

# COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

## GUIDANCE STATEMENT : THRESHOLDS FOR *IN VIVO* MUTAGENS.

### Introduction

1. The general advice of the COM when considering the risk assessment of chemicals which are mutagenic *in vivo* has been that it is prudent to assume a non threshold dose response. Thus it is assumed that any exposure to an *in vivo* mutagen is associated with some damage to DNA and consequently an increased risk of mutation leading to an increased risk of adverse health effects albeit that this may be small. In such instances the Committee has recommended that exposures be reduced to a low as is reasonably practicable. The Committee has previously considered specific chemicals, on a case-by-case basis, with regard to deviations from its general approach to *in vivo* mutagens.(COM., 2001)

2. The Committee considered draft guidance statements at its June and October meetings in 2009 and its March 2010 meeting. The Committee agreed that advice should be given on terminology used to define threshold *in vivo* mutagens, some examples of *in vivo* mutagens considered to exhibit a threshold should be provided, as well as information on an approach to the identification of threshold doses for *in vivo* mutagens.

### Thresholds for *in-vivo* mutagens

#### *Definitions*

3. A number of definitions are used in this document and in the published literature regarding thresholds for *in vivo* mutagens.(Kirsch-Volders et al., 2009) .

*True Threshold:* A point in the dose response relationship for mutagenicity where a slope of zero changes into a slope of greater than zero.

*Threshold dose:* The actual dose below which there is no increase in effect over the background level. (Severe practical issues make the unequivocal experimental identification of the threshold dose difficult and a practical threshold is often derived.)

*Practical Threshold:* The point where the experimentally derived dose response relationship first exceeds the background variability in a suitably designed well conducted study.

*Biologically meaningful*

*threshold:* The threshold dose (most often an experimentally derived practical threshold dose) for a substance where there is also appropriate supporting evidence to conclude that a threshold mode of action occurs. A Biologically meaningful threshold dose can be used in subsequent hazard/risk assessment evaluations.

*Threshold mode of action.*

The plausible biological explanation of a threshold dose for mutagenicity (most often for an experimentally derived threshold dose).

*NOEL* The No Observed Effect Level. The highest dose in an experiment where no statistically significant increase over the concurrent negative control has been identified. The NOEL is dependent upon the experimental design sample size, dose spacing, statistical methods used, etc. The NOEL should not be equated with a threshold dose because the failure to show statistical significance can mean that a real but smaller effect is below the level of detection.

4. It is important to remember that there are many sources of endogenous mutation including physico-chemical processes, free radicals and enzymatic processes controlling DNA damage and repair which influence the spontaneous mutation frequency and are reflected in the background mutation level.(Morley and Turner, 1999)

5. The unambiguous experimental demonstration of a biologically meaningful threshold for mutagenicity requires extensive dose-response and mode of action data to be provided. The acceptance of a non-linear dose response extrapolation for *in-vivo* mutagens requires a biological mechanistic rationale derived from mode of action investigations for a threshold regarding mutagen-cellular target interactions, experimental data from the best available testing methods, coupled with thorough statistical analysis of the data and modelling to describe the non-linear dose-response relationship.(Kirsch-Volders et al., 2003)

6. In a number of instances, a biologically meaningful threshold for genotoxicity has been established (e.g. see discussion of aneugens acting by tubulin inhibition in paragraphs 10-12 below),

*Indirect modes of action for mutagens*

7. There are a number of indirect modes of mutagenic action that have not been considered in detail in this guidance statement which include oxidative stress (leading to oxidative DNA damage), inhibition of DNA repair systems resulting in genomic instability and deregulation of cell proliferation by inactivation of growth controls such as tumour suppressor genes. These modes of action explain, in part, the mutagenicity of metal ions (such as arsenic, antimony, beryllium, cadmium, chromium, cobalt, lead, nickel and vanadium). (Beyersmann and Hartwig, 2008) It has also been postulated that the observed *in vitro* genotoxicity seen with chloroform and carbon tetrachloride is due to oxidative DNA damage resulting from glutathione depletion. (Beddowes et al., 2003) These particular indirect modes of mutagenic action may have a threshold but the data have not been considered in detail by COM and no conclusions have been reached.

#### *In-vivo mutagens considered by COM with thresholds*

8. A short overview is given below of some chemicals that have been considered by the COM to provide examples of a threshold mode of action for mutagenicity. Phenol has been included as an example where there is no clear evidence for a threshold mode of genotoxic action, but rapid detoxification in the gastro intestinal tract results in a threshold upon oral administration. An overview of the COM conclusions for acrylamide is also provided as an example of a mutagen with multiple modes of action for genotoxicity. Some, but not all of these modes of action of acrylamide, and of its genotoxic metabolite glycidamide, have been shown to exhibit a threshold. Information on paracetamol has been included as an example of a compound with genotoxic effects for which all possible modes of genotoxicity are considered to exhibit a threshold. The information reviewed can be used to provide generic advice on an approach to *in vivo* mutagens.

9. The section outlining example chemicals and modes of action by which genotoxic thresholds could be identified for some chemicals is followed by a consideration of the types of evidence needed to assess the potential for an *in vivo* genotoxic threshold effect.

#### Non DNA targets

##### *Aneugens acting by tubulin inhibition*

10. Benomyl, carbendazim and thiophanate-methyl belong to the methyl benzimidazole carbamate (MBCs) class of chemicals. Compounds in the MBC class of chemicals are used in approved pesticide products as fungicides and also in veterinary medicines in particular as antihelmintics in both food producing and companion animals. These chemicals act by interfering with microtubule formation during mitosis. The COM has provided advice to U.K regulatory Authorities namely the Pesticides Safety Directorate (PSD) (now part of the Chemicals Regulation Directorate) and the Veterinary Medicines Directorate (VMD) (an Executive Agency of the Department for Environment, Food and Rural Affairs) on the most appropriate approach for the risk assessment of MBCs. (COM., 1993, 1995, 1997)

11. The COM has previously agreed that it is reasonable to assume that aneuploidy inducing chemicals (particularly those that function by interfering with the spindle apparatus of cell division) may have a threshold mode of action.(COM., 1993) Subsequently *in-vitro* studies of micronuclei formation, chromosome loss and gain and non-disjunction in bi-nucleate human lymphocytes were undertaken to identify NOELs which could aid in risk assessment.(Elhajouji et al., 1995, Unpublished, 1996, Elhajouji et al., 1997, Bently et al., 2000) The design of studies included a sufficient number of exposed cells and dose levels to identify a threshold and dose-response. The COM reached conclusions regarding NOELs for benomyl, carbendazim and thiophanate-methyl.(COM., 1995, 1997) Data were published on a range of spindle inhibitors including benomyl, carbendazim, mebendazole and nocodazole.(Elhajouji et al., 1995, Elhajouji et al., 1997, Bently et al., 2000)

12. The subsequent risk assessment of MBC pesticides at EU level took into account all of the available toxicological and genotoxicity data. The lowest No Observed Adverse Effect Level (NOAEL) [the highest administered dose at which no adverse effect has been observed] used for risk assessment was not based on mutagenicity.

#### *Topoisomerase inhibitors*

13. The clastogenicity of topoisomerase inhibitors is considered to be due to the transient stabilisation of the topoisomerase enzyme with DNA during the catalytic cycle, leading to DNA strand breaks. Experimental evidence for the occurrence of a threshold for micronucleus induction by a number of topoisomerase inhibitors in L5178Y cells has been published.(Lynch et al., 2003) The experimental approach included investigations into the mode of action of clastogenicity, scoring large numbers of cells for micronuclei and statistical modelling of the dose-response curves.

#### *Antimetabolites; methotrexate.*

14. Methotrexate acts as a substrate for dihydrofolate reductase and blocks the reduction of folate to dihydrofolate and tetrahydrofolate, resulting in inhibition of nucleoside triphosphate production(Jackson et al., 1996) A shortage of deoxy-thymidinetriphosphate (dTTP) and purine nucleotides results in a reduction in the repair of spontaneous DNA lesions and leads to a progressive accumulation of DNA strand breaks in post-replication DNA..(Choudhury et al., 2000) Methotrexate is an *in vitro* clastogen in mammalian cells and an *in vivo* mutagen in rodents and humans.(Choudhury et al., 2000, Kirkland et al., 2005) The data on methotrexate is consistent with a threshold mode of action for genotoxicity.

#### Protective mechanisms

*Rapid detoxification of orally administered hydroquinone and phenol*

15 In 1994 and 2000, the COM concluded that both hydroquinone and phenol should be regarded as somatic cell *in vivo* mutagens. There was good evidence from appropriate toxicokinetic studies that two protective mechanisms (namely rapid conjugation and detoxification via the glutathione pathway) would substantially reduce systemic exposure to any active metabolites formed. Therefore the Committee agreed that there was potential for a threshold of activity for these two compounds when administered by the oral route.(COM., 2000)

16. The COM considered phenol in 2008 when data investigating the potential mode of genotoxic action *in vivo* was discussed, specifically the proposal that phenol-induced hypothermia was responsible for the *in vivo* mutagenicity reported in rodents.(Spencer et al., 2007) The COM concluded that phenol is mutagenic *in vitro* in mammalian cells giving rise to gene mutation and chromosomal damage in the presence and absence of exogenous metabolic activation. The mode(s) of action had not been fully elucidated although there was evidence that effects were in part due to oxidative DNA damage. The COM also concluded that phenol should be regarded as an *in vivo* somatic cell mutagen. The additional studies suggested a role, but not necessarily causality, for phenol-induced hypothermia in the formation of micronuclei. The COM concluded that all the available data on phenol suggested phenol should be regarded as a non-threshold *in vivo* systemic mutagen. There is insufficient evidence to support a threshold approach to risk assessment of systemic phenol.(COM., 2008) Thus the threshold mode of action for hydroquinone and phenol is route-specific relating to detoxification in the gastro-intestinal tract before systemic absorption.

*DNA repair of adducts formed from small molecular weight alkylating agents.*

17. Direct-acting small molecular weight alkylating agents produce a spectrum of DNA adducts.(Kaina et al., 1998, Jenkins et al., 2005) The potential for mutagenic effects such as point mutations and clastogenicity varies between the different DNA adducts formed. Thus methyl methane sulphonate (MMS) predominantly produces clastogenicity as a result of  $N^7$ -methylguanine formation whereas methyl nitrosourea (MNU) predominantly produces mutations as a result of  $O^6$ -methylguanine formation. DNA repair (e.g.  $O^6$ -methylguanine-DNA methyltransferase (MGMT), members of the base excision repair and mismatch repair pathways) can protect against mutagenic and clastogenic events due to the formation of  $O^6$ -alkylguanine and other DNA lesions (e.g at N7G, N3A positions).(Kaina et al., 1998, Christmann et al., 2003, Drablos et al., 2004) The COM considered a discussion paper on DNA repair at low doses for a number of alkylating agents (methyl- $N$ -nitro- $N$ -nitrosoguanidine (MNNG), ethyl methane sulphonate (EMS), and MMS) in 2004. Most information was available on the  $O^6$ -methylguanine transferase repair system, mainly in bacterial systems but some information was also available in mammalian cells. The Committee agreed that these data provided some evidence for thresholds in the adaptive

state (where protective mechanisms are upregulated) that could be attributed to the induction of the MGMT.(COM., 2004)

18. The Committee considered a commentary (Jenkins et al., 2005) on thresholds for genotoxic alkylating agents at its October 2006 meeting. Some evidence regarding a threshold for formation of  $O^6$ -ethylguanine in *S.typhimurium* following treatment with ethyl nitrosourea (ENU) existed. Evidence for a threshold for mutagenic (in *hprt* and *tk* loci in AHH-1 cells) and clastogenic effects (in MCL-5 cells) was reported for MMS.(Jenkins et al., 2005) Doak and colleagues published dose-response data for micronucleus (MN) formation and *hprt* mutations in AHH-1 cells for four alkylating agents (ENU, MNU, EMS and MMS).(Doak et al., 2007) Evidence for a threshold dose level was reported in these assays for EMS and MMS but not for ENU and MNU. The authors attributed the NOELs observed for micronucleus induction with EMS and MMS to repair of  $N^7$ -alkyl guanine whereas repair of  $O^6$ -alkyl guanine could give rise to the NOELs displayed with these compounds in the *HPRT* assay. In subsequent experiments evidence for induction of MGMT following treatment of AHH-1 cells with MMS at doses below the NOEL for *hprt* mutations was interpreted as evidence for enhanced repair of  $O^6$ -methylguanine adducts.(Doak et al., 2008)

#### *Evidence for in vivo threshold for EMS*

19. The COM considered pre-publication papers which demonstrated a NOEL for *in vivo* mutagenicity of EMS in mouse bone marrow MN formation and point mutations in LacZ gene in Muta<sup>TM</sup> mouse studies (using bone marrow, gastrointestinal tract and liver).(Gocke et al., 2009, Gocke and Muller, 2009, Gocke and Wall, 2009, Lave et al., 2009, Muller et al., 2009) Dose-response modelling and statistical analyses provided evidence for thresholds for all the observed *in vivo* mutagenic effects of EMS. One useful approach used in these studies was fractionation of a mutagenic dose of EMS (350 mg/kg bw, p.o) into 12.5 mg/kg bw/day p.o for 28 days. No mutagenic effect was reported in animals given repeated doses of EMS which provided supporting evidence for a threshold dose level for EMS induced mutagenicity. (Gocke et al., 2009, Gocke and Muller, 2009, Gocke and Wall, 2009, Lutz and Lutz, 2009, Muller et al., 2009) A risk assessment which compared reported NOELs for mutagenicity in rodents with estimated maximal human doses or compared estimated AUC or  $C_{max}$  values in rodents at NOEL doses with estimated values for humans at the maximal estimated intake was undertaken.(Muller et al., 2009)

20. The COM agreed that a threshold had been demonstrated for EMS mutagenicity and there was an adequate margin of exposure between the NOEL for mutagenicity and likely maximum exposures in patients that ingested EMS-contaminated Viracept tablets(Muller et al., 2009).

#### Overload of detoxification pathways

##### *Paracetamol*

21. Paracetamol induces chromosomal damage *in vitro* in mammalian cells at high concentrations and *in vivo* (bone marrow chromosomal aberrations in mice and micronuclei in bone marrow of rats and mice, and chromosomal aberrations in peripheral blood lymphocytes of volunteers administered paracetamol).(Muller and Kasper, 1995, Bergman et al., 1996) Three possible modes of action of paracetamol-induced genotoxicity have been identified, namely (i) inhibition of ribonucleotide reductase, (ii) increase in cytosolic and intranuclear Ca<sup>2+</sup> levels, and (iii) DNA damage caused by N-acetyl-p-benzoquinone imine after glutathione depletion.(Bergman et al., 1996) All three modes of action are considered to have a threshold below which mutagenic effects will not occur. DNA damage following glutathione depletion is an example of a threshold mode of action where the protective mechanism of a detoxification pathway is overloaded leading to mutagenic effects.

#### *In vivo* mutagen with multiple modes of action of genotoxicity

##### *Acrylamide*

22. The COM published a review of the genotoxicity of acrylamide in January 2009.(COM., 2009) The COM concluded that acrylamide is an *in vivo* mutagen. Assessment of the genotoxic potential of acrylamide is complicated by multiple potential modes of action that include extensive protein binding / enzyme inhibition, oxidative stress and DNA adduct formation. It is plausible that each of these modes of action may contribute to the genotoxicity of acrylamide and are not mutually exclusive. The default assumption is that there is no level of exposure to this genotoxic carcinogen that is without some risk. In order to move away from this assumption, it will be necessary to identify evidence of a threshold with supporting mechanistic data for all of the potential genotoxic modes of action of acrylamide in somatic cells and germ cells. (COM., 2009)

#### **Evidence for Threshold for *in vivo* mutagens**

23. The Committee agreed that evidence for a plausible threshold mode of action for genotoxicity was a prerequisite before conducting studies to identify threshold doses. Mutagenic effects that have been reported only at dose levels inducing a high level of toxicity or mortality should not be included in any evaluation for threshold dose levels, as the observed genotoxicity may not reflect a true mutagenic mode of action for the chemical under consideration. The biological significance of high dose positive *in vivo* mutagenic effects needs to be assessed on a case-by-case basis.

#### Identification of Threshold modes of action for *in vivo* mutagens

24. In 2001, the Committee reviewed evidence that threshold dose responses could involve exposure to redundant or multiple cellular targets with inactivation or modification before a toxic response is produced.(Parry et al., 2000) Lists of potential cellular targets for threshold-related genotoxicity could include protein targets such as microtubules, DNA polymerase and

topoisomerases.(Henderson et al., 2000) The COM agreed that data must be considered on a chemical-by-chemical basis.

25. Three main modes of action have been reviewed by COM that can lead to genotoxic thresholds as discussed in paragraphs 8-19 above; i) involvement of non-DNA targets, (e.g. aneugen inhibition of microtubules) ii) the contribution to protective mechanisms (e.g. repair of DNA adducts formed from small molecular weight alkylating agents).(Jenkins et al., 2005) and iii) overload of detoxification pathways (e.g. paracetamol). Appropriate data should be provided on a chemical-by-chemical basis for all potential genotoxic modes of action for each chemical investigated.

### Experimental Approaches to identification of NOELs

26. The statistical power of a study should be considered with regard to the detection of genotoxic effects and the identification of NOELs. Statistical analysis of dose-response data from genotoxicity studies cannot exclude the possibility of effects at doses below a NOEL that has been derived using statistically significant differences. Experimental evidence for a mode of action is, therefore, a prerequisite before investigating the dose-response for a possible threshold for a mutagenic effect. Dose-response investigations should include doses selected around the presumed NOEL together with appropriate replicate investigations.(Lovell, 2000) The more experimental/dose levels the better the dose-response relationship will be characterized especially when there are multiple dose levels in the area of interest.(Lovell, 2000, 2008). Approaches such as the use of flow cytometrics for micronuclei evaluation have the potential to increase the power of studies to detect genotoxic effects.

27. A range of statistical models can be used to fit curves to data. The use of any specific statistical models should be justified on a case-by-case basis. One model, the threshold or 'Hockey stick' model is, for example, a piece-wise linear regression model designed to detect discontinuities, change points or inflections in a dose-response curve.(Lovell, 2008) Lutz and Lutz have recently described the development of a 'Hockey stick' threshold model for use with genotoxicity data.(Lutz and Lutz, 2009) They note though that when the 'Hockey stick' model fits the data significantly better than linearity, the threshold-like appearance of the dose-response curve will have to be corroborated by mechanistic considerations and experimental testing of the respective hypothesis.(Lutz and Lutz, 2009) It should be noted that while a Hockey Stick model may be a better fit than a linear model to a set of data, a non-thresholded non-linear model could also be as good or better fit to the experimental data.

### **Conclusions**

28 i) The COM reaffirmed the default position that for *in vivo* mutagens, in the absence of mechanistic data to infer a threshold, it is prudent to assume that there is no threshold for mutagenicity.

ii) If there is good reason to consider that a threshold mode of action is appropriate, then it is necessary to investigate the biologically meaningful threshold for all genotoxic effects that have been reported.

iii) An appropriate strategy should be devised for each chemical under consideration to identify threshold dose levels or NOELs for all potential thresholded modes of action of genotoxicity, which may include either *in vitro* or *in vivo* studies.

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## **References**

- Beddowes E, Faux S, Chipman K. Chloroform, carbon tetrachloride and glutathione depletion induce secondary genotoxicity in liver cells via oxidative stress. *Toxicology* 187:101-115.2003.
- Bentley K, Kirkland D, Murphy M, Marshall R. Evaluation of thresholds for benomyl and carbendazim-induced aneuploidy in cultured human lymphocytes using fluorescein in situ hybridisation. *Mutation Research* 464.41-51,2000..
- Bergman K, Muller L, Wegberg Teigen S. The genotoxicity and carcinogenicity of paracetamol: a regulatory (re)view. *Mutation Research* 349:263-288.1996.
- Beyersmann D, Hartwig A. Carcinogenic metal compounds: recent insight into molecular and cellular systems. *Archives of Toxicology* 82:493-512.2008.
- Choudhury R, Ghosh S, Palo A. Cytogenetic toxicity of methotrexate in mouse bone marrow. *Environmental Toxicology and Pharmacology* 8:191-196.2000.
- Christmann M, Tomicic M, Roos W, Kaina B. Mechanisms of human DNA repair: an update. *Toxicology* 193:3-34.2003.
- COM. Annual Report of the Committees on Toxicity, Mutagenicity, Carcinogenicity of Chemicals in Food, Consumer Products and the Environment.1993.
- COM. Annual Report of the Committees on Toxicity, Mutagenicity, Carcinogenicity of Chemicals in Food, Consumer Products and the Environment .1995.
- COM. Annual Report of the Committees on Toxicity, Mutagenicity, Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. 1997.
- COM. Mutagenicity of hydroquinone and phenol. COM statement. COM/00/S1.2000.

- COM. Risk Assessment of *in-vivo* mutagens and genotoxic carcinogens. MUT/01/S3.2001.
- COM. Annual Report of the Committees on Toxicity, Mutagenicity, Carcinogenicity of Chemicals in Food, Consumer Products and the Environment.2004.
- COM. Update Statement (2008): Mutagenicity of Phenol. COM/08/S2.2008.
- COM. Statement on the Genotoxicity of Acrylamide. COM/09/S1.2009.
- Doak S, Brusehafer K, Dudley E, Quick E, Johnson G, Newton R, Jenkins G  
No-observed effect levels are associated with up-regulation of MGMT following MMS exposure. Mutation Research 648:9-14.2008.
- Doak S, Jenkins G, Johnson G, Quick E, Parry E, Parry J. Mechanistic Influences for Mutation Induction Curves after Exposure to DNA-Reactive Carcinogens. Cancer Research 67:3904-3910.2007.
- Drablos F, Feyzi E, Aas P, Vaagbo C, Kavli B, Bratlie M, Pena-Diaz J, Otterlei M, Slupphaug G, Krokan H. Alkylation damage in DNA and RNA-repair mechanisms and medical significance. DNA Repair 3:1389-1407.2004.
- Elhajouji A, Tibaldi F, Kirch-Volders M. Indications for thresholds of chromosome non-disjunction versus chromosome lagging by spindle inhibitors *in-vitro* in human lymphocytes. Mutagenesis 12:133-140.1997.
- Elhajouji A, Van Hummelen P, Kirsch-Volders M (Indications for a threshold of chemically induced aneuploidy *in-vitro* in human lymphocytes. Environmental and Molecular Mutagenesis 26:292-304.1995).
- Gocke E, Ballatyne M, Whitwell J, Muller L (MNT and Mut<sup>TM</sup>Mouse studies to define the *in vivo* dose response relations of the genotoxicity of EMS and ENU. Toxicology Letters 190:286-97, 2009.
- Gocke E, Muller L. *In vivo* studies in the mouse to define a threshold for the genotoxicity of EMS and ENU. Mutation Research 678:101-7, 2009.
- Gocke E, Wall M. *In vivo* genotoxicity of EMS: Statistical assessment of the dose response curves. Toxicology Letters: 190: 298-302, 2009.
- Henderson L, Albertini S, Aarema M. Thresholds in genotoxicity responses. Mutation Research 464:123-8.2000.
- Jackson M, Stack H, Waters M. Genetic activity profile of anticancer drugs. Mutation Research 355:171-208.1996.
- Jenkins G, Doak S, Johnson G, Quick E, Waters E, Parry J. Do dose response thresholds exist for genotoxic alkylating agents? Mutagenesis 20:389-398.2005.
- Kaina B, Fritz G, Ochs K, Haas S, Grombacher T, Dosch J, Christmann M, Lund P, Gregel C, Becker K. Transgenic systems in studies on genotoxicity of alkylating agents: critical lesions, thresholds and defense mechanisms. Mutation Research 405:179-191.1998.
- Kirkland D, Aardema M, Henderson L, Muller L. Evaluation of the ability of a battery of three *in vitro* genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and relative predictivity. Mutation Research 584:1-256.2005.
- Kirsch-Volders M, Vanhauwaert A, Eichenlaub-Ritter U, Decordier I. Indirect mechanisms of genotoxicity. Toxicology Letters 140-141:63-74.2003.
- Kirsch-Volders M, Gonzalez L, Carmichael P, Kirkland D (Risk assessment of genotoxic mutagens with thresholds: A brief introduction. Mutation Research 678: 72-5, 2009.

- Lave T, Paehler A, Grimm H, Gocke E, Müller L. Modelling of patient EMS exposure: translating pharmacokinetics of EMS in vitro and in animals into patients. *Toxicology Letters* 190: 310-6, 2009.
- Lovell D. Dose-response and threshold-mediated mechanisms in mutagenesis: statistical models and study design. *Mutation Research* 464:87-95.2000.
- Lovell D. Experimental Design and Statistical Analysis of Studies to Demonstrate a Threshold in genetic Toxicology: A Mini-review. *Genes and Environment* 30:139-149.2008.
- Lutz W, Lutz R. Statistical model to estimate a threshold dose and its confidence limits for the analysis of sublinear dose-response relationships, exemplified for mutagenicity data. *Mutation Research* 678: 118-22, 2009.
- Lynch A, Harvey J, Aylott M, Nicholas E, Burman M, Siddiqui A, Walker S, Rees R. Investigations into the concept of a threshold for topoisomerase inhibitor-induced clastogenicity. *Mutagenesis* 18:345-353.2003.
- Morley A, Turner D. The contribution of exogenous and endogenous mutagens to in-vivo mutations. *Mutation Research* 428:11-15.1999.
- Müller L, Cocke E, Lave T, Pfister T. Ethyl methanesulfonate toxicity in Viracept- A comprehensive human risk assessment based on threshold data for genotoxicity. *Toxicology Letters* 190: 317-29, 2009.
- Müller L, Kasper P. OTC Pharmaceuticals and Genotoxicity Testing: The Paracetamol, Anthraquinone and Griseofulvin Cases. *Archives of Toxicology* 17:312-325.1995.
- Parry J, Jenkins G, Haddad F, Bourner R, Parry E. In vitro and in vivo extrapolations of genotoxin exposures: consideration of factors which influence dose-response thresholds. *Mutation Research* 464:53-63.2000.
- Spencer P, Gollapudi B, Waechter JJ. Induction of Micronuclei by phenol in the mouse bone marrow:1. Association with chemically induced hypothermia. *Toxicological Sciences* 97:120-127.2007.
- Unpublished Report Thiophanate-methyl induction of aneuploidy in cultured peripheral blood lymphocytes. Final Report submitted in confidence to COM 1996.