Cancer and Metastasis Reviews 6: 113–154, 1987

Cancer risk from inorganics

Sabine H.H. Swierenga[1], John P.W. Gilman[2] and John R. McLean[3]
[1] Bureau of Drug Research, Health and Welfare, Canada; Genetic Toxicology Section, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario KIA OL2 Canada (address for offprints); [2] Biomedical Sciences Department, University of Guelph, Canada; [3] Bureau of Radiation and Medical Devices Health and Welfare, Canada

Keywords: carcinogenesis, mineral fibers, synthetic fibers, metal carcinogenesis, DNA mutations & repair, radio-pharmaceuticals

Abstract

Inorganic metals and minerals for which there is evidence of carcinogenicity are identified. The risk of cancer from contact with them in the work place, the general environment, and under conditions of clinical (medical) exposure is discussed. The evidence indicates that minerals and metals most often influence cancer development through their action as cocarcinogens.

The relationship between the physical form of mineral fibers, smoking and carcinogenic risk is emphasized.

Metals are categorized as established (As, Be, Cr, Ni), suspected (Cd, Pb) and possible carcinogens (Table 6), based on the existing *in vitro,* animal experimental and human epidemiological data. Cancer risk and possible modes of action of elements in each class are discussed. Views on mechanisms that may be responsible for the carcinogenicity of metals are updated and analysed.

Some specific examples of cancer risks associated with the clinical use of potentially carcinogenic metals and from radioactive pharmaceuticals used in therapy and diagnosis are presented. Questions are raised as to the effectiveness of conventional dosimetry in accurately measuring risk from radiopharmaceuticals.

Introduction

Approximately one sixth of all the human carcinogens designated by the International Agency for Research on Cancer (IARC) are inorganics. Almost all of these are minerals and metals, which act either as true chemical carcinogens or are carcinogenic because of their radioactivity.

Through the first half of this century fibers and non-radioactive metals were not recognized as a major cause even of occupational cancers and, with the possible exception of arsenic, were disregarded outside the work place.

Recognition of human cancer risk from inorganics has been based mainly on epidemiological data obtained from occupational studies. In recent years however, there has been an increased recognition of cancer risk from contaminants in the general environment, including excesses of certain inorganics in drinking water, ambient atmosphere and occasionally, the food supply.

Clinical exposure to metals in either very high concentration or prolonged duration is another category of cancer risk from inorganics that will be considered. Modern medical practice has also introduced a further potential cancer risk with the use of radioactive inorganic injectibles in diagnostic procedures.

1. Mineral fiber carcinogenesis

Asbestos is the major mineral cancer hazard in man's environment. Early reports of a positive association between exposure to mineral fibers and cancer are concerned with the incidence of carcinoma of the lung among asbestos workers [1, 2]. Interestingly, the report by Breslow *et al.* [1] points to the now well established critical role of cigarette smoking in lung cancer risk from asbestos exposure.

The causal relationship between asbestos and malignant mesothelioma became apparent in the 1960's while currently an increased risk assocation with gastrointestinal cancer is also suspected. Several other fibrous minerals in addition to asbestos are implicated in these processes (reviewed by Craighead and Mossman [3], McConnell *et al.* [4], Wagner [5]).

Experimental and epidemiological studies provide strong evidence of a major co-carcinogenic role for mineral fibers in both respiratory tumor and mesothelioma induction. Fibers also appear to contribute to the tumor initiation process through the mechanical transport of polycyclic aromatic hydrocarbons (PAH) and their intracellular transfer for adduct formation with DNA [6]. Injected asbestos, glass and quartz fibers also interact as co-carcinogens with inhaled radon in both lung tumor and mesothelioma development in rats. The remote promotion of the lung tumors observed may involve immunosuppression or growth factor release [7].

Although the carcinogenicity of several types of natural mineral fibers is well established, their risk assessment is still the subject of controversy [5]. Whether or not man-made mineral fibers are carcinogenic is particularly equivocable, although the probability is that prolonged industrial exposure constitutes a lung cancer risk [8].

1.1 Properties of mineral fibers

The biological properties of mineral fibers are not well understood, despite numerous studies on asbestos-related disease. The physical properties of these fibers, particularly their dimensions and *in vivo* durability appear to bear a critical relationship to the cancer risk that they pose [5, 9, 10].

1.1.1. Asbestos. The family of commercially exploited naturally occurring fibers grouped under this name has been classified according to physical characteristics. A great deal of variance in these characteristics exists within each class, dependent to some extent on the geographic source of the ore. The characteristics of some of these classes, because of their wide use and potential impact on public health, are briefly listed: (Reviews: 3, 5)

a) *Serpentines – Chrysotile* fiber deposits are widely distributed and constitute over 90% of the world's commercial production. Their magnesium and silica-rich sheets are curled into relatively blunt hollow fibers.

b) *Amphiboles – Crocidolite* and *Amosite* are mined mostly in South Africa and Western Australia. These were very extensively used into the 1940's and '50's but are much less so now. Fibers are solid, straight and iron rich, with less exposure surface and greater penetrance than chrysotiles. *Anthophyllite* and *Tremolite* are calcium- and magnesium-containing amphiboles that are used less commercially, but are environmental/agricultural contaminants. They may be present in various amounts in chrysotile asbestos and in talc.

1.1.2. Zeolites and clays. These are the most important mineral fibers other than asbestos. Zeolites are lattice-shaped silicates used in filtration and for catalysis, while absorbent clays have many uses including being a component of some cat litters.

1.1.3. Synthetic mineral fibers. All man-made mineral fibers have a glassy structure and are formed from liquid melts at from 1000° C to 1500° C. They are of various diameters but are usually coarser than their natural counterparts. Synthetic insulating (Rockwool) fibers have a 1–6 μm diameter. Ultra-fine fibers are synthesized for filter paper manufacturing [5].

1.2. Respiratory tract cancer

Reports of a causal association between asbestos, interstitial pulmonary fibrosis (asbestoses), and an elevated incidence of lung cancer date back for fifty years and are by now very numerous. Workers in the textile industry are at particularly high risk. Risk in other asbestos related manufacturing is somewhat lower, being least in asbestos cement-production and mining [5, 11].

1.2.1. Cigarette-smoking factor. Selikoff and Hammond [12] reported the following striking relative lung cancer increases among asbestos workers compared to persons who did not smoke and were unexposed to asbestos:

Asbestos exposure, non-smokers: 5 fold risk elevation

No asbestos exposure, smokers: 11 fold risk elevation

Asbestos exposure, smokers: 53 fold risk elevation

This multiplicative model is still widely accepted [13], although it has also been shown that after correcting for the effect of smoking on lung cancer the relative risk due to the asbestos exposure is highest for non-smokers, intermediate for ex-smokers and lowest for smokers. The trend is statistically significant [14]. A review of data from several other studies, showing a 1.8 fold relative risk increase attributable to asbestos *per se* in non-smokers, should be accepted with caution [14]. Actual lung cancer risk was nonetheless much elevated for the smoker groups.

In animal experiments polycyclic aromatic hydrocarbons show a marked synergism with asbestos in respiratory tumor induction in rats. B(a)P-coated fiber penetration of the lining epithelial cells facilitated tumor induction, while the fibers alone show little or no tumorigenic activity [6].

1.3. Malignant mesotheliomas

Mineral fiber-induced mesotheliomas are most often associated with occupational exposure to crocidolite, less often to amosite and least often to crysotile fibers [3]. Tremolite asbestos fibers and zeolite fibers have also been causally linked to both lung plaques and mesothelioma induction [5]. Mesothelioma risk appears to be independent of smoking habits [14].

A dose response relationship between mesothelioma incidence and occupational asbestos exposure has been reported [15, 16]. Nonoccupational (environmental) exposure to mineral fibers has also been related to increased risk of pleural and peritoneal mesotheliomas in the general population [5].

Mesotheliomas are usually characterized by prolonged latencies, in the 20–50 year range. This fact presents the distinct possibility of an outbreak of such cases occurring between now and early in the next century as a result of the wide spread often excessive industrial exposures of the 1940–60 period [17].

1.3.1. Fiber dimensions factor. Durability, shape, and size of a very wide range of mineral fibers correlate consistently with the induction of fibrous sarcomas in rats [9]. Evidence from pathogenesis studies on fiber size, shape, and the role of phagocytosis in fibrous plaque and mesothelioma development clearly support the importance of these factors [3].

The generally accepted conclusion concerning the critical dimensions of fibers in risk enhancement is that risk is highest for those fibers measuring $\leq 0.25\,\mu m$ by $>8\,\mu m$. Relatively high correlations have been shown, however, for fibers up to $1.5\,\mu m$ diameter and as low as $4\,\mu m$ long [3, 5, 9, 18]. Bronchiogenic carcinomas and mesotheliomas have been recorded as associated with zeolites, particularly fine fibered erionites from the Eastern Mediterranean. Epidemiological studies demonstrate much elevated risk from environmental exposure to small erionite fibers ($<0.25\,\mu m$) in two widely separated villages in Turkey which recorded exceptionally high fiber-related cancer incidences, with relatively few cases from adjacent communities [19, 20]. These findings are confirmed in at least one animal study [21] although animal studies on erionite from New Zealand and on mordenite in Japan have proven negative [5].

1.4. Gastro-intestinal cancer

Epidemiological studies on occupational asbestos exposure in relation to cancers of the GI tract are suggestive of an association; but supportive findings from tissue analysis and dose response evaluations are inconclusive, as are animal experiments [22].

1.5. In vitro toxicity

Activity of various asbestos fibers in some *in vitro* assays is shown in Table 1. Some form of cytotoxicity has been observed for all classes of asbestos fibers (for review see reference 23). Fibers tend to be phagocytosed by a wide variety of cell types including macrophages (reviewed by Hei *et al.* [24]). EM studies have shown that thin fibers penetrate membranes, phagosomes and lysosomes, nuclei and chromosomes [25]. Toxicity varies with fiber type and physical properties. For example, chrysotile, but not crocidolite, causes growth inhibition in a concentration-dependent manner in rat pleural mesothelial cells [26]. For rodent and human fibroblast and epithelial cell types chrysotile fibers are generally more reactive than amphiboles [27]. Long, thin fibers appear to induce morphological transformation more efficiently in cultured cells [28, 29]. Glass fibers with diameters of 0.1 to 0.2 μm were also positive in transformation assays, but not after milling, which reduced the fiber size 10 fold [28]. Hei *et al.* [24] show that acid leaching of fibers to remove chemical contaminants did not reduce their toxicity for mouse $C^3H1OT1/2$ fibroblasts.

The presence of macrophages in asbestos-exposed tissue may contribute to cellular injury. Production and release of proteolytic enzymes such as plasminogen activator, RNAase, β-galactosidase, and protease have been observed in macrophages exposed to asbestos fibers [30–32], as well as enhanced release of hydrogen peroxide and superoxide anion [33]. The hydrogen peroxide present in the supernatants of asbestos-elicited macrophages has anti-protease inhibitor activity [33].

Cell specificity for asbestos fiber injury has also been demonstrated *in vitro*. Lechner *et al.* [34]

Table 1. Activity of asbestos fibers in *in vitro* short-term tests.[a]

	Chrysotile	Amosite	Crocidolite	Other* *
In vivo/In vitro Tests[b]				
Cytotoxicity assays[c]	+[33]	+[24] ⊕ [34]	+[24]	+[24]
DNA Effects: biochemical assays[d]				
DNA Damage – Bacteria[e]				
DNA Damage – Mammalian cells[f]	−[44]		−[44]	
DNA Synthesis inhibition – Mammalian cells[g]	+[26]	+[24]	−[26] +[24]	
DNA Repair induction – Mammalian cells[h]				
DNA Repair inhibition – Bacteria[i]				
DNA Repair inhibition – Mammalian cells				
Chromosome aberrations – Plant cells				
Chromosome aberrations – Mammalian cells	+[29] +[37]	−[29] ⊕ [34]	+[29] +[38]	−[24]
Sister chromatid exchanges		−[24]	−[38]	−[24]
Mutations: Bacteria[j]	−[47]			
Yeast and plant cells				
Drosophila				
Mammalian cells				
Mammalian cell transformation[k]	+[29] +/−[43] +[78]	+/−[43] −[24] ⊕ [34]	+[29] +/−[43] +[28]	+/−[43]
Enhanced virus transformation[l]				
Enhanced genotoxicity[m]	+[41] +[43] +[42]	+[46] +[43] +[41]	+[46] +[43] +[41] +[24]	+[43] +[24]

* For explanation of assays, see footnotes for Table 3.

* * Unspecified, or anthophyllite (43)

show 10- to 100-fold greater cytotoxicity of fibers for human mesothelial than bronchial or fibroblast cells. Woodworth *et al.* [35] consider the possibility of enhanced damage in foci of metaplastic squamous epithelial cells observed in respiratory tissue of smokers, and find that the metaplastic, but not the normal ciliated hamster tracheal cells, in organ culture showed fiber uptake. Ginns *et al.* [36] show an impairment of natural killer cell activity in smokers and asbestos workers, suggesting a role in immune suppression.

Chromosome aberrations in mammalian cells have been observed after chrysotile [29 (review), 37] and crocidolite [29, 38] exposure. Amosite fibers have been reported as both negative in rat fibroblasts [24], and positive in human mesothelial cells [34]. Oshimura *et al.* [37] report a specific, non random chromosome change in Syrian hamster embryo cells transformed with chrysotile fibres. Six of eight lines exhibited trisomy II; other common abnormalities include specific chromosome deletions. The induction of aneuploidy may play a role in neoplastic progression for both metal and asbestos carcinogenesis.

Metal and fiber – exposed cultured mammalian cells have several other features in common. As for metal toxicity (see section 2.3), some alteration of cytoskeletal integrity has been shown for asbestos-treated cells. Mechanical disruption of cytoskeletal networks [25], and morphological changes that are mediated by cytoskeletal components such as spreading, multinucleation, and vacuolation [26, 39] have been observed in fibroblasts and pleural mesothelial cells. Altered differentiation expression such as enhanced squamous metaplasia [35] and mucin secretion [40] in hamster trachea cells has also been observed. Finally, as for metals, synergism with benzo(a)pyrene or γ-irradiation toxicity has been observed in the presence of asbestos fibers: for B(a)P uptake and DNA alkylation in hamster trachea cells [41], mutagenesis in *S. typhimurium* [42], sister chromatid exchanges and morphological transformation in rodent fibroblasts [24, 43]. Enhanced B(a)P toxicity is not accompanied by an increase in DNA strand breaks [44] or by inhibition of metabolic cooperation [45].

1.6. Risk assessment

Relative risks from exposure to the many different types of mineral fibers encountered in the environment are still a matter of controversy. Industrial risk varies widely from different types of exposure. Fiber sizes are altered, tending to get smaller with processing, hence the greater degree of risk encountered in industries such as textiles in comparison with mining [48]. Industrially–used asbestos is usually mixed and varies depending on source and market factors. An increase in the proportion of amphiboles present will tend to enhance risk [48].

The difficulties of risk assessment are compounded for non-industrial environmental exposure. Fibers are ubiquitous in the general environment, and almost everyone has some fibers in their lungs [3, 18].

Dose relatedness of risk is the key to implementing intelligent precautionary safeguards. Unfortunately dose response relationship evidence appears often not to be clearly understood or properly interpreted. Recently Levine [22] has thoroughly and provocatively reviewed this topic.

All asbestos exposures are not equivalent to those of 30 or 40 years ago. Worker exposure of 0.5 fibers/ml for 40 years is being considered by OSHA as a permissible level.

Relative risk from various levels of asbestos exposure and other hazards should be kept in mind in the decision-making process. For example, the potential risk to workers exposed for 40 years to 0.5 mixed asbestos fibers per ml air, is 8200 lifetime excess cancers per million exposed. The risk to children in schools containing asbestos products, resulting in exposure to 0.001 mixed asbestos fibers over 6 years is 5 lifetime excess cancers per million, or 1.5 lifetime excess cancers per million if the exposure is to chrysotile fibers only [11]. Childhood annual death rates of 14 per million from bicycle accidents (10–14 year olds), 10 per million from high school football, and 5–20 per million from whooping cough vaccination are presented as comparison [11].

These sorts of risk ratios and the concomittant risk of atmospheric pollution with fibers on demolition should be weighed against the very low risk

from properly maintained buildings. Scientifically defensible exposure levels comparable to those developed by OSHA for industry should be drawn up for the guidance of local decision makers.

1.7. Conclusions and unanswered questions

Mineral fibers act primarily as co-carcinogens and are probably relatively weak carcinogens in the absence of organic or other initiating agents. Cigarette smoke in particular, other PAH, and irradiation encountered in the environment interact with various types of mineral fibers to induce cancer. This view is supported by *in vitro* evidence of increased cytotoxicity from fiber interaction with PAH and γ irradiation and the fiber-enhanced mutagenesis of *S. typhimurium*.

The physical characteristics of fibers are critical to their carcinogenic potential with the long thin more durable ones presenting the greatest hazard.

Cytotoxic effects *in vitro* result from the penetration of organelle and nuclear membranes and disruption of the cytoskeleton. Chromosomal aberrations and aneuploidy are induced by both mineral fibers and metals. The frequently expressed view that asbestos is not mutagenic seems based on lack of published positive mutation assay results, which may simply reflect the lack of sufficient attempts.

Questions on the mechanism(s) by which mineral fibers promote carcinogenesis in the whole animal require further study in the light of *in vitro* findings. The suggestion that the depression of NK cell activity (as seen *in vitro*) may be involved in the fiber-mediated promotion of remote tumors should be pursued. The mechanism(s) by which fibers influence the initiation process also remain largely unresolved.

Other major unanswered questions include (a) The carcinogenicity of man-made fibers; (b) The role of mineral fiber ingestion in gastrointestinal tract carcinogenesis (if any); (c) The possible identification of a pattern of *in vitro* cytotoxic response that is indicative of tumor promotion.

2. Metal carcinogenesis

The literature dealing with the carcinogenicity of a wide range of nonradioactive metallic elements has been reviewed in detail up to 1984 [49–51]. Metals are classified by Gilman and Swierenga as being either *established, suspected* or *possible* carcinogens. This classification is based on a weighting of the evidence from epidemiological studies, animal experiments and short term test reports and will again be used in this review. By careful consideration of short term test data and co-carcinogenesis factors, this system of classification permits a greater number of 'possible carcinogens' than does the more conservative IARC evaluation [52]. Classification criteria are shown in Table 2.

Arsenic, beryllium, chromium and nickel have been classed as established carcinogens. Cadmium

Table 2. Criteria for classification of metal carcinogens

Carcinogen class	Epidemiological evidence[a]	Animal experiments[b]	Short term test results[c,d]
Established	+	+	+
Suspected	±	+	+
Possible	(±)	±	+ or ±

[a] + Evidence considered conclusive
± Evidence indicative but inconclusive
(±) Inconclusive evidence of co-carcinogenesis.
[b] + Tumor induction in 2 or more species (preferably one non-rodent)
± positive results limited to one species.
[c] + activity in all classes of tests
± limited data available but including activity in DNA damage and chromosome tests in more than one species including mammals.
[d] Authors' designation of criteria. Risk evaluation guidelines are lacking.

is designated a suspected carcinogen, as also is lead on the basis of recent reports on its role in renal carcinogenesis and its role as a promoting agent. Iron on the other hand has been reclassified from suspected to possible carcinogen, on the basis of recent evidence of the causal role of radon daughters in iron (hematite) related carcinogenesis and the lack of evidence for Fe_2O_3 carcinogenicity in experimental animals.

A number of metals may be classed as possible carcinogens. Recent experimental observations are presented for some of these metals, with particular emphasis on their interactions and possible promoting activity.

2.1. Recent human and animal studies

2.1.1. Established carcinogens. Arsenic, chromium VI, nickel and berrylium are generally recognized as metals carcinogenic to man and are classed as established carcinogens [50]. By no means all compounds of these elements are equally carcinogenic or even carcinogenic at all. Variations observed are thought to be related to the chemical and physical form of the compounds.

Arsenic. Despite the fact that arsenic was the first metal to be recognized as presenting a cancer risk to man (iatrogenic skin cancers from Fowler's solution of arsenic), its exact role in the carcinogenic processes with which it is causally associated is both unclear and controversial. Occupational and other forms of environmental exposures to inorganic arsenicals are invariably combined with mixtures of other agents as in smelter dusts, pesticide manufacture and application, contaminated water supply etc.

Difficulties in identifying a suitable experimental animal model for arsenic have probably been exacerbated by the persistent use of the more traditional methods of exposure (diet, skin painting), test species (mice and rats), and pure compounds rather than mixtures (reviewed by Gilman and Swierenga [50]).

More attention has recently been directed towards metal interaction with other inorganic and organic chemicals [53, 54], and it seems quite possible that interaction with other agents may be necessary for the process of arsenic carcinogenesis. Investigations should, on the basis of *in vitro* findings, be extended to include the interaction of arsenic with physical agents [55, 56].

Arsenic-containing ore dust [57] and insecticide mixtures [58] instilled by the intratracheal route have been reported to induce lung carcinomas in rats. Arsenic trioxide administered to Syrian hamsters by the same route at two different dose levels shows a dose response relationship in adenoma induction [59]. Adenomas have also been induced in other species with calcium arsenate and, equivocably, with arsenic trisulfide, both compounds showing high retention in lung on occupational exposure [60]. In another experiment Pershagen G. *et al.* [61], exposed hamsters to arsenic trioxide and/or benzo(a)pyrene, inducing significant incidences of pulmonary tumors and evidence of a synergistic enhancement in numbers of lung tumors from the interaction of arsenic and benzo(a)pyrene. This is suggested as being comparable to the interaction between occupational exposure and smoking.

Several of the more recent epidemiological studies establish a clear dose response causal relationship between occupational exposure (most often to trivalent arsenic) and lung cancer incidence in smelter workers [62–64].

A recent well designed case-control study of men living in the vicinity of a copper smelter records an increased relative lung cancer risk for those within 20 km of the point of emission [63], although earlier similar studies had given conflicting results. There is a need for improved, (possibly standardized) epidemiological study methods to define more precisely and monitor risk from potentially carcinogenic atmospheric pollutants; particularly as such hazards are presumably controllable.

A dose response relationship has been established between several types of cancer, including bladder, kidney, skin, lung and the proportion of the water supply derived from high arsenic artesian wells in several Taiwan villages. The study was conducted in an endemic area of black foot disease (an arsenic-related syndrome) which shows a com-

Table 3. Activity of frequently tested metals in *in vitro* short-term tests.[a]

	As	Be	Cr	Ni	Cd	Pb	Co	Cu	Fe	Li	Mn	Mg	Hg	Mo	Pt	Se	Ag	Tc	Sn	Zn
In vivo/In vitro tests[b]	⊗	+	☒	×	⊗	☒	+						⊗		×	⊗		+	×	+
Cytotoxicity assays[c]	+		+	×	+	+		+			+		⊗		+		+	+		
DNA Effects: biochemical assays[d]	+	×	×	×	×	×	×	×	+		×	−	+			−	+			+
DNA Damage – Bacteria[e]	×	+	×	+	×	+	+	−	+	−	+	−	+	×	×	×	∅	+	−	+
DNA Damage – Mammalian cells[f]	⊗		⊗	+	+	×	⊕				+		+		×	⊕			⊕	⊖
DNA synthesis inhibition – Mammalian cells[g]	+[168]		+	⊗[161]	+		+						+		+	+			+	
DNA Repair induction – Mammalian cells[h]	⊕		⊗	−											+	+				
DNA Repair inhibition – Bacteria[i]	×		+		+									+	×					
DNA Repair inhibition – Mammalian cells	⊕			+	+	+							⊕		+					+
Chromosome aberrations – Plant cells	×	+	×	×	×	+	×	×	×	×	+	−	×	+	+		+		+/−	×
Chromosome aberrations – Mammalian cells	☒	⊕	⊗	⊗[160]	⊗	⊗	⊖		+		+		⊗		⊗	×		⊕	⊖	⊕
Sister chromatid exchanges	⊗	⊕	⊗	☒	⊕	⊕	⊕		−		⊕		⊕		⊗	⊗				
Mutations: bacteria[j]	×	+[157]	×	+	×	−	+[156]	+	+		×		−	+	×	×	−	−		+
Yeast and plant cells	+	+	+	+	+		+	+	+		×		+			+				+
Drosophila			+	−	+	+							+		+					+
Mammalian cells	+	+	×	×	×	+[170]	+	+	+		×	−	+	+[170]	×	−	+			+[170]
Mammalian cell transformation[k]	⊕[162]	×	⊗[162]	⊗[160,162]	×	⊕[162]	⊗	+	+	+	+	+	+		+		+			+
Enhanced virus transformation[l]	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+		+			+
Enhanced genotoxicity[m]	+[168]			+	+[158]				+[42]											
Reduced genotoxicity[n]											+					+[169]				

[a] Summarized from previous reviews (50, 51, 144), plus recent experimental data (referenced by superscripts).
[b] Analysis following *in vivo* exposure, including teratogenesis, host-mediated assay, dominant lethal test, cytogenetic tests including in exposed humans.
[c] E.g. analysis of plasma or lysosomal membrane damage in mammalian cells, spindle effects.
[d] Infidelity of DNA structure; or DNA, RNA synthesis in cell-free systems.
[e] Differential growth sensitivities of wild-type and repair-deficient strains of *B. subtilis* (rec-assay) or *E. coli* (Pol A$^-$/Pol A$^+$ assay).
[f] DNA binding, adducts, crosslinks, single and double strand breaks.
[g] Includes continuing depression of DNA synthesis after carcinogen removal.
[h] Unscheduled DNA synthesis (UDS).
[i] Inhibition of excision or SOS repair in *E. coli*.
[j] In *E. coli* or strains of *S. typhimurium* (Ames test).
[k] Altered growth properties *in vitro*. Transformed colonies induce tumors in appropriate hosts.
[l] Enhancement of SA-7 virus-induced neoplastic transformation of Syrian hamster embryo cells or of leukosis virus transformation of human fibroblasts.
[m] Additive or synergistic responses when used in combination with other carcinogens (metals, B(a)P, ionizing irradiation).
[n] Ability to reduce the toxicity of other carcinogens.

Symbols + positive response in 1 or 2 reports
× positive response in 3 or more reports
O includes data for human cells: 1 or 2 reports; □ 3 or more reports
− negative response reported: 1 or 2 reports; ∅ 3 or more reports
+/− weak positive response reported.

parable dose response relationship [65]. Similar relationships to regional endemic chronic arsenic intoxication from drinking water are shown for skin, respiratory and digestive tract tumors in regions of Japan and South American countries [66].

The experimental animal studies referred to above show promise of providing an acceptable animal model system for arsenic carcinogenesis. A wider range of species should be investigated, with arsenic-containing materials more closely reflecting those so clearly related causally to cancer in man. Animal experiments using long term exposure to the Taiwan artesian well water in different species do not appear to have been reported and might profitably be undertaken.

The approach of measuring and relating arsenic and other metal concentrations in various tissues to the tumor incidence in experimental animal studies should be undertaken as it has been in autopsy tissues from smelter workers [67]. In that study, arsenic in the lungs of smelter workers is 7-fold greater than that in matched controls from 50 km away, but no different for smelter workers dying of cancer than from other causes. However the highest concentrations of other metals (Sb, Cd, Pb etc.) are always found in the lung tissues of the lung cancer cases. This is taken to support the theory of a multifactorial cause for excess lung cancers in these workers.

The epidemiological term 'confounding factors' should be recognized to have relevance to our desire, but inability, to pin down a single uncomplicated causal factor in environmental carcinogenesis. In fact much of the evidence reviewed above, as well as that available for other occupational and environmental cancer risk situations (see below), seem to point to interactions and synergisms between a principal 'exciting' causal element and various 'confounding' factors. In the case of arsenic there is evidence showing its causal role may be exerted mostly as a late stage effect in a multistage mechanism of carcinogenesis [68].

Beryllium. Epidemiological studies on workers exposed to beryllium and on populations living in the neighbourhood of extensive beryllium emissions demonstrate an excessive risk of lung cancer (Reviewed by Kuschner [69] and Costa *et al.* [70]).

BeO is the common exposure form of beryllium for humans. It seems unlikely that beryllium oxides are encountered in the atmosphere in sufficent concentration from the combustion of fossil fuels to constitute a cancer hazard on their own, as they may under occupational conditions [71]. The combustion of coal is a major source of atmospheric beryllium oxides [72]. The epidemiological demonstration of a causal relationship between the products of fossil fuel combustion and lung cancer in the general population is extremely difficult [73].

At least thirteen different beryllium compounds are carcinogenic to a variety of animal species. Osteosarcomas are induced by parenterally injected beryllium while respiratory carcinomas result from inhalation in both rats and monkeys [74].

Despite convincing evidence in support of classifying beryllium as an established lung carcinogen in humans, it is still frequently referred to as a chemical for which the carcinogenicity to humans is uncertain [52]. In view of this persisting uncertainty it is surprising to find that there is not a single study on beryllium among the 487 selected abstracts from 1982–85 that are included in the NCI Oncology Overview on occupational respiratory tract cancers [75].

Nickel and Chromium. These are the two most thoroughly investigated and universally accepted of the established carcinogens. The extensive evidence for the carcinogenicity of nickel has been reviewed in recent international symposia [75, 77]. In addition, specific aspects of nickel carcinogenesis are the subjects of recent update reviews [78, 79].

Chromium is somewhat less extensively reviewed in recent publications; however several concise overviews on the carcinogenicity of chromium are available [80, 83].

The numerous early observations on the increased risk of lung and nasal cavity cancers in nickel refinery workers have generally been confirmed for the pyrometallurgical and electrolytic refining processes. The former process results in exposure to nickel sulfides and oxides while the latter involves nickel chloride and sulfate emissions.

Encouragingly, workers at a hydro metallurgical nickel refinery have shown no excess of respiratory cancer and no cases of nasal or sinus tumors [84]. The study did not support the implication that nickel concentrate or metallic nickel are respiratory carcinogens in man. However, the refinery did not commence operations until 1954 which allowed for a maximum of 30 years from first exposure of any of the workers. It seems probable that the observation times are still too short for definitive conclusions on the absence of risk.

A negative report on increased risk for respiratory cancer among 2,861 high nickel alloy production workers, at 12 different plants in the USA, notes significantly increased standard mortality ratios (SMR's) of 182 and 233 respectively for liver and large intestine carcinomas. No causal association with nickel exposure could be made. The large intestine cancers occur only in non-white males which seems to suggest other causal factors; however both types are associated with long exposure and latency [85].

Several recent epidemiological studies on chromate production workers support the earlier evidence for an increased respiratory cancer risk [86, 87]. Neither the number of lung cancers nor the total cancer deaths among nickel/chromium alloy foundry men are significantly elevated over the expected rates, in a study recorded by Cornell and Landis [88], although an excess of lung cancer occurs among white males aged 65–99. Lung cancer rates tended to increase with increasing length of foundry employment and it is suggested that the pattern of excess might be associated with length of foundry employment rather than with nickel/chromium. The data give no evidence of increased risk from nickel/chromium exposure.

The high exposure levels to both nickel and chromium VI that were common to primary refinery industries, fortunately are drastically reduced, however there are still very large numbers of workers in secondary industries who are exposed to lesser levels of nickel or chromium VI. There is evidence of a mild excess of respiratory cancers among welders as a whole and it appears likely that most of the excess is in those exposed to aerosols from welding high alloys and stainless steel [89]. This probability is supported by *in vivo* and *in vitro* genotoxicity tests and clearly warrants further investigations using a specifically designed uniform epidemiological protocol [90]. Welding with coated electrodes produces a higher proportion of CrVI compounds and shows a higher cancer risk than other welding processes [91]. It would seem necessary to attempt to determine the relative risk from nickel, chromium and combined nickel/chromium fumes by initially using short term tests.

The identification of a specific causative chromium or nickel compound carcinogenic to man by epidemiological studies is unlikely because of the numerous confounding agents that will inevitably be present. A process (e.g. nickel refining) may be such more readily identified as a cause of cancer in the workplace, than a specific compound (e.g. Ni_3S_2). Animal experiments and more recently, short term assays, are a means of gathering information on the carcinogenicity/mutagenicity of specific compounds, but cannot provide conclusive evidence on their effect in man. The ambivalence of this situation has recently been used to argue that only Ni_3S_2 and, debatably, nickel carbonyl should be classed under IARC group 2B, as possibly carcinogenic to humans. It is proposed that all other nickel compounds should be listed as IARC class 3 chemicals for which there is insufficient evidence to classify even as possibly carcinogenic [92].

This plea for a negative risk classification for nickel oxide and metallic nickel is based on the fact that pure metallic nickel dust and nickel oxide fail to induce malignancies by inhalation or intratracheal instillation [92]. Neither substance has been exhaustively tested by these routes nor has a variety of species been used. In view of the paucity of genotoxicity data for NiO, and the importance of the compound as a catalyst, further *in vitro* and animal tests should be undertaken to clarify its carcinogenic status.

The extensive current literature on animal experiments and their relevance to possible mechanisms of nickel carcinogenesis are reviewed by Sunderman [51, 78]. Sunderman [93] has recently completed a series of studies on 18 nickel compounds injected intramuscularly at an equivalent

dose of 14 mg nickel per rat. The compounds fall into five different histopathological categories. These results include a significant correlation between the mass of nickel per compound and its sarcoma incidence [93].

There is a need for renewed attempts to define which chromium compounds are carcinogenic by means of coordinated short term and animal testing programs. This approach should concern itself particularly with an evaluation of the possible carcinogenic role of trivalent chromium, its ability to cross cell membranes and the relationship of compound solubility including lipid solubility to carcinogenesis.

2.1.2. Suspected carcinogens. This category contains those elements for which convincing short term test and experimental animal evidence of mutagenicity and carcinogenicity exist, but for which the human epidemiological evidence is inconclusive:

Cadmium. A review on cadmium carcinogenesis up to 1980 by Piscator [94] includes a brief but thorough discussion of the physiology, metal metabolism and possible roles of cadmium, hormonal imbalance and zinc on cancer of the prostate.

The IARC places cadmium and certain cadmium salts into category 2A, in which animal evidence of carcinogenicity is considered acceptable but for which insufficient human data are available [52]. Epidemiological reports at that time were in fact limited to a few that were suggestive of an association between prostatic cancer and heavy occupational exposures and only one or possibly two showing a clear association with respiratory cancer [95].

Massive injections of cadmium induce sarcomas at the site of injection in rats, and some distant (systemic) tumors in the interstitial cells of the testes in both rats and mice. Chronic low level dietary exposure to cadmium has proven negative, as have the several earlier experiments on rodents designed to investigate the relationship of cadmium to cancer of the prostate [96]. The induction of prostatic carcinomas in the rat has recently been achieved by the injection of $CdCl_2$ into the ventral prostate lobe [97]. Five of 100 one year-old rats developed prostate invasive carcinomas within 270 days of injection, 11 more developed an atypical hyperplasia with severe dysplasia, and 67 others showed less severe hyperplasias. No severe dysplasia or carcinoma developed in any of the controls which included 20 animals injected with $ZnCl_2$ in the prostate. The rat experiment should perhaps be repeated in younger animals and possibly a longer living species to permit better simulation of the prolonged latency common to metal occupational cancer. A histologically more appropriate model for human prostatic cancer would be provided by the dog or the monkey.

The induction of carcinomas in rats following inhalation of cadmium chloride aerosols [98] strengthens the evidence for a cadmium role in occupational respiratory cancer. This experiment definitely warrants confirmation and extension to other laboratory species. The results demonstrate a clear dose response relationship and underscore the necessity for lifetime observations in chronic experiments on metal carcinogenesis.

Intratracheal instillations of up to 75 micrograms of cadmium oxide in rats fail to induce respiratory tract tumors [99].

A recent epidemiological report on cancer risk for production workers in a U.S. cadmium recovery plant shows a statistically significant dose response relationship between lung cancer mortality and cumulative exposure to cadmium [100]. A study on Swedish nickel-cadmium battery plant workers also shows a consistant SMR increase with dose and latency for both lung and prostate cancers though the increase is not statistically significant. This cohort was also exposed to nickel hydroxide, and as one case of nasopharangeal cancer occurred the causal involvement of nickel cannot be ruled out.

An overview of all the evidence to date would seem to support the view that an association exists between heavy exposures to cadmium and increased risk of both lung and prostatic cancers. This view is not universally held however [100] and further carefully designed epidemiological as well as animal studies are needed.

Lead. Lead is widely distributed in the human environment, particularly in urban areas, and lead poisoning remains one of the most common forms of industrial intoxication.

There are very few thorough epidemiological studies on lead as an occupational carcinogen. Those that are available have proven difficult to interpret due usually to the paucity of information on the role of confounding factors such as smoking habits [101], other metals such as arsenic and copper [102], and lack of detail on real levels of lead exposure [103]. The consensus of the available reports is that there is relatively little excess cancer risk to man from industrial or urban environmental exposure [103]. The latter form of environmental pollution results mostly from fossil fuel combustion; and, having been considerably reduced by anti-pollution device requirements, is unlikely to pose a cancer risk in the urban atmosphere [71].

The several studies on a cohort of U.S. smelter workers and battery manufacturing employees reported by Cooper [101] have been reviewed and reevaluated [70]. Costa notes that several of Cooper's tables indicate elevated SMR's, suggesting that the evaluation of the relationship between human neoplasia and lead must await further well controlled epidemiological studies.

Recent animal experiments on the carcinogenicity of lead are concerned with investigating its possible role as a cofactor in carcinogenesis. Lead acetate acts as a promoter in 2-(ethylnitrosamine)ethanol (EHEN) induced renal tumors [104]. At the dose of lead used, its promoting action is not associated with histologically detectable toxicity related changes or compensatory regeneration of the interstitial kidney tissues.

Lead acetate, when added to diets containing the renal carcinogen N'-(4'fluro-4-biphenyl)acetamide (FBPA), markedly accelerates the onset of those preneoplastic lesions considered indicative of tumor promotion [105]. Further evidence for the promoting action of lead acetate has been reported [106]. Lead acetate and calcium acetate interact to increase significantly ($p<0.03$) the number of renal tumors over that induced by lead alone. Calcium acetate increases the toxicity and carcinogenicity of lead while decreasing lead accumulation in the kidney [107].

Lead acetate in drinking water of Sprague-Dawley rats results in 81% incidence of renal tumors. When the rats are fed ethyl urea and sodium nitrite (precursors of ethylnitrosourea) along with lead acetate the renal tumor incidence is only 50% and no renal tumors develop from the EU/$NaNO_2$ diet. However the last mentioned diet induces tumors other than renal in 100% of the exposed animals. The EU/$NaNO_2$ tumors are mostly lymphosarcomas. Lead acetate is not syncarcinogenic with ENU probably because of their different target organs [108]. The specificity of lead for renal cancers in the rat plus the fact that these tumors are histologically comparable to those occurring spontaneously in man provides a useful experimental model.

Besides being a renal carcinogen, lead acetate acts as an enhancing or promoting agent for other chemical carcinogens, for virus induced neoplasms [109] and for spontaneous tumors in other animals [110].

These effects of lead may possibly be caused by its inhibitory action on the immune system, thus indirectly encouraging tumor development in the target organ [111].

The action of lead *in vitro* (Table 3) is compatible with its tumor promoting and enhancing roles in the whole animal. Further animal experiments should be designed to clarify the effects of lead on the immune response as these may relate to its cocarcinogenic role. Current animal experiments suggest the need for epidemiological studies designed to consider the possible tumor promoting role of lead and to look more closely at its interaction with organic carcinogens and other metals in the environment.

2.1.3. Possible carcinogens. Little or no positive epidemiologic evidence and only limited animal experiment data have been reported, but considerable *in vitro* evidence for carcinogenic risk exists for this group of compounds.

A complete coverage of all inorganics that might meet the above criteria for possible carcinogens has not been attempted; thus mercury, manganese, several of the precious metals etc. for which little new animal or epidemiological data could be

found, have been omitted. Most of these elements have been recently reviewed [50].

Cobalt. There is no epidemiological evidence that cobalt exposure enhances the risk of neoplasia in man, though relatively few studies have been undertaken specifically on this metal [112].

Cobalt is used mostly to produce alloys, and in the manufacture of tungsten carbide hard metal products, which may also contain other metals such as titanium and vanadium. Cobalt is obtained through the smelting of arsenic-containing mixed ores of copper, chromium, lead and nickel.

The smelting conditions are conducive to metal interactions and make the drawing of specific conclusions on the role of any one metal from epidemiological studies most difficult. Thus a necropsy study on 66 copper smelter workers, with a mean exposure of 30 years, showed a fourfold increase in chromium and twofold increases in cobalt and lanthanum in their lung tissues over that of 14 controls. Almost 1/3 of the smelters had died of cancer (10% respiratory), as opposed to no cancer deaths in controls. None of the metals showed any decrease through time from terminal exposure and it was concluded that the cancer incidence could not be linked to a single factor [113].

Animal experiments demonstrate cobalt to be a local-acting carcinogen only, inducing sarcomas at site. Its possible role as a cocarcinogen has not been adequately investigated, particularly in view of the implantation of cobalt containing alloys such as vitallium (Co, Ca, Mo, Ni) in surgical prostheses [50]. Long term follow up studies on patients with these prosthetic devices could provide much needed information in this regard.

Specific short term and animal studies should be initiated to determine whether or not cobalt enhances the mutagenicity and carcinogenicity of the metals with which it is commonly mixed in the work place (As, Cr, Ni, Ti, V). Pending clarification of its interactive role with these other metals, cobalt should be considered a possible carcinogen.

Copper. Numerous epidemiological studies have shown an enhanced cancer risk among copper smelter workers and agricultural (vineyard) workers exposed to copper and arsenic-containing spray materials (Reviewed by Gilman and Swierenga [50], Enterline and Marsh [114]). These reports invariably ignore the possibility of copper itself being involved in the carcinogenesis, attributing the increased risk to the presence of other elements such as arsenic, lead and sulfur dioxide in the work place atmosphere [64].

The unknown but possible influence of Cu in the experimental induction of lung carcinomas in 60% of the rats exposed to a pesticide mixture containing As and Cu has been emphasized [58]. The possible interaction of copper and calcium with arsenic and other carcinogens in the lung cancer incidence associated with chronic arsenic poisoning in vineyard workers [115] warrants much more attention than it has received to date.

A recent study shows exogenous copper chelates promote tumorigenesis and alter the characteristics of a copper-dependent transplantable melanoma in mice [116]. Copper compounds have been shown to be mutagenic, to cause morphological transformation and to affect DNA synthesis in short term assays (Table 3).

While there is no evidence that copper is a primary carcinogen, its role needs to be reassessed in terms of metal interactions. The assumption that copper is in no way involved in the increased cancer risk associated with copper smelting and the application of arsenic-containing, copper-rich, agricultural chemicals certainly needs investigation.

Iron and radon daughters. Iron carbohydrate complexes induce sarcomas at the site of multiple subcutaneous or intramuscular injections in rats, mice, hamsters and rabbits. Sarcoma of the buttocks in man is, questionably, attributed to these complexes (reviewed by Gilman and Swierenga [50]).

Underground hematite mining is occupationally responsible for an increased respiratory tumor incidence in essentially all parts of the world where iron is mined. Surface iron miners do not show an elevated risk.

Reports of respiratory tumors in iron and steel foundry workers from England and Finland, show definite cancer excesses but values are not corrected for smoking and other factors [117].

The role of Fe_2O_3 in the increased risk of lung cancer in underground hematite miners is unclear. There is no evidence of Fe_2O_3 itself being a primary chemical carcinogen either in man or experimental animals [118], although it is almost certainly involved in the carcinogenic processes resulting in the tumor excess seen among hematite miners, either as a physical (mechanical) or chemical co-factor. There is extensive experimental animal evidence for the cocarcinogenic role of iron oxide [50, 119].

Short lived radioactive daughters from decayed radon attach to dust particles (Fe_2O), cigarette smoke, gases etc, and are inhaled; their alpha radiations may affect the bronchial epithelium. Radon, in various amounts enters the mines by underground streams, crushed ore etc. Poor ventilation enhances their concentration [120]. Exposure to radon daughters is the generally acknowledged primary (initiating) cause of cancers in iron miners.

A dose response relationship exists between radon daughter exposure and the level of particle inhalation, and there is an additive effect from smoking. There is evidence that cigarette smoke in this situation plays a promoting role in multistage carcinogenesis [120].

A Swedish study on the role of radon daughters in iron miner lung cancer with a smoke-specific analysis shows that the absolute risk coefficient for smokers is 21.8 excess cases per 10^6 person-years per working level month (WLM), while for non smokers it is 16.3 excess cases, and 19.0 for the group as a whole [121]. The combined effect of smoking and radon daughters is nearly additive. The report also suggests that while underground exposure is the main cause, home exposure to radon daughters also gives rise to a number of lung cancer cases in the general population. The risk of lung cancer occurs with radon daughter exposure rates that are close to the occupational exposure limit for underground mining of 4 WLM per year in the USA and Sweden [121].

A French prospective study on hematite miners for a five-year period shows three times the expected number of lung cancer deaths [122]. All lung cancer deaths are in underground workers who were heavy smokers. The lung cancer fatalities show a higher incidence of bronchitis and functional defects than the other deaths. Cigarette consumption, age and underground work duration are all greater in the lung cancer group. Radioactivity in these mines is considered low. Iron oxide dust, silica, and gases including SO_2 and diesel fumes, are all present. Probably no one of these factors alone is responsible for the excess lung cancer mortality, which more likely results from multifactor interaction [122].

Zinc. There is no epidemiological evidence that zinc itself is carcinogenic. However studies on chromium pigment production workers both in Britain and in Norway show that zinc chromate pigment production gives rise to a severe risk of lung cancer, while lead chromate does not, even at highly toxic exposure levels [123, 124]. Another study on zinc and lead chromate pigment workers in the USA notes an increased incidence of stomach and pancreatic, as well as lung cancers [125].

In animal studies, $ZnCl_2$ injections into the testes has induced teratomas in several species. Development of these tumors involves the indirect stimulation of pituitary gonadotropin release [112, 126].

Zinc acetate has recently been reported to act as a promoting agent in the induction of brain tumors in rats, giving a three-fold increase in tumor incidence over that induced by the carcinogen NMU alone. Zinc acetate alone induces no tumors [127].

Zinc has also been reported to have an anti carcinogenic activity under certain conditions including the inhibition of organic chemical carcinogenesis by dietary zinc [50].

All these data strongly suggest that zinc may be an important carcinogenic cofactor and that further studies on its interactive role may prove helpful in determining mechanisms in metal carcinogenesis. In addition, its neuro-oncogenic role deserves further investigations, as experimental models for this class of tumor are rare.

Aluminum. Aluminum has been considered non mutagenic and a non carcinogenic metal, although neurotoxic effects from aluminum have been associated with renal dialysis and the neurodegenerative changes of Alzheimers disease [70].

An investigation on mortality patterns of employees with 5 or more years exposure from 14 aluminum reduction plants in the USA does not reveal any excess in lung cancer. However there is a higher than expected mortality from pancreatic, genito-urinary, and lymphohematopoietic system cancers [128].

A cohort of coworkers in the primary aluminum industry in Norway shows no overall cancer incidence increase but does reveal a significant increase in cancer of the lung almost entirely within two processing department subgroups. Difficulties in selection of an appropriate reference population and incomplete occupational and life style histories require that caution be exercised in interpreting the result at this point [129].

Exposure to high levels of aluminum dust in smelting operations results in pulmonary fibrosis in workers using the Soderberg process [130]. A 2.9-fold increase in the relative risk of bladder cancer is reported in this study and is attributed to B(a)P exposure, with cigarette smoking as a significant contributing factor. The relative risk after 20 or more years exposure rises to 12–38 and the authors conclude that aluminum smelting is causally associated with bladder cancer.

These epidemiological studies suggest that the possibility of aluminum interaction with organic initiating agents in lung and bladder cancer induction should be looked into in more detail using both short term and animal bioassays.

Until further data become available it seems prudent to regard aluminum as a potential carcinogenic cofactor under conditions of industrial exposure.

Molybdenum and tungsten. The addition of molybdenum to the drinking water of rats significantly inhibits the induction of N-nitroso-N-methylurea-induced mammary gland carcinomas [131]. A comparable inhibitory effect has also been shown for molybdenum in the chemical induction of esophageal and forestomach tumors in rats [132]. In both of the above studies tungsten at 150–200 ppm in the drinking water significantly reduces the tumor inhibitory effect of molybdenum.

Molybdenum apparently exerts its effect on the promotional stage of the carcinogenic process [131]. The investigators postulate that this may involve the blocking of estrogen receptors by molybdenum in the target organ, thus reducing the effectiveness of the promoting action of estrogen. They further suggest that the counteracting effect of tungsten is exerted through its physiological antagonism to molybdenum.

Epidemiological studies on a population from a district in China in which several tungsten mines are located are quoted by Wei et al. [131] as showing a breast cancer mortality risk of 13.8- and 2.5-fold greater than that of the national average for males and females respectively.

Molybdenum shows positive in several short-term tests (Table 3), while few if any *in vitro* tests on tungsten have been reported. The experimental results reviewed above do not seem to rule out the possibility of tungsten acting as a cocarcinogen or promoter in mammary tumorigenesis. The interactions reported between these two metals and organic chemical carcinogens should be the subject of further studies.

Selenium. Early animal experiments give contradictory evidence for the carcinogenicity of selenium in rats and mice [50, 133]. The reports generally fail to stand up to critical evaluation [117]. Nevertheless selenium sulfide is listed by the EPA carcinogenesis assessment group as a chemical having substantial evidence for carcinogenicity based on a National Cancer Institute carcinogenesis testing program report, in which selenium sulfide induced carcinomas of the liver in F344 rats and female B6C3, F_1 mice, and carcinomas and adenomas of the lung in female mice [134]. There seems to be no epidemiological evidence of selenium involvement in occupational cancers, though few studies have been published.

A far greater number of studies have recently been directed towards elucidating the apparent antitumor role of selenium. Here again the reported experimental evidence is contradictory, with implications of metal interactions between selenium and its antagonists such as zinc [135].

One recent report shows that 1,2-dimethylhydrazine (DMH) induced intestinal adenocarcinomas

in male Sprague-Dawley rats are unaffected by either Se deficient or supplemented diets [136]. Another study on the effects of graded levels of selenium in the diet on mammary carcinogenesis from 7-12-dimethylbenz(a)anthracene (DMBA) in Sprague-Dawley rats, shows selenium has an effect on the initiation stage of tumor induction. However selenium deficiency significantly enhances DMBA mammary carcinogenesis when maintained through the promotion (post initiation) period. Conversely an excess selenium diet inhibits tumor development only if it is maintained after DMBA initiation [137]. Other reports supporting a role for selenium in tumor promotion have appeared (reviewed by Willett and MacMahon [138]).

Comparison of analyses of selenium levels in tissues of smelter workers who died from cancers, with those of workers who died from other causes, reveals lower selenium levels ($p<0.05$) in the former group. There is no difference in selenium levels between lung cancer and other cancer deaths, however. Tissues from the non smelter environment workers have sgnificantly lower selenium levels ($p<0.05$) [67]. An extension of this study shows lung cancer in those workers with the lowest selenium lung tissue levels compared to controls and to workers dead from other causes [113].

Animal experiments, particularly on the influence of selenium on tumor promotion, should be extended. The possible carcinogenicity of selenium in animals should not, on the existing evidence, be discounted but should be investigated further. The relative lack of occupational studies on the several possible effects of this metal should also be addressed, as it is widely used in the chemical, pigment, glass, and refining industries.

8. Titanium. There have been no reported studies on occupational exposure to titanium dusts in industry [49, 117].

An experimental study on rats exposed to TiO_2 inhalation over a 2 year period (6 hrs/day; 5 days/wk) reports bronchioalveolar adenomas and cystic keratanizing squamous cell carcinomas at a 250 $\mu g/m^3$ dust exposure level; no tumors occur at lower concentrations. The investigators consider the tumors not to be relevant to human cancer due to the differences in their anatomic location and histological type [139]. While obviously not providing a suitable model for human metal respiratory carcinogenesis, the assumption of irrelevance of these observations seems somewhat arbitrary. Experimental observations on other metal-induced tumors at locations and of histological types quite different from those encountered in human exposure have proven to be relevant bioindicators of an enhanced human risk (e.g. rhabdomyosarcomas from implants of Ni_3S_2 in rats).

Another experimental study on the tumorigenicity of titanium shows a 10% (2 fibrosarcomas and 3 lymphosarcomas) tumor incidence from intramuscular implants in rats. No tumors are induced in an equal number of controls implanted with either copper or iron dusts [140].

TiO_2 is co-carcinogenic with B(a)P in the induction of respiratory tract tumors in hamsters [141].

These findings are of interest as titanium alloys are frequent components of such medical devices as surgical prostheses and titanium coated materials for the correction of jaw defects [142]. Although such devices have not been associated with an increased cancer risk [117], their wide use is relatively recent. Life time follow ups on patients with these devices seems advisable.

The inconclusive but suggestive nature of the animal data on this metal, its use in medical practice, and activity in short term assays (Table 4) indicate the need for further studies.

2.2. Activity of metals in in vitro bioassays

The rapid development of a variety of short-term assays for toxicity during the past decade has facilitated the otherwise insurmountable task of screening vast numbers of chemical compounds for mutagenicity and possible carcinogenicity. The results of these assays contribute a rational basis for decisions and priorities in regard to long-term animal testing and epidemiological studies.

A wide variety of short-term *in vitro* assays has been applied to metal toxicity testing. Detailed description of some of these assays and their application to the testing of metals can be found in a

review by Stout and Rawson [143]. Several comprehensive reviews have appeared recently on the activity of metal compounds in *in vitro* short-term assays [50, 51, 144] as well as reviews of selected metals [145–147]. The genotoxic effects of nickel [78, 148, 149] chromium [83, 150], arsenic [83, 151] and lead [152] have been reviewed separately.

Short-term *in vitro* assays in microbial, plant, and mammalian cells are designed to detect genetic damage either as DNA damaging events, as seen for example by DNA strand breaks and repair; or as heritable lesions, as seen by chromosome changes, mutations, and cell transformation. The heritable lesions may be considered to be of greater importance to the organism, and should thus have more weight in safety considerations. Chromosomal changes such as aneuploidy are associated with animal and human neoplasms. Single point mutations result in oncogene activation [153]. Cell transformation *in vitro*, including by carcinogenic metals, is associated with tumor formation in athymic nude mice [154] whereas DNA damage and repair assays are usually, but not necessarily, indicative of deleterious genetic effects, since repair observed after DNA damage may represent an appropriate detoxification mechanism, if such repair is error-free.

2.2.1. Overview and update. An overview summary of the activities of metals in the various *in vitro* tests, combined from information of recent reviews [50, 51, 144] as well as new reports in the literature is shown in Tables 3 and 4. For details concerning specific compounds or species, the appropriate references should be consulted. A variety of assays, including cell transformation, indicate that most metals have some potential for genotoxicity. Positive responses for various forms of metal compounds are far more frequent than carcinogenic events in animal studies. It is not clear at this point whether this is attributable to 'false negative' responses in the *in vitro* test systems, or to their greater sensitivity. It should be kept in mind that certain artifacts exist in culture conditions such as imperfect (in relation to the intact animal) detoxification (per cell) and excretory (per culture) systems, as well as (frequently) proliferative vs terminally differentiated conditions *in vitro*. On the other hand, far more animal than human (via epidemiological evidence) carcinogens have also been documented to date, partly because of differing test strategies. *In vitro* studies, particularly as they can utilize human cells and relevant (to human exposure) test concentrations, may turn out to be useful early warning systems for carcinogenicity of specific metal compounds [see 2.2.1.,d below]. Further, *in vitro* studies may be particularly well suited for identifying those forms of a specific metal that pose the greatest risk to human health [147] and they are proving to be invaluable for investigating mechanisms of metal carcinogenesis. With a comprehensive overview and new test data, several trends are becoming apparent from the *in vitro* studies (Tables 3 and 4):

a) With the exception of chromium (VI) compounds, metals have generally been considered to be relatively inactive in bacterial genotoxicity, particularly mutagenicity, assays [51, 144, 155]. The apparent lack of agreement between eukaryotic and prokaryotic assay results may have been a major barrier for evaluation of metals *in vitro* [145]. Reasons cited for the discrepancy include differing culture media compositions, permeability or metabolic factors, repair mechanisms; or, the concept that mutagenesis, at least in bacterial terms, may not be an important event in metal carcinogenesis. This viewpoint may no longer apply. As shown in the updated summary (Table 3), metals are indeed active in all of the bacterial test systems tried. In some instances positive responses have been observed by 3 or more different research groups, as in the case of bacterial DNA damage assays (Rec/Pol assay, *E. coli*) for the metals arsenic, chromium, cadmium, molybdenum, platinum and selenium. In fact, possibly the most information about the mutagenicity of heavy metals may have been obtained with microbial assays, with the majority of studies using either the *S. typhimurium* or the *E. coli* reverse mutation systems (for review, see Babich *et al.* [146]). Study results have been inconsistent, however, which possibly accounts for the discrepancy. Newer studies have adjusted test protocols to be more sensitive to metal toxicity. For example, Schultz *et al.* [156] show 4 out of 16 or-

Table 4. Activity of less frequently tested metals in *in vitro* short-term tests.[a *]

	Al	Sb	Ba	Cs	F	Ir	Os	Pd	Rh	Ru	Sr	Ta	Tl	Ti	V
In vivo/*In vitro* tests[b]	+	+	+										+		
Cytotoxicity assays[c]			−											+	+
DNA Effects: Biochemical assays[d]	−														
DNA Damage – Bacteria[e]		+		+		+	+		+	−			+		+
DNA Damage – Mammalian cells[f]															
DNA Synthesis inhibition – Mammalian cells[g]															
DNA Repair induction – Mammalian cells[h]															
DNA Repair inhibition – Bacteria[i]															
DNA Repair inhibition – Mammalian cells					+[172]										
Chromosome aberrations – Plant cells		−		+			+			+	+	+	+	+	
Chromosome aberrations – Mammalian cells													+		
Sister chromatid exchanges															
Mutations: Bacteria[j]		−		−		−			+	+			−		+
Yeast and plant cells			+								+				
Drosophila					+										
Mammalian cells											−				
Mammalian cell transformation[k]	−	+												−	
Enhanced virus transformation[l]	−	+										+	+		
Enhanced genotoxicity[m]					+[171]										
Reduced genotoxicity[n]															

* Footnotes as for Table 3.

ganic cobalt compounds to be positive in the *S. typhimurium* mutation assay (Ames Test) with up to 50× enhanced activity over control values, by increasing the lability of the compounds via a methyl substitution on the organic ligands. Arlauskas *et al.* [157] found cadmium, beryllium, chromium, and vanadium compounds to be positive in a variation of the Ames test in which cultures are grown in suspension (fluctuation test), whereas only chromium (VI) and selenium (VI) were positive in the standard Ames test. Beryllium has not to the best of our knowledge, been shown previously as positive in a bacterial mutation assay. Increased activity has been shown when metals were tested in combination with other carcinogenic agents: for example, synergism was observed in the Ames test with cadmium and the nitrosamines MNNG and MNU [158], and enhanced UV mutagenesis in *E. coli* by $CuCl_2$, $MnCl_2$, and $NaMoO_4$ [159], suggesting interference by metals with repair mechanisms (see 2.2.1. below). In other test systems, a lack of positive responses may simply reflect inadequate data. Rossman and Molina [159] conclude that many metal carcinogens are positive in bacterial assays provided that the assay endpoint is broad enough to detect any possible damage to DNA, and that the exposures are done under sub-toxic and long-term growth conditions.

b) A second trend in genotoxicity assays with metals is the increasing use of human cells. For most of the metals shown in Table 3 positive responses for DNA damage, chromosome aberration and sister chromatid exchange have been observed in human cells. The cells used are usually peripheral lymphocytes, fibroblasts, or primary epithelial cells such as kidney [160] or bronchial [161] cells. *In vivo* exposures have also been thoroughly documented, by evaluation of chromosome damage in white blood cells of workers exposed to arsenic, chromium, cadmium, lead, mercury, and selenium (Table 3). Recent studies on the toxicity of metals to human cells have added the following observations to the data base: inhibition of DNA synthesis in bronchial epithelial cells by $NiSO_4$ [161]; chromosome damage in a variety of cell types including kidney by beryllium, nickel, cadmium, cobalt, manganese, and mercury compounds (160, and reviewed by Sunderman [51]); and induction of transformation markers including anchorage independence in fibroblasts and epithelial cells by compounds of arsenic, chromium, nickel, and lead [162].

c) Clusters of positive responses induced by metals are appearing for certain classes of tests (Tables 3 and 4), in particular the biochemical (cell-free) assays which include studies on fidelity of DNA conformation, replication, and transcription. Chromosome tests including aberrations in plant and animal cells as well as sister chromatid exchange (SCE) assays in mammalian cells (for review see Sunderman [51]) and the mammalian cell transformation assays. Positive responses for the latter have until lately been described mainly for Syrian hamster embryo cultures but recent studies have added metal-induced transformation in cell types including human fibroblasts [162] and epithelial cells [160], C3H10T½ mouse fibroblasts [154], and T51B rat liver epithelial cells [199]. These additional observations have usually been achieved by modification of standard test protocols. For example, transformation markers in epithelial cell cultures treated with nickel compounds appeared after long-term (weeks to months) exposure to subtoxic concentrations [161, 199].

The appearance of a greater frequency of positive responses for specific assays is of interest. It may be that more effort has been concentrated in these areas; alternatively these tests may be pinpointing important lesions in metal carcinogenesis. This will be discussed in greater detail in the section on 'Mechanisms'.

d) The data shown in Table 3 contains a note of warning. Those metals classified as established human carcinogens by the International Agency for Research on Cancer (IARC) are heavily positive, in many molecular forms, in all of the short-term assays, in many cases with human cells. The table shows that similar results are already in place for cadmium, mercury, and platinum, with several others following closely. In all cases, chromosome aberrations – a potentially important lesion – have also been observed in human cells. Leopold and coworkers [163] have cautioned, based on data from animal experiments, that the use of cis-platinum (II) complexes for chemotherapy may result

in increased risk of secondary cancers (see: 3.1.1. Platinum complexes below).
e) Negative responses have also been observed in some instances. These may be significant, such as the inability of nickel compounds to induce DNA repair (see: 2.2.2. Recent experimental data, below), or may reflect an ineffective concentration used in the test system, as is probably the case for the assays showing negative responses for magnesium compounds (Table 3). Magnesium is up to 2 orders of magnitude less toxic than the other metals [164]. Several workers have shown that the genotoxic potencies of metal compounds are equal at equi*toxic* doses [51, 165–167]. However equitoxic (with carcinogenic metals) *in vitro* concentrations of magnesium would have very little relevance to human exposure levels.

2.2.2. Recent experimental data. Some additional new information on metal effects *in vitro* is shown in Table 5. Further evidence has been presented for genotoxicity (not *in vivo* carcinogenicity) of nickel and chromium compounds where these are tested at LD_{50} concentrations [162, 166]. Other new data include gene mutations in hamster fibroblasts after prolonged exposure to lead, molybdenum, and zinc compounds [170], and examples of specific chromosome damage [161, 173] and altered gene expression [161, 166, 174]. Some of these observations will be discussed further in section 2.3. under Mechanisms of Metal Carcinogenesis.

Interest should be focused on accumulating evidence that various metals, but possibly not chromium (VI), can inhibit DNA excision repair (see also Rossman [175]). Hexavalent but not trivalent chromium compounds have been shown to be proficient inducers of three SOS repair genes in *E. coli* [176]. An interference with DNA repair mechanisms was probably first demonstrated for bacterial systems, in which metal toxicity has frequently been measured as enhanced mutations or killing of repair – deficient strains of *E. coli,* or *B. subtilis* [147]. Rossman & Molina [159] recently demonstrated the ability of arsenic, cobalt, copper and molybdenum to interfere with UV-induced excision repair in one such strain. A similar effect of arsenic was observed in human epidermal cells by Jung *et al.* [177]. With the exception of chromium, only isolated reports of DNA repair induction in mammalian cells have appeared, for arsenic, plati-

Table 5. New information from *'in vitro'* experiments (1984–1986)

Compound	Target cells	Observed response	Reference
KH_2AsO_4, $NaAsO_2$, $NaHAsO_4$	human fibroblasts	Anchorage independence at LD_{50} concentrations	[162]
$NaAsO_2$	*E. coli*	Interference with excision repair	[159]
$Be(NO_3)_2$ $BeSO_4.4H_2O$	*S. typhimurium*	Mutations	[157]
Cr_2S_3, $CrCl_3$, CrO_3, KCr_2O_7 $CaCr_2O_7$, $PbCrO_4$, CrO_3	human fibroblasts	Anchorage independence at LD_{50} concentrations	[162]
$CdSO_4$	mouse hepatocytes	Double minute chromosomes, expression of metallothionein gene	[174]
Cu^{2+}	mouse lymphoma cells	Cytotoxicity of trace amounts	[180]
$NiSO_4$	human bronchial epithelial cells	Inhibition of DNA synthesis, marker chromosomes, altered terminal differentiation	[161]
$Ni(CH_3COO)_2$, NiO, $NiSO_4$, Ni_3S_2	human fibroblasts	Anchorage independence at LD_{50} concentrations	[162]
Ni_3S_2, $NiCl_2$	rat liver cells	Mutations, inhibition of DNA synthesis and repair, altered cytokeratin, altered differentiation	[166]
NiS, $NiCl_2$	hamster fibroblasts	Selective damage to heterochromatin	[173]
Pb $(NO_3)_2$ Na MoO_4 Zn Cl_2	Chinese hamster lung cells	Mutations (5 day exposure at maximum non-toxic concentrations)	[170]

num, and selenium (Table 3). Bianchi *et al.* [150] reported Cr (VI) to be positive in all tests of a battery of bacterial and mammalian cells except for DNA damage and UDS in human fibroblasts. Nickel has been reported as negative in the UDS assay [166]. Inhibition of repair or repair enzymes, on the other hand, has been shown for arsenic, nickel, cadmium, lead, zinc, mercury and selenium (Table 3). The remarkable ability of nickel compounds to inhibit excision repair, rendering the cell vulnerable to other DNA damaging agents, is illustrated in Fig. 1. A distinction should be made between excision and strand break repair. Strand break rejoining after DNA damage has been observed for nickel compounds [217], in the absence of excision repair visualized autoradiographically (UDS assay). Klein *et al.* [172] demonstrated the inhibition of both excision (via endonuclease/polymerase effects) and strand break (ligase effects) repair, in mouse spleen cells by fluoride and cadmium ions. Evidence for enhanced genotoxicity in the presence of other carcinogens described in numerous recent reports [42, 55, 158, 168, 171] would be a logical consequence of DNA repair inhibition.

Loch-Caruso and co-workers [178] have recently demonstrated that compounds of arsenic, cadmium, mercury, lead, and nickel, but not aluminum or zinc inhibit metabolic cooperation (intercellular communication), a property of tumor promoters, in cultured V-79 cells. A hypothesis for the role of failed intercellular communication in the cancer process has been proposed [179].

2.3. *Mechanisms of metal carcinogenesis*

The ultmate mechanism, if there is a single one, by which metals initiate the carcinogenic process is still unknown. However, many hypotheses have emerged, based for the large part on molecular events observed in *in vitro* (whole-cell and cell-free) systems; for example, on mechanisms of metal-DNA interactions. These hypotheses have been reviewed and updated at regular intervals, as new information becomes available (e.g. 49, 51, 79). Major concepts and new findings will be briefly reviewed below. Recent work has included studies on the role of bioavailability of metals for intracellular targets, cross linking of DNA with specific proteins, and metal-induced oncogene activation.

2.3.1. Bioavailability factors. The intracellular bioavailability of carcinogenic metals for target molecules depends on the chemical properties of the particular metal compound and their effect on membrane transport, microsomal metabolism, and cellular detoxification mechanisms (for a concise and lucid overview of the properties and coordination chemistry of metal ions in relation to their carcinogenic potencies, see ref. [181]). Metal compounds, depending on their size, charge, oxidation state, etc., can penetrate cell membranes by passive perfusion or mediated permeation such as the phosphate or sulfate transport systems and phagocytosis (for reviews see [51, 146, 182]). For example, correlations have been proposed for carcinogenic potencies of various nickel compounds with surface charge, serum dissolution rates, and phagocytic indices (reviewed by Sunderman [51]). Whereas phagocytosis and intracellular translocation of particulate nickel compounds may be important toxic events in some cell types *in vitro* (hamster fibroblasts [183], rat liver cells [166], macrophages and rhabdomyosarcoma cells [184]), and possibly contribute to enhanced metal ion bioavailability, correlations with carcinogenicity have not been established *in vivo*. Kuehn *et al.* [185] find no significant correlation between phagocytosis of nickel and sarcoma induction in rat muscle. Sunderman and co-workers report investigating the induction of renal cancers by 17 nickel compounds, and finding no correlation between tumor induction and mass fraction of nickel in the 17 compounds, dissolution half times, or phagocytic indices [186]. *In vitro* electron microprobe analysis of rhabdomyosarcoma cells treated with various nickel compounds suggests that phagocytosis is not correlated with tumor induction but serves as an excretory mechanism [187], however, differing behaviour of tumor vs. normal cells can not be ruled out. Hansen & Stern [167] conclude that toxicity may arise at any cellular location of nickel, whether membrane-bound, phagocytosed, or available from solution.

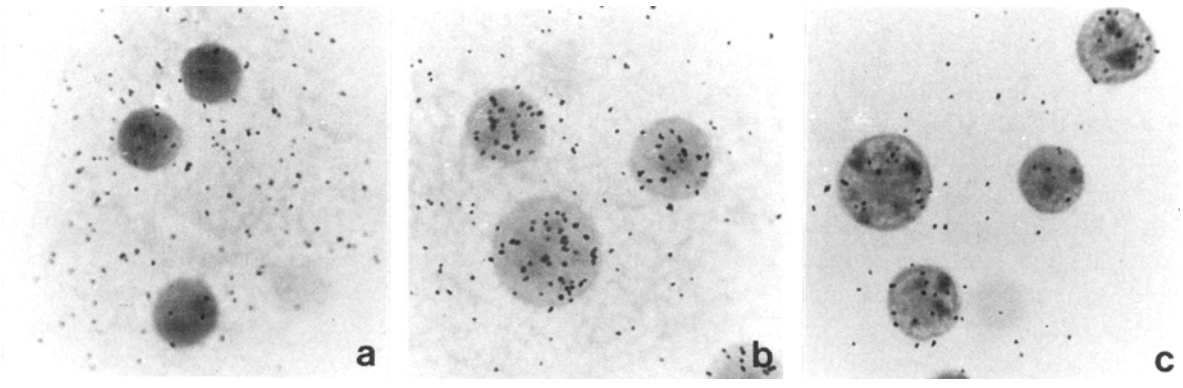

Fig. 1. DNA repair, visualized as grains over nuclei of freshly isolated rat hepatocytes. (a) $NiCl_2$-treated cells showing an apparent negative response; (b) positive response following treatment with methylmethanesulfonate (MMS); (c) inhibition of MMS-induced response in the presence of $NiCl_2$. 400 ×

Microsomal metabolism may play a role in the genetic activity of some metals. The presence of heavy metals can influence the metabolism of other carcinogens (see: 2.3.4. Altered cellular detoxification). In addition, metals may themselves be metabolized by microsomal enzymes. Differing behaviour of tri and hexavalent chromium compounds in cellular metabolism is an interesting example of differing bioavailability patterns (reviewed by Sunderman [51], de Flora *et al.* [188], Babich *et al.* [146]). Chromium (VI) but not chromium (III) compounds are considered human carcinogens, and similarly are very active in genotoxity tests, whereas Chromium (III) compounds are usually unable to cross cell membranes. On the other hand, trivalent chromium compounds induce DNA damage in isolated nuclei, purified DNA, or in intact cells if entry is made possible by suitable ligands or endocytosis [188]. Rat liver cells and microsomal preparations can reduce Cr (VI) to Cr (III) through the P-450 system (reviewed by Babich *et al.* [146]), suggesting that this step is a prerequisite for interaction of chromium with DNA [189]. Rafetto *et al.* [165], and de Flora *et al.* [188], however, provide evidence that Cr (VI) and Cr (III) both interact with DNA in bacteria but by different mechanisms, i.e., by unbalancing nucleotide pools, and by cross-linking activities, respectively [188]. Differences have also been observed in microsomal metabolism of Cr (VI) compounds with different tissues (rat liver vs. rat lung microsomes [155]). Thus, genotoxic activity by both forms of chromium, where these are able to enter cells, is likely.

Under culture conditions, the genotoxic potency of metal compounds appears to depend on the bioavailability of the metal or metal ion, independent of source or uptake mechanism. Different metals and different compounds of the same metal have widely differing cytotoxicities at equimolar concentrations. By means of a sensitive cytotoxicity assay based on neutral red incorporation into intact lysosomes of mouse fibroblasts, Borenfreund and Puerner [190] have ranked metals by cytotoxicity; for cations (as chlorides) in the order Cd > Hg > Ag > Zn > Mn > Cu > Co > Ni > Cr (III) and for anions (as sodium or potassium salts) in the order $Cr_2O_7 > CrO_4 > AsO_2 > AsO_4 > SeO_3 > SeO_4 > MnO_4$. However, at equitoxic concentrations, different metals, including compounds of carcinogenic metals with no apparent *in vivo* carcinogenic potency, have similar genotoxic potencies. The critical cytotoxic concentration for the induction of heritable toxic events in cultured mammalian cells for several assays appears to be ≤50% survival. Other factors such as metal-induced pH changes in the culture milieu can influence genotoxicity, and should be considered.

2.3.2. Cross-linking. Metal cations are powerful binding and cross-linking agents of DNA and proteins. Evidence is accumulating that the resulting

structural and conformational distortions of DNA, chromatin, or enzyme matrices can account for most, if not all, the observed toxic effects of metals, such as inhibition or infidelity of scheduled and unscheduled DNA replication, altered enzyme activities, structural and behavioural chromosome changes, and altered gene expression. In addition to conformational changes, cross-links can result in single-strand breaks resulting in mis-repair and single-point mutations.

a) Cross-linking, DNA structure and behaviour

Binding. The ability of metals to bind to DNA, RNA, and protein has been well documented (for further detail, see [51, 182, 191]). In general, transition metals interact with DNA and polynucleotides at electron donor sites such as the oxygen and nitrogen atoms of the bases, and by complexing with the phosphate groups in the DNA backbone [182, 192, 193], leading to cross-linking with adjacent DNA strands, intercalation, and cross-linking with adjacent proteins. Persisting DNA-protein and metal-DNA adducts, with partial or no repair, have been demonstrated [191, 194].

Conformation changes. Several studies have shown that carcinogenic metals can induce structural anomalies in the DNA molecule (for review see Bourtayre *et al.* [195]). Carcinogenic and mutagenic nickel compounds as well as Mg^{2+}, Mn^{2+}, and Co^{2+} induce a right–to–left handed helix conformation (B→Z) transition of double-stranded poly (dG-dC), regardless of the form of nickel [196]. In the Z form, the phosphodiester chain adopts a zig-zag shape (hence the name Z-DNA). Nickel ions appear to stabilize this structure. Bourtayre *et al.* [195] postulate that this stabilization, which is also induced by the organic carcinogen acetylaminofluorene and is preventable by an antitumor agent, may be a carcinognic event. Diminished template efficiency by the Z form has been shown for *E. coli* RNA polymerase. Antibodies to the Z form are available for further studies [197].

Interstrand cross-linking would further be expected to result in altered cleavage specifications of endonucleases, altered condensation of DNA into chromosomes (see also: 2.3.2., Inhibition of DNA synthesis), and altered templates [182] leading to errors in replication, transcription and protein synthesis, possibly through interfering with DNA unwinding for normal replication or repair. These events could account, at least in part, for the observed well-documented effects of metals on fidelity of DNA and RNA synthesis (reviewed by Sunderman [51, 78]). Direct effects of metals on DNA and RNA polymerases have also been reported frequently (reviewed by Sunderman [78]). For example, Be (II) forms a strong complex with DNA polymerase I [182]. Copper (II), lead (II), and cadmium (II) reversibly inhibit human DNA polymerase by direct interaction with the enzyme, but not at the catalytic magnesium, manganese or zinc sites [192].

Inhibition of DNA synthesis. Toxic metals (As, Ci, Ni, Cd, Co, Sn, Hg, Pt, Se) cause prolonged, that is, persisting after fresh medium changes, inhibition of DNA synthesis in cultured cells. A direct effect on essential enzymes, for example at metal-sensitive sulfhydryl sites or by substitution of metal catalysts, is possible. Divalent nickel is capable of substituting itself for other divalent metals in sites in enzymes and proteins [191]. Inhibition could also be mediated via structural changes (though cross links); directly, at enzyme levels, or indirectly, by a switching-off of proliferation signals. Switching-off of proliferation signals could occur via changes in gap junctions [179], cytoskeletal structure, or membrane receptors for growth factors (see: 2.3.2. (c)). Evidence for such a mechanism is coming from observations such as differentiation induction *in vitro* after exposure to metals [161, 198, 199] and inorganic fibers [35]. Interestingly, these observations usually involve altered differentiation of cytokeratins, a feature of many human carcinomas.

b) DNA-protein cross-links

Cis and trans platinum, chromium (VI) and chromium (III), nickel compounds, and beryllium (reviewed by Sunderman [51]), have been shown to crosslink DNA and cellular proteins. Advances are being made in identifying specific proteins that are cross-linked to DNA by metal ions, especially by

nickel. Complexing with histone and non-histone nuclear proteins by nickel or beryllium has been shown by several workers [51]. Ono *et al.* [200] suggest that the cross-linked proteins may include nuclear matrix and chromosome scaffold proteins. This was confirmed by Borochov *et al.* [193], who showed chromatin cross-linking and compacting by first row transition metals manganese, cobalt, nickel, and copper. Patierno *et al.* [201] show that sub-toxic concentrations of nickel induce DNA-protein crosslinks during late S-phase in Chinese hamster (CHO) cells. The molecular weights of the crosslinked proteins range from 20 to 95 K daltons, and are thought to be nonhistone chromosomal protein, DNA binding proteins, and histone-1. Additional experiments suggest that the nickel preferentially bound to heterochromatin [202]. Recently, in an elegant set of experiments, Wedrychowski *et al.* [203] show (by electrophoretic separation of cross-linked proteins followed by their identification with specific monoclonal antibodies) that chromium and cis-platinum (cis-DDP) cross-linked DNA and the same 3 major cytokeratins (of approximately 39, 49, and 56 K Da) in intact Novikoff hepatoma cells. These cytokeratins co-isolated with chromatin. A differential ability of cytokeratins to associate with DNA was observed.

c) DNA-keratin crosslinks, chromosome changes, and altered gene expression

Fractionation of tissues from animals exposed to radioactive metal compounds frequently shows association of DNA with metal, possibly due to the presence of crosslinked proteins, but autoradiographic or electron microprobe analyses of metal-exposed cells *in vitro* do not show significant localization of metal in the nucleus [187, 200]. On the other hand, metals are strongly clastogenic (Table 3). Interaction of metals with keratins offers a possible explanation for the observed chromosome-damaging effects. The affinity of metal ions for cytoplasmic cytoskeletal components has been known for some time [204, 205]. Nearly 20 years ago Pearce [206] described a histochemical method for keratin staining of tissue sections using nickel. Recent technical advances allow visualization of intact intermediate filaments in extracted whole mount preparations of cultured cells by electron microscopy. These have shown that cytoplasmic keratin filaments (identified by immunogold precipitation), the major class of intermediate filaments of epithelial cells, adjoin at the pore complexes of the nuclear membrane [207, 208]. The nuclear membrane during interphase is lined by heterochromatin [209] and lamin [210, 211], another filament-metal binding candidate which is within cross-linking distance of cytokeratin filament ends. Heterochromatin contains a high proportion of reiterative DNA sequences and is susceptible to metal binding [209] and damage [173]. Heterochromatin and lamin are thought to play a role in organizing spindle microtubules and folding patterns of the chromosomes during condensation; possibly via essential metals [193]. Inappropriate cross-linking at these sites may thus result in chromosome aberrations [173] or altered gene activation by transposition. Chromosome aberrations indicative of gene amplification, such as double minutes have been induced by cadmium [174], nickel [160], and arsenic [56]. Direct action of toxic metals on spindle proteins can also contribute to chromosome abnormalities such as aneuploidy.

Binding of metal ions to cytokeratin filaments can result in abnormal morphologies of filament networks of treated cells. This has been observed recently by immunofluorescence with specific antibodies during transformation of rat liver cells by nickel compounds [166]. Altered expression of intermediate filaments has also been observed after oncogene transfection in these cells (N. Marceau, personal communication). Changes in morphology and expression of intermediate filaments after nickel treatment are accompanied by altered differentiation patterns in these cells [199]. Blobel [212] has postulated a role for nuclear filaments and the nuclear pore complex in maintenance and control of distinct three-dimensional structures related to expression of differentiation states (gene gating). Distortions of nuclear pore complexes by metal-keratin cross-linking would profoundly affect such a mechanism, possibly leading to aberrant differentiation, which may play a role in the carcinogenic process. For example, Huber *et al.* [213] suggest that increased oncogene expression early in

liver carcinogenesis is differentiation, not proliferation, related. The tumor growth factor TGFβ promotes differentiation in a human squamous carcinoma line [214]. A high degree of differentiation is a common feature of nickel-induced animal tumors.

2.3.3. DNA strand breaks and mutations. Metal-induced DNA strand breaks have been observed in a variety of cell types by alkaline elution, density gradient, and fluorescence analysis of DNA unwinding techniques (for review see Sunderman [51]). Strand breaking efficiencies by different metals appear to be variable; tin (II) for example, but not tin (IV) appears to resemble γ radiation in its efficiency for strand break induction [215]; whereas induction by nickel or mercury compounds has appeared equivocal [51], often requiring cytotoxic concentrations [216]. Tin-induced strand breaks are repaired within minutes by rat liver cells [217] whereas nickel damage may persist, illustrating differing mechanisms and significance of metal-induced breaks. Dose-dependent strand break induction at increasingly toxic doses may reflect lethality rather than significant genotoxicity [166].

The fact that mutations and transformations of mammalian cells by metals has frequently been observed at $>LD_{50}$ concentrations suggests that strand break induction with genetic consequences may not be of primary importance in metal carcinogenesis. However, breaks do occur, whether by direct or indirect (e.g., endonuclease action for crosslink or adduct removal) action, and it can be argued that misrepair (e.g., through polymerase infidelity) can result in important single point mutations leading to oncogene activation. Cellular oncogenes have been identified in many human chromosomes, frequently at breakpoints (sensitive sites) that occur in certain malignancies (reviewed by Reith and Brogger [149]). Oncogene activation is thought to occur after qualitative (e.g., single-point mutation [218]) or quantitative change in gene expression, and has been observed after tumor induction with various carcinogens including ionizing radiation (strand break inducer) in rat skin [219], and cisplatin (crosslinking agent) in mouse skin [220].

2.3.4. Other contributing factors

a) Free radicals. Free radicals are highly reactive molecules that have an odd number of electrons. Attention has been focused recently on the role of free radical damage in carcinogenesis. Many transition metals can act either as metal-enzymes or catalysts of oxidation of other compounds to produce peroxides, epoxides, and free radicals such as the hydroxyl ion, which can cause DNA strand breaks, mutation and cell death or lead to lipid peroxidation [182, 221–223]. Metal-induced or catalyzed lipid peroxidation allows changes in membrane structure and function including disruption of intercellular junctions and cytoskeletal anchorage sites leading to altered signal transmission [179, 224]. *In vivo* evidence for metal induced lipid peroxidation occurs as chromolipid granules in various organs of animals treated with iron, copper, lead, bismuth, mercury, silver, and gold (reviewed by Squibb and Fowler [221]). Lipid peroxidation is discussed further in Section 3.4.

A contribution of macrophage activity in metal tumor induction should also be considered. Macrophages are often conspicuous in inflammatory lesions caused by metals and inorganic fibers. Factors released by initiated macrophages such as neutral proteases, complement components, endogenous pyrogens, oxygen metabolites, prostaglandins, and growth factors may all contribute to toxicity or altered behaviour of adjacent cells (reviewed by Hamilton [32]). A role for macrophages in oxidative damage induction is well documented [222, 225, 226].

b) Altered cellular detoxification. It has been shown that hepatic microsomal P_{450} synthesis is inhibited by many divalent metal ions including cobalt (II), cadmium (II), mercury (II), copper (II), nickel (II) and lead (II) [182], suggesting a further role for metals in mediating carcinogenesis induced by other initiating agents (e.g., from cigarette smoke; see also Inhibition of Repair, Section 2.2.2.). Enhanced cell killing, genotoxicity, and tumor formation (co-carcinogenesis) by metals has been observed especially for benzo(a)pyrene and nitrosamine carcinogenesis (Tables 3 and 4, Nordberg and Anderson [53] – review). Altered benzo(a)-

pyrene metabolism is observed in perfused rat lungs after exposure to nickel and cadmium, but not cobalt [227]. After asbestos and iron treatment microsomal enzymes from rat liver also alter benzo(a)pyrene [42, 228]. Altered rates of uptake of benzo(a)pyrene into microsomes is considered a factor.

Cellular non-protein sulfhydryls, consisting essentially of glutathione and other aminothiols such as cysteine and cysteamine, are thought to have significant free-radical scavenging abilities [229]. Changes in reduced glutathione and concomitant enhanced cytotoxicity have been observed by a number of SH reagents including metals [230]. This type of toxicity, however, may be restricted to hypoxic cells. Hei *et al.* [229] showed that non-protein sulfhydryl depletion did not result in additional cytotoxicity, sister chromatid exchanges, or cell transformations following γ-irradiation of aerated mouse fibroblasts. Enhanced radiosensitivity has been shown following cisplatin treatment under anoxic conditions, suggesting a mechanism for the observed synergism between cisplatin and radiotherapy for anoxic tumor tissues [231]. Similarly, Popenoe and Schmaeler [192] show that inhibition of human DNA polymerase B by copper, lead, and cadmium was not affected by pretreating the enzyme with a sulfhydryl reagent. Effects of metals on another major cellular detoxification mechanism – DNA repair was discussed in section 2.2.2.

2.4. Conclusions and unanswered questions

The metals discussed in section 2 are classed according to their weighted carcinogenic potential in Table 6.

The risk of cancer from environmental exposure to specific metals is largely restricted to occupational exposures.

The concentration of specific (single) metals in the general environment is rarely sufficient to constitute a measurable risk. However as cancer risk from most inorganics is through their interactions with other carcinogenic agents, the actual risk from such sources as the combustion of fossil fuels may be considerably greater than the individual atmospheric metal measurements might indicate.

Reviews of animal and epidemiological evidence show that the carcinogenicity of arsenic often results from interaction with other carcinogenic agents and that the effects of arsenic may be exerted in the later stages of carcinogenesis.

The causal association of cadmium with occupational prostate and lung cancers is still unresolved. The dose-response related induction of lung tumors from cadmium acetate inhalation in rats and its consistent positive activity in *in vitro* assays, makes it strongly suspect.

Elements listed in the possible carcinogen category are almost all co-carcinogens. The carcinogenic risk from these elements is probably dependent on rather specific environmental and/or physiological conditions, thus:

a) Iron oxide is apparently inactive as a chemical carcinogen but interacts with radon daughters and probably PAH in inducing lung cancer in the underground hematite mining environment.

b) Conflicting tumorigenic and anti tumorigenic reports on zinc and selenium can probably be attributed to their being essential trace metals, the effects of which will be profoundly affected by their intracellular availabilities and the physiological state of the host organisms.

Although not reviewed in section 2.1., platinum is another element that should be considered a possible carcinogen pending the long term findings from its use in chemotherapy as discussed in section 3.1.1.

The most pressing single requirement in environmental carcinogenesis research is the elucidation of the interactive role of specific metals. Clarification of this role in relation to other inorganic, organic and physical carcinogens encountered in the environment is essential to rational risk assessment and prevention.

In vitro bioassays should point the way to the appropriate animal experiments and epidemiological studies required. Choice of the proper animal model for the investigation of specific inorganic carcinogens deserves more attention than it has generally received.

It appears likely that cellular neoplastic transformation by various metal compounds results from an interaction of events related to the ability of metals to bind and crosslink cellular proteins, especially structural proteins. These interactions can lead to intracellular toxicity, altered signal transmission, altered gene expression, and altered cellular detoxification mechanisms which enhance the vulnerability of the cell for organic carcinogens. Chromosomal effects may be more important for neoplastic progression in mammalian cells than strand breaks and mutations unless these result in deleterious misrepair or oncogene activation. The type of metal induced damage observed *in vitro* may be a reflection of concentrations used.

Further understanding of mechanisms will come from new oncogene studies. The identification of specific genes that are turned on in response to carcinogenic metals is already in progress. Determining which of these lead to neoplastic transformation is expected to follow. A role for aberrant differentiation in epithelial cells, possibly mediated by altered keratin expression, in the initiation of carcinogenesis should be explored further.

The importance of *in vitro* studies in providing information about the risk of metal compounds is established. The importance of positive *in vitro* results in the absence of other data; for example for essential metals such as zinc [170] should be considered. Information concerning the toxicity of metals *in vitro* should be extensively expanded for cells from human tissues.

Table 6. Carcinogenicity classification of inorganic metals

Carcinogen class	Inorganic element	Epidemiological studies[a]	Animal experiments[b]	Short term assays[c,d]
Established	As	+	±	+
	Be	+	+	±
	Ca	+	+	+
	Ni	+	+	+
Suspected	Cd	±	+	+
	Pb	±	+	−
Possible	Al	(±)	±	
	Co		±	+
	Cu	(±)	±	±
	Fe	(±)	±	±
	Se		±	±
	Ti		±	±
	Zn	(±)	±	+
	Mo			±
	W	(±)		
	Pt		±	+
	Hg			+
	Mn			+

[a] + Evidence considered conclusive
± Evidence indicative but inconclusive
(±) Inconclusive evidence of co-carcinogenesis
[b] + Tumor induction in 2 or more species (preferably one non-rodent)
± positive results limited to one species
[c] + activity in all classes of tests
± limited data available but including activity in DNA damage and chromosome tests in more than one species including mammals.
[d] Authors' designation of criteria. Risk evaluation guidelines are lacking

3. Risk from clinical exposure to inorganics

3.1. Therapeutic procedures

Several pharmaceuticals in which metals form all or part of the active ingredients are currently in use in North America and elsewhere. The two most widely used drugs for which concerns have been raised in the literature, regarding a posible link with induction of new cancers, are the antineoplastic cis-platinum coordination complexes such as cisplatin (generic name), and the drug lithium, which is used in the treatment of leukopenia and in the chronic drug therapy of psychiatric patients.

3.1.1. Platinum complexes. Platinum coordination complexes are currently being used in the treatment of human cancers. Cis-diaminedichloroplatinum (II), which was first discovered by Rosenberg and co-workers (reviewed by Rosenberg [231]) to inhibit bacterial cell division but not growth (the bacterial rods grew into long filaments), and cisplatin combinations are presently the first choice treatment of patients with disseminated testicular carcinoma and ovarian cancer [232].

Cisplatin is thought to interact directly with DNA by forming DNA interstrand and DNA protein crosslinks, mainly at regions that are rich in guanosine and cytosine [231, 233]. Eastman [233] showed crosslinks adducts at GG, AG, and GNG sequences by separation of platinum-treated nucleosides by enzymatic digestion and HPLC. It is likely that such lesions can constitute carcinogenic events (see: Section 2.3.2.). Platinum coordination complexes are active in all genotoxicity assays (Table 3) and have caused tumors in experimental animals [50, 163]. Their use in chemotherapy, with intravenous, high dose administration should be considered as constituting a risk factor for secondary cancers in long-term survivors; thus involving a risk/benefit decision.

At chemotherapeutic doses cisplatin is nephrotoxic. Reduced glomerular filtration rates, increased BUN and serum creatinine values, and severe renal proximal tubular necrosis have been observed in rats receiving equitherapeutic doses [234, 235]. Mikheal *et al.* [236] have recently reported extensive pathological changes in kidneys of 30 patients after cisplatin treatment that included degeneration and necrosis of the proximal convoluted tubules, dilation of distal tubules, and glomerular abnormalities. Certain procedures such as hydrating the patient or treatment by slow infusion apparently ameliorate the nephrotoxicity of cisplatin without affecting its antitumor activity [231]. Biochemical approaches to toxicity reduction are in progress. Bark and Magin [237] report that binding of platin with thiosulfate or selenite reduces nephrotoxicity in mice without affecting (in the case of selenite) its antineoplastic activity. Perez-Soler *et al.* [238] reduce the toxicity of a cisplatin analogue by liposome encapsulation.

Toxic response to cisplatin chemotherapy, including that of developing secondary cancer, varies widely among individuals. Consideration might profitably be given to the development and validation of a chromosome analysis or other screening procedure for the identification of high risk individuals, particularly in the case of non terminal younger patients.

Cisplatin treatment can also induce leukopenia and thrombocytopenia [235], as well as damage to the lining of the intestine [231, 234]. To overcome these toxic effects as well as tumor resistance to cisplatin, other analogues are being investigated, such as N-methyl-iminodiacetate-diaminocyclohexane platinum II (MIDP) [234], tetraplatin [235], and carboplatinum [239]. Data are lacking on their potential genotoxicity and carcinogenicity.

Other metals as potential chemotherapeutic agents. Since some evidence indicates that dietary selenium supplement can reduce the incidence of virally and chemically induced tumors (reviewed by Gilman and Swierenga [50], see also 2.1.3.) and can reduce the growth of tumor cells *in vitro* [240], selenium compounds have been proposed as potential chemotherapeutic agents. Fico *et al.* [240] show that selenium compounds result in 10–75% growth inhibition at 0.075 μg/ml in canine mammary tumor, but not non-neoplastic cultured cells. The cytotoxic potency of the various selenium com-

pounds is: selenodiglutathione > sodium selenite > selenocystine > selenomethionine, and is thought to be due to altered RNA transcription and inhibition of protein synthesis [240]. Buell [241] reviews the animal and human toxicity data and suggests that selenium chemotherapy should be viewed with caution until the complex metabolic interactions of selenium especially selenomethionine, are better understood. In the light of considerable genotoxicity (including chromosome aberrations) and animal carcinogenicity data, as well as the continuing uncertainty of the role of dietary selenium in cancer prevention (see section 2.1.3.), this seems prudent.

Organo-gold complexes represent a novel class of antitumor agents. Bis[1,2-bis-(diphenylphosphino)ethane] gold(I)chloride suppresses growth of transplantable murine tumors without nephrotoxicity [242], and of human colon adenocarcinoma cells *in vitro* [243]. DNA single strand breaks and DNA-protein crosslinks, along with S-phase-specific inhibition of growth are observed [243].

Titanium compounds have also been tested in animal tumor suppression models. Titanocene dichloride and titanocene dibromide are more effective with less toxicity, than cisplatin against a human colon carcinoma transplanted into athymic mice [244], suggesting their possible use in chemotherapy for tumor types not easily treatable with platinum complexes. The titanium compounds appear to increase the immunity of the host against the tumor cells [245].

3.1.2. Lithium. Lithium carbonate and other lithium compounds are widely used in the long-term treatment of mental disorders such as mania and depression [246], schizophrenia [247], and alcoholism [248]. In addition, lithium is used in the treatment of leukopenia, for example, after systemic chemotherapy, as it has been shown to cause leukocytosis via a selective increase in the number of circulating neutrophils (reviewed by Leber [249]). Although such an effect has been shown in cancer patients by several researchers; for example, in the treatment of small cell carcinoma of the lung [250], it is not observed by others, as in the case of acute myeloid leukemia therapy [251, 252]. In fact, in one study, the incidence of total remission is lower in lithium-treated patients [252]. Several reports have suggested that lithium therapy does not contribute to the longevity of the patient [252, 253]. The mechanism of lithium induced leukocytosis is thought to involve a direct stimulation of granulocyte production [249]. Several experimental studies have shown altered hemopoietic stem cell differentiation after lithium exposure [254, 255].

Adverse effects of lithium therapy include nephrotoxicity [256, 257], neurotoxicity [258], thyroid function effects [259] sinus node dysfunction [260], and sudden death [253].

There is some concern that lithium therapy may also stimulate the production of abnormal (leukemic) stem cells. Various (>15 in the last 8 years) anecdotal reports have appeared that link lithium induced leukocytosis with the induction or reinduction of acute and chronic monocytic leukemia [249, 261–264]. No increased evidence of leukemia is observed in one group of patients on long-term lithium therapy for manic depressive illness [265], and no enhanced association with lithium treatment is observed in two retrospective studies of leukemia and psychiatric patients [266, 267]. Nevertheless several investigators share the view that the frequency of hematological malignancy in patients on lithium may be greater than chance [261] and that there is a need for a well designed prospective study that specifically follows up each subject [267].

Carcinogenicity and genotoxicity data are lacking for lithium compounds except for numerous reports on chromosome aberrations in plant cells (Table 3). Since some uncertainty exists about the efficacy of adjunct lithium treatment in chemotherapy [252, 253], perhaps it should be viewed with caution.

3.1.3. Other drugs. Numerous prescription and over-the-counter drugs for general health care contain potentially hazardous metals. Ointments and lotions for various skin irritations such as acne or poison ivy may contain considerable amounts of titanium, zinc, mercury, or lead. Mercury compounds are frequently found in ophthalmic solu-

tions, and in some diuretic pharmaceuticals. Dental materials, particularly those used in root canal therapy may contain titanium, bismuth, zinc, and even arsenic and creosote. Chromium chelates are found in popular 'vitamin supplements,' including in pre-natal preparations where they seem to seem to present an unnecessary risk. Usually chromium is present in small amounts as part of multi-vitamin complexes, but sometimes in larger amounts as separate supplements. Presumably the Cr (III) form is used here and is not readily absorbed intracellularly; however it could be argued that inflammatory conditions such as chronic inflammation of the gut with the presence of phagocytic cells could contribute to individual risk (for a discussion of the genotoxicity of Cr (III), see Section 2.3.1.). Similarly, the use of metals in ointments that are in contact with skin lesions could contribute to risk by means of intracellular toxicity. Frequently, the metal components of these pharmaceuticals may have a trivial purpose, such as providing colour, where they are present in far greater than the required essential trace amounts. A more conservative use of metals in pharmaceuticals should perhaps be considered.

3.2. Diagnostic procedures with radioactive pharmaceuticals

Irradiation by radium or X-ray treatment for very benign or non-disease conditions [268], such as X-irradiation for enlarged thymus glands in children, ringworm of the scalp, inflammatory diseases of the breast, and acne vulgaris, has occurred as recently as the 1950's. This excessive and unnecessary exposure should be kept in mind when considering safety of the current uses of radiopharmaceuticals.

Radiopharmaceuticals are radiolabelled compounds that are used as injectibles for diagnosis or therapy. The gamma photons they release from locations inside the body are converted by external scintillation detectors (gamma-cameras) to medically useful images or scintigraphs.

Many radiodiagnostics are inorganic compounds. With the exception of iodine-131 (^{131}I), all of the popular radiodiagnostics decay by electron-capture.

In this process, the nucleus captures an orbital electron and concomitantly releases one or more gamma photons as a new daughter nuclide is formed [269]. The daughter subsequently emits characteristic X-rays and Auger electrons during de-excitation as the orbital vacancies are filled [269].

The dosimetry of the gamma photons released by radiopharmaceuticals and the risk associated with them is well established. However, the contribution made by the low energy Auger electrons to absorbed dose (and hence to risk) cannot be assessed adequately by conventional means, and their contribution may have been underestimated (see below).

3.2.1. The present method of expressing risk from radiopharmaceuticals

a) Effective dose equivalent (EDE). Radiopharmaceuticals are generally considered to provide a net benefit to society at a cost of a small increase in the estimated number of radiation-induced cancers and unwanted genetic effects. The effective dose equivalent (EDE) is the current means of expressing the risk from a radiopharmaceutical. It is a single quantity, derived from average organ doses, which expresses the total risk (cancer and genetic) from an exposure. Risk evaluation therefore begins with an assessment of the average radiation dose absorbed by specific organs.

The magnitude of any organ dose depends on the initial ditribution of the radiopharmaceutical in the body, the number of nuclear disintegrations which occur in each organ and the energy imparted to each organ per nuclear disintegration. This energy is a function of organ mass (for the gamma photons only). These factors are then combined by application of methods developed by the medical internal radiation dose (MIRD) committee of the Society of Nuclear Medicine to give an estimate of average organ dose [270].

After the dose has been established a quality factor (Q) of 1 for X, gamma and beta radiations, and for Auger electrons[1], is applied to each organ

[1] Recent evidence (discussed in a subsequent section) indicates that at intracellular locations, Auger electrons should be assigned a Q which is considerably greater than 1.

dose to obtain a mean organ dose equivalent [271]. This quantity is multiplied by a weighting factor to reflect the radiosensitivity of each organ and then all weighted mean organ dose equivalents of the most radiosensitive organs are summed to give a mean whole body dose equivalent, the EDE. The EDE is then related to currently accepted risk estimates which for a 1 Gy whole body exposure to low-LET radiation is 1–2% risk of a fatal cancer, 1–2% risk of a curable cancer and 0.2% risk of a serious genetic defect in offspring [272].

The EDE was originally introduced by the International Commission on Radiological Protection as a means of assigning total risk to radiation workers [271]. But the concept also provides a means of expressing the total risk to patients from exposure to radiopharmaceuticals and allows the relative safety of various radiopharmaceuticals to be compared.

b) Shortcomings of the EDE when applied to radiopharmaceuticals. The two different types of emissions released by diagnostic radiopharmaceuticals, highly penetrating gamma photons and nonpenetrating Auger electrons, differ in their distribution in target sites. A gamma photon has the potential to interact at sites in many cells along its path. Energy transfer from a gamma photon to an organ or cell is random and independent of the location of the site of decay in the body. With relatively high concentrations of radioactivity, all cells in an exposed organ should receive an equal exposure to photon energy (equal dose). An average organ dose would then be a reasonable description of individual cell doses and hence of risk.

In contrast, Auger electrons, because of their limited range and the large localized dose they impart, have the potential to affect only a single cell. But in order to produce biologically relevant damage, the Auger electrons must irradiate intracellular sites. Their ability to reach these sites depends, not on random processes as for gamma photons, but on the chemical and physical properties of the radiopharmaceutical. The intracellular location of the radiopharmaceutical must be known before any dose approximation or risk assessment can be attempted.

The distribution of energy from Auger electrons is highly non-uniform at the organ level, as each decay affects only a single cell. The number of cells at risk from Auger electrons therefore depends on the number that take up the agent.

This leads to a dilemma: for equal uptake (i.e., equal numbers of cells at risk) the organ with the largest mass would appear to have received the lowest dose. The contribution made by Auger electrons may be understated if the average organ dose is used as a basis for estimating risk.

3.2.3. The general nature of low-level radiation injury. Radiation damage at low doses starts with injury to a single cell. Each cell is assumed to contain a number of radiosensitive structures (sites) which can be permanently altered by interaction with ionizing radiation. A radiosensitive site can sustain a hit (energy deposition event) up to a certain size without being permanently altered, that is, the resulting lesion can be spontaneously either reversed or repaired by an error-free system. Beyond this threshold, the hit is sufficient to produce damage which cannot easily undergo error-free repair. Such damage, if unrepaired, could result in cell death or mutation if acted on by error-prone repair systems [273]. The number of radiosensitive sites that must be hit, and the number of hits needed to produce a response is not known with certainty.

For low-LET radiation a hit size of about 100 eV deposited in a radiosensitive site of about 3 nm in diameter is needed to produce a biological response in a mammalian cell [273, 274]. For high LET radiation, the critical energy event must be greater than 300 eV in a similar sized sphere. Similar hits to nonsensitive sites of a cell would be inconsequential. For low doses of radiation, it is the cell (not the organ) that responds to a radiation exposure [275].

3.3. Documented effects of tissue-incorporated Auger electron emitters

Current studies, while very preliminary, imply that Auger electrons contribute substantially to the bio-

logical effects of tissue-incorporated radiopharmaceuticals. Such effects are probably the result of the high localized doses they impart to radiosensitive intracellular structures. Three experimental lines of evidence, directly or indirectly, support this contention. These are measurements of (1) various biological endpoints (cell death, mutation or malignant transformation) produced in cells treated *in vitro* with a number of Auger electron emitters, (2) radiobiological effects from *in vivo* treatments with pure beta or Auger electron emitters, and (3) various biological endpoints induced in cells irradiated *in vitro* with ultrasoft x-rays.

3.1.1. Cells treated in vitro with Auger electron emitters

a) Iodine-125. Iodine-125 releases 17 low energy electrons of 15 to 475 eV with ranges of 1 to 25 nm. Such particles deposit, in spheres of 1 nm radius, 6 eV/(nm)^3 which is equivalent to 10^8 rads [276]. Such an energy deposition is sufficient to produce irreparable damage in radiosensitive structures [277]. In one study, the decay of ^{125}I incorporated into DNA was eleven times more effective at inducing mutations and cell inactivations than external 170 kvp x-rays and would probably exceed the RBE or high-LET radiation of 80–110 keV/μm [278].

In another study, involving ^{125}I bound to DNA, each decay produced 25 times more malignant transformations and 12 to 16 times more cell killing than similarly bound tritium (^{3}H) [279]. At low doses of ^{125}I, where there was no cell killing, malignant transformation frequencies equalled those induced by 3 to 5 Gy of x-rays [279]. The D_{37} values (the mean lethal dose at 37% survival) for ^{125}I, Bromine 77 (^{77}Br) and ^{3}H, when incorporated into DNA, was found to be 0.037, 0.13 and 1.64 pCi/cell respectively [280]. The D_{37} for x-rays is considered to be about 580 rads. When ^{125}I was only sequestered near DNA, the D_{37} was 0.5 pCi/cell [281] and when able to freely diffuse in and out of mammalian cells (not bound to any cellular macromolecule), ^{125}I was still 10–11 times more lethal than external radiation [282].

In bacteria, ^{125}I bound to DNA was 10 times more lethal than each decay of phosphorus 32 (^{32}P) [283]. When the lethal effects of nuclear bound ^{3}H, ^{131}I and ^{125}I were compared, ^{131}I and ^{3}H caused an identical amount of cell killing and produced survival curves which were characteristic of low-LET radiation (x and gamma rays) [284]. In contrast, ^{125}I was 4–5 times more toxic than either ^{3}H or ^{131}I and resulted in a survival curve that was characteristic of high-LET radiation [284].

b) Radionuclides other than Iodine-125. The average dose to the cell nucleus was calculated for exposures arising from selenium 75 (^{75}Se) (an Auger electron emitter) and sulfur 35 (^{35}S) (a pure beta emitter) after their incorporation into cytoplasmic proteins [285]. It was found that radiotoxicity could be related to the average nuclear dose from the 9.5 keV K Auger electron of ^{75}Se and from the beta emissions of ^{35}S [285, 286]. The low energy Auger electrons, which deliver the high localized doses, produced no discernible biological effect from their location in the cytoplasm [285]. Similar results were found when gallium (^{67}Ga) was sequestered in the cytoplasm [287] and for ^{125}I, when located on the plasma membrane or in the cytoplasm [288].

Indium (^{111}In) has been used as a label for human leukocytes and other cellular elements of blood. After labelling 10^7 cells with 9 μCi of ^{111}In, it was found that the chromosome aberration frequency was equivalent to that produced by 2–2.5 Gy of x-rays [289]. The aberration frequency rose to 95% at ^{111}In concentrations needed to prepare human lymphocytes for use in scintigraphic imaging (about 150 μCi/10^8 cells) [289]. Similar leukocyte labelling using Technetium (^{99m}Tc) was less damaging, as determined by the frequency of micronuclei [290]. A dose equivalent to 0.6 Gy of 250 kvp x-rays was produced in cells when labelled with 242 μCi of ^{99m}Tc [290] in direct contrast to the dose produced by ^{111}In.

3.3.2. Potential radiobiological effects from in vivo treatments.

The effect of Auger electron emitters, thallium (^{201}Tl) and iron (^{55}Fe) on sperm cell survival was compared with their beta emitting counterparts, ^{204}Tl and ^{59}Fe [291]. The Auger electron emitters (^{201}Tl and ^{55}Fe) were found to be three times more effective at killing sperm cells than their

average (conventional) dose estimates would indicate [291]. The failure of conventional dosimetry to account fully for the Auger electrons that gain access to the cell nuclei results in an understatement of dose and risk (292, see section 3.2.1.). In a similar study of cytoplasmic bound ^{75}Se (an Auger electron emitter) and ^{35}S and ^{3}H (pure beta emitters), the survival fraction of sperm cells correlates with the conventionally calculated average dose to the testes [293, 294]. This further indicates that Auger electrons released from cytoplasmic decay sites are biologically irrelevant and should not unduly influence dose and consequently the estimate of risk.

3.3.3. Biological effects from ultrasoft X-rays. Evidence that the release of Auger electrons at intracellular sites could have substantial significance in radiation protection can be inferred from experiments involving ultrasoft x-rays. Ultrasoft x-rays are efficient in inducing biological damage in spite of the fact that they produce only very low energy, short-ranged electrons in cells [295]. The total energy of a 0.28 keV ultrasoft carbon K x-ray is deposited along a pathlength of not more than 7 nm [295]. This indicates that the sensitive target for radiation action is <7 nm in size and that a single deposition of ≤ 280 eV is sufficient to produce significant biological damage. The Auger electrons of all radionuclides of medical interest are capable of equalling or exceeding this threshold energy density.

3.4. Other radiation-sensitive targets in cells

Throughout this discussion, it has been assumed, but not explicitly stated, that chromosomal DNA is the critical target for radiation injury. But DNA is not the only radiosensitive organelle in the cell. Biological membranes are also extremely radiosensitive [296]. Membranes represent a considerable volume for energy absorption and contain many easily reactable entities that mediate innumerable cellular processes. Alteration of membrane-systems by radiation can therefore disrupt normal cell function and contribute to the expression of radiation effects. The molecular target for radiation damage in mammalian membranes may be the polyunsaturated fatty acids (PUFA). Their presence disposes membranes to oxidation reactions and thus to be sensitive to radiation. The G-value (the number of affected molecules per 100 eV energy absorbed) for lipids is considerably higher than for nucleic acids [297]. The oxidation of PUFA by radiation-induced radicals leads to the formation of lipid hydroperoxides. The concentration of lipid hydroperoxides increases inversely with the dose rate [296] which suggests that a chain reaction amplifies and sustains the initial chemical change [298].

The degradation of lipid hydroperoxides can produce malondialdehyde, and other low molecular weight intermediates [299] which can damage DNA in the cell's own nucleus or in nuclei of surrounding cells [300]. Lipid hydroperoxides given subcutaneously to rats produce tumors at distant sites [301]. This may be due to the diffusion of long-lived free radicals to areas which have more favorable conditions for tumor growth and development. It is apparent, therefore, that peroxidation and decomposition of membrane lipids has carcinogenic potential. In fact malondialdehyde has been referred to as the ultimate carcinogen [301] and is both cytotoxic and mutagenic in murine cells *in vitro* [302].

3.5. Conclusions and unanswered questions

Conventional organ dosimetry, based on average absorbed energy, may not account for the biological effects produced by the intracellular distribution of tissue-incorporated radionuclides. The distribution of dose at the organ level (macroscopic energy distribution) is a reasonable approximation of the energy deposited by photons or energetic beta emissions and hence average organ dose can be used as a basis to estimate their risk.

Auger electrons deliver a highly localized dose to one cell (microscopic energy distribution). The distribution of energy is therefore highly nonhomogenous at the organ level and conventional dosimetry therefore is not a good index of risk.

To properly reflect risk, radiopharmaceutical dosimetry must account for the microscopic distribution of dose at the cellular level. More data is needed on the subcellular distribution of radiopharmaceuticals to determine if the Auger electrons are biologically relevant. A major contribution in this regard would be an answer to questions as to the extent to which the increased cell transformation and mutagenesis from *in vitro* treatment with intracellular Auger electron emitters actually reflect the *in vivo* events.

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