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MUT/06/03

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

A Comparison of the Relative Sensitivities of the *in vivo* Rat Liver UDS Assay and the *in vivo* COMET Assay.

Introduction:

1. The Committee considered the need for a comparison of the liver UDS assay vs the COMET assay in their horizon scanning exercise in October 2005 (COM/05/21). It was agreed that an evaluation of the two assays would be useful, but noted the amount of data available would be limited. In the first instance the secretariat asked to identify UDS positives and look for concordance with COMET (liver). It was agreed that there may be little data available, but some wider considerations of the COMET assay could be considered if possible.
2. This paper was drafted following a literature search that identified compounds which have data from both the UDS assay and the COMET assay available.
3. Assessment of the UDS studies retrieved showed that the assay protocols were largely in accordance with the OECD guideline (OECD 482; 1986). Criteria for a positive response were not explicitly stated. COMET assay protocols were more variable, although the majority were performed using standardised procedures (Sekihashi et al 2002, Sasaki et al 1997). Positive responses were judged based on statistical analysis.

Discussion:

4. The data retrieved are provided in Table 1. There were 16 compounds which were considered to have been adequately tested in both assays. The majority of the data were obtained from a limited number of papers which expressly aimed to examine the sensitivity and general applicability of the assays (UDS Mirsalis et al 1982, COMET Sasaki et al 1997, Sekihashi et al 2002). Most of the compounds are known carcinogens and several are frequently used as positive controls in these assays.
5. Direct comparisons between the two assays are difficult to make; for several compounds UDS data is available only from rats and COMET data from mice. Furthermore, for many, dose levels or route of exposure differs. COMET data obtained from the liver was generally available to compare with the liver UDS. Additionally, Sekihashi et al (2002) examined multiple organs in mice and rats.
6. Concordant results: Of the 16 chemicals selected, 11 produced results in agreement across both assays (positive or negative responses). Known carcinogens such as aflatoxin B₁, diethylnitrosamine (DEN), 2-acetylaminofluorene (2-AAF), 2,4-diaminotoluene, 1,2 dimethylhydrazine (DMH), methyl methanesulphonate (MMS), and benzidine gave clear-cut positive results in both assays. Compounds negative in both assays included 2,6-diaminotoluene (a non-carcinogen), 7,12-dimethylbenzanthracene (DMBA) and cyclophosphamide (CP). For DMBA and CP, COMET data was

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only available from lymphocytes (Vrzoc and Petras 1997) and the possibility of positive results being achieved from other target organs cannot be ruled out. However, CP was shown to be positive in a different COMET study using circulating lymphocytes (Franke et al 2005); the data are not presented as the COMET scoring system used was unconventional.

7. Results for Acrylamide from liver UDS and liver COMET assays were clearly negative. In other organs assessed using the COMET assay results were borderline positive or equivocal (including the brain and testes; Maniere 2005). Acrylamide is genotoxic *in vitro* in mammalian cells, produces structural chromosome aberrations *in vivo* in somatic and germ cells and is classified as probably carcinogenic to humans (IARC <http://www-cie.iarc.fr/htdocs/monographs/vol60/m60-11.htm>), and therefore a positive response in the COMET would be expected. It is noted that the magnitude of the positive response induced by the positive control chemical used in the COMET study (MMS) was also quite small. It is possible that the lack of unequivocal positive responses with Acrylamide are the consequence of a poorly performing assay.
8. Discordant results: Five /16 chemicals assessed gave different answers in the two assays. Benzyl acetate, classified as carcinogenic to the mouse liver and stomach was negative in the UDS assay and the liver after COMET assessment in both species, but positive in the stomach of both rats and mice. MNNG, a direct acting carcinogen, reported as negative in the mouse bone marrow micronucleus assay (Janssen and Ramel 1980) and here as equivocal in the UDS assay, is demonstrated to be clearly positive in several tissues in the COMET assay (liver presented here, also kidney and lung). Chlorodibromomethane and Benzo(a)pyrene did not induce positive responses in the liver UDS assay, but were clearly positive in the liver following COMET evaluation. Chlorodibromomethane was considered by COM in 1994/1995; it was concluded that it was not mutagenic *in vivo*, based on the results of a bone marrow micronucleus assay and the rat liver UDS assay. The latter assay was specifically undertaken at the request of the COM. It is not known whether these data presented here represent a COMET false positive. Benzo(a)pyrene, which induces site of contact tumours, was not examined in the stomach in the report examined here and thus a conclusion on target organ specificity cannot be drawn .
9. *o*-anisidine was negative in the liver UDS assay and in the liver following COMET assessment, in accordance with previous reports of its non-genotoxicity to rodents (Ashby et al 1991). However, it was shown to be clearly positive in the bladder following COMET assessment, its target organ for carcinogenesis, providing evidence of the usefulness of this target organ assay.

Conclusions:

10. Due to protocol differences it was not possible to directly compare the sensitivities of the two assays. In general, there was good concordance between assays. For chemicals with differing results, it appears that the COMET is either more sensitive particularly if evaluation of specific target organs were assessed.
11. Questions:
 - Do the Committee consider the two assays evaluated (Liver UDS and COMET) to have broadly similar sensitivities?

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- Do the data retrieved on chlorodibromomethane alter the prior conclusion on it's genotoxicity?
- Is a further evaluation of data on Acrylamide warranted?

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Table1: Comparison of data from liver US+DS assay with the COMET assay:

UDS data presented as net nuclear grain counts

Comet data presented as μm DNA migration unless otherwise stated

Where a variety of timepoints were examined, the maximum increases are given

All chemicals given as single oral doses unless otherwise stated.

All animals male unless otherwise stated

COMPOUND	Liver UDS data	COMET data
2AAF	Rat (F344) 50mg/kg Control 12h : -4.4 ± 0.5 Treated: 45.0 ± 11.3 Mirsalis et al 1982 Rat (Wistar): n=16 Control -3.0 ± 0.7 2AAF 25mg/kg: $+10.9 \pm 3.7$ Ashby and Beije 1985	Mouse: CD-1 Dose 400mg/kg ip Liver at 3h: Control: 2.43 ± 9.83 Treated: 13.90 ± 18.3 p<0.01 Sasaki et al 1997
Acrylamide (AA)	Rats (Fischer344 male) n=5 Control: 2h -1.3 ± 0.2 12h -3.0 ± 0.2 4h(5d) -3.1 ± 0.2 AA: 100mg/kg 2h -2.3 ± 0.2 12h -2.5 ± 0.3 AA: 30mg/kg for 5d 4h -2.0 ± 0.2 Butterworth et al 1992	Rats (SD male) 54mg/kg Liver: Control : 5h 3.14 ± 1.05 24h 5.28 ± 2.10 * AA 5h: 6.46 ± 3.44 (not stat sig, positive for % DNA in tail) 24h 7.55 ± 2.00 Leukocytes Control : 5h 0.87 ± 0.19 24h 0.84 ± 0.24 AA 5h: 2.36 ± 1.21 (p<0.05) 24h 2.04 ± 0.79 p<0.05 Maniere et al 2005
Aflatoxin B ₁	Rat (F344) 2mg/kg Control 2h: -5.1 ± 0.5 Treated: 32.3 ± 3.9 Mirsalis et al 1982	Mouse (ddY) 5mg/kg Liver: Control: 1.66 ± 0.74 Treated 17 8h: 5.58 ± 1.18 (not stat sig) 3.4-fold increase at 3 h (not stat sig) Colon: Control: 5.46 ± 0.54 Treated at 3h: 52.7 ± 4.10 p<0.001 (also positive in stomach, bladder, lung, brain) Rat: 5mg/kg Liver: Control : 1.11 ± 0.39 Treated at 3h: 37.5 ± 4.31 p<0.001 Colon: Control: 9.69 ± 0.62 Treated at 8h: 36.7 ± 8.07 p<0.001 (also positive in stomach, kidney, bladder, lung)

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		Sekihashi et al 2002
COMPOUND	Liver UDS data	COMET data
Benzidine	<p>Rat (F344) 200mg/kg Control 2h: -5.10.5 12h: -4.4±0.5 Treated: 2h 6.0 ± 2.1 12h 20.7 ± 9.6</p> <p>Mirsalis et al 1982 Ashby et al 1990</p>	<p>Mouse at 160mg/kg Liver: Control: 2.40±0.69 Treated at 8h: 11.8±1.22 4.9-fold increase at 8h</p> <p>Rat at 200mg/kg Liver : Control: 1.11±0.39 Treated at 8h: 11.6±1.69 10.5-fold increase at 8h</p> <p style="text-align: right;">Sekihashi et al 2002</p>
Benzyl acetate	<p>Rat (F344) 1000mg/kg Control 12h: -5.6±0.4 Treated: -4.6±0.3 (negative) Mirsalis 1989</p>	<p>Mouse at 1600mg/kg Liver: Control: 1.40±0.84 Treated at 3h: 3.00±0.90 2.1-fold increase ; not stat sig</p> <p>Stomach: Control: 4.16±0.71 Treated at 3h: 47.2±3.71 11.3-fold increase</p> <p>Rat at 1500mg/kg Liver : Control: 2.50±0.42 Treated at 24h: 6.40±0.96 2.6-fold increase not stat sig</p> <p>Stomach: Control: 11.9±1.87 Treated at 24h: 29.5±6.57 2.5-fold increase p>0.001 Sekihashi et al 2002</p>
Benzo (a) pyrene	<p>Rat ((F344): 100mg/kg ip Control at 12h : -4.4±0.5 Treated at 12h: -3.6±1.2</p> <p>Mirsalis et al 1982</p>	<p>Mouse BALB/c: DNAlength/width ratio Control 16h: 1.026±0.019 Treated 25mg/kg: 1.074±0.038 Treated 100mg/kg: 1.066±0.016 (both p<0.05) Vrzoc and Petras 1997</p> <p>Mouse: CD-1 Dose 250mg/kg ip Liver at 3h: Control: 2.22±9.02 Treated: 11.70±18.1 p<0.001 Sasaki et al 1997</p>
Chlorodibromomethane	<p>Rat: (SD): 2000mg/kg Control 2h: -2.3 Treated 2h: -4.1 Control 14h: -2.3 Treated 14h: -2.6 (negative) Stocker et al 1997</p>	<p>Mouse (ddY): 400mg/kg Liver: Control: 1.47±0.35 Treated at 8h: 8.54±0.57 5.8-fold increase p>0.001</p> <p>Rat: 200mg/kg Liver: Control: 1.810.83 Treated at 8h: 16.3± 6.33</p>

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		9.0-fold increase at 8h p>0.05 Sekihashi et al 2002
COMPOUND	Liver UDS data	COMET data
Cyclophosphamide	Rat (Alpk) : 20mg/kg (doses in excess of this were toxic) Control 12h: -4.4±0.5 CP 12h: -1.7 ± 0.9 Ashby and Beije 1985	Mouse (BALB/c) n=4 Lymphocytes DNA length/width ratio Control 16h: 1.159±0.167 200mg/kg at 16h: 1.362±0.298 50mg/kg at 16h: 1.195±0.178 not stat sig Vrzoc & Petras 1997
2,4 diaminotoluene	Rat (F344) 150mg/kg: Control: -5.1±0.5 Treated at 2h: 15.9±4.8 Mirsalis et al 1982	Mouse (ddY): 60mg/kg Liver: Control 2.35±0.49 Treated at 8h: 12.5±0.45 p<0.001 5.3-fold increase Kidney: Control: 2.27±0.62 Treated at 3h: 7.57±1.45 p<0.001 3.3-fold increase Rat: 130mg/kg Liver : Control: 1.19±0.44 Treated at 3h: 3.61±0.63 3.0-fold increase(not stat sig) Kidney: Control: 3.12±0.78 Treated at 8h: 21.7±3.69 7.0-fold increase at 8h Sekihashi et al 2002 Mouse: 240mg/kg Liver 3h : Control 2.22±9.02 Treated: 17.30±6.73 Kidney: Control 2.22±8.69 Treated 24h: 26.60±13.6 Sasaki et al 1997
2,6 diaminotoluene (non-carcinogenic)	Rat (F344) 150mg/kg Control 2h: -5.1±0.5 Treated at 2h: -3.5±0.7 Mirsalis et al 1982	Mouse : 60mg/kg Rat: 250mg/kg Clearly negative in all tissues examined. Sekihashi et al 2002
7,12 -DMBA	Rat (F344): 200mg/kg po Control : -5.1±0.5 Treated at 2h: -0.8±2.0 (negative) 150mg/kg ip: Control -4.4±0.5 Treated at 12h: -2.6±0.4 (negative)	Mouse: 600mg/kg Lymphocytes : (DNA length/width ratio) Control 16h: 1.057±0.007 Treated: 1.066±0.042 (negative) Vrzoc and Petras 1997

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COMPOUND	Liver UDS data	COMET data
1,2-dimethylhydrazine (DMH)	<p>Mouse (NMRI): n=4 Data from 11 independent studies Control : -0.67 ± 0.34 DMH 40mg/kg 2h n=5: 7.50±4.6 3h n=5: 18.43±5.4 – 29.58±8.1 4h n=1: 38.47±14.0</p> <p>Rats (Wistar): n=3-4 Data from 7 independent studies Control: -0.87±0.3 DMH 40mg/kg: 2.46±0.2 – 4.13±0.6 80mg/kg: 2.36±0.8 Brendler-Scwab et al 2002</p> <p>Rats: (male Alpk) n=15-23: DMH 30mg/kg: Control: -2.8±0.9 Treated at 2h +14.4±4.0 Control: -3.0±0.7 Treated at 16h +19.4±5.6 Kennelly 1995</p>	<p>Mouse: 30mg/kg Liver: Control: 2.29±0.57 Treated at 3h: 41.3±4.42 p<0.001 18-fold increase at 3h</p> <p>Rat: 100mg/kg Liver: Control: 2.50±0.42 Treated at 3h: 52.8±1.99 p<0.001 21.1-fold increase at 3h Sekihashi et al 2002</p> <p>Mouse CD-1: n=4 Liver: Control: 2.29±0.57 30mg/kg 3h: 46.4±8.53 30mg/kg 24h: 20.6±4.68</p> <p>Colon: 11.3±1.06 30mg/kg 3h: 25.8±2.33 30mg/kg 24h: 32.7±7.58</p> <p>Sasaki et al 1998</p>
DEN	<p>Rat: (F344): 50mg/kg Control 2h: -4.8±0.8 Treated at 2h: 30.8± 3.4</p> <p>Mirsalis et al 1982</p>	<p>Mouse (ddY): 160mg/kg Liver: Control at 3h: 2.29±0.57 Treated at 3h: 89.8±3.06 p<0.001 39-fold increase</p> <p>Rat (Wistar): 160mg/kg Liver: Control at 3h: 2.17±0.77 Treated at 3h: 51.5±3.89 p<0.001 23.7-fold increase</p> <p>Sekihashi et al 2002</p>
MMS	<p>Rat (F344): Control 2h: -4.8±0.8 Treated: 20mg/kg 2h: 9.1 ± 2.2 100mg/kg 2h: 38.0±2.9</p> <p>Mirsalis et al 1982</p> <p>Mouse (muta mouse) 100mg/kg Control 2h: -4.2±0.47 MMS 2h: 24.61±1.94</p> <p>Tinwell et al 1998</p>	<p>Mouse male (ddY): 80mg/kg Liver: Control at 3h: 2.07±0.53 Treated at 3h: 54.7±3.78 p<0.001 26-fold increase</p> <p>Rat (Wistar): 80mg/kg Liver: Control at 3h: 2.38±0.49 Treated at 3h: 38.8±1.37 p<0.001 16.3-fold increase Sekihashi et al 2002</p> <p>Mouse (BALB/c) 100mg/kg n=4</p>

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		Lymphocytes (DNA length/width ratio) Control 16h: 1.066±0.013 MMS 16h: 2.593±0.192 (p<0.001) Vrzoc & Petras 1997
COMPOUND	Liver UDS data	COMET data
MNNG	Rat (F344): 50mg/kg ip Control 12h: -3.0±1.7 MNNG 12h: 1.2±3.5 (classified as weakly positive; 21% cells in repair) Mirsalis et al 1982	Mouse: CD-1 Dose 100mg/kg ip Liver: Control: 2.50±8.99 Treated: 12.50±17.9 p<0.001 Sasaki et al 1997
o-anisidine	Rat (Alpk ApfSD) 4 experiments Control 2h: -3.27- -3.97 Treated: 1104mg/kg -3.39 690mg/kg -3.45 Control 12h: -3.15 - - 5.19 Treated: 1104mg/kg -3.21±0.24 690mg/kg -3.05±0.41 Ashby et al 1991	Mouse : 690mg/kg Liver: Control: 2.19±0.68 Treated at 3h: 2.620.45 1.2-fold increase at 3h (negative) Bladder: Control: 7.46±0.68 Treated at 3h: 33.0±1.97 p<0.001 4.4-fold increase at 3h Rat: 1000mg/kg Liver: Control: 2.58±0.38 Treated at 3h: 4.21±0.40 2.4-fold increase at 8h (not stat sig) Bladder: Control: 9.87±0.52 Treated at 8h: 45.98±2.74 p<0.001 4.7-fold increase at 8h Sekihashi et al 2002

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