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CC/2004/6

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)

TOXICOGENOMICS/PROTEOMICS/METABONOMICS: UPDATE ON LITERATURE RETRIEVED DURING 2003

Introduction

1. The COT/COC/COM held a joint symposium on the use of genomics and proteomics in toxicology on 8 October 2001. A statement outlining the conclusions reached has been published on the Committee websites and a full write up of the meeting was published in *Mutagenesis*. 2003 May;18(3):311-7. This paper provides a brief overview of the literature relevant to use of these techniques in carcinogen risk assessment published since the joint meeting. It is pertinent to concentrate on new data where COT/COC/COM can provide relevant advice to assist Government Departments in the assessment of toxicological data using these new approaches.

Conclusions reached at October 2001 symposium (statement appended at end of paper for ease of reference).

2. These are reproduced from the published statement below;
 - i) We *recognise* the future potential of proteomics and genomics in toxicological risk assessment.
 - ii) We *note* that these techniques may serve as adjuncts to conventional toxicology studies, particularly where proteins under investigation are known to be causally related to the toxicity.
 - iii) However, we *consider* that research and validation is required before these techniques can be considered for routine use in regulatory toxicological risk assessment. In particular there is a need for more research leading to development of genomic/proteomic databases, methods in bioinformatic and statistical analysis of data and pattern recognition and information on the normal range of gene expression.

Outline of discussion papers for COT/COC/COM

3. An update paper was considered by the COT in February 2003 who concluded that there was no need to amend the conclusion reached in October 2001. It was acknowledged that the application of genomics, (this term is used in this paper instead of the term transcriptomics) proteomic and metabonomics to toxicology was rapidly developing and

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thus COT asked for an annual update paper to be prepared. The most recent draft review for the February 2004 COT meeting has been forwarded to COC members as background information. (This latter paper also includes the first summary of metabonomic data presented to COT.) This is a relatively bulky paper and in order to assist COC members, key outcomes of the review of published papers retrieved for COT during 2003 are given below ;

- a) There is extensive research ongoing regarding the use of toxicogenomic approaches in toxicology. Most concern transcriptomic studies with fewer data available on proteomic and metabonomic approaches.
 - b) The most useful application of toxicogenomic data is the development of hypotheses for mechanistic interpretation of toxicological data.
 - c) The results of the initial screening studies (for specific target organ effects e.g. nephrotoxicity, hepatotoxicity) indicate that cDNA microarrays may be of value in early screening for potential effects but there are considerable areas of further work required dealing with reproducibility both within and between studies or laboratories and the development of bioinformatic approaches to aid data evaluation.
 - d) The application of Principle Component Analysis (PCA) has shown promise as a useful method for displaying complex data in a visually interpretable form. This approach uses analysis of the principle sources of variance in data and displays this information graphically, either 2-dimensionally or 3-dimensionally, e.g. PC1vPC2.
 - e) There is little available research which provides a comparison of the outcome of genomics with proteomics. It was not possible to draw any definite conclusions from the one available study of the acute hepatotoxicity of bromobenzene in rats. Newer developments in proteomic methods (SELDI-TOF) may assist in such work in the future.
 - f) There is little comparative data on the use of high density cDNA microarrays (e.g. with thousands of genes) and low density cDNA arrays (with small numbers of genes targeted for a limited number of toxic mechanisms). In general high density arrays are difficult and expensive to develop and it is difficult to interpret the data. Low density arrays are cheaper, easier to use and evaluate, but may miss novel mechanisms and have limited coverage.
 - g) It is noted that many journals require the results of cDNA microarray experiments to be replicated by methods involving quantitative mRNA analysis (e.g. RT-PCR).
4. The COM considered an update paper at its 5 February 2004 meeting. The paper focused on the ILSI/HESI research and other published research aimed at primarily screening for genotoxicity and for identification of mechanisms for false positive responses in assays. Almost all the available work related to L5178Y mouse lymphoma cells (p53 deficient) and human TK6 cells (p53 proficient). Overall COM members considered that the data did not support the use of transcriptomics for mutagenicity screening.

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5. The ILSI/HESI trial didn't consider carcinogenicity and thus this paper is based on the available published literature (essentially from 2002 onwards). Five areas of investigation were identified in literature searches;
 - a) Transcriptomic profiling of stages in the development of tumours induced by model carcinogens. (Experiments where a key objective was to monitor gene expression changes during the carcinogenic process have been placed in annex 1)
 - b) Investigation of transcript profiles of chemical induce tumours (e.g. liver, mammary gland) in rodents (Experiments where a key objective was to define clusters of genes associated with carcinogenicity and which might help as biomarkers have been placed in annex 2)
 - c) Comparison of gene expression changes in rodents and humans using a model genotoxic carcinogen (MNNG) (annex 3)
 - d) Investigation of effects of low dose effects of phenobarbital on DEN initiation of hepatocarcinogenesis in rats (annex 4)
 - e) Early identification of genotoxic carcinogens (in-vivo) (Annex 5)

6. An overview of the information is given below. Relevant papers are appended.

Evaluation of retrieved publications

Transcriptomic profiling of stages in the development of tumours induced by model carcinogens. (annex 1)

Chemical/Experimental approach	cDNA microarrays used	Results	Comments
Alachlor. Dietary administration for up to 18 months (= 126 mg/kg bw/day). This regime is known to result in defined series of histological lesion of olfactory epithelium leading to adenoma (6 months) and adenocarcinoma (at 11 months).	Affymetrix U34 A (4777 probes). cDNA analysis of samples from two animals at 2,3,4,5 months and one animal at 1 day, 4 days and 1 month (one sample analysed in duplicate). Tumour tissues were analysed from two rats at 18 months. A total of 26 cDNA microarray analyses reported (i.e. most analyses were undertaken as replicates at each time point).	In total 1265 probes (genes and ESTs) were differentially expressed (ca 1.8x or 0.5x in at least 2 of the samples). In hierarchical clustering 148 probes exhibited up-regulation of 3x or more at 1 day, 4 days, or 1 month. This included changes to control of extracellular matrix, oxidative stress, cell proliferation and cell cycle regulation and calcium homeostasis. There were 417 genes/ESTs with at least a 2 fold upregulation in olfactory tumours. The pattern was reported to be markedly different from pre-neoplastic lesions. Key changes included altered immune response gene expression and the wnt signalling pathway. (Up-regulation of wnt was confirmed by immunohistochemical staining for β -catenin	The authors used different criteria for identifying gene changes during pre-neoplastic and neoplastic phases. Little information on statistical aspects of design were provided and RT-PCR was not used to confirm gene expression changes. However differences in patterns between pre-neoplastic and neoplastic lesions were identified. Inclusion of a genotoxic chemical inducing olfactory tumours would have provided valuable additional information.

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<p>Nitroso-morpholine administered via drinking water (200 mg/l) for up to 20 weeks. Regime designed to result in liver tumours. Hepatocytes (foci) isolated by perfusion after 5 weeks. Tumours microdissected.</p>	<p>Authors initially undertook cDNA using rat microarray but failed to get results using phosphoroimaging technique. Subsequent investigations used a human cDNA platform and fluorometric analysis (2345 cDNAs). A 2 fold change in expression of treated hepatocytes and tumours compared to normal hepatocytes/ liver tissue was taken as the criteria for altered gene expression.</p>	<p>Results of cDNA microarray experiments comparing hepatoma and normal liver tissue reported. Up-regulation of 553 probes and downregulation of 55 probes in hepatoma cells compared to normal liver was reported. The authors considered that the up-regulation of genes associated with protein synthesis in hepatoma cells was noteworthy.</p>	<p>The authors accepted that use of human cDNAs would result in some degree of mismatch. The approach to cluster analysis was not reported and RT-PCR was not used to validate the results. No comment on the effect of culturing hepatomas on gene expression was made. Overall no conclusions can be reached from this study.</p>
<p>2,3,5-tris(glutathion-S-yl) hydroquinone (TGHQ). Isolated renal epithelial cells were transformed by treatment with TGHQ (QT RRE). In further studies Eker rats (which bear a mutation in allele of the tuberous sclerosis 2 (Tsc-2) gene predisposing to formation of renal tumours) were treated with TGHQ over 11 months using a regime designed to result in renal tubule tumours.</p>	<p>Authors undertook cDNA microarray studies of renal tissue from 1 or 2 rats treated for 1 d, 4,d, 1 month, 2,3,4,5 months and in 2 animals renal tubular adenocarcinomas at 18 months of age. The Atlas 1.2 cDNA microarray was used. Most samples were analysed in duplicate. A threshold of 3 fold change in gene expression was used as the criteria for reporting.</p> <p>Isolated hepatocytes were passaged for up to 36 times in culture. Gene expression changes for 11 selected genes were confirmed by RT-PCR. Western Blot analysis of annexin I and II was undertaken.</p>	<p>Transformed QT RRE cells showed 40 genes with altered expression (from -11 fold to +10 fold). It was reported that gene expression was relatively constant in passage 4 and 36 cells. The most evident gene expression changes in transformed cells involved genes associated with invasion and metastasis. In renal tumours a total of 50 genes had altered expression (-27 to +14 fold). Results in the two tumours analysed were reported to be similar (data not presented). Genes involved in phase II metabolism and GSH metabolism were down regulated or could not be identified in tumours. Genes up-regulated in tumours included 4 members of annexin family, some genes related to control of cell-cycle, extracellular signalling. The concordance between altered gene expression in transformed cells and neoplasms was small (ca 15%).</p>	<p>The authors considered that the results of the study were essentially hypothesis generating and considered that differentiation between transformed renal cells and renal tumours was possible.</p> <p>The use of RT-PCR validates the results to some degree. The small number of replicate analyses limits the conclusions that can be reached.</p>
<p>Diethylnitrosamine (with 2-AAF and partial hepatectomy). A treatment regime was designed to result in significant numbers of GST-P positive liver foci within 6 weeks.</p>	<p>Authors undertook cDNA microarray analysis using a platform supplied by Agilent Technologies. Experimental design was unusual in that RNA was extracted from foci identified from immunostained slides of liver tissue.</p>	<p>Authors noted that immunostaining did reduce RNA levels. However they did identify some gene expression changes associated with the production of GST-P foci and confirmed a number by RT-PCR. These included upregulation of metabolising enzymes previously associated with pre-neoplastic changes induced by liver carcinogens such as aflatoxin B₁. It was suggested this approach might be useful for mechanistic work and for identifying novel biomarkers</p>	<p>The isolation of RNA from fixed/stained tissue needed more evaluation in order to assess in key gene expression changes were lost through this technique. The authors noted that RT-PCR (for 5 genes) did confirm cDNA microarray analyses except for the expression of GST-P.</p>

6. In these studies, the investigators attempted to use cDNA microarrays to monitor the progression from initiated cell to tumours using a number of model carcinogens (both genotoxic and non-genotoxic). The limitations of these studies resolve around the application of cDNA microarrays (e.g processing of tissue, use of appropriate platforms, numbers of replicate analyses, methods used to undertake cluster analysis, and validation of results). The data suggest a number of gene targets for the carcinogenic process for a number of tumours in rodents and that it may be possible to delineate gene expression changes during carcinogenesis using this approach. However the data aren't sufficiently robust to draw any conclusions at present. Members may also wish to note the paper from Deng WG et al, (World J Gastroenterology vol 10, 46-52, 2004.). These authors used RT-PCR, DNA sequencing for mutations to examine the role of p53 expression and specific mutation in p53 during hepatocarcinogenesis induced by 3-methyl-4-dimethylaminoazobenzene. The approach used a targeted (hypothesis driven) approach to evaluation

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of gene expression changes whereas the cDNA approaches used in the above studies examine global gene expression changes to identify hypotheses for further evaluation.

Investigation of transcript profiles of non-genotoxic and genotoxic carcinogens in rodents (annex 2)

Non-genotoxic carcinogens

7. Iida M and colleagues (Carcinogenesis, 24, 757-770, 2003.) undertook an evaluation of gene expression changes in the early stages of carcinogenesis using two model mouse hepatocarcinogens (oxazepam and Wy-14,643, a peroxisome proliferator). cDNA microarray analysis (using NIEHS mouse chip 8736 genes and ESTs), RT-PCR and western blot analysis (for selected genes) and proteomic analysis (using 2D-gel separation and MALDI/TOF mass spectrometry) were undertaken at a number of time points up to 6 months of dietary administration. Dose levels were designed to result in hepatocellular hypertrophy and would eventually lead to hepatocellular carcinogenesis. The approach taken in this study showed how using a variety of techniques provided complimentary data and aided the elucidation of the gene-expression changes seen during the early response to administration of 2 model non-genotoxic hepatocarcinogens. It is notable that RT-PCR only partly confirmed the cDNA microarray results. This may be due in part to the methods used to generate and analyse cDNA microarray data, although the number of replicates used is commensurate with the best publications currently available using this technology.
8. The authors concluded that Cyp2b20, growth arrest and damage inducible gene (Gadd 45), tumour necrosis factor α inducible protein 2 and insulin-like growth factor binding protein protein 1 genes and proteins were all up regulated by **oxazepam**. Cyp2b20, cyclinD1, PCNA, Igfbp5 Gadd 45 and cell death inducing DNA fragmentation factor α subunit like effector exhibited up-regulation after **Wy-14,643**. The authors noted that no effects on gene expression of p53, Fos, Myc and Rb genes were found in this study. In addition there was no evidence of increased wnt signalling in this study with either chemical (unlike alachlor induced olfactory tumours as reported above).
9. Overall there appeared to be more distinct than common changes between the two compounds under study. It is noteworthy that there remained a number of identified protein changes which could be the subject of future study.
10. The original intention was to attempt to identify common gene expression changes in early carcinogenesis which might assist in developing an approach to early in-vivo detection of carcinogenesis. The results suggest that this would be a difficult task and the available data did not support the use of cDNA microarrays for this purpose.

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Investigation of a peroxisome proliferator and a genotoxic carcinogen in mouse liver

11. The authors noted that PPAR α hyperactivity in AOX^{-/-} mice resulted in the formation of hepatocellular carcinomas (HCC) between 10-15 months of age. The initial objective was to identify gene clusters associated with peroxisomal proliferation modulated hepatocarcinogenesis. cDNA microarray investigations were undertaken using HCCs derived from AOX^{-/-} mice (n=26) and from HCCs derived from ciprofibrate (n= 3) and diethylnitrosamine (DENA n =3) animals. Tumours from 6 wild type C57BL/6J mice (aged 5,7 or 15 months, pooled tissue) were used as controls. Hybridisations (duplicate runs) were undertaken using the NIH mouse array (9180 genes and ESTs). A hierarchical clustering approach to analysis was used. Northern blotting analysis was used to confirm selected gene expressions.
12. It was reported that each treatment revealed a distinct pattern of gene expression changes but there was more in common between AOX^{-/-} and ciprofibrate than either of these two groups had with DENA. The authors examined commonality between between all three groups and reported RIEKN cDNA, Ly-6D and lipocalin 2 might be potential molecular markers for liver carcinogenesis. The authors clearly identified genes associated with PPAR α activation in tumours from AOX^{-/-} and ciprofibrate treated animals but not in tumours from DENA treated animals. (examples include up-regulation of as cyp 4a14, 4a10, carnitine acyltransferase, fatty acid binding protein and Per11 α). Northern blot analysis (semi-quantitative) confirmed the selected gene expression changes.
13. The authors didn't present the results of gene expression changes in DENA tumours in any detail.

Comment on studies investigating hepatocarcinogenicity

14. It is interesting to attempt to compare the results of these two studies which used different very different approaches to examining gene expression changes resulting from exposure of mice to peroxisome proliferators. However the Wy-14,643 experiment considered early changes during the first 6months of exposure whilst the ciprofibrate experiment focused on changes in hepatocellular tumours. Each experiment used a different approach to cDNA microarray analysis and to validation of the results. Overall it is suggested that no comparison can be made. More information on the gene expression changes induced by the model genotoxic hepatocarcinogen DENA would have been useful. In addition, in view of the limited number of chemicals examined to-date, the

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variation in cDNA microarray approaches used, it is also suggested there is a need for these results to be independently reproduced before any weight can be given to use of such data to identify gene expression changes during hepatocarcinogenesis.

Investigation of transcript profiles of mammary tumours in rats induced by genotoxic carcinogens (end of Annex 2).

15. Four-DMBA and three-PhIP induced mammary carcinomas were analysed by cRNA hybridisation experiments with Affymetrix U34 A using fluometric analysis of ₁₀transformed filtered for presence in 6/7 carcinomas. The data were subject to hierarchical clustering analysis. Quantitative RT-PCR analysis was undertaken for 12 genes selected to reflect the cluster patterns reported.
16. The authors described four distinct clusters which reflected commonality and differences between tumours. The coefficient of correlation between DMBA and PhIP was 0.63. Correlation between carcinomas within each group ranged from 0.78-0.95. The authors suggested that gene expression profiles in mammary tumours might reflect the aetiology and suggested the aetiology of cancer might be retrospectively estimated from their expression profiles.

Comparison of gene expression changes in rodents and humans using a model genotoxic carcinogen (annex 3)

17. Male ACI rats were given MNNG via the drinking water (83 mg/l) from age 8 weeks up to 40 weeks of age. Animals were killed at 65 weeks. 22/28 treated animals had stomach carcinomas. cRNA microarray analysis was undertaken using a gene expression change of >20 as a cut off for further evaluation. Using a more stricter criterion of >20 fold change, there were 50 genes/ESTs up-regulated and 25 down-regulated. Up-regulated genes were involved in maintenance of the extra cellular matrix, immune response and ossification. Down-regulated genes were involved in hydrocarbon metabolism, gastric mucous and gastric hormone production. A number of known oncogenes were up-regulated, PDGF receptor α and Gro1. The tumour suppressor gene metastasis-suppressor gene Kangai 1 was down-regulated. RT-PCR confirmed these results. The authors noted that if a cut off of >2 fold change was used then it was possible to obtain discordant results between microarray experiments and RT-PCR.
18. Comparison with published gene-expression changes in human stomach cancer revealed a number of changes in common between rat and human stomach cancers. Observed in common up-regulated genes were related to the extracellular matrix and cellular trafficking. Commonly down-regulated genes were those related to differentiated phenotypes of stomach such as production of HCl and proteases for food digestion.

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However there were few gene changes in common when rat stomach and mammary gland carcinomas were compared.

Other studies comparing gene expression in animal tumours and human tumours (end Annex 3)

19. A number of investigators have attempted to compare gene expression changes in rodent tumours and equivalent tumours in humans. One key objective for most of these studies is to identify potential common gene changes in rodents and humans and to use this information to derive potential molecular targets for new developments in therapy. A number of observations from these studies which may be of relevance for the evaluation of chemical induced tumours in rodents and their relevance to human tumours have been identified. Some of these taken from a review of investigations on mammary gland cancer are briefly summarised below;

Normal architecture of rodent and human equivalent tissues can vary considerably

Comparisons of gene expression data between normal and tumour tissue have to be considered in the light of the structural changes during the carcinogenic process which for can also include non-neoplastic changes (e.g changes in epithelial, fat and stromal cells in mammary glands).

There may be technical impediments to comparing cDNA microarray experiments between rodents and humans, including relatively few homologous genes on platforms, imperfections in public databases, and the number of unknown/uncertain gene sequences (i.e. Expressed Sequence Tags, ESTs).

20. An abstract of a paper providing information on gene expression profiles from normal lung tissue, lung adenoma and adenocarcinomas from mice and a comparison with tissues from human biopsies where equivalent histological diagnoses were reported is included at the end of Annex 3 (Bonner AE et al Oncogene, vol 23, 1166-1176, 2004). Overall, the authors are suggesting that mouse and human tumour gene expression profiles can be clustered into groups where expression is similar. Relatively little information or conclusions can be derived from this abstract. Key parts to evaluation of the work would be the statistical design of the microarray experiments and approaches to hierarchical clustering and analysis of derived patterns.
21. Similar factors would be important in assessing the significance of the comparison between MNNG induce stomach tumours in rats and human stomach cancers.

Investigation of effects of low dose effects of phenobarbital on DEN initiation of hepatocarcinogenesis in rats (annex 4)

22. The authors reported previous results in rats which suggested that a low dose of phenobarbital (administered via the diet at 2 ppm) reduced the

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formation of hepatic preneoplastic foci but at higher levels (ca 15 or 200 ppm) enhanced the number of foci formed. In the current experiment levels of 8-hydroxy-2'-deoxyguanosine (8-OhdG) formation were suppressed in an initiation/promotion design study in rats using a phenobarbital dose level of 2 ppm. The authors also undertook cDNA microarray investigations of liver tissue from animals subjected to various initiation/promotion experiments. The identified up regulation of oxoguanine glycosylase 1 (Ogg1) a repair enzyme involved with repair of 8-OhdG. They also identified up-regulation of glutamine acid decarboxylase (GAD65) and speculated on the possible involvement of this gene in suppressing the formation of hepatocellular foci. The cDNA microarray experiments were useful in suggesting hypothesis for further investigation in this experiment.

COC discussion

23. The COC update paper on application of toxicogenomics differs from the COT/COM papers in that the ILSI/HESI venture on screening for toxicological mechanisms only considered hepatotoxicity/renal toxicity and genotoxicity.
24. Two basic approaches have been used to applying transcriptomic analyses to carcinogenicity. The first involves attempting to screen gene expression changes during early and late stages of carcinogenesis so that the data derived can inform on mechanisms (by allowing hypothesis generation) and also potentially be of use in carcinogen identification. No clear conclusions can be reached from the limited information available as relatively few chemicals have been examined and there were differing approaches to and standards of validation used in the cDNA microarrays and evaluation of the data produced. The studies using alachlor and 2,3,5-tris(glutathione-S-yl)hydroquinone suggest it is possible to distinguish between different phases of the carcinogenic process. The potential application of early changes to screening would need considerably more data before any conclusions could be reached.
25. One additional paper was considered by the COM (Annex 5) during its review. The authors evaluated gene expression profiles in the liver of rats treated with four genotoxic liver carcinogens for periods of up to 14 days. Chemicals used were dimethylnitrosamine, 2-nitrofluorene, aflatoxin B1 and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK). The changes seen in this in-vivo study were consistent with the predicted effects of genotoxic carcinogens in rat liver and reflected the observed histology changes. RT-PCR analyses undertaken for a few selected genes confirmed the microarray experimental findings.
26. The second approach identified in this review has been to investigate if tumours resulting from the administration of chemicals carcinogens have consistent gene-expressions for tumours with the same histological diagnosis, to examine whether the aetiology of a particular tumour can be

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diagnosed from the gene expression pattern and whether gene expression patterns of rodent tumours and equivalent human tumours are similar.

27. It is evident that these studies were based on examination of comparatively few tumours.
28. However a number of interesting observations can be made. The lack of apparent concordance of gene expression for hepatocellular tumours induced by peroxisome proliferators appeared to be an unexpected finding. Confirmation of this observation would raise interesting questions regarding potential differences between chemicals within the peroxisome proliferator group although it is now generally accepted that these rodent tumours have little, if any, relevance to humans. At present it is too early to draw definite conclusions from these data.
29. The suggestion that the aetiology of mammary tumours might be chemical-specific also warrants further investigation. However, again it is too early to draw conclusions and repetition of these results would be useful. It is noteworthy of the lack of common gene expression changes between mammary and stomach tumours in rats.
30. The finding of concordance between gene expression of stomach tumours in rats and humans would suggest that the rat is a reasonable model for human carcinogenesis or alternatively if the aetiology hypothesis has any credence that the human tumours might be associated with MNNG or other similar nitrosating agent? As with previous comments, repetition of these studies would aid in placing weight on the results. The discussion of comparison of rodent and human tumours also indicates the need for agreement on the designs and statistical approaches to interpretation of complex gene expression patterns.
31. Members will be interested to note that one suggestion from the COT was for a review of the statistical and bioinformatic approaches to interpretation of data generated by these new approaches to investigating gene expression. [The secretariat has asked Dr D Lovell from Surrey University to prepare a paper on this aspect. It is hoped a paper will be presented to COT in the near future.]

Overall question for COC.

33. Members are asked to consider if a revision to the existing statement is warranted and to agree that any further work should be undertaken in conjunction with COT and COM and should cover all aspects of toxicogenomics.

Secretariat February 2004

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Addendum to CC/2004/6

**COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD
CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)**

**TOXICOGENOMICS/PROTEOMICS/METABONOMICS: UPDATE ON
LITERATURE RETRIEVED DURING 2003**

**Additional paper with analysis of tumours for gene expression changes.
(Should be considered as part of Annex 2 to CC/04/6).**

Liu J et al. (2004). Toxicogenomic analysis of aberrant gene expression in liver tumours and non-tumorous liver of adult mice exposed in-utero to inorganic arsenic. Toxicological Science, 77, 249-257.

The authors had previously demonstrated the transplacental carcinogenicity of sodium arsenate (Waalkes MP et al, Toxicol Appl Pharm, 164, 161-170, 2003) in C3H mice. The in-life phase consisted of administration of drinking water containing either 42.5 ppm or 85 ppm As on days 8-18 of gestation. Litters were culled to 8 on PND 4. Animals were weaned at 4 weeks of age. Post-mortems and histology were undertaken at week 74. A marked increase in hepatocellular carcinomas was reported, particularly in males. Thus the incidence of hepatocellular carcinoma in control mice at 74 weeks was 12% compared to 61% in mice treated with 85 ppm arsenic *in-utero*. Tissue (tumour and surrounding non-tumour tissue) was taken from 4 animals treated with As and from 4 males with spontaneous tumours (3 adenomas/1 hepatocellular carcinoma (HCC)). Pooled RNA from these animals was used for cDNA microarray investigations. A custom made cDNA microarray containing 600 genes was used. Analysis of platforms used ³²P-labelling. Hybridisations were undertaken three-five times. Selected genes (n=31) were analysed by RT-PCR. Statistics employed used Students's *t*-test for comparison between groups. A two fold change in expression between arsenic induced tumour and normal tissue was used for reporting effects.

For arsenic treated animals there were 56 up regulated and 26 down regulated genes in HCC compared to normal tissue. Spontaneous tumours showed 25 gene up regulated and 21 genes down regulated compared to normal tissue. A comparison of normal tissues from arsenic treated animals and controls showed 60 genes with differential expression.

Up-regulation of α -fetoprotein, c-myc, cyclin D1, proliferation-associated protein (PAG) and cytokeratin-18 in arsenic induced hepatocellular carcinoma compared to spontaneous tumours. Up-regulation of α -fetoprotein, c-myc were also documented in non-tumour tissue in arsenic exposed animals. RT-PCR confirmed the effects on α -fetoprotein and cyclin D1 expression and partially confirmed the effects on c-myc. Overall, the effects seen in the cDNA microarrays were confirmed by the RT-PCR analyses.

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The authors considered it remarkable that gene expression changes could be identified in adults whose only exposure to arsenic was during gestation.

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