

Section A6.12.2(5)

Annex Point II A6.12.2

Human Case Report*specify subsection number***273.4 Examinations****A6.12.2(5): Indian Childhood Cirrhosis***give type of examination and time after exposure for each examination*

Percutaneous liver biopsy specimens were obtained from 19 children in India diagnosed with a variety of liver diseases. Eight children were from Pune, 8 from Madras, and one from Benares, Bombay, and Vellore.

Part of the specimen was fixed and transported in 10% formol saline. Histological examination was done by B.P. without knowledge of the clinical details. Staining methods were described in a previous publication.

A second part of the specimen was placed in an acidwashed vial, deep frozen within 12 h, and freeze-dried. The liver cores were weighed and digested in concentrated nitric acid. Copper content was estimated by atomic absorption spectrophotometry with a flame technique and expressed as $\mu\text{g/g}$ dried tissue.

A preliminary study showed that there was negligible contamination of samples with copper from the biopsy needle ('Trucut', Travenol Laboratories).

*give any available information on the medical treatment of intoxicated persons***273.5 Treatment**

Treatment was not discussed in this publication.

273.6 Remarks

None

274 RESULTS*Describe findings. If appropriate, include table.
Describe any relevant effects observed***274.1 Clinical Signs**

Clinical signs were not reported.

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specify subsection number

A6.12.2(5): Indian Childhood Cirrhosis

274.2 Results of examinations

Describe the results of e.g. clinical chemistry, blood analysis and urinalysis

A histological diagnosis of Indian Childhood Cirrhosis (ICC) was made in 5 of the 19 patients. Clinically, these children formed a homogeneous group. All were Hindu children aged 14-21 months in whom abdominal distension had been first noticed 2-3 months previously. One child was anicteric, while the other 4 had had jaundice for 1-4 weeks before biopsy. All had hepatosplenomegaly; the liver edge was 4-10 cm below the costal margin and sharp, and the liver was strikingly hard. All were anaemic (Hb 5.5-9.5 g/dl).

The other 14 children had a variety of hepatic disorders (see Table A.6.12.2(4)-1).

Orcein Staining and Copper Content

The 5 patients with ICC, but none of the others, showed massive intracellular granular orcein staining. In all 5 cases, the hepatic copper concentration was >1000 µg/g dry weight (1045-2303). Normal values for adults and for children aged 1-2 years do not exceed 55 µg/g dry weight.

Hepatic copper was slightly raised in 2 of the remaining 14 children. In patient 16, a 6-year-old girl with chronic active hepatitis and cirrhosis, in whom the copper level was 292 µg/g dry tissue, scanty orcein-staining granules were confined to the periportal regions and the periphery of regenerating nodules. In patient 9, a 16-year-old boy with post-hepatitic scarring, in whom the copper level was 170 µg/g dry tissue, only very occasional orcein-stained granules were seen.

Hepatic copper concentration was normal in the other 12 patients. Orcein-stained granules were not observed, although in 2 patients the presence of hepatitis B surface antigen was demonstrated by homogeneous cytoplasmic staining of scattered hepatocytes.

274.3 Effectivity of medical treatment

Treatment was not discussed in this publication.

274.4 Outcome

Describe outcome / manifestation of symptoms / disease

Outcome was not discussed in this publication.

274.5 Other

Describe any other significant observations

None.

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A6.12.2(5): Indian Childhood Cirrhosis

**275.1 Materials and
methods**

275 APPLICANT'S SUMMARY AND CONCLUSION

Briefly describe circumstances

Percutaneous liver biopsy specimens were obtained from 19 children in India diagnosed with a variety of liver diseases. Eight children were from Pune, 8 from Madras, and one from Benares, Bombay, and Vellore.

Part of the specimen was fixed and transported in 10% formol saline. Histological examination was done by B.P. without knowledge of the clinical details. Staining methods were described in a previous publication.

A second part of the specimen was placed in an acid-washed vial, deep frozen within 12 h, and freeze-dried. The liver cores were weighed and digested in concentrated nitric acid. Copper content was estimated by atomic absorption spectrophotometry with a flame technique and expressed as $\mu\text{g/g}$ dried tissue.

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A6.12.2(5): Indian Childhood Cirrhosis

275.2 Results and discussion

Summarize relevant results

The hepatic copper concentration of $1389 \pm 525 \mu\text{g/g}$ dry weight in the 5 patients with ICC greatly exceeds both the expected value in normal infants in the second year of life ($<55 \mu\text{g/g}$ dry weight), and the values in the 14 Indian children with other types of liver disease ($3\text{-}292 \mu\text{g/g}$). Only in these 5 patients were there wide spread and massive orcein-stained granules. These results are thus further evidence that the orcein-stained material is a copper-binding protein, and that orcein staining is a reliable indicator of hepatic copper content.

In hepatic disorders in which cholestasis is a prominent feature, failure of biliary excretion of copper causes hepatic accumulation, the copper concentration being proportional to the duration of cholestasis. Cholestasis, however, cannot be the reason for the findings reported here, since none of the 5 cases had been jaundiced for more than 4 weeks and none had cholestasis apparent on histological examination.

Despite its earlier age of presentation and its more rapid progression ICC bears some resemblances to Wilson's disease. The copper values presented here are similar to those found by other researchers. Renal tubular abnormalities and haemolysis are common to the two conditions. The familial incidence of ICC suggests that it may resemble Wilson's disease in being an inherited metabolic disorder. However, the familial incidence could equally well be caused by environmental factors common to siblings, and this is made more likely by the absence of the condition in Indian children born outside the Asian subcontinent. It is therefore important to investigate the possibility that ICC is a disease caused by increased copper ingestion, perhaps similar to "copper toxicosis" of sheep; when sheep graze on copper-contaminated pastures there is a long phase of symptomless hepatic copper accumulation which ends in crisis as copper is released from the liver. This mechanism, which has also been postulated in patients with Wilson's disease who present with acute hepatic failure and haemolysis is an attractive model for the rapid clinical deterioration which characterises the late stages of ICC. There are only scattered reports of chronic copper poisoning in man, but one example, a child who died at 18 months of cirrhosis due to copper overload believed to be caused by a high copper intake in drinking water bears many resemblances to patients with ICC. A search for the causes of excessive copper ingestion is therefore urgently

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required: drinking water, traditional or Ayurvedic medicines which are given to infants and pregnant mothers, soil, and food cooked in *bittall*, tinned-copper vessels, are obvious

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*specify subsection number***A6.12.2(5): Indian Childhood Cirrhosis****275.3 Conclusion***Give general conclusions*

Tanner *et al* (1979) studied 19 children in India who had liver disease and investigated the hepatic copper concentration from liver biopsy samples. Five of the children were diagnosed as having Indian Childhood Cirrhosis (ICC). Liver disease in the remaining 14 cases included post-hepatic scarring, hepatitis, hepatic fibrosis and a possible case of glycogen storage disease. The hepatic copper content in the five ICC cases was strikingly high (1045–2303 $\mu\text{g/g}$ dry weight) the normal range being 15–55 $\mu\text{g/g}$. In the remaining 14 cases, two subjects had slightly elevated hepatic copper levels (170 and 292 $\mu\text{g/g}$ dry weight) and the rest were within the normal range. The authors concluded that the high hepatic copper concentration may be caused by excessive copper ingestion or an abnormality of copper metabolism. No data were provided in this study regarding copper intake of the ICC cases.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[REDACTED]
Materials and Methods	[REDACTED]
Results and discussion	[REDACTED]
Conclusion	[REDACTED]
Reliability	[REDACTED]
Acceptability	[REDACTED]
Remarks	
COMMENTS FROM ... (specify)	
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>Discuss if deviating from view of rapporteur member state</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

Table A.6.12.2(4)-1 Histological features and hepatic copper concentration in 19 cases of liver disease in Indian children.

Histological Diagnosis	Patient's age	Orcein staining	Copper (µg/g dry weight)
ICC			
1	1.5	Massive deposits	1121
2	1.2	Massive deposits	2303
3	1.75	Massive deposits	1363
4	0.9	Massive deposits	1112
5	1.5	Massive deposits	1045
Post-hepatic scarring			
6	6	Negative	15
7	7	Negative	9
8	5	Negative	45
9	16	Very occasional small granules	170
10	6	HBsAg in scattered cells	3
11	8	Negative	25
12	2.25	Negative	22
13	1.5	Negative	42
Chronic active hepatitis			
14	10	Negative	45
15	3	HBsAg in scattered cells	27
16	6	Scanty periportal deposits	292
Acute unresolved hepatitis			
17	9	Negative	24
Congenital hepatic fibrosis			
18	2.8	Negative	21
Possible glycogen storage disease			
19	2	Negative	30

Section A6.12.2(6)

Annex Point IIA6.12.2

Human Case Report*specify subsection number***A6.12.2(6): Acute self poisoning**Official
use only**276.1 Reference****276 REFERENCE***Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages)
If necessary, copy field and enter other reference(s).*

Chuttani HK, Gupta PS, Gulati S, & Gupta DN. (1965) Acute Copper Sulfate Poisoning. Am J Med, 39: 849-854 (published).

**277 GUIDELINES AND QUALITY ASSURANCE
(NOT APPLICABLE)****2***Form (e.g. powder, grain size, particle size/distribution), purity*

Copper Sulphate

278.1 Substance**MATERIALS AND METHODS***Give any available information on substance/product, such as identity, physical***278.2 Persons exposed**

278.2.1 Sex

Of the 48 patients who sought admission to the Irwin Hospital New Delhi:
32 males and 16 females

278.2.2 Age/weight

Of the 48 patients who sought admission to the Irwin Hospital New Delhi:
The age range was 14 to 60 years (average 23.6 years). The majority (71%) of patients were between 16 and 25 years of age.

No information on weight of patients is given.

278.2.3 Known Diseases

State if any; state, if healthy

Not reported.

278.2.4 Number of persons 53 (see point 3.2.5)

278.2.5 Other information The study comprises 53 subjects: 48 who sought admission to the Irwin Hospital, New Delhi and 5 from whom autopsy material was made available after they had died of acute copper sulphate poisoning in other hospitals in the city.

278.3.2 Frequency of exposure

278.3 Exposure

Oral

*Indicate respective route, delete other routes*278.3.1 Reason of exposure *occupational, accidental, abuse, attempted suicide*

Attempted suicide.

Single/multiple specify frequency

Not reported.

278.3.3 Overall time period *if applicable*

278.3.3 Overall time period *if applicable*
of exposure Not reported.

278.3.4 Duration of single *if applicable*
exposure Not reported.

278.3.5 Exposure concentration/dose *measured, estimated, not available*
give all available information
Reliable information regarding the quantities of copper sulphate ingested was not available. According to statements obtained from the patients, the amount varied from 1g to 4 ounces (113g). The poison, in the form of crystals or powder, was swallowed with water.

278.3.6 Other information None.
give type of examination and time after exposure for each examination

278.4 Examinations

For each patient admitted to the hospital a detailed form was used to record the clinical features and to maintain regular progress notes. Efforts were made to determine the quantity of copper sulphate ingested. In each patient vomit was tested for the presence of copper sulphate, using ammonium hydroxide; the urine was examined for various abnormal constituents, especially urobilinogen and haemoglobin; and blood was tested for haemoglobin, red blood cell count, total and differential leukocyte counts, reticulocyte count, blood urea, serum bilirubin, zinc sulphate turbidity, alkaline phosphatase, serum glutamic oxalacetic transaminase and prothrombin time. The direct-reacting (ionic) fraction of plasma copper, total serum copper and whole blood copper were also estimated. A fractional test meal was given to 20 patients and a barium meal study carried out in 16. A liver biopsy was performed in 33 patients and a kidney biopsy, was performed in 4. Autopsy material was available in 9 cases.

278.5 Treatment

give any available information on the medical treatment of intoxicated persons
Treatment procedures are not discussed.

278.6 Remarks

Background

At the time of this publication acute copper sulphate poisoning was common in Delhi and was implicated in about one-third of the cases of poisoning encountered in the Irwin Hospital, New Delhi. Copper sulphate was a cheap, readily available substance used mostly in whitewashing and the leather industry. It was quite commonly employed as a suicidal poison among people in the lower income group in India.

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Human Case Report

specify subsection number

A6.12.2(6): Acute self poisoning

279 RESULTS

Describe findings. If appropriate, include table.

279.1 Clinical Signs

Describe any relevant effects observed

See point 4.2 for clinical signs.

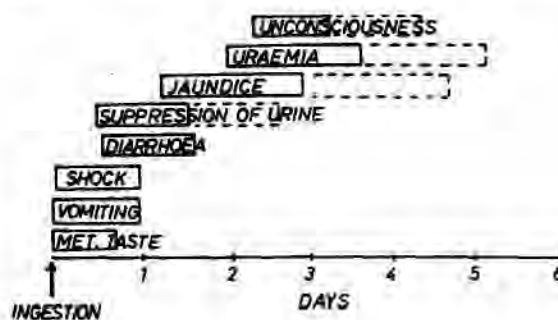
279.2 Results of examinations

Describe the results of e.g. clinical chemistry, blood analysis and urinalysis

The symptoms presented by patients are given in the table below:

Clinical feature	No of cases	% of cases
Metallic taste, nausea, vomiting, and burning in epigastrium	48	100
Diarrhoea	14	29.1
Jaundice	11	23.0
Haemoglobinuria and/or haematuria	14	29.1
Anuria	13	27.0
Oliguria	5	10.3
Hypotension	4	8.3
Coma	4	8.3
Melaena	1	2.1

A diagrammatic representation of the order of appearance of various manifestations after ingestion of copper sulphate is presented in the figure below:



Detailed description of each clinical finding follows:

Gastrointestinal Symptoms

Metallic taste, nausea, burning in the epigastrium and repeated vomiting of greenish material were the chief symptoms.

The episodes of vomiting ranged from 2 to 10. In the first few episodes the vomit was greenish blue due to the presence of copper sulphate. The vomiting usually subsided with stomach washout, although nausea and anorexia persisted.

Diarrhoea occurred in 14 patients, usually appearing on the first or second day and lasting for a maximum of 24 hours. On average, 4 or 5 loose stools were passed. Only one patient had frank melaena.

The cause of the gastrointestinal symptoms is presumed to be injury of the mucosa by copper sulphate. The fractional

test meal given to 20 patients on the third or fourth day showed the presence of blood in all patients and decreased free and total acidity in 10. Barium meal studies performed in 16 patients during the second week of the hospitalisation did not demonstrate any roentgenologic evidence of injury; however, erosions of gastric mucosa, if there were any, could have healed by this time. The gastric mucosa from patients who had died was stained green and showed superficial and deep erosions.

The small intestine showed areas of haemorrhage. Microscopic examination of the stomach disclosed haemorrhage in the mucosa as well as oedema and congestion of the vessels in the sub mucosa; mucosal ulcers were also observed.

Jaundice

Jaundice appeared in 11 patients (23%). It invariably occurred on the second or third day. In those who died the jaundice had deepened gradually until the time of death, but in the other patients it lasted for only 2 to 5 days. These patients can be divided into two groups. In the first group consisting of 5 patients jaundice was deep, the liver was enlarged and tender, the average serum bilirubin was 11.2 ± 8.9 mg/100 ml and the results of liver function tests showed gross derangement. The liver biopsy in these patients showed centrilobular necrosis and biliary stasis. In the one patient in this group who died, examination of the liver at autopsy revealed severe histologic changes.

In the second group consisting of 6 patients jaundice was mild, the liver was not palpable or just palpable, and non-tender. The results of liver function tests were within the normal range but the reticulocyte count was slightly increased and the urobilinogen was markedly increased or haemoglobinuria was present. In these patients the biopsy specimen of liver was normal histologically. In one patient in this group who died, the liver on microscopic examination showed dilatation of the sinusoids and central veins. In the first group the predominant cause of the jaundice appeared to be toxic liver injury, whereas in the second group it was intravascular haemolysis.

There was a third group of eight patients in whom jaundice was not present and the serum bilirubin was within normal limits but the urobilinogen was increased markedly and the reticulocyte count was increased (average 3.5 ± 1.3 %). Biopsy specimens of liver in these patients showed no

pathologic changes. The haemolytic reaction may have been so mild that it did not give rise to an increase in serum bilirubin.

Haemoglobinuria and Haematuria

The urine became smoky or red-coloured on the second or third day in 14 patients. Examination revealed the presence of red blood cells (6 patients) and haemoglobin (8 patients). Both manifestations usually cleared by the fourth or fifth day. Eight patients also had jaundice and in all 14 suppression of urine developed with a fatal outcome in 3.

Suppression of Urine

Significant oliguria was observed in 5 patients and anuria occurred in 13. Anuria appeared within 24 to 48 hours after the ingestion of copper sulphate and lasted from 12 to 36 hours (average 23.7 ± 7.9 hours) in patients who survived. The blood urea increased in 7 patients and ranged from 47 to 395 mg/100 ml (average 118.1 ± 122.1 mg/100 ml). Before the onset of anuria and even subsequently the blood pressure remained normal. Therefore, hypotension could not have been the cause of the suppression of urine. Biopsy specimens of kidney tissue showed swelling or necrosis of the tubular cells in 2 patients. In the four patients in the group with suppression of urine who died, the kidneys on gross examination were swollen and congested. On histologic examination there was congestion of glomeruli, necrosis and denudation of tubular cells, and in some instances haemoglobin casts were present. The presence of haemoglobin casts suggests that the kidney lesion could have been due to excessive haemolysis.

Hypotension

Hypotension occurred in four patients, on the first day except in one patient. The exact cause of shock is not known. It could have been due to oligoemia resulting from excessive vomiting and diarrhoea. Plasma volume studies were not carried out in these patients, but the vomiting and diarrhoea were not of a degree severe enough as ordinarily results in hypotension. Shock was of poor prognostic significance as three patients of the four died.

Coma

Coma occurred in four patients, developing on the second day or later. Probably it was due to uraemia resulting from renal damage. The blood urea values in these patients were high (mean 118 mg/ 100 ml). Two patients recovered and

the other two died. It seems unlikely that copper sulphate has a direct toxic effect on the brain as those patients in whom uraemia did not develop remained conscious even though serum copper levels were high. None of the patients investigated in this study showed any evidence of extrapyramidal lesions, suggesting that acute copper sulphate poisoning does not injure basal ganglia.

Deaths

Of the 48 patients who sought admission to the Irwin Hospital New Delhi, seven died (14.6 %). Deaths occurring within 24 hours after ingestion were due to shock, whereas deaths occurring at a later stage appeared to be due to hepatic or renal complications or both.

279.3 Effectivity of medical treatment

Medical treatment was not discussed in this publication.

279.4 Outcome

Describe outcome / manifestation of symptoms / disease

Seven of the 48 patients who sought admission to the Irwin Hospital New Delhi died.

279.5 Other

Describe any other significant observations

Copper Estimation

Serum total copper was estimated in 41 patients, whole blood copper in 20 and direct reacting (ionic) copper in 14. Although the serum total copper levels were increased in patients with copper sulphate poisoning, no correlation could be established between the levels of serum copper and the severity of symptoms. However, a significant relationship was found between the levels of whole blood copper and the severity of the disease, as the values of whole blood copper in mild cases were $287 \pm 126.8 \mu\text{g} / 100 \text{ ml}$ and in severe cases $798 \pm 396.4 \mu\text{g} / 100 \text{ ml}$.

The values for serum ionic copper were about seven times higher in patients with poisoning than in normal subjects. The levels of serum ionic copper were much higher ($257.2 \pm 144.2 \mu\text{g} / 100 \text{ ml}$) when determined within 12 hours after ingestion of the poison than the values obtained ($23.4 \pm 12.4 \mu\text{g} / 100 \text{ ml}$) when the tests were performed at a later time. Furthermore, the initially high levels of serum ionic copper were considerably lower when tests were repeated on subsequent days.

280.1 Materials and methods

280 APPLICANT'S SUMMARY AND CONCLUSION

Briefly describe circumstances

The study comprises fifty-three subjects: forty-eight who sought admission to the Irwin Hospital, New Delhi and five from whom autopsy material was made available after they had died of acute copper sulphate poisoning in other hospitals in the city.

For each patient admitted to the hospital a detailed form was used to record the clinical features and to maintain regular progress notes. Although efforts were made to determine the quantity of copper sulphate ingested, the information provided often was not reliable because those who had attempted suicide hesitated to divulge the exact quantities they had taken.

In each patient vomit was tested for the presence of copper sulphate, using ammonium hydroxide; the urine was examined for various abnormal constituents, especially urobilinogen and haemoglobin; and blood was tested for haemoglobin, red blood cell count, total and differential leukocyte counts, reticulocyte count, blood urea, serum bilirubin, zinc sulphate turbidity, alkaline phosphatase, serum glutamic oxalacetic transaminase and prothrombin time.

The direct-reacting (ionic) fraction of plasma copper, total serum copper and whole blood copper were also estimated in 14, 41 and 20 patients respectively.

A fractional test meal was given to 20 patients and a barium meal study carried out in 16. A liver biopsy was performed in 33 patients and a kidney biopsy, was performed in 4. Autopsy material was available in 9 cases.

Summarize relevant results

The most frequent symptoms observed in subjects were metallic taste, nausea, epigastric burning and vomiting. In addition, diarrhoea was reported in 14 patients (29%). Biopsy examination of fatalities indicated deep erosions in gastric mucosa, haemorrhage in the stomach and small intestine and oedema in the sub mucosa. Jaundice of variable severity occurred in 11/48 cases (23%). In the more severe cases, palpable liver enlargement, significantly elevated serum glutamic oxaloacetic transaminase (SGOT, 252.4 ± 142 IU) and elevated bilirubin (112 ± 8.9 mg/litre) were observed. Biopsy examination of liver tissue from fatalities showed centrilobular necrosis and biliary stasis. Postmortem examination also indicated swollen and congested kidneys with glomerular swelling and necrosis of tubular cells. Anuria was reported in 13/48 patients (27%) and oliguria in 5/48 (10%). Red discoloration of urine was observed, with haemoglobinuria confirmed in some patients. These findings suggest haemolysis and are consistent with other reports.

Author's discussion: Although the serum total copper levels were increased in patients with copper sulphate poisoning, no correlation could be established between the levels of serum copper and the severity of symptoms. However, a significant relationship was found between the levels of whole blood copper and the severity of the disease. The values for serum ionic copper were about seven times higher in patients with poisoning than in normal subjects. The initially high levels of serum ionic copper were considerably lower when tests were repeated on subsequent days. From these observations it was concluded that after ingestion of copper sulphate, copper is absorbed quickly from the gut and starts to move almost immediately at a rapid rate into the red blood cells where it achieves a very high concentration.

**280.2 Results and
discussion**

280.3 Conclusion

Give general conclusions

This study investigates 48 cases, including 7 fatalities, admitted to one hospital in New Delhi and 5 fatalities reported to other New Dehli hospitals. Special reference was made to clinical features and to biochemical and histopathologic injury. An autopsy was performed in nine fatal cases.

The most frequent symptoms observed in subjects were metallic taste, nausea, vomiting and epigastrical burning. In addition diarrhoea was reported in 14 patients. There were superficial or deep ulcerations of gastric and intestinal mucosa. Histologic examination of the liver revealed dilatation of central veins, varying degrees of liver cell necrosis and bile thrombi. In the kidneys there was congestion of glomeruli, swelling or necrosis of tubular cells, and in some cases haemoglobin casts were observed.

The levels of serum total copper, serum ionic copper and whole blood copper were found to be much higher in these patients than in normal subjects. A significant correlation was found between the levels of whole blood copper and the severity of manifestations.

Estimated quantities of copper ingested were based on patients accounts and therefore are unreliable.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	██████████
Materials and Methods	██
Results and discussion	██
Conclusion	██
Reliability	█
Acceptability	██████████
Remarks	

COMMENTS FROM ... (specify)

Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>Discuss if deviating from view of rapporteur member state</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>

Conclusion

Discuss if deviating from view of rapporteur member state

Remarks

Human Case Report

specify subsection number

A.6.12.2(7): Chronic self poisoning

Official
use only

281.1 Reference

281 REFERENCE

*Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages)
If necessary, copy field and enter other reference(s).*

O'Donohue JW, Reid MA, Varghese A, Portmann B, Williams R (1993) Micronodular cirrhosis and acute liver failure due to chronic copper self-intoxication. Eur. J. Gastroenterol. 5:561-562. (published).

X

282 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

283.1 Substance

283 MATERIALS AND METHODS

Give any available information on substance/product, such as identity, physical form (e.g. powder, grain size, particle size/distribution), purity

Copper tablets by mail order (Nature's Best, Tunbridge Wells, Kent, UK).

283.2 Persons exposed

283.2.1 Sex

Male

283.2.2 Age/weight

26 years old, weight not reported.

283.2.3 Known Diseases

State if any; state, if healthy

283.2.4 Number of persons 1

283.2.5 Other information None

283.3 Exposure

Oral

Indicate respective route, delete other routes 283.3.1 Reason of exposure
occupational, accidental, abuse, attempted suicide

Exaggerated use of copper as a dietary supplement.

283.3.2 Frequency of exposure

*Single/multiple
specify frequency*
Multiple

Multiple

283.3.3 Overall time period of exposure

if applicable
ca. 3 years.

283.3.4 Duration of single exposure

if applicable
A tablet was taken orally each day.

283.3.5 Exposure
concentration/dose

*measured, estimated, not available give all
available information*

30 mg/day during ca. 2 years, followed by 60 mg/day during ca. 1 year.

283.3.6 Other information Prior to taking the copper tablets, the patient took
a 3 month course of zinc tablets.

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specify subsection number

A.6.12.2(7): Chronic self poisoning

give type of examination and time after exposure for each examination

283.4 Examinations

The following examinations were performed (prior to liver transplantation):

- Physical examination,
- Laboratory investigations: serum bilirubin, albumin, alkaline phosphate, aspartate amino transferase, γ glutamyl transpeptidase, prothrombin time, serum copper, serum caeruloplasmin,
- Tests for hepatitis A and B antibodies and autoantibodies,
- Ultrasonography,
- Urinary copper excretion,
- Histology of the explanted liver.

give any available information on the medical treatment of intoxicated persons

283.5 Treatment

Penicillamine (1.5 g/day) was commenced with increasing cupriuresis of 281 μ mol on day 3.

Emergency orthotopic liver transplantation was performed (7 weeks after presentation).

283.6 Remarks

None.

284 RESULTS

*Describe findings. If appropriate, include table.
Describe any relevant effects observed*

284.1 Clinical Signs

The patient had a 6 weeks history of malaise, jaundice and abdominal swelling.

On admission to the hospital the patient was thin, jaundiced, with moderate ascites, splenomegaly and with Kayser-Fleischer rings and sunflower cataracts visible on slit-lamp examination.

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**284.2 Results of
examinations**

Human Case Report

specify subsection number

A.6.12.2(7): Chronic self poisoning

Describe the results of e.g. clinical chemistry, blood analysis and urinalysis

Laboratory investigations revealed serum bilirubin 28 mmol/l, albumin 24 g/dl, alkaline phosphate 257 IU/l (normal <90 IU/l), aspartate amino transferase 401 IU/l (normal <40 IU/l), γ -glutamyl transpeptidase 356 IU/l (normal <50 IU/l). Prothrombin time was 23 s (control 14 s).

Serum copper was 22.6 mmol/l (normal range 12.6-26.7 mmol/l); serum caeruloplasmin 0.24 mmol/l (normal 0.21-0.49 mmol/l). [Authors' note: it is suspected from the "normal" value cited that these units should be μ mol and the stated units are a typographical error]

Tests for hepatitis A and B antibodies and auto-antibodies were negative. Ultrasonography showed hepatomegaly and splenomegaly. Twenty four hour urinary copper excretion was 204 μ mol (normal <1.2 μ mol/day).

**284.3 Effectivity of
medical treatment**

Penicillamine (1.5 g/day) was commenced with increasing cupriuresis of 281 μ mol on day 3 but because of the development of haemolytic anaemia, the drug was discontinued.

Emergency orthotopic liver transplantation was performed (7 weeks after presentation). The patient made a good postoperative recovery.

284.4 Outcome

Describe outcome / manifestation of symptoms / disease

Six weeks after presentation, because of deteriorating encephalopathy and jaundice, the patient was transferred to King's College Hospital, London, UK. The prothrombin time rose to 130 s and he developed respiratory tract sepsis and acute renal failure. Emergency orthotopic liver transplantation was performed 1 week after transfer and the patient made a good postoperative recovery. Histology of the explanted liver was indistinguishable from that seen in Indian Childhood Cirrhosis (ICC) and Wilson's disease.

Mean liver copper was 3230 μ g/g dry weight (normal 20-50 μ g/g). Liver zinc was normal. Postoperative urinary copper collections showed a steady decline in cupriuresis, with urinary copper excretion 47.4 μ mol on day 12, 2.8 μ mol on day 17 and 2.0 μ mol on day 61. Kayser-Fleischer rings were still present on day 65, but had started to fade by 4 months.

284.5 Other

Describe any other significant observations

No other significant observations.

Section A6.12.2(7)

Annex Point IIA6.12.2

285.1 Materials and methods

Section A6.12.2(7) Human Case Report

specify subsection number

A.6.12.2(7): Chronic self poisoning

Annex Point IIA6.12.2

285 APPLICANT'S SUMMARY AND CONCLUSION *Briefly describe circumstances*

A 26 year old man presented to Coleraine Hospital with a 6 week history of malaise, jaundice and abdominal swelling. Three years before, he had developed a cough following jogging which he attributed to trace metal deficiency, having read about this in health magazines. He took a 3 month course of zinc tablets but felt no better and assuming his symptoms were due to copper deficiency, purchased copper tablets by mail order (Nature's Best, Tunbridge Wells, Kent, UK). These he took for 2 years in a dose of 30 mg (maximum recommended dose was 3 mg). Subsequent occasional feelings of lethargy resulted in a progressive dose escalation to 60 mg daily the year before presentation

Summarize relevant results

On examination the patient was thin, jaundiced, with moderate ascites, splenomegaly and with Kayser-Fleischer rings and sunflower cataracts visible on slit-lamp examination. Laboratory investigations revealed serum bilirubin 28 mmol/l, albumin 24 g/dl, alkaline phosphate 257 IU/l (normal <90 IU/l), aspartate amino transferase 401 IU/l (normal <40 IU/l), γ -glutamyl transpeptidase 356 IU/l (normal <50 IU/l). Prothrombin time was 23 s (control 14 s).

Serum copper was 22.6 mmol/l (normal range 12.6-26.7 mmol/l); serum caeruloplasmin 0.24 mmol/l (normal 0.21-0.49 mmol/l). [Authors' note: it is suspected from the "normal" value cited that these units should be imol and the stated units are a typographical error]

Tests for hepatitis A and B antibodies and auto-antibodies were negative. Ultrasonography showed hepatomegaly and splenomegaly. Twenty four hour urinary copper excretion was 204 μ mol (normal <1.2 μ mol/day).

Six weeks after presentation, because of deteriorating encephalopathy and jaundice, the patient was transferred to King's College Hospital, London, UK. The prothrombin time rose to 130 s and he developed respiratory tract sepsis and acute renal failure. Emergency orthotopic liver transplantation was performed 1 week after transfer and the patient made a good postoperative recovery. Histology of the explanted liver was indistinguishable from that seen in Indian Childhood Cirrhosis (ICC) and Wilson's disease.

Mean liver copper was 3230 μ g/g dry weight (normal 20-50 μ g/g). Liver zinc was normal. Postoperative urinary copper collections showed a steady decline in cupriuresis, with urinary copper excretion 47.4 μ mol on day 12, 2.8 μ mol on day 17 and 2.0 μ mol on day 61. Kayser-Fleischer rings were still present on day 65, but had started to fade by 4 months.

Screening of the patient's parents and siblings indicated that their urinary copper levels were normal.

Authors discussion:

In this case, there are striking similarities both clinically and histologically to fulminant Wilson's disease and to the late stages of ICC.

It is likely that exogenous copper was the sole aetiological factor; an underlying heterozygote or homozygote genotype for Wilson's disease could theoretically have been

unmasked by such copper load, although the patient's
normal caeruloplasmin level and the absence of subclinical Wilson's
disease in all family members conflict with this theory. Kayser-
Fleischer rings have been described in



Section A6.12.2(7)

Annex Point IIA6.12.2

Human Case Report*specify subsection number***A.6.12.2(7): Chronic self poisoning****285.3 Conclusion***Give general conclusions*

O'Donohue *et al* (1993) describe a case of chronic copper intoxication leading to micronodular cirrhosis and acute liver failure. The case was that of a 26 year old male who had taken copper tablets on a regular basis for approximately three years (30 mg/day for two years, rising to 60 mg/day during the third year). Initial signs and symptoms included malaise, jaundice and abdominal swelling. Clinical investigations indicated acute liver failure. Levels of several enzyme markers of hepatotoxicity were substantially elevated. Laboratory investigations revealed normal serum copper and serum caeruloplasmin levels but very high urinary excretion of copper (204 $\mu\text{mol}/24\text{ h}$) compared to the normal ($< 1.2\ \mu\text{mol}/24\text{ h}$). Hepatomegaly and splenomegaly were demonstrated by ultrasound. With further deterioration, liver transplantation was performed and the patient made a good recovery. Histopathology of the explanted liver showed advance micronodular cirrhosis. The concentration of copper in explanted liver tissue was 3230 $\mu\text{g/g}$ dry weight (normal 20-50 $\mu\text{g/g}$). Screening of the patient's parents and siblings indicated that their urinary copper levels were normal. The authors noted striking similarities both clinically and histologically to fulminant Wilson's disease and to the late stages of ICC. This single case study provides evidence of hepatotoxicity in an adult associated with long term oral exposure to copper at 30 or 60 mg/day.

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	██████████
Reference	• ██████████ ██
Materials and Methods	██
Results and discussion	██
Conclusion	██
Reliability	█
Acceptability	████████████████
Remarks	

COMMENTS FROM ... (specify)

Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>Discuss if deviating from view of rapporteur member state</i>

Section A6.12.2(7)

Annex Point ΠA6.12.2

Human Case Report

specify subsection number

A.6.12.2(7): Chronic self poisoning

Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Remark	

Section A6.12.4(1)**Epidemiological Study**

Annex Point IIA6.12.4

*state type of study (cohort study, case control ~, cross-sectional ~)***A.6.12.4(1): Cohort study - Vineyard sprayer's lung**Official
use only**291 REFERENCE****291.1 Reference***Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages)
If necessary, copy field and enter other reference(s).*

Plamenac P, Santic Z, Nikulin A, & Serdarevic H (1985) Cytologic changes of the respiratory tract in vineyard spraying workers. Eur J Respir Dis, 67: 50-55 (published).

291.2 Data protectionNo, published data.
*(indicate if data protection is claimed)
Give name of company*

291.2.1 Data owner

Not applicable.

Give name of company/companies which have the right to use these data on behalf of the data owner (see TNsG in support of Annex VI)

Not applicable.

Choose one of the following criteria (see also TnsGon Product Evaluation) and delete the others:

Not applicable, published data.

292 GUIDELINES AND QUALITY ASSURANCE *Not applicable***293 MATERIALS AND METHODS****293.1 Test material***As given in section 2
or give name used in study report*

Bordeaux mixture: contains 1.5% copper sulphate solution, neutralised with hydrated lime.

293.1.1 Lot/Batch number *List lot/batch number if available*

Not reported.

293.1.2 Specification

*As given in section 2**Deviating from specification given in section 2 as follows
(describe specification under separate subheadings, such as the following;
additional subheadings may be appropriate):*

Not reported.

293.1.2.1
nDescription *If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)*

Not reported.

293.1.2.2

Purity

Give purity in % of active substance

293.1.2.3

Stability

Describe stability of test material
Not reported.**293.2 Type of study**

Cohort study.

Section A6.12.4(1)
Annex Point IIA6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~)
A.6.12.4(1): Cohort study - Vineyard sprayer's lu

293.3 Method of data collection

*Interviewer
(State if blind)
Self administered questionnaire
Record review
Or other (specify)*

Sputum samples were collected from the sprayers on three consecutive days.

293.4 Test Persons / Study Population

Non-entry field

293.4.1 Selection criteria

*cohort study and cross sectional study:
give population exposed, e.g. workers at company producing compound under consideration
if available: special exposure situations/workplaces analysed
case-control-study:
give selection criteria for cases (source of cases)
specify type of disease
diagnostic procedure
disease incident or prevalent*

Vineyard sprayers who showed no signs of respiratory disease. No other selection criteria provided.

293.4.2 Number of test persons per group/cohort size

*specify, if there are differences for example for treatment and recovery groups
Give number
52*

293.4.3 Sex sprayers'

Not specified, experimental group described as 'vineyard

X

293.4.4 Age

35-55 years old.

293.4.5 Diseases

State if any; state, if healthy

All subjects were healthy adults with normal chest roentgenograms. At the time of investigations subjects showed no sign of any pulmonary or other disease.

293.4.6 Smoking status

smokers or non-smokers

Results were reported separately for smokers (defined as smoking for at least 5 years) and non-smokers.

293.5 Controls

Yes

293.5.1 Type of control

*cohort or cross-sectional study:
National population of
Regional population of
Company with different exposure:
Persons from the same company not exposed: (give type of work)
case-control-study:
selection criteria (type of disease)
matching criteria (sex, age, smoking habits)*

Inhabitants of the same environment who were not vineyard workers

293.5.2 Number of test persons per group/cohort size

*Give number
specify number of matched persons
per case*

51

293.5.3 Sex

Not specified, experimental group described as 'workers'.

Section A6.12.4(1)**Epidemiological Study****Annex Point II A6.12.4***state type of study (cohort study, case control ~, cross-sectional ~)***A.6.12.4(1): Cohort study - Vineyard sprayer's lung**

293.5.4 Age	22-55 years.	X
293.5.5 Diseases	<i>State if any; state, if healthy</i> All subjects were healthy adults with normal chest roentgenograms. At the time of investigations subjects showed no sign of any pulmonary or other disease.	
293.5.6 Smoking status	<i>smokers or non-smokers</i> Results were reported separately for smokers (defined as smoking for at least 5 years) and non-smokers.	
293.6 Administration/ Exposure	No Entry field	
293.6.1 Exposure Route	<i>Oral/Inhalation/Dermal/Combined/ Or other</i> Inhalation	
293.6.2 Exposure Situation	<i>Workplace or other; specify if other</i> <i>If workplace specify exposure conditions, e.g.emptying, filling, cleaning,</i>	
293.6.3 Exposure concentration(s)	<i>transferring, packaging, maintenance or other</i> Authors assume that inhalation exposure to 'Bordeaux mixture' occurred during vineyard spraying operations. Subjects had been vineyard sprayers for on average 9 years.	
293.6.4 Method(s) to determine exposure		
293.6.5 Postexposure period	<i>Information available / information not available Measured / estimated for all considered chemicals</i> No information available. <i>Give a short description of the analytical method(s) including limit of detection, delete unused lines</i> <i>Area air sampling</i> <i>material sampling</i> <i>personal sampling</i> <i>exposure pads</i> <i>Or other</i> Not applicable. <i>State period of time elapsed between last exposure / first examination or last exposure / last examination respectively</i> Information not provided. <i>No Entry field</i>	
293.7 Examinations		
293.7.1 Type of disease	<i>Specify disease and tumour sites give ICD-Numbers (and Number of ICD-Revision)</i> Sputum samples were used to detect cytological changes in the respiratory tract. <i>E.g. hematology, lung function</i>	
293.7.2 Parameters	Sputum specimens were obtained by morning cough on 3 consecutive days. Only expectorated material containing	

pulmonary macrophages was accepted as sputum. After fixation in 75% alcohol, the sputa were embedded in paraffin and the sections were stained with haematoxylin and eosin. These were then tested for iron and for copper with rubeanic acid and benzidine.

293.8 Further remarks None.

Section A6.12.4(1)

Annex Point IIA6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~)

A.6.12.4(1): Cohort study - Vineyard sprayer's lung

294 RESULTS AND DISCUSSION

Describe findings. If appropriate, include table. Sample tables are given below.

294.1 Exposure

No exposure data is available.

294.1.1.1 Number of measurements

Not applicable.

294.1.1.2 Average concentrations

arithmetic, geometric mean or median and/or 95-Percentile

Not applicable.

294.1.1.3 Standard deviation

Not applicable.

294.1.1.4 Date(s) of measurement(s)

Not applicable.

294.1.2 Other

Not applicable.

- 294.2 Number of cases for each disease / parameter under consideration** *No effects / describe significant effects referring to data in results table including confidence intervals*
- 294.3 SMR (Standard mortality ratio), RR (relative risk), OR (Odds ratio)** Cytological changes of the respiratory tract were investigated in 52 exposed subjects and 51 control subjects.
No effects / describe significant effects referring to data in results table including confidence intervals
- 294.4 Other Observations** Not applicable.
Describe any other significant effects. None

295 APPLICANT'S SUMMARY AND CONCLUSION

Give concise description of method

295.1 Materials and methods

Cytological changes in the respiratory tract were investigated in vineyard spraying workers employed in former Yugoslavia. The study population comprised 52 vineyard sprayers exposed by inhalation on a regular basis to 'Bordeaux mixture'. Subjects had worked as sprayers for an average of 9 years and none of them showed any signs of respiratory or other disease. Sputum samples were collected from the sprayers on three consecutive days. Samples were also collected from 51 workers from the same region not engaged in spraying who acted as the control group. Sputum samples were examined for cytological changes indicative of changes in the respiratory epithelium. Presence of copper in sputum samples was investigated by means of histochemical methods, specifically using rubeanic acid to test for positive responses in macrophages. Copper content of sputum samples was not quantified. Results were reported separately for smokers (defined as smoking for at least 5 years) and non-smokers.

Section A6.12.4(1)

Annex Point IIA6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~)

A.6.12.4(1): Cohort study - Vineyard sprayer's lung

295.2 Results and discussion

Summarize relevant results

For the incidence of cytological findings in professional vineyard sprayers sputa see Table A6.12.4(1)-1.

The incidence of cytological abnormalities among non-smoking vineyard sprayers was as follows (expressed as percentage of subjects affected, with incidence in non-smoking controls in parentheses): abnormal columnar cells 82% (10%); atypical squamous metaplasia 5% (0%); eosinophilia 42% (3%); copper-containing macrophages 42% (0%); respiratory spirals 68% (7%), plus increased sputum production. Among vineyard sprayers who were smokers, the cytological changes were generally more prevalent (incidence in smoking controls in parentheses): abnormal columnar cells 100% (95%); atypical squamous metaplasia 29% (5%); eosinophilia 43% (19%); copper containing macrophages 64% (0%); respiratory spirals 79% (43%). The results were not subjected to statistical analysis in this report. Nevertheless the reported cytological changes in the sputum of non-smoking vineyard sprayers provide evidence of changes in the respiratory epithelium.

295.3 Conclusion

Plamenac *et al* (1985) investigated cytological changes of the respiratory tract in vineyard spraying workers employed in former Yugoslavia. The study population comprised 52 rural workers who had worked as vineyard sprayers for an average of 9 years, none of whom showed any signs of respiratory or other disease. Sputum samples were collected from the sprayers, and from 51 workers from the same region not engaged in spraying who acted as the control group, on three consecutive days. Samples were examined for cytological changes indicative of changes in the respiratory epithelium, and for the presence of copper. Results were reported separately for smokers and nonsmokers. Results revealed that the macrophages of control subjects contained no copper whereas copper was detected in 64% of the vineyard sprayers. In both groups abnormal findings were more frequent in smokers than in nonsmokers. The results revealed cytological changes in the sputum of non-smoking vineyard sprayers, providing evidence of changes in the respiratory epithelium. The authors concluded that it was not possible to say with certainty which one of the possible mechanisms (toxic, allergic or inflammatory) was responsible for the changes in the respiratory epithelium. The study report provided little information on the cases studied, for example basis of selection. No information on confounding variables was given. Only limited information was given on the 'Bordeaux mixture' and no exposure data was provided.

Section A6.12.4(1)

Annex Point IIA6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~)

A.6.12.4(1): Cohort study - Vineyard sprayer's lung

295.3.1 Reliability

Based on the assessment of materials and methods include appropriate

reliability indicator 0, 1, 2, 3, or 4 4

295.3.2 Validity

Discuss critical points i.e.: bias / confounding (especially smoking) / study size /quality of exposure measurements /duration of exposure / latency

Due to the deficiencies mentioned under point 5.3.3 this study has no value for risk assessment purposes.

295.3.3 Deficiencies

Yes

The study report provided little information on the cases studied, for example basis of selection. No information on confounding variables was given. Only limited information was given on the 'Bordeaux mixture' and no exposure data is provided.

(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)

295.4 Other

None

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

[REDACTED]

Materials and Methods

[REDACTED]

[REDACTED]

[REDACTED]

Results and discussion

[REDACTED]

Conclusion

[REDACTED]

Reliability

[REDACTED]

[REDACTED]

[REDACTED]

Acceptability

[REDACTED]

Remarks

Section A6.12.4(1)

Annex Point IIA6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~)

A.6.12.4(1): Cohort study - Vineyard sprayer's lung

	COMMENTS FROM ...
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

Table 6.12.4(1)-1: Incidence of cytological findings in professional vineyard sprayers sputa

	Age	No of subjects	Sputa with abnormal columnar cells	Squamous metaplasia		Macro-phages with copper	Eosinophilia	Respir. spirals	Saliva (lack of expectoration)
				without atypia	with atypia				
Vineyard sprayers <i>Nonsmokers</i>	25-35	38	31 (82%)	22 (58%)	2 (5%)	16 (42%)	16 (42%)	26 (68%)	7 (18%)
		14	14 (100%)	5 (36%)	4 (29%)	9 (64%)	6 (43%)	11 (79%)	0
Control Group <i>Nonsmokers</i>	25-55	30	3 (10%)	0	0	0	1 (3%)	2 (7%)	27 (90%)
		21	20 (95%)	3 (14%)	1 (5%)	0	4 (19%)	9 (43%)	1 (5%)

Section A6.12.4(2)
Annex Point IIA6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~)

A6.12.4(2): Case control study - Indian Childhood Cirrhosis

Official
use only

296.1 Reference		296 REFERENCE <i>Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages)</i> <i>If necessary, copy field and enter other reference(s).</i> Tanner MS, Kantarjian AH, Bhawe SA, Pandit AN. (1983) Early introduction of copper-contaminated animal milk feeds as a possible cause of Indian Childhood Cirrhosis. Lancet 2: 992-995 (published).
296.2 Data protection		No, published data. <i>(indicate if data protection is claimed)</i> <i>Give name of company</i>
296.2.1	Data owner	Not applicable.
296.2.2	Companies with letter of access	<i>Give name of company/companies which have the right to use these data on behalf of the data owner (see TNSG in support of AnnexVI)</i>
296.2.3	Criteria for data protection	Not applicable. <i>Choose one of the following criteria (see also TnsGon Product Evaluation) and delete the others:</i> Not applicable, published data.
297 GUIDELINES AND QUALITY ASSURANCE <i>Not applicable</i>		
298.1 Test material		298 MATERIALS AND METHODS <i>As given in section 2</i> <i>or give name used in study report</i> Copper
298.1.1	Lot/Batch number	<i>List lot/batch number if available</i> Not relevant.
298.1.2	Specification	<i>As given in section 2</i> <i>Deviating from specification given in section 2 as follows (describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):</i> Not relevant. <i>If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)</i>
298.1.2.1	Description	Not relevant.
298.1.2.2		<i>Give purity in % of active substance</i> Purity [REDACTED]
298.1.2.3	Stability	<i>Describe stability of test material</i> Not relevant.
298.2 Type of study		Case control study.

Section A6.12.4(2)

Epidemiological Study

Annex Point IIA6.12.4

state type of study (cohort study, case control ~, cross-sectional ~)

A6.12.4(2): Case control study - Indian Childhood Cirrhosis

298.3 Method of data collection

*Interviewer
(State if blind)
Self administered questionnaire
Record review
Or other (specify)*

A feeding history was obtained by the admitting paediatrician. A home visit and second interview were performed by a social worker, who also examined the house, water supply, and utensils used for cooking and storage.

298.4 Test Persons / Study Population

Non-entry field

298.4.1 Selection criteria

*cohort study and cross sectional study:
give population exposed, e.g. workers at company producing compound under consideration
if available: special exposure situations/workplaces analysed
case-control-study:
give selection criteria for cases (source of cases)
specify type of disease
diagnostic procedure
disease incident or prevalent*

132 children with ICC (101 boys, 31 girls) were compared with 70 children with other liver disorders (49 boys, 21 girls).

Three groups of control homes and healthy children were similarly assessed:

- Prune controls: 50 houses around the home of 1 case of ICC seen in the Naravan Peth area of Prune (66 children),
- Miraj controls: 35 houses in the vicinity of Miraj, 150 km south of Prune where the incidence of ICC is reportedly low and in which no cases were seen (140 children),
- Srirampur controls: 5-10 homes in the vicinity of each of 18 cases ICC in villages surrounding the town of Srirampur, an area of high incidence (total 137 houses; 171 children).

298.4.2 Number of test persons per group/cohort size

*Give number
specify, if there are differences for example for treatment and recovery groups*

298.4.3 Sex

See point 3.4.1.

298.4.4 Age

Age of introduction of animal milk in children was investigated (see Table A6.12.4(2)-3). The age at which children were diagnosed with liver disease is also discussed (see point 5.2).

298.4.5 Diseases

State if any; state, if healthy

132 children were diagnosed with Indian Childhood Cirrhosis (ICC). 70 children were diagnosed with other liver disorders.

298.4.6 Smoking status

Only infants and children are investigated in this study.

298.5 Controls

Yes
See point 3.4.1.

Section A6.12.4(2)

Annex Point II A6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~)

A6.12.4(2): Case control study - Indian Childhood Cirrhosis

298.5.1 Type of control	<i>cohort or cross-sectional study:</i> <i>National population of</i> <i>Regional population of</i> <i>Company with different exposure:</i> <i>Persons from the same company not exposed: (give type of work)</i> <i>case-control-study:</i> <i>selection criteria (type of disease)</i> <i>matching criteria (sex, age, smoking habits)</i>
298.5.2 Number of test persons per group/cohort size	See point 3.4.1. <i>Give number</i> <i>specify number of matched persons per case</i>
See point 3.4.1.	
298.5.3 Sex	Not reported.
298.5.4 Age	See point 3.4.4.
298.5.5 Diseases	<i>State if any; state, if healthy</i> 377 healthy children
298.5.6 Smoking status study.	Only infants and children are investigated in this study.
298.6 Administration/ Exposure	No Entry field
298.6.1 Exposure Route	<i>Oral/Inhalation/Dermal/Combined/ Or other</i> Possible oral exposure.
298.6.2 Exposure Situation	<i>Workplace or other; specify if other</i> <i>If workplace specify exposure conditions, e.g.emptying, filling, cleaning, transferring, packaging, maintenance or other</i> Possible oral exposure to children through animal milk feeds contaminated with copper from copper and brass storage and cooking vessels.
298.6.3 Exposure concentration(s)	<i>Information available / information not available / estimated for all considered chemicals</i> No quantitative data is available on exposure concentrations. However, In order to gain information on possible sources of copper contamination, various milk and water samples were taken (see point 4.6.4).

Section A6.12.4(2)**Epidemiological Study****Annex Point II A6.12.4***state type of study (cohort study, case control ~, cross-sectional ~)***A6.12.4(2): Case control study - Indian Childhood Cirrhosis***Give a short description of the analytical method(s) including limit of detection, delete unused lines**Area air sampling**material sampling**personal sampling**exposure pads**Or other*

In order to gain information on possible sources of copper contamination, the following samples were taken:

- Samples of milk and water were taken from the houses of 30 cases of ICC and 30 age-matched ward controls with other hepatic disorders. Milk samples were freeze-dried, transferred to Leicester (UK), re-dried, and weighed. After digestion and reconstitution, copper was assayed by atomic absorption spectrophotometry. Water copper rations were assayed without pre-treatment.
- Two copper and two brass utensils were purchased in Prune. One of each was tinned by a local “kallai wallah”, a process in which a coating of tin (kallai) is applied by heating the vessel over an open flame, with a flux of ammonium chloride. A specimen of kallai was analysed by mass spectrometry. Copper concentrations in cow’s and in tap water were estimated before and after storage and in these vessels under various conditions.

State period of time elapsed between last exposure / first examination or last exposure / last examination respectively

X

No information available.

298.7 Examinations

No Entry field

298.7.1 Type of disease

*Specify disease and tumour sites
give ICD-Numbers (and Number of ICD-Revision)*

132 children with Indian Childhood Cirrhosis (ICC) were compared with 70 children with other liver disorders, and controls.

298.7.2 Parameters

E.g. hematology, lung function

A feeding history was obtained by the admitting paediatrician. A home visit and second interview were performed by a social worker, who also examined the house, water supply, and utensils used for cooking and storage.

298.8 Further remarks None.

Section A6.12.4(2)

Annex Point IIA6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~)

A6.12.4(2): Case control study - Indian Childhood Cirrhosis

299 RESULTS AND DISCUSSION

Describe findings. If appropriate, include table. Sample tables are given below.

299.1 Exposure

- 299.1.1.1 Number of measurements No quantitative exposure data are available concerning copper intake of the subjects which specifically featured in this investigation. See points 3.6.4 and 4.1.2.
- 299.1.1.2 Average concentrations *arithmetic, geometric mean or median and/or 95-Percentile*
See point 4.1.1.1.
- 299.1.1.3 Standard deviation See point 4.1.1.1.
- 299.1.1.4 Date(s) of measurement(s) See point 4.1.1.1.

299.1.2 Other

Water Copper Concentrations

124 samples were obtained from the houses of 30 cases of ICC, and 30 age-matched ward controls. Copper concentrations ($\mu\text{g}/\text{dl}$) were:

- well-water (26 ICC, 9 control) 1.86 ± 1.99 ,
- tap-water (8 ICC, 8 control) 1.54 ± 1.16 ,
- water in a copper vessel (4 ICC, 6 control) 9.3 ± 5.1 ,
- brass vessel (20 ICC, 20 control) 7.5 ± 5.7 ,
- earthen storage container (17 ICC, 7 control) 2.8 ± 2.6 .

There were no differences between samples from ICC and control households within these various categories. Leicester (where the UK researchers were based) tap-water contained $5.1 \pm 0.6 \mu\text{g}/\text{dl}$ copper.

Milk Copper Concentrations

Values in English dairy milk ($n=14$) were $9.3 \pm 3.0 \mu\text{g}/\text{dl}$. A sample of milk stored in a brass vessel was available from 16 of 30 ICC households visited. The copper concentration in these samples (13-975, mean $273 \mu\text{g}/\text{dl}$) did not significantly differ from concentrations in 8 samples taken from brass vessels in control households (39-546, mean $130 \mu\text{g}/\text{dl}$).

Laboratory Studies (see Table A6.12.4(2)-1)

Experimentally, the copper concentration in tap water boiled in glass, tinned copper, or tinned brass, showed no change. Large increases occurred after storage in copper vessels, or after boiling in copper or brass vessels.

Milk took up copper from utensils much more avidly than water did. The copper concentration of milk increased after storage in tinned copper or brass vessels, whilst storage or boiling in untinned copper or brass caused gross copper contamination of milk.

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~)

Section A6.12.4(2)

A6.12.4(2): Case control study - Indian Childhood Cirrhosis

Annex Point HA6.12.4

No effects / describe significant effects referring to data in results table including confidence intervals

299.2 Number of cases for each disease / parameter under consideration

See point 3.4.1.

No effects / describe significant effects referring to data in results table including confidence intervals

299.3 SMR (Standard mortality ratio), RR (relative risk), OR (Odds ratio)

Not applicable.

Describe any other significant effects

299.4 Other Observations

None.

300.1 Materials and methods

300 APPLICANT'S SUMMARY AND CONCLUSION *Give*

concise description of method

During a prospective study of chronic liver disease in children in Pune (formerly Poona), 132 children with ICC (101 boys, 31 girls) were compared with 70 children with other liver disorders (49 boys, 21 girls). A feeding history was obtained by the admitting paediatrician. A home visit and second interview were performed by a social worker, who also examined the house, water supply, and utensils used for cooking and storage. Three groups of control homes and healthy children were similarly assessed: 50 houses around the home of 1 case of ICC seen in the Naravan Peth area of Prune; 35 houses in the vicinity of Miraj, 150 km south of Prune where the incidence of ICC is reportedly low and in which no cases were seen; and 5-10 homes in the vicinity of each of 18 cases of ICC in villages surrounding the town of Srirampur, an area of high incidence (total 137 houses).

Samples of milk and water were taken from the houses of 30 cases of ICC and 30 age-matched ward controls with other disorders. Milk samples were freeze-dried, transferred to Leicester (UK), re-dried, and weighed. After digestion and reconstitution, copper was assayed by atomic absorption spectrophotometry. Water copper rations were assayed without pre-treatment.

Two copper and two brass utensils were purchased in Prune. One of each was tinned by a local "kallai wallah", a process in which a coating of tin (kallai) is applied by heating the vessel over an open flame, with a flux of ammonium chloride. A specimen of kallai was analysed by mass spectrometry. Copper concentrations in cow's and in tap water were estimated before and after storage and in these vessels under various conditions.

300.2 Results and discussion

Summarize relevant results

Results

76% of children with ICC and 41% of those with other liver disorders came from rural areas.

Of 107 households with a child with ICC, 96 (90%) used brass vessels for milk, and in 72 of these a brass vessel was used to boil milk. Brass vessels were used as containers for milk by most ICC families and adjacent households in the Srirampur area. Households in Srirampur used brass vessels as milk containers significantly more often than did

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~)

A6.12.4(2): Case control study - Indian Childhood Cirrhosis

households with children with other hepatic disorders and Miraj and Pune control groups. (see Table A6.12.4(2)-1)

All of the brass vessels used for milk seen in the urban Pune families, except that in the family with a case of ICC, were well coated with kallai, whereas 38% of those used in ICC for boiling milk were uncoated, and in the remainder and in Srirampur controls the kallai was usually abraded and yellow. Kallai was found to consist of tin containing (in µg/g) lead 500; antimony 90; bismuth 40; arsenic 200; copper 120; silver 0.8; barium 0.4.

X

Copper or brass utensils were universally used to carry water from the well or tap and to store it within the house in ICC families, in families where children had other liver disorders, and in the 2 rural control groups, but not in urban Pune.

Feeding History (see Tables A6.12.4(2)-2/3/5 and Figure A6.12.4(2)-1)

No child with ICC was exclusively breast-fed. All 132 patients received cow, buffalo, or goat's milk. There was no difference in the mean age at which animal milk was introduced to male and female cases of ICC (males 4.4±4.4 months; females 3.7±4.3 months). By contrast, of 171 healthy control children in adjacent houses in the Srirampur area, 54 were exclusively breast-fed before the addition of solid foods, such as rice or dal.

The median age of introduction of animal milk in children with ICC was 3.0 months. By contrast, the median age of introduction of animal milk in Srirampur control children was 9.0 months. The distribution of age at introduction of animal milk differed significantly in these two groups.

There were also significant differences in the number of children exclusively breast-fed, and the age of introduction of animal milk in the remainder between ICC and children with other hepatic disorders, and between ICC and Miraj controls.

ICC children and urban Pune controls had similar feeding histories.

The age at which children with ICC presented to hospital correlated with the age at which animal milk had been introduced.

Although there was a tendency for the children with ICC to start solid foods earlier than those with other liver disorders (11.1±7.8 months and 15.1±8.3 months, respectively), this difference was not significant.

Authors discussion

The results of this study suggest that ICC is related to early introduction of animal milk feeds which have been contaminated with copper from brass utensils. This study was retrospective and milk intake could not be measured, but clear differences in feeding practices emerged between the groups examined.

ICC predominantly affected rural rather than urban families. Of the urban Pune households visited, only a minority used brass or copper utensils. The brass cooking pots seen in urban households were well coated with kallai, because the

Section A6.12.4(2)

Annex Point IIA6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~)

A6.12.4(2): Case control study - Indian Childhood Cirrhosis

kallai wallah regularly visited. Milks used for infant feeds were either proprietary formulae, or government dairy milk sold in bottles, which had been heated in stainless steel or aluminium vessels. This population did resemble the ICC group, however, with respect to the prevalence of artificial feeding and age at which animal milk feeds had been started.

By contrast, neighbouring families in the villages around Srirampur differed from those affected by ICC, not in their use of brass and copper vessels, but in their use of artificial feeds. Children with ICC had an "urban" pattern of early, or total, artificial feeding, whereas in village control children 32% did not receive animal milk at all before solids were introduced. Reasons for inadequate breast feeding of children in whom ICC later developed included: maternal death or illness; twins (4 pairs); and early return of the mother to agricultural work, with the baby being left in the care of elderly relatives.

In the Mira area the incidence of ICC was low, copper and brass vessels were little used for milk, and extended breast feeding was almost universal. A heterogeneous group of children with other hepatic disorders differed from the ICC group in the same way as controls in the Miraj area did.

It is likely that copper contamination of milk is more important than that of water or of solid foods. Firstly, there was a correlation between the ages of starting on animal milk and presenting with disease. There was an interval of approximately 16 months between the two events.

Secondly, concentrations of copper in samples of milk kept in brass containers under various experimental conditions were higher than those reached in samples of water under the same conditions. Nevertheless, 11 children with ICC were stated not to have been fed with milk from brass utensils, and, in these, all had had copper-contaminated water, and solid foods had been started between 8 days and 10 months.

The copper content of mature Indian breast milk in the present study (12-35 µg/dl) and a previous report (29±1 µg/dl) did not differ from that reported in the west. Assuming a milk intake of 150 ml/kg/day, this would supply 15 to 60 µg copper/kg/day. Feeding similar amounts

Section A6.12.4(2)

Annex Point II A6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~)

A6.12.4(2): Case control study - Indian Childhood Cirrhosis

of animal milk which had been stored, or boiled then stored, in brass utensils would supply 400 ± 90 , or 930 ± 36 μg copper/kg/day, respectively.

Whether hepatic copper loading alone is responsible for the liver damage of ICC, or whether a second hepatic insult is necessary to initiate disease in a copper-loaded liver, we believe that avoidance of excess copper ingestion will prevent the disease.

300.3 Conclusion

Tanner et al (1983) investigate the hypotheses that brass and copper household utensils are a possible source of gross hepatic copper accumulation characteristic of Indian Childhood Cirrhosis (ICC). Feeding histories were established for 132 children with ICC, 70 children with other liver disorders, and 377 healthy children from three communities where cases of ICC had occurred.

In families with a child with ICC, the use of copper or brass for water storage and of brass for milk storage or milk boiling resembled that of neighbouring village control families. However, the feeding history of 132 children with ICC differed from that of 70 children with other hepatic disorders and 311 children in 2 rural control groups. No child with ICC was exclusively breast-fed whereas 10%, 32%, and 25% of the control children were. Duration of breast-feeding was shorter in children with ICC and animal milk was introduced earlier. Fifty seven percent were started on animal milk before 3 months of age. The age at introduction of animal milk correlated with the age at presentation with ICC. Sixty six urban Pune children had a feeding history similar to those with ICC, but in these families brass vessels were not used for milk.

The concentration of copper was determined in water samples ($n=124$) collected from houses of 30 ICC cases and 30 age-matched controls with other liver disorders. No significant differences in copper levels were found between the ICC and control households.

Contamination of milk and water with copper was examined under experimental conditions using utensils purchased in one of the regions in the study. Copper content of either cow's milk or water was determined before and after storage and/or boiling in the vessels. Milk was shown to take up copper more readily than water. The authors estimated that the copper concentration in milk samples obtained from ICC households, and those obtained experimentally, would supply a copper intake 6 to 20 times greater than that of the breast-fed infant. The findings of this study provide some indication that the occurrence of ICC could be associated with the early introduction of animal milk feeds contaminated with copper from copper and brass utensils. However, it is not clear if hepatic copper loading alone is responsible for the liver damage. No quantitative data are available concerning copper intake of the subjects featured in this investigation.

Based on the assessment of materials and methods include appropriate

reliability indicator 0, 1, 2, 3, or 4

300.3.1 Reliability

Section A6.12.4(2)

Annex Point IIA6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~)

A6.12.4(2): Case control study - Indian Childhood Cirrhosis

300.3.2 Validity

Discuss critical points i.e.: bias / confounding (especially smoking) / study size / quality of exposure measurements / duration of exposure / latency

Due to the deficiencies mentioned under point 5.3.3 this study has no value for risk assessment purposes.

300.3.3 Deficiencies

Yes

This study provides no quantitative data concerning copper intake of the subjects which featured in this investigation. A relationship between copper loading and liver is not shown.

(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)

X

300.4 Other

None

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[REDACTED]
Materials and Methods	[REDACTED]
Results and discussion	[REDACTED]
4) Conclusion	[REDACTED]
Reliability	[REDACTED]
Deficiencies	[REDACTED]
Acceptability	[REDACTED]
Remarks	[REDACTED]

Table A6.12.4(2)-2 Feeding history of children with ICC, children with other hepatic disorders, and control groups

Section A6.12.4(2) Annex Point HA6.12.4 Study Group	Epidemiological Study				
	Children	None given before 2 years of age (no [%])	Animal milk introduction (mean, median \pm SD) in months	None given (no[%])	Breast milk Duration (mean, median \pm SD) in months
ICC	132	0	4.3, 3.0 \pm 4.3	21(16)	10.6, 11.0 \pm 7.0
Other hepatic disorders	70	7(10) [†]	9.1, 9.0 [†] \pm 5.7	4(6)	17.1, 15.0[†] \pm 9.1
Srirampur controls	171	54 (32) [†]	9.4, 9.0 [†] \pm 5.5	2(1)	
Miraj controls	140	35(25) [†]	10.5, 12.0 [†] \pm 6.1	5(4)*	15.7, 12.0 [†] \pm 6.0
Prune controls	66	0	5.0, 3.0 \pm 4.5	8(12)	

Values which differ significantly from corresponding value for ICC are indicated by *(p<0.01) or [†](p<0.001)

Table A6.12.4(2)-3 Age of introduction of animal milk

Age (months)	ICC	Srirampur
0-3	75	14
4-6	26	32
7-9	16	16
10-12	10	44
13-15	1	2
16-18	4	4
19-21	0	1
22-24	0	4
>24	0	0
Above overall median*	81	24
Below overall median*	31	72

*Median age for ICC + Srirampur is 6 months
 $X^2=46.31$, p<0.001 in K sample median test

Table A6.12.4(2)-4 Copper concentrations (μ g/dl) in samples of water and milk (n=4) after storage, and after boiling then storage in various utensils

	Water		Milk	
	Standing at	Boiled, then	Standing at	Boiled, then

Section A6.12.4(2)

Epidemiological Study

Annex Point IIA6.12.4

state type of study (cohort study, case control ~, cross-sectional ~)

A6.12.4(2): Case control study - Indian Childhood Cirrhosis

	21°C		stored at 21°C for 6 h		21°C		stored at 21°C for 6 h	
	10 min	6 h	After boiling	After 6h	10 min	6 h	After boiling	After 6h
Glass	4.2 ±0.5	4.1 ±0.5	6.3 ±0.4	4.9 ±0.3	8.6 ±1.4	10.4 ±3.7	11.7 ±4.9	13.5 ±3.3
Tinned copper	4.4 ±0.7	5.7 ±0.8	7.0 ±1.0	6.7 ±0.4*	12.5 ±4.8	23.9 ±9.1	10.8 ±2.5	13.2 ±3.4
Tinned brass	5.5 ±0.8	5.7 ±1.0	6.2 ±0.5	7.5 ±1.0*	13.6 ±4.2	19.3 ± 5.0	16.1 ±3.8	18.6 ±5.6
Copper	8.2 ±0.7†	68.0 ±7.0†	39.2 ±3.0†	34.7 ±10.1†	187.5 ±9.5†	1118 ±223†	773 ±83†	628 ± 151†
Brass	5.0 ±0.9	12.5 ±1.5†	7.6 ±0.5†	32.2 ±1.3†	28.0 ±3.6†	266 ± 62†	405 ±77†	625 ± 24†

Values which differ significantly from pre-treatment values for water (5.1±0-6) or milk (11.5±3.6) on Student's t test are shown by * (p<0.01) or †(p<0.001).

COMMENTS FROM ...

Date

Give date of comments submitted

Materials and Methods

Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.

Discuss if deviating from view of rapporteur member state

Results and discussion

Discuss if deviating from view of rapporteur member state

Conclusion

Discuss if deviating from view of rapporteur member state

Reliability

Discuss if deviating from view of rapporteur member state

Acceptability

Discuss if deviating from view of rapporteur member state

Remarks

Table A6.12.4(2)-1 Utensils used to store and boil milk and to carry and store water in houses of children with ICC, other liver disorders, and controls groups

Study group	Houses	Milk		Water
		Houses with Brass vessels (%)	Houses with brass to boil milk (%)	Houses with Cu or brass (%)
ICC	107	90	67	97
Other hepatic disorders	48	42*	23*	88
Srirampur controls	137	78	67	77
Miraj controls	35	8*	0*	92
Prune controls	50	19*	15*	19*

*Values which differ significantly from corresponding value for ICC on χ^2 analysis are indicated ($p < 0.001$)

Table A6.12.4(2)-2 Feeding history of children with ICC, children with other hepatic disorders, and control groups

Study Group	Children	Animal milk		Breast milk	
		None given before 2 years of age (no [%])	Age of introduction (mean, median \pm SD) in months	None given (no [%])	Duration (mean, median \pm SD) in months
ICC	132	0	4.3, 3.0 \pm 4.3	21(16)	10.6, 11.0 \pm 7.0
Other hepatic disorders	70	7(10)†	9.1, 9.0† \pm 5.7	4(6)	17.1, 11.0 \pm 9.1
Srirampur controls	171	54 (32)†	9.4, 9.0† \pm 5.5	2(1)	
Miraj controls	140	35(25)†	10.5, 12.0† \pm 6.1	5(4)*	15.7, 12.0† \pm 6.0
Prune controls	66	0	5.0, 3.0† \pm 6.1	8(12)	

Values which differ significantly from corresponding value for ICC are indicated by *($p < 0.01$) or †($p < 0.001$)

Table A6.12.4(2)-1 Age of introduction of animal milk .

Age (months)	ICC	Srirampur
0-3	75	14
4-6	26	32
7-9	16	16
10-12	10	44
13-15	1	2
16-18	4	4
19-21	0	1
22-24	0	4
>24	0	0
Above overall median*	81	24
Below overall median*	31	72

*Median age for ICC + Srirampur is 6 months
 $X^2=46.31$, $p<0.001$ in K sample median test

Table A6.12.4(2)-4 Copper concentrations ($\mu\text{g}/\text{dl}$) in samples and milk (n=4) after storage, and after boiling in various utensils

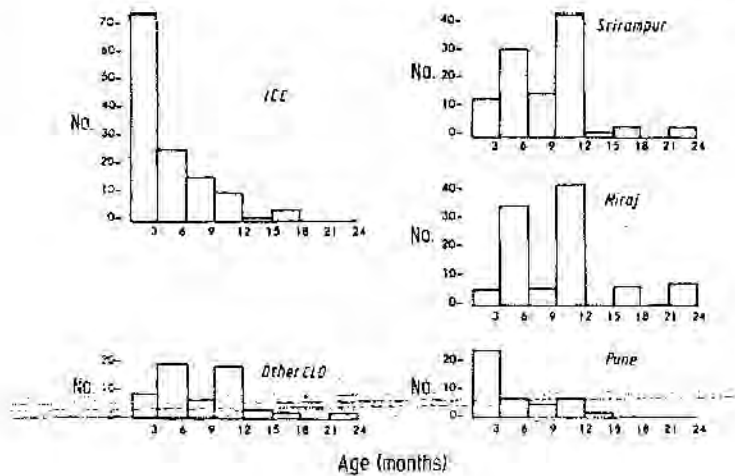
	Water				Milk			
	Standing at 21°C		Boiled, then stored at 21°C for 6 h		Standing at 21°C		Boiled, then stored at 21°C for 6 h	
	10 min	6 h	After boiling	After 6h	10 min	6 h	After boiling	After 6h
Glass	4.2 ± 0.5	4.1 ± 0.5	6.3 ± 0.4	4.9 ± 0.3	8.6 ± 1.4	10.4 ± 3.7	11.7 ± 4.9	13.5 ± 3.3
Tinned copper	4.4 ± 0.7	5.7 ± 0.8	7.0 ± 1.0	6.7 $\pm 0.4^*$	12.5 ± 4.8	23.9 ± 9.1	10.8 ± 2.5	13.2 ± 3.4
Tinned bras	5.5 ± 0.8	5.7 ± 1.0	6.2 ± 0.5	7.5 $\pm 1.0^*$	13.6 ± 4.2	19.3 ± 5.0	16.1 ± 3.8	18.6 ± 5.6
Copper	8.2 $\pm 0.7^\dagger$	68.0 $\pm 7.0^\dagger$	39.2 $\pm 3.0^\dagger$	34.7 $\pm 10.1^\dagger$	187.5 $\pm 9.5^\dagger$	1118 $\pm 223^\dagger$	773 $\pm 83^\dagger$	628 $\pm 51^\dagger$
Brass	5.0 ± 0.9	12.5 $\pm 1.5^\dagger$	7.6 $\pm 0.5^\dagger$	32.7 $\pm 1.3^\dagger$	28.0 $\pm 3.6^\dagger$	266 $\pm 62^\dagger$	405 $\pm 77^\dagger$	625 $\pm 24^\dagger$

Values which differ significantly from pre-treatment values for water (5.1 ± 0.6) or milk (11.5 ± 3.6) on Student's t test are shown by * ($p<0.01$) or \dagger ($p<0.001$).

Table A6.12.4(2)-5 Summary of feeding history in children with ICC and 3 healthy control groups.

	Copper contamination of animal milk by brass vessels unlikely	Copper contamination of animal milk by brass vessels likely
Exclusive breast feeding, or late introduction of	Miraj controls	Srirampur controls
Early introduction of animal milk	Urban Pune controls	Indian childhood cirrhosis

Figure A6.12.4(2)-1 Age of introduction of animal milk feeds in children with ICC, children with other hepatic disorders (CLD), and healthy children in 2 rural areas (Srirampur and Miraj) and urban Pune



Section A6.12.4(3)
Annex Point IIA6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~) **A6.12.4(3):**
Ecological (correlational) study - Idiopathic Copper Toxicosis

Official
use only

301.1 Reference

301 REFERENCE

*Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages)
If necessary, copy field and enter other reference(s).*

Scheinberg, I.H.; Sternlieb, I. (1994) Is non-Indian childhood cirrhosis caused by excess dietary copper? Lancet, 344: 1002-1004. (published)

301.2 Data protection

No, published data.

(indicate if data protection is claimed)

Give name of company

301.2.1 Data owner

Not applicable, published data.

301.2.2 Companies with letter of access

Give name of company/companies which have the right to use these data on behalf of the data owner (see TNSG in support of AnnexVI)

Not applicable, published data.

301.2.3 Criteria for data protection

Choose one of the following criteria (see also TnsGon Product Evaluation) and delete the others:

Not applicable, published data.

302 GUIDELINES AND QUALITY ASSURANCE *Not applicable*

303.1 Test material

303 MATERIALS AND METHODS

As given in section 2

or give name used in study report

Copper

303.1.1 Lot/Batch number *List lot/batch number if available*

Not applicable.

303.1.2 Specification

As given in section 2

Deviating from specification given in section 2 as follows (describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):

Not applicable

Section A6.12.4(3)**Annex Point IIA6.12.4****Epidemiological Study***state type of study (cohort study, case control ~, cross-sectional ~) A6.12.4(3):***Ecological (correlational) study - Idiopathic Copper Toxicosis****Section A6.12.4(3)****Epidemiological Study***state type of study (cohort study, case control ~, cross-sectional ~) A6.12.4(3):***Ecological (correlational) study - Idiopathic Copper Toxicosis****Annex Point IIA6.12.4**

303.1.2.1 Description *If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)*

Not applicable.

303.1.2.2 Purity

303.1.2.3 Stability

Describe stability of test material

Not applicable.

*Cohort study***303.2 Type of study***Case control study**Cross-sectional study**Or other*

Ecological (correlational) study: this study aims to test the hypothesis that Indian Childhood Cirrhosis (ICC) is an entirely environmental condition by investigating the incidence of this finding in populations where drinking water was known to contain high levels of copper.

303.3 Method of data collection*Interviewer**(State if blind)**Self administered questionnaire**Record review**Or other (specify)*Mortality data

Information on cause of deaths between 1969 and 1991 in the 0-5 year old populations of three Massachusetts towns was obtained from the Massachusetts Department of Public Health.

Copper concentrations

Analyses of copper concentrations in the drinking water of the towns of Duxbury, Wrentham, and Bellingham were made between July and December, 1992. One litre of water was drawn from a kitchen or bathroom cold-water tap in which water had been motionless for at least 6 h. Sixty homes were sampled in each town.

303.4 Test Persons / Study Population*Non-entry field*

303.4.1 Selection criteria

*cohort study and cross sectional study:**give population exposed, e.g. workers at company producing compound under consideration**if available: special exposure situations/workplaces analysed**case-control-study:**give selection criteria for cases (source of cases)**specify type of disease**diagnostic procedure**disease incident or prevalent*

The populations of three Massachusetts towns were chosen as the drinking water in these areas was known to contain high levels of copper.

Section A6.12.4(3)**Annex Point IIA6.12.4****Epidemiological Study**

*state type of study (cohort study, case control ~, cross-sectional ~) A6.12.4(3):
Ecological (correlational) study - Idiopathic Copper Toxicosis*

303.4.2 Number of test persons per group/cohort size	<p><i>Give number specify, if there are differences for example for treatment and recovery groups</i></p> <p>The average population aged 0-5 over the 23 year period investigated was as follows:</p> <p style="padding-left: 40px;">Duxbury: 805 Wrentham: 563 Bellingham: 1420</p> <p>(This corresponds to 64,124 child years of exposure to the drinking water presumed to contain ca. 8.5 mg Cu/L).</p>
303.4.3 Sex	Males and females.
303.4.4 Age	0-5 years.
303.4.5 Diseases	<p><i>State if any; state, if healthy</i></p> <p>The overall incidence of mortality and mortality related to any form of liver disease was investigated.</p>
303.4.6 Smoking status	<p><i>smokers or non-smokers</i></p> <p>Not applicable.</p>
303.5 Controls	
303.5.1 Type of control	<p><i>cohort or cross-sectional study: National population of Regional population of Company with different exposure: Persons from the same company not exposed: (give type of work)</i></p> <p><i>case-control-study: selection criteria (type of disease) matching criteria (sex, age, smoking habits)</i></p> <p>Not applicable. No control.</p>
303.5.2 Number of test persons per group/cohort size	<p><i>Give number specify number of matched persons per case</i></p> <p>Not applicable.</p>
303.5.3 Sex	Not applicable.
303.5.4 Age	Not applicable.
303.5.5 Diseases	<p><i>State if any; state, if healthy</i></p> <p>Not applicable.</p>
303.5.6 Smoking status	<p><i>smokers or non-smokers</i></p> <p>Not applicable.</p>
303.6 Administration/ Exposure	
303.6.1 Exposure Route	<p><i>Oral/Inhalation/Dermal/Combined/ Or other</i></p> <p>Via drinking water.</p>
303.6.2 Exposure Situation	<p><i>Workplace or other; specify if other If workplace specify exposure conditions, e.g.emptying, filling, cleaning, transferring, packaging, maintenance or other</i> Exposure to domestic drinking water.</p>

Section A6.12.4(3)

Annex Point IIA6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~) A6.12.4(3):

Ecological (correlational) study - Idiopathic Copper Toxicosis

303.6.3 Exposure concentration(s)

*Information available / information not available
Measured / estimated for all considered chemicals*

No data was available on copper levels in drinking water during the period investigated (1969-1991).

Samples were taken from Duxbury, Wrentham, and Bellingham in 1992 to measure the copper levels in the drinking water.

303.6.4 Method(s) to determine exposure

Give a short description of the analytical method(s) including limit of detection, delete unused lines

*Area air sampling
material sampling
personal sampling
exposure pads
Or other*

The water in Bellingham and Wrentham was analysed by Matrix Analytical Laboratories; that in Duxbury by Oliveira Environmental Laboratories. No information was provided on the methods used.

303.6.5 Postexposure period

State period of time elapsed between last exposure / first examination or last exposure / last examination respectively

Not applicable.

303.7 Examinations

No Entry field

303.7.1 Type of disease

*Specify disease and tumour sites
give ICD-Numbers (and Number of ICD-Revision)*

No examinations were conducted within the context of this study. This study relies on Massachusetts Department of Public Health records

303.7.2 Parameters

E.g. hematology, lung function

Not applicable.

303.8 Further remarks Background

Over the past 20 years reports have appeared of an infantile disorder indistinguishable from Indian Childhood Cirrhosis (ICC) but occurring in several other countries. This disorder (which the authors of this report term Idiopathic Copper Toxicosis or ICT) and Indian Childhood Cirrhosis are characterised by the onset in the first few years of life of hepatic insufficiency caused by cirrhosis with hepatocellular cytoplasmic hyalin, a normal or raised serum caeruloplasmin concentration, and hepatic copper concentrations of 400-4000 µg Cu/g dry weight (neonatal normal 295 µg/g dry weight).

Section A6.12.4(3)

Annex Point IIA6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~) A6.12.4(3):

Ecological (correlational) study - Idiopathic Copper Toxicosis

304 RESULTS AND DISCUSSION

Describe findings. If appropriate, include table. Sample tables are given below.

For each of the three towns, the drinking water of 60 homes was sampled.

arithmetic, geometric mean or median and/or 95-Percentile

Copper levels (mg/L) in the drinking water from samples were as follows:

Duxbury: 8.8

Wrentham: 8.7

Bellingham: 8.5 Not reported.

Measurements were made in 1992.

304.1.2 Other

No data was available on copper levels in drinking water during the period investigated (1969-1991).

304.2 Number of cases for each disease / parameter under consideration

No effects / describe significant effects referring to data in results table including confidence intervals

Over the 23 year period, there were 135 deaths among young children from all causes and no deaths from any form of liver disease.

304.3 SMR (Standard mortality ratio), RR (relative risk), OR (Odds ratio)

No effects / describe significant effects referring to data in results table including confidence intervals

Not applicable.

304.4 Other Observations

Describe any other significant effects None

305 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.12.4(3) Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~) A6.12.4(3):

Ecological (correlational) study - Idiopathic Copper Toxicosis

Copper concentrations in drinking water

Analyses of copper concentrations in the drinking water of the towns of Duxbury, Wrentham, and Bellingham were made between July and December, 1992.

One litre of water was drawn from a kitchen or bathroom cold-water tap in which water had been motionless for at least 6 h. Sixty homes were sampled in each town. The water in Bellingham and Wrentham was analysed by Matrix Analytical Laboratories; that in Duxbury by Oliveira Environmental Laboratories.

The high concentration of copper in water may have been due to the concentration of copper in the towns wells, the interaction of acidic well-water and the copper plumbing between the wells and taps, or both.

Cause of death in children 0-5 years

Data was obtained on all deaths and deaths from any form of liver disease in children aged 0-5 year in the three towns from the Massachusetts Department of Public Health.

Summarize relevant results

Results from the current investigation

Cause of death in children 0-5 years

The following table shows total deaths and deaths from liver disease in the 0-5 year-old populations in Duxbury, Wrentham, and Bellingham between 1969 and 1991.

Town	Copper (mg/L)	Average population aged 0-5*	All deaths	Liver deaths†
Duxbury	8.8	805	30	0
Wrentham	8.7	563	32	0
Bellingham	8.5	1420	73	0

*US Bureau of the Census data. †Caused by acute and subacute necrosis of the liver, chronic liver disease and cirrhosis, liver abscess and sequelae of chronic liver disease, or other disorders of liver.

Over the 23 year period, there were 135 deaths among young children from all causes and no deaths from any form of liver disease.

Copper levels in drinking water

The copper concentrations of water from the Massachusetts towns exceeded 8 mg/L (see above table).

Section A6.12.4(3)**Annex Point IIA6.12.4****Epidemiological Study***state type of study (cohort study, case control, cross-sectional)* **A6.12.4(3):****Ecological (correlational) study - Idiopathic Copper Toxicosis**

The authors note that the concentrations of copper in water in the reported cases of ICT were all less than 6.8 mg/L.

Results from published reports of cases of ICT

The table below lists all published reports of cases of ICT where the copper concentration of the family's tap water was known, and one report where this information is irrelevant.

Authors	Sex	Age at onset	Age at death	Liver Cu ($\mu\text{g/g}$ dry weight)	Cu in water (mg/L)
Lim and Choo ¹	M	2 yr	NK	1200	0.05
Müller-Höcker et al ² , Weiss et al ³	F	7 mo	10 mo	1485	0.4-5.5
Müller-Höcker et al ⁴	F	9 mo	13 mo	2154	2.2-3.4
	M	5 mo	NK	2800	2.2-3.4
Walker-Smith and Blomfield ⁵	M	14 mo	16 mo	3360	0.3-6.8
Adamson et al ⁶	M	2 yr	32 mo	1500	1-13
Aljajeh et al ⁷	F	1-3 mo	15 mo	425	Breast-fed

NK=not known.

Authors discussion:

Two objections may be raised to our conclusion that ingestion by children under the age of 6 years of water containing about 8.5 mg Cu/L does not cause ICT.

First, since analyses of copper in the towns water were made in 1992, it can be argued that similar concentrations may not have existed in the preceding two decades: 99% of instances of copper contamination of town water systems in the USA originate from corrosion of copper pipes, generally by acidic water; 1% are due to copper present in the water before it enters copper plumbing. Wrentham and Bellingham are contiguous, with Duxbury about 35 miles away. Their proximity to each other suggests that the wells from which their water is drawn are fed from a common source. With no reason to assume that these towns water mains, or the plumbing of homes, differ significantly from those of other Massachusetts towns, their water is probably of unusually low pH or contains unusually high concentrations of copper. Moreover, the fact that most lifelong residents of the towns have always drunk tap water whereas many who arrive in adult life find its slightly metallic taste unacceptable and drink bottled water, suggests that the copper content of the water has not changed appreciably over past decades.

Second, if ICT, or other severe liver disease, had developed in a child in one of these small towns, it is possible that the child would have been admitted to hospital in Boston. If death, however, had occurred in Boston, Massachusetts law requires the death certificate to record the event as a death of the town of residence. Moreover, staff at Boston's Massachusetts General Hospital, Children's Medical Center, and New England Medical Center know of no cases of ICT.

These data, we believe, rule out the possibility that copper alone in drinking water caused the illnesses and deaths of the seven patients.

Section A6.12.4(3)

Annex Point IIA6.12.4

The author

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~) A6.12.4(3):

Ecological (correlational) study - Idiopathic Copper Toxicosis

s also discussed various published cases of ICT (including those mentioned above) and the evidence that a genetic defect played a part in the patients ICT.

305.3 Conclusion

In order to test the hypothesis that Idiopathic Copper Toxicosis (ICT) is an entirely environmental condition, Scheinberg & Sternlieb (1994) reported on three Massachusetts, USA, towns where drinking water was known to contain high levels of copper (8.5-8.8 mg Cu/L on first-draw samples after 6 h of stagnation).

Information on cause of deaths between 1969 and 1991 in children under 6 years old was obtained from the Massachusetts Department of Public Health. The authors concluded that the finding of no deaths from any form of liver disease among these children after the equivalent of 64,124 child-years of exposure to drinking water containing ca. 8.5 mg Cu/L, make it highly unlikely that the hepatic illnesses and deaths of the patients with Idiopathic Copper Toxicosis (reported previously by other authors) were due solely to drinking water containing less than 6.8 mg Cu/L. They propose that ICT develops only in children with a genetic defect.

Analyses of copper concentrations in the drinking water of the towns investigated were made in 1992. No information is available on levels of copper in drinking water during the investigated period (1996-1991). The sample size of this study was insufficient to fully test the proposed hypothesis.

305.3.1 Reliability

Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4 4

305.3.2 Validity

Discuss critical points i.e.: bias / confounding (especially smoking) / study size / quality of exposure measurements / duration of exposure / latency

This study is not considered valid for risk assessment purposes due to the deficiencies mentioned under point 5.3.3.

305.3.3 Deficiencies

Yes

No information is available on levels of copper in drinking water during the investigated period.

The sample size of this study was insufficient to fully test the proposed hypothesis (i.e. that copper content in drinking water is not the sole aetiological factor for previously reported cases of Idiopathic Copper Toxicosis (ICT)).

(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)

305.4 Other

None.

Section A6.12.4(3)
Annex Point IIA6.12.4

Epidemiological Study

state type of study (cohort study, case control ~, cross-sectional ~) **A6.12.4(3):**
Ecological (correlational) study - Idiopathic Copper Toxicosis

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[REDACTED]
Materials and	[REDACTED]
Methods Results and	[REDACTED]
discussion Conclusion	[REDACTED]
Reliability	[REDACTED]
Acceptability	[REDACTED]
Remarks	
	COMMENTS FROM ...
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state</i>
Results and	<i>Discuss if deviating from view of rapporteur member state</i>
discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	

Table A6.9(01)-1. Effects of Copper on Brain Biogenic Amines in Rats Fed a 10% or 21% Casein Diet.

Dietary and treatment groups	DA (µg/g)	% change	NE (µg/g)	% change	5-HT (µg/g)	% change
21% casein (control)	0.708 ± 0.033	--	0.381 ± 0.014	--	0.455 ± 0.021	--
21% casein + Cu ²⁺	0.818 ± 0.014	+16 ^a	0.447 ± 0.016	+17 ^a	0.465 ± 0.017	0
10% casein (control)	0.753 ± 0.031	--	0.405 ± 0.017	--	0.430 ± 0.014	--
10% casein + Cu ²⁺	0.763 ± 0.034	0	0.423 ± 0.019	0	0.366 ± 0.016	-20 ^b

Values represent mean ± S.E. of 6 rats. Difference between control and experimental animals: ^a *P* < 0.02; *P* < 0.01.

Table A6.9(01)-2. Effects of Copper on Brain Metal Contents in Rats Fed a 10% or 21% Casein Diet.

Dietary and treatment groups	Copper (µg/g fresh weight)	% change	Calcium (µg/g fresh weight)	% change	Zinc (µg/g fresh weight)	% change
21% casein (control)	2.69 ± 0.05	--	236.50 ± 13.13	--	18.12 ± 1.10	--
21% casein + Cu ²⁺	7.33 ± 0.04	+172 ^c	153.22 ± 5.28	-35 ^c	15.20 ± 0.48	-16 ^a
10% casein (control)	2.69 ± 0.05	--	214.08 ± 6.34	--	21.59 ± 0.68	--
10% casein + Cu ²⁺	7.38 ± 0.24	+174 ^c	164.96 ± 7.01	-30 ^b	14.19 ± 0.89	-22 ^a

Values represent mean ± S.E. of 6 rats. Difference between control and experimental animals: ^a *P* < 0.05; *P* < 0.01; ^c *P* < 0.001

Section A7
Annex Point A3.1.2

A7, ECOTOXICOLOGICAL PROFILE INCLUDING ENVIRONMENTAL FATE AND BEHAVIOUR

Detailed justification:

No Documents IIIA CAR are available for the Environment sections, as the assessment for this part of the dossier is taken directly from the Copper Voluntary Risk Assessment (published by the European Copper Institute, 2008).

All reports and assessments related to the copper Voluntary Risk Assessment are available from:
<http://echa.europa.eu/web/guest/information-on-chemicals/transitional-measures/voluntary-risk-assessment-reports> and it is owned and copyrighted by the European Copper Institute. A letter of access is required from the European Copper Institute for any regulatory purpose.

Section A8
IUCLID: 8.1-8.8

A8, Measures necessary to protect man, animals and the environment

Official
use only

Subsection
(Annex Point)

Ensure adequate ventilation and avoid

- 8.1 Recommended methods and precautions concerning handling, use, storage, transport or fire (IIA8.1)** generation of dusts Not combustible
Store in a dry place and keep container tightly closed
In the event of fire, carbon dioxide, carbon monoxide and copper oxides may be released.
- 8.2 In case of fire, nature of reaction products, combustion gases, etc (IIA8.2)**
In the event of fire wear self-contained breathing apparatus and use a suitable extinguishing medium e.g. water, foam, carbon dioxide and dry powder.
- 8.3 Emergency measures in case of an accident (IIA8.3)**
Following contact with skin, wash off immediately with plenty of water.

In case of contact with eyes immediately flush with plenty of water.

In case of ingestion, drink plenty of water and if you feel unwell seek medical attention.

Following inhalation, move to fresh air. Symptoms of toxicity may occur after several hours, therefore, medical observation of breathing may be necessary

In case of accidental spillage avoid dust formation and use personal protective equipment.

Environmental precautions – do not allow to enter drains
- 8.4 Possibility of destruction or decontamination following release in or on the following: air, water, including drinking water and soil (IIA8.4)**
Since the copper compounds are insoluble with very low vapour pressures, significant contamination of air should not occur as the compounds will quickly precipitate from the atmosphere to soil and water compartments.
Contamination of water may occur in the case of leakage at the manufacturing plant. In this case the contaminated water is collected and may be recycled by re-introducing it in the manufacturing process.
Should any contamination of water occur outside the plant, the contaminated water should be collected or contained with clean-up via suction and filtering.

If surface waters are contaminated, insoluble copper compounds are deposited to the sediment where it is durably bound by different complexation and adsorption processes. The buffer capacity of

sediment can be enhanced by addition of organic matter or increasing the pH. Sediments can also be dredged and removed to an approved dumping site.

If the substance is spilled in soil, soil has to be collected and removed to an incineration plant or approved landfill site.

Non entry field

8.5 Procedures for waste management of the active substance for industry or professional users (IIA8.5)

Contamination of water may occur in the case of leakage at the manufacturing plant. In this case the contaminated water is collected and may be recycled by re-introducing it in the manufacturing process.

8.5.1 Possibility of re-use or recycling (IIA8.5.1)

Neutralisation with chemicals is not necessary as the copper compounds are not toxic.

8.5.2 Possibility of neutralisation of effects (IIA8.5.2)

Under Hazardous Waste Directive (91/689/EEC) surplus copper carbonate and contaminated materials (including sawdust) must be classified as "Special Waste". Disposal must be in accordance with these regulations and requirements set out in the Integrated Pollution Prevention and Control Directive (91/6/EC).

x

8.5.3 Conditions for controlled discharge including leachate qualities on disposal (IIA8.5.3)

The active substance can be disposed to an approved landfill site as specified by the local or country authorities. Due to the high binding capacity of copper to soil any release from an approved landfill site would not leach significantly into surrounding soil.

8.5.4 Conditions for controlled incineration

The recommended method of disposal is by chemical landfill rather than controlled incineration.

8.6 Observations on undesirable or unintended side-effects e.g. on beneficial and other non-target organisms (IIA8.6)

In view of the tightly controlled conditions under which the active ingredient is produced, handled and formulated into wood preservation products, it is considered that there is no potential for undesirable or unintended effects on beneficial and other non-target organisms. Similarly, given that the formulated active ingredient is applied to wood in an essentially closed system, there is no potential for adverse effects as a result of this process. The active ingredient in treated wood is effectively fixed into the article and does not impact on non-target organisms.

8.7 Identification of any substances falling within the scope of List I or List II of the Annex to Directive 80/68/EEC on the protection of groundwater against pollution caused by certain dangerous substances

Copper and its compounds are included in List II of Council Directive 80/68/EEC of 17 December 1979 on the protection of groundwater against pollution caused by certain dangerous substances.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[REDACTED]
Materials and methods	[REDACTED]
Conclusion	[REDACTED]
Reliability	[REDACTED]
Acceptability	[REDACTED]
Remarks	[REDACTED]
COMMENTS FROM ...	
Date	<i>Give date of comments submitted</i>
Results and discussion	<i>Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	