LITERATURE DATA

Copper

1. Antiseptic effects of copper

Copper is a trace element required for several essential biological processes that exhibits remarkable structural and functional conservation from bacteria to humans. The fact that copper exists in two oxidation states, cuprous Cu(I) (reduced) and cupric Cu(II) (oxidized), contributes to its ability to serve as a catalytic cofactor in diverse biological systems including microorganisms.
Copper is an essential element; however, in low or excessive amounts copper can have deleterious effects. **In excess, copper affects the biochemistry of macromolecules and reportedly leads to a rapid decline in membrane integrity** [HONG, 2012; IBRAHIM, 2011].

Currently, the antimicrobial properties of metallic copper surfaces are being tested against numerous pathogens. Humans have used copper as far back as the 5th and 6th millennia; however, it was not until 2008 that its ability to inactivate microbes upon contact was identified. Subsequently, rigorous testing by the US Environmental Protection Agency (EPA) **led to the registration of copper alloys as antimicrobial agents**. EPA support has resulted in increased interest in the use of copper as an antimicrobial agent. **The human pathogens that have been the most widely tested against copper include Methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile*, *Escherichia coli*, *Listeria monocytogenes, Influenza A (H1N1), Aspergillus niger, and Pseudomonas aeuroginosa*, as reviewed by GRASS [2011].**

Table 1 summarizes the species tested, test procedures and killing kinetics [GRASS, 2011].

Table 1. Contact killing of microbes by copper surface [GRASS, 2011]

Species	Application method	Killing time, RT[a]
Salmonella enterica	Wet, 4.5×10^6 CFU[b]	4 h
Campylobacter jejuni	Wet, 4.5×10^6 CFU[b]	8 h
Escherichia coli O157	Wet, $(3–4) \times 10^7$ CFU[c]	65 min
Escherichia coli O157	Wet, 2.7×10^7 CFU[c]	75 min
MRSA[d] (NCTC10442)	Wet, $(1–1.9) \times 10^7$ CFU[c]	45 min
EMRSA-1[e] (NCTC11939)	Wet, $(1–1.9) \times 10^7$ CFU[c]	60 min
EMRSA-16[e] (NCTC13143)	Wet, $(1–1.9) \times 10^5$ CFU[c]	90 min
Listeria monocytogenes Scott A	Wet, 10^7 CFU[c]	60 min
Mycobacterium tuberculosis	Wet, 2.5×10^7 CFU[f]	5 to 15 days[g]
Candida albicans	Wet, $>10^5$ CFU[f]	60 min
Klebsiella pneumoniae	Wet, $>10^7$ CFU[f]	60 min
Pseudomonas aeruginosa	Wet, $>10^7$ CFU[f]	180 min
Acinetobacter baumannii	Wet, $>10^7$ CFU[f]	180 min
MRSA	Wet, $>10^7$ CFU[f]	180 min
Influenza A virus (H1N1)	Wet, 5×10^5 viruses[h]	6 h, 4-log decrease
C. difficile (ATCC 9689) vegetative cells and spores	Wet, 2.2×10^5 CFU[c]	24–48 h
C. difficile NCTC11204/R20291 vegetative cells	Wet, $(1\text{-}5) \times 10^6$ CFU[i]	30 min
C. difficile dormant spores	Wet, 8×10^6 CFU[i]	Unaffected in 3 h
C. difficile germinating spores	Wet, 8×10^6 CFU[i]	3 h, 3-log decrease
Pseudomonas aeruginosa PAO1	Wet, 2.2×10^7 CFU[j]	120 min
MRSA NCTC 10442	Wet, 2×10^7 CFU	75 min, 7 log decrease
Escherichia coli W3110	Dry, 10^9 CFU[k]	1 min
Acinetobacter johnsonii DSM6963	Dry, 10^9 CFU[k]	A few minutes
Pantoea stewartii DSM30176	Dry, 10^9 CFU[k]	1 min
Pseudomonas oleovorans DSM 1045	Dry, 10^9 CFU[k]	1 min
Staphylococcus wamerii DSM20316	Dry, 10^9 CFU[k]	A few minutes
Brachybacterium conglomeratum DSM 10241	Dry, 10^9 CFU[k]	A few minutes
Aspergillus flavus	Wet, $(2–300) \times 10^5$ spores[c]	120 h
Aspergillus fumigatus	Wet, $(2–300) \times 10^5$ spores[c]	>120 h
Aspergillus niger	Wet, $(2–300) \times 10^5$ spores[c]	> 576 h
Fusarium culmonium	Wet, $(2–300) \times 10^5$ spores[c]	24 h
Fusarium oxysporum	Wet, $(2–300) \times 10^5$ spores[c]	24 h
Fusarium solani	Wet, $(2–300) \times 10^5$ spores[c]	24 h
Penicillium crysogenum	Wet, $(2–300) \times 10^5$ spores[c]	24 h
Candida albicans	Wet, $(2–300) \times 10^5$ spores[c]	24 h
Enterococcus hirae ATCC 9790	Wet, 10^7 CFU[c]	90 min
Different *Enterococcus* spp.	Wet, 10^6 CFU[f]	60 min
Candida albicans	Dry, 10^6 CFU[k]	5 min
Saccharomyces cerevisiae	Dry, 10^6 CFU[k]	30 s

[a] RT, room temperature; only the values for the most efficient alloy are reported.
[b] Inoculation with 1.5 ml of culture (4.5×10^6 CFU), kept under humid conditions.
[c] Inoculation with a 20-μl drop of culture.
[d] Methicillin-resistant *Staphylococcus aureus*.
[e] Epidemic methicillin-resistant *Staphylococcus aureus*.
[f] Twenty microliters of culture spread on coupons.
[g] Time before strain became culture positive in Bactec 12B growth medium after exposure to copper.
[h] Inoculation with 20 μl of virion suspension.
[i] One hundred microliters of dilute culture.
[j] Twenty-five microliters of culture spread on coupons with a glass spreader.
[k] Thin film applied with a cotton swab.

In general, microbes were inactivated on copper within hours, but such parameters as the inoculation technique, incubation temperature, and copper content of the alloy used were not usually investigated in a systematic way and are difficult to compare between studies. Nevertheless, a few general principles appear clear: higher copper content of alloys, higher temperature, and higher relative humidity increased the efficacy of contact killing. Treatments that lowered corrosion rates, e.g., application of corrosion inhibitors or a thick copper oxide layer, lowered the antimicrobial effectiveness of copper surfaces [GRASS, 2011].

These findings indicate that copper can have antibacterial effects in applications where control of these germs would be beneficial. Currently, copper is used in numerous effective antimicrobial agents, including fungicides, antifouling paints, antimicrobial medicines, oral hygiene products, **hygienic medical devices**, and antiseptics [Airey, 2007 cited by IBRAHIM, 2011].

Antimicrobial effects of copper

No study on the efficacy of cupric sulfate as antimicrobial agent following nasal administration is available in the literature. Two studies assessing the anti-bacterial effects of a solution of $CuSO_4$ on dental plaque are presented. Dental plaque is a heterogeneous accumulation adherent to the surface of teeth or situated in the dental gingival spaces. **Plaque consists of a community of anaerobic and aerobic bacteria** enclosed in an intercellular matrix of mucoprotein polymers of microbial and salivary origin. The deposit forms in a few hours [ROBERT, 1999].

Characteristics of these studies are summarized in the table below:

Efficacy of cupric sulfate on microorganisms		
WAERHAUG, 1984 Double blind cross over study	14 dental students (modified experimental gingivitis model) 20-26 years old	$CuSO_4$: 2.2mM Chlorhexidine 2.2mM 2 mouth rinses/ day for 21 days Wash-out period: 14 days
WALER, 1982 Double blind cross over study	5 volunteers	$CuSO_4$: 1.1mM Chlorhexidine 1.1mM Silver nitrate 1.1mM Placebo 2 mouth rinses (10mL – 1min)/day for 4 days Wash-out period: 3 days

$CuSO_4$ (2.2mM) was shown to inhibit plaque formation due to bacteria and development of gingivitis in a modified experimental gingivitis model [WAERHAUG, 1984].
Fourteen dental students were included in this double blind cross-over study and treated by 2 daily mouth rinses of 2.2mM $CuSO_4$ or 2.2mM chlorhexidine gluconate versus tap water (negative control) for 21 days without mechanical oral hygiene. The wash-out period between the 2 treatments was 14 days to avoid carry over effects. Prior to each experiment period, the participant teeth were scaled and polished in order to remove all supragingival deposits. The gingival index (GI) and the plaque index were recorded (PII). Impressions and side effects, if any were also reported. Results were statistically analyzed (t-test).

The results were as follows:
$CuSO_4$ showed a significant inhibiting effect upon plaque formation as well as the development of gingivitis but these effects were not as marked as those of chlorhexidine.

Subjects Mean ± SD	Plaque index			Gingival index		
	CHX	$CuSO_4$	Water	CHX	$CuSO_4$	Water
	0.29±0.18	0.79±0.26	1.25±0.26	0.57±0.24	0.83±0.32	1.02±0.32

The effect of $CuSO_4$ on the prevalence of PI (score 1) was negligible whereas the effect upon the prevalence of visible plaque (PII score 2) and moderate gingivitis (GI score 2) seemed to approach that of chlorhexidine.

The test subjects, in general complained about the taste of the test substance. Cupric sulfate seemed to be more acceptable in this respect than chlorhexidine and did not cause any lasting interference with taste. Only a certain dryness of the mouth was experienced by some of the participants after both chlorhexidine and $CuSO_4$ rinses.

In conclusion, these results evidenced the efficacy of $CuSO_4$ (2.2mM, twice daily) on the bacteria constituting the dental plaque modified experimental gingivitis model.
Some teeth coloration was also noted for both tested products in some patients, the coloration was darker for chlorhexidine [WAERHAUG, 1984].

These results are in line with those of WALER [1982] who assessed the effects of chlorhexidine, copper ($CuSO_4$) and silver ($AgNO_3$) solutions in a group of 5 volunteers using sucrose rinses to enhance plaque formation in a double blind cross-over study. The plaque index (PI) was measured after rinsing with the test solutions twice daily (10mL) for 4 days and no mechanical oral hygiene allowed. There were at least 3 days of habitual oral hygiene and use of NaF tablets between each experimental period.

The results are displayed in the table below:

Plaque Index (PI)	Sucrose rinses	1.1mM CHX +sucrose rinses	1.1mM $CuSO_4$ +sucrose rinses	1.1mM $AgNO_3$ +sucrose rinses
Mean ± SD	1.57±0.14	0.32±0.11	0.37±0.06	1.26±0.29

CHX: chlorhexidine

Chlorhexidine and copper, reduced similarly plaque score (no statistically significant difference between these 2 rinses). Silver nitrate also showed a plaque inhibiting effect, statistically significant from the placebo, but the effect was also significantly less than when chlorhexidine or copper were used.
The anionic parts of the salts are known to have significance for the plaque inhibiting effect as long as the salts are soluble in water, so the plaque inhibition could conceivably be caused by the cationic metal in the same way as by the cationic chlorhexidine molecule.

In conclusion, these results evidenced the efficacy of $CuSO_4$ (1.2mM, twice daily) on the bacteria constituting the dental plaque modified experimental gingivitis model [WALER, 1982].

Some studies assessed the efficacy of copper in *in vitro* studies and evidenced its mechanism of action as antibacterial agent.
HONG [2012] assessed the survival, phospholipids oxidation (TBARS levels), and DNA degradation in *E. coli* exposed to copper alloy surfaces containing 60 to 99.90% copper or in medium containing $CuSO_4$ (4 to 8mM).
Exposure of *Escherichia coli* to increasing concentrations of $CuSO_4$ correlated with decreased survival and increased TBARS production. Little to no killing was seen at the 4 and 5mM $CuSO_4$ concentrations, although no significant increase in the number of CFU was observed either. It appears that at these low concentrations $CuSO_4$ copper ions were bacteriostatic but not bactericidal.
This is in line with other literature data evidencing that the presence of copper ions in the medium inhibited proliferation of wild-type strains of *E. coli* at concentrations as low as 0.2 to 2.5mM [*Grass, 2001; Outten, 2001* cited in HONG, 2012] but may not be killing the cells. At 6 mM$CuSO_4$, a low rate of killing was observed, about 3 logs of killing over the 60-min time course. At 7 to 8mM, this rate appeared to reach a maximum of about 7 to 8 logs of killing, leaving few to no survivors after 1 h.
It should be noted that TBARS levels increased even at the lowest concentrations of 4 to 5mM, which did not appear to kill cells.
Taken together, these results suggest that copper alloy surface-mediated killing of *E. coli* is triggered by non-enzymatic oxidative damage of membrane phospholipids that ultimately results in the loss of membrane integrity and cell death.

IBRAHIM [2011] evaluated the antibacterial activity of copper surfaces and copper powder against members of the *Burkholderia cepacia* complex (Bcc) consisting of 17 closely related multidrug resistant bacterial species difficult to eradicate. This group is an important causative agent of opportunistic and nosocomial infections. The antibacterial activity of different copper surfaces was evaluated by incubating them with Bcc strain suspensions (5×10^7 CFU/ml) in dry or wet sample. The bacterial survival counts were calculated and the data for various copper surfaces were compared to the data for stainless steel and polyvinylchloride, which were used as control surfaces. The antibacterial activity of copper powder (0.25mg/disk) was determined with the diffusimetrical technique and the zone of inhibition was evaluated with paper disks. A single cell gel electrophoresis assay, staining assays, and inductively coupled plasma mass spectroscopy were performed to determine the mechanism responsible for the bactericidal activity.

The results showed:

- a significant decrease in the viable bacterial count after exposure to copper surfaces. The minimal time recorded for complete *B. seminalis* death was 1 min on dry copper surfaces and 14 h on moist copper surfaces. In contrast, *B. cepacia, B. multivorans, B. cenocepacia, B. arboris, B. dolosa*, and *B. lata* required a minimum of 8h of copper exposure. The physical appearance of the copper surfaces changed during the antibacterial copper sheet assays, where the copper sheets turned a dark brown color with time and the bacterial suspensions became a pale blue color, this being caused by Cu++ ion release. Over time, this pale blue color became deepened.
- that the copper powder produced a large zone of inhibition
- a significantly higher influx of copper ions into the bacterial cells that were exposed to copper surfaces compared to the controls.

The SCGE (single cell gel electrophoresis) test was used to study the Bcc bacterial DNA after dry metallic copper exposure. Comet tails or DNA fragmentation was observed for the cells exposed for 1 h but not for those exposed for 15 sec or to the PVC control surface. These results indicate that the bacterial DNA is lethally damaged by copper after 1 h of exposure but not after 15 s.

Using general bacterial staining method, cell disintegration resulting in visible cellular debris was evidenced after 15 s of exposure to the dry metallic copper surface. In contrast, the control cells exposed to PVC remained unaffected. These results indicated that bacterial exposure to copper led to severe structural damage and cell lysis.

In conclusion, the present study demonstrates that copper (surfaces or powder) has an antibacterial effect against Bcc bacteria and that copper adversely affects the bacterial cellular structure, thus resulting in cell death. These data, along with the existing literature, suggest that bacterial death via copper is preceded by cell membrane damage, copper influx into the cells, oxidative damage, and DNA damage.

FAN [2013] evaluated the effectiveness of copper sulfate ($CuSO_4$), chlorine, potassium permanganate (KMnO4), hydrogen peroxide (H_2O_2) and ozone on the cell integrity and densities of *Microcystis aeruginosa*. $CuSO_4$ was used in this study as a bench mark to compare with other water treatment technologies for cyanobacteria control. Copper concentrations of 0.0 (control), **0.5, 1.0 and 1.5 mg/L were tested.** Three replicates for each concentration were conducted. Samples were taken after copper incubation on days 1, 2, 3 and 7 for analyses.

The cell membrane of cyanobacteria was compromised to varying degrees depending on the tested products. Chlorine showed the strongest ability to impair the cell integrity with a

majority (≥88%) of the cells compromised within the first minute and with the cell lysis rates ranging of 0.640-3.82/h during 1-60 min. Ozone dose of 6 mg/L also could induce 90% lysis of the cyanobacterial cells in 5 min and the cell lysis rate of KMnO4 (10 mg/L) was 0.829/h. H_2O_2 and $CuSO_4$ and could not only destroy the viability of cyanobacterial cells but also showed algistatic potential over the 7 day treatment.

Only the results concerning $CuSO_4$ are detailed below:
The optical properties of cells shift as their physiology changes in response to chemical treatment. The group of M. aeruginosa cells shifted after 1 day $CuSO_4$ treatment. Addition of $CuSO_4$ to the M. aeruginosa culture gave rise to a second population of cyanobacterial cells.
The population of lysed cells increased with increasing $CuSO_4$ concentration. The percentage of compromised cyanobacterial cells almost achieved 100% with highest $CuSO_4$ concentration (1.5 mg/L) treatment. Other oxidants presented similar population shifting in the flow cytometric dot-plots and histogram when the cells lost membrane integrity (e.g. for H_2O_2). 13% of the cyanobacterial cells lost membrane integrity with copper concentration of **0.5 mg/L** treatment after 1 day. The percentage of compromised cells increased with time during the first 3 days (33% for 0.5mg/L). Approximately 70% of cells exposed to **1.0 mg/L $CuSO_4$** lost membrane integrity after 1 day with 98% of the cells impaired after 2 days treatment.

In conclusion, lowest $CuSO_4$ concentration of 0.5 mg/L led to lysis in 33% of the population after 3 days copper treatment. This is in agreement with the study by *Kenefick* [1993 cited by FAN, 2013] which demonstrated that 0.5 mg/L copper resulted in loss of membrane integrity in *M. aeruginosa* cells [FAN, 2013].

Antiviral effects of copper

Copper is a promising candidate for virus inactivation [IMAI, 2012]. Several studies have shown that copper reduced the infectivity of the enveloped or non-enveloped DNA or RNA viruses, including influenza virus, with different intensities. Copper has potent virucidal properties, and copper's neutralization of infectious bronchitis virus, poliovirus, human immunodeficiency virus type 1 (HIV-1), and other enveloped or non-enveloped single- or double-stranded DNA or RNA viruses has been reported [BORKOW, 2005].

Copper was also shown to inhibit influenza viruses. The infectivity of the H9N2 virus to MDCK cells was time-dependently inhibited by copper solutions ($CuSO_4$ and $CuCl_2$) in *in vitro* testing at concentrations of 2.5-250 µM [HORIE, 2008]. In 25 µM $CuSO_4$ solution, the virus titer decreased by approximately 3 and 4 log within 3 and 6 h, respectively.
The H9N2 virus hemagglutinin activity was not affected by 2.5-250 µM Cu^{2+} ($CuSO_4$ and

$CuCl_2$ solutions). The H9N2 virus neuraminidase (NA) activity was drastically reduced by 25 mM Cu^{2+} ($CuSO_4$ and $CuCl_2$) marginally reduced by 250 µM Cu^{2+}, and not affected by 25 µM Cu^{2+}.
Thus, copper ions suppress the infectivity of influenza virus at lower concentrations at which neither NA nor hemagglutination inhibition occurs. Electron microscopic analysis revealed morphological abnormalities of the Cu^{2+}-treated H9N2 virus.

BORKOW [2007] reported the capacity of copper oxide-containing filters (25mg/300µmole of copper oxide/ filter - 2.5 cm radius) to reduce infectious titers of a panel of viruses spiked into culture media.
Among these viruses, several are implicated in ORL infections such as:

- Rhinovirus 2 causing 50% of common colds [Santé Canada Rhinovirus, 2011]
- Influenza A virus responsible for seasonal flu epidemics [CDC Influenza, 2014]
- Parainfluenza virus 3 causing a spectrum of respiratory illnesses, 30–50% of which are complicated by otitis media. Most children are infected by parainfluenza virus type 3 (PIV-3) by the age of two years [WHO Parainfluenza 3, 2008].
- Respiratory syncytial virus (RSV) that is the most common cause of bronchiolitis and pneumonia among infants under 1 year of age [SA Health, RSV, 2016].
- Adenovirus type 1 causing respiratory infections such as rhinopharyngitis occurring mainly in young children [Santé Canada, Adénovirus, 2014].

Enveloped, non-enveloped, RNA, and DNA viruses were affected, suggesting the possibility of using copper oxide-containing devices to deactivate a wide spectrum of infectious viruses found in filterable suspensions.
As summarized in Table 2, a passage of the viruses through the filters containing copper oxide resulted in a significant reduction of the infectious viral titers, ranging from 0.47 $\log_{10}$ to 4.6 $\log_{10}$ depending on the virus tested. Regarding the virus especially involved in ORL pathologies, a significant reduction of the infectious viral titers, ranging from 1.1 $\log_{10}$ to 2.2 $\log_{10}$.
A further decrease in infectious viral titers could be achieved by reduction of the flow rate. Approximately 1.7-fold increase in HIV-1 neutralization was achieved by decreasing the flow rate by 2.5-fold. Residual virus was undetectable by further reducing the flow rate by twofold.

Table 2. Reduction of infectious viral titers by copper oxide-containing filters

Virus	Viral strain	Viral family	Genome	E/NE[a]	Cells used in assay	Virus titer control[b]	Virus titer Cu filter[b]	Log_{10} reduction (mean ± SD)[c]	*P* value[d]
Rhinovirus 2	HGP	*Picornaviridae*	RNA	NE	HeLa Ohio-1	4.3/7/7	3.3/3/6	2 ± 1.7	0.2
Yellow fever virus	17D	*Flaviviridae*	RNA	E	Vero-76	6.3/6.3/5.9	5.7/4.7/4.8	1.1 ± 0.5	<0.05
Influenza A virus	Panama/2007/99 H3N2	*Orthomyxoviridae*	RNA	E	MDCK	7.5/7.5/6.8	6.7/5/4.8	1.77 ± 0.87	<0.001
Measles virus	Chicago	*Paramyxoviridae*	RNA	E	CV-1	3.67/3.67/3.67	0/0/0	≥3.67	<0.001
Respiratory syncytial virus	A2	*Paramyxoviridae*	RNA	E	MA-104	4/4/4	3/2/2.5	1.5 ± 0.5	<0.01
Parainfluenza virus 3	14702	*Paramyxoviridae*	RNA	E	MA-104	8/8/8	7.33/6.33/7	1.11 ± 0.5	<0.05
Punta Toro virus	Adames	*Bunyaviridae*	RNA	E	LLC-MK2	7/6.6/6.6	3.5/6/5.5	1.73 ± 1.55	0.09
Pichinde virus	AN 4763	*Arenaviridae*	RNA	E	BSC-1	7.5/7.6/7	4.5/5.6/6.9	1.7 ± 1.47	0.08
HIV-1	IIIB	*Retroviridae*	RNA	E	MT2	6/6.5/6	0.8/2.5/1.5	4.6 ± 0.6	0.001
Adenovirus type 1	Ad-HIVluc	*Adenoviridae*	DNA	NE	cMAGI	5/5/5.2	2.5/3.2/2.9	2.2 ± 0.36	0.001
Cytomegalovirus	AD169	*Herpesviridae*	DNA	E	Fibroblasts	6/6/6	2/1.5/1.6	4.3 ± 0.26	<0.001
Vaccinia virus	WR	*Poxviridae*	DNA	E	Vero-76	7.4/7.6/7.6	7.4/6.7/7.1	0.47 ± 0.45	0.095

[a] E, enveloped viruses; NE, nonenveloped viruses.
[b] Log_{10} PFU/ml for vaccinia virus; log_{10} $CCID_{50}$/ml for the other viruses.
[c] The log reduction was calculated as log_{10} $CCID_{50}$/ml of the titer obtained from the control filter minus log_{10} $CCID_{50}$/ml of the filter containing copper oxide. The values are the mean and standard deviation obtained in three separate experiments.
[d] *t* test between viral titers obtained after filtration in control filters not containing copper versus copper-containing filters.

In conclusion, these in vitro and human studies support the efficacy of copper (Cu^{2+}) and $CuSO_4$ as solid or in solution for decreasing and/or stabilizing bacterial and viral populations including viruses directly related to ORL diseases. In addition, they suggested that a minimal contact time is required for the copper effect on infectious viral titers. Concentrations of $CuSO_4$ as low as 2.5µM were found efficacious for inhibiting influenza virus in vitro.

In this way $CuSO_4$ can help to limit microorganism populations in nasal cavities for patients suffering from URTI that likely to be surinfected or to contaminate other persons. Indeed, virus or bacteria are spread when an infected person talks, coughs or sneezes small droplets containing infectious agents into the air. The droplets in the air may be breathed in by those nearby. Infection may be spread by contact with hands, tissues and other articles soiled by infected nose and throat discharges.

2. Safety of copper/cupric sulfate

Copper is a natural element that is an essential micronutrient to ensure the wellbeing of all aerobic life forms. It plays a vital part in the development and performance of the human nervous and cardiovascular systems, as well as the skin, bone, immune and reproductive systems, including gene transcription.
The recommended daily intakes are established from 0.4mg/d for infants to 1.5-2mg/day for adults. For preterm, the RDI has to be nearly doubled [AFSSA, 2001]. Some toxicological studies performed in humans following oral administration allowed a NOAEL to be established at 0.042mg/kg/d for copper sulfate with respect to gastrointestinal effects [ATSDR, 2016]. Suspected early atrophic changes in the nasal mucosa only occurred after long term exposure (>10 years) to copper dusts containing 92% of copper as different compounds [ASKERGREN, 1975]. Contact sensitivity of metal salt including CuSO4 (10% in ethanol 20%) was evidenced in animal studies [IKARASHI, 1992].

Studies

Three experimental human studies and two community-based studies have examined the oral toxicity of copper in healthy humans. The primary focus of these studies was examination of the potential of low doses of copper to induce hepatic effects in adults [*Araya, 2003; Pratt, 1985* cited in ATSDR, 2016] or infants [*Olivares, 1998; Zietz, 2003a, 2003b* cited in ATDSR, 2016]; no adverse effects were found.
The Araya study [2003] also assessed the potential for gastrointestinal effects in adults and found significant increases in the incidence of effects as a function of dose/duration. **A NOAEL was established in humans as 0.042mg/kg/d for copper sulfate in this 2 month study** [ATSDR, 2016].

Exposure to excess levels of copper has been associated with adverse health effects in infants and children [ATSDR, 2016]. There is an extensive body of literature on two syndromes that have been associated with exposure to high levels of copper, Indian childhood cirrhosis and idiopathic copper toxicosis. Both are characterized by severe liver damage in infants and children (<5 years of age). In the case of Indian childhood cirrhosis, excessive copper exposure has been traced to the use of brass or copper containers for storage and heating of milk. Additionally, no alterations in serum biomarkers of liver damage (alanine aminotransferase activity, aspartate aminotransferase activity, gamma glutamyl transferase activity, and total bilirubin levels) were observed in infants ingesting water containing **2 mg/L copper** (0.319 mg/kg/day) [*Olivares, 1998* cited in ATDSR, 2016] or infants living in households with tap water copper levels of 0.8 mg/L [*Zietz, 2003a, 2003b* cited in ATDSR, 2016].

Copper sulphate is a powerful oxidizing agent, which is corrosive to mucous membranes [SARAVU, 2007]. *In vitro* studies suggest that copper is poorly absorbed through intact skin. Less than 6% of copper deposited on ex vivo human skin samples was absorbed; copper chloride was absorbed to a higher extent than copper sulfate [ATSDR, 2016]. ASKERGREN [1975] evaluated changes in the nasal mucosa after exposure to copper salt dust. Ten metal workers with varying exposure to complex copper salts in dust form, 6 other metal workers not exposed and 9 construction workers in other occupational categories also unexposed to the salts were compared with respect to history and mucosal change in the nose.
The same physician examined all subjects. The mucous membrane of throat and nose were examined first and their state was noted.

The constituents of the patina dust according to the manufacturer analysis are displayed in the table below.

Constituents of the patina dust	Percentage
Copper hydroxide nitrate: $Cu(NO_3)_2.3Cu(OH)_2$	26
Copper hydroxide sulfate: $CuSO_4.3Cu(OH)_2$	25
Copper hydroxide chloride: $CuCl_2.3Cu(OH)_2$	1
Copper hydroxide carbonate: $CuCO_3.\ 3Cu(OH)_2$	1
Copper silicate: $CuSiO_3$	22
Copper oxide: CuO	17
Ferric oxide: Fe_2O_3	4
Water, H_2O	4

Four compounds dominated the patina dust in this study: copper hydroxide nitrate, copper hydroxide sulfate, copper silicate and cupric oxide known to act as local irritants.
The results are displayed in the table below:

Subject	*Age (years)*	*Occupation*	*Years in occupation*	*Work with patinated sheeting*		*Exposure in number of years with ordinary copper sheeting*	*Normal mucosa*	*Livid mucosa*	*Mucoid secretion*	*Crusts*	*Dry mucosa*	*Thin mucosa*
				Duration (months)	Latest exposure							
1	33	Sheet-metal worker	19	7	1971	7	+					
2	39		17	8	1971	5		+				
3	50		35	6	1972	5	+					
4	51		32	1	1969	18		+	+			
5	52		30	9	1972	12					+	
6	60		45	7	1972	5	+					
7	60		45	60	1972	20						+
8	60		40	18	1968	12		+				+
9	61		47	12	192	3					+	+
10	61		43	60	1972	16						+
11	26		10			½	+	+				
12	33		19			4						+
13	50		34			10	+					
14	51		36			25		+				
15	61		50			50			+	+		
16	61		20			6					+	
17	33	Carpenter	18				+					
18	39	Painter	22								+	
19	50	Concrete worker	30					+	+			
20	51	Construction	2						+			

		worker										
21	52	Concrete + laborer	35				+					
22	56	Laborer	35								+	
23	60	Carpenter	43						+			
24	60	Laborer	43					+	+			
25	61	Concrete + supervision	40				+		+			

+: presence of this symptom

Four of the ten exposed sheet-metal workers displayed atrophic mucosal changes. These four subjects were also the ones with the longest exposure time. It is notable that one of the examined subjects had not been exposed to the substances for at least 6 months prior the examination. One patient even reported a previous exposure occasion 5 years earlier. The fact that one man with thin mucosa was found among the 15 unexposed subjects should hardly have any effect on the picture as whole. The number of cases with livid mucosa (mainly on the conchae) and the distribution of these cases among the three occupational groups and age groups did not permit the drawing of any conclusions; neither could conclusions be drawn concerning the finding of dry mucosa.
In conclusion, suspected early atrophic changes in the nasal mucosa occurred after long term exposure to copper dusts containing 92% of copper as different compounds.

Conclusion: Copper is a natural element that is an essential micronutrient to ensure the wellbeing of all aerobic life forms. The recommended daily intakes are established from 0.4mg/d for infants to 1.5-2mg/day for adults. For preterm, the RDI has to be nearly doubled [AFSSA, 2001]. Some toxicological studies performed in humans following oral administration allowed a NOAEL to be established at 0.042mg/kg/d for copper sulfate with respect to gastrointestinal effects [ATSDR, 2016].

3. REFERENCES

Literature

AFSSA. Apports nutritionnels conseillés pour la population française, 3ième édition, 2001. P 158. Editions, Tec&Doc.

Agence de la Santé publique du Canada. Adénovirus (type 1, 2, 3, 4, 5 et 7) – Fiche techniqe santé-sécurité : agents pathogènes, 2014

Agence de la Santé publique du Canada. Rhinovirus– Fiche techniqe santé-sécurité : agents pathogènes, 2011

Askergren A, Mellgren M. Changes in the nasal mucosa after exposure to copper salt dust. A preliminary report. Scand J Work Environ Health. 1975 Mar;1(1):45-9.

ATSDR, 2016; available at https://www.atsdr.cdc.gov/toxprofiles/tp132-c3.pdf.

Borkow G, Sidwell RW, Smee DF, Barnard DL, Morrey JD, Lara-Villegas HH, Shemer-Avni Y, Gabbay J. Neutralizing viruses in suspensions by copper oxide-based filters. Antimicrob Agents Chemother. 2007 Jul;51(7):2605-7.

Borkow G, Gabbay J. Copper as a biocidal tool. Curr Med Chem. 2005;12(18):2163-75.

CDC. Influenza (Flu) viruses, 2014.

Fan J, Ho L, Hobson P, Brookes J. Evaluating the effectiveness of copper sulphate, chlorine, potassium permanganate, hydrogen peroxide and ozone on cyanobacterial cell integrity. Water Res. 2013 Sep 15;47(14):5153-64.

Grass G, Rensing C, Solioz M. Metallic copper as an antimicrobial surface. Appl Environ Microbiol. 2011 Mar;77(5):1541-7.

Hong R, Kang TY, Michels CA, Gadura N. Membrane lipid peroxidation in copper alloy-mediated contact killing of Escherichia coli. Appl Environ Microbiol. 2012 Mar;78(6):1776-84.

Horie M, Ogawa H, Yoshida Y, Yamada K, Hara A, Ozawa K, Matsuda S, Mizota C, Tani M, Yamamoto Y, Yamada M, Nakamura K, Imai K. Inactivation and morphological changes of avian influenza virus by copper ions. Arch Virol. 2008;153(8):1467-72.

Ibrahim M, Wang F, Lou MM, Xie GL, Li B, Bo Z, Zhang GQ, Liu H, Wareth A. Copper as an antibacterial agent for human pathogenic multidrug resistant Burkholderia cepacia complex bacteria. J Biosci Bioeng. 2011 Dec;112(6):570-6.

Ikarashi Y, Tsuchiya T, Nakamura A. Detection of contact sensitivity of metal salts using the murine local lymph node assay. Toxicol Lett. 1992 Aug;62(1):53-61.

Imai K, Ogawa H, Bui VN, Inoue H, Fukuda J, Ohba M, Yamamoto Y, Nakamura K. Inactivation of high and low pathogenic avian influenza virus H5 subtypes by copper ions incorporated in zeolite-textile materials. Antiviral Res. 2012 Feb;93(2):225-33.

Robert JC. Flore buccale, plaque dentaire et parodonthopathies. Actualités Pharmaceutiques 1999,

376:9-13.

S.A. Health. Respiratory syncytial virus (RSV) infection – including symptoms, treatment and prevention, 2016.

Saravu K, Jose J, Bhat MN, Jimmy B, Shastry B A. Acute ingestion of copper sulphate: A review on its clinical manifestations and management. Indian J Crit Care Med 2007;11:74-80.

Waerhaug M, Gjermo P, Rølla G, Johansen JR. Comparison of the effect of chlorhexidine and CuSO4 on plaque formation and development of gingivitis. J Clin Periodontol. 1984 Mar;11(3):176-80.

Wåler SM, Rölla G. Comparison between plaque inhibiting effect of chlorhexidine and aqueous solutions of copper- and silver-ions. Scand J Dent Res. 1982 Apr;90(2):131-3.

Who. Parainfluenza 3. Immunization, vaccines and biologicals, 2008