

IN VITRO/IN VIVO SCREENING TESTS

IN VITRO

RJR used five in vitro screening assays for comparison of Premier with KY reference cigarette 1R4F:

Mutagenicity

- Salmonella (Ames) - Bacterial Assay.
- Chinese Hamster Ovary (HGPRT) - Mammalian Cell Assay.

Chromosome Damage

- CHO/Chromosome Aberration (CA) - Chromosome Aberrations.
- CHO/Sister Chromatid Exchange (SCE) - Chromosome Rearrangements.

DNA Markers.

- Rat Hepatocyte Unscheduled DNA Synthesis (UDS) - Repair of Direct DNA Alterations.

Their rationale for choice of this battery of assays included:

- Should represent a number of variables such as gene mutation, chromosomal changes and DNA alterations.
- Should include bacterial and mammalian cells.
- Should be composed of routine, widely used and available assays.
- Should not give high percentages of false positive or negative results.
- Should be widely recognized as appropriate for developing a genetic toxicology profile.
- Should be positive for reference (1R4F) cigarette smoke condensate.

In overview their choices seem sensible, but in hindsight one can argue that Ames and either CA or SCE would have been sufficient as a screening battery, with the possible inclusion of UDS.

BATUKE currently can employ the Ames and chromosome aberration (mammalian cell type under review) assays for screening. These two assays complement each other and are widely used in the UK community.

Similar arguments should apply to selection of screening assays for other marketplaces, but specific choices could be a function of local considerations (e.g., CA vs SCE assay, choice of mammalian cell type, etc.).

The attached Table 1 summarizes usage and status of prominent in vitro screening assays.

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Regarding in vitro screening in the U.S., the four assays listed in Table 1 as NTP "routine" provide the basic set from which to select a screening test battery. It is recognized that much recent research addresses variations of these four as well as attempts to develop other assays. However, with the exception of some reported automation, no widely accepted "improvements" have materialized and it is the majority view of those in the field that these four will continue as the core screening tests with the best "scientific" basis. Further the largest data bases exist for these four.

Of these four assay types, Ames and SCE seem the most appropriate and complementary pair for U.S. application to cigarette additives (pure or in the context of smoke condensate). Mammalian cell mutagenicity screening (specifically CHO HGPRT) does not give positive response to smoke condensate. As a chromosome damage assay, SCE gives broader response than CA. Cultured CHO cells are by far the most widely used for SCE assays; some labs are working with other cell types, but these appear to offer no real scientific advantage over CHO. The CHO data base is by far the largest of the cell types. Table 2 compares the four basic assays along several parameters. Figure 1 outlines an in vitro screening scheme, which includes, but goes beyond the Ames and SCE tests.

Of the core assay types, Ames is traditionally the easiest and "scoring" can be semiautomated. The other three require continued maintenance of cell cultures and "scoring" is usually done manually; automation possibilities are being pursued.

IN VIVO

RJR employed the following in vivo genetic toxicology screening assays following animal inhalation studies:

- SCE - rat bone marrow cells.
- CA - rat bone marrow cells.
- Micronucleus - rat bone marrow cells.
- Rat urine mutagenicity (Ames).

Criteria for assay selection were:

- Should be widely used and acceptable to majority of toxicologists.
- Should be appropriate for use with rats.

Regarding additive screening, in vivo screening tests of this sort would only be considered after completion of an in vitro screening battery.

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1607p

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TABLE 1
IN VITRO SCREENING TESTS

	BAT	RJR (PREMIER)	OVERALL COMPETITION	NTP ("ROUTINE")	NTP ("RESEARCH")
MUTAGENICITY					
Bacterial					
Salmonella (Ames)	✓	✓	✓	✓	✓
Mammalian Cell					
Chinese Hamster Ovary (HGPRT)		✓	✓		
Mouse Lymphoma (MOLY)			✓	✓	✓
Human Lymphocyte					✓
CHROMOSOME DAMAGE					
Chromosome Aberration (CA)					
CHO	(✓)	✓	✓	✓	✓
Human Lymphocyte	✓		✓		✓
Animal Lymphocyte					✓
Animal Embryo Lung Cell	((✓))				
Sister Chromatid Exchange (SCE)					
CHO	(✓)	✓	✓	✓	✓
Human Lymphocyte					✓
Animal Lymphocyte					✓
DNA MARKERS					
Unscheduled DNA Synthesis (UDS)					
Rat Hepatocyte		✓	✓		✓

() = Used or evaluated in past.

(()) = Under evaluation.

WHD/11p
1604p

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TABLE 2

COMPARISON OF THE CORE IN VITRO SCREENING ASSAYS

ASSAY TYPE	RELATIVE STAFF*	RELATIVE TIME*	TRUE POSITIVE RESULTS**		FALSE POSITIVE RESULTS**	
	PER RESULT	TO GET RESULT	NO.	%	NO.	%
Ames	1	1	20	45	4	14
MOLY	2.5	2-4	31	70	16	55
CA	2.0	2-3	24	55	9	31
SCE	2.0	2-3	32	73	16	55

*Based on traditional methodologies; semi-automation could reduce MOLY, CA and SCE efforts and times by one third. Information obtained from 3 toxicology testing labs plus literature sources.

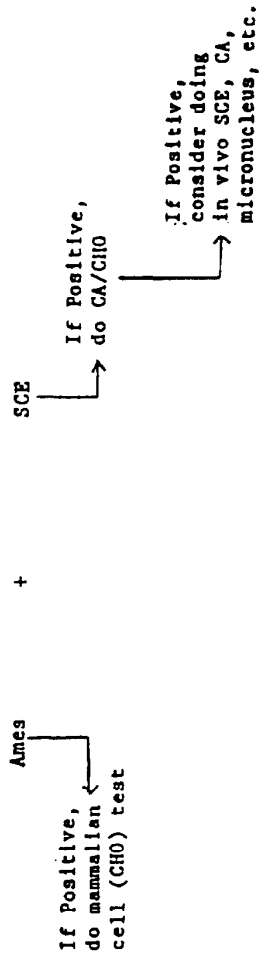
**Information derived from National Toxicology Program Data.

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FIGURE 1

AN IN VITRO SCREENING SCHEME



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Aromatic amine (18)	Nonsmokers (25)		Tobacco type			
			Blond (43)		Black	
2-Naphthylamine (4.9)	11.6		(1.1)	17.2	(1.8)	21.3
0-Toluidine	188	(19)	290	(19)	329	(22)
m-Toluidine	1141	(138)	1097	(108)	1140	(138)
p-Toluidine	209	(24)	306	(35)	415	(73)
2-Ethylaniline	38	(3)	70	(10)	80	(12)
3-Ethylaniline	102	(16)	115	(22)	129	(34)
2,5-Dimethylaniline	50	(6)	67	(10)	70	(14)
2,4-Dimethylaniline	40	(5)	73	(10)	114	(17)
2,6-Dimethylaniline	264	(90)	86	(15)	98	(30)
2,3-Dimethylaniline	52	(8)	56	(11)	64	(19)
3,5-Dimethylaniline	93	(12)	112	(31)	135	(31)
3,4-Dimethylaniline	47	(11)	46	(11)	65	(19)
3-Aminobiphenyl	1.2	(0.4)	13.8	(1.9)	12.9	(3.0)
4-Aminobiphenyl	51	(18)	176	(14)	288	(21)

* Values expressed in pg amine/g haemoglobin

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Compound	Adduct	Reference
N nitrosornicotine [NNN]	Rats/haemoglobin	4
4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone [NNK]	Rats/haemoglobin	4
NNN	Rats/DNA	4
NNK	Rats/DNA	4
NNK	Rats/7 Methylguanine	4
NNK	Rats/0 ⁶ Methylguanine	4
4 aminobiphenyl	Humans/haemoglobin	5
3 aminobiphenyl	Humans/haemoglobin	5
0-toluidine	Humans/haemoglobin	5
p-toluidine	Humans/haemoglobin	5
2,4-dimethylaniline		
& isomers	Humans/haemoglobin	5
2-&3-ethylamine	Humans/haemoglobin	5
2-naphthylamine	Humans/haemoglobin	5
Benzo[a]pyrene	Humans/DNA	6
Ethylene ? or)	Humans/Haemoglobin	7
Ethylene Oxide?)		
or C ₂ H ₃ radical)		

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