

The significance of chemical-induced mutation for human health

Executive summary:

Introduction:

1. The Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) is an independent body that advises Governmental departments and agencies on the potential genetic toxicity of natural and man-made chemicals. The Committee has the following terms of reference as detailed on the COM website page

1. To assess and advise on the mutagenic risk to man of substances
2. To advise on important general principles or new scientific discoveries in connection with mutagenic risks, to co-ordinate with other bodies concerned with the assessment of mutagenic risks and to present recommendations for mutagenicity testing.

2. The COM remit involves providing advice on a wide range of chemicals including food contaminants (those that are naturally occurring or those from pesticide or veterinary medicines which potentially are present as residues in food), chemicals that are used in manufacturing processes or in household goods or those that may become environmental pollutants. Additionally, the COM is also concerned with providing generic advice on testing methods and strategies and has recently published a Guidance document on a strategy for the testing and evaluation of chemical mutagens

Basic concepts and general principles:

Genotoxicity:

3. Genotoxic (or genotoxicity) refers to chemicals that interact with or damage the DNA and/or the cellular apparatus which regulates the fidelity of the genome. It is a broad term that includes heritable effects such as point mutation, structural chromosomal damage (clastogenicity), numerical chromosomal damage (aneuploidy, polyploidy) damage to DNA or the production of DNA adducts, by the chemical itself or its metabolites. Genotoxic effects also include effects which are lethal to a cell, and effects on nuclear genetic material such as DNA strand breakage, unscheduled DNA synthesis (UDS), sister chromatid exchange (SCE) and mitotic recombination in yeast. However the detection of such effects does not in itself provide direct evidence of inherited mutations. These effects are termed modes of genotoxic action (MoGA).

Mutation and mutagenicity:

4. Mutation is defined as a permanent change in the amount or structure of the genetic material of an organism. This may result in change to a single

44 nucleotide in a single gene or a regulatory DNA sequence. In turn, this may
45 cause deregulated gene expression and a heritable change in the
46 characteristics of the cell/organism. Mutagenicity is the capacity to induce
47 these permanent changes which manifests in a number of ways, principally
48 through an alteration in the nucleotide sequence of the DNA coding for a gene
49 or through a change in the physical arrangement of a chromosome (i.e.
50 MoGA, as described above). The process is mutagenesis.

51 5. Point mutations are small changes in individual genes. A base
52 substitution mutation is the replacement of a single nucleotide by an incorrect
53 base, a frame-shift mutation arises when a deletion or insertion of a nucleotide
54 base results in an alteration in the 'reading frame' (i.e the three base pair
55 sequence encoding each amino acid). Examples are the deamination of
56 cytosine to thymine resulting in G:C to A:T transition.

57 6. Chromosome abnormalities which give rise to mutational alterations
58 can be either a structural or a numerical change. A structural rearrangement
59 can arise from a whole chromosome breakage, which then becomes
60 misaligned when the chromosome is rejoined. Translocation of chromosomal
61 material can be balanced (an even exchange of material with no genetic
62 information extra or missing) or unbalanced (where the exchange of
63 chromosome material is unequal resulting in additional or missing genetic
64 material) – unbalanced translocations are more likely to result in deregulated
65 gene expression.

66 7. Aneuploidy is the acquisition of an incorrect number of chromosomes.
67 It can manifest during cell division if the chromosomes do not separate
68 correctly between the two cells. Chemicals which have the potential to
69 damage the cell's spindle apparatus, critical for the accurate progression of
70 DNA replication and cell division, or those which interfere with enzymes
71 involved with critical stages of DNA replication such as the topoisomerases,
72 all have the potential to cause clastogenicity/genotoxicity and hence
73 mutagenic change.

74 8. Further details and discussion of these basic principles can be found in
75 a number of published reference text books (Lynch 2009, Turnpenny and
76 Ellard 2005, Preston and Hoffman 2008)

77

78 **Consequences of mutagenesis**

79 9. A mutation that occurs in a non-coding region of the DNA may result
80 only in a change to the genetic material, a genotypic change, where the
81 mutation does not manifest as an alteration in the functionality of the cell.
82 When a mutation occurs in a region of DNA where the code is translated to
83 produce functional proteins, this can alter the expression of affected genes,
84 resulting in the creation of a new character or trait not demonstrated in the
85 parent cell. This is termed a phenotypic change. (Phillips and Arlet 2009).

86 10. Mutations arise from DNA damage caused by radiation (ionizing and
87 non—ionizing), exogenous chemicals (those that do not occur naturally in the
88 body) or endogenous molecules, such as reactive oxygen species. The
89 principal remit of the COM is to examine the potential for chemicals in food,
90 consumer products or the environment to cause mutation. However,

91 understanding the hazard and risk of such chemicals is inextricably linked to
92 the damage caused by mutations generally, including those which arise as a
93 consequence of endogenously generated chemicals.

94 11. Chemical-induced mutagenicity occurs by a variety of mechanisms. An
95 alkylating agent can transfer small groups such as methyl or ethyl moieties, to
96 reactive oxygen or nitrogen species in the DNA bases generating an altered
97 nucleotide. This can lead to an incorrect reading of the base when the DNA
98 strand is transcribed during cell division. Examples of alkylated bases are O⁶-
99 methyl or ethyl guanine, generated by chemicals which donate these groups
100 (e.g dimethyl nitrosamine, DMN; ethylnitrosourea ENU). *N*-Methyl-*N'*-nitro-*N*-
101 nitrosoguanidine, (MNNG) (Wyatt and Pittman 2006) or *N*⁷-ethyl guanine.
102 These cause G:C to A:T transversions (Boysen et al 2009)

103 12. Bulky DNA adducts can form as the consequence of the covalent
104 binding of an entire chemical, or commonly, its electrophilic metabolite, with a
105 nucleophilic site on a DNA base. Chemical molecules can also form
106 links/bridges between bases (termed intercalating agents). The presence of
107 an adduct can hinder or cause transcription errors in the process of DNA
108 replication. A good example of a class of chemicals which cause mutation in
109 this manner are the polycyclic aromatic hydrocarbons (PAH's) (Luch 2005).
110 These chemicals are ubiquitous environmental contaminants, derived from
111 coal, tar, oil and the combustion of these products. When metabolised to
112 reactive species, they are able to form stable adducts with DNA primarily with
113 N² of guanine.

114 13. The detection and measurement of chemical DNA adducts can be
115 utilised as an effective exposure biomarker for mutagenic/carcinogenic
116 chemicals (Phillips 1996, Farmer 2004). However, the formation of an adduct
117 does not automatically translate to the generation of a mutation. It is possible
118 that this lesion will be repaired, by excision repair mechanism (see DNA
119 repair) and the DNA strand will ultimately be restored to its correct
120 confirmation. Therefore detection of an adduct is a demonstration of
121 genotoxicity but not necessarily mutagenicity.

122 ***Mutagenesis - the role of DNA repair***

123 14. DNA is intrinsically fragile and it is estimated that, in a human body,
124 10³-10⁶ potentially mutagenic lesions occur per cell every day (Ames and Gold
125 1990). If DNA damage were left unrepaired then this damage could give rise
126 to altered gene expression, leading to genomic instability and impaired cellular
127 functioning. To ensure a continued maintenance of DNA (genomic) integrity,
128 complex DNA repair pathways exist which respond to a variety of different
129 types of DNA damage. Pathways include mismatch repair pathway (MMR)
130 which repairs mismatched bases (e.g G instead of T, A instead of C);
131 nucleotide excision repair (NER) which has the capacity to remove bulky
132 adducts generated by large mutagenic molecules. Base excision repair (BER)
133 repairs bulky, helix-distorting lesions – by a series of well defined
134 enzymatically controlled steps, either single bases or a short series of bases
135 are replaced (termed short and long patch repair respectively) (Wyatt and
136 Pittman 2006). A more specific target for a DNA repair pathway is the methyl
137 group present as part of the O⁶-alkylguanine lesion; this is removed by the

138 enzyme O⁶-methylguanine-DNA methyltransferase (MGMT) (DNA (Dixon and
139 Kopras 2004).

140 15. These processes are readily activated to repair the damage induced by
141 endogenous and exogenous genotoxic agents similarly and are likely to be
142 detectably elevated following a chemical genotoxic insult. An increase in DNA
143 repair is one end-point measured in the genotoxicity test battery as it is
144 considered to represent an increase in chemical induced DNA damage
145 (unscheduled DNA synthesis; UDS). Fidelity of the DNA repair processes is
146 paramount to cell function and survival, and their ability (or not) to repair
147 lesions induced by exogenous chemicals will in part determine the potency of
148 a mutagen.

149 ***Mutagenesis - the role of apoptosis-***

150 16. Apoptosis, also termed programmed cell death, is a process by which
151 cells are removed from an organism in a controlled, regulated manner. It
152 differs from necrosis which is cell death caused by toxins or infection; this
153 involves the breakdown of affected cells and is usually detrimental to the
154 organism. In contrast during apoptosis, the cell is broken into fragments
155 which are easily engulfed, thereby avoiding spillage of the cell contents and
156 damage to surrounding cells. Apoptosis is an integral aspect of the
157 maintenance of cell and tissue growth and integrity, and is regulated by
158 complex signal transduction pathways. Gene families involved include
159 p53/BCL-2, tumour necrosis factor (*TNF*) and FAS ligand activated pathways.
160 The presence of DNA damage can trigger apoptosis via these pathways
161 hence removing the opportunity for the damage to be replicated at the next
162 cell division, resulting in a mutation (Rich et al 2010).

163

164 **Testing strategies:**

165 17. Assays designed to evaluate the genotoxic/mutagenic potential of
166 chemicals routinely, were initially developed in the 1970's, the most notable
167 being the *in vitro* bacterial mutagenicity assays of Bruce Ames and colleagues
168 (Ames et al 1973, 1975). This test is based on the ability of mutagenic
169 chemicals to cause a reverse mutation in a previously altered histidine gene of
170 *Salmonella* (and latterly *Escherichia coli*) strains of bacteria, which is readily
171 identified when treated bacterial cultures are incubated in histidine free growth
172 medium. This assay remains central to the current testing strategy.

173 18. A number of other *in vitro* test systems utilising cultured cell lines or
174 freshly isolated human lymphocytes were simultaneously developed and
175 refined. These assays use structural chromosomal aberrations (reviewed in
176 Scott et al 1990) or gene mutations in Chinese hamster ovary cells (CHO) or
177 Mouse lymphoma cells (reviewed in Cole et al 1990) as the genotoxicity
178 endpoints. Between them, these *in vitro* assays are able to detect a
179 spectrum of chemically-induced genotoxic damage and thus a battery of
180 different tests assessing different end-points was considered the optimum
181 approach to identifying a wide range of genotoxic agents and incorporated
182 into the original guidance (DHSS 1981, UKEMS 1983). Considerable
183 research and regulatory effort has been applied to validating and improving

184 these tests and these principles have under-pinned genotoxicity testing
185 strategies ever since (Muller et al 1999, Kirkland 2000).

186 19. The measurement of *in vivo* genotoxicity has evolved similarly,
187 although a number of the early tests using whole animals proved to be
188 cumbersome and time consuming and are no longer in general use (e.g
189 mouse spot test, dominant lethal assay). The mouse bone marrow
190 micronucleus assay started to be used widely in the 1980's and has since
191 been shown to be a robust and predictive of the mutagenic potential of a wide
192 variety of known human genotoxic carcinogens and accordingly has attained
193 the status as a front line *in vivo* assay (see Guidance). The advantage of
194 using whole animals is that absorption, distribution, metabolism and clearance
195 factors of a chemical can be taken into account when assessing the hazard
196 (for example, direct acting mutagens which are conjugated and detoxified
197 before entering the systemic circulation) which enables a more accurate
198 assessment of whether a genotoxic event observed in an *in vitro* test will
199 translate to a genotoxic hazard in humans.

200 20. Many assays have been developed which examine different endpoints
201 *in vivo*. The COM is constantly reviewing new and developing assays, with
202 view to understanding the role that they may play in the testing strategy. For
203 example, the COMET assay, an assay developed in the 1990's, which
204 measures DNA strand breaks as a surrogate marker for genotoxic damage
205 has increasingly become recognised as a sensitive assay for *in vivo*
206 genotoxicity (Sasaki et al 1997, Burlinson et al 2007). Assays using mice
207 engineered to contain a transgene (*Lac I/Z*) which can be isolated from
208 tissues from treated animals and analysed for gene mutations, have been
209 developed and validated in recent years (Thybaud et al 2003). Both the
210 COMET assay and transgenic rodent bioassays (BigBlue, Mutamouse) are
211 now included as front-line assays for the first time in the new COM testing
212 strategy. The use of these *in vitro* assays are also discussed in a COM
213 statement. <http://www.iacom.org.uk/statements/TargetOrganMutagenicity.htm>

214

215 **Understanding hazard and risk**

216 21. Classical definitions of hazard and risk state that: a hazard is 'the
217 potential to cause harm' and risk is 'the likelihood of the harm occurring in a
218 given circumstance'. Furthermore, a conventional chemical risk assessment
219 comprises of hazard identification, hazard characterization, exposure
220 assessment and risk characterization [for more detail see the Health
221 Protection Agency website :

222 <http://www.hpa.org.uk/ProductsServices/ChemicalsPoisons/ChemicalRiskAssessment/RiskAssessment>.

224 22. With regards to the current assessment of genotoxicity, the
225 demonstration of the intrinsic ability of a chemical to cause DNA damage
226 using the *in vitro* and *in vivo* test battery satisfy's the hazard elements of the
227 risk assessment process. Risk takes into account a number of other factors
228 including the extent and duration of exposure, the likelihood of the mutagenic
229 moiety reaching the DNA of the target tissues and the chances that the lesion
230 is repaired or removed.

231 23. Traditionally the risk assessment is based on a linear dose response
232 for a mutagenic chemical; theoretically a single hit could result in a mutagenic
233 lesion. This is described in COM/01/S3 [link]. This conservative approach
234 is generally applied. However there are examples of where there is threshold
235 for the response; where it has been mechanistically demonstrated that there is
236 a low dose below which, genotoxicity will not be observed. The COM have
237 published advice on mutagens for which there are threshold modes of action.
238 <http://www.iacom.org.uk/statements/COM01S3.htm>.

239

240 **Biomarkers of exposure and/or of effect**

241 24. Many of the endpoints examined in the genotoxicity testing battery are
242 also used to evaluate DNA damage in humans following exposure to
243 potentially genotoxic substances in the workplace, the environment or
244 following treatment with cytostatic medicines (Bonassi et al 2005). These
245 investigations are principally conducted using peripheral blood cells.

246 25. Biomarkers for which there is adequate data include chromosome
247 aberrations and micronuclei, single strand breaks (as measured in the
248 COMET assay) and DNA adducts. Investigations using biomarkers such as
249 DNA adducts are often designed with the aims of establishing whether there
250 are associations between exposure, DNA damage and disease, cancer in
251 particular (Phillips et al 1996, Farmer et al 2005, Boffetta 2010). These links
252 have been demonstrated but as yet, these data do not give conclusive
253 evidence that biomarkers can be used as prognostic or diagnostic tools for
254 human disease. However, there are rapid developments in this field, including
255 proteomic techniques and it is likely that the role of these methods in
256 understanding the effect of genotoxic chemicals on human health will increase
257 accordingly.

258 <http://www.iacom.org.uk/statements/COM04S5.htm>.

259

260 **Significance of mutations for human health**

261 26. As described earlier, a genotoxic (mutagenic) chemical may cause a
262 variety of different DNA lesions by a number of different modes of action and
263 this damage may be repaired by DNA repair pathways. If the damage is not
264 repaired and the lesion is recognised as different from the original and copied
265 during the next round of cell division, then there is a possibility that changes in
266 gene expression will occur. A majority of these will result in a mutational
267 change which is lethal to the cell, or the cell is stimulated to undergo
268 apoptosis and is removed. However some may persist; a mutation in the
269 germ cells of sexually reproducing organisms may be transmitted to the
270 offspring whereas a mutation that occurs in a somatic cell will be transferred
271 only to the daughter cells.

272

273 **Carcinogenesis**

274 27. DNA damage in a somatic cell resulting in mutation may lead to
275 malignant transformation (cancer). Cancer is defined as 'uncontrolled,

276 abnormal cell growth' – a process during which a single somatic cell gives rise
277 to a de-differentiated mass of cells which have escaped the confines of
278 normal cell cycle control. From this de-differentiated pre-neoplastic lesion a
279 malignant (neoplastic) tumour arises (tumourigenesis). This escape is widely
280 attributed to deregulation of genes encoding proteins responsible for the
281 regulation and maintenance of normal cell proliferation, cell death (apoptosis)
282 and DNA repair. Activated genes are often termed oncogenes. Genes which
283 when de-activated result in malignant change, are called tumour suppressor
284 genes (reviewed in Pitot and Dragan 2008, Dixon and Kopras 2004, Little
285 2010).

286 28. The process of carcinogenesis is considered to be multi-stage. A
287 simplistic model of this process comprises of: an initiation event, thought to
288 involve a mutation in an oncogene or tumour suppression gene which
289 provides a growth advantage compared to a normal cell. This cell may be
290 removed by apoptosis and is thus considered to be a reversible step. The
291 next stage, promotion, is generally associated with increased cell proliferation
292 and the clonal expansion of the initiated cell which further increases the
293 likelihood of DNA replication errors. This stage is not necessarily
294 characterised by mutational events. Further progression of the initiated foci of
295 cells involves the accumulation of a number of different mutations which
296 ultimately leads to the acquisition of characteristics which determine the
297 malignant phenotype. These include insensitivity to growth inhibitory signals,
298 defects in cell cycle checkpoint control and deregulation of DNA repair
299 mechanisms. There is an hypothesis which suggests that a 'mutator
300 phenotype' is acquired which triggers further cascades of mutations resulting
301 in destabilisation and tumour formation (Wogan et al 2004).

302 29. However, in the context of assessing the impact of environmental
303 chemicals on tumour development, it is generally not possible to distinguish
304 between mutations which are attributable to the action of endogenous or
305 exogenous factors.

306 ***Specific mutations in cancer - the human cancer /tumour genome***

307 30. Considerable endeavour is directed towards unravelling the genetic
308 alterations in human tumours and the patterns of somatic mutations in the
309 human cancer genome (Greenman et al 2009, Stratton et al 2010, Bell 2010).
310 Currently this information is most widely used to elucidate the dynamics of
311 tumour growth with view to developing therapies. However there is a potential
312 that the evaluation of altered genomic fingerprints will provide insight into the
313 aetiology of specific tumours and the role that exogenous chemicals play in
314 their formation.

315 31. The affected genes are invariably elements of pathways implicated in
316 the maintenance of the cell cycle or which affect the cell's ability to undergo
317 apoptosis which is a mechanism used to remove the damaged cell from the
318 body. The p53 oncogene, a tumour suppressor gene, is the most extensively
319 researched gene with regards to cancer and is implicated in a majority of
320 cancer types (Nigro et al 1989, Levine and Oren 2009). Homozygous loss of
321 p53 is found in 70% of colon cancers, 30-50% breast cancers and 50% of
322 lung cancers. p53 genes with normal functioning act to induce cell cycle
323 arrest to allow either repair and survival of the cell or apoptosis to discard a

324 damaged cell. Transcription of p21 (H-RAS) and inhibition of Rb
325 (retinoblastoma gene) are regulated via p53, which in normal cells confers cell
326 cycle progression at the G1- S-phase transition.

327 32. Genes which code for growth factors or growth factor receptor proteins
328 are also potential oncogenes. Again these factors are essential for the control
329 of normal cell proliferation so in turn, deregulation of these pathways may lead
330 to uncontrolled growth. Examples of these are epidermal growth factor (EGF)
331 and its receptor, a tyrosine protein kinase coded by *erb-B*, transforming
332 growth factor – β and its receptor.

333 33. Further down the signalling pathways signal transduction proteins such
334 as RAS (*h-ras*), mitogen activated protein kinases (MAPK, ERK), cyclins and
335 cyclin dependent kinases (CDK) , all factors implicated in cell cycle control
336 pathways are regarded as potential oncogenes as deregulation of the genes
337 coding for these proteins are known to disrupt normal growth control. There
338 is evidence that some of these genes may be aberrant in the cancer genome,
339 although currently not identified as strongly and universally in tumours as the
340 p53 gene (Vogelstein and Kinzler 2004),

341 34. BRCA 1 and 2 are genes which are associated with breast cancer, the
342 presence of mutations in these genes are associated with an increased risk of
343 the disease. However to date, there is no association between these
344 mutations and chemical exposure.

345 ***Specific examples of cancers caused by mutagenic chemicals***

346 35. The ability of a chemical to induce mutations can be rigorously
347 examined in *in vitro* systems and in animal models, and from these data it is
348 possible to predict chemicals that may cause cancer in humans. However in
349 man there is relative paucity of experimental data enabling these
350 extrapolations to be confirmed as unsurprisingly, it is often difficult to
351 demonstrate conclusively, or quantify, human exposures to specific
352 chemicals. Epidemiological studies underpin these assessments, where
353 human populations with known potential exposures (e.g an accidental
354 chemical exposure, an occupational exposure) are compared with un-exposed
355 groups. These studies need to account meticulously for confounding factors
356 which may also contribute to cancer burden (such as smoking habits, age,
357 dietary or other lifestyle factors) and be appropriately statistically powered
358 (Bailey et al 2006).

359 36. The International Agency for Research on Cancer (IARC) is a World
360 Health Organisation affiliated agency which examines dietary, environmental
361 and occupational factors which may contribute to cancer risk in humans.
362 Epidemiological investigations, if available, underpin these analyses but mode
363 of action data extrapolated from animals is of significant value in deriving the
364 classifications. These analyses are available as a series of detailed
365 monographs where chemicals (or agents, mixtures and exposures) are
366 categorized into five different classifications <http://monographs.iarc.fr/>

367 37. Epidemiological data indicating increases in cancers in populations
368 exposed to particular chemicals complimented with a plausible mechanism
369 derived from experimental data provide weight of evidence for carcinogenic
370 mode of action. Although there are examples of exposures classified as

371 Group 1 (carcinogenic to humans) which are not genotoxic, the primary mode
372 of action of the majority of chemicals is mutagenicity.

373 38. The WHO International Programme on Chemical Safety (IPCS) has
374 recently established a human relevance mode of action framework which is
375 designed to systematically consider data on the mode of action of chemical
376 carcinogens. The demonstration of a genotoxic MOA from the assessment of
377 the standard battery of genotoxicity tests will count robustly towards human
378 relevance if the chemical in question has been shown to be a carcinogen in
379 life-time studies in rodents.

380 <http://www.iacoc.org.uk/papers/documents/cc083.pdf>

381 **Some examples of mutagenic carcinogens**

382 **Aflatoxin-B1**

383 39. Aflatoxin B1 is a naturally occurring mycotoxin produced by species of
384 the fungus *Aspergillus*. Human exposure occurs following the ingestion of
385 crops contaminated with the fungus, notably cereals and nuts. Aflatoxin B1 is
386 metabolised to a reactive intermediate in the liver where it binds to hepatocyte
387 DNA. Gross liver damage (cell death) is one outcome but chronic low-level
388 exposure is associated more with an increase in liver cancer. It is one of the
389 most potent genotoxins identified, generating tumours at very low doses in
390 animals ($\mu\text{g}/\text{kg}$ quantities) and there is a large body of data detailing its
391 genotoxic MOA (IARC, Smela 2001).

392 **Chromium VI**

393 40. Notable human exposure to Chromium VI (Cr-VI) is principally via
394 industry but as it is a naturally occurring element there is also documented
395 low-level exposure from environmental sources including air. IARC considers
396 that there is sufficient evidence to classify hexavalent chromium compounds
397 as carcinogenic in humans. The epidemiological evidence for this is the
398 observed increased risk of respiratory cancers in workers occupationally
399 exposed to Cr VI compounds (US ATSDR study 1993). The exposures in
400 these circumstances were high and there is no evidence of this increased risk
401 from low, environmental exposures (de Flora 2000). However the MOA
402 framework case study concluded that the weight of evidence was towards a
403 mutagenic MOA for Cr (VI) and thus it is assumed that there is no threshold
404 when performing the oral risk assessment (Mc Carroll et al 2010).

405 **Cyclophosphamide**

406 41. Cyclophosphamide is an anti-neoplastic pharmaceutical used for the
407 treatment for a range of cancers including lymphomas, leukaemia and
408 neuroblastomas. It is an established carcinogen in life-time rodent studies
409 and has a genotoxic MOA; it acts as an alkylating agent, generating dose
410 related adducts in the target tissues of treated animals (McCarroll et al 2008).
411 There is also epidemiological evidence that it induces tumours in exposed
412 humans, particularly bladder tumours (IARC 1981).

413 **1,3-butadiene**

414 42. 1,3-butadiene is used principally in the production of rubber and
415 synthetic polymers and workers in these industries are potentially exposed. It

416 is carcinogenic in rats and mice but molecular epidemiology studies
417 examining bio-markers such as adduct formation or chromosome damage
418 yield inconclusive results. Genotoxicity tests yield generally positive results
419 and mutations of p53 and ras were identified in tumours from mice treated
420 with butadiene (Kirman et al 2010). A comprehensive review of the
421 epidemiological data gathered in occupational settings revealed increases in
422 haemopoietic and -lymphatic cancers and it was classified by IARC as
423 'carcinogenic to humans' (IARC). However, in humans the dose response
424 analyses suggest that a MOA may be subject to a threshold mechanism, and
425 that the observed carcinogenesis is dependent on more than 1,3-butadienes
426 mutagenic potential.

427 **4-aminobiphenyl (4-AB)**

428 43. 4-AB is another known human carcinogen which induced a high
429 incidence of bladder tumours in exposed workers. In mice, it causes an
430 increase in the occurrence of bladder and liver tumours and it is clearly
431 genotoxic, positive in all the standard genotoxicity assays, *in vitro* and *in vivo*.
432 4-AB and a number of other structurally similar chemicals (aromatic amines)
433 are also constituents of cigarette smoke. DNA adducts derived from 4-AB
434 metabolites have been detected in human bladder tumours and protein
435 adducts are found in the haemoglobin of smokers (Vineis 1992).

436

437

438 **Germ cell mutagenesis**

439 44. If a mutation arises in a germ cell (i.e. an oocyte or sperm cell) it is
440 possible that this mutation will be transferred to the off-spring. Heritable
441 genetic damage has been suitably demonstrated for a number of well known
442 mutagenic chemicals in animal models (e.g. antineoplastic drugs, acrylamide,
443 ethylene oxide) (Shelby et al 1993). Investigations using experimental
444 animals show there can be gender specific effects which are likely a
445 consequence of structural and functional differences during gametogenesis
446 (the last meiotic division of germ cell generation) or epigenetic influences (e.g
447 DNA methylation) (Eichenlaub-Ritter et al 2007). Generally male gametes
448 are more sensitive - paternally transmitted chromosomal structural aberrations
449 have been demonstrated in mouse zygotes for a large number of mutagens
450 (Marcehetti and Wyrobek 2005). In human studies, there is evidence that the
451 sperm of smokers have elevated levels of oxidative DNA damage and
452 benzo(a)pyrene diol epoxide adducts and evidence that the adducts are
453 transmitted to embryos (Chang 2008) (*association with child cancers?*)

454 45. Germ cells are particularly susceptible to aneuploidy during meiosis
455 and there are notable examples of chemically induced aneuploidy in animal
456 models (e.g bisphenol A in female mice; Hunt et al 2003, acrylamide in mouse
457 sperm, Adler, I-D et al; 2002) cyclophosphamide; Pacchierotti et al 1983). For
458 most chromosomes, aneuploid fetuses are not viable. However, exceptions
459 exist, and in man aneuploidy gives rise to a number of well characterized
460 syndromes; for example, an additional chromosome 21 is diagnosed as
461 Down's syndrome, a missing X chromosome is Turner's syndrome (Hassold et
462 al 2007). To links have been made with other risk factors, maternal age in

463 particular but there is no convincing evidence for an association of these
464 conditions with maternal chemical exposures through occupational, medicinal
465 or environmental factors, including cigarette smoking.

466 46. Overall, there is no convincing evidence for chemically- induced
467 heritable germ cell mutations in human populations despite a large number of
468 studies examining the effects of mutagenic carcinogens on germ cell
469 endpoints. However; it is anticipated that emerging molecular epidemiological
470 methods and techniques may help to improve protocol designs and provide
471 insight into this aspect of potential mutagenesis (Verhofstad et al 2008).

472 **Teratogenicity –**

473 47. Teratogenicity is a sub-section of developmental toxicity which
474 encompasses foetal malformations, embryo lethality, growth retardation and
475 functional impairment following *in utero* exposure to a chemical. Although
476 many of the well established human chemical teratogens do not act via a
477 mutagenic mechanism (e.g thalidomide, diethylstilbestrol), there is evidence
478 that a number of *in vivo* mutagens produce birth defects if administered to
479 pregnant women. These include anti-cancer drugs busulfan, 6-mercaptopurine
480 and daunorubicin (Bishop et al 1997). The effects reported are largely
481 congenital abnormalities such as cleft palate, hypospadias and limb defects.
482 Although these chemicals are known mutagens, the precise MOA's for
483 teratogenesis are largely uninvestigated. Understanding the causal
484 associations between chemical exposures and teratogenic effects is
485 complicated by the knowledge that exposures at different gestational times
486 may produce a different spectrum of effects and the complexity of what
487 normal variations in congenital defects are. There are no significant reports of
488 an increase in the induction of diseases (e.g atherosclerosis) in exposed off-
489 spring nor evidence of germ cell effects in which mutational effects are passed
490 on to future generations.

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492

493

494 **Summary:**

495 48. The COM has a remit to provide advice on and evaluate information
496 relating to chemical- induced mutations. The new Guidance provides a frame
497 work for the detailed investigation of the induction of mutations in *in vitro* and
498 *in vivo* for a wide variety of chemicals. (*link again?*)

499 49. Mutagenesis in man is principally associated with cancer – there are
500 clear links between exposure to some mutagens and the increased incidence
501 of tumours. Therefore there are restrictions on exposure to such chemicals.
502 The impact of chemicals on germ cell mutagenesis and human health is less
503 well understood, although currently there are no clear associations. The COM
504 are constantly reviewing data available on all aspects of the potential of
505 chemicals to induce mutations, from animal and human studies , with the clear
506 view to understanding and limiting their impact on human health .

507

508 References:

- 509 Adler, I-D., Schmid, T.E., Baumgartner, A. (2002) Induction of aneuploidy in
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641 **Glossary** (*to be inserted at next draft*).