MOLECULAR TOXICOLOGY STUDIES

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MOLECULAR TOXICOLOGY STUDIES

Scientific Basis

- Altered Molecular Function

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- Altered Cellular Function

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- Altered Organ Function

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- Disease

MOLECULAR TOXICOLOGY STUDIES
Approaches

- Evaluate effect of test agents on biologically important molecules or key cellular functions.
- Quantify absorbed and/or biologically effective dose of test agents in living systems.

MOLECULAR TOXICOLOGY STUDIES

Model Systems

- *In vitro* Studies
  - Condensate
  - Whole smoke
- Rodent Studies
  - Condensate dermal studies
  - Whole smoke inhalation studies
- Studies in Smokers

MOLECULAR TOXICOLOGY STUDIES

Dose Selection

- Experimental design should be comparative.
- Test and Reference cigarettes are evaluated at concentrations where the Reference cigarettes induce a concentration-dependent positive response.

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Model Systems

- ***In vitro* Studies**
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  - Condensate dermal studies
  - Whole smoke inhalation studies
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Preparation of Mainstream Cigarette Smoke Condensate for *In Vitro* Studies

- Cigarettes were smoked under standard FTC conditions and the particulate matter collected on Cambridge pads.
- DMSO was added to yield a concentration of 10 mg TPM/ml DMSO; the pads were shaken for 25 minutes.
- The pads were removed to provide the dosing solution.

In Vitro Studies

- Cytotoxicity
- Genotoxicity

In Vitro Cytotoxicity Rationale

- Mechanistically important step in several chronic disease processes

  (e.g. carcinogenesis, emphysema)
- Determination of appropriate exposure concentrations for *in vitro* genotoxicity assays
- Minimizes use of animals for toxicological assessment

Cytotoxicity Endpoints

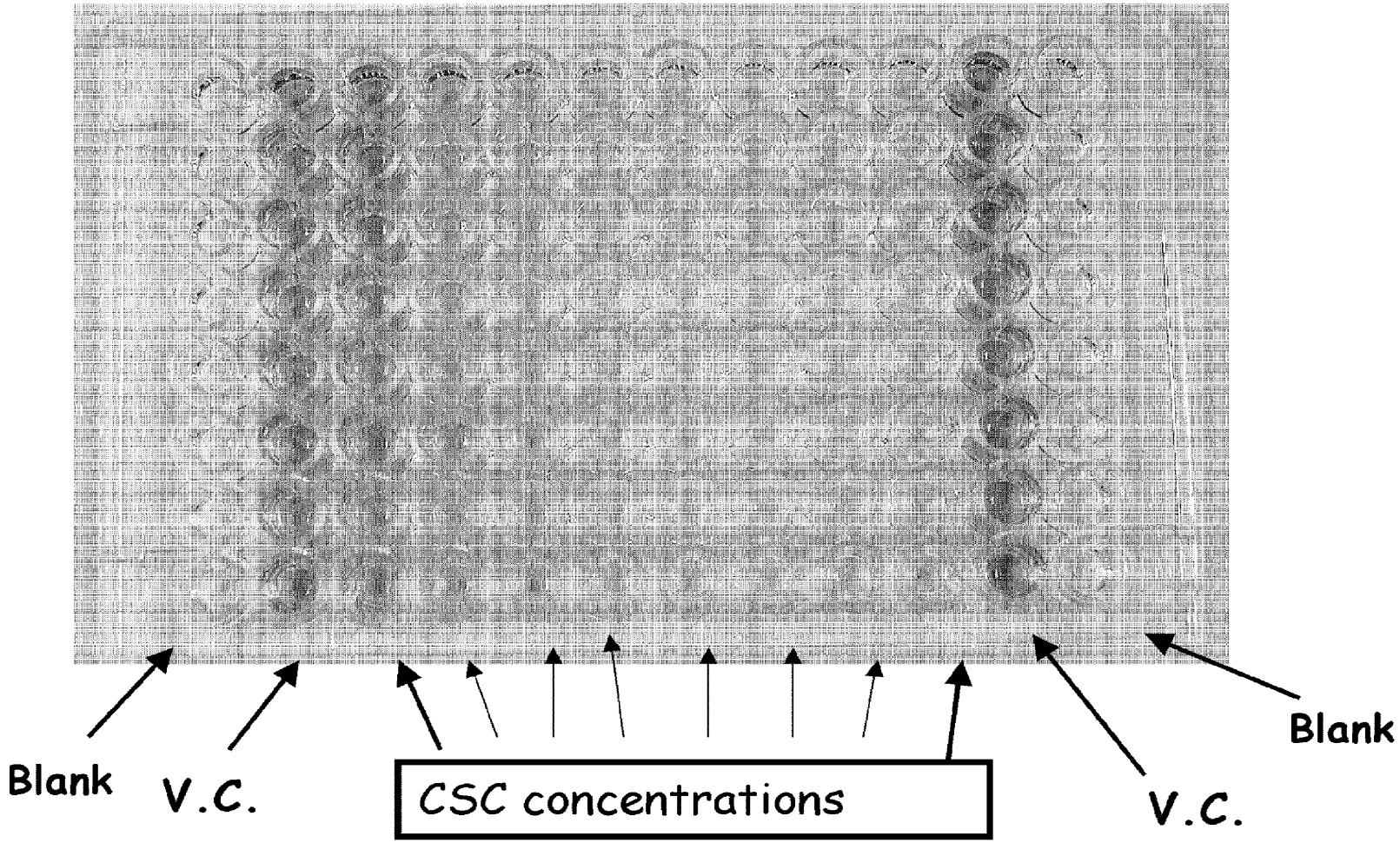
- Lactate Dehydrogenase
  - Measures structural damage to the cellular plasma membrane.
  - Most useful for short term exposure to test agent (ca. 1 hr.)

Cytotoxicity Endpoints

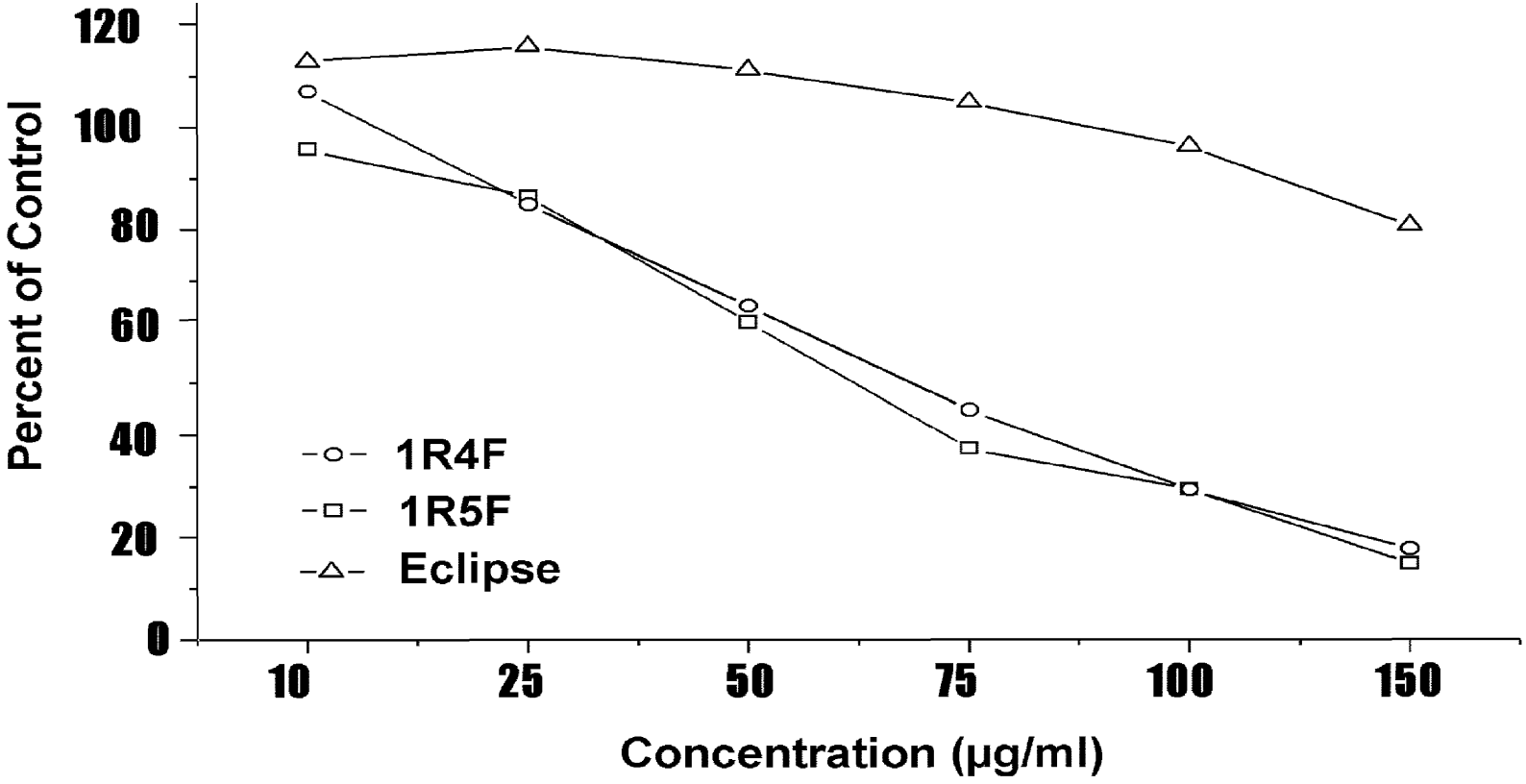
- Neutral Red Assay
  - Measures cell killing and/or reduction in cell growth
  - Most useful for longer term exposure to test agent (ca. 24 hr.)
  - Methodology recommended by ICCVAM *(Interagency Coordinating Committee on the Validation of Alternative Methods)*

Neutral Red Uptake Assay

Example of plate



Neutral Red Cytotoxicity Assay of CSC CHO Cells



GENETIC TOXICOLOGY

The Study of the Interaction of Test Agents with DNA

OBJECTIVE: To characterize and define the potential of test agents to adversely affect the structure or function of DNA molecules.

Genetic Toxicology Rationale

- Genotoxic potential has been associated with carcinogenic potential.

- DNA damage in germ cells may produce heritable defects.

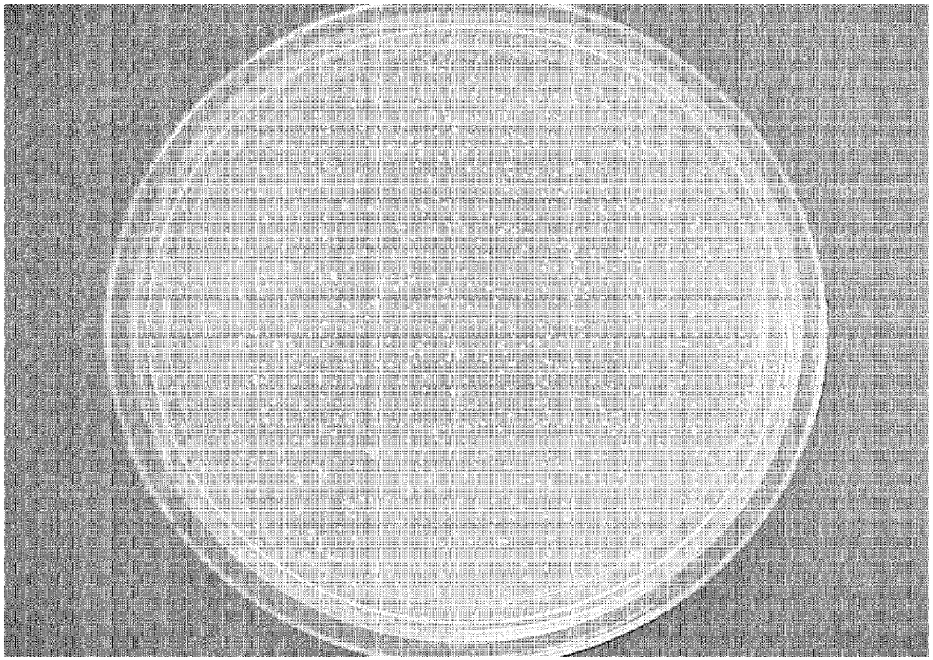
- ICH Tripartite* guidelines on genotoxicity testing of pharmaceuticals for human use
  - Bacterial reverse mutation test
  - An *in-vitro* test with cytogenetic evaluation of chromosomal damage in mammalian cells
  - An *in-vivo* test for genetic damage

[*European union, Japan and USA]

Genetic Toxicology Testing Program

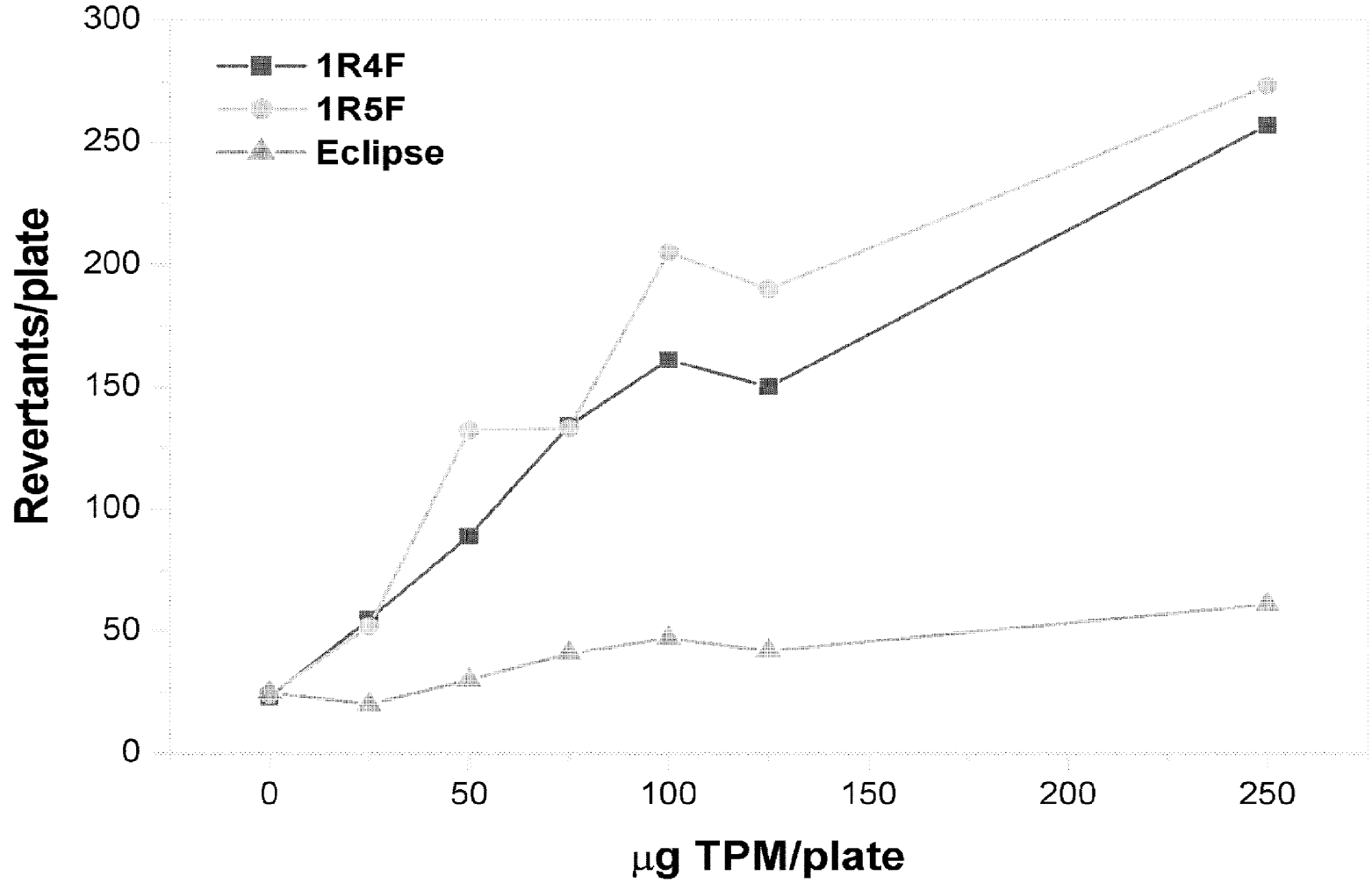
- ***In vitro* Studies (effects)**
  - Bacterial Cells
  - Mammalian Cells
- *In vivo* Studies (dosimetry)
  - Rodents
  - Smokers

The Ames Assay

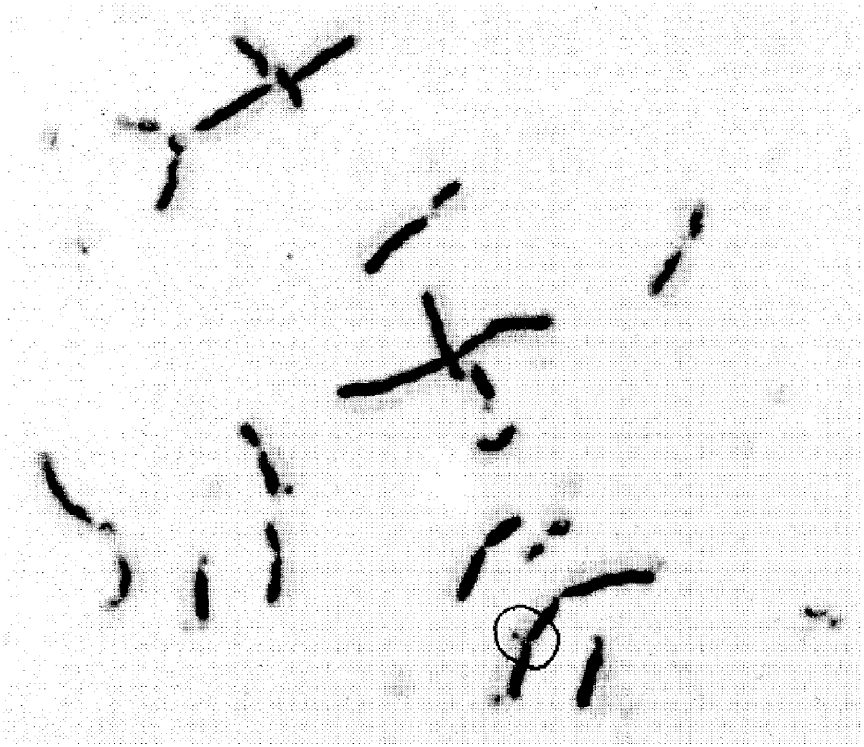


- Measures ability of test substances to produce mutation(s) in specific strains of *Salmonella* or *E. coli* bacteria
- OECD Guideline # 471, Bacterial Reverse Mutation Assay

Ames Test
TA98 with S9 Metabolic Activation

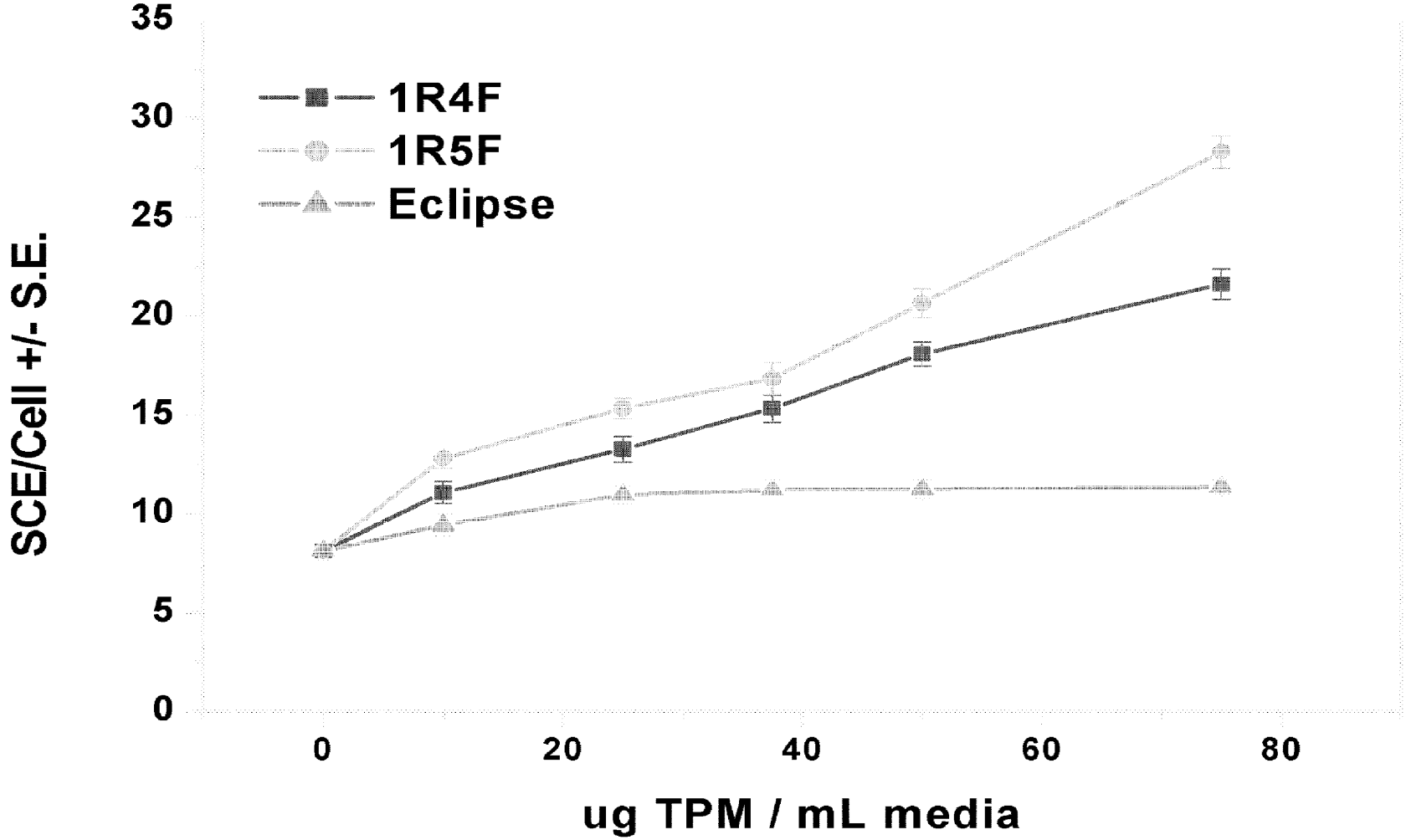


Sister Chromatid Exchange (SCE) Assay

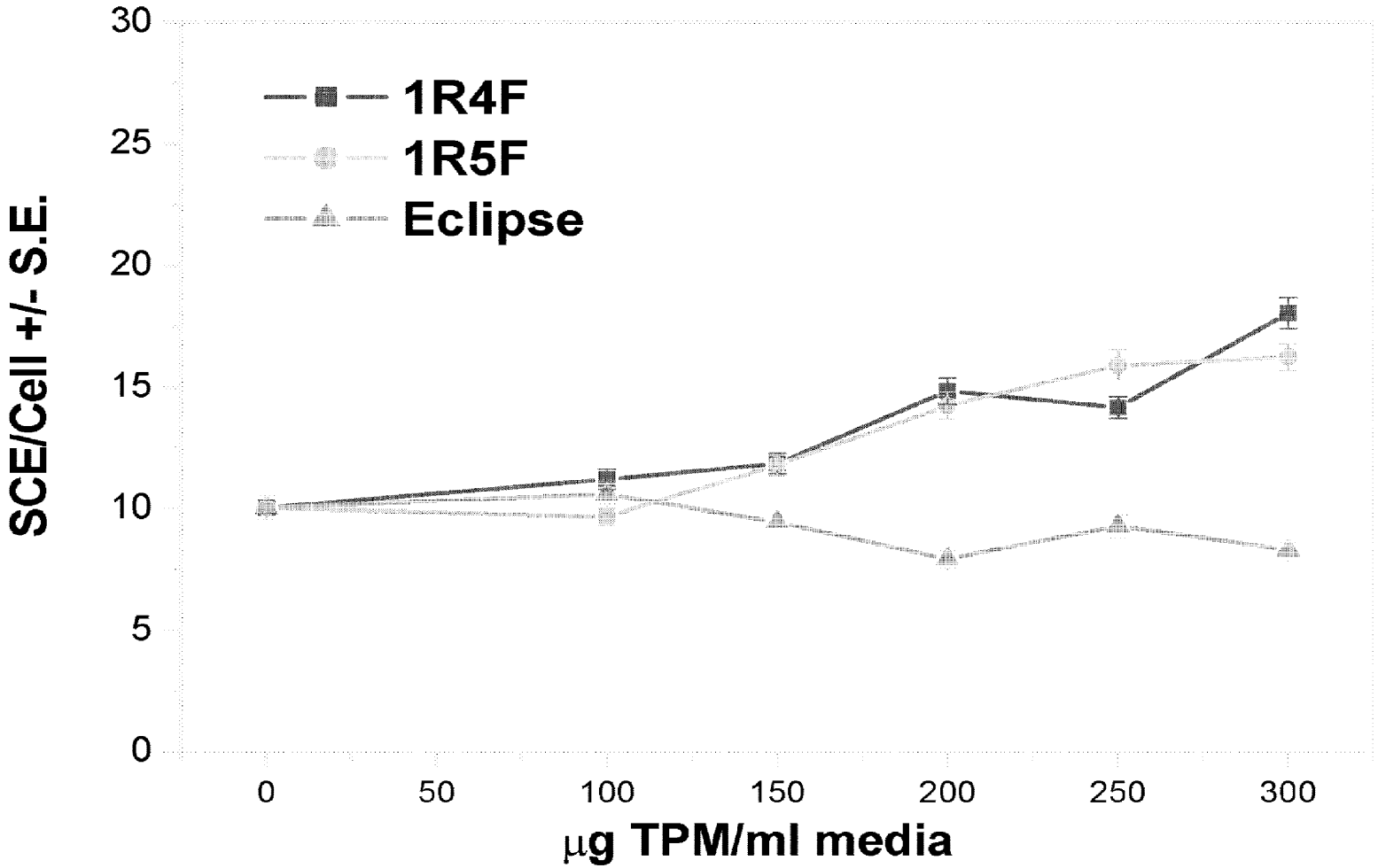


- Measures ability of test substances to cause exchange of genetic material between sister chromatids of a chromosome
- Method by Shelia Galloway *et al.*, 1985 Env. Mut 7, 1-51.

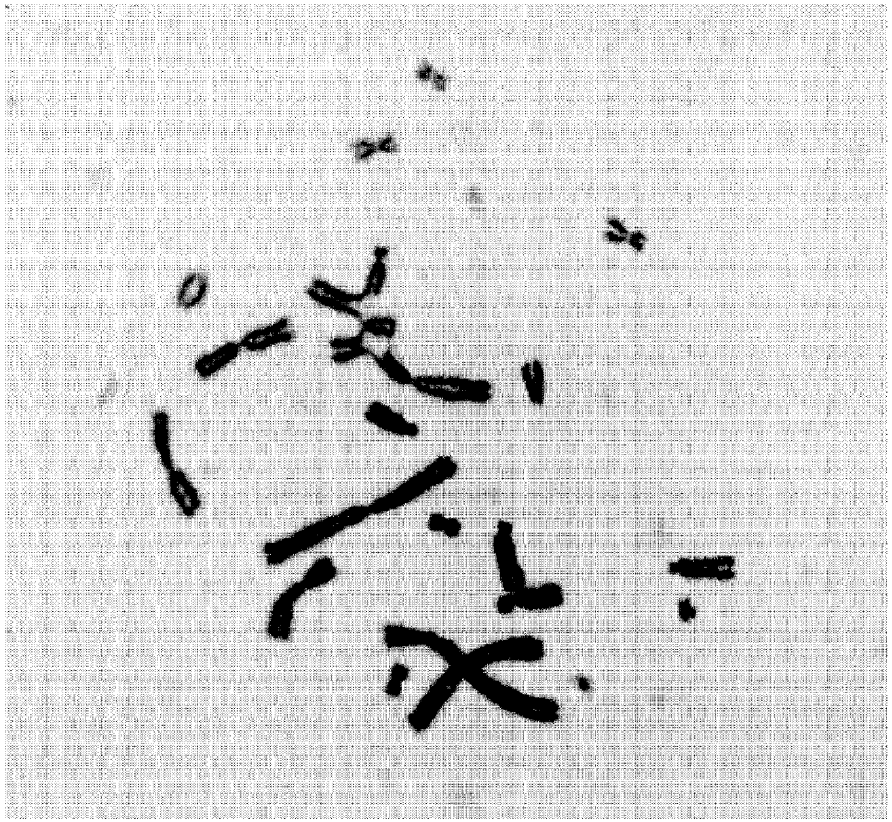
SCE Assay without S9 Activation



SCE Assay with S9 Activation

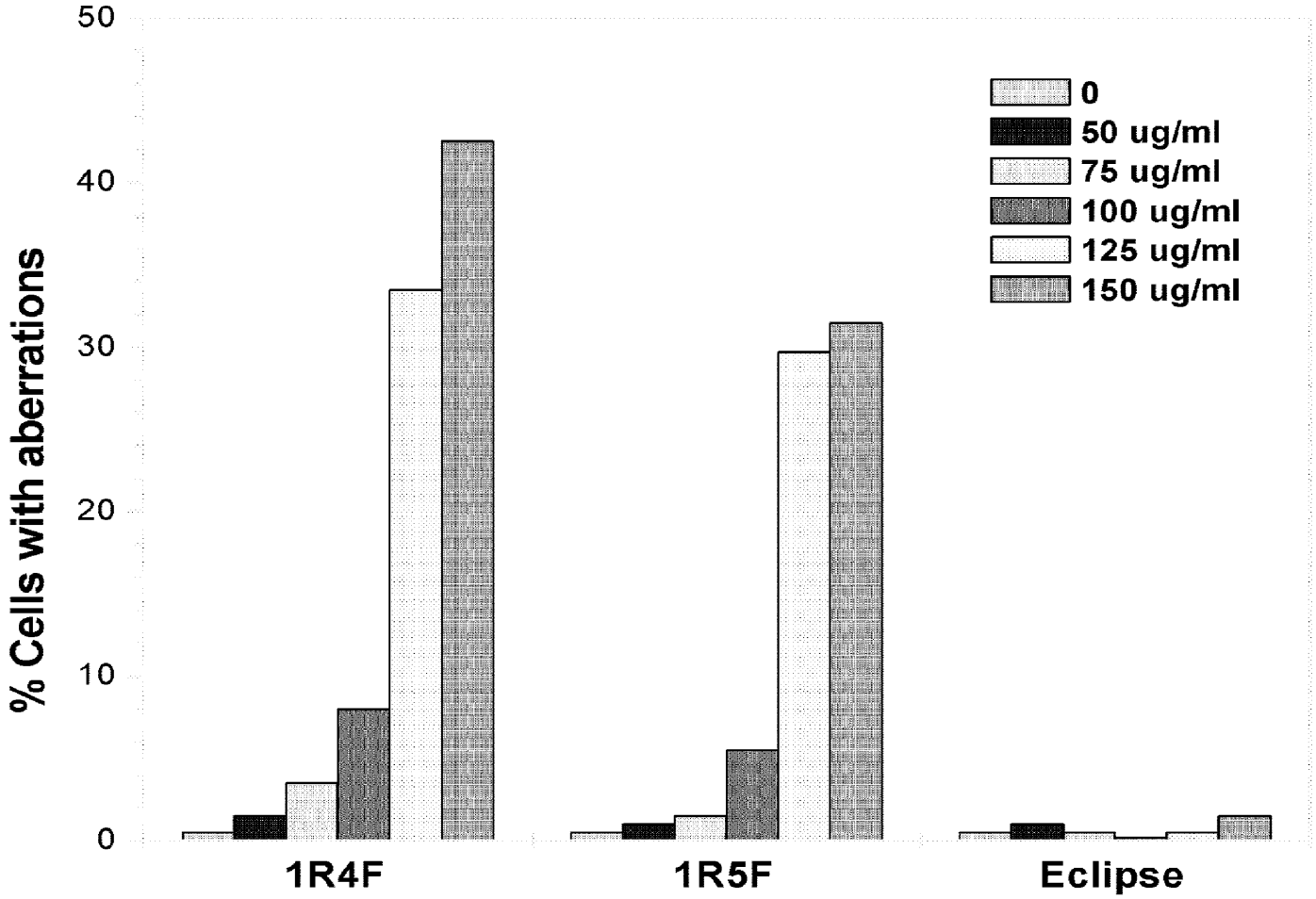


Chromosome Aberration Assay

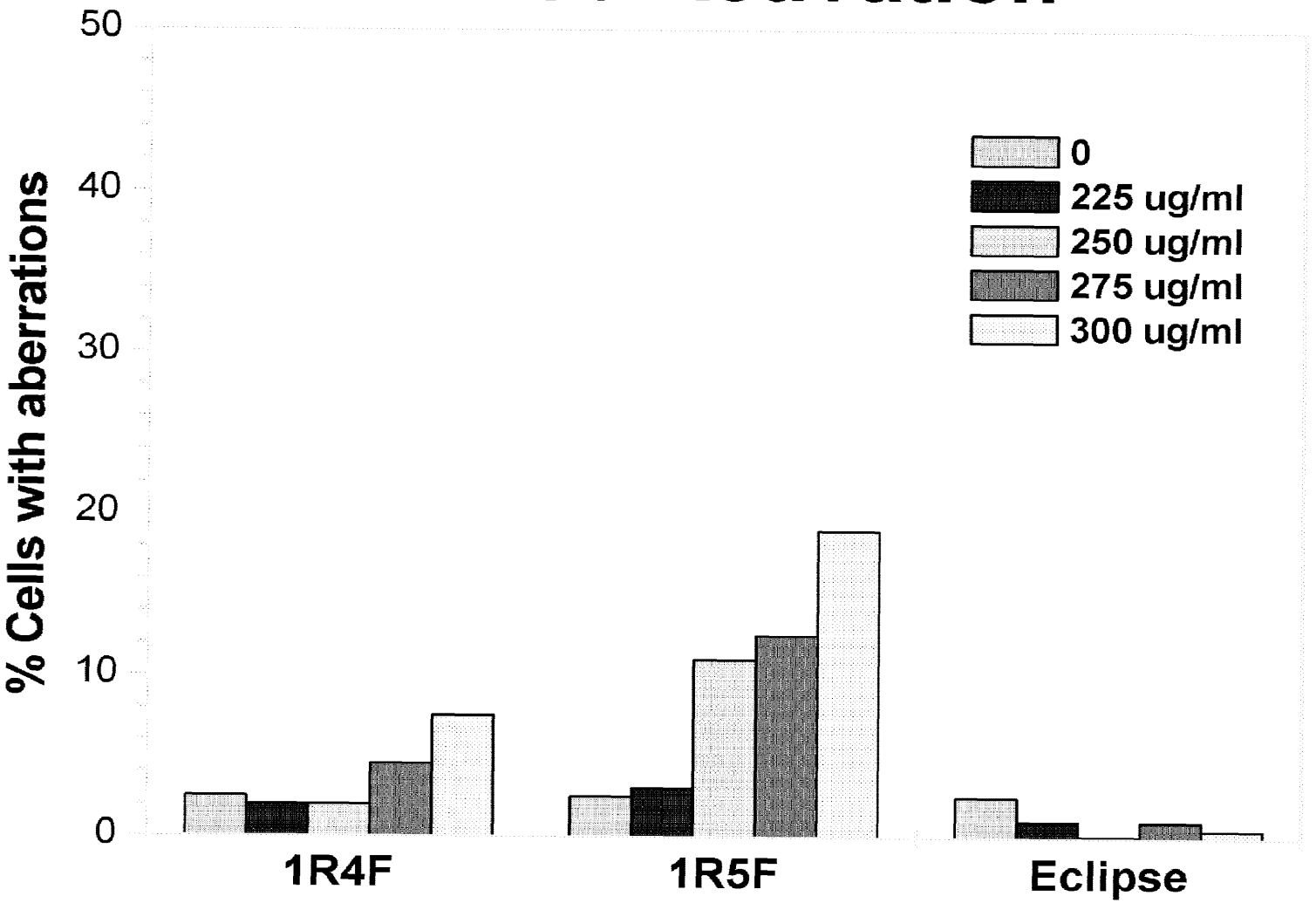


- Measures ability of test substances to cause structural rearrangements in chromosomes
- OECD guideline 473

Chromosome Aberration Assay without S9 Activation



Chromosome Aberration Assay with S9 Activation



In Vitro Genetic Toxicology Tests Summary Cigarette Smoke Condensate**

	Ames	Sister Chromatid Exchange	Chromosome Aberration
Eclipse	--+	--+	---
1R4F Reference	+++++	+++++	+++++
1R5F Reference	+++++	+++++	+++++

**Comparisons made on a "per mg" basis

Genetic Toxicology Testing Program

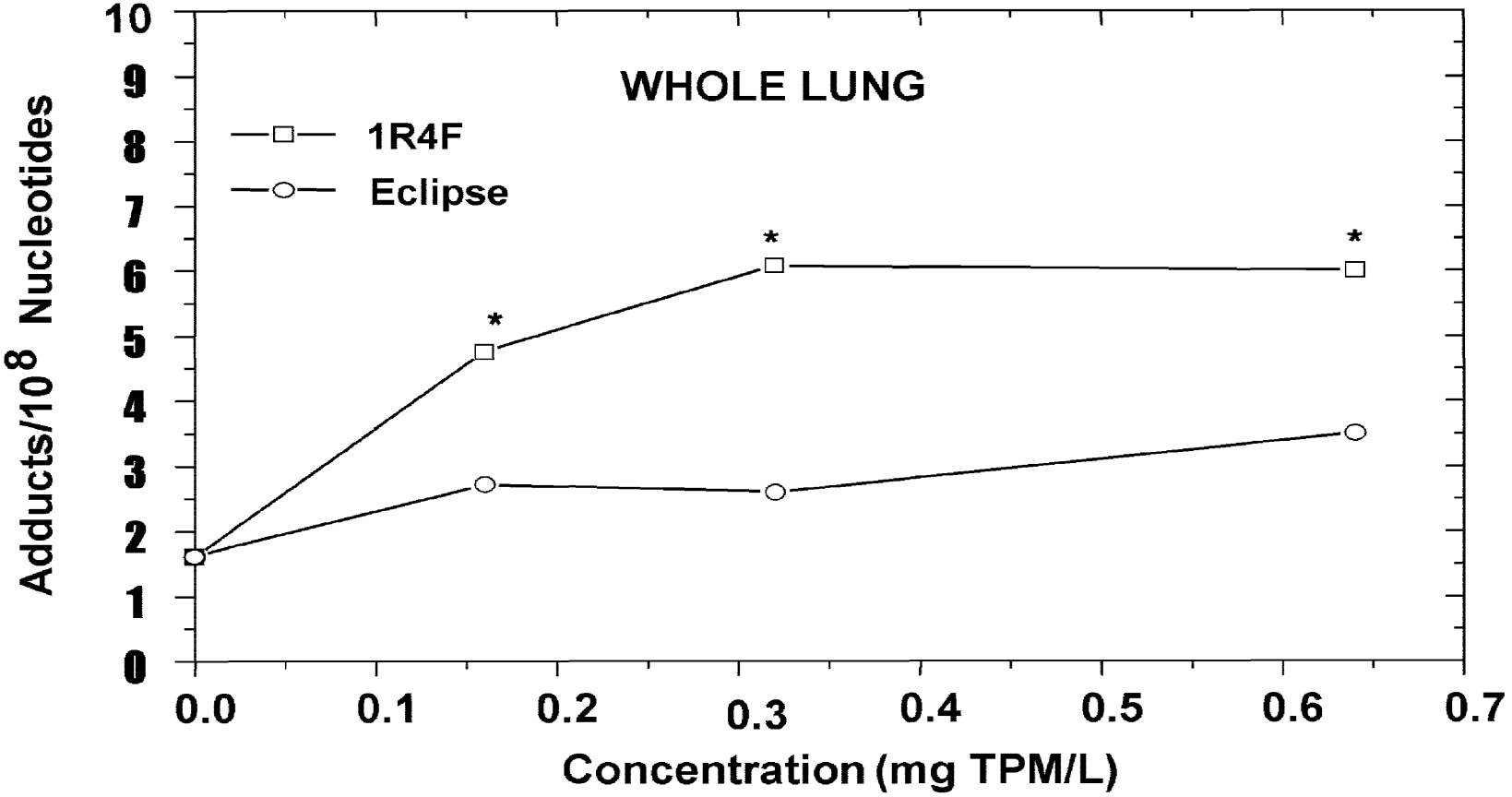
- *In vitro* Studies (effects)
  - Bacterial Cells
  - Mammalian Cells
- ***In vivo* Studies (dosimetry)**
  - **Rodents**
  - Smokers

DNA ADDUCTS

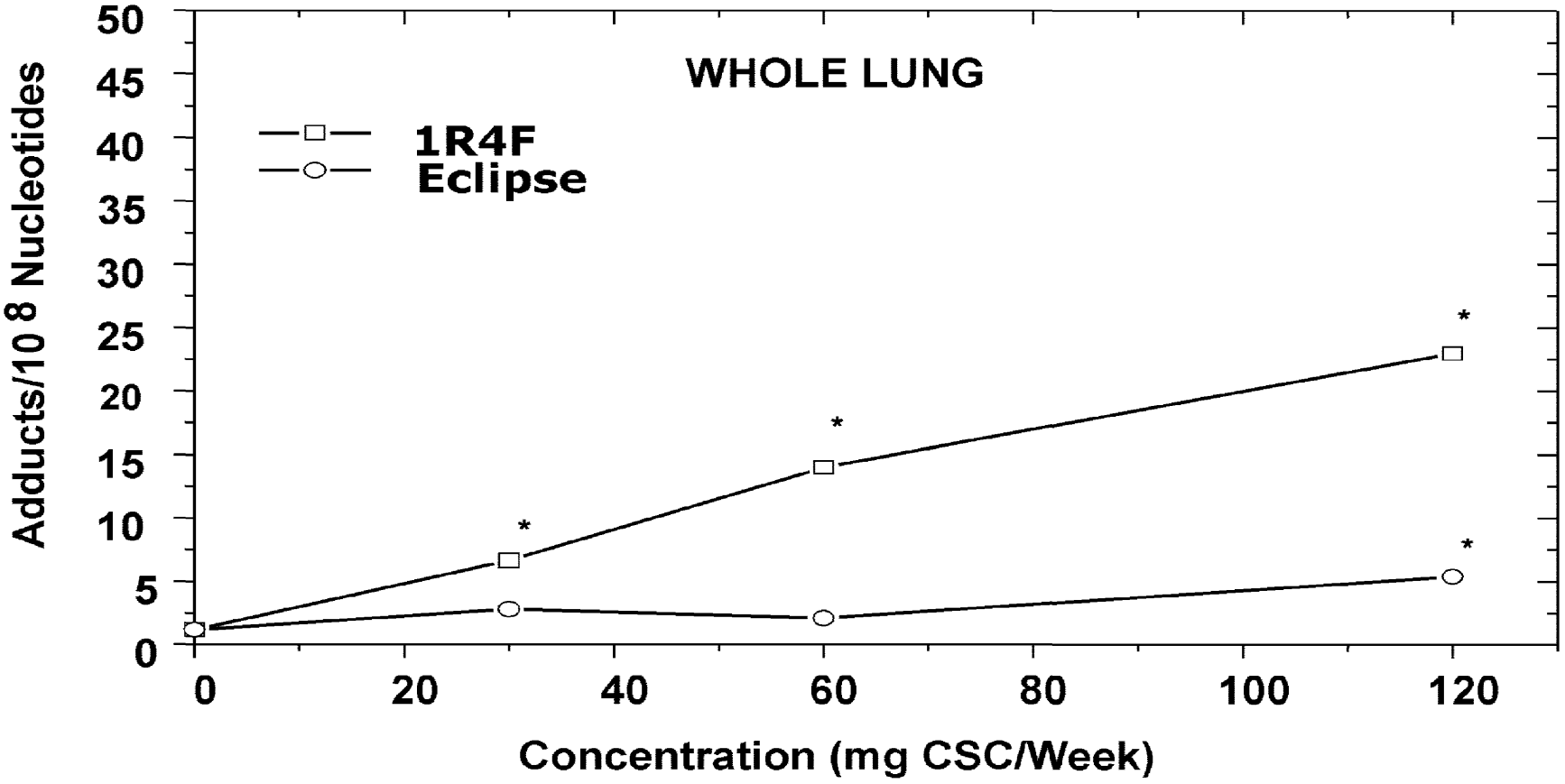
Dosimeter for Chemical Exposure of DNA in Specific Target Tissues

^{32}P Post-Labeling

Sub-Chronic Inhalation Exposure
90 Days
B6C3/F1 Male Mice



29 Week Dermal Exposure
SENCAR Female Mice
Lung DNA Adducts



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URINE MUTAGENICITY

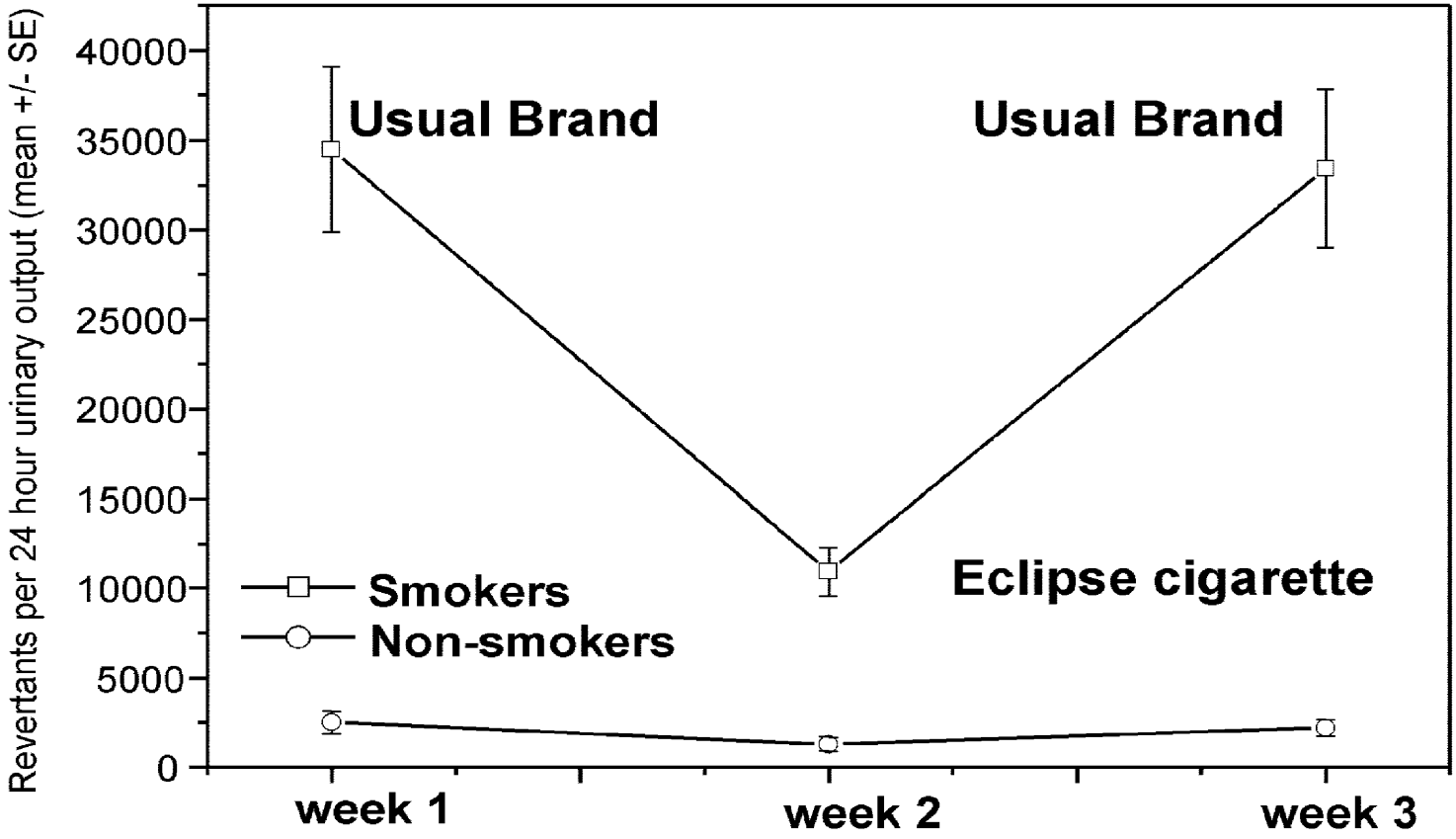
- Urine is a major excretion route for most xenobiotics.
- Urine is collected, concentrated and tested in AMES bacterial mutagenesis assay.
- Urine mutagenicity reflects an individual's recent exposure to mutagens.

Experimental Design

		Week No.		
	n	**1**	**2**	**3**
Smokers	**23**	Usual brand	Eclipse	Usual brand
Non-smokers	**10**	None	None	None

NOTE: All subjects remain on a controlled diet for a 3-week period.

Urinary Mutagenicity Study
TA98 5% S9



RESULTS

- Usual brand and *Eclipse* cigarette consumption per day was not statistically different.
- Salivary cotinine levels for subjects when smoking usual brand or *Eclipse* were not statistically significantly different.

CONCLUSION

A battery of appropriate molecular toxicology assays is effective for evaluating and comparing the toxicity of smoke from different cigarettes.

Effective Strategy for Assessing Risk Reduction in Cigarettes

Reduction of Chemical Substances

- ✓ Smoke Chemistry Studies

Less Toxicity

- ✓ *In Vitro* Toxicity Studies
- ● Animal Toxicity Studies

Reduced Effects in Smokers

- ● Smoking Behavior/Exposure Assessment
- ● Clinical Studies using Markers