 male and female weanlings at 1 500 ppm. No effects on number of foetuses, numbers of early and late embryonic deaths, or on sternebral ossification.	Study report 2005, as cited in (ECHA, 2026)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
		mg Cu/kg/day P1 females during gestation; 3.39, 17.7, 33.8 and 55.7 mg Cu/kg/day P1 females during the first 2 weeks of lactation; 2.25, 11.5, 23.5 and 36.1 mg Cu/kg/day F1 males during premating; 2.65, 13.3, 26.7 and 43.8 mg Cu/kg/day F1 females during premating; 1.69, 8.5, 17.1 and 26.5 mg Cu/kg/day F1 females during gestation; 3.27, 17.6, 35.2 and 55.4 mg Cu/kg/day F1 females during the first 2 weeks of lactation. Copper sulphate pentahydrate		
Pre-natal developmental toxicity study Oral (not specified) Non-TG	Mouse, Swiss Albino Female Number of animals not specified	0 and 100 mg/kg bw/d Daily for 7 days on GD 7 to GD 12 Animals sacrificed on GD 18 Copper sulfate	No effects on the number of implantations, viability of embryos, resorption, mean body weight of embryos and frequency of anomalies	(Kadammattil et al., 2018)
Neonatal developmental toxicity study	Mouse, C3H/HeN	Group 1: 6 ppm copper in	Foetal growth	(Kasama and Tanaka, 1988)

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Method, route	Species, strain, sex, number/ group	Doses, duration of exposure, test material	Results	References
Oral (drinking water) Non-TG	Female Number of animals not specified	drinking water from GD 13 to delivery and 5 ppm copper in drinking water during lactation period Group 2: 6 ppm copper in drinking water from GD 13 to delivery and tap water during lactation period Group 3: tap water from GD 13 to delivery and tap water during lactation period.	• Copper had no effects Postnatal growth ↓ • Maternal exposure to copper during lactation reduced postnatal growth • Protein concentration ↓ in liver and kidney Organ copper concentration • Concentration in foetal cerebral ↑ Hepatic concentration ↑ only when copper exposure was during gestation and lactation.	
Copper hydroxide				
Pre-natal developmental toxicity (OECD TG 414) Oral (gavage)	Rabbit, New Zealand White, Female (pregnant) 22 animals per sex per dose	6, 9, 18 mg Cu/kg bw/day Exposure on days 7-28 of gestation.	Maternal toxicity, including deaths (at top dose), diarrhoea, bw loss, reduced food consumption. Lower mean foetal weight, delayed ossification of skull and pelvic bones, increased incidences of hemivertebra, and supernumerary ribs in foetuses.	Study report 2003, as cited in (ECHA, 2026)

7.8.3 Summary

Human evidence suggests a possible adverse effect of occupational copper exposure on male reproductive function, including altered reproductive hormones and erectile dysfunction, but the evidence is insufficient and limited by few small studies, co-exposure to other metals and lack of quantitative exposure data. In women, no association between plasma copper concentrations and time to pregnancy or subfertility was observed. Studies with male animals report effects on seminiferous tubules and sperm. However, no effects on functional fertility were observed in two-generation and combined repeated-dose and reproductive/developmental toxicity studies.

Overall, the available evidence for developmental toxicity of copper is inconsistent to establish a causal relationship between copper exposure and developmental toxicity in humans. The reported findings, particularly for preterm birth and birth defects, warrant consideration, but interpretation is complicated by copper being an essential element, differences in exposure

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biomarkers and timing, co-exposure to other metals, and residual confounding by nutritional, inflammatory/infectious and socioeconomic factors.

8. Other considerations

8.1 Mode of action (MoA) considerations

Copper is an essential trace element required for numerous physiological processes; however, excessive copper exposure can disrupt cellular homeostasis and result in toxicity. The copper ion itself is the effective agent in the inorganic copper compounds (DFG, 2014).

Under physiological conditions, intracellular copper concentrations are tightly regulated by membrane transporters (ATP7A and ATP7B), copper chaperone proteins and metallothionein, which maintain very low concentrations of free copper ions (Bull et al., 1993, Linder and Hazegh-Azam, 1996, Vulpe et al., 1993). Adverse health effects occur when these homeostatic mechanisms are overwhelmed, leading to an increase in the intracellular pool of unbound copper capable of participating in redox reactions (ATSDR, 2024, SCOEL, 2014).

The primary mechanism underlying copper toxicity is oxidative stress (Gaetke et al., 2014). Excess intracellular copper catalyses the formation of ROS through redox cycling, resulting in lipid peroxidation, protein oxidation and oxidative DNA damage (WHO, 1998). In addition, copper binds to sulfhydryl groups on proteins, altering enzyme activity, impairing mitochondrial function and disrupting cellular energy metabolism, which may ultimately lead to apoptosis or necrosis (Gaetke and Chow, 2003, Linder and Hazegh-Azam, 1996). The liver is particularly susceptible to these effects because it is the primary organ responsible for copper storage and homeostasis, although the kidneys and central nervous system may also be affected when copper accumulation exceeds normal regulatory capacity, as demonstrated in Wilson disease (Bull et al., 1993, WHO, 1998).

For inhalation exposure, which is primarily relevant in occupational settings, the initial adverse effects are considered to result from the direct interaction of deposited copper-containing particles with the respiratory epithelium. Local oxidative stress and inflammatory responses are thought to underlie the irritation of the upper respiratory tract and the reversible respiratory symptoms reported in exposed workers, including coughing, sneezing, nasal irritation and thoracic discomfort (Askergren and Mellgren, 1975, Suciu et al., 1981). Occupational biomonitoring studies have shown that systemic copper concentrations generally remain within the normal physiological range despite airborne exposure, indicating that homeostatic mechanisms effectively limit systemic accumulation under typical workplace conditions (Finelli, 1981, Kossowska et al., 2010, Kossowska et al.). More recent investigations in copper smelter workers have likewise found no clinically relevant impairment of lung function or persistent inflammatory responses at contemporary exposure levels, supporting the conclusion that the critical effects of occupational copper exposure are primarily local and concentration-dependent rather than systemic (Haase et al., 2021, Haase et al., 2022). Overall, the available evidence indicates that copper toxicity results from disruption of physiological homeostasis and subsequent oxidative cellular injury rather than from a specific receptor-mediated mechanism.

8.2 Lack of specific scientific information

No sub-chronic or chronic *in vivo* inhalation studies were found.

8.3 Groups at extra risk

No groups at extra risk were identified.

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9. Evaluation and recommendations

9.1 Derived Occupational Exposure Limit (OEL) values

9.1.1 Published approaches to establish OELs

9.1.1.1 DFG (2014)

Deutsche Forschungsgemeinschaft ((German Research Foundation (DFG)) (DFG, 2014) reviewed scientific data and concluded that local effects on the respiratory tract are the critical effects after inhalation exposure to copper compounds.

(DFG, 2014) noted the report of (Gleason, 1968) in workers, which presented that no effects were to be expected at copper concentrations of 0.008 mg/m^3. (DFG, 2014) considered that this acute exposure study of workers (Gleason, 1968), the 28-day rat inhalation study ((ICA, 2010, as cited in (DFG, 2014, AGS, 2025, SCOEL, 2014)) [10], together with the five or 10-day inhalation study in mice by (Drummond et al., 1986) were all in agreement with regard to the target organ (the lung, and in particular the alveolar macrophages), and the marked effects elicited in the same concentration range (0.12–0.36 mg Cu/m^3).

(DFG, 2014) selected the 28-day rat inhalation study with dicopper oxide as the key one and identified a LOAEC of 0.17 mg Cu/m^3 for lung inhalation and a calculated NOAEC of 0.06 mg Cu/m^3 (using an assessment factor of three). This was considered as a point of departure (PoD). The human equivalent concentration (HEC)-PoD was calculated on the basis of this study using the Multiple-Path Particle Dosimetry (MPPD) model at 0.012 mg Cu/m^3. (DFG, 2014) assumed slow clearance and large differences in retention between rats and humans (T½ of 400 days for humans and 60 days for rats). Regarding the alveolar surface area (ASA) values, (DFG, 2014) used the ratio of human to rat as 193 (ASA_{Rat}: 2 970/ASA_{Human}: 572 200).

Based on the considerations above, (DFG, 2014) established an 8h-TWA value for the respirable fraction for copper and its inorganic compounds of 0.01 mg/m^3 (no additional assessment factors used). An 8h-TWA for the inhalable fraction was not proposed because "*it is not possible to give a conclusive assessment of the effects in the nose which occurred at 0.2 mg Cu_2O/m^3 (0.17 mg copper/m^3) in the 28-day inhalation study with rats (ICA, 2010)*". No value was recommended for the inhalable fraction.

For short-term exposure (peak limitation), (DFG, 2014) set category II (i.e., substances with absorption) with an exceedance factor of 2, meaning that transient peaks should not exceed twice the 8-h TWA of 0.01 mg Cu/m^3.

According to (DFG, 2014), copper and its inorganic compounds do not necessitate a "skin" notation.

9.1.1.2 SCOEL (2014)

The European Commission's Scientific Committee on Occupational Exposure Limits (SCOEL) reviewed the toxicological data available and recommended an 8 h-TWA for copper and its inorganic compounds.

SCOEL noted that the acute-exposure human study by (Gleason, 1968) established a NOAEC of 0.008 mg Cu/m³ but was not considered robust for 8h-TWA derivation. A 28-day inhalation study in rats exposed to dicopper oxide was used instead ((ICA, 2010, as cited in (SCOEL, 2014)). The most sensitive parameter was an increase in neutrophils in the bronchoalveolar lavage fluid (indicator of lung inflammation) (ICA, 2010). From this study, SCOEL considered a LOAEC of 0.178 mg Cu/m^3 for lung inflammation at all exposure levels, and a NOAEC of 0.067 mg Cu/m^3 as PoD was calculated (after applying an assessment factor of three). Further, SCOEL calculated a HEC of 0.012 mg Cu/m^3 using the MPPD model.

[10] A recent publication (Poland et al. 2022) describes a short-term 28-day inhalation study (OECD TG 412) study on dicopper oxide in rats. DFG, 2014; SCOEL, 2014 and AGS, 2025 refer to the same study as ICA, 2010.

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No further assessment factors were used and an 8h-TWA of 0.01 mg Cu/m^3 for the respirable fraction was established. This value should not be applied to copper nanoparticles due to their particularly high toxicity in pulmonary epithelial cells (SCOEL, 2014). The same 8h-TWA value for the respirable fraction was established by (DFG, 2014).

A potential OEL for the inhalable fraction was considered based on subacute nasal inflammation observed in one male rat at 0.17 mg/m^3 ((ICA, 2010, as cited in (SCOEL, 2014)). It was however concluded that recommending an inhalable OEL was not feasible "*because: (1) only five animals per group were examined; (2) a time-related increase cannot be ruled out, since no animals were affected at the 3-week interim sacrifice up to 1.7 mg/m^3; and (3) the study used the respirable fraction, whereas higher nasal deposition would be expected with the inhalable fraction.*"

Furthermore, a NOAEC of 0.36 mg/m^3 for acute sensory irritation in humans (Gleason, 1968) was considered by SCOEL. However, the metal fume fever-like symptoms observed in employees exposed to copper dust at 0.12–0.36 mg/m^3 were considered to be primarily dependent on concentration or on total dose (concentration * time product). Due to numerous uncertainties, no STEL was recommended.

SCOEL considered the dermal uptake of copper compounds to be low. A "skin" notation was therefore not recommended.

SCOEL did not set any biological limit values because "*First, there is a large range of variation of mean and individual background serum or plasma copper concentrations in the European population. Second, inhalation exposure to copper concentrations up to 1 mg Cu/m^3 did not result in significantly or dose-dependent elevated copper plasma concentrations compared to controls (Finelli et al., 1981, Kossowska 2010 as cited in (SCOEL, 2014)). After oral exposure up to 6 mg/l drinking water, there was no significant elevation of serum copper levels. This concentration already produced local effects. Therefore, copper levels in blood are no suitable biomarker, presumably due to tight copper homeostasis. Also in the case of urinary copper, there is a wide variation in urinary copper levels among normal (occupationally unexposed) population ((Minoia et al., 1990 as cited in (SCOEL, 2014)). No data were available on the relationship between occupational exposure and urinary copper levels.*"

9.1.1.3 AGS (2025)

More recently, Ausschuss für Gefahrstoffe (AGS) reviewed the available evidence on copper inhalation effects and established 8h-TWAs for both respirable and inhalable fractions using animal and human data (AGS, 2025).

For the inhalable fraction (E-dust), occupational studies with long term follow-up were considered for 8h-TWA OEL derivation. In the cohort study of Haase et al. (2021), 326 copper smelter workers were employed for 22 years and exposed to a mean cumulative concentration of inhalable dust of 4.61 mg Cu/m³ × years. Their average exposure was 0.21 mg Cu/m^3, which was considered as PoD for the OEL derivation. No significant exposure-related respiratory effects were identified at this concentration. Therefore, an 8h-TWA for the inhalable fraction of 0.2 mg Cu/m^3 was set. In addition, an exceedance limit of 4 for short-term exposure to the inhalable fraction was set.

For the respirable fraction (A-dust), the 28-day inhalation study with dicopper oxide ((ICA, 2010, as cited in (AGS, 2025)) was used with a PoD of 0.2 mg/m^3 (considered as a NOAEC, contrary to (SCOEL, 2014, DFG, 2014) who interpreted this dose (0.17 mg/m^3) as a LOAEC). Using a HEC approach and MPPD modelling a HEC value of 0.09 mg Cu/m^3 was derived. For HEC modelling, the specific parameters (i.e., rat ventilatory parameters, alveolar deposition rate, and normalization values) were similar to (DFG, 2014), with a lower ratio of ASA between rats and humans. Yet, contrary to (DFG, 2014), a clearance factor of 1 for soluble particles was used. For the HEC value of 0.09 mg Cu/m^3, a default assessment factor of 6 was applied to extrapolate from sub-acute to chronic exposure, resulting in a value of 0.015 mg Cu/m^3. However, for the setting of a limit value, human studies (Haase et al., 2021, Haase et al., 2022), were given preference. An 8h-TWA for the respirable fraction was derived from the inhalable fraction 8h-TWA (E-dust) of 0.2 mg Cu/m^3, adjusted for a respirable-to-inhalable dust ratio of 23% as

determined by (Haase et al., 2021). The final 8h-TWA for the respirable fraction resulted in 0.045 mg Cu/m^3. An exceedance limit of 8 for short-term exposure to the respirable fraction was set.

9.1.2 8h-TWA value

The local lung effects observed after repeated inhalation exposure are considered the critical effects to be considered for the setting of an 8h-TWA value (IOELV) for copper and inorganic compounds. For this type of effects, and considering different occupational settings and types of exposure, it could be useful to introduce separate IOELVs for the respirable and inhalable fractions.

9.1.2.1 Respirable fraction

No human studies that could be used for the derivation of an 8h-TWA value for respirable copper were identified. In addition, no chronic or sub-chronic animal studies with copper or inorganic copper compounds could be found. Therefore, the 28-day rat inhalation study with dicopper oxide (add ref) can be used as the key study for the 8h-TWA derivation. Effects were reported already at the lowest dose of 0.2 mg/m^3, corresponding to 0.18 mg Cu/m^3, (see section 7.3.2.2) which can be considered a LOAEC and can be used as a starting point. This dose was converted to a NOAEC by applying a standard factor of 3 for LOAEC to NOAEC, resulting in 0.06 mg Cu/m^3.

Following a similar approach as earlier taken by (AGS, 2025, DFG, 2014, Poland et al., 2026, SCOEL, 2014), HEC was calculated from NOAEC 0.06 mg Cu/m^3. The MPPD model (version 3.04) was used to calculate the human deposited dose rate and inhaled dose per day using the same specific modelling parameters (including, e.g., ASA_{Rat}: 4 000 cm^2/ASA_{Human}: 1 430 000 cm^2) as described in the Supplementary information of the paper by (Poland et al., 2026).

From the model, human-equivalent doses of 0.92 mg Cu/day in males and 0.7 mg Cu/day were derived (average 0.81 mg Cu/day).

Using the average dose of 0.1 mg/Cu/day as PoD, the 8h-TWA value calculations include the following steps:

1) Considering the standard workday inhalation volume of 10 m^3:

HEC: 0.81 mg Cu/day / 10 m^3/day = 0.081 mg Cu/m^3.

2) Assessment factor proposed to be applied: a standard factor of 6 for extrapolation for the duration from sub-acute to chronic exposure.

Application of this factor results in an **8h-TWA value for the respirable fraction** based on local respiratory effects as follows:

8h-TWA value = 0.081 mg Cu/m^3 / 6 = 0.0135 mg Cu/m^3 ≈ **0.01 mg Cu/m^3**

Considering the human occupational cohort study data (Haase et al., 2021, Haase et al., 2022), in which no exposure-related respiratory effects were reported at an average exposure level of 0.21 mg Cu/m^3 (as inhalable fraction; corresponding to a respirable fraction of 0.045 mg Cu/m^3 on the basis of the respirable:inhalable dust ration of 23% as reported by the authors), no additional assessment factors are proposed.

As the effects are expected to be caused by the copper ion, this 8h-TWA value can be applied to the respirable fraction of copper and inorganic copper compounds. However, with reference to the considerations by (Poland et al., 2026), the HEC approach and derived value might not be appropriate for nanosized copper due to the potentially higher lung deposition of such small particles.

9.1.2.2 Inhalable fraction

As explained above and in section 7.3.1.1., recent human study data (Haase et al., 2021, Haase et al., 2022) found no significant effect of chronic exposure on lung function or inflammation at

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average exposure levels of 0.21 mg Cu/m^3 (inhalable fraction). The studies have some limitations in particular concerning the study design and exposure assessment (see section 7.3.1.1), but in the absence of other robust data for the inhalable fraction (including animal studies), the NOAEC of 0.21 mg Cu/m^3 (inhalable) could be used as PoD.

For the derivation of an 8h-TWA value for the inhalable fraction, an assessment factor of 2 is proposed to cover uncertainties related to the study design.

Application of this factor results in an **8h-TWA value for the inhalable fraction** based on local respiratory effects as follows:

8h-TWA value = 0.21 mg Cu/m^3 / 2 = **0.1 mg Cu/m^3**

As the effects are expected to be caused by the copper ion, this 8h-TWA value can be applied to the inhalable fraction of copper and inorganic copper compounds.

9.1.3 OEL – Short-Term Exposure Limits (STELs)

The available data does not indicate a need to propose a STEL.

9.1.4 Biological Limit Value (BLV)

There are methods available to measure blood and urinary levels of copper, but biomonitoring has not been commonly used at workplaces. The quantitative data on the absorption of inhaled copper in humans and correlations between workplace air concentration and copper concentrations in urine or blood are limited. It is assumed that due to the homeostatic regulation of copper, no increased blood copper or urine are expected for exposures in the range of the air limit value. No BLV is proposed.

9.1.5 Biological Guidance Value (BGV)

If a biological guidance value (BGV) is recommended, it should relate to background copper exposure in the general population and not those occupationally exposed to copper. Copper is an essential element and is subject to strong homeostatic regulation; consequently, concentrations in blood and urine do not directly reflect external exposure over a wide exposure range.

The HBM4EU Rapid Response Mechanism compiled human biomonitoring data from 13 European countries (with significant heterogeneity between datasets including different sampling methods, age distributions, and an overrepresentation of women, including pregnant women) and found that copper concentrations in the general population were generally within established clinical reference ranges (HBM4EU, 2018) (see section 6.2.1).

Therefore, urinary copper appears to provide the more appropriate matrix for describing population background levels, although it is also influenced by urine dilution and excretion rate. Based on the available HBM4EU data, a BGV of 60 µg/L urinary copper can be proposed as a pragmatic upper reference level corresponding to the upper limit of the established normal range and encompassing the P95 concentrations observed in almost all general-population datasets.

9.2 Notations

The dermal absorption of copper and inorganic copper compounds is low and therefore no ‘skin’ notation is proposed.

No evidence of skin or respiratory sensitisation has been reported in humans occupationally exposed to copper or in animal studies. Therefore, no ‘skin sensitisation’ or ‘respiratory sensitisation’ notation is proposed.

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Appendix 1. List of REACH registrations on copper and its inorganic compounds

Table 30: REACH registered Copper and its inorganic compounds and estimated tonnage bands.

EC/List number	Chemical name	CAS number	Estimated tonnage band [11] (in tonnes)
208-883-6	Copper cyanide	544-92-3	100 to 1 000
215-269-1	Copper oxide	1317-38-0	10 000 to 100 000
215-270-7	Dicopper oxide	1317-39-1	1 000 to 10 000
215-271-2	Copper sulphide	1317-40-4	100 to 1 000
215-572-9	Dicopper chloride trihydroxide	1332-65-6	1 000 to 10 000
215-582-3	Tetracopper hexahydroxide sulphate	1333-22-8	100 to 1 000
221-838-5	Copper dinitrate	3251-23-8	1 000 to 10 000
231-159-6	Copper	7440-50-8	1 000 000 to 10 000 000
231-210-2	Copper dichloride	7447-39-4	100 to 1 000
231-674-6	Copper iodide	7681-65-4	100 to 1 000
231-842-9	Copper chloride	7758-89-6	100 to 1 000
231-847-6	Copper sulphate	7758-98-7	10 000 to 100 000
232-131-6	Copper bromide	7787-70-4	intermediate use only
232-167-2	Copper dibromide	7789-45-9	10 to 100
233-279-4	Diphosphoric acid, copper salt	10102-90-6	10 to 100
234-634-6	Copper dichromium tetraoxide	12018-10-9	100 to 1 000
235-285-2	Dicopper hydroxide phosphate	12158-74-6	10 to 100
243-815-9	Copper dihydroxide	20427-59-2	1 000 to 10 000
244-842-9	Dicopper sulphide	22205-45-4	100 to 1 000
253-959-4	Copper(2+) tetrafluoroborate(1-)	38465-60-0	10 to 100
266-964-1	Cement copper	67711-88-0	10 000 to 100 000
266-967-8	Matte, copper	67711-91-5	100 000 to 1 000 000
269-053-7	Copper chromite black spinel	68186-91-4	1 000 to 10 000
273-744-9	Scale (coating), copper	69012-45-9	intermediate use only
273-752-2	Electrolytes, copper-manufg., spent	69012-54-0	10 000 to 100 000
273-836-9	Speiss, copper	69029-97-6	10 000 to 100 000
273-925-2	Lead, dross, copper-rich	69227-11-8	1 000 to 10 000
439-590-3	copper(2+) hydroxide nitrate	12158-75-7	1 000 to 10 000

[11] Calculated from the Article 10 registration data taking into account the manufactured tonnage, imports and exports, the tonnage used as intermediate and confidentiality requests.

EC/List number	Chemical name	CAS number	Estimated tonnage band [11] (in tonnes)
701-554-2	Copper and silver nitrates and nitric acid from electrochemical refining of silver anodes	-	intermediate use only
884-197-6	Copper(I, II) sulfite	15293-86-4	1 to 10
910-356-7	Reaction mass of copper oxide and manganese dioxide	-	10 to 100
910-437-7	Reaction mass of copper oxide and zinc oxide	-	10 to 100
910-628-5	Reaction mass of copper oxide and dicopper oxide		intermediate use only
910-853-9	A mixture, with or without stabilising agents, of calcium hydroxide and copper(II) sulphate	-	100 to 1 000
912-664-7	Reaction mass of cobalt and copper and iron	-	intermediate use only
912-666-8	Reaction mass of copper and iron and zinc	-	1 to 10
918-168-7	Anode, copper	-	100 000 to 1 000 000
918-452-0	black copper, copper smelting	-	10 000 to 100 000
919-583-6	cupro, copper processing	-	10 000 to 100 000
922-642-9	Cement, purification of copper electrolyte	-	intermediate use only
943-528-5	Precipitate from tellurium containing acid solutions by copper metal cementation	-	100 to 1 000
944-726-4	Reaction mass of copper oxide and magnesium oxide and silicon dioxide and calcium oxide		intermediate use only
951-951-1	sodium copper sulphate, sodium zinc chloride, zinc sulphate, recovery products from copper and zinc dusts	-	intermediate use only
951-963-7	copper sulphide and zinc chloride sulphate hydroxide,		intermediate use only

EC/List number	Chemical name	CAS number	Estimated tonnage band [11] (in tonnes)
	recovery products from copper and zinc dusts		
955-817-3	Amorphous barium copper zinc borobismuthate	-	1 to 10
955-840-9	copper zinc trihydroxide nitrate	-	1 to 10

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