Section A6.12.2(4) **Human Case Report**

Annex Point IIA6.12.2 *Specify subsection number*

A.6.12.2(4): Vineyard sprayer's lung

Official use only

266 REFERENCE

266.1 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).
Pimentel JC, Menezes AP. (1977) Liver disease in vineyard sprayers. Gastroenterology 72:275-283. (published).

267 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

268 MATERIALS AND METHODS

268.1 Substance

Give any available information on substance/product, such as identity, physical form (e.g. powder, grain size, particle size/distribution), purity
'Bordeaux Mixture': [redacted]

268.2 Persons exposed

268.2.1 Sex: Not specified, subjects described as 'rural workers'.

268.2.2 Age/weight: No information provided.

268.2.3 Known Diseases

State if any; state, if healthy
All workers had "Vineyards sprayer's lung".

[Vineyard Sprayers lung is described by Pimentel JC & Marques F (1969) 'Vineyard sprayer's lung': a new occupational disease. Thorax, 24: 678-688, see section A.6.12.2(1)].

In most cases examination of livers were conducted at autopsy. No further information on the health status is provided. However, the authors indicate that all cases with other possible causes of liver damage, such as hepatitis, alcoholism, and exposure to hepatotoxic substances, including pesticides containing inorganic arsenic, were excluded.

268.2.4 Number of persons 30 vineyard workers.

268.2.5 Other information None.

268.3 Exposure

Indicate respective route, delete other routes
Inhalation
Although no exposure data is available it is assumed that inhalation to the 'Bordeaux mixture' occurred during spraying.

268.3.1 Reason of exposure Occupational

268.3.2 Frequency of exposure

Single/multiple
Multiple
Spraying was carried out from 15 to 100 days per year.

Section A6.12.2(4) **Human Case Report**

Annex Point IIA6.12.2

Specify subsection number

A.6.12.2(4): Vineyard sprayer's lung

268.3.3 Overall time period of exposure

if applicable

Mean duration of 18 years (range 3-45 years).

268.3.4 Duration of single exposure

if applicable

No information available.

measured, estimated, not available give all available information

Bordeaux mixture contained 1-2% copper sulphate.

268.3.5 Exposure concentration/dose

268.3.6 Other information 600 litres of mixture were sprayed each day by each worker.

give type of examination and time after exposure for each examination

268.4 Examinations

The livers of 30 rural workers who sprayed vineyards with Bordeaux mixture were studied. The spleens of 4 of them were also examined.

The morphological changes in the liver were classified according to predominant aspects, and sometimes, the clinical and laboratory findings.

The specimens were fixed in a 10% neutral formaldehyde solution and embedded in paraffin. The following staining techniques were used: haematoxylin-eosin, van Gieson, Wilder, periodic acid-Schiff, Perls, Unna-Pappanheim, Gomori for fungi, and Ziehi-Neelsen. The sulphide-silver (slightly modified), rubeanic acid, and benzidine methods were used for the histochemical localization of copper. Conventional, polarized light, phase contrast, and interference microscopy were used in these examinations. In the case of angiosarcoma, hepatic copper levels were estimated by atomic absorption spectrophotometry in tumour and in tumour free portions of the liver.
Normal livers were used as controls.

268.5 Treatment

give any available information on the medical treatment of intoxicated persons

Treatment is not discussed in this study; most samples were taken at autopsy.

Section A6.12.2(4)	Human Case Report
Annex Point IIA6.12.2	*Specify subsection number* **A.6.12.2(4): Vineyard sprayer's lung**

268.6 Remarks

Experimental work

Experimental work was carried out in young male guinea pigs (three groups of 6), fed on a diet appropriate for their development and kept at 18 to 22°C. These animals were exposed daily for 5 min to aerosols of 0.4% aqueous solutions of two fungicide formulations: group 1 received copper oxychloride (containing 50% copper) and group 2 received oxychloride (37.5% Cu) plus zineb (16%). The flow of the nebulizer was 0.2 to 0.3 ml per min. A third group represented control animals. The animals were killed after 60, 120, 200, 270, and 420 periods of exposure. The histological techniques used were the same as those applied to the human material.

After 70 days of exposure, animals showed copper inclusions within swollen Kupffer cells and histiocytes in the portal tracts and subcapsular areas. In 3 animals killed after 270 days of exposure, a close association was noted between the lesion reported and perisinusoidal and portal fibrosis.

269 RESULTS

Describe findings. If appropriate, include table.

269.1 Clinical Signs

Describe any relevant effects observed

Clinical signs were not reported.

269.2 Results of examinations

Examination of liver tissue samples from subjects, taken either at autopsy or surgical biopsy, revealed the following morphological changes:

- focal or diffuse swelling and proliferations of Kupffer cells in all 30 cases,
- histiocytic or sarcoid like granulomata (7 seven cases),
- fibrosis of variable degree in the perisinusoidal, portal and subcapsular areas, accompanied by atypical proliferation of the sinusoidal lining cells (8 cases),
- micronodular cirrhosis (3 cases),
- idiopathic portal hypertension (2 cases),
- angiosarcoma of the liver (1 case).

Abundant deposits of copper were revealed by histochemical techniques within hepatic and pulmonary lesions in these patients.

269.3 Effectivity of medical treatment

Treatment was not discussed in this report.

Section A6.12.2(4) **Human Case Report**

Annex Point IIA6.12.2 *Specify subsection number*

A.6.12.2(4): Vineyard sprayer's lung

269.4 Outcome *Describe outcome / manifestation of symptoms / disease*

Outcome was not investigated in this report. In most cases samples were examined at necropsy.

269.5 Other *Describe any other significant observations*

None

270 APPLICANT'S SUMMARY AND CONCLUSION

270.1 Materials and methods *Briefly describe circumstances*

The livers of 30 rural workers who sprayed vineyards with Bordeaux mixture for periods that varied from 3 to 45 years (mean 18 years) were studied. The spleens of 4 of them were also examined.

The morphological changes in the liver were classified according to predominant aspects, and sometimes, the clinical and laboratory findings. All cases with other possible causes of liver damage, such as hepatitis, alcoholism, and exposure to hepatotoxic substances, including pesticides containing inorganic arsenic, were excluded.

One case of proliferation of Kupffer cells, 2 of granuloma, and 3 of liver fibrosis were studied by percutaneous biopsy. In 2 cases of idiopathic portal hypertension surgical biopsies were done and, in all other cases, the liver was examined at autopsy. The spleen was studied after splenectomy in 2 cases of idiopathic portal hypertension and at autopsy in the other cases.

Section A6.12.2(4)	**Human Case Report**
Annex Point IIA6.12.2	*Specify subsection number* **A.6.12.2(4): Vineyard sprayer's lung**
270.2 Results and discussion	*Summarize relevant results* Liver disease with inclusions of copper was identified in all 30 cases. The following changes were seen: diffuse or focal swelling of Kupffer cells with granular inclusions of copper (30 cases), histiocytic or sarcoid-type granulomas containing variable amounts of copper (7 cases), liver fibrosis (8 cases), cirrhosis (3 cases), idiopathic portal hypertension (2 cases) and liver angiosarcoma (1 case). Author's discussion: Copper is a normal component of hepatic parenchymal cells. It may occur there in higher than normal levels in various conditions such as Wilson's disease, cryptogenic cirrhosis, and primary biliary cirrhosis. Copper may also occur within the reticuloendothelial elements of the liver or in granulomatous lesions. Our observations in vineyard sprayers demonstrate that copper enters the body via a nonphysiological route (inhalation), and in a non physiological form ('Bordeaux mixture'), is deposited inside the reticuloendothelial cells of the liver, and produces different kinds of lesions. Copper particles inhaled during the spraying of copper sulphate may reach the liver by the bloodstream. The digestive route does not seem to be involved, as exposure occurs almost exclusively by inhalation. The identification of copper in other areas, such as the lymphatic system, spleen, or kidney may be explained also by a haematogenous dissemination of the inhaled material. The observations on the human and experimental material suggest an etiological relationship between exposure to copper sulphate and the lesions described. A morphological resemblance was noted between the liver disease of vineyard sprayers and the hepatic lesions reported in workers exposed to inorganic arsenic and to vinyl chloride.

Section A6.12.2(4)	**Human Case Report**
Annex Point IIA6.12.2	*Specify subsection number* **A.6.12.2(4): Vineyard sprayer's lung**
270.3 Conclusion	*Give general conclusions*

Pimentel and Menezes, (1977) investigated liver disease in a group of former vineyard workers with 'vineyard sprayer's lung'. Thirty subjects were investigated who had sprayed vineyards with 'Bordeaux mixture' for a mean duration of 18 years (range 3-45 years). Liver tissue samples from subjects, taken either at autopsy or surgical biopsy showed: diffuse or focal swelling of Kupffer cells with granular inclusions of copper occurred in all 30 cases, histiocytic or sarcoid-type granulomas containing variable amounts of copper (7 cases), liver fibrosis (8 cases), cirrhosis (3 cases), idiopathic portal hypertension (2 cases) and liver angiosarcoma (1 case). The authors conclude that their observations "suggest an etiological relationship between exposure to copper sulphate and the lesions described". The study report provided little information on the cases studied, for example the basis of selection. Only limited information was given on the 'Bordeaux mixture' and no exposure data was provided.

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	[redacted]
[redacted]	

	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*

Section A6.12.2(4)	**Human Case Report**
Annex Point IIA6.12.2	*Specify subsection number*

A.6.12.2(4): Vineyard sprayer's lung

Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Official use only

271 REFERENCE

271.1 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).
Tanner MS, Portmann B, Mowat AP, Williams R, Pandit AN, Mills CF, Bremner I (1979) Increased hepatic copper concentration in Indian Childhood Cirrhosis. Lancet 1:1203-5 (published).

272 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

273 MATERIALS AND METHODS

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

273.2 Persons exposed

273.2.1 Sex: The proportion of male and female children was not reported.

273.2.2 Age/weight: 0.9 - 16 years (10 children between age 1 and 3 years). See Table A.6.12.2(4)-1.

273.2.3 Known Diseases
State if any; state, if healthy
All children had been diagnosed with liver disease.

273.2.4 Number of persons: 19

273.2.5 Other information: All children were from India: 8 children were from Pune, 8 from Madras, and one from Benares, Bombay, and Vellore.

273.3 Exposure
Oral/Inhalation/Dermal
Indicate respective route, delete other routes
Exposure is not investigated in this study.

273.3.1 Reason of exposure
occupational, accidental, abuse, attempted suicide
ee point 3.3.

S

273.3.2 Frequency of exposure
Single/multiple
specify frequency
See point 3.3.

273.3.3 Overall time period of exposure
if applicable
See point 3.3.

273.3.4 Duration of single exposure
if applicable
See point 3.3.

273.3.5 Exposure concentration/dose
measured, estimated, not available
give all available information
See point 3.3.

273.3.6 Other information: None

A6.12.2(5): Indian Childhood Cirrhosis

273.4 Examinations

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

273.5 Treatment

give any available information on the medical treatment of intoxicated persons

Treatment was not discussed in this publication.

273.6 Remarks

None

274 RESULTS

274.1 Clinical Signs

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

Clinical signs were not reported.

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.

275 APPLICANT'S SUMMARY AND CONCLUSION

275.1 Materials and methods

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 μg/g dried tissue.

Section A6.12.2(5)	**Human Case Report**
Annex Point IIA6.12.2	*specify subsection number* **A6.12.2(5): Indian Childhood Cirrhosis**
275.2 Results and discussion	*Summarize relevant results* The hepatic copper concentration of 1389±525 µg/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 µg/g dry weight), and the values in the 14 Indian children with other types of liver disease (3-292 µg/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 sub continent. 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 h 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 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

X

Section A6.12.2(5)	**Human Case Report**
Annex Point IIA6.12.2	*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 ìg/g dry weight) the normal range being 15-55 µg/g. In the remaining 14 cases, two subjects had slightly elevated hepatic copper levels (170 and 292 ìg/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	*Give date of comments submitted*
Materials and Methods	*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*
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 scaring			
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
Possibleglycogen storage disease			
19	2	Negative	30

Official use only

276 REFERENCE

276.1 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).
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)

278 MATERIALS AND METHODS

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

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 Exposure
Oral
Indicate respective route, delete other routes

278.3.1 Reason of exposure *occupational, accidental, abuse, attempted suicide*
Attempted suicide.

278.3.2 Frequency of exposure
Single/multiple
specify frequency
Not reported.

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

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

Section A6.12.2(6)	**Human Case Report**
Annex Point IIA6.12.2	*specify subsection number*

A6.12.2(6): Acute self poisoning

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.

278.4 Examinations

give type of examination and time after exposure for each examination

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.

279 RESULTS

Describe findings. If appropriate, include table.

279.1 Clinical Signs

Describe any relevant effects observed

See point 4.2 for clinical signs.

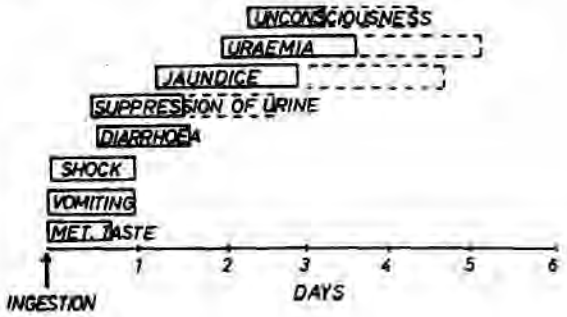
279.2 Results of examinations

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 oligaemia 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 ± 126.8 µg /100 ml and in severe cases 798 ± 396.4 µg /100 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 ± 144.2 µg /100 ml) when determined within 12 hours after ingestion of the poison than the values obtained (23.4 ± 12.4 µg /100 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 APPLICANT'S SUMMARY AND CONCLUSION

280.1 Materials and methods

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.

280.2 Results and discussion

Summarize relevant results

The most frequent symptoms observed in subjects were metallic taste, nausea, epigastrial 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 discolouration 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.

280.3 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 epigastrial 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 haemogiobin 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	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*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*
Remarks	

Section A6.12.2(7) **Human Case Report**

Annex Point IIA6.12.2 *specify subsection number*

A.6.12.2(7): Chronic self poisoning

Official use only

281 REFERENCE

281.1 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).

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 MATERIALS AND METHODS

283.1 Substance

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.

Section A6.12.2(7)	**Human Case Report**
Annex Point IIA6.12.2	*specify subsection number* **A.6.12.2(7): Chronic self poisoning**
283.4 Examinations	*give type of examination and time after exposure for each examination* The following examinations were performed (prior to liver transplantation): • Physical examination, • Laboratory investigations: serum bilirubin, albumin, alkaline phosphate, aspartate amino transferase, γglutamyl transpeptidase, prothrombin time, serum copper, serum caeruloplasmin, • Tests for hepatitis A and B antibodies and auto-antibodies, • Ultrasonography, • Urinary copper excretion, • Histology of the explanted liver.
283.5 Treatment	*give any available information on the medical treatment of intoxicated persons* Penicillamine (1.5 g/day) was commenced with increasing cupriuresis of 281 μmol on day 3. Emergency orthotopic liver transplantation was performed (7 weeks after presentation).
283.6 Remarks	None.

284 RESULTS

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

Section A6.12.2(7)	**Human Case Report**
Annex Point IIA6.12.2	*specify subsection number*
	A.6.12.2(7): Chronic self poisoning
284.2 Results of examinations	*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 ìmol 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 µmol (normal <1.2 µmol/day).
284.3 Effectivity of medical treatment	Penicillamine (1.5 g/day) was commenced with increasing cupriuresis of 281 µmol 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 µg/g dry weight (normal 20-50 µg/g). Liver zinc was normal. Postoperative urinary copper collections showed a steady decline in cupriuresis, with urinary copper excretion 47.4 µmol on day 12, 2.8 µmol on day 17 and 2.0 µmol 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)	**Human Case Report**
Annex Point IIA6.12.2	*specify subsection number* **A.6.12.2(7): Chronic self poisoning**

285 APPLICANT'S SUMMARY AND CONCLUSION

285.1 Materials and methods

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.

Section A6.12.2(7)	**Human Case Report**
Annex Point IIA6.12.2	*specify subsection number* **A.6.12.2(7): Chronic self poisoning**

285.2 Results and discussion

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 ìmol 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 µmol (normal <1.2 µmol/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 µg/g dry weight (normal 20-50 µg/g). Liver zinc was normal. Postoperative urinary copper collections showed a steady decline in cupriuresis, with urinary copper excretion 47.4 µmol on day 12, 2.8 µmol on day 17 and 2.0 µmol 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)	**Human Case Report**
Annex Point IIA6.12.2	*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 ìmol/24 h) compared to the normal (< 1.2 µmol/24 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 ìg/g dry weight (normal 20-50 µg/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	[redacted]
Reference	• [redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*

Section A6.12.2(7)	Human Case Report
Annex Point IIA6.12.2	*specify subsection number* **A.6.12.2(7): Chronic self poisoning**
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Official use only

286 REFERENCE

286.1 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).

Pratt, W.B., Omdahl, J.L. and Sorenson, R.J., (1985). Lack of Effects of Copper Gluconate Supplementation. The American Journal of Clinical Nutrition, **42:** 681 – 682 (Published).

287 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

288 MATERIALS AND METHODS

288.1 Substance

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

[redacted]

288.2 Persons exposed Non-entry field

288.2.1 Sex: Male and female.

288.2.2 Age/weight: Mean age 42 years.

288.2.3 Known Diseases: *State if any; state, if healthy*
All the subjects suffered from back pain.

288.2.4 Number of persons 7 (3 men and 4 women). 288.2.5 Other information None X

288.3 Exposure Oral

Indicate respective route, delete other routes 288.3.1 Reason of exposure
As part of a study of back pain management.

288.3.2 Frequency of exposure: Multiple doses. Subjects were dosed twice a day.
specify frequency

288.3.3 Overall time period of exposure: *if applicable*
12 weeks.

288.3.4 Duration of single exposure: *if applicable*
Not applicable.

288.3.5 Exposure concentration/dose: Subjects received 5 mg of copper twice a day in capsule form.
give all available information

288.3.6 Other information: Control subjects received placebo capsules.

288.4 Examinations

give type of examination and time after exposure for each examination

Subjects were seen every 2 weeks to evaluate their progress. Blood, serum, urine and hair samples were collected at the beginning of the study, after 6 weeks of supplementation and at the end of the 12 week study.

288.5 Treatment

give any available information on the medical treatment of intoxicated persons

Not applicable; there were no adverse effects on treated individuals. The study was approved by the Human research Review Committee.

288.6 Remarks

The study was double blind.

289 RESULTS

Describe findings. If appropriate, include table.

289.1 Clinical Signs

Describe any relevant effects observed

There were no clinical signs associated with treatment.

289.2 Results of examinations

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

There was no significant change in the level of copper, zinc or magnesium of the serum, urine or hair samples of the seven subjects during the 12 weeks of the study. There was also no significant change in the haematocrit, mean corpuscular volume, serum cholesterol, serum triglyceride, SGOT, serum alkaline phosphatase, serum GGT, or serum LDH (**Table A6.12.2(01)-1**). Serum potassium did change from a mean of 4.3 mEq/L to 4.0 mEq/L ($p < 0.05$). The incidence of nausea, diarrhoea, and heartburn was the same in the seven subjects receiving the copper gluconate as it was among the seven other subjects receiving the placebo capsules.

289.3 Effectivity of medical treatment

Not applicable to the present study.

289.4 Outcome

Describe outcome / manifestation of symptoms / disease

It was found that 10 mg/day of copper as copper gluconate had no detectable effect on the seven subjects. It was concluded that treated individuals excrete excess amounts of absorbed copper not needed to meet tissue needs or to maintain liver stores under homeostatic conditions.

289.5 Other

Describe any other significant observations

None.

290 APPLICANT'S SUMMARY AND CONCLUSION

290.1 Materials and methods

Briefly describe circumstances

As part of a double-blind study of back pain management, 7 adult patients received an oral dose of 5 mg copper (as copper gluconate capsules) twice a day for 12 weeks. Seven others received a placebo capsule over the exposure period.

	Subjects were seen every 2 weeks to evaluate their progress. Blood, serum, urine and hair samples were collected at the beginning of the study, after 6 weeks of supplementation and at the end of the 12 week study. Parameters assessed were haematocrit, mean corpuscular volume, serum cholesterol, serum triglyceride, SGOT, serum alkaline phosphatase, serum GGT, serum LDH and serum potassium. Copper, zinc and magnesium levels were also assessed in serum, urine and hair.
290.2 Results and discussion	*Summarize relevant results* There was no toxicologically significant change in the level of copper, zinc or magnesium of the serum, urine or hair samples of the seven subjects during the 12 weeks of the study. Similarly, there was no toxicologically significant change in the haematocrit, mean corpuscular volume, serum cholesterol, serum triglyceride, SGOT, serum alkaline phosphatase, serum GGT, or serum LDH. Serum potassium changed from a mean of 4.3 mEq/L to 4.0 mEq/L. The incidence of nausea, diarrhoea, and heartburn was the same in subjects receiving copper gluconate as it was in the control group. It was concluded that 10 mg/day of copper as copper gluconate had no detectable adverse effect on the seven test subjects.
290.3 Conclusion	*Give general conclusions* 10 mg copper/day, administered orally as copper gluconate for 12 weeks, had no detectable adverse effect on the livers or gastrointestinal tracts of the seven test subjects.

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]

Section A6.12.2(1)	**Human Case Report**
Annex Point IIA6.12	*specify subsection number*
IUCLID : 5.9/01	**A6.12.2(1) Copper Tolerance**

Acceptability	[redacted]
Remarks	

	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*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*
Remarks	

Table A6.12.2(01)-1. A comparison of serum levels before and after 12 weeks of supplementation with 10 mg of copper/da

	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Copper (µg/100 ml)	Zinc (µg/100 ml)	Magnesium (mg/100 ml)
Before Cu supplement	199.6 ± 26.8 mg/dL	112.4 ± 37 mg/dL	126 ± 22 µg/100 ml	1.47 ± 0.12 µg/100 ml	18.4 ± 1.5 mg/100 ml
After 12 week supplement	212.6 ± 40.7 mg/dL	101.8 ± 40 mg/dL	123 ± 16 µg/100 ml	1.44 ± 0.38 mg/ml	19.4 ± 1.6 mg/100 ml

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

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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 protection

No, published data.

(indicate if data protection is claimed)
Give name of company

291.2.1 Data owner

Not applicable.

291.2.2 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.

291.2.3 Criteria for data protection

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

Not applicable, published data.

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 Description *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*

[redacted]

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 lung**	
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	Not specified, experimental group described as 'vineyard sprayers'.	
293.4.4 Age	35-55 years old. *State if any; state, if healthy*	X
293.4.5 Diseases	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) **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**	
293.5.4 Age	22-55 years.	X
293.5.5 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.	
293.5.6 Smoking status	*smokers or non-smokers* Results were reported separately for smokers (defined as smoking for at least 5 years) and non-smokers.	
293.6 Administration/ Exposure	No Entry field	
293.6.1 Exposure Route	*Oral/Inhalation/Dermal/Combined/ Or other* Inhalation	
293.6.2 Exposure Situation	*Workplace or other; specify if other* *If workplace specify exposure conditions, e.g.emptying, filling, cleaning, transferring, packaging, maintenance or other* Authors assume that inhalation exposure to 'Bordeaux mixture' occurred during vineyard spraying operations. Subjects had been vineyard sprayers for on average 9 years.	
293.6.3 Exposure concentration(s)	*Information available / information not available* *Measured / estimated for all considered chemicals* No information available.	
293.6.4 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* Not applicable.	
293.6.5 Postexposure period	*State period of time elapsed between last exposure / first examination or last exposure / last examination respectively* Information not provided.	
293.7 Examinations	*No Entry field*	
293.7.1 Type of disease	*Specify disease and tumour sites* *give ICD-Numbers (and Number of ICD-Revision)* Sputum samples were used to detect cytological changes in the respiratory tract.	
293.7.2 Parameters	*E.g. hematology, lung function* 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.

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

Cytological changes of the respiratory tract were investigated in 52 exposed subjects and 51 control subjects.

294.3 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.

294.4 Other Observations

Describe any other significant effects

None.

295 APPLICANT'S SUMMARY AND CONCLUSION

295.1 Materials and methods

Give concise description of method

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)	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**
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)	**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**
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]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

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

	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 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 expect-oration)
				without atypia	with atypia				
Vineyard sprayers	25-35								
Nonsmokers		38	31 (82%)	22 (58%)	2 (5%)	16 (42%)	16 (42%)	26 (68%)	7 (18%)
Smokers		14	14 (100%)	5 (36%)	4 (29%)	9 (64%)	6 (43%)	11 (79%)	0
Control Group	25-55								
Nonsmokers		30	3 (10%)	0	0	0	1 (3%)	2 (7%)	27 (90%)
Smokers		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 REFERENCE

296.1 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).
Tanner MS, Kantarjian AH, Bhave 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.
(indicate if data protection is claimed)

296.2.1 Data owner

Give name of company
Not applicable.

296.2.2 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.

296.2.3 Criteria for data protection

Choose one of the following criteria (see also TnsGon Product Evaluation) and delete the others:
Not applicable, published data.

297 GUIDELINES AND QUALITY ASSURANCE *Not applicable*

298 MATERIALS AND METHODS

298.1 Test material

As given in section 2
or give name used in study report
Copper

298.1.1 Lot/Batch number *List lot/batch number if available*
Not relevant.

298.1.2 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 relevant.

298.1.2.1 Description

If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)
Not relevant.

298.1.2.2 Purity

Give purity in % of active substance
[redacted]

298.1.2.3 Stability

Describe stability of test material
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)	**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**
298.5.1 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)* See point 3.4.1.
298.5.2 Number of test persons per group/cohort size	*Give number* *specify number of matched persons per case* See point 3.4.1.
298.5.3 Sex	Not reported.
298.5.4 Age	See point 3.4.4.
298.5.5 Diseases	*State if any; state, if healthy* 377 healthy children
298.5.6 Smoking status	Only infants and children are investigated in this study.
298.6 Administration/ Exposure	No Entry field
298.6.1 Exposure Route	*Oral/Inhalation/Dermal/Combined/ Or other* Possible oral exposure.
298.6.2 Exposure Situation	*Workplace or other; specify if other* *If workplace specify exposure conditions, e.g.emptying, filling, cleaning, transferring, packaging, maintenance or other* 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)	*Information available / information not available* *Measured / estimated for all considered chemicals* 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).

X

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**
298.6.4 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* 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. X
298.6.5 Postexposure period	*State period of time elapsed between last exposure / first examination or last exposure / last examination respectively* 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.

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 (µg/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 (were the UK researchers were based) tap-water contained 5.1±0.6 µg/dl copper.

Milk Copper Concentrations

Values in English dairy milk (n=14) were 9.3 ±3.0 µg/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 µg/dl) did not significantly differ from concentrations in 8 samples taken from brass vessels in control households (39-546, mean 130 µg/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.

X

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**
299.2 Number of cases for each disease / parameter under consideration	*No effects / describe significant effects referring to data in results table including confidence intervals* See point 3.4.1.
299.3 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.
299.4 Other Observations	*Describe any other significant effects* None.
	300 APPLICANT'S SUMMARY AND CONCLUSION *Give concise description of method*
300.1 Materials and methods	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

X

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 04. 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

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

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 in 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.

300.3.1 Reliability

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

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**	
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

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

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

		Milk		Water
Study group	Houses	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 x^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

		Animal milk		**Breast milk**	
Study Group	**Children**	**None given before 2 years of age (no [%])**	**Age of introduction (mean, median ±SD) in months**	**None given (no[%])**	**Duration (mean, median ±SD) in months**
ICC	132	0	4.3, 3.0 ±4.3	21(16)	10.6,11.0 ±7.0
Other hepatic disorders	70	7(10)†	9.1, 9.0†±5.7	4(6)	17.1, 11.0±9.1
Srirampur controls	171	54 (32)†	9.4, 9.0†±5.5	2(1	
Miraj controls	140	35(25)†	10.5, 12.0†±6.1	5(4)*	15.7, 12.0†±6.0
Prune controls	66	0	5.0. 3.0†±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	Strirampur
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 (µg/dl) in samples and milk (n=4) after storage, and after boiling in various utensils

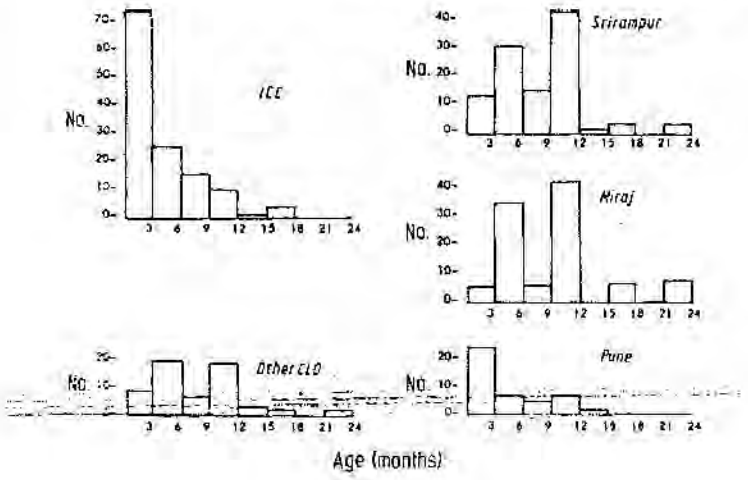
	Water				Milk			
	Standing at 21°C		Boiled, then stored at 21°C for 6 h		Standing at 21°C		Boiled, then stored at 21°C for 6 h	
	10 min	6 h	After boiling	After 6h	10 min	6 h	After boiling	After 6h
Glass	4.2 ±0.5	4.1 ±0.5	6.3 ±0.4	4.9 ±0.3	8.6 ±1.4	10.4 ±3.7	11.7 ±4.9	13.5 ±3.3
Tinned copper	4.4 ±0.7	5.7 ±0.8	7.0 ±1.0	6.7 ±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 ±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 ± 51†
Brass	5.0 ±0.9	12.5 ±1.5†	7.6 ±0.5†	32.7 ±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).

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 cirrosis

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 REFERENCE

301.1 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).

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 MATERIALS AND METHODS

303.1 Test material

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**
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	*Give purity in % of active substance* [redacted]
303.1.2.3	Stability	*Describe stability of test material* Not applicable.
303.2 Type of study		*Cohort 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)* <u>Mortality data</u> 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. <u>Copper concentrations</u> 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	*Give number* *specify, if there are differences for example for treatment and recovery groups* The average population aged 0-5 over the 23 year period investigated was as follows: Duxbury: 805 Wrentham: 563 Bellingham: 1420 (This corresponds to 64,124 child years of exposure to the drinking water presumed to contain ca. 8.5 mg Cu/L).
303.4.3 Sex	Males and females.
303.4.4 Age	0-5 years.
303.4.5 Diseases	*State if any; state, if healthy* The overall incidence of mortality and mortality related to any form of liver disease was investigated.
303.4.6 Smoking status	*smokers or non-smokers* Not applicable.
303.5 Controls	
303.5.1 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)* Not applicable. No control.
303.5.2 Number of test persons per group/cohort size	*Give number* *specify number of matched persons per case* Not applicable.
303.5.3 Sex	Not applicable.
303.5.4 Age	Not applicable.
303.5.5 Diseases	*State if any; state, if healthy* Not applicable.
303.5.6 Smoking status	*smokers or non-smokers* Not applicable.
303.6 Administration/ Exposure	*No Entry field*
303.6.1 Exposure Route	*Oral/Inhalation/Dermal/Combined/ Or other* Via drinking water.
303.6.2 Exposure Situation	*Workplace or other; specify if other* *If workplace specify exposure conditions, e.g.emptying, filling, cleaning, transferring, packaging, maintenance or other* Exposure to domestic drinking water.

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).

304 RESULTS AND DISCUSSION

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

304.1 Exposure

304.1.1.1 Number of measurements: For each of the three towns, the drinking water of 60 homes was sampled.

304.1.1.2 Average concentrations

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

304.1.1.3 Standard deviation: Not reported.

304.1.1.4 Date(s) of measurement(s): 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)

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**

305.1 Materials and methods

Give concise description of method

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.

305.2 Results and discussion

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).

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 (µg/g dry weight)	Cu in water (mg/L)
Lim and Chod[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[2]	F	9 mo	13 mo	2154	2·2–3·4
	M	5 mo	NK	2800	2·2–3·4
Walker-Smith and Blomfield[3]	M	14 mo	16 mo	3360	0·3–6·8
Adamson et al[4]	M	2 yr	32 mo	1500	1·13
Aljaleh et al[5]	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.

	The authors also discussed various published cases of ICT 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 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.

Subsection (Annex Point)		Official use only
8.1 Recommended methods and precautions concerning handling, use, storage, transport or fire (IIA8.1)	Ensure adequate ventilation and avoid generation of dusts Not combustible Store in a dry place and keep container tightly closed	
8.2 In case of fire, nature of reaction products, combustion gases, etc (IIA8.2)	In the event of fire, carbon dioxide, carbon monoxide and copper oxides may be released.	
8.3 Emergency measures in case of an accident (IIA8.3)	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. 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.

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

Non entry field

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

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

8.5.2 Possibility of neutralisation of effects
(IIA8.5.2)

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

8.5.3 Conditions for controlled discharge including leachate qualities on disposal (IIA8.5.3)8.5.4 Conditions for controlled incineration

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

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

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

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

8.7 Identification of any substances falling

In view of the tightly controlled conditions under which the active gredient is produced, handled and formulated into wood preservation oducts, it is considered that there is no potential for undesirable or ...intended 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.

Copper and its compounds are included in List II of Council

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	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	
Acceptability	
	[redacted]
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*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*
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	