REVIEW ON TOXICITY OF STAINLESS STEEL

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PREAMBLE

Stainless steels are a complex group of iron-based alloys containing at least 10.5% chromium and a maximum of 1.2% carbon. Chromium makes stainless steels corrosion resistant. As stainless steels are composed of several metals, chemical legislation [for example, REACH and CLP (Classification, Labelling and Packaging Regulation) in the EU] and the GHS (Globally Harmonized System of Classification and Labelling of Chemicals) consider these alloys as preparations or mixtures of substances. However, chromium and other alloying elements limit the release of constituent metals from the stainless steel matrix, which significantly affects its toxicological properties when compared to the effects of its constituent metals.

The current study reviews available *in vitro* and *in vivo* data, including the metal release data, conducted on stainless steel in order to assess the toxicological relevance of this data to human health, to draw conclusions about the toxicity of stainless steel, and to give recommendations for the classification and labelling of stainless steel according to GHS. Special interest is taken in toxic endpoints like sensitization, respiratory tract toxicity, mutagenicity and carcinogenicity. The hypothesis of the study has been that the toxicity of stainless steels cannot to be predicted on the basis of the bulk content of individual elements in stainless steel, but, rather, on the basis of the metal release from the stainless steel matrix.

The review is composed of the following parts:

- A short overview on the production and designation of stainless steels
- The release of metal constituent from stainless steels
- A review of the toxicological data on stainless steels
- A discussion of the available data on the human health hazards of stainless steels and recommendations for classification and labelling.

The study focuses on the toxicity of stainless steel as such in order to conclude whether stainless steel should be considered as a hazardous material. Welding of stainless steel is beyond the scope of this paper. Likewise, we do not include the risks related to different fumes formed during the manufacture and processing of stainless steel. Furthermore, the study does not include any comprehensive review of the manufacture and uses of stainless steels or the occupational exposures.

The study was commissioned by the International Stainless Steel Forum (ISSF) and the European Confederation of Iron and Steel Producers (EUROFER) with the objectives of a high scientific quality as well as an independent and transparent data review and assessment. An independent research institution, the Finnish Institute of Occupational Health (FIOH), performed the study.

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0. EXECUTIVE SUMMARY

Stainless steels are a group of iron-based alloys containing at least 10.5% chromium and a maximum of 1.2% carbon. Chromium makes stainless steels corrosion resistant.

As stainless steels are composed of several metals, chemical legislation [for example, REACH and CLP (Classification, Labelling and Packaging Regulation) in the EU] and the voluntary GHS (Globally Harmonized System for Classification and Labelling of Chemicals) consider these alloys as preparations or mixtures of substances. However, chromium and other alloying elements limit the release of constituents from the stainless steel matrix, which significantly affects its toxicity when compared to the toxicity of its constituents.

The aim of this report is to assess the toxicological relevance of the available data to human health, draw conclusions about the toxicity of stainless steel, and give recommendations for the classification and labelling of stainless steel according to GHS. Special interest is taken in toxic endpoints like sensitization, respiratory tract toxicity, mutagenicity and carcinogenicity.

STUDIES ON METAL RELEASE FROM STAINLESS STEEL

Metal release from stainless steel sheets and particles, as well as from consumer products and medical devices, has been determined in numerous studies. Studies on the release of chromium and nickel from kitchenware made of stainless steel have provided inconsistent results. In some studies, the chromium or nickel concentration in foods has increased, for example when acidic food had been prepared in new stainless steel pans and bowls, whereas, in other studies, researchers did not observe a remarkable increase. However, in any case, the measured releases have been very low compared to the intake of chromium and nickel via the food.

Some studies show that chromium and nickel may be released from stainless steel medical implants or appliances like orthodontic appliances, although the results have been inconsistent. Scientists have observed a large variation in the chromium and nickel concentrations in saliva. However, the ingested amount of chromium or nickel released from orthodontic appliances seems to be well below the daily dietary intake levels.

Not much data are available on the metal release from stainless steel prosthetic implants. The conflicting results seen in these studies may be related to analytical challenges.

Several *in vitro* studies focus on the release of metallic constituents from stainless steel in different synthetic body fluids. Because of the risk of skin

sensitization caused by nickel, previous studies have focused particularly on nickel release from stainless steel in synthetic sweat.

When measuring the nickel release from various stainless steel materials into synthetic sweat, blood and urine, scientists observed that the surface finish of the materials significantly affected the nickel release. From the polished materials, the nickel release into each of the test fluids was generally very low. In the case of stainless steel plates with a matt or mirrored finish, the release of nickel appeared increase and, in some cases, the release of nickel into urine and blood plasma was more than twice as high as into artificial sweat.

The metal ion release from stainless steel 316L particles of various sizes and from metal sheets of various grades, into artificial sweat has also been studied. The total release into artificial sweat is generally very low. Scientists have only observed higher release rates with the resulphurated grade 303 steel.

Researchers have also studied the release of metals from stainless steel in other artificial body fluids to mimic inhalation or gastrointestinal exposure scenarios. When the releases of different metal constituents of stainless steel are compared, iron is usually released at higher amounts than chromium and nickel. However, in all cases the release of metal ions is very low. Usually less alloyed ferritic grades release more metals, but the increase is attributed to the release of iron. The differences in release rates between different grades or surface finishes of stainless steel are usually small (for example, twofold).

Significant differences have been seen when the release from stainless steel has been compared to the release from pure metals. In one study, which compared the release from 316 sheets of stainless steel to the release from plain nickel and iron in artificial lysosomal fluid, researchers observed thousand-fold differences in iron and nickel release. The releases of chromium were on the same level both from stainless steel and from pure chromium metal. These *in vitro* studies suggest that, while chromium bioaccessibility from stainless steel is similar to that from metallic chromium, the iron and nickel bioaccessibility from stainless steel is significantly lower than from plain (unalloyed) iron and nickel particles. Thus, stainless steel containing 17.2% chromium and 10.7% nickel behaves as a mixture of chromium with <0.1% iron and nickel. These results strongly support the conclusion that the health effects of stainless steel cannot be estimated solely on the basis of its bulk contents of iron and nickel. This can be explained by the chromium oxide passivation layer, which comprises most of the stainless steel surface. The enrichment of chromium oxide in the surface occurs during *in vitro* incubation in artificial biological fluids and decreases release rates. This most likely also occurs *in vivo*.

TOXICOKINETICS

No studies have been performed to specifically investigate the toxicokinetic parameters of stainless steel. Two studies presenting limited human toxicokinetic data, based on workers in stainless steel production, have been published. However, the data do not provide much information on the toxicokinetic profile of stainless steel.

IN VITRO CYTOTOXICITY

The cytotoxicity of stainless steel has been studied *in vitro* neutral red uptake tests and the toxicity has been low. These tests are currently being validated by ECVAM (European Centre for the Validation of Alternative Methods) for their applicability when establishing the starting dose for acute oral toxicity tests. It is, therefore, not possible to draw conclusions for instance about the oral acute toxicity based on these available test data.

ACUTE TOXICITY

Although there are no data on acute toxicity studies of stainless steels, the long-term use and subacute studies strongly suggest that no acute toxicity via inhalation, dermal or oral exposure is expected. Also, none of the constituent metals is known to be acutely toxic.

IRRITATION AND CORROSION

The low solubility of stainless steel makes it an improbable irritant. There are no reports of skin or eye irritation by stainless steel. We have not found any studies of eye irritation by metallic nickel. Metallic chromium has not been tested for irritation. Chromium always has, however, a coating of Cr_2O_3, which has been found to be non-irritating and non-corrosive to the skin and eyes.

Also, the fact that stainless steel has extensively been used in objects which come into contact with the skin, and even with people's eyes, without resulting in any reports of irritation, supports the assumption that stainless steel can be regarded as non-irritant.

SKIN SENSITIZATION

Nickel is a common contact allergen, and therefore the potential of stainless steel to cause sensitization is of interest.

Release tests of stainless steel samples into various artificial body fluids generally show low release rates of nickel. EU has restricted the release of nickel into synthetic sweat to 0.5 µg/cm^2/week (0.2 µg/cm^2/week for piercing

post assemblies), which is also the limit for sensitization classification according to the CLP.

Study reports on nickel release from different grades of stainless steels clearly show that even in the worst cases, the Ni release from stainless steels is usually clearly below the limit of 0.5 µg/cm^2/week. Only studies with one grade (AISI 303, high sulphur content) have shown release rates above the limit. One current study indicates that from unfinished or unpolished stainless steels twice as much nickel may be released into urine and blood plasma as compared with artificial sweat.

Chromium is released from stainless steel as non-sensitizing trivalent chromium. Chromium(VI) (which is a known sensitizer) has not been detected in release tests.

A clear decrease in frequency of nickel allergy was observed when comparing groups of young women before and after the Ni release restriction came into force. The same results were also obtained when comparing groups who had their ears pierced before/after the restriction was implemented. These studies strongly support the assumption that the limit 0.5 µg/cm^2/week can protect consumers from Ni sensitization.

The potential of stainless steel to elicit reactions in nickel-sensitized persons has been tested in a number of studies. The results clearly show that no allergic reactions occur, and based on this, stainless steels can be regarded as safe even in persons with nickel allergy.

The frequency of nickel sensitivity among patients with stainless steel orthopaedic prostheses is not increased, and allergy tests performed before and after implantations do not show any signs of inducing nickel sensitivity. Studies on patient groups with stainless steel coronary stents indicate that implanting stainless steel stents obviously does not significantly induce nickel sensitivity, but the role of nickel allergy in stent restenosis cannot be excluded. The amount of available data is insufficient for final conclusions, but, based on the most extensive studies, the use of stainless steel in coronary stents seems to be safe.

Based on the low release rates of nickel, sensitization caused by stainless steel can be regarded as unlikely. Also its widespread use and the low number of confirmed cases of nickel allergy, even in persons previously sensitized to nickel, support the conclusion that stainless steel is not a potential sensitizer.

REPEATED DOSE AND LONG-TERM EXPOSURE TOXICITY

A 28-day repeated inhalation study, performed with stainless steel, clearly indicates a lack of toxicity. The doses used in the stainless steel study were markedly higher than those used in the corresponding nickel metal study. In the study researchers saw no adverse effects, even at the highest concentration of stainless steel (1 mg/L), whereas another 28-day study with nickel metal showed that the lowest nickel dose (0.004 mg/L) already resulted in clear signs of toxicity. Available data on animal or human long-term exposure via metallic implants do not indicate any adverse local or systemic effects caused by stainless steel.

MUTAGENICITY

In *in vitro* genotoxicity studies stainless steel has been negative. There is no relevant *in vivo* data on the mutagenicity of stainless steel but the negative data from *in vitro* mutagenicity studies and the lack of clear mutagenicity of the main metallic components of stainless steel support the conclusion that stainless steel is not genotoxic. Regarding nickel, only soluble nickel compounds have been classified at mutagenicity cat 2 within the EU according to the CLP system, whereas nickel metal has not been classified. Although the current data does not warrant a mutagen classification for nickel metal, even if metallic nickel were to be classified, the substantially lower release of nickel from stainless steel compared to nickel metal supports the non-classification of stainless steel.

CARCINOGENICITY

Animal studies on the carcinogenicity of stainless steel include studies evaluating the ability of different stainless steel implants to induce local cancers at the place of implantation. No indication of carcinogenicity has been seen in these studies. Human data on occupational exposure to stainless steel, for example in grinding and polishing tasks, have not raised concerns about the potential carcinogenicity of stainless steel. Although there are few case reports on the stainless steel implants and local tumours near them, analytical epidemiological studies on the carcinogenicity of various implants have not shown any evidence of increased cancer risk. The IARC has concluded that stainless steel implants are *not classifiable as to their carcinogenicity to humans (Group 3).*

Based on older studies of local cancers after local injection/instillation of nickel metal at various sites, metallic nickel has been classified within the EU at CLP cat 2 for its carcinogenicity. However, *in vitro* studies on the release of nickel

from stainless steel and a recent *in vivo* repeated-dose inhalation toxicity study show that nickel plays a significantly lower role in the toxicity of stainless steel than can be predicted on the basis of its bulk concentration. This conclusion is strongly supported by negative animal studies evaluating the ability of different stainless steel implants to induce local cancers at the place of implantation. In addition, negative stainless steel genotoxicity data and available human data on, for example, the grinding and polishing of stainless steel and the use of stainless steel implants, do not raise concerns about the carcinogenicity of stainless steel. Thus, the weight of evidence supports the non-carcinogenicity of stainless steel regardless of the possible carcinogenicity of nickel.

REPRODUCTIVE TOXICITY

No data exist on the reproductive or developmental toxicity of stainless steel. None of the main metallic components of stainless steel has shown reproductive toxic properties. Some soluble nickel compounds have been classified at Category 1B for developmental toxicity within the EU according to the CLP system. Recent EU risk assessment on nickel concluded that, since the developmental toxicity of nickel compounds is related to the systemically available nickel, this effect should be considered as relevant for metallic nickel as well, but, because the potential dose of nickel from metallic nickel is substantially lower than from the soluble compounds, it was agreed that metallic nickel should not be classified for this effect. Based on the low nickel release from stainless steel, it is very unlikely that it would cause reproductive or developmental toxicity. Testing of stainless steel for these properties is considered irrelevant and inadvisable.

CONCLUSIONS

One conclusion suggested by the data is that stainless steel is likely to exert very low toxicity. Based on the GHS classification and labelling criteria for mixtures, many stainless steels should be classified as specific target organ toxicants and/or category 2 carcinogens within the EU because of their nickel content. However, available stainless-steel-specific data provides solid evidence that this kind of classification is misleading.

In vitro release tests show that nickel release from stainless steel in artificial lung fluids is substantially lower than from nickel metal due to the chromium(III) oxide enrichment at the surface of stainless steel. A recent 28-day study on stainless steel inhalation toxicity showed low inhalation toxicity from stainless steel compared to nickel powder. Therefore, no classification for target organ toxicity in repeated exposure to stainless steel is proposed. A low

dissolution of metallic constituents and available toxicological data do not support classification for mutagenicity or carcinogenicity either.

The small differences in metal release from different grades of stainless steels under various pH conditions are considered negligible when compared to the difference seen in the release of nickel from pure nickel and from stainless steel.

Certain resulphurated stainless steels (for example, AISI 303) may release nickel above 0.5 µg/cm^2/week in artificial sweat. Although the actual threshold for the induction of nickel allergy is unknown, it has been seen in Europe that the limit set within the EU for nickel containing alloys in direct or prolonged contact with the skin has significantly decreased the prevalence of nickel allergies. In the case of sulphur-rich stainless steels like AISI 303, the risk of sensitization is higher. Therefore, these grades of stainless steel should be considered potentially sensitizing in close and prolonged skin contact. Nowadays, within Europe, these grades of steel are not recommended for use in continuous contact with the skin. Experts have not found cases of skin sensitization when these grades of steel are used in nuts and bolts, bushings, shafts, aircraft fittings, electrical switchgear components, gears, valve bodies and valve trim. This can be explained by the limited exposure time.

The data presented in this review shows that the toxicity of stainless steel cannot be predicted solely from the bulk concentrations of constituents, but their actual release plays an essential role. This must be taken into account in the hazard assessment and classification of stainless steel as indicated above.

The applicability of this same approach to other alloys has to be, however, considered separately, taking into account the specific properties of the alloy. This demands further studies and validation of release tests for different kinds of alloys.

We do not propose any further toxicity testing with stainless steels. The main hazards of stainless steels are related to the processing of stainless steel, especially welding, and, therefore, future emphasis should be on the assessment and management of these risks.

1. STAINLESS STEELS

The European Standard EN 10088 (EN 2005) defines stainless steels as iron based alloys containing at least 10.5% chromium and a maximum of 1.2% carbon. Chromium makes this large and complex group of alloys corrosion resistant. Stainless steels may contain up to 38% nickel as another major alloying element. The properties of stainless steels can be adjusted with several alloying elements in addition to chromium and nickel. These elements include carbon, sulphur, aluminium, molybdenum, tungsten, nitrogen, copper, titanium, niobium, zirconium, cerium, manganese, calcium and silicon. The key to the corrosion resistance of stainless steel is its chromium content: under the influence of oxygen from air or water, the chromium rapidly forms a very thin, chromium (III)-rich oxide film on the surface of the steel. This passivation layer very effectively separates the material from the surroundings. It is adherent, coherent and insoluble under normal conditions. The layer self-heals immediately in contact with oxygen from air or water, if it is broken for instance by scratching. Any effects of other elements are only to influence the effectiveness of chromium in forming or maintaining the film (for instance nickel promotes re-passivation, especially in reducing environments; and molybdenum stabilises the passive film in the presence of chlorides). Increasing the chromium content, from the minimum of 10.5% necessary for "stainless steel", to 17 to 20%, greatly increases the stability of the passive film. Elements such as copper, nitrogen, nickel and molybdenum help steel resist corrosion but their effect is limited if chromium is not present.

1.1 PRODUCTION OF STAINLESS STEELS

Stainless steel production predominantly uses electric arc furnaces (EAF). The EAF is charged with recycled materials (scrap) and ferroalloys including ferrochromium. Typically, an EAF with a 90 MVA transformer and a capacity of 130 tonnes will melt a 125 ton charge in 1 hour at a temperature of 1650°C. The molten charge is transferred from the EAF to an argon-oxygen decarburisation (AOD) vessel for refining. An argon- oxygen mixture is blown through tuyeres in the bottom of the AOD vessel to reduce the carbon content of the melt. Adjustments to the chemical composition of the charge are also made by controlled additions of alloying elements like nickel to the AOD vessel. One method is to tap the molten charge into a ladle arc furnace, to make final adjustments to the chemical composition of the charge (this could occasionally include addition of small, kilogram amounts, of chromium metal) and to bring the temperature to a suitable range for casting. Temperatures are raised by the

use of an electric arc or reduced by bubbling argon through the charge. For more information, see ISSF (International Stainless Steel Forum) www.worldstainless.org/About+stainless/How+is+ss+produced/).

The carbon content can also be reduced by the vacuum oxygen decarburisation (VOD) process, where the molten metal charge is treated under reduced pressure. The chamber pressure can be lowered to about 100 Pa. The decarburization is achieved by injecting oxygen through a lance, while small amounts of an inert gas can be blown through tuyeres in the converter bottom. The advantages of the VOD process are:

- The attainment of very low carbon levels (less than 0.01%), which is useful for high chromium stainless steels (about 30% Cr) and some duplex and "super-austenitic" grades (although in the latter case these alloys contain over 50 % non-ferrous elements, these materials are in fact defined as stainless steels and they have a superior resistance to pitting and crevice corrosion in environments containing halides).
- The attainment of very low sulphur contents (less than 0.002%)
- Reduced refractory wear in the tuyere zone.

Continuous casting is the preferred production route for slabs and billets. The continuous caster receives liquid steel into a tundish, which feeds a reciprocating, water-cooled copper or aluminium mould. As the steel cools, a retractable plug at the base of the mould is lowered and a continuous strand of steel is formed. After cooling, and prior to moving on to the hot rolling mill, the continuously cast strand is cut to length with a flame cutter and surface defects (sometimes the whole surface) are removed by grinding the whole surface. The hot rolling process is used to make large reductions in the cross-sectional area of the cast slabs or billets and to change its cast structure to a wrought (more refined) structure. Although hot rolling does not produce top quality surfaces or close dimensional tolerances, hot rolled coil and plate are suitable for some applications. However, the majority of hot rolled material is sent to the cold rolling plant for further treatment. Cold rolling is used to further reduce the cross-sectional area of the steel and to further refine its metallurgical structure. Annealing (softening) and pickling are an integral part of the process. Cold rolling allows close control of both dimensional tolerances and surface finish in the resulting stainless steel coil and sheet. Sheets are cut from cold rolled coil by a variety of techniques like mechanical cutting, shearing, plasma cutting and sometimes press tools or water jets are used. Laser cutting is used mainly for profile cutting of steel sheets in downstream applications.

According to the ISSF (International Stainless Steel Forum), the world stainless steel production in 2009 was 24.6 million tons. This is clearly below the previous peak production of 28.4 million tons in 2006. The world economic crisis and dramatic stock changes have influenced the stainless steel markets over the last 3 years.

The global stainless steel markets have seen dramatic changes in the last nine years regionally and by volume. China has become the by far biggest stainless steel producer in the world, in 2001 this country ranked only as number 11. Japan has lost its dominance in stainless steel production - other Asian countries like Korea, India and Taiwan are picking up tremendously.

Data on stainless production compiled by International Stainless Steel Forum (ISSF) are presented in Table 1, Table 2 and Table 3.

Table 1.Stainless and Heat Resisting Steel Crude Steel Production (Ingot/Slab Equivalent) in 1000 metric tonnes (Figures in italics denote estimates). Data from ISSF (International Stainless Steel Forum)

Country/Region	2001	2003	2005	2007	2008	2009 p
Austria	**42**	**35**	**45**	**51**	**64**	**30**
Belgium	**644**	**888**	**1,032**	**1,521**	**1,471**	**1,045**
Finland	**557**	**1,074**	**1,124**	**975**	**957**	**726**
France	**1,093**	**1,026**	**658**	**308**	**297**	**202**
Germany	1,600	1,597	1,592	1,505	1,574	1,320
Italy	1,288	1,447	1,606	1,558	1,471	1,216
Spain	1 ,1 81	1,172	1,127	1,105	998	693
Sweden	789	718	639	645	574	445
United Kingdom	500	440	408	351	340	224
EU 15	7,694	8,397	8,231	8,017	7,744	5,902
USA	1,817	2,223	2,238	2,171	1,925	1,617
Brazil	322	493	450	433	390	324
Japan	3,868	4,113	3,983	3,882	3,567	2,607
South Korea	1,546	1,987	2,292	1,942	1,660	1,677
Taiwan, China	1,212	1,505	1,514	1,515	1,297	1,468
China	730	1,780	3,160	7,206	6,943	8,805
India	1,048	1,260	1,549	1,655	1,544	1,379
Others [1)]	950	1,083	875	1,015	860	783
WORLD	19,187	22,840	24,292	27,836	25,930	24,562

[(1)] Czech Republic, Slovenia, Canada, Cuba, South Africa, Russia, Ukraine, Provided by: ISSF

High volatility of raw material costs - especially for nickel - has changed the grade mix of stainless steel production, partially enforced by the weak performance of the global automotive industry as one of main users of ferritic stainless steels.

Table 2. Global Stainless Crude Steel Melting by Main Grades in 1,000 metric tons. Coverage: ISSF members only. Data from ISSF (International Stainless Steel Forum).

Grade Category	2001	2003	2005	2007	2008	2009 p
"200" series: mod♣ified	846	1,168	1,985	2,813	2,653	2,611
200 series standard	41	46	174	205	243	132
300 series (total)	11,434	13,232	13,833	15,152	13,565	12,943
thereof 304	8,113	9,292	9,967	11,726	10,136	8,072
316	1,098	1,389	1,367	1,945	1,672	1,304
other molybdenum containing grades	306	348	228	454	341	387
Duplex grades	73	89	133	308	306	159
400 series (total)	3,723	4,120	5,439	7,482	6,678	6,318
thereof 409	1,161	1,351	1,285	2,530	2,202	1,227
430	1,338	1,575	1,870	2,915	2,948	2,541
martensitic grades	532	448	601	432	432	215
molybdenum containing grades	47	126	366	454	446	192
others	83	83	386	354	300	79
Grand total	16,201	18,739	21,933	26,334	23,724	22,242

Table 3. Crude Stainless Steel Production 2009 (Preliminary results). Data from ISSF (International Stainless Steel Forum).

Grade Category	Europe/Africa		Americas		Asia (incl. China)		Global Consolidation	
Grade	**1000 t**	**%**	**1000 t**	**%**	**1000 t**	**%**	**1000 t**	**%**
"200" series: modified (non AISI standard)	6	0.1	0	0.0	2,605	17.8	2,611	11.7
200 series AISI standard	36	0.6	96	5.0	0	0.0	132	0.6
300 series (total)	4,222	73.0	1,188	61.4	7,624	52.1	12,943	58.2
thereof 304	3,018	52.2	912	47.2	4,207	28.7	8,072	36.3
316	722	12.5	190	9.8	414	2.8	1,304	5.9
other molybdenum containing grades	129	2.2	35	1.8	226	1.5	387	1.7
Duplex grades	126	2.2	9	0.5	29	0.2	159	0.7
400 series (total)	1,351	23.4	625	32.3	4,364	29.8	6,318	28.4
thereof 409, 410	172	3.0	289	14.9	768	5.2	1,227	5.5
430	729	12.6	206	10.7	1,016	6.9	1,947	8.8
stabilized grades	272	4.7	48	2.5	274	1.9	594	2.7
martensitic grades	48	0.8	18	0.9	165	1.1	215	1.0
molybdenum containing grades	39	0.7	20	1.0	133	0.9	192	0.9
others	39	0.7	17	0.9	23	0.2	79	0.4
Grand total of analysis	5,780	100	1,935	100	14,645	100	22,242	100
Total crude steel production	6,449		1,942		15,935		24,562	
Representation of analysis	89.6		99.6		91.9		90.6	

1.2 DESIGNATION OF STAINLESS STEELS

There are several different systems currently used to designate stainless steels. Common designations include the AISI (American Iron and Steel Institute) system, used in the USA, and the European Standard (EN 2005), adapted for use in the European Union. Other national designations are also used. In the AISI system, austenitic grades are in the 200 and 300 series; martensitic and ferritic grades are in the 400 series. Steels of the 200 series were originally developed as a cheaper alternative to the 304 series to conserve nickel by replacing it with manganese at a ratio of 2% manganese for each percentage of nickel replaced. Duplex grades are austenitic-ferritic stainless steels like 2205 alloy (22% Cr, 5.5% Ni, 3% Mo, 0.02% C, and 0.14% N) that have nitrogen added to improve corrosion resistance and strength. Examples of stainless steel grades, with corresponding AISI and European Standard designations are presented in Table 4.

Table 4.Examples of AISI and European Standard designations for stainless steels (Industry information)

Stainless steel family	AISI designation	European Standard designation	
		Name	Number
Austenitic	201	X12CrMnNiN17-7-5	1.4372
	202	X12CrMnNiN18-9-5	1.4373
	301	X10CrNi18-8	1.4310
	301L	X2CrNiN18-7	1.4318
	304	X5CrNi18-10	1.4301
	304L	X2CrNi 18-9	1.4307
	305	X4CrNi18-12	1.4303
	316	X5CrNi Mo17-12-2	1.4401
	316L	X2CrNiMo17-12-2	1.4404
	321	X6CrNiTi18-10	1.4541
Martensitic	410	X12Cr13	1.4006
	420	X30Cr13	1.4028
Ferritic	430	X6Cr17	1.4016
	409	X2CrTi 12	1.4512
	434	X6CrMo17-1	1.4113
	441	X2CrTiNb18	1.4509
	436	X6CrMoNb17-1	1.4526

The austenitic grades account for about 75% of stainless steel production, much of this represented by AISI 304. The ferritic grades of stainless steels account for most of the remaining quarter of the production. The identity and the properties of the main types of stainless steels are compiled and described below (Keegan 2001).

Austenitic stainless steels consist of chromium (16–28%), nickel (6–38%) and iron. Carbon content is usually kept low (< 0.08%). Other alloying elements (molybdenum, for example) may be added or the alloy content modified to the lower nickel content of

the 200 series depending on the desired properties of derivative grades. The austenitic group contains more grades that are used in greater quantities than any other category of stainless steel. Their applications include chemical processing equipment, food processing and handling equipment, domestic appliances, hospital equipment, dairy equipment, beverage equipment, pharmaceutical equipment, petrol refining equipment, architectural trim, vehicle wheel covers, railway car bodies, street furniture, and even coronary stents, which are small metallic tubes implanted in heart arteries to prevent them closing up.

Austenitic stainless steels exhibit superior corrosion resistance to both ferritic and martensitic stainless steels. Corrosion performance may be varied to suit a wide range of service environments by careful alloy adjustment like modifying the carbon or molybdenum content.

These stainless steels are not subject to an impact transition at low temperatures and they possess high toughness at cryogenic temperatures. They exhibit greater thermal expansion and heat capacity, with lower thermal conductivity than other stainless or conventional steels. They are generally readily welded. Austenitic stainless steels are often described as non-magnetic, but may become slightly magnetic when machined or worked.

Martensitic stainless steels consist of carbon (0.2–1.0%), chromium (10.5–18%) and iron. These stainless steels may be heat treated, in a similar manner to conventional steels, to provide a range of mechanical properties, but offer better hardenability and have different heat treatment temperatures. Their corrosion resistance may be described as moderate (their corrosion performance is poorer than other stainless steels of the same chromium and alloy content). They are ferromagnetic, subject to impact transition at low temperatures and possess poor formability. Their thermal expansion and other thermal properties are similar to conventional steels. They may be welded with caution, but cracking can be a feature when matching filler metals are used. Applications include bolts, nuts, screws, cutlery, scissors, knife blades, surgical equipment, springs and beater bars for paper mills.

Ferritic stainless steels consist of chromium (10.5 to 20.0%, but typically 12.5% or 17%) and iron. These materials contain very little carbon and usually less than 1% nickel (as an impurity), although a few special grades may contain nickel up to a maximum of 2.5%. They are non-heat treatable, but have superior corrosion resistance to martensitic stainless steels and possess good resistance to oxidation. They are ferromagnetic and although subject to impact transition (becoming brittle) at low temperatures, possess adequate formability. Ferritic stainless steels are readily welded in thin sections, but suffer grain growth with consequential deterioration of properties when welded in thicker sections. Applications include vehicle mufflers, exhaust system and catalytic converters, hot water tanks, food and beverage containers (when lined for

instance with tin or polymers), vehicle trim, kitchen trim and equipment, drums and tubs for washing machines, drums for dryers, heat exchangers, oil burner parts and interior architectural trim.

Duplex stainless steels consist of chromium (18-30%) nickel (1.35-8%), molybdenum (0.1–4.5%), copper and iron. Nitrogen is added to improve corrosion resistance and strength and other alloying elements (such as copper) may be added as well. These stainless steels have a microstructure consisting of austenite and ferrite, which provides a combination of the corrosion resistance of austenitic stainless steels and greater strength. Duplex stainless steels are weldable. They are ferromagnetic and subject to an impact transition at low temperatures. Formability is reasonable, but higher forces than those used for austenitic stainless steels are required. Applications include marine uses, desalination plants, heat exchangers, pipe and tube applications, petrochemical equipment, pulp and paper processing machinery and equipment.

Precipitation hardening stainless steels develop high strength and hardness through low temperature (500–800°C) heat treatment. They are sub-divided into semi-austenitic and martensitic alloys, the latter formed by heat treatment of austenitic grades before precipitation hardening. The chemical composition of the 17–7 PH (semiaustenitic) is 0.08% carbon, 17% chromium, 7% nickel and 1% aluminium; and that of 17–4 PH (martensitic) is 0.05% carbon, 16% chromium, 4.5% nickel, 3.5% copper and 0.3% niobium. Applications include springs, clips, pressure tanks; and these steels are used in the aerospace and other high-technology industries.

2. RELEASE OF METALS FROM STAINLESS STEEL

Stainless steel is used in a variety of domestic applications and thus consumers are likely to experience exposure to the released metal ions. Articles such as watches, jewellery and fasteners on clothing may be in direct and prolonged skin contact. Use of cooking utensils may result in exposure via the food. Use of stainless steel in medical appliances may result in systemic exposure to stainless steel components. Inhalable particles may be formed in occupational settings for instance when stainless steel is ground.

Since the health effects are related to the metal components released from stainless steel, the release of metals from articles has been studied both *in vivo* and *in vitro*. The dissolution/corrosion properties of the steels can also be enhanced by various surface treatments to strengthen the protective properties of the passivation layer. The data on the release of metals provides information on the potential bioaccessibility of components. Bioaccessibility means the potential for a substance to come in contact with a living organism and interact with it (IUPAC Glossary of terms used in Toxicology). Bioaccessibility may lead to absorption (bioavailability).

2.1 RELEASE OF METALS FROM STAINLESS STEEL COOKING UTENSILS

Accominotti et al. (Accominotti, Bost et al. 1998) measured the chromium and nickel levels in meals cooked in stainless steel pans. Two tested pans contained 17 and 18% of chromium (grades 436 and 304, respectively), and the pH of meals ranged from 7.0 to 8.7. Slightly increased levels of chromium and nickel were detected in several of the meals. Some meals prepared in stainless steel saucepans contained more chromium and nickel than the meals prepared in glass saucepan, but the results were not consistent. The concentration ranges of chromium in meals prepared in glass or stainless steel saucepans were 7–47 µg/kg and 7–79 µg/kg, respectively. The comparable concentrations of nickel were 8—93 µg/kg and 10–132 µg/kg. The increase in the chromium and nickel contents during cooking was small compared with their natural contents in the meals.

Flint and Packirisamy (Flint and Packirisamy 1995) measured the release of chromium and nickel from pans, when food items of relatively low pH (rhubarb, apricots, lemon marmalade, green tomato chutney and potatoes) were cooked. These foods were selected since they were supposed to be aggressive to stainless steel; the pH ranged from 2.8 to 5.9. The pans contained 19% chromium and 9% nickel (grade S30400). The highest releases occurred, when new pans were used for the first time. With repeated use the release from pans decreased to negligible levels. According to the authors these findings show that release of chromium or nickel from cooking utensils contributes only negligible amounts of these metals to the diet compared with background or normal dietary levels.

Kumar et al. (Kumar, Srivastava et al. 1994) investigated the releases of iron, chromium and nickel from utensils (tumblers and bowls) following storage of foods and food simulants in stainless steel containers for several hours. The chromium release in 5% acetic acid (pH 2.11) varied between 8 and 75 pg/L. The respective concentrations for iron were 11 and 3420 pg/L and for nickel 4 and 170 pg/L. In the alkaline solution (5% of sodium carbonate, pH 11.50), the chromium content varied between 5 and 120 pg/L, iron content between 180 and 880 pg/L and nickel between 5 and 310 pg/L. Distilled water did not leach out any of the metals. With lemon pickle (pH 2.6), chromium was detected at levels of 131–247 pg/ kg, nickel 136—540 pg/kg and iron 570—1310 pg/kg, whereas no leaching of chromium or nickel was detected in milk, tea and coffee (pH 6.5–6.9). The iron release in the foods varied between 30–161 pg/kg. The limit of detection was not given. The chromium content of the stainless steel of the utensils ranged from 9.7–20.8% and the nickel content ranged from 2.3–9.3%. The grades of the stainless steels were not indicated.

Agarwal et al. (Agarwal, Srivastava et al. 1997) studied leaching of chromium and nickel from stainless steel (grades not given) pans, bowls and tumblers using mild acidic

solutions (0.1 N) of citric, tartaric and lactic acids. Boiling periods in frying pans and "storing" in bowls were 10 minutes and 1 hour, respectively. The level of chromium in the acidic leachate varied from 60 to 130 µg/L and the level of nickel 20-70 µg/L. When some Indian curds and juices were used, the leached chromium content was 170–550 µg/L and the nickel content 120–200 µg/L. Agarwal et al. (Agarwal, Srivastava et al. 1997) concluded that not only acidity, but also the complexation of chromium ions with organic acid anions, affects the extent of leaching.

The release of metals from seven different stainless utensils (grades not given) as well as from cast iron, mild steel, aluminium and enamelled steel was determined by Kuligowski and Halperin (Kuligowski and Halperin 1992). The chromium content of the stainless utensils was 18% and the nickel content 8–10%. The materials were exposed to 5% boiling acetic acid solution. Nickel was the major corrosion product from the stainless steel utensils; chromium and iron were also detected. The chromium content in 5% acetic acid solution from 9 samples, which were boiled for 5 minutes varied between 10 and 315 pg/kg. The authors calculated that 500 g of acidic food would give a dietary intake of 0.015 mg (15 µg) of chromium, 0.05 mg (50 µg of nickel, and 0.15 mg (150 µg) of iron.

Berg et al. (Berg, Petersen et al. 2000) found that chromium was not released from electric kettles and coffee machines (grades of stainless steel not given) in any significant amount. Electric kettles with heating elements made of stainless steel, or with elements efficiently coated by gold or Teflon, did not release nickel in quantities of any significance, whereas kettles with elements of nickel-plated copper and some of chromium-plated copper released measurable amounts.

In conclusion, the studies on the release of chromium and nickel from kitchenware made of stainless steel provide inconsistent results. In some studies, the chromium or nickel concentration in foods has increased when acidic food was prepared in new stainless steel pans and bowls, whereas no remarkable increase was observed in other studies. However, the measured releases have been very low when compared to the normal intake of chromium and nickel from food.

Chromium content is quite variable among different lots of foods. In one study, self-selected diets were composited for 7 days and analysed for chromium content. The mean chromium intake of 10 adult men was 33 µg/day (range 22 to 48 µg/day), and the chromium intake for 22 women was 25 µg/day (range 13 to 36 µg/day)(Anderson and Kozlovsky 1985). The chromium content of 22 daily diets, designed by nutritionists to be well balanced, ranged from 8.4 to 23.7 µg/1,000 kcal with a mean of 13.4 µg/1,000 kcal (Anderson, Bryden et al. 1992). In another study, a group of adults self-selected a mean chromium intake of 14.4 µg/1,000 kcal (Anderson, Polansky et al. 1991), and lactating mothers consumed foods containing 18.8 µg/1,000 kcal (Anderson, Bryden et al. 1993).

In WHO Environmental Health Criteria the chromium intake from the diet and water was estimated to vary considerably between regions; typically, levels lie within the range 50–200 µg/day (WHO 1988).

An U.S. committee (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes 2001) has summarized that there is not sufficient evidence to set an Estimated Average Requirement for chromium. Therefore, an Adequate Intake (AI) was set based on estimated mean intakes. The AI is 35 µg/day and 25 µg/day for young men and women, respectively. Few serious adverse effects have been associated with excess intake of chromium from food. Therefore, a Tolerable Upper Intake Level (UL) was not established.

The dietary intake of nickel is on the level of some 100–400 µg/day (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes 2001). Nickel intake from the Danish diet was estimated to be 150 µg/person/day on average. Roots and vegetables, meal, grain and bread relatively supply the average diet with much nickel. Certain food items such as cocoa and chocolate, soya beans, oatmeal, nuts and almonds, fresh and dried legumes, have high nickel contents. Consumption of these items in larger amounts may increase the nickel intake to 900 µg/person/day or more (Flyvholm, Nielsen et al. 1984).

The Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals. U.S. level is 1.0 mg/day of soluble nickel salts for adults over 19 years. The U.S. committee also states: ‘The risk of adverse effects resulting from excess intakes of nickel from food and supplements appears to be very low at the highest intakes noted above. Increased risks are likely to occur from environmental exposures or from the consumption of contaminated water.’ (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes 2001).

An extensive study of Flint et al. (Flint and Packirisamy 1997) indicated that apart from aberrant metal release values of new pans on first use, the contribution made by stainless steel cooking utensils to chromium and nickel in the diet is negligible. New pans, if first used with acid fruits, showed a greater pick-up of chromium and nickel, ranging from approximately 1⁄20 to 1⁄3 and 1⁄20 to 1⁄2 of the normal daily intake of chromium and nickel respectively. This situation did not recur in subsequent usage, even after the pan had been cleaned by abrasion. The rate of chromium and nickel release in new pans was related to surface finish, since treatment of the surface of a new pan was partly, and in the case of electropolishing, wholly effective in eliminating their initial high release.

2.2 RELEASE OF METALS FROM STAINLESS STEEL MEDICAL APPLIANCES

2.2.1 ORTHODONTIC APPLIANCES

Orthodontic bands, brackets and wires, made of stainless steel contain approximately 8–12% nickel and 17–22% chromium, were studied for metal releases (Kocadereli, Atac et al. 2000). Chromium and nickel concentrations were measured in the saliva of 45 patients who had fixed orthodontic appliances. Four samples of stimulated saliva were collected from each patient: before insertion of the appliance, and 1 week, 1 month and 2 months after insertion. A large variation in nickel (0.07–3.32 µg/mL) and chromium concentrations (0.29–8.0 µg/mL) in saliva was observed. No significant differences were found between the samples obtained before and after insertion of the appliances. Thus, the study provided no indication that fixed orthodontic appliances affect chromium concentrations of saliva during the first 2 months of treatment. A number of variables like time of the day, diet and salivary flow rate may also affect the composition of saliva.

Agaoglu et al. (Agaoglu, Arun et al. 2001) measured the concentrations of nickel and chromium ions in salivary and serum samples from patients with fixed orthodontic appliances. The stainless steel in the appliances contained approximately 8% nickel and 18% chromium. Samples were collected from 100 patients prior to and 1 week, 1 month, 1 year, and 2 years after appliance insertion. Before the insertion and two years afterwards, the mean nickel levels in saliva were 8.36 and 10.27 ppb while the chromium levels in serum were 6.21 and 10.98 ppb, respectively. These increases were statistically significant. In saliva samples, both nickel and chromium reached the highest levels in the first month (means 11.53 ppb Ni and 1.53 ppb Cr) and decreased to their initial levels within two years. The authors conclude that fixed orthodontic appliances released measurable amounts of nickel and chromium into the mouth. The serum nickel and chromium levels reported in this study are markedly higher than those found by Kocadereli et al. (Kocadereli, Atac et al. 2000) and thus there may have been analytical problems, (e.g. contamination of samples by stainless steel venipuncture needle mentioned by the authors), which impair the reliability of the results.

Barrett et al. (Barrett, Bishara et al. 1993) evaluated the *in vitro* corrosion rate of chromium and nickel from standard orthodontic appliances consisting of bands, brackets in either stainless steel or nickel–titanium wires. The appliances were immersed for 4 weeks in a prepared artificial saliva medium at 37°C and Cr and Ni arising from corrosion products were analysed. Ten identical sets were used, each simulating a complete orthodontic appliance. Chromium and nickel releases were quantified at days 1, 7, 14, 21, and 28. The results indicate that orthodontic appliances made of stainless steel released measurable amounts of chromium and nickel, when placed in an artificial saliva medium. The concentrations of chromium in artificial saliva

on day 1, 14, and 28, were 21.2, 125.5 and 233.1 ppb respectively. The nickel concentrations were 2865, 5220, and 1262 ppb, respectively. The estimated daily releases were 0.7 µg chromium and 13.05 µg nickel. Over the total study period the nickel concentration averaged 37 times higher than the chromium concentration. The release rates of nickel from stainless steel and nickel-titanium arch wires were not significantly different.

Grimsdottir et al. (Grimsdottir, Gjerdet et al. 1992) measured *in vitro* release of chromium and nickel from various orthodontic appliances, such as face-bows, brackets, molar bands, and arch wires, most of which were made of stainless steel, typically containing 18% Cr and 8% Ni. Release of chromium was measured after the appliances had been kept in 0.9% sodium chloride for 14 days. The total accumulated amount of chromium released from face-bows was from 3.2 to 13.9 µg and that of nickel from 0.5 to 10.4 µg; this range of nickel is near to that reported by Barrett et al (Barrett, Bishara et al. 1993), but the amount of chromium significantly higher. Releases from 0 to 3.2 µg Cr and from 0 to 5.0 µ Ni were measured for molar bands, brackets, arch wires.

Kerosuo et al. (Kerosuo, Moe et al. 1997) studied the nickel and chromium concentrations in saliva of patients who had different types of fixed appliances. Stainless steel alloys containing 8–12% nickel and 17–22% chromium were used for the appliances. Four saliva samples were collected from each of the 47 patients, with a mean age of 12.4 years: before insertion of the appliance; then 1 to 2 days; 1 week, and 1 month after insertion of the appliance. Large variations were seen in the chromium (0–320 ng/mL) and nickel (0–440 ng/mL) levels in saliva from the subjects, but no significant differences were found between the control samples and samples obtained before and after insertion of the appliances. Kerosuo et al. conclude that only when the rate of corrosion is high (like in the case of chromium cobalt dentures), will metal concentrations in saliva possibly reach a level that overrules the natural variation of chromium concentrations in saliva. In this study, the accuracy of analysis may have been compromised, since the chromium concentration in 11 blanks varied between 0 and 115 ng/mL and those of nickel between 0 and 65 ng/mL.

Park and Shearer (Park and Shearer 1983) found that the *in vitro* release rate of chromium from full-mouth orthodontic appliances was 36 µg/day and the release of nickel 40 µg/day.

The nickel content in oral mucosa cells was determined in three dental patients (Jensen, Lisby et al. 2003). Oral mucosal cells from two patients were harvested for nickel content analysis before and one week after the insertion of dental braces (different metallic alloys; 3–18% Ni). The third patient had 6 braces made of stainless steel (DIN no. 1.4542) implanted for three weeks. From this patient, oral mucosal cells were harvested before attachment, and every second day until day 21. No measurable

amounts of nickel were found in any of the mucosal samples from the three patients, either before or after the attachment of dental braces (Ni detection limit was 0.5 µg/L).

2.2.2 PROSTHETIC IMPLANTS

Stainless steel was previously quite a common material for prosthetic implants but nowadays, in developed countries, it has been replaced by other metals like unalloyed titanium and titanium alloys and other chromium alloys like cobalt-chromium and cobalt-chromium-molybdenum and also with ceramics.

Ryhänen et al. (Ryhänen, Niemi et al. 1997) measured the release of nickel from orthopaedic surgical materials (nickel-titanium-alloy Nitinol, stainless steel AISI 316 LVM). Initially, Nitinol released more nickel (129–87 mg/L) into the cell culture media than stainless steel (7 mg/L), but after 2 days the concentrations were about equal (23–5 mg/L versus 11–1 mg/L).

Diaz et al. (Diaz, Sevilla et al. 2008) studied if increasing the thickness of the passivation layer on stainless steel 316L by anodization would decrease the leaching of Ni and Cr ions. The Ni and Cr release in simulated body fluid at 37ºC were detected at times of 1, 6, 11, and 15 days by means of atomic absorption in a graphite furnace. However, these anodization methods released 2–10 times more nickel and chromium than the original stainless steel, depending on the method used. The anodization did not improve the long-term behaviour of the stainless steel for its application as cardiovascular stents.

Assad et al. (Assad, Lemieux et al. 1999) measured the release of nickel from nickel-titanium (NiTi), pure nickel (Ni) and pure titanium (Ti) powders, and also from stainless steel (316L SS). The release rate of Ni in the different semiphysiological solutions decreased in order: pure Ni, 316L SS, NiTi, Ti, and controls.

2.3 RELEASE OF METALS FROM STAINLESS STEEL IN CONTACT WITH SKIN

On daily basis, consumers touch stainless steel items (like door handles, bottle openers, key rings, kitchen worktops, sinks and drainers, handrails, luggage racks, internal trim), but the contacts are usually short. Use of stainless steel spectacle frames, watches, clothing fastenings and jewellery makes the exposures prolonged. Concerns about nickel and its skin sensitization have mostly fuelled the investigations on the exposures to stainless steel (Cross, Beach et al. 1999).

Nickel release from stainless steels into blood plasma, urine and artificial sweat was studied by the EN1811 standard method (LGC Limited 2003). The study also investigated the effects of surface finish on release rates. The tested samples are presented in Table 5.

Table 5. Materials studied by LGC Ltd for nickel release. Adapted from LGC ltd 2003

Material	Finishing

Stainless steel plates		
	316LVM	plate with matt finish
	316L	**polished surface with dull finish**
	316L	plate with mirrored finish
	1.4435 (316S13)	plate with matt finish
Stainless steel wires		
	1.4404 wire Ø1.6mm	wire with silver mirrored finish
	316LVM ground and polished bar Ø2.0 mm	wire with silver mirrored finish
Stainless steel piercing post assemblies		
	316L ear studs	post with silver mirrored finish
	316L gold plates ear studs	post with gold mirrored finish
	30200 butterflies	butterfly with silver mirrored finish

The surface finish of the materials significantly affected the nickel release. When the metal surface was polished or worked, the nickel release decreased. The nickel release from polished objects into the test fluids was predominately below the detection limit of <0.01 µg/cm^2/week. The release of nickel from stainless steel plates with matt or mirrored finish was about 0.4–0.5 µg/cm^2/week in artificial sweat. In urine and blood plasma, the release rates were significantly higher. The nickel release from plates with matt surface was up to 1.15 µg/cm^2/week in plasma and urine, and from one polished plate with dull finish the release into urine was 6.5 µg Ni/cm^2/week and to plasma 4.6 µg/cm^2/week, compared with 0.2 µg/cm^2/week into artificial sweat. To show the importance of surface finish, some plates which had high release rates in urine were polished and retested. The Ni release into urine was about half of the rate observed from the original material. The testers speculated that the reasons for the higher release into urine or plasma are due to biological complexation of the metal ions and the organic components. The pH was very similar for all test solutions (artificial sweat pH 6.5, urine pH 6, and blood plasma pH 7) and thus the differences cannot be explained by acidity/alkalinity.

Hedberg et al. (Hedberg, Midander et al. 2010) performed an EN1811 standard test (CEN 1998) on nickel release from stainless steel 316L particles (particle size <45 µm) and ultrafine particles (<4 µm). After the 168 h exposure period in artificial sweat (pH 6.5), the nickel concentration in the test medium was below the limit of detection (0.5 µg/L, measured by graphite furnace atomic absorption spectroscopy GF-AAS) in the case of <45 µm particles, corresponding to a release rate of <0.007 µg/cm^2/week. With the ultrafine 316L particles the Ni concentration was measurable, showing a release rate of <0.01 µg/cm^2/week. Ni release into artificial tear fluid (pH 8.0) was also measured. The Ni release rates after 24 and 168 h of exposure were <0.007 µg/cm^2/week both for the fine and ultrafine particles. As a comparison, Ni released from ferrochromium alloy (bulk nickel concentration 0.4%) was also <0.007 µg/cm^2/week.

The same study (Hedberg, Midander et al. 2010) also revealed the release of other constituent metals. More metals were released into artificial sweat than into tear fluid, but the amounts released were very low in both cases. After 168 h in artificial sweat, 0.008% of the 316L particle mass was released into the fluid. The corresponding value for ferrochromium was 0.02%. The chromium amounts released both from stainless steel 316L and ferrochromium into artificial sweat or tear fluid were low (<0.01 µg/cm^2 after 168 h of exposure), and at the same level as from samples of pure chromium metal or chromium (III) oxide. The chromium amounts from ferrochromium were slightly higher than from the stainless steel. Chromium was released as Cr (III) and no Cr (VI) was detected. The Cr release rate was significantly higher in artificial sweat than in the artificial tear fluid. Iron release from stainless steel particles was significantly lower than from pure Fe metal. The average release rate of Fe after 168 h exposure in artificial sweat was 0.1 µg/cm^2/week, for stainless steel and ferrochromium, and 8.4 µg/cm^2/week for pure iron particles.

The resulphurated grades of stainless steel that contain approximately 0.3% of sulphur are the only grades with high nickel release rates. The higher content of sulphur renders these stainless steels easier to machine but more susceptible to corrosion and nickel release, particularly in chloride-containing media.

Studies on nickel release from different grades of stainless steel (AISI 303, 304, 304L, 316, 316L, 310S, 430) in artificial sweat showed that AISI 303 (high sulphur grade) was the only grade for which the rate was close to or above 0.5 µg/cm^2/week (Haudrechy, Foussereau et al. 1994; Haudrechy, Mantout et al. 1997). In the standard testing conditions (pH 6.6), the nickel release from AISI 303 was only 0.3 µg/cm^2/week, but lowering the pH of the test medium to 4.5 increased the releases up to 1.4 µg/cm^2/week. The pH of 4.5 was chosen as it was the lowest pH of sweat quoted in the literature. All other grades of stainless steel released nickel clearly below the limit. Lowering the pH to 3.0 or increasing the chloride concentration increased nickel release. The release rates from stainless steels were significantly lower than from pure nickel or nickel-plated steel (100 µg/cm^2/week). The higher release rates obtained with AISI 303, as compared with the other grades, are due to its sulphur content, which in combination with manganese may initiate pitting corrosion. Haudrechy et al. concluded that the use of high-sulphur stainless steels in contact with skin is not to be recommended. This is also the recommendation of European stainless steel industry. Previously (before the EU nickel directive), AISI 303 was used for instance in the backs of wrist watches. Haudrechy et al. stated that the other stainless steel grades can be regarded as safe when considering nickel sensitization.

He et al. (He, Pan et al. 2002) compared the metal releases of AISI 303 and 304L in synthetic sweat in pH 6.9. The release rates of 0.036 µg/cm^2/week and 0.011 µg/cm^2/week were obtained for grades 303 and 304L, respectively. However, in a pilot

study at pH 6.5, a release of 3.3 µg/cm^2/week was obtained for AISI 303 whereas the release from AISI 304L was comparable to the release observed at pH 6.9. This release observed at 6.5 was even higher than the release (1.4 µg/cm^2/week) observed by Haudrechy et al. (Haudrechy, Foussereau et al. 1994) at pH 4.5. He et al. hypothesized that the distribution and composition of inclusions in the material could be the main reason for these large differences in nickel release from AISI 303. Moreover, parameters like structure, size and preparation of the sample, pH and temperature of the solution, and even illumination, may have affected the Ni release from AISI 303.

The release of nickel from four different stainless steel alloys into artificial saliva (pH 5.1) or artificial sweat (pH 6.5) was measured by Jensen et al. (Jensen, Lisby et al. 2003). Artificial saliva was used to mimic physiological conditions. The lower pH of saliva results in potential corrosion of alloys and release of metal ions. The tested stainless steel grades were AISI 305, 321, and two different 316L (DIN 1.4404 and 1.4435). The released nickel was measured after 1, 3, 7, 14 and 21 days. The results showed that in artificial sweat the nickel release was <0.05 µg/cm^2/week from all samples. In the artificial saliva <0.13 µg Ni/cm^2/week was detected.

Some reports present data on nickel release from stainless steel jewellery (Fischer, Fregert et al. 1984; Kanerva, Sipilainen-Malm et al. 1994). However, no details on composition of stainless steels were presented, and the value of these studies is therefore limited.

2.4 RELEASE OF METALS FROM STAINLESS STEEL IN OCCUPATIONAL INHALATION SCENARIOS

When stainless steel is processed, airborne particles may arise and the inhalation exposure is possible. Some inhaled particles are removed from respiratory tract to gastrointestinal tract. Therefore, the metal release from stainless steel has been investigated in artificial lung fluids and gastric fluid. Some studies have been performed in phosphate buffered saline (PBS), which represents neutral physiological environment, like blood. Both stainless steel sheets and particles have been used in these studies.

2.4.1 STAINLESS STEEL SHEETS

He et al. (He, Pan et al. 2002) studied the metal release from AISI 303 and 304L grades of stainless steel in four media. These were artificial sweat (results described above), phosphate-buffered saline, artificial interstitial fluid (Gamble's solution) and artificial intracellular fluid (‘cytosol’). The latter two solutions simulated the biological environment of lung alveoli. Stainless steel samples were commercial long rods with a diameter of 20 mm. Exposure periods of 8 h, 1 d and 1 and 4 weeks were chosen and the releases of chromium, nickel and iron were measured by atomic absorption

spectroscopy. Analysis of the surface with X-ray photoelectron microscopy was performed and samples were also evaluated visually. Pitting corrosion was observed frequently on AISI 303 exposed to synthetic sweat and artificial intracellular fluid whereas in the case of 304 this was observed only in one sample. Metal release rates were highest in the beginning of the incubation and reached a steady state after 1 week. Iron was the most abundant metal released. Highest releases of all metals were observed in artificial intracellular fluid and the lowest in Gamble's solution. Synthetic sweat was the second most aggressive fluid. Release of nickel and chromium from both grades was in all cases below 0.5 µg/cm^2/week, when the release of iron was up to 6.5 µg/cm^2/week (AISI 303, artificial intracellular fluid). Nickel release was significantly higher from 303 than 304 only in artificial intracellular fluid. In addition, iron release was significantly higher from 303 than 304 only in artificial intracellular fluid. In other fluids no significant differences were seen between the grades. Measurement of the momentary corrosion rate was also performed by electrochemical polarization and impedance spectroscopy. These techniques revealed that after the initial high corrosion rate, a relatively constant low level was reached during the first week of exposure. The studies on surface chemistry showed that Cr was enriched on the surface, resulting in the formation and growth of a protective passive layer. This explains the decrease in the corrosion rate with time and the onset of a steady state.

Seven stainless steel grades were studied *in vitro* to see whether there are differences in the release rates (Herting, Odnevall Wallinder et al. 2007). Grades 2205 (duplex), austenitic steels 201, 304, 310, 316L and ferritic steels 409, 430 were studied. Chromium contents varied between 11.4% (grade 409) and 24.2% (grade 310) and nickel contents between 0.11% (430) and 19.1% (310). Three different surface finishes (2R, 2B and shot-blasted) were present. Each grade was studied as received and with the surface abraded. Stainless steel sheets, approximately 6–7 cm^2, were immersed for 8, 24, 48 and 168 h in Gamble's fluid (pH 7.4) or artificial lysosomal fluid (pH 4.5) mimicking conditions in lung alveolar spaces before and after phagocytosis. Although stainless steel in massive form cannot be inhaled, it was considered appropriate to conduct screening tests to compare the dissolution of metals from different grades and surface finishes and to understand the parameters affecting the releases. The released metals (Cr, Ni, Fe, and Mo) were measured by GF-AAS. In addition, surface analysis by X-ray photoelectron spectroscopy was performed before and after incubation in order to see the effects on surface composition. The results show that the total release of metals was very low from all grades of stainless steel (<5 µg/cm^2/week). Iron release rates were higher than the release rates of other metals (<0.067 and <4.4 µg/cm^2/week; vs. Cr<0.003 and <0.18; Ni<0.011 and <0.08 in Gamble's solution and ALF, respectively). Ferritic grades had the highest total release rates, but this was caused only by the release of iron. No nickel and only small amounts of chromium were released from these grades. Even though the chromium and nickel content of these

grades varied between 11.4% and 24.2%, and 0.11% (430) and 19.1% (310), respectively, the release rates were remarkably similar. In ALF the release rate was higher than in Gamble's solution, but all grades showed a similar pattern. Highest release rates of nickel were seen with 316L in ALF, although its bulk nickel content is lower (10.6%) than that of 310 (19.1%). Table 6 summarizes the results of the screening test. The results can be explained by the fact that the increased chromium content or alloying with nickel or molybdenum improves the corrosion resistance.

Table 6. Release of chromium, nickel, iron and molybdenum from different stainless steel. The release rates in µg cm^{-2} $week^{-1}$. Adapted from Herting et al. 2007.

Grade	2205		201		304		310		316L		409		430	
Solution	G	ALF	G	ALF	G	ALF	G	ALF	G	ALF	G	ALF	G	ALF
Cr	0.003	0.15	0.003	0.15	0.003	0.15	0.003		0.002	0.1	0.001	0.18	0.003	0.13
Ni	0.011	0.024	0.009	0.01	0.002	0.37	0.003		0.006	0.08	–	–	–	–
Fe	0.031	1.2	0.067	1.1	0.037	0.98	0.006	0.22	0.075	1.2	0.05	4.4	0.05	1.8
Mo	0.002	0.033	–	–	–	–	–	–	0.001	0.026	–	–	–	–

G = Gamble's solution
ALF = alveolar lavage fluid

The total release rate was highest in the beginning of exposure (during the first 8 hours) and decreased with time in longer incubation. This occurred with all grades and may be explained by increasing chromium content of the surface film. Good correlation with the chromium bulk content and total metal release was shown (Herting, Odnevall Wallinder et al. 2007).

Herting et al. (Herting, Wallinder et al. 2008b; Herting, Wallinder et al. 2008a) examined the release of the main components of manganese-chromium containing stainless steels in ALF. Two grades, containing either 16 wt% Cr, 9.7 wt% Mn and 1.0 wt% Ni or 16.1 wt% Cr, 7.2 wt% Mn and 4 wt% Ni, were tested. The total release rate of metals was very low from both grades (<3 µg/cm^2/week). Iron release rates were higher than the rates of other metals. Release rates (estimated from the article's graphics) varied between 1–2 µg/cm^2/week for iron and between 0.25–0.4 µg/cm^2/week for Cr and between 0.2–0.5 µg/cm^2/week for Mn. Nickel release was very low. Low-nickel grade showed somewhat higher total metal release and the release of iron, chromium and manganese, but the differences between very low quantities were less than two-fold. The highest rate was during the first 8 hours of exposure. Manganese was also present in the surface film at a relative amount (Mn/Cr+Fe+Mn+Ni) 0.03–0.08.

Herting et al. (Herting, Odnevall Wallinder et al. 2006) studied the impact of surface finish (2B, 2D, and BA) on the release of chromium, nickel and iron from the sheets of steel grade 304 after 8-hour or 1-week incubation in ALF. As in the previous studies, the release of metals from all samples during one week was very low, with a total release

(Cr, Ni, and Fe) varying from 0.7 to 1.1 µg/cm^2/week. Iron was released at a 10-fold higher rate than nickel and chromium. The release rate was higher in the early part of incubation (at 8 h time point), and decreased later. Nickel releases from stainless steel with different surface finishes did not vary whereas some variation was seen in iron and chromium releases. The variation in chromium oxide content of the surface, in the thickness of the passive film, surface roughness, or the geometric surface area did not correlate with release rates, whereas the variation in electrochemically active surface correlated. The differences between surface finishes were, however, very small and are unlikely to have any impact on the toxicity of stainless steel.

This study (Herting, Odnevall Wallinder et al. 2006) on the effects of surface finishes was repeated using 5 different surface finishes BA, E, EP, 2B, 2D (Herting, Leygraf et al. 2009). The results showed that depending on surface finish, the total metal release during 1 week of incubation in ALF varied between ≈0.5 and 1.1 µg/cm^2/week. Again iron was released at highest rate and it accounted for the differences in total metal release. All finishes showed similar low release of nickel. Chromium was released at similar rates from BA, E and EP and at slightly higher rates from 2B and 2D.. Analysis of surface layer composition showed that observed small differences in release rates cannot be explained solely by changes in chromium content of the surface. In addition, surface roughness did not explain the differences. The main determinant correlating with release rates was electrochemically active surface area. From the health hazard perspective, one important feature to notice is that in all cases the nickel release remains at the same, very low level.

2.4.2 RELEASE FROM THE STAINLESS STEEL PARTICLES

Midander et al. (Midander, Pan et al. 2006) studied metal release from particles in artificial biological media, using stainless steel 316L particles (>86.6% of the particles less than 44 µm and 6.1% <11 µm in size and a specific surface area of 0.069 m^2/g, measured by BET-analysis). If the powder is assumed to consist of spherical particles of equal size, this surface area corresponds to a particle diameter of 11 µm. PBS and artificial lysosomal fluid (pH 4.5) were used to mimic conditions in neutral physiological environment and in lungs after phagocytosis of the particles by alveolar macrophages. 0.2, 2 or 20 g/L of stainless steel powder was incubated in Erlenmayer flasks either for two weeks or for a week under agitation at 37^{o}C. After exposure, particles were removed and the metal concentrations in the solutions were analysed by ICP-MS. The surface of the particles was characterized by X-ray photoelectron microscopy, which gave information on the composition of the outermost 5 nm surface layer. Only a small fraction of metals were released from stainless steel particles. After 1-week of incubation and with the loading of 0.2 g/L (showing highest release) the soluble fraction was 0.065 wt% in ALF and <0.004 wt% in PBS. Iron was the dominant released

element with a tenfold higher release rate than chromium or nickel. All metals were released at ten times higher rates in ALF than in PBS. When the release from stainless steel particles was compared to the release from stainless steel sheets, slightly lower release rate/surface area was seen from particles. The surface of particles consisted mainly of iron and chromium oxides, and the relative chromium content increased after the one week exposure in ALF. The preferential dissolution of iron oxide compared to dissolution of chromium oxide may explain the observation. Chromium oxide is enriched at the surface of stainless steel in contact with biological fluids, which affects the release of metals from the alloy.

Midander et al. (Midander, Pan et al. 2007) has also measured the metal release from stainless steel particles of different sizes. An ultrafine powder, two fine powders and two coarse powders of stainless steel 316L were used and the results were compared with the results of Herting et al.(Herting, Odnevall Wallinder et al. 2007), which were obtained using massive sheets. Specific surface areas of the tested particles varied from 0.07–0.7 m^2/g. ALF and Gamble's fluid were used to mimic the conditions in lung alveolar spaces. Incubation duration was one week and the release of chromium, nickel and iron was measured. The release rates of chromium and nickel per surface area were not significantly different between different sized powders in ALF, whereas the release rate of iron from ultrafine particles was almost fourfold that from coarse particles. A decreasing trend in iron release with increasing particle size was seen when ultrafine particles were compared to fine and coarse particles. However, the release rates from fine and coarse particles were very similar to that from massive sheets. Only ultrafine particles showed twofold release rates when compared to massive sheets. The overall metal release was about ten times lower in Gamble's fluid than in ALF and no clear correlation was seen to particle size/specific surface area. When the particles were exposed for 8 h to Gamble's fluid followed by 158 h exposure to ALF, the metal release was somewhat lower than after exposure to ALF only. Ultrafine particles showed higher release rate compared to coarse particles. The differences were, however, very small. From the health hazard perspective, the metal release rates from stainless steel particles were very low. Thus, the small differences observed in release rates have hardly any biological importance.

2.4.3 METAL RELEASE FROM STAINLESS STEEL COMPARED TO THE METAL RELEASE FROM PURE METALS

From the health hazard perspective, the studies (Herting, Wallinder et al. 2008b; Herting, Wallinder et al. 2008a) demonstrating large differences in metal release rates between stainless steel and pure metals are of special importance. Stainless steel (316L, with as-received 2B surface and abraded surface), pure chromium, nickel and iron sheets were immersed in artificial lysosomal fluid (ALF) and the metal release rates were evaluated by AAS after incubation from 1 h to 168 h. Surface composition

changes were evaluated by XPS. The results of this study show again that iron is the main constituent released from stainless steel, with about 10–45-times higher release than that of nickel and chromium. The release of these constituents is not proportional to bulk composition or surface composition of stainless steel. Large differences were seen when the release of chromium, nickel and iron from stainless steel was compared with their releases from pure metal particles. Whereas chromium was released almost at similar rates from both stainless steel and chromium metal particles, the releases of nickel and iron from stainless steel were approximately 1000 times lower than from the respective pure metals (see Table 7). The release of iron from iron particles was 4.58 mg/cm^2/week and nickel from nickel particles 1.98 mg/cm^2/week, whereas from the stainless steel the release was 0.001–0.004 and 0.0001–0.0004 mg/cm^2/week, respectively. Thus, the release of nickel from stainless steel was only 0.005–0.02% of the release of nickel from pure nickel and the release of iron was only 0.02–0.08% of the release of iron from pure iron. This data indicates that 316 grade of stainless steel containing 17.2% chromium and 10.7% nickel behaves as a mixture of chromium with 0.02–0.08% iron and 0.005–0.02% nickel (Table 7).

Table 7. Release of iron, chromium and nickel from stainless steel and pure metals after 168 hours immersion in ALF. The released rates are given as mg.cm^{-2}week^{-1}. .

Element	316 as-received	316 abraded	Pure Fe	Pure Cr	Pure Ni
Fe	0.001±0.00005	0.004±0.002	4.6±0.2	–	–
Cr	0.00003±1.0e-6	0.0001±0.0001	–	0.00003±2.0e-6	–
Ni	0.0001±0.00001	0.0004±0.0001	–	–	2.0±0.05

This difference can be explained by the chromium oxide passivation layer formed at the surface of stainless steel. When the surfaces of the stainless steel sheets were studied by XPS after the immersion of different periods, it was observed that the increase of chromium surface contents during immersion slowed down the metal release. The decrease in release rates during the incubation in biological fluids has been shown also in other studies. These observations are important when the health hazards of stainless steel are evaluated. Because the release of iron and nickel from stainless steel is thousand-fold lower than from respective metals, their bioaccessibility is also much lower. Therefore, the health hazards of stainless steel cannot be directly predicted from the bulk concentrations of the constituents. Chromium release is similar from both stainless steel and pure chromium, suggesting similar bioaccessibility and also similar toxicity. The chromium oxide-rich passivation layer makes the stainless steel and chromium metal surfaces similar.

Additional studies of the same phenomenon have been carried out recently with stainless steel and ferrochromium particles in different synthetic fluids (Ullmann 2009; Hedberg, Midander et al. 2010; Midander, Frutos et al. 2010). The release of iron and chromium from ferrochromium and stainless steel particles (316L with 87% of particles

<45 µm, and 316L with 93% of particles <4 µm), and pure particles of iron and chromium, as well as chromium (III) oxide and iron (II,III) oxide, was studied in synthetic sweat (pH 6.5) and tears (pH 8.0) after 24 or 168 hours of incubation The results support the earlier results with stainless steel sheets, and indicate that the release of chromium from stainless steel (316L) particles is close to the release of chromium from chromium particles, whereas the release of iron from stainless steel is almost 100-fold lower than from iron particles. Ferrochromium with 67 wt% chromium and 25 wt% of iron shows chromium and iron releases very similar to stainless steel 316L with 17.2% chromium and 10.7% nickel. A summary of the results is presented in Table 8. Nickel release from ferrochromium and stainless steel in these experiments was very low and remained below the detection limit (0.5 µg/L) expect in the case of ultrafine (<4 µm) stainless steel particles showing a detectable release of <0.01 µg Ni/cm^2/week in both fluids. Chromium and iron release during 1 week incubation can be seen in Table 6. Chromium(III) oxide released slightly higher amounts of chromium than metallic chromium or stainless steel but the differences were small. When these two different fluids were compared, artificial sweat was more aggressive showing higher releases for all the particles. However, it should be noted that overall amounts released in both fluids were very small. Chemical speciation of released chromium showed that chromium is released in the form of chromium(III) and no chromium(VI) is formed.

Table 8. The release of metals from coarse (<63 µm) particulate ferrochromium, chromium, iron and stainless steel in 168 h incubation in artificial sweat. The releases are given as µg/cm^2/week. Adapted from .

Material	FeCr	316SL	C fine	Fe
Fe	0.08±0.02	0.11±0.06	–	8.62±5.1
Cr	0.08±0.02	0.11±0.06	–	8.62±5.1

Ferrochromium contained 67% Cr and 25%Fe

Midander, Frutos et al. (Midander, Frutos et al. 2010) compared the release rates of iron and chromium from ferrochromium and 316L stainless steel particles to release rates from pure iron and chromium particles in different fluids (GST, pH 1.5; ALF, pH 4.5; Gamble's fluid, pH 7.2; and PBS, pH 7.4). Chromium releases from ferrochromium, stainless steel and chromium particles were very low and similar to each other, whereas iron was released at significantly higher levels from iron particles than from stainless steel particles. Stainless steel revealed lower release of iron than ferrochromium due to more protective passivation layer.

In their cytotoxicity studies on stainless steel and elemental nickel dusts, Landolph (Landolph 2001) measured a 20 times lower bioavailability of nickel from SS316L steel than from elemental nickel powder of similar particle size and shape. The nickel dissolution measurements confirmed this result: The release of Ni^{++} ion from stainless

steel sample was at least 100-fold lower than from elemental nickel. The release of nickel ions from particles was measured after 48 hours of incubation in cell culture medium (Eagles Basal Medium containing 10% foetal calf serum) at 37°C.

Similarly, Assad, Lemieux et al. (Assad, Lemieux et al. 1999) compared nickel release from stainless steel 316L rods and nickel powder in RPMI cell culture media and found out that during 24 hours incubation nickel release from nickel powder was 30-fold higher than from stainless steel. The smaller difference in nickel release between stainless steel and pure nickel seen in these last two studies compared to the study by Herting et al. (Herting, Wallinder et al. 2008b) may be explained at least partly by the shorter incubation time used in the studies by Assad, Lemieux et al. (Assad, Lemieux et al. 1999) and Landolph (Landolph 2001). As demonstrated by Herting et al. (Herting, Wallinder et al. 2008b) the release of nickel from stainless steel is highest in the beginning of the incubation but decreases to almost undetectable levels after the first initial flush during the first 4 hours. This observation can be explained by the changes in surface oxide film of stainless steel with enrichment of chromium in the surface and increase in its protective ability (Herting, Wallinder et al. 2008b; Herting, Wallinder et al. 2008a).

SUMMARY AND CONCLUSIONS ON METAL RELEASE FROM STAINLESS STEEL

In conclusion, studies on the release of chromium and nickel from stainless steel kitchenware have provided inconsistent results. In some studies, the chromium or nickel concentrations in foods have increased, for example when acidic food was prepared in new stainless steel pans and bowls, whereas in other studies researchers did not observe any remarkable increase in chromium or nickel concentrations in foods. However, the measured releases have been very low compared to the intake of chromium and nickel via food (at least one order of magnitude lower), and so the Council of Europe Guidelines on metals and alloys used as food contact materials (Council of Europe 2001) concluded that 'numerous studies of corrosion in various media and of uptake of metals by foods cooked in stainless steel pans give rise to no concern for health due to excessive intakes of nickel or chromium from the stainless steels'. Regarding chromium, the Council of Europe Guidelines state: 'anthropogenic chromium in foodstuffs is not a toxicological problem because the recommended intake is higher than actual (intake) values.' The council also states that 'the migration of nickel to foodstuffs should be as low as reasonably achievable and no more than: 0.1 mg/kg as a general limit of migration into foodstuffs

and 0.05 mg/L from electric kettles'. In the case of stainless steel, these values can safely be reached if, before initial cooking (first use of new items), the food contact items are exposed to boiling water and the water is discarded. The Council of Europe Guidelines regards stainless steel as resistant to corrosion by foods.

There are studies showing that some chromium and nickel may be released from stainless steel medical implants or appliances like orthodontic appliances, although other studies detected no significant increases in chromium or nickel levels in saliva in patients with orthodontic appliances. In general, researchers have observed a significant variation in the concentrations of chromium and nickel in saliva. The ingested amount of chromium or nickel released from orthodontic appliances cannot be quantified using the currently available release data, but it is well below the daily dietary intake levels (Sfondrini et al. 2009).

Not much data are available on the metal release from stainless steel prosthetic implants. The conflicting results seen in these studies may be related to analytical challenges.

There are several in vitro studies on the release of metallic constituents from stainless steel in different synthetic body fluids. Because of the risk of skin sensitization caused by nickel, several studies on nickel release from stainless steel in synthetic sweat are available.

LGC (2003) measured the nickel release from various stainless steel materials (plates, wires, and piercing post assemblies) into synthetic sweat, blood and urine. The results showed that the surface finish of the materials significantly affected the nickel release. The nickel release from the polished materials into all the test fluids was predominantly below 0.01 µg/cm^2/week. In the case of stainless steel plates with a matt or mirrored finish, the release of nickel appeared to be about 0.4–0.5 µg/cm^2/week in artificial sweat. In in vitro studies on urine and blood plasma, the release rates were clearly (in some cases more than twofold) higher. The authors speculated that the reason for the higher release into urine or plasma was most likely due to the biological complexation of the metal ions and the organic components. In the assessment by LGC, it was suggested that the nickel release limit from piercing post

assemblies should be 0.2 µg/cm^2/week in order to take into account the higher nickel release into blood. This limit for piercing post assemblies was adopted in the European Commission directive 2004/96/EC (EC 2004).

The metal release from stainless steel 316L particles of various sizes into artificial sweat has also been studied. The total metal release into artificial sweat is generally very low (<0.008% of the total particle mass dissolved). The released amounts of both nickel and chromium are therefore very low. For nickel, the levels have generally been <0.007 µg/cm^2/week and, in the 'worst case scenario' (ultrafine particles <4 µm in diameter), they have been <0.01 µg/cm^2/week. The chromium release after one week was <0.01 µg/cm^2. When testing the nickel release from metal sheets into artificial sweat, the rates are generally very low. Researchers have only observed high release rates for the resulphurated grade 303 steel and mainly in test situations at pH 4.5 (instead of the standard pH 6.5; Ni release 1.4 µg/cm^2/week), but also in a pilot study at pH 6.5 (3.3 µg Ni/cm^2/week).

The release of metals from stainless steel has also been studied in other artificial body fluids in order to mimic inhalation or gastrointestinal exposure scenarios. These fluids include Gamble's fluid, which mimics extracellular conditions in lungs, artificial lysosomal fluid (ALF) and artificial gastric fluid. The metal release has usually been highest in aggressive fluids like ALF. When the releases of different metal constituents of stainless steel are compared, iron is usually released at higher amounts than chromium and nickel. However, in all cases the release of metals is very low (<5 µg/cm^2/week) and is comprised mostly of iron. Particle size has a small effect on the release rate of different metals per cm^2, which is seen as a slightly higher release in the case of smaller particles. When seven different grades of stainless steels were compared to each other, the differences in release rates between different grades of stainless steel were very small. Usually less alloyed ferritic grades released more metals, but the increased release was attributed to the release of iron. Even if the chromium and nickel content of these different grades varied between 11.4% and 24.2%, and 4.2% (201) and 19.1% (310), respectively, the release rates were remarkably similar. The highest release rates for

nickel were seen with 316L particles in ALF, although its bulk nickel content is lower (10.6%) than that of 310 grade steel (19.1%). This can be explained by the higher content of chromium in grade 310 steel. In addition, when the nickel release from stainless steel is compared to the nickel release from ferrochromium (containing <1% nickel), the release rate is very similar. Thus, although there are wide differences in the bulk content of nickel, the release rates do not show remarkable differences. In addition, when comparing different surface finishes, researchers noticed some differences, but usually the differences were small (for example, twofold).

However, very significant differences have been seen when the metal release from stainless steel has been compared to the metal release from pure metals. In a study in which researchers compared the release from sheets of grade 316 stainless steel to the release from pure nickel and iron metals during one week incubation in artificial lysosomal fluid, they noticed differences of over a thousand-fold in iron and nickel release. On the other hand, the release of chromium was at the same level both for stainless steel and for pure chromium metal. This finding is very important from the health hazard point of view. These in vitro studies suggest that, when chromium bioaccessibility from stainless steel is similar to the chromium bioaccessibility from metallic chromium, the iron and nickel bioaccessibility from stainless steel is a thousand-fold lower than from pure iron and nickel particles. This means that 316 grade stainless steel behaves as a mixture of chromium with <0.1% iron or nickel.

These results strongly support the conclusion that the health effects of stainless steel cannot be estimated solely on the basis of the bulk content of the elements. This effect can be explained by the chromium oxide passivation layer, which comprises most of the stainless steel surface. In vitro studies have shown that chromium oxide enrichment of the surface occurs during incubation in artificial biological fluids, resulting in highly decreased release rates after the first initial flush, and at a very low level steady state release during prolonged exposure. This is likely to occur also in vivo.

3. TOXICITY OF STAINLESS STEELS

3.1 TOXICOKINETICS OF STAINLESS STEEL

No standard toxicokinetic studies have been conducted on the absorption, distribution, metabolism or excretion of stainless steel. Many dissolution tests have indicated that the release of the constituents from these alloys is very low because of oxidized passivation layer on the surfaces. Therefore, also the bioavailability of potentially harmful constituents can also be assumed to be very low.

Chromium levels in the blood and urine of 31 workers from a stainless steel smelting shop and 35 workers in a cold rolling mill were studied by Huvinen et al (Huvinen, Kiilunen et al. 1993). The chromium exposures were low in these parts of the plant. In the steel smelting shop the average total dust concentration was 1.8 mg/m^3 of which 2–4% was chromium. The average total chromium content in the breathing-zone personal samples was 30 µg/m^3. In the cold rolling mill, the concentrations in the total dust were as low as 0.3–0.5% and the personal air samples had no measurable chromium. Biomonitoring measurements revealed slightly elevated urinary chromium levels in pre- and post-shift samples, indicating that some absorption may occur. However, no accumulation of chromium was observed, and so biological monitoring is not suitable as a routine method for exposure assessment in modern production facilities with low levels of exposure.

Huvinen et al. also investigated the retention of dust in the lungs of stainless steel workers in a smelting shop and cold rolling mill by magnetopneumography (Huvinen, Oksanen et al. 1997). The smelting shop workers (n=30) had slightly elevated lung loads of magnetic material compared to the control group (n=5). No differences were observed between the cold rolling mill workers (n= 32) and the controls. These results do not give much information on toxicokinetics.

SUMMARY AND CONCLUSIONS ON TOXICOKINETICS

No studies have been performed in order to specifically investigate the toxicokinetic parameters of metallic stainless steel.

Two studies presenting limited human toxicokinetic data based on workers at an integrated stainless steel production plant have been published. In the studies, researchers observed some chromium absorption among the workers, but the levels were generally very low. Another study focusing on dust retention in the lungs of stainless steel workers showed a slightly elevated lung load of magnetic material. However, this data does not provide much

information with respect to the toxicokinetic profile of stainless steel.

3.2 CYTOTOXICITY *IN VITRO*

In vitro methods that use mammalian cell cultures and various cytotoxicity endpoints have been proposed as alternatives to *in vivo* acute oral systemic toxicity tests that use rodents. *In vitro* cytotoxicity test methods that measure basal cytotoxicity (general cytotoxicity that affects structures or processes intrinsic to all cell types) are not currently regarded as suitable replacements for rodent acute oral toxicity tests. However, some methods have been validated for establishing the starting dose for acute oral toxicity tests so as to reduce and refine the use of animals for such testing. In February 2008, ICCVAM forwarded recommendations (ICCVAM - Interagency Coordinating Committee on the Validation of Alternative Methods 2006) on the use of neutral red uptake *in vitro* test methods for estimating starting doses for acute oral systemic toxicity tests. ICCVAM recommended that these test methods be considered before using animals for acute oral systemic toxicity testing, and that the methods should be used where considered appropriate. Data from the test methods should be used in a weight-of-evidence approach for determining starting doses for *in vivo* studies. Using these *in vitro* methods where appropriate is expected to reduce the number of animals required for each toxicity test.

Stainless steel powder has been tested for cytotoxicity with Neutral Red uptake test in human alveolar epithelial (A549) and human monocyte (THP-1) cells. THP-1 cells were used also to test for the phagocytic uptake of the powder (VITO - Vlaamse Instelling voor Technologisch Onderzoek 2006). The cytotoxicity appeared to be slight. The conclusion of the testing laboratory was 'Based on the neutral red uptake results, the stainless steel powder causes cytotoxicity. The IC_{20} concentration (grade 1 reactivity) of 0.06 mg/mL can be considered as safe'. In the phagocytosis test monocytes did take up stainless steel particles. The ability to be phagocytised does not without any further data allow conclusions on the toxicity of the particles.

The cytotoxicity induced by stainless steel, nickel, and various nickel compounds was determined by treating cultured 10T1/2 cells (Landolph 2001). The samples of stainless steel powders of various spherical particle size were only weakly cytotoxic even at very high concentrations. The cytotoxicity differences were remarkable between the elemental spherical nickel particles (LC_{50} = 1.2 µg/mL) and the stainless steel SS 3.5 particles (LC_{50} = 201 µg/mL) of similar size (mean particle diameter 3.3 and 4.2 µm, respectively). If nickel behaved similarly in both samples, it could be expected that the LC_{50} of the SS 3.5 sample containing 12% nickel would be ≈ 10 µg/mL. However, the LC_{50} for the SS 3.5 powder was ≈ 200 µg/mL, 20-fold the predicted level. This result indicates a 20-fold lower bioavailability of nickel ion from SS316L as compared with

elemental nickel powder of similar particle size and shape. The nickel ion release measurements confirmed this result by showing at least 100-fold lower release of Ni^{2+} ion from stainless steel sample SS 3.5 than expected based on the behaviour of the elemental nickel powder of similar particle size and shape.

Ryhänen et al. (Ryhänen, Niemi et al. 1997) compared the proliferation of osteoblasts and fibroblasts incubated for 10 days with test discs of nickel-titanium (Nitinol) and stainless steel 316 LVM. The proliferation of fibroblasts was 108% (Nitinol), and 107% (316 LVM), compared to the control cultures. The proliferation of osteoblasts was 101% (Nitinol), and 105% (316 LVM) when compared to the controls. The authors concluded that Nitinol and 316 LVM have good *in vitro* biocompatibility with human osteoblasts and fibroblasts.

Good, similar biocompatibilities of stainless steel, Nitinol, and Ti-6Al-4V –alloy were also observed by Ryhänen et al. (Ryhänen, Kallioinen et al. 1998) after 2, 4, 8, 12, and 26 weeks from the implantation of the test specimens into paravertebral muscle and near the sciatic nerve of rats.

Montanaro et al. (Montanaro, Cervellati et al. 2006) investigated the cytotoxicity and biocompatibility of a nickel-reduced stainless steel, Böhler P558, in comparison to the conventional stainless steel AISI 316L. The neutral red (NR) uptake and the Amido Black (AB) tests were performed on L929 fibroblasts and MG63 osteoblasts. The results indicated the absence of cytotoxicity in both materials.

Toxicity and loss of viability in three-dimensional reconstructed human oral epithelium cell cultures induced by point-welded, laser-welded, and silver-soldered orthodontic stainless steel wires have also been studied (Vande Vannet, Hanssens et al. 2007). Histological evaluation of toxicity and measurement of viability in the 3D cell cultures did not show severe effects for any of the wires.

Costa et al. (Costa, Lenza et al. 2007) studied the cytotoxic effects of their corrosion products of AISI 304 stainless steel and manganese stainless steel (low-nickel) brackets in artificial saliva on L929 cells. The bracket extracts did not alter cell viability or morphology. The AISI 304 -bracket extracts decreased cellular metabolism slightly. The results indicated that the low-nickel SS has slightly better *in vitro* biocompatibility than AISI brackets.

The heat treated stainless steel orthodontic wires retain their high corrosion resistance and low ion release rate. The cytotoxicity of the ions released into the artificial saliva was low (Oh and Kim 2005).

Two full sets of stainless steel orthodontic brackets were immersed in 0.9% saline solution for a month. Human periodontal ligament fibroblasts and gingival fibroblasts were exposed to various concentrations of the immersion media. None of the

orthodontic materials-derived media had any effect on the survival and DNA synthesis of either cells (Eliades, Pratsinis et al. 2004).

In vitro cytotoxicity testing is under development to possibly substitute acute *In vivo* testing. One modification of Neutral Red Uptake, the 3T3 NRU PT is based on the OECD test guidelines and is designed to detect the phototoxicity induced by the combined action of a test article and light by using an *in vitro* cytotoxicity assay with the Balb/c 3T3 mouse fibroblast cell line. The test identifies aqueous-soluble compounds (or formulations) that have the potential to exhibit *in vivo* phototoxicity after systemic application.

SUMMARY AND CONCLUSIONS ON *IN VITRO* CYTOTOXICITY

Stainless steel has shown only slight in vitro cytotoxicity in tests like neutral red uptake test. Those tests are currently undergoing validation to be applicable for establishing the starting dose for acute oral toxicity tests in order to reduce and refine the use of animals for such testing. The tests, however, use different cells than those which have been used to study stainless steel cytotoxicity. It is not possible to draw definitive conclusions about the oral acute toxicity based on these studies. Some additional uncertainty also stems from the fact that the culture media and the gastric juice have differing acidities.

3.3. ACUTE TOXICITY OF STAINLESS STEELS

No studies exist where the acute toxicity of metallic stainless steels has been specifically investigated.

3.3.1 *IN VIVO* ORAL AND DERMAL TOXICITY

The low metal release rate of stainless steel materials suggests that the acute toxicity can be expected to be negligible. The solubility of the components is very low, and for instance chromium(III) oxide, which forms the passive surface layer, has an oral LD_{50} of over 5000 mg/kg bw (WHO 2009). Animal testing would be unnecessary and unethical.

Very low acute oral and dermal toxicity is also apparent from the use of stainless steel in cooking utensils, cutlery, tableware and orthodontics. There are no acute toxicity reports from the skin contact with stainless steel tools or jewellery.

3.3.2 *IN VIVO* INHALATION TOXICITY

The acute inhalation toxicity of stainless steel is very low. This can be concluded from the subacute study of SafePharm Laboratories (SafePharm Laboratories 2008). In their

inhalation study, rats were nose-only exposed to stainless steel powder for a period of five consecutive days and two rest days, for twenty-eight days at dose levels of up to 1.0 mg/L. This exposure had no adverse effects. The 'No Observed Adverse Effect Level' (NOAEL) was considered to be 1 mg/L. LC_{50} limits to classify an agent to acute inhalation toxicity Category 4 are 1 and 5 mg/L in 4 hours. It seems to be quite safe to evaluate from the subacute data that LD_{50} for stainless steel dust most likely is higher than 5 mg/L.

The above study can be compared to the study with nickel powder. In a range finding subacute study WIL Research Laboratories, Inc. (WIL Research Laboratories 2002) concluded that for nickel the Lowest-observed-effect level (LOEL) for whole-body inhalation exposure of metallic nickel to rats five/days/week for four consecutive weeks was 0.004 mg/L. In the exposed rats, granulomatous inflammation and mucoid exudate were high for all nickel exposure groups (males and females). However, the severity of these findings was exposure concentration-dependent. There were, however, no clinical signs indicative of respiratory distress for any animal.

SUMMARY AND CONCLUSIONS ON ACUTE TOXICITY

Although no available data on acute toxicity studies of stainless steels exist, long experience on its use as well as subacute studies strongly suggest that they are not acutely toxic via inhalation, or indeed, via dermal or oral exposure.

3.4 IRRITATION OF THE SKIN AND EYES

The low metal release rate of stainless steel makes it an improbable irritant. There are no reports on skin or eye irritation by stainless steel.

Extensive and long continuous use of stainless steel objects on the skin, and even in human eyes has not caused irritation.

3.5 SENSITIZATION

Nickel is the main constituent of concern when considering the sensitization potential of stainless steel.

In developed countries, nickel is the most common contact allergen (Thyssen, Linneberg et al. 2007). As a preventive measurement to reduce the sensitizing effects of articles containing nickel, the release rate of nickel from products intended to come into direct and prolonged contact with the skin was restricted in the EU to a maximum of 0.5 µg/cm^2/week (limit specified in nickel Directive 94/27/EEC) (EC 1994). 0.5 µg Ni/cm^2/week is also the limit value for sensitization classification of nickel containing alloys according to the EU CLP (Classification, Labelling and Packaging of substances

and mixtures) regulation (EC 2008b; EC 2008a). The standard test method for nickel release in Europe is EN1811 (CEN 1998). In addition to the general release limit, a specific limit of 0.2 µg/cm^2/week, for nickel release from piercing post assemblies, was applied in 2004 (EC 2004).

The same limit of 0.5 µg Ni/cm^2/week was set in Denmark in 1991 and implemented in 1992. Studies performed before and after the Danish release limit came into force show a definite decrease in the incidence of nickel allergy among the younger, most commonly exposed population (Johansen, Menne et al. 2000; Veien, Hattel et al. 2001; Jensen, Lisby et al. 2002). In children (0–18 years) the frequency of nickel allergy decreased from 24.8%, in the study period 1985–1986, to 9.2% in 1997–1998. Among young women under the age of 20, the frequency of nickel sensitivity was also significantly decreased. During the first period (1986–1989), 155 of 702 women (22.1%) in this age group, who were patch tested, showed positive results to nickel. In 1996–1999 324 women were tested, of whom 54 (16.7%) were nickel-positive. A clear correlation was also seen between nickel allergy and the time-point of having their ears pierced (before/after 1992).

A number of studies have focused on the metal release from stainless steel (see section 2.3 and 2.4). The study results clearly indicate low release of all constituent metals (Herting, Odnevall Wallinder et al. 2007; Herting, Wallinder et al. 2008b; Herting, Wallinder et al. 2008a). Ni release studies performed according to the EN1811 standard show that the nickel release from 316L grade samples into various artificial body fluids (pH 1.5–8.0) is significantly below the release limit, even in studies performed with fine stainless steel particles (Hedberg, Midander et al. 2010; Midander, Frutos et al. 2010). In a Ni release study performed with different grades of stainless steel at pH 4.5, the high sulphur containing AISI 303 grade was the only one which showed release rates >0.5 µg/cm^2/week (Haudrechy, Foussereau et al. 1994; Haudrechy, Mantout et al. 1997). Previously (before the EU nickel directive) AISI 303 was used for example for the back of wrist watches, but currently it is mainly used for products, which are not in continuous contact with the skin, like nuts and bolts, bushings, shafts, aircraft fittings, electrical switchgear components, gears, valve bodies and valve trim.

The release of nickel from stainless steel plates, wires and ear studs into blood plasma and urine has also been assessed by the EN1811 protocol, and compared with release into sweat (see section 2.3) (LGC Limited 2003). The results indicate that in some cases twice as much nickel can be released into urine and blood plasma *in vitro*, compared with artificial sweat. In addition, the surface finish is crucial for the release of nickel. The authors of the test report concluded that in the case of piercing post assemblies, the release limit of 0.5 µg Ni/cm^2/week in artificial sweat should be adjusted, in order to account for the higher rate of nickel release into blood plasma. In the report, a nickel migration limit of 0.2 µg Ni/cm^2/week, according to the EN1811 methodology, was

suggested for all post assemblies. This limit of 0.2 µg Ni/cm^2/week was adopted by EU in 2004 (EC 2004).

Compared to the results obtained by LGC, Samitz and Katz observed lower release into plasma than to synthetic sweat (Samitz and Katz 1975). They tested the nickel release from stainless steel prostheses, suture wires and screws (including grades 302, 303 316L; for some of the items no data on grade was presented) in sweat, blood, plasma and PSS. The nickel concentrations measured in the different media after one week varied a lot, and as the data on testing material was insufficient, the reliability of this study remains unclear.

Chromium is released from stainless steel as non-sensitizing trivalent chromium, and no measurable amounts of potentially sensitizing chromium(VI) have been detected (Hedberg, Midander et al. 2010; Midander, Frutos et al. 2010).

3.5.1 *IN VIVO* DATA

A study with hairless descendants of Mexican hairless dogs claims that a constant contact with stainless steel cages may cause contact hypersensitivity (Kimura 2007). Six male hairless dogs affected with severe chronic dermatitis were studied. All dogs were individually housed in (allegedly) stainless steel cages. The first signs of dermatitis were observed when the animals were 8–12 months of age. Patch testing was performed with standard series as well as with metal salts. No positive reactions were seen with nickel, but positive results were obtained with potassium dichromate. The report does not indicate whether the chromate test was positive in each of the animals. No data was available on the grade of stainless steel used for the cages, or if the material even was stainless steel at all, and therefore the informative value of the study remains unclear. Chromium metal and trivalent chromium do not cause sensitization (WHO 2009) and it is unlikely that the sensitization would have been caused by exposure to stainless steel.

The potential of stainless steel AISI 316 LVM to produce an allergic response was tested in guinea pigs (Wever, Veldhuizen et al. 1997). Metals released from 7 stainless steel cylinders with an area of 94 cm^2, which were kept in 30 mL 0.9% NaCl solution at 50°C for 72 h under gentle movement. Woven patches were saturated with the extract (0.5 mL) and applied to a clipped area on the dorsal side of ten animals, covered by an occlusive dressing, and kept for 6 h. This induction phase was repeated twice with one-week intervals. After two weeks of rest, the animals were challenged on untested pre-shaved skin. The challenge was carried out on the ten guinea pigs in the stainless steel group, and on five non-induced control animals. The test sites were scored for erythema and oedema formation 24 h and 48 h after removal of the challenge patches. No erythemas or oedemas were observed at the test sites of any of the animals in the treatment group or among the control animals. The metal ion concentrations in the

extract used for the test were not measured. According to available data on the poor solubility of stainless steel (see sections 2.3 and 2.4) it is likely that the patch test concentrations were very low. Therefore the information value of this report is not clear. However, the study circumstances are likely to be comparable to real situations, because the metal concentrations in the tested extract are probably at the same low levels as in occupational or consumer exposure situations.

3.5.2 HUMAN DATA

ELICITATION OF SKIN RESPONSES IN SUBJECTS PREVIOUSLY SENSITIZED TO NICKEL

Patch test results with different grades of stainless steel (AISI 303, 304, 304L, 316, 316L, 310S, 430) on 50 patients already known to be sensitive to nickel were presented by Haudrechy et al. (Haudrechy, Foussereau et al. 1994; Haudrechy, Mantout et al. 1997). The tests were performed with the same stainless steel grades as used in the release tests, as well as with nickel plated steel. Circular samples (diameter 1.5 cm) of each test material were applied to the back of the test persons, and the results were assessed after two or three days. 96% of the patients were intolerant to nickel plated samples and 14% (7/50 persons) reacted to the AISI 303 samples. No positive skin reactions were elicited by the other stainless steel grades. These results correlate well with the nickel release studies, showing higher nickel release into synthetic sweat from AISI 303 than from the other grades tested (see section 2.3) (Haudrechy, Foussereau et al. 1994; Haudrechy, Mantout et al. 1997).

The sensitizing potentials of four different stainless steels (and 17 other alloys) were studied by patch testing on 100 nickel-positive persons and 20 non-nickel sensitized control persons (Liden, Menne et al. 1996). 13 of the persons were also chromium-positive. The patch tests with four different stainless steels on the upper back showed no significant numbers of positive reactions. With three of the grades (stainless steel surgical (ISO 5832; AISI 317), stainless steel 18/8 (ISO 683 XIII, AISI 304) and stainless steel 142382 (<0.5% Ni)) there were no cases of positive patch test results. The fourth grade (stainless steel 18/8 (ISO 683 XIII), gold plated) showed positive reactions in four of the 100 patients. This result was not statistically significant ($p>0.05$) when compared with the control group. The patch testing on the ear-lobes of 20 nickel positive subjects was negative for all three stainless steels tested. Based on these results, it was concluded that the use of stainless steel is safe, even for persons with chromium or nickel allergy.

Four different stainless steel grades (AISI 305, 321, two different 316L) used for example in dental brackets were patch tested on 31 nickel-sensitive persons (Jensen, Lisby et al. 2003). The test evaluations were performed at different time points, two times within five days after removal of the discs. None of the 31 subjects reacted to any of the stainless steels tested.

Sixty six persons who were previously sensitized to nickel were patch-tested with 15 metals and alloys (nickel content 0–100%), including stainless steel (Menne, Brandup et al. 1987). Two of the tested persons showed a weak positive response to stainless steel. The following trend was observed: alloys with Ni release >1.0 µg/cm^2/week elicited positive reactions in >50% of the subjects previously sensitized whereas alloys with Ni release <0.5 µg/cm^2/week elicited positive reactions in <30% of the subjects with prior sensitization.

Sensitivity to stainless steel was tested in 52 metal-allergic subjects and in 48 persons without a history of metal allergy (no patch test reactivity to Ni, Co or Cr) (Summer, Fink et al. 2007). The allergic group consisted of 41 nickel-, 16 cobalt- and 12 chromium-positive individuals. 15 of the 52 persons were allergic to more than one metal. Stainless steel (ISO 5832-9; 20.8% Cr, 9.8% Ni) was patch-tested in all subjects. In addition, a CoCrMo alloy was also tested. No patch test reactions were observed with stainless steel in either of the groups. Five of the metal-allergic individuals reacted to the CoCrMo alloy.

SENSITIZATION VIA MEDICAL DEVICES

Stainless steel has for decades been used in various types of medical devices, including orthopaedic prostheses, dental brackets and coronary stents. A number of prospective and retrospective studies have been carried out with groups of patients.

493 patients who were going to have a stainless steel implant to fix a fracture or osteotomy were studied by Swiontkowski et al. (Swiontkowski, Agel et al. 2001). Metal sensitivity (chromium, nickel, cobalt) was investigated by patch tests. The prevalence of metal allergy was re-assessed in 241 of the patients by a second patch test, which was carried out at a mean interval of 187 days. In the initial group, the prevalence of sensitivity was 0.2% for chromium, 1.3% for Ni, and 1.8% to cobalt. In the group of 241 patients being followed-up, the incidence of conversion from negative to positive was 2.7% for Cr, 3.8% for Ni and 3.8% for Co. However, the desensitization rate (conversion from positive to negative result) was 1% for Cr, 2.1% for Ni, and 3.8% for Co. No statistical analysis was carried out, but based on the results of the study long-term exposure to stainless steel did not increase the prevalence of metal allergy.

In a prospective study of 60 patients who received stainless steel implants (Cr 15–20%, Ni 10–14%) in operations of extremity fractures (Hindsén, Carlsson et al. 1993), the patients were tested epicutaneously and intracutaneously with chromium and nickel within 2 days of surgery and were followed for one year. Forty eight patients participated in the follow-up. Three patients tested positive to nickel both in the primary and secondary test. The remaining 45 patients, who were retested, were negative in the nickel and chromium tests both at the time of surgery and one year later. There were no dermatologic or orthopaedic complications due to the materials

used. This study did not reveal any cases of contact allergy or dermatitis from metallic surgical implants.

Eighteen patients with a contact allergy (chromium, cobalt and/or nickel) were followed up (on average 6.3 years) after implantation of a stainless steel orthopaedic device (Carlsson and Möller 1989). The stainless steels used in the devices contained chromium and nickel, chromium and cobalt, or chromium, nickel and cobalt. Clinical and radiographic examinations as well as epicutaneous and intracutaneous tests were carried out to see any dermatologic or orthopaedic complications. No complications attributable to metal allergy were observed after exposure during many years, and no new allergies were recognized. Carlsson et al. concluded that stainless steel is safe for orthopaedic implants, and the sensitizing potential of these alloys is very low.

Metal stent implantation is an effective method for the treatment of atherosclerotic disease. Stainless steel grade 316 L is the most commonly used material, both for bare stents and stents with a coating material (Mani, Feldman et al. 2007). Some 10–30% of the patients, however, suffer from restenosis (re-narrowing of a coronary artery). The reasons for restenosis are unclear so far, but allergy to metal ions dissolved from the stent has been suggested to be a risk factor.

In a retrospective study, 484 patients with endovascular coronary stents were patch tested with a broad series of test preparations such as the European baseline series, metals, preservatives and fragrances (Ekqvist, Svedman et al. 2007). 314 patients had implantations of unplated stainless steel (316L) stents, and 170 of the patients had steel stents plated with 99.9% gold. There was no statistically significant difference in contact allergies to nickel between the patients and the controls. However, the frequency of nickel allergy was higher among women with unplated stainless steel stents compared with the controls (33.8% versus 20.4%), but this finding was not statistically significant ($p=0.07$). On the contrary, the patch testing showed that contact allergy to gold was markedly higher in the group of patients with gold-plated stents than in the control group ($p<0.001$). There was also a clear correlation between contact allergy to gold, gold stent and restenosis. No such correlation was observed regarding nickel allergy and stainless steel stents. The rate between Ni sensitive patients with restenosis and non-allergic patients with restenosis, was not statistically significant.

Contrary to the results observed by Ekqvist et al. (Ekqvist, Svedman et al. 2007) and Svedman et al. (Svedman, Ekqvist et al. 2009). Saito et al. (Saito, Hokimoto et al. 2009) concluded that nickel was a major factor for in-stent restenosis (ISR). In a group of 128 patients with stainless steel (316L) coronary stents, 60 of the patients had second ISR, and 68 patients were without second ISR. All subjects were patch tested with nickel, chromium and manganese. Twenty four subjects were nickel-positive (19%). Of these nickel-positive subjects, 18 (30%) were in the study group (second ISR) and 6 (9%) in the control group (stent patients without second ISR). Statistical analysis of the data

indicated a significant correlation (p=0.0033) between nickel sensitivity and second ISR. No significant differences in chromium or manganese sensitivity were observed between the two groups.

The frequency of metal allergies in patients with coronary stents was also studied in a German population (Hillen, Haude et al. 2002). Retrospective patch testing was carried out in 20 patients, 3–12 months after application of the coronary stents. A second group consisted of 7 patients, who were observed prospectively – they were patch tested prior to their first coronary catheterization. In the retrospective group 2 patients (10%) showed sensitization to nickel. In-stent restenosis occurred in 6⁄18 nickel-negative and 1⁄2 nickel-positive patients. Only one patient in the group investigated prospectively was nickel sensitive. Restenosis was observed in 2 patients (29%), neither of whom had nickel allergy. Together the results of this study bring little evidence for the role of metal allergy in restenosis. The authors concluded that it cannot be excluded that metal allergy may play a role in restenosis.

CASE STUDIES

There are a number of case reports on reactions to objects like surgical or dental prosthetic devices, internal surgical clips, ear-piercing kits, as well as to wearing a stainless steel watchstrap or wrist strip (Schriver, Shereff et al. 1976; Cramers and Lucht 1977; Fischer, Fregert et al. 1984; Olerud, Lee et al. 1984; Widstrom, Bergstrom et al. 1986; Fine and Karwande 1990; Gawkrodger 1993; Räsänen, Lehto et al. 1993; Fisher 1994; Kanerva, Sipilainen-Malm et al. 1994; Takazawa, Ishikawa et al. 2003; Ehrnrooth and Kerosuo 2009). However, these do not provide much useful information on the sensitization potential of stainless steel, because the extent of the use of articles at issue remains unclear.

SUMMARY AND DISCUSSION ON SENSITIZATION

Nickel is the most common contact allergen in developed countries and, therefore, the potential of stainless steel to cause sensitization is also of interest.

Release tests of stainless steel samples into various artificial body fluids generally show very low release rates for nickel. Within the EU, the release of nickel into synthetic sweat has been restricted to 0.5 µg/cm^2/week (0.2 µg/cm^2/week for piercing post assemblies), which is also the limit for sensitization classification according to the CLP system.

Available study reports on nickel release from different grades of stainless steels clearly show that in most cases the release increases

when the pH of the test medium decreases. In addition, in the worst case scenarios, the Ni release from stainless steels is usually clearly below the limit of 0.5 µg/cm^2/week. Only studies with one grade of stainless steel (AISI 303, high sulphur content) have shown release rates above the limit. One current study indicates that twice as much nickel may be released into urine and blood plasma from unfinished or unpolished stainless steels compared to artificial sweat.

Chromium is released from stainless steel as non-sensitizing trivalent chromium. Chromium(VI) (which is a known sensitizer) has not been detected in release tests.

A clear decrease in the frequency of nickel allergy was observed when comparing groups of young women in Denmark before and after the Ni release restriction came into force. The same results were also obtained when comparing groups who had their ears pierced before and after the restriction was implemented. These studies strongly support the assumption that the 0.5 µg/cm^2/week limit can protect people from Ni sensitization.

The potential of stainless steel to elicit reactions in previously nickel-sensitive persons have been patch tested in a number of studies. The results clearly show that no allergic reactions occur and, based on this, stainless steels can be regarded as safe, even in persons with nickel allergy.

The frequency of nickel sensitivity among patients with stainless steel orthopaedic prostheses has not increased, and allergy tests performed before and after implantations do not show any signs that the orthopaedic prostheses induce nickel sensitivity. Studies on patient groups with stainless steel coronary stents show conflicting results on the frequency of nickel allergy and the correlation between nickel allergy and cases of restenosis among the patients. The implantation of stainless steel stents obviously does not significantly induce nickel sensitivity, but the role of nickel allergy in stent restenosis cannot be excluded. The available data is insufficient for final conclusions, but, based on the most extensive studies published (Ekqvist et al. 2007; Svedman et al. 2009), the use of stainless steel in coronary stents seems to be safe.

In conclusion, nickel release from stainless steel is the critical sensitization factor. However, many studies show very low release rates of nickel and, therefore, sensitization caused by stainless steel can be regarded as unlikely. In addition, its widespread use and the low number of confirmed cases of nickel allergy, even in persons previously sensitized to nickel, support the conclusion that stainless steel is not a potential sensitizer.

3.6 REPEATED OR LONG TERM EXPOSURE

3.6.1 ANIMAL STUDIES

SafePharm Laboratories has carried out a 28-day repeated dose nose-only inhalation study with rats using stainless steel powder (grade 316L) (SafePharm Laboratories 2008). The study complies with the requirements of EU and OECD (OECD 412) guidelines. Four groups of five female and five male Wistar rats were exposed to stainless steel powder at concentrations of 0.01, 0.10, 0.30, and 1.00 mg/L by inhalation. The particle size (MMAD) was between 2.50 µm (0.10 mg/L group) and 3.04 µm (1.00 mg/L group). A control group (five female and five male rats) was treated in the same way as the study groups, but exposed to air only at the same air-flow rates as in the 1.00 mg/L group. The exposures were carried out for 6 hours/day, 5 days/week, followed by two days of rest, for a period of four weeks (28 days). All animals were sacrificed at day 29. There were no deaths or clinical signs of toxicity during the exposure period. The treatment did not affect body weight, blood parameters, or food or water consumption. All animals were sacrificed at the end of the exposure period (day 29), organ weights were measured, and histopathological examinations were carried out. Elevated relative lung weights were observed for male and female rats treated with 0.3 mg/L and 0.1 mg/L stainless steel as compared with the controls. Accumulation of black pigment in the lung lobes was observed for all exposed animals, being more severe at the highest exposure level. Alveolar macrophages, phagocytising pigment, were found in high amounts in the lungs, but no signs of inflammatory responses, tissue degeneration or necrosis could be observed. Small amounts of black pigment were found in the nasal cavities at all exposure levels, being most severe at 1.0 mg/L. No signs of inflammation, tissue degeneration or necrosis could be observed. Accumulations of black pigment were also seen in the trachea, larynx, pharynx and mediastinal lymph nodes of animals exposed to the highest dose of stainless steel powder (1.0 mg/L). However, in no instance was there any associated inflammatory response. Based on the lack of adverse effects in this study the 'No Observed Adverse Effect Level' (NOAEL) was therefore considered to be 1.0 mg/L.

The results of the 28-day stainless steel inhalation study can be compared with results obtained in a similar OECD 412 test with nickel metal (WIL Research Laboratories 2002). Groups of five male and five female albino rats (strain: Crl:WI (Glx/BRL/Han)IGS BR) were exposed to 0.004, 0.008 and 0.024 mg/L (4, 8, 24 mg/m^3) of nickel powder. The control group (five male and five female rats) were exposed to filtered air on a comparable regimen. 90% of the particles had a diameter <1.84 µm. The exposure pattern was the same as in the 28 day stainless steel study; 6 hours/day, 5 days/week, followed by two days of rest, for a period of four weeks. At the end of the exposure period animals were killed and the standard examinations were carried out. No exposure-related deaths occurred. Lower mean body weight gains were observed at all exposure levels. The mean food consumption was lower than in controls at week one in all exposure groups, at week 2 in the 0.008 and 0.024 mg/L groups, and at week 3 in the 0.024 mg/L group. Furthermore, the absolute and relative lung weights increased in all groups in an exposure-dependent manner. Macroscopic effects were seen in the lungs and lymph nodes at all dose levels. Microscopic examinations revealed granulomatous inflammation, proteinaceous and/or mucoid exudate and black pigment in the lungs, and granulomatous inflammation, hyperplasia and black pigment in the lymph nodes after exposure to 0.004, 0.008 or 0.024 mg/L of nickel powder. Based on the test results the NOAEL for metallic nickel was <0.004 mg Ni/L and the LOAEL was 0.004 mg/L (4 mg/m^3).

The effects of implanted stainless steel wires (orthodontic stainless steel wire "Tru-Chrome"; 68% Fe, 19% Cr, 13% Ni; 16 mm length, 1.05 mm diameter) were studied in rabbits (Gjerdet, Kallus et al. 1987). The wires were implanted subcutaneously both in previously nickel-sensitized and unsensitized animals, and left in place for 31 days. As a control material, the same animals had polytetrafluoroethylene (PTFE) tubing implanted. The animals were sacrificed and the surrounding tissues examined histopathologically at the end of the study period. The only observed local response was surrounding collagen capsules containing fibroblasts and fibrocytes. This effect was similar both for the stainless steel and the PTFE material. No signs of material-dependent local toxic effects were seen.

McGeachie et al. implanted 5 mm long pieces of surgical grade stainless steel wire (no data on identity of the material) into the leg muscle of 36 mice (McGeachie, Smith et al. 1992). The local tissue responses of the leg muscle were histopathologically examined at eight different time-points, 3 days to 12 weeks after the insertion of the implant. The results were compared with those obtained from mice with similar titanium implants. The animals, which were sacrificed and examined three days to 2 weeks after the implantation, had initial inflammation responses and formation of a collagen capsule was observed. However, there were no marked changes in animals after 3 weeks and later, which indicates a rapid regeneration of muscle fibres. The

results were similar for the stainless steel and titanium metal. An additional group of 16 mice had implants (8 with stainless steel and 8 titanium) inserted in both legs and had a tritiated thymidine radiolabel injection. The autoradiography of tissues from these animals confirmed that muscle fibres regenerated rapidly and there was no myogenesis inhibition.

Escalas et al. studied local tissue reactions caused by 26 different materials in rabbits (Escalas, Galante et al. 1976). The test materials were metals and ceramics considered as possible joint-prostheses materials. Stainless steel 316L (rods and powder) served as control. Solid implants were introduced surgically in the paravertebral muscle of rabbits and powders were applied to a pocket dissected in the paravertebral muscle fibres. Six animals were used for the evaluation of each material, and each of the animals had three solid and three powder implants. The rabbits were sacrificed after six months, and the surrounding muscle was examined. Also the liver, lung, kidney and spleen were inspected. A mild tissue response was observed in the examined muscle tissue after stainless steel implantation, including minimal inflammation and some fibrosis. All implanted materials caused the same type of reactions, at various severities, and therefore they can be considered as non-specific reactions which are related to insertion of foreign material and not to the chemical composition of the material.

Elicitation of local tissue responses were observed in guinea pigs with stainless steel (ASTM F55, F138-139, 2.7 mm diameter) and cobalt chromium screws inserted in the right and left proximal tibiae (Lewin, Lindgren et al. 1982; Lewin, Lindgren et al. 1987). The test was carried out with groups of animals previously sensitized to nickel or chromium. After four months the animals were sacrificed and the bone was examined. All screws were well fixed in the tibia and no differences in bone density, histology, or general parameters like body weight gain were observed among either the sensitized or non-sensitized animals. The sensitized animals showed eczematous changes in the skin. Based on these results, contact allergy appears to be non-significant for the fate of orthopaedic implants.

Ferreira et al. (Ferreira, de Lourdes Pereira et al. 2003) investigated toxic effects of stainless steel on spleen histochemical and immunohistochemical parameters in mice. The animals were injected with a suspension of AISI 316L stainless steel containing Fe 490 mg/L, Cr 224 mg/L and Ni 150 mg/L, which was obtained by electrochemical dissolution. 0.5 mL of the obtained suspension was administered subcutaneously to groups of 5-10 mice every 72 h for 3, 10, 14 or 30 days. Examination of the spleens showed several pronounced alterations in the spleen architecture, as well as depletion of T4 and B cells. The results indicate that the immune system may be hampered by metallic elements, and thus affect the defence mechanisms of the body. Due to the low bioavailaibility of stainless steel components, such a situation is, however, not very likely in real life.

3.6.2. HUMAN DATA

Huvinen et al. investigated long-term health effects among workers engaged in the manufacture of stainless steel in a factory an integrated with chromite mine, stainless steel smelter and rolling mill (Huvinen, Uitti et al. 1996; Huvinen, Uitti et al. 2002). The study groups consisted of 36 chromite miners, 109 workers in the furnace department of a ferrochromium plant and steel melting shop, 76 workers from the sintering and crushing department of the FeCr plant, and 95 workers from a cold-rolling mill. The average duration of exposure was 18 years in the first study, and the results were followed up five years later. The cold-rolling mill stainless steel worker group served as a control group, as the levels of chromium were low in that area of plant. The study focused on the relationship between respiratory health and exposure to chromium in the various processes. The results showed that lung function was somewhat impaired among the chromite workers, but no symptoms were observed in the group containing FeCr furnace and stainless steel melting shop workers as compared with the cold-rolling group. As the study focused on health effects caused by chromium, and the workers were grouped according to Cr-exposure, it is not possible to make any conclusions for the workers solely engaged in stainless steel production. In addition, the roles of exposures other than chromium remain unclear. Moreover, the cold-rolling mill workers cannot be considered as true unexposed controls.

Local immunological reactions were measured in 19 patients having stainless steel or titanium miniplates and screws inserted after mandibular fractures (Torgersen, Moe et al. 1995). The devices were removed after 15-47 weeks (stainless steel mean 32 weeks), after which samples of local soft tissue and bone tissue were examined histologically. The results showed mild tissue reactions, including scattered T lymphocyte clusters and small numbers of macrophages. No marked changes were observed in bone tissue.

A case report described a patient who had had retinal tacks of stainless steel for 21 years to attach detached retina without tissue effects. This report suggests that intraocular stainless steel may cause minimal or no retinal toxicity during long-term follow-up (Javey, Schwartz et al. 2009).

SUMMARY AND DISCUSSION ON REPEATED DOSE AND LONG-TERM EXPOSURE TOXICITY

The 28-day repeated inhalation study performed with stainless steel clearly indicates a lack of toxicity. The doses used in the stainless steel study were markedly higher than those used in the corresponding nickel study (maximum stainless steel dose 1 mg/L = 1000 mg/m^3; maximum nickel dose 0.024 mg/L = 24 mg/m^3). No adverse effects were seen, even at the highest concentration of

stainless steel, whereas the lowest nickel dose (0.004 mg/L) already resulted in clear signs of toxicity in a 28-day nickel inhalation study.

These results show that the metallurgical properties of the alloy play an extremely important role in its potentially toxic effects. In the case of stainless steel, the in vivo inhalation test showed no signs of adverse effects, although researchers would assume repeated dose toxicity if they were to exclusively consider only the nickel content of the material. These findings strongly support the hypothesis that, in the case of alloys, the bioaccessible fraction rather than the elemental nominal composition is the main factor causing toxicity/non-toxicity.

Available data on animal or human long-term exposure via metallic implants do not indicate any adverse local or systemic effects caused by stainless steel.

3.7 MUTAGENICITY

3.7.1 *IN VITRO* MUTAGENICITY

Mutagenicity of stainless steel (316L) has been tested *in vitro* in standard Ames tests following OECD guideline 471 with strains TA98, 100, 102, 1535 and 1537 with and without metabolic activation. Stainless steel powder (size of the particles; 90% <4 microns) was dissolved in dimethylsulphoxide (DMSO) and tested at concentrations of 0.25–5 mg/plate. No mutagenicity was observed in any of the strains, whereas positive controls were clearly mutagenic (VITO - Vlaamse Instelling voor Technologisch Onderzoek 2006).

In another study (CTL 2006), grade 316L stainless steel powder, diameter (MMAD) 2 µm, was dissolved in DMSO and evaluated in a bacterial mutagenicity assay according to OECD 471 over a range of concentrations (100–5000 µg/plate) and using four strains of Salmonella typhimurium (TA1535, TA1537, TA98 and TA100) and one strain of Escherichia coli (WP2 uvrA (pKM101)) in the presence and absence of metabolic activation system (S9-mix). The study was performed following GLP. Positive and negative controls were used separate assays with each strain; the test substance did not induce any significant, reproducible increases in the observed number of revertant colonies, either in the presence or absence of S9-mix. The positive controls for each experiment induced the expected responses.

The same sample of stainless steel was tested also in standard chromosomal aberration tests performed according to OECD 473 and following GLP (CTL 2006). Human

lymphocytes were treated either for 3 hours both in the presence and absence of S9-mix, or for 20 hours in the absence of S9-mix. The 3-hour experiment with metabolic activation was repeated twice with slightly different doses. All cultures were harvested 68 hours after culture initiation. The doses used were 78, 313, 1250 µg/mL for the first 3 hour experiment with or without S9 mix, and 313, 625 and 1250 µg/mL for the second 3 hour experiment with S9 mix. In the 20 hours experiments without S9 mix, doses of 156, 313, 625 µg/mL were used. Significant reductions in mean mitotic activity were observed at the highest concentrations used. In the absence of metabolic activation, stainless steel caused a small, but significant increase at the highest dose (1250 µg/mL) after 3 hours treatment. However, no increase was seen after 20 hours treatment at the doses up to 625 µg/mL. In addition, the level observed (2.5% aberrant cells excluding gaps) at 1250 µg/mL was within the concurrent control range. In the presence of S9 mix, no increase in chromosomal aberrations (CA) was seen in the first experiment after 3 h treatment, but in the second experiment a statistically significant increase (5.5% aberrant cells excluding gaps) was seen. This value was, however, equal to the upper level of historical control range and since it was not reproducible, it was not considered biologically significant. Positive controls, cyclophosphamide and mitomycin C showed clear increases in the number of CA.

Stainless steel samples were tested to determine whether they induce morphological transformation in cultured C3H/10T1/2 mouse embryo cells (Landolph 2001). Three different stainless steel 316L samples were studied; particles with a mean size of 3.54 µm (SS3.5), 8.45µm (SS8.5) and 2.4 µm (SS<3). Concentration ranges tested were 100–275, 50–250 and 200–500 µg/mL, respectively, depending on the cytotoxicity of the particles. Five different concentrations were used. Nickel subsulfide at concentrations of 0.03–0.5 µg/mL and nickel oxide at 2 µg/mL were tested for comparison. Water insoluble particles were suspended in 0.5% acetone. 3-Methylchlorantrene was used as a positive control. Nickel subsulfide caused a dose-dependent increase in the number of transformed foci and number of dishes with foci. Total number of transformed foci (type II and III foci) was 26/20 dishes at 0.5 µg/mL. Ni(II) oxide at 2 µg/mL caused 4.5–19.2 transformed foci/20 dishes scored (2 separate experiments). Methylcholanthrene (1 µg/mL) caused 9–22 transformed foci/20 dishes scored. Stainless steel sample 3.5 caused some increases in the number of transformed foci, but no dose-response was seen. The levels were 4.7, 2.4, 4.6, 0.9, 2.9 transformed foci/20 dishes, scored at 100, 150, 200, 250, 275 µg/mL, respectively. The levels of 2.9–4.7 were statistically significantly increased when compared to the levels in concurrent acetone controls (0.4). The three highest doses showed clear cytotoxicity with less than 50% survival of the cells. With samples SS8.5 and SS<3 no increases in the number of transformed foci were seen. The control levels of transformed foci varied between 0.0–0.5 (medium only control) to 0.0–4.6 transformed foci/20 dishes (0.5% acetone). As a conclusion, stainless steel induced only very weak or no cell transformation in this assay, at the

concentrations which were >100 -fold higher than those which caused clear cell transformation with nickel subsulfide.

Induction of chromosomal aberrations was also studied in C3H10T1⁄2 mouse embryo cells (Landolph 2001). The cultured cells were treated for 48 hours with stainless steel particles with a mean size 3.54 µm (SS3.5), 8.45µm (SS8.5), and 2.4 µm (SS< 3) at 5–7 different concentrations varying from 50–250 µg/mL for sample SS3.5, 10–200 µg/mL for sample SS8.5 and 12.5–250 µg/mL for sample SS< 3.0. Mitomycin-C was used as a positive control and nickel subsulfide at concentrations of 0.03 – 1µg/mL was also tested. Sample SS3.5 caused a statistically significant increase (12%) of chromosomal aberrations at 250 µg/mL. In addition, some increase was seen at 200 µg/mL, but this was not statistically significant. However, samples SS8.5 and SS3.0 caused no or only slight increases in the numbers of chromosomal aberrations. No clear dose response was seen. The highest levels observed with these particles were 4.6 (100 µg/mL) for sample SS8.5 and 6.2 for sample SS< 3 (250 µg/mL). The increases seen at sample SS8.5 were said to be not statistically significant. No information on the statistical significance of the increase seen in the highest dose of sample SS< 3 was given. The control levels of chromosomal aberrations varied between 1.5–3.9% (medium only control) and 1.0–3.0% (acetone 0.5% control). The historical range for chromosomal aberrations was 0.5–5% for acetone controls. Nickel subsulfide caused some increases in the number of chromosomal aberrations (highest level of CA was 9.2% at 0.25 µg/mL. However, no dose-response was seen. At the highest dose (1.0 µg/mL) the percentage of chromosomal aberrations was 7.4%. The positive control, 1 µg/mL MMC (4 h), caused 10–22% of chromosomal aberrations. Overall, the results of this study on the ability of stainless steel to cause chromosomal aberrations are rather equivocal and no clear conclusions compared to nickel subsulfide can be made.

Eight metals (cobalt, chromium, nickel, iron, molybdenum, aluminium, vanadium and titanium) and their alloys (stainless steel, chromium alloy, and titanium–aluminium–vanadium alloy) were tested for cell transforming effect in C3H T½ mouse fibroblasts (Doran, Law et al. 1998). The cells were exposed to solutions of the metal salts, to metal, or alloy particles (particle size ≤ 5 µm). Cell transformation was observed with soluble forms of cobalt, chromium (VI), nickel and molybdenum. Soluble nickel chloride caused a statistically significant increased incidence of cell transformation at 10 and 20 µg/mL concentrations, but no cell transformation was seen with metallic nickel or stainless steel. Other particulate metals (like chromium and iron) and alloys failed to induce cell transformation (Doran, Law et al. 1998)- Differences in cytotoxicity (measured as plating efficiency) of different metals/alloys was also noted: nickel showed marked cytotoxicity at 50 µg/mL, chromium at 100 µg/mL and iron at 500 µg/mL. With stainless steel marked cytotoxicity was evident at 100 µg/mL.

Wever et al. (Wever, Veldhuizen et al. 1997) studied the genotoxicity of the extracts of nickel-titanium alloy and stainless steel 316L alloy by Ames test and chromosomal aberration test *in vitro*. Extracts were produced by incubating 94 cm^2 pieces of test materials in 30 mL 0.9% NaCl in aqua bidets at 37°C for 72 h under gentle movement. Five concentrations of 20, 40, 60, 80 and 100% of the sample extracts were tested. Four strains (TA1535, 100, 1537 and 98) were used for the Ames test. The chromosomal aberration test was performed with Chinese hamster fibroblast cell line V79. Both tests were performed with and without metabolic activation. Appropriate positive controls and a negative solvent control were included in the assays. No increases in the number of revertants were observed in Ames test in any of the concentrations or strains tested by both alloys. None of the alloys caused any increase in the number of chromosomal aberrations when compared to negative controls. Positive controls showed clear genotoxic reaction. The results can be explained by low dissolution of the metallic components from the alloys. On the other hand, even nickel compounds considered as clearly carcinogenic haven't been clearly positive in Ames test (EC 2008a).

Montanaro et al. (Montanaro, Cervellati et al. 2005; Montanaro, Cervellati et al. 2006) studied the genotoxicity of the extracts of AISI 316L and new, nickel free P558 stainless steel by Ames test, sister chromatid exchange and chromosomal aberration test. Extracts were produced by incubating 33 mm discs in either HAM F12 Medium or Minimum Essential Medium (MEM) with Earle's salt at 37°C for 72±2 h. All tests were performed with and without metabolic activation. Chromosomal aberration and sister chromatid exchange tests were performed by Chinese hamster epithelial-like CHO K1 cells by adding 500 µl/mL of sample extracts or negative or positive control substances (MEM/HAM F12 or mitomycin C/cyclophosphamide) into the culture medium and incubating for 90 minutes. Strains TA 1535, 100, 102, 1537 and 98 were used for Ames test, and 0.1 mL of sample extract or positive (4-nitro-o-phenylenediamine/2-antramine/sodium azide/mitomycin C/ICR) or negative control substances were added to top agar. Neither of the alloys caused any increases in the number of revertants in Ames test. In addition, both alloys caused no increases in the number of chromosomal aberrations or sister chromatid exchanges when compared to negative controls. Positive controls showed clear genotoxic reaction. Treatment caused no effects on cell viability measured by MTT assay. As noted above, the results can be explained by low dissolution of the metallic components from the alloys. On the other hand, even clearly carcinogenic nickel compounds haven't been clearly positive in Ames test (EC 2008b; EC 2008a).

Extracts of stainless steel 316L, nickel-titanium alloy and pure titanium were produced following ISO 10993-3 sample preparation conditions, and human peripheral blood lymphocytes were exposed *in vitro* to these extracts for 70 hours (Assad, Yahia et al.

1998). In situ end labelling (ISEL) with immunogold staining and electron microscopic evaluation was employed to detect DNA single strand breaks (SSB). Results show no increased incidence of DNA SSB after exposure to extracts from NiTi or Ti alloy, but the stainless steel 316L caused non-significant increase in labelling in interphase nuclei and a statistically significant labelling in metaphase nuclei indicating the increase in SSB. The levels were: mean 122 and 238 immunogold particles/μm^2 chromatin, respectively, whereas in negative controls the levels were 94 and 157 immunogold particles/μm^2chromatin. Methylmethanosulfonate, used as a positive control caused 397 and 346 immunogold particles/μm^2 chromatin.

Assad et al. (Assad, Lemieux et al. 1999) also compared nickel powder, SS316L, NiTi and Ti with the same methodology. Cellular chromatin exposition to pure Ni and 316L SS demonstrated a significantly stronger gold binding than exposition to NiTi, pure Ti, or the untreated control. In addition, the release of Ni from the materials took the following descending order in the semiphysiological solutions: pure Ni, 316L SS, NiTi, Ti, and controls. Nickel release from stainless steel was 30-times lower than from nickel powder during 24 h incubation. It seems, however, that there is some variation in the comparative nickel release between SS 316L and Ni-Ti alloy, since in the study of Ryhänen et al. (Ryhänen, Niemi et al. 1997), the release of nickel from SS 316L and Ni-Ti alloy in two different cell culture media was rather similar; Ni-Ti alloy releasing slightly higher amounts of nickel than stainless steel.

Nickel has been the cause of concern for its mutagenicity. A recent EU risk assessment report proposed to classify the soluble nickel compounds nickel sulphate, nickel chloride, nickel nitrate, and nickel carbonate to mutagenicity Category 3 (R68, according to Dangerous Substances Directive 67/548/EEC) corresponding the CLP Regulation (1272/2008) mutagen Category 2. Only very few data are available on insoluble nickel compounds or metallic nickel, which precludes the classification of these substances. However, it seems that nickel compounds cause mainly chromosomal type damage, since even readily soluble nickel compounds have been negative in bacterial assays with S. typhimurium and E. coli. In addition, although positive results for gene mutations have been obtained in mammalian cell lines, they have often been only weak positives (EU, 2008). *In vitro* chromosomal aberration tests with soluble nickel compounds have been positive. There is only one study available on nickel metal, showing negative results ((Paton and Allison 1972). Cell transformation studies with different cell lines have given positive results for soluble nickel compounds and both positive and negative results for nickel powder (Costa, Abbracchio et al. 1981; Costa, Simmons-Hansen et al. 1981; Doran, Law et al. 1998).

3.7.2 *IN VIVO* MUTAGENICITY

No *in vivo* animal genotoxicity data is available on stainless steel.

Aneuploidy (studied by centromeric fluorescence in situ hybridization of chromosomes 1 and 2) and chromosomal translocations (by painting of chromosomes 1, 2, and 3) in cultured peripheral lymphocytes were evaluated in British patients (n=31) having revision arthroplasty of the hip, predominantly metal-on-plastic total hip replacement and compared to the patients with primary arthroplasty (n=30) (Doherty, Howell et al. 2001). Patients with cobalt-chrome prostheses (n=11) had a 2.5-fold increase in aneuploidy and a 3.5-fold increase in chromosomal translocations; six patients with stainless steel prostheses did not differ from the primary arthroplasty controls. Smoking status and age and gender were taken into account in the statistical analyses.

Ten patients, who had stainless steel fracture fixation devices, were compared with 15 matched control subjects with no implants, and the sister chromatid exchanges (SCEs) in cultured peripheral lymphocytes were evaluated (Savarino, Stea et al. 2000). A statistically significant increase in the mean number of SCEs per cell was seen in patients with implants (3.5 versus 4.9). In addition, the mean number of cells with a high frequency of SCEs was statistically significantly higher in the patients (9.8) than in controls (3.2). Chromium concentration in the serum was, on average, 1.01±0.77 ng/mL in the patients, as compared with 0.19±0.27 ng/mL in the controls, and the respective values for nickel were 1.71±1.49 and 0.72±0.52 ng/mL. Cr level in serum correlated significantly with the number of cells with a high frequency of SCEs, but not with individual mean number of SCEs/cell, while serum nickel had an inverse correlation with individual mean number of SCEs/cell.

Faccioni et al. (Faccioni, Franceschetti et al. 2003) and Westphalen et al. (Westphalen, Menezes et al. 2008) have evaluated the ability of stainless steel fixed orthodontic appliances to induce DNA damage in oral mucosa. First of these is the study of Faccioni et al. (2003) in which DNA damage in oral mucosa of 55 patients having worn fixed orthodontic appliances for 2-4 years was compared to 33 controls. The study groups were matched for smoking, drinking, age etc. However, whereas brackets and bands of these patients were made of stainless steel (316 and 304, respectively), archwires were Ni-Ti, chromium-cobalt-nickel alloy, or stainless steel. DNA damage was evaluated by comet assay and cell viability by the trypan blue exclusion method. Nickel and cobalt levels in buccal cells were 3.4 and 2.8-fold higher in patients than in controls. An increase in the number of comets and a decrease in cell viability were seen when compared to the controls. These changes correlated with increased metal levels. The effects seen in this study cannot, however, be attributed to stainless steel. In the study of Westphalen et al. (Westphalen, Menezes et al. 2008), in which 20 healthy patients were followed for the induction of DNA damage after placement of stainless steel (15.5–17.5% Cr, 3–5% Ni and Cu, 1% Mn and Si, 0.15–0.45% niobium +tantalum) orthodontic appliances, no increase in the number of comets was seen 10 days after the placement, when compared to the situation before the placement. However, an

increase in the number of micronuclei in buccal cells was seen 30 days after the treatment (5 out of 20 patients had 1–3 micronuclei/1000 cells). Analysis of micronuclei in epithelial cells may be prone to bias since only a small proportion of cells in epithelial smears divide and the induction of micronuclei requires cell division. No conclusions on the genotoxicity of stainless steel can be made based on these studies.

Thus, *in vivo* data on the genotoxicity of stainless steel is inconclusive. Soluble nickel compounds have shown positive responses in *in vivo* genotoxicity tests (EC 2008b; EC 2008a) but very few data is available on insoluble nickel compounds or metallic nickel. A study by Zhong et al. (Zhong, Li et al. 1990) on micronucleus, CA and SCE induction after single intratracheal administration of metallic nickel suggests that it is genotoxic *in vivo*, but the conclusion is questionable since no information on statistical significance of the changes is given.

SUMMARY AND CONCLUSIONS ON MUTAGENICITY

Researchers have tested the mutagenicity of the stainless steel particles in guideline-based bacterial reverse mutation tests. These tests have not shown mutagenic effects. In addition, extracts of stainless steel have not caused any mutagenicity in bacterial tests. There are studies on the ability of stainless steel to induce chromosomal aberrations and cell transformation in mammalian cells. Studies on cell transformation have shown clear differences between stainless steel and soluble nickel compounds. Stainless steel powder has been negative in these studies, but soluble nickel compounds have caused clear positive responses. There are also some data suggesting that metallic nickel powder may cause cell transformation in vitro. A mechanistic link from cell transformation to cancer is unknown, although it has been shown to correlate with the carcinogenic ability of some substances.

The results of chromosomal aberration tests in vitro also suggest a lack of clear genotoxic effects. In one study with stainless steel particles, researchers detected no induction of chromosomal aberrations (CTL 2006b), whereas in another study (Landolph 2001) the results were inconclusive. In addition, studies with stainless steel extracts have not shown an induction of chromosomal aberrations, sister chromatid exchanges or micronuclei. In vitro chromosomal aberration tests with soluble nickel compounds (classified as Cat 2 mutagens within the EU according to the CLP system) have been mainly positive, whereas bacterial tests have been negative. Not

much data are available on insoluble nickel or nickel metal, which has precluded the classification of these substances.

There are no in vivo data on the mutagenicity of stainless steel. The few human studies which have been done on the mutagenic effects of stainless steel prostheses or orthodontic appliances do not allow for any firm conclusions. In in vitro metal release studies, researchers have shown that nickel is released at substantially lower levels from stainless steel than from nickel metal, suggesting substantially lower bioaccessibility and bioavailability. When summarising the negative findings from in vitro genotoxicity studies with stainless steel, the significantly lower bioaccessibility of nickel from stainless steel than from nickel metal, and the lack of clear mutagenicity of the other dissolved components (iron, chromium) of stainless steel, the conclusion is that stainless steel is very unlikely to be genotoxic.

3.8 CARCINOGENICITY

3.8.1 ANIMAL DATA

Stanton and Wrench (Stanton and Wrench 1972) implanted intrapleurally 40 mg ball-mill steel fragments, pulverized (nickel-chrome steel, not further specified, 10–50 µm in diameter) or nickel metal 1 mg (< 3 µm particles) in female rats (n=80 and 34/group, respectively) and followed animals for 25 months. A control group of 90 animals with gelatin pledget implantation and asbestos was used. Steel particles produced no mesotheliomas at a dose equal to that of the highest level of asbestos. A similar 40 mg dose of pure nickel was extremely toxic; all rats died with haemorrhagic pneumonitis within 60 days, and therefore the dose of 1 mg was used, and this dosage caused no mesotheliomas either. However, only 25 out of 80 steel treated rats and 18 out of 34 nickel 1 mg treated rats survived for the whole observation period of 2 years.

A stainless steel pellet (316L, personal communication; Newarkwire 1 November 2006) was placed via a tracheotomy in the left bronchus of 139 male and female Porton-Wistar rats (6-8 week old) and left there for about 2 years (Levy and Venitt 1986). This group served as a control group for the other animals, which were treated with pellets loaded with 2 mg of different trivalent and hexavalent chromium compounds. The rats were killed and the lungs were removed, distended and fixed with Bouin's fluid at the end of the study. Histopathological changes of the bronchial epithelium (inflammation, hyperplasia, dysplasia and carcinoma in situ) were studied. No local or lung tumours were found in animals treated only with stainless steel pellets (Levy and Venitt 1986).

Incidence of squamous metaplasia was 8.6%, which was similar to the incidence observed with trivalent chromium (chromium(III) oxide, chromite ore, chromium(III) chloride hexahydrate) whereas the incidence of metaplasia was significantly higher in groups exposed to Cr[VI]-materials, and in rats exposed to the reference carcinogen 3-methylcholanthrene. Cr[VI] compounds with sparing aqueous solubility also developed bronchial squamous carcinoma at statistically significant levels. Similarly, the animals treated with cholesterol filled stainless steel pellets did not show any dysplasia, carcinoma in situ or carcinomas of the lung or malignant lymphomas. Only a single pheochromocytoma was observed. In histopathology, however, bronchial inflammation as a tissue response to implanted pellets was a frequent finding. Squamous metaplasia was found in 7 out of 100 cases (rats). No controls without any treatment were included in these studies.

IARC (IARC 1999) summarized the experimental studies done to evaluate the ability of stainless steel implants to induce local cancers at the place of implantation. The summary of the studies is in Table 9.

Table 9. Studies of stainless steel implants and the induction of local tumours in animals (adapted from IARC 1999).

Stainless steel composition (grade)	Route of administration/size/dose	Species	Duration of exposure	Local tumour outcome, No of tumour bearing animals (%)	References
Fe59Cr14Ni7C4Al2Mn1 Fe68Cr13C3Al2	Intratracheal administration of dust (size 3-5 µm) 12 x 3 mg 12 x 9 mg 12 x 9 mg	Hamster	26-30 months	/63 (0%) 0/62 (0%) 0/56 (0 %)	
Stainless steel composition/grade unspecified	Implantation of intramuscular discs (diameter 18, 12, 4 mm, thickness 1.5 mm)	Guinea-pig Rat	>30 months	0/47 (0%) 6/59 (10%)	
Fe65Cr17Ni14Mo2 (316L)	Implantation of intramuscular rod (length 8 , diameter 1.6 mm)	Rat	2 years	0/40 (0%)	
Fe65Cr17Ni14 (316 L) Fe65Cr16Ni13 (316L)	Implantation of intraosseus rod (4 mm length, diameter 1.6 mm) or powder (< 28 and 28-44 µm), 40 mg	Rat	30 months	0/26 (0%) 0/52 (0%)	
Fe70Cr15Ni12	Intrabronchial implantation of stainless steel 28 gauge surgical wire (10 mm long)	Rat	24 months	0/32 (0%)	

Table 9 indicates that almost all of the studies have not shown induction of tumours.

GHS classification and labelling criteria for mixtures state that any mixture containing a Cat 2 carcinogen substance should also be classified to carcinogen Category 2 if the limit of >1% of component is exceeded. In compliance with this, stainless steel, containing more that 1% of nickel (currently classified as CLP Cat 2 carcinogen in EU) should also be classified as a carcinogenic substance. Classification of nickel metal as carcinogen is based on the studies involving local injection of nickel metal at various sites or for instance intratracheal instillation of nickel and the production of local tumours (EC 2008a).

There is, however, a new, standard two year inhalation carcinogenicity bioassay on nickel powder (Oller, Kirkpatrick et al. 2008). The study was performed according to the OECD 451 test guideline and GLP. Wistar rats (n=50 animals/sex) were exposed by whole body inhalation 6 hours/day, 5 days/week, for up to 104 consecutive weeks to target exposure levels of 0.1, 0.4 and 1.0 mg/m^3 of respirable metallic nickel powder (MMAD 1.7–1.8 microns). The highest dose was clearly toxic and resulted in the early termination of the exposure. The other results did not show increased incidence of respiratory tract neoplasms, but the exposure resulted in lung toxicity including proteinosis, alveolar histiocytosis, chronic inflammation and bronchio-alveolar hyperplasia. Only tumours with increased incidence were adrenal pheochromocytomas in males, and combined adrenal adenomas/carcinomas in females. These were, however, considered secondary to lung toxicity and hypoxia resulting in increased catecholamine release.

3.8.2 HUMAN DATA

The human data available related to stainless steel and cancer comes only from the production of stainless steel or from the handling like welding, grinding and polishing of products. All these techniques are associated with exposures to several different dusts and fumes formed in the process. This fact makes it practically impossible to draw any conclusions on the cancer risk of metallic stainless steel or stainless steel powders from these observations. Some of these studies with focus on production and grinding or polishing of stainless steel, are summarized here. Because of the special, complex characteristics of welding, studies on welding are excluded.

Grinding and polishing of stainless steel may involve exposure to the dusts of stainless steel, the dusts derived from the grinding equipment or the mists of polishing oils and wax. Svensson et al. (Svensson, Englander et al. 1989) examined a cohort of 1,164 male workers in an industry that produced stainless steel articles. Measurements of the total dust in the workroom air showed the presence of chromium (on average 10% of the dust) and nickel (on average 5%) during grinding and polishing. They (Svensson, Englander et al. 1989) found an increased morbidity from colon-rectum cancer (observed cases=11, SMR = 283, CI 1.4 7–5.19, in the period 1958–1983), with at least

5 years exposure and allowing for a 20-year latency period. Total cancer or lung cancer morbidity did not increase. Whether the cause of the cancers was the grinding material, grinding agents, stainless steel, or some other factors is not possible to differentiate.

Hansen et al. (Hansen, Lauritsen et al. 1996) studied the cancer incidence among stainless steel workers in a small subcohort of stainless steel grinders (n=521). Only non-significant increases in the incidences of total cancer, respiratory tract cancer, and male genital organ cancer were seen. The small size of the subcohort precludes any reliable conclusions.

Jakobsson et al. (Jakobsson, Mikoczy et al. 1997) performed a retrospective cohort study of cancer morbidity and mortality among the workers grinding stainless steel in the manufacture of sinks and saucepans. Workers (n=727) employed for at least 1 year were involved and a 15 years observation period was included. Control cohorts were other industrial workers and fishermen. The results showed lower overall mortality, cancer mortality, and cancer morbidity among the stainless steel workers when compared to control cohorts. Also respiratory tract cancer morbidity was lower among the exposed group. Statistically insignificant excess of colon tumours (sigmoidal part of the colon) was found. It was related to longer employment time (1–14 years: four observed cases vs. 2.3 expected, SIR 1.7, 95% confidence interval (95% CI) 0.4 to 4.5; >15 years: three observed cases vs. 0.7 expected, SIR 4.3, 95% CI 0.9 to 13). This was considered causally related to exposures in stainless steel grinding. The limited size of the study precludes making any firm conclusions on the results.

Moulin et al. (Moulin, Portefaix et al. 1990) studied French workers who produced stainless steel and metallic alloys. The lung cancer mortality was increased, but the causal link was stronger to polycyclic aromatic hydrocarbons in ferrochromium production than to exposures in stainless steel manufacturing. Another study of Moulin et al. (Moulin, Wild et al. 1993) was aimed at assessing the potential risk of lung cancer from occupational exposures when producing stainless steel. No significant excess of lung cancer was seen in the melting and casting of stainless steel, but a significant excess (SMR = 334, CI 119–705) was observed among workers with more than 30 years of employment in the foundry area. The process, however, involved exposure to several carcinogens, like crystalline silica, asbestos and polycyclic aromatic hydrocarbons as well as nickel and chromium. No conclusions on the carcinogenicity of stainless steel can be made based on these results.

In an update of these French studies of Moulin et al. (Moulin, Clavel et al. 2000), the risk of lung cancer due to exposure to metals in stainless steel manufacturing was investigated in a cohort follow-up of 4900 workers from 1968 to 1992. Occupational exposure was assessed through the complete job histories and a special job-exposure matrix. The manufacture of ferroalloys and stainless steel generates a complex mixture

of particles, fumes, and chemicals, among which iron, nickel, trivalent and hexavalent chromium are present. The cohort study did not show any trend for lung cancer mortality among the workers. In a case-control study nested in the cohort, no excess mortality from lung cancer was observed. The analysis was restricted to workers whose smoking habits were known and adjusted for smoking and other known potential confounders. The study failed to demonstrate any relationship between lung cancer and exposure to metals in stainless and alloyed steel manufacturing.

Some other studies, including (Park and Shearer 1983) and (Ahn, Park et al. 2006), are also available on steel manufacturing, including stainless steel manufacturing, but because of exposure to multiple agents, no conclusions on the role stainless steel can be made.

Carcinogenicity of different implants, including those made from stainless steel has been evaluated by IARC (IARC 1999). Based on the data of the time, IARC concluded that there is inadequate evidence in humans for the carcinogenicity of metallic implants and metallic foreign bodies. IARC also concluded that there is inadequate evidence in experimental animals for the carcinogenicity of implants of chromium metal, stainless steel, titanium metal, titanium-based alloys and depleted uranium. Therefore, according to IARC, implanted foreign bodies of metallic chromium or titanium and cobalt-based, chromium-based and titanium-based alloys, stainless steel and depleted uranium are not classifiable as to their carcinogenicity to humans (Group 3). The data available on the carcinogenicity of stainless steel implants included mainly case reports on the local cancers near the implantation site. Several analytical (mostly cohort) studies are also available on the risk of cancer after orthopaedic implantation. IARC summarised those, which were published before 1999. None of these separated stainless steel prostheses from those made of other materials, and so these studies cannot be used for the assessment of the carcinogenicity of stainless steel. Overall, however, these studies had not found increased risk of cancer.

SUMMARY AND CONCLUSIONS ON CARCINOGENICITY

Animal studies available on the carcinogenicity of stainless steel include studies evaluating the ability of different stainless steel implants to induce local cancers at the place of implantation. The weight of evidence from these studies supports non-carcinogenicity of stainless steel. Human data on occupational exposure involves multiple exposures and the effect of stainless steel cannot usually be differentiated. However, the studies from stainless steel grinding, polishing and manufacturing have not raised any major concerns on the potential carcinogenicity of stainless steel. From the use of stainless steel implants there are few case reports on local tumours

near the site of implantations. However, analytical studies on the cancer of different implants have not shown evidence on increased cancer risk. IARC has concluded that stainless steel implants are not classifiable as to their carcinogenicity to humans (Group 3). Metallic nickel has shown local cancers in studies involving local injection/instillation of nickel metal at various sites. A recent inhalation carcinogenicity study did not show any increased risk of lung tumours with metallic nickel. The only tumour type which was increased was adrenal tumours, including pheochromocytomas. Their biological significance and relationship to the systemically available nickel ion is questionable (Oller, Kirkpatrick et al. 2008).

However, since nickel metal is currently considered as a suspected carcinogen, carcinogenicity of stainless steel in relation to nickel metal should be considered. According to in vitro dissolution tests in synthetic body fluids, the release of nickel from stainless steel is substantially lower than the release of nickel from nickel metal. In one study, the release of nickel from stainless steel was >1000-fold lower than from nickel metal whereas the release of chromium from stainless steel was similar to the release of chromium from pure chromium metal. This means that stainless steel (316L, containing ≈ 11% of nickel) behaves as a mixture of chromium with less than 0.1% of nickel. This suggests that nickel plays a significantly lower role in the toxicity of stainless steel than could be predicted on the basis of its bulk concentration. This is actually supported by repeated dose toxicity data showing 1000-fold lower inhalation toxicity of stainless steel when compared to metallic nickel powder (see section repeated dose toxicity). Based on this, it can be assumed that also in the carcinogenicity of stainless steel, nickel plays a significantly lower role than could be predicted on the basis of its bulk concentration. The lack of carcinogenic potency of stainless steel is also demonstrated by negative animal implantation studies and supported by the lack of epidemiological evidence on the tumours in patients with different stainless steel prostheses. The other main components of stainless steel (metallic chromium, iron) have not shown any carcinogenic potency, either.

3.9 REPRODUCTIVE TOXICITY

There are no standard reproductive or developmental toxicity studies available on stainless steel. The sole available data comes from a 28-days inhalation exposure study (nose only), which SafePharm Laboratories (SafePharm Laboratories 2008) carried out with rats using stainless steel powder (grade 316L). The study complied with the requirements described in EU and OECD412 guidelines. Four groups, each containing five female and five male Wistar rats were exposed to stainless steel powder at concentrations of 0.01, 0.10, 0.30, and 1.00 mg/L by inhalation. The particle size (MMAD) was between 2.50 µm (0.10 mg/L group) and 3.04 µm (1.00 mg/L group). A control group was exposed to air only. The exposures were carried out for 6 hours/day, 5 days/week for a period of 28 days. All animals were sacrificed at day 29. No effects on absolute or relative testicular, epididymal or ovarian weights were seen. In standard histopathology, no accumulation of inflammatory cells in prostate was seen. In female reproductive organs some minimal dilation of the horns of the uterus was seen in animals exposed to 1 mg/L of stainless steel. The clinical relevance of this finding and its relationship to stainless steel exposure is unclear. Histopathology of these organs was performed only in animals treated at the highest dose level. No other data on the effects on reproductive organs are available.

Metallic nickel has not been studied for the reproductive and developmental toxic effects. Soluble nickel species like nickel chloride and sulphate have not caused fertility effects at doses up to 50 mg Ni/kg bw/day, although in some limited studies effects on male sex organs in rodents have been reported after oral, inhalation or subcutaneous administration (EC 2008a). However, soluble nickel species have shown consistent evidence of developmental toxicity in rats at dose levels not causing maternal toxicity. The EU Risk Assessment Report (EC 2008a) consequently proposes that the soluble and sparingly soluble nickel compounds (nickel sulphate, nickel chloride, nickel nitrate and nickel carbonate) should be classified for developmental toxicity in Category 2 according to DSD (R61, meaning Repr. Cat 1B in GHS-CLP). Regarding metallic nickel, it was concluded: ‘The developmental toxicity of nickel compounds is related to the systemic available nickel and therefore the effect should be considered as relevant for metallic nickel as well. However, the potential release and absorption of nickel from metallic nickel is substantially lower than for the soluble compounds via all routes, and the TC C&L have agreed that metallic nickel should not be classified for this effect.’(EC 2008a).

SUMMARY AND CONCLUSIONS ON REPRODUCTIVE TOXICITY

There are no data on the reproductive or developmental toxicity of stainless steel. In a 28 days repeated dose inhalation toxicity study, no changes in reproductive organ (testis, epididymis, ovarian)

weights were seen. Furthermore, no biologically meaningful histopathological alterations were seen in ovaries or prostate of the exposed animals. These limited data cannot, however, provide any evidence on the presence or absence of effects on reproductive function. Of the main constituents of stainless steel, iron has not caused any concerns for reproductive toxicity. Reproductive toxicity of metallic chromium and trivalent chromium has been recently evaluated by ICDA (2006) and WHO (2009) resulting in a conclusion of the lack of these effects in insoluble chromium(III) oxide. This same conclusion applies to metallic chromium (ICDA, 2006; WHO, 2009). Soluble nickel compounds have shown evidence of the developmental toxic effects, and nickel sulphate, nickel chloride, nickel nitrate and nickel carbonate have been classified for developmental toxicity in Category 1B according to CLP in EU. In the EU Risk assessment report on nickel it was concluded that since the developmental toxicity of nickel compounds is related to the systemically available nickel, this effect should be considered as relevant for metallic nickel as well, but since the potential release and absorption of nickel from metallic nickel is substantially lower than for the soluble compounds metallic nickel should not be classified for this effect (EU, 2008). In vitro studies on the nickel release from stainless steel show that nickel is released from stainless steel at substantially lower levels than from nickel metal in different synthetic biological fluids. This supports substantially lower bioaccessibility and bioavailability of nickel from stainless steel than from nickel metal powder. Thus, these developmental toxic effects are not considered relevant for stainless steel.

4. DISCUSSION ON HUMAN HEALTH HAZARDS AND PROPOSAL FOR CLASSIFICATION

4.1 DISCUSSION ON HUMAN HEALTH HAZARDS

For all the countless applications of stainless steel over many decades, harmful toxic effects have not been reported. The only reported health hazard has been sensitization from the nickel released from some stainless steel grades after close and prolonged contact. In some cases, however, it is even questionable if the material causing the sensitization has even been proper stainless steel. The inertness of such things as stainless steel jewellery, tableware, cooking utensils, orthopaedic and orthodontic appliances is well known. For instance, the release of nickel and chromium from

cookware is insignificant when compared to the normal dietary intake of these elements. Chromium is an acknowledged essential dietary element. It has not been found to have any harmful effects on set dietary uptake levels. The essentiality of nickel is questionable, but its uptake limit significantly exceeds the daily dietary intakes. In addition, the alloying properties of stainless steels make its bioavailability very low. The chromium oxide in the covering passivation layer similarly makes the dissolution of other alloying materials like iron, molybdenum, and manganese very low.

The toxicological effects of stainless steels are determined by the release of ions from its constituent metals. This is very well demonstrated in the case of skin sensitization caused by nickel, for which the limit set by the EU directive for nickel release has significantly reduced the risk of skin sensitization. Based on the studies reviewed in this paper, the limit of 0.5 µg Ni cm^2/week (0.2 µg/cm^2/week, if used in piercing post assemblies) can be considered reasonable.

Metal ion release tests of various grades of stainless steel show that, in most cases, the nickel release is clearly below 0.5 µg/cm^2/week. Only some high sulphur containing stainless steel grades have shown nickel releases above this limit. For these, a potential risk of skin sensitization exists and, therefore, they should not be used in applications including close or prolonged skin contact.

In the case of other toxicological effects, there are no rules for how the release of metals should be taken into account when assessing the hazards and risks of stainless steel. GHS considers alloys to be mixtures. The European Chemicals Regulation (REACH) states, in its annex 1, that *'When assessing the risk of the use of one or more substances incorporated into a special preparation (for instance alloys), the way the constituent substances are bonded in the chemical matrix shall be taken into account.'* However, there is currently no official guideline for this. The EIMAG (European Industry Metallic Alloys Group) has drafted a document which provides a guideline for this, but it has not been accepted yet (EIMAG, 2009). *In vitro* metal release studies with different artificial body fluids have shown that the release of metals from stainless steel may be significantly lower when compared to the release of metals from pure metals. In a study by Herting et al (2008), the release of nickel from nickel metal in artificial lysosomal fluid was 1.98 mg/cm^2/week, whereas from stainless steel it was ≈ 0.0004 mg/cm^2/week during a one-week incubation period. Thus, in this study, the nickel release from pure nickel metal was approximately 5000-fold higher than the nickel release from stainless steel. The release of chromium from stainless steel was, however, similar to the release of chromium from pure chromium metal. This means that 316 grade stainless steel behaved as a mixture of chromium with an effective concentration of only 1⁄5000 (=0.02%) nickel. In some other studies with a shorter incubation time (24–48 h), thirty-fold and a hundred-fold differences in nickel release between nickel metal and stainless steel have been seen. The same trend has been

observed in the case of iron release from stainless steel versus pure iron metal. This phenomenon can be explained by the protective surface oxide film of stainless steel, which is composed mostly of chromium(III) oxide. Thus, based on these data, we can predict that the impact of nickel ion on the toxicity of stainless steel is significantly lower than what could be expected on the basis of the bulk composition. This can also be seen in some toxicological studies performed with stainless steel.

The cytotoxicity of stainless steel particles has been compared with nickel and various nickel compounds. Stainless steel has been significantly less cytotoxic than could be expected from the release of nickel ions and their bioavailability. However, the cytotoxicity tests have not yet been properly validated to serve as a surrogate for, for example, acute toxicity testing. Therefore, based on these studies, no definitive conclusions on the *in vivo* toxicity of stainless steel can be drawn. Various products have been in frequent and continuous contact with many parts of the human body for decades without significant signs of irritation. Thus, irritation is a condition unlikely to be caused by stainless steel.

Stainless steel has been tested in animals for its repeated-dose toxicity by inhalation. The results clearly indicate a lack of toxicity, even after exposure to high doses. Nickel, in contrast, has clear toxic effects, even at low concentrations. These results clearly indicate that, in the case of stainless steel, the release of ions from constituent metals rather than the elemental nominal composition is crucial for the toxicity caused by repeated exposure.

In bacterial mutagenicity tests stainless steel has been negative. On the other hand, not even nickel has caused positive responses in bacterial tests. Studies on cell transformation have shown differences between stainless steel and soluble nickel compounds. When stainless steel powder has been negative in these studies, soluble nickel compounds have caused positive responses. Some positive responses have also been obtained with nickel metal. *In vitro* chromosomal aberration tests with soluble nickel compounds have shown positive responses, whereas only a limited amount of data is available on insoluble nickel or nickel metal. Soluble nickel compounds have been classified at mutagenicity cat 2 within the EU according to the GHS-CLP system, whereas nickel metal has not been classified. There are no relevant *in vivo* data on the mutagenicity of stainless steel, but the negative data from *in vitro* mutagenicity studies and the lack of clear mutagenicity of the main metallic components of stainless steel support the conclusion that stainless steel is not genotoxic.

No standard carcinogenicity bioassay has been performed with stainless steel. Regarding the metallic components of stainless steel, nickel metal is currently classified as a CLP cat 2 carcinogen within the EU based on old reports on local cancers in studies involving the local injection/instillation of nickel at various sites. However, *in vitro* studies on the release of nickel from stainless steel and a recent *in vivo* repeated-dose

inhalation toxicity study suggest that nickel plays a significantly lower role in the toxicity of stainless steel than can be predicted on the basis of its bulk concentration. This conclusion is strongly supported by several negative animal studies evaluating the ability of different stainless steel implants to induce local cancers at the place of implantation. The negative stainless steel genotoxicity data and available human data on the handling like grinding and polishing of stainless steel, as well as on the use of implants do not raise concerns about the carcinogenicity of stainless steel. Thus, the weight of evidence supports the non-carcinogenicity of stainless steel regardless of the possible carcinogenicity of nickel. The IARC has concluded that stainless steel implants are not classifiable as to their carcinogenicity to humans (Group 3).

There are no data on the reproductive or developmental toxicity of stainless steel. None of the main metallic components of stainless steel is considered to be a reproductive toxicant. Soluble nickel sulphate, nickel chloride, nickel nitrate and nickel carbonate have been classified at Category 1B for developmental toxicity within the EU according to the CLP system. The EU Risk Assessment Report considered this effect to be relevant for metallic nickel as well, but, since the release and absorption of nickel from metallic nickel is substantially lower than from soluble nickel compounds, it was concluded that metallic nickel should not to be classified for this effect. Since stainless steel releases substantially lower levels of nickel than metallic nickel, these developmental toxicity effects should not be considered relevant for stainless steel

4.2 PROPOSALS FOR CLASSIFICATION ACCORDING TO GHS

ACUTE TOXICITY

No classification is needed for acute toxicity. The acute oral toxicities of nickel and chromium(III) oxide, which forms the stainless passivation layer on chromium and stainless steel surfaces, are very low. The acute inhalation toxicities of nickel and chromium oxide are also low. No acute dermal stainless steel toxicity has been reported.

IRRITATION

No classification is needed. Stainless steel objects have been in frequent and continuous contact with human skin and eyes. No confirmed cases of irritation have been reported. The absence of irritation can be expected from the presence of very inert passivation layer, which oxidised chromium (chromium(III) oxide) generates in concentrations required for stainlessness.

SENSITIZATION

Nickel is a known sensitizing substance. The mixture classification, according to the GHS, requires sensitization classification if the proportion of the sensitizing agent in the mixture is at 1 wt% or greater.

According to the Classification, Labelling and Packaging Regulation (CLP) system for the EU, nickel containing alloys do not have to be classified as skin sensitizers if the nickel release in a standard *in vitro* test is <0.5 µg/cm^2/week. For metals used in piercing post assemblies, the limit is 0.2 µg/cm^2/week. This limit has been shown to decrease the prevalence of nickel allergy markedly.

Release studies of various stainless steels reviewed in this paper generally show release rates clearly below <0.5 µg/cm^2/week. Based on the positive experiences of applying the release limits within EU, and on the basis of the studies referenced in this review, *no sensitization classification is suggested for stainless steels with a nickel release <0.5 µg/cm^2/week in artificial sweat* (and <0.2 µg/cm^2/week if used in piercing post assemblies).

REPEATED DOSE TOXICITY

Within the EU, nickel metal is classified for repeated dose toxicity (STOT RE 1, H372). For repeated-dose toxicity via inhalation, mixtures containing >10 wt% of a classifiable substance (GHS) should be classified as STOT RE 1, and those containing 1–10 wt% as STOT RE 2. This means that stainless steels with >1% nickel have to be classified for repeated-dose toxicity according to the GHS system.

Based on the lack of toxicity in a GLP repeated-dose toxicity study with stainless steel, no classification for repeated-dose toxicity is suggested. This is supported by *in vitro* release studies showing that the release of nickel from stainless steel 316L is substantially lower (<0.1%) than the nickel release from pure nickel metal. Therefore, stainless steel containing 11% nickel behaves as a mixture of chromium and iron with <0.1% Ni.

MUTAGENICITY

In *in vitro* genotoxicity studies stainless steel has been negative. There are no relevant *in vivo* data on the mutagenicity of stainless steel but the negative data from *in vitro* mutagenicity studies and the lack of clear mutagenicity of the main metallic components of stainless steel support the conclusion that stainless steel is not genotoxic. Regarding nickel, only soluble nickel compounds have been classified at mutagenicity cat 2 within EU according to the CLP system, whereas nickel metal has not been classified. Although the current data does not warrant a mutagen classification for nickel metal, even if nickel was classified as a cat 2 mutagen, the substantially lower

release of nickel from stainless steel compared to nickel metal supports the non-classification of stainless steel.

CARCINOGENICITY

Nickel metal is currently classified as a CLP cat 2 carcinogen within EU based on the old reports on local cancers in studies with injection/instillation of nickel at various sites. According to the classification criteria for mixtures, any mixtures containing >1% of category 2 carcinogens should be classified as such. However, *in vitro* studies on the release of nickel from stainless steel and a recent *in vivo* repeated-dose inhalation toxicity study show that nickel plays a significantly lower role in the toxicity of stainless steel than can be predicted on the basis of its bulk concentration. The *in vitro* genotoxicity of stainless steel has been negative. The evidence regarding the non-carcinogenicity of stainless steel comes from the animal studies evaluating the ability of different stainless steel implants to induce local cancers at the place of implantation. Available human data on, for example, the grinding and polishing of stainless steel, and on the use of stainless steel implants do not raise concerns about the carcinogenicity of stainless steel. Therefore, it can be concluded that the weight of evidence supports the non-carcinogenicity of stainless steel. No classification of stainless steel for carcinogenicity is proposed.

REPRODUCTIVE TOXICITY

There is no data on the reproductive or developmental toxicity of stainless steel. None of the main metallic components of stainless steel has shown reproductive toxic properties. Soluble nickel sulphate, nickel chloride, nickel nitrate and nickel carbonate have been classified in Category 1B for developmental toxicity in EU according to CLP. In the EU Risk assessment report this effect is considered as relevant for metallic nickel as well, but since the release and absorption of nickel from metallic nickel is substantially lower than from soluble nickel compounds it was concluded that metallic nickel should not to be classified for this effect. Since stainless steel releases substantially lower levels of nickel than metallic nickel, these developmental toxic effects should not be considered relevant for stainless steel. No classification for reproductive toxicity is proposed. Taking the data on dissolution of metals from stainless steel into account, testing of stainless steel for these properties is considered irrelevant and inadvisable.

4.3 DATA GAPS IDENTIFIED

Most studies on the dissolution of the constituent elements or biological properties of stainless steels have been done with AISI 316. How well these studies represent other stainless steels can, of course, be debated. Nickel has been seen as the potential for possibly causing harm to stainless steels, and, with the exception of resulphurised

grades, its bioavailability seems to be quite similar among different grades. Even in these cases, the only relevant harmful effect seems to be sensitization. It does not seem likely that other toxicities arise from other stainless steels. The number of different grades is, however, huge. *In vitro* release data would help us to judge whether the bioaccessibility of constituent metals from less common grades of stainless steel differs substantially from AISI 316, which has mostly been used to study the biological effects of stainless steel. According to currently available data, the differences in release rates of metal constituents between different grades of stainless steel are, however, very small.

The sensitization hazard can probably be detected quite easily via the approved nickel dissolution test, EN 1811.

The greatest health hazards related to stainless steel have been and will continue to be the fumes caused by welding work, and there is still scientific work to be done for the assessment and management of these risks.

5. CONCLUSIONS AND FURTHER STUDY NEEDS

In conclusion, metallic stainless steel is likely to exert very low toxicity. Based on GHS-CLP classification and labelling criteria for mixtures, many stainless steels should be classified as specific target organ toxicants and/or category 2 carcinogens because of their nickel content. However, available stainless-steel-specific data provide enough evidence to show that this kind of classification is misleading.

In vitro release tests show that the nickel release from stainless steel in artificial lung fluids is substantially lower than from nickel particles due to chromium(III)oxide enrichment at the surface. The existence of low inhalation toxicity, compared to nickel powder, is supported by a recent 28-day stainless steel inhalation toxicity study. Therefore, no classification for target organ toxicity in repeated exposure to stainless steel is proposed. In addition, based on the low dissolution of nickel from stainless steel and that the available stainless-steel-specific data raised no concerns for carcinogenicity, no classification for carcinogenicity is proposed.

Although some grades of stainless steel show a somewhat higher release of nickel than grade AISI 316L (which is the grade mostly used in toxicity tests), the differences between grades are low when compared to the differences seen in the release of nickel from pure nickel and stainless steel. Thus, these conclusions can be regarded as applying to all common grades of stainless steel, including grade 303, with the highest nickel release.

Certain stainless steels with a sulphur addition (for example, AISI 303) may release nickel in artificial sweat at more than 0.5 µg/cm^2/week. Although the actual threshold for the induction of nickel allergy is unknown, it has been experienced in Europe that

this limit (set within the EU for nickel-containing alloys in direct or prolonged contact with skin) and the use of standard *in vitro* release tests has significantly decreased the number of cases of nickel-related skin allergies. In the case of sulphurated stainless steels like AISI 303, the risk of skin sensitization after prolonged skin contact is higher. Therefore, these grades should be considered potentially sensitizing in situations of continuous skin contact. Nowadays, within Europe, using these grades of steel is not recommended for applications involving continuous contact with the skin. In the case of uses like nuts and bolts, bushings, shafts, aircraft fittings, electrical switchgear components, gears, valve bodies and valve trim, no cases of skin sensitization have been described. This can be explained by the limited exposure time.

The data presented in this review clearly shows that the toxicity of stainless steel cannot be predicted solely on the basis of the bulk concentration of elemental constituents, but that the release of the constituents plays an essential role in the toxicity of stainless steel. This has to be taken into account in the hazard assessment and classification of stainless steel as indicated above.

However, the applicability of a similar approach to other alloys must be considered separately by evaluating the specific properties of the alloy. This demands further studies and validation of release tests for different kinds of alloys. The general applicability of this approach is currently being considered under the HERAG program, with the aim of producing a general guideline on the human health risk assessment of alloys (www.herag.net).

No further toxicity studies of stainless steel are proposed. The main hazards of stainless steels are related to some uses of the material, especially welding. Future emphasis should be on the assessment and management of these risks.

ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
AISI	American Iron and Steel Institute
ALF	Artificial lysosomal fluid
AOF	Argon-oxygen decarburisation
BET	Brunauer Emmet Teller surface analysis
CA	Chromosomal aberration
CLP	Classification, Labelling and Packaging of substances and mixtures, EC Regulation 1272/2008
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DSD	Dangerous Substances Directive; 67/548/EEC
EAF	Electric arc furnace
GF-AAS	Graphite Furnace Atomic Absorption Spectroscopy
GHS	Globally Harmonized System for Classification and Labelling of Chemicals
GLP	Good Laboratory Practice
IARC	International Agency for Research on Cancer
IC_{20}	Inhibitory concentration; concentration of a substance that causes a defined inhibition (percentage as a subscript) of a given system.
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ISR	In-stent restenosis
LC_{50}	Lethal concentration; concentration of a substance in an environmental medium that causes 50% death following a certain period of exposure.
LD_{50}	Lethal dose; amount of a substance or physical agent (e.g. radiation) that causes 50% lethality when taken into the body.
LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
MMAD	Mass median aerodynamic diameter
MTT	3- (4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NOAEL	No-observed adverse effect level
PBS	Phosphate buffered saline
PTFE	Polytetrafluoroethylene
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals, EC Regulation 1907/2006
SCE	Sister chromatid exchanges
SSB	Single strand break
STOT RE	Target organ toxicity after repeated exposure
UL	Tolerable Upper Intake Level
VOD	Vacuum oxygen decarburisation
XPS	X-ray photoelectron spectroscopy

GLOSSARY

Main source: IUPAC Glossary of Terms Used in Toxicology, Unites States National Library of Medicine, National Institutes of Health; http//sis.nlm.nih.gov/enviro/iupacglossary/glossarym.html

acute toxicity	Adverse effects of finite duration occurring within a short time (up to 14 d) after administration of a single dose (or exposure to a given concentration) of a test substance or after multiple doses (exposures),
adrenal tumour	Benign and malignant neoplasms of the adrenal gland, several of which are notable for their tendency to overproduce endocrine hormones.
bio-accessibility	Potential for a substance to come in contact with a living organism and then interact with it.
bio-availability	Extent of absorption of a substance by a living organism.
biomonitoring	Continuous or repeated measurement of any naturally occurring or synthetic chemical, including potentially toxic substances or their metabolites or biochemical effects in tissues, secreta, excreta, expired air or any combination of these in order to evaluate occupational or environmental exposure and health risk by comparison with appropriate reference values based on knowledge of the probable relationship between ambient exposure and resultant adverse health effects.
carcinogenicity	Process of induction of malignant neoplasms, and thus cancer, by chemical, physical or biological agents.
cytotoxic	Causing damage to cell structure or function.
epicutaneous	Literally, on the skin, referring to introduction of biologic material or drugs into the skin by shallow, bloodless piercing with small-gauge needles through drops of solution.
epididymis	Part of male reproductive organs; a convoluted tube situated along the posterior margin of each testis, in which spermatozoa are stored
genotoxic	Capable of causing a change to the structure of the genome.
histochemistry	Study of the chemical composition of tissues by the means of specific staining reactions
immunohistochemistry	Histochemical localization of immunoreactive substances using radiolabelled antibodies as reagents.
intracutaneous	Introduction of material in the skin, particularly the dermis.
intrapleural	Administered by entering the pleura or pleural cavity (body cavity that surrounds the lungs).
in vitro	In glass, referring to a study in the laboratory usually involving isolated organ, tissue, cell, or biochemical systems.
in vivo	In the living body, referring to a study performed on a living organism.

mesothelioma	Malignant tumour of the mesothelium of the pleura, pericardium or peritoneum.
mortality	Death as studied in a given population or subpopulation.
mutagenicity	Ability of a physical, chemical, or biological agent to induce (or generate) heritable changes (mutations) in the genotype in a cell as a consequence of alterations or loss of genes or chromosomes (or parts thereof).
pheochromocytoma	tumour that usually starts in the cells of adrenal glands
phototoxicity	Adverse effects produced by exposure to light energy, especially those produced in the skin.
pneumonitis	Inflammation of lung tissue.
reproductive toxicology	Study of the nonheritable adverse effects of substances on male and female reproductive function or capacity and on resultant progeny.
restenosis	Re-narrowing of a coronary artery.
sensitization	Immune response whereby individuals become hypersensitive to substances, pollen, dandruff, or other agents that make them develop a potentially harmful allergy when they are subsequently exposed to the sensitizing material (allergen).
squamous metaplasia	Benign (non-cancerous) changes in the epithelial linings of certain organs within the body
tibiae	The large bone between the knee and the foot.
toxicokinetics	Generally, the overall process of the absorption (uptake) of potentially toxic substances by the body, the distribution of the substances and their metabolites in tissues and organs, their metabolism (biotransformation), and the elimination of the substances and their metabolites from the body.

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