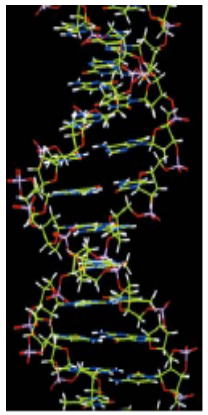
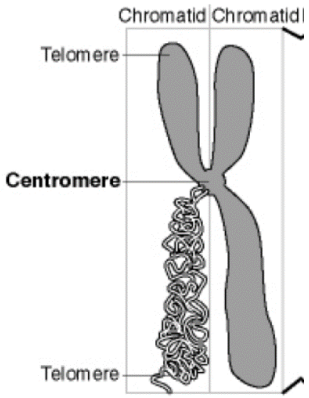
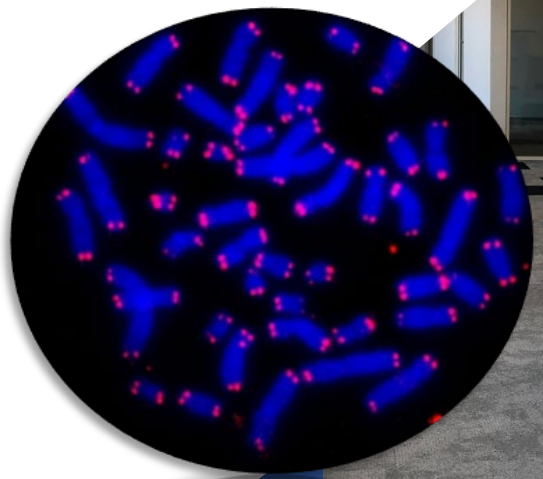
GENEVOLUTION

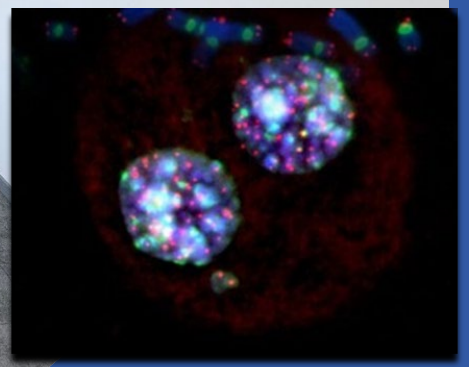
# **Société de services pour prévenir des risques du "cancer" (toxique pour l'ADN) et** pour les perturbateurs endocriniens

Un CRO avec une expertise en génotoxicité





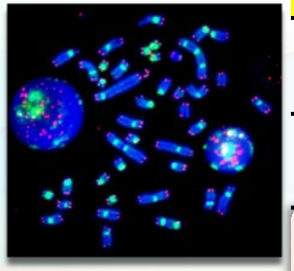


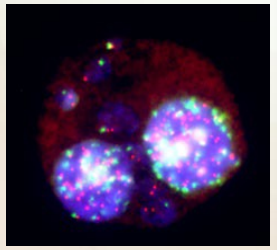


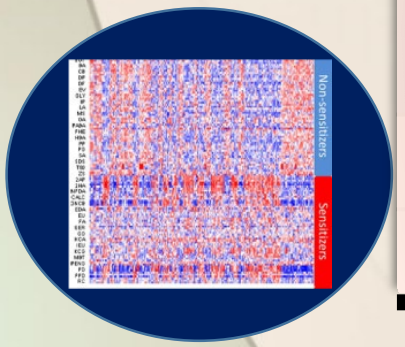

***BPL conformité ANSM & COFRAC***

SEQENS

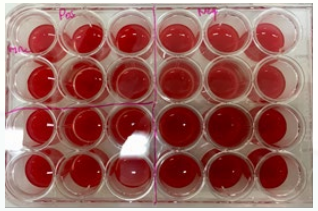
# Tests *in vitro* pour la caractérisation génotoxique

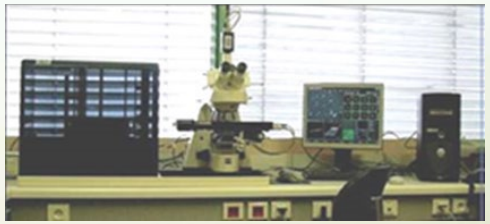


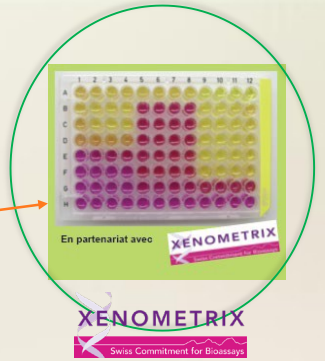





| Test Names Guidelines | Description |
|---|---|
| Ames Test OECD 471 | Detection des composés mutagènes |
| HLCA test (telo/centromère) OECD 473 | Etude aberration Chromosomique |
| Micronucleus test (telo/centromere) OCDE 487 | Detection des produits clastogénes/ aneugénes |
| *in vitro* Phototoxicity OECD 432 | Sun effect on compound and on human skin |
| *in vitro* Cytotoxicity OECD 432 | *In vitro* toxicity on cell lines |
| *in vitro* irritation/ corrosion OECD 431 /439 | Irritation on reconstructed human epiderminis |
| *GARD (Genomic Allergen Rapid Detection) in vitro* (LLNA alternative) | Hypersensitivity of pure test article or mixture in partnership with SENZAGEN |
| *in vitro* Endocrine disruptions | YES / YAS transfected yeasts in partenership with XenometriX |
| Expertise | Analyze the process of the test of the customer and propose tests or specific solutions |



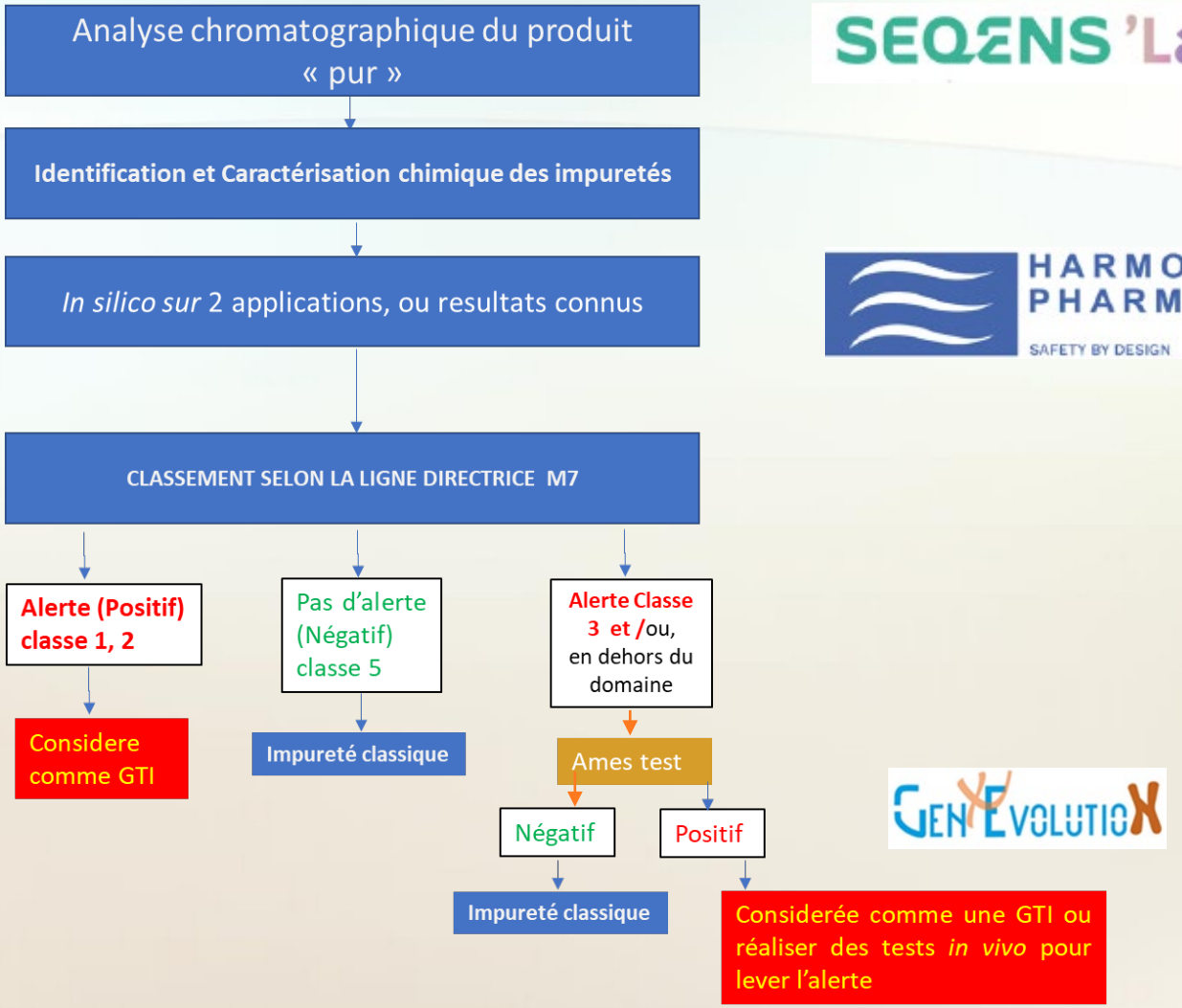




# Liste non exhaustive où les tests de génotoxicité sont utilisés ….

- Echantillons environnementaux air, sol et eau
- Produits Chimiques Reach Annexe VII etc.
- Produits cosmétiques
- Préparation botanique
- Extraits plantes médicinales
- Equipements médicaux
- Alimentaire
  - Animaux
  - Humains
- Produits pharmaceutiques et impuretés pharmaceutiques (animal et humain)

# Produits pharmaceutiques : les impuretés Génotoxiques
# Rappel arbre décisionnel simplifié ligne directrice M7




Rapport , expert

# Historique des différents types de test d'Ames OCDE 471 (St Louis)

| Assay type | Top concentration | Compound require (mg) | Throughput (assay/week)[a] | Predictivity: GLP-Ames |
|---|---|---|---|---|
| Mini Ames (6-well plates) | 1000 µg/well (~5 mg/plate) | 25 | 7/week | ~100% |
| Micro Ames (24-well plates) | 250 µg/well (~5 mg/plate) | 15 | 4/week | NA |
| Mini Ames mix strains (100 mm plates) | 2000 µg/well | 45 | 6/week | 95% |
| BioLum Ames | ~5 mg/plate | 2.5–5 | 50/week | 95% |
| Ames II ™ | 1000–5000 µg/mL | 5–25 | 30/week | ~92% |
| Ames MPF | 5000 µg/mL | 40 | 10/FTE | 78% |
| 5-Fluorouracil | 5000 µg/mL | 10 | 2/week | 95% |
| SOS/umu | 5000 µg/plate | 5 | 4/week | 83% |
| SOS Chromotest | 1000 µg/mL | 2 | 30/FTE | 82% |

Plus récemment le test « umu » refait surface

**Table 1** Comparison of *umu* test results and chemicals tested for rodent carcinogenicity [22]

| Carcinogenicity | *umu* test | | | |
|---|---|---|---|---|
| | + | — | ± | Total |
| + | 119 | 72 | 2 | 193 |
| — | 11 | 39 | 0 | 50 |
| ± | 0 | 5 | 0 | 5 |
| Total | 130 | 116 | 2 | 248 |
| Sensitivity | 65 % | (119/193) | | |
| Specificity | 78 % | (39/50) | | |
| Accuracy | 65 % | (158/243) | | |

**Table 2** Standard methods for the determination of the genotoxicity of water and wastewater using *umu* test

| Year | Description | Country |
|---|---|---|
| 1993 | An official method of the water supply test method | Japan |
| 1995 | A standard method of the genotoxicity of water and wastewater (DIN 38415T3) [24] | Germany |
| 1997 | An official method of the wastewater test method | Japan |
| 2000 | A genotoxicity test of water and wastewater in International standardization Organization (ISO) Standards (ISO/CD 13829) [25] | ISO |
| 2008 | A standard method of the genotoxicity of water and wastewater (MS ISO13829) | Malaysia |

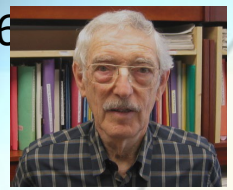
Oda *Genes and Environment* (2016) 38:24
DOI 10.1186/s41021-016-0054-8

Ger

REVIEW

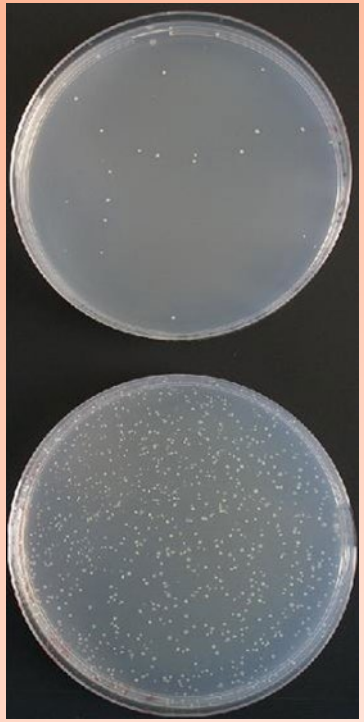
Development and progress for three decades in *umu* test systems

Yoshimitsu Oda



# STANDARD AMES TEST : METHOD (OECD 471)

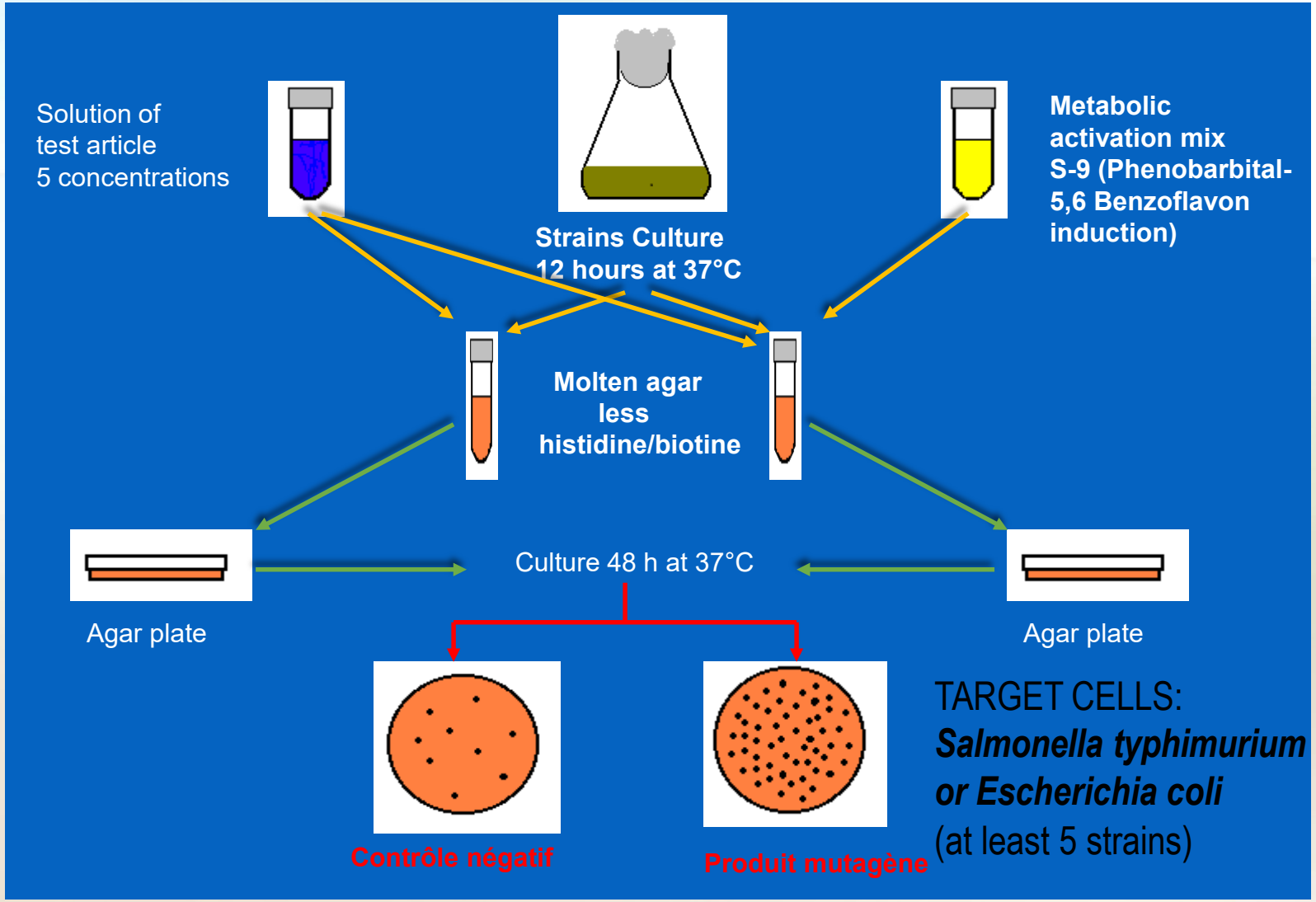
HISTIDINE ($HIS^-$) AUXOTROPHIC
[mutants selected by Ames, without histidine the strains did not growth ]



MUTAGENS AGENT
+/- METABOLISM

REVERSE MUTATION

HISTIDINE ($HIS^+$)
PROTOTROPHIC , [mutation => back to wild type]



# Les NITROSAMINES dans le médicament

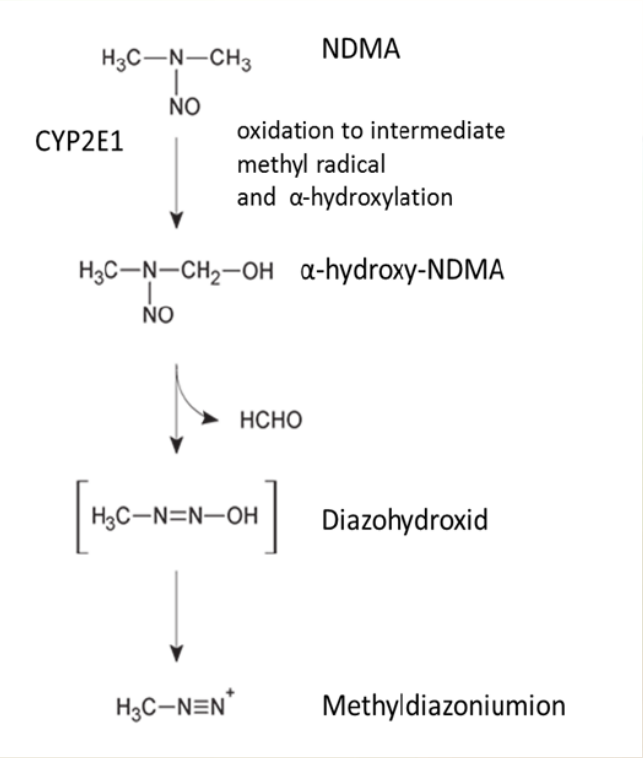
## MECANISME GENOTOXICITE DES NITROSAMINES

- « *Les nitrosamines sont métabolisées principalement par les **CYP2A6 et CYP2E1** en mutagènes réactifs à l'ADN par méthylation (alkylation) à la position O6 de la guanine, à la position O4 de la thymidine et à d'autres lésions moins mutagène. Lorsqu'elle n'est pas corrigée, l'O6-méthylguanine peut être reconnue à tort comme étant de l'adénine, causant **des mutations de substitution GC>AT** pendant la réplication, cette mauvaise reconnaissance peut entraîner **des mutations TA>CG**.* »

- « *Les agents cancérigènes mutagènes agissent souvent au moyen de mécanismes multiples. La NDMA et la NDEA sont également clastogènes et hépatotoxiques et peuvent causer des dommages secondaires par la production de formaldéhyde et d'espèces réactives d'oxygène (Organisation mondiale de la Santé, 2002). Cependant, l'adduction **O6-alkyl-guanine est le mécanisme le plus répandu et le plus puissant lié au cancer.***

- *George E. Johnson et al, « Permitted daily exposure limits for noteworthy N-nitrosamines » Environ Mol Mutagen 2021 Jun 5. doi: 10.1002/em.22446.*

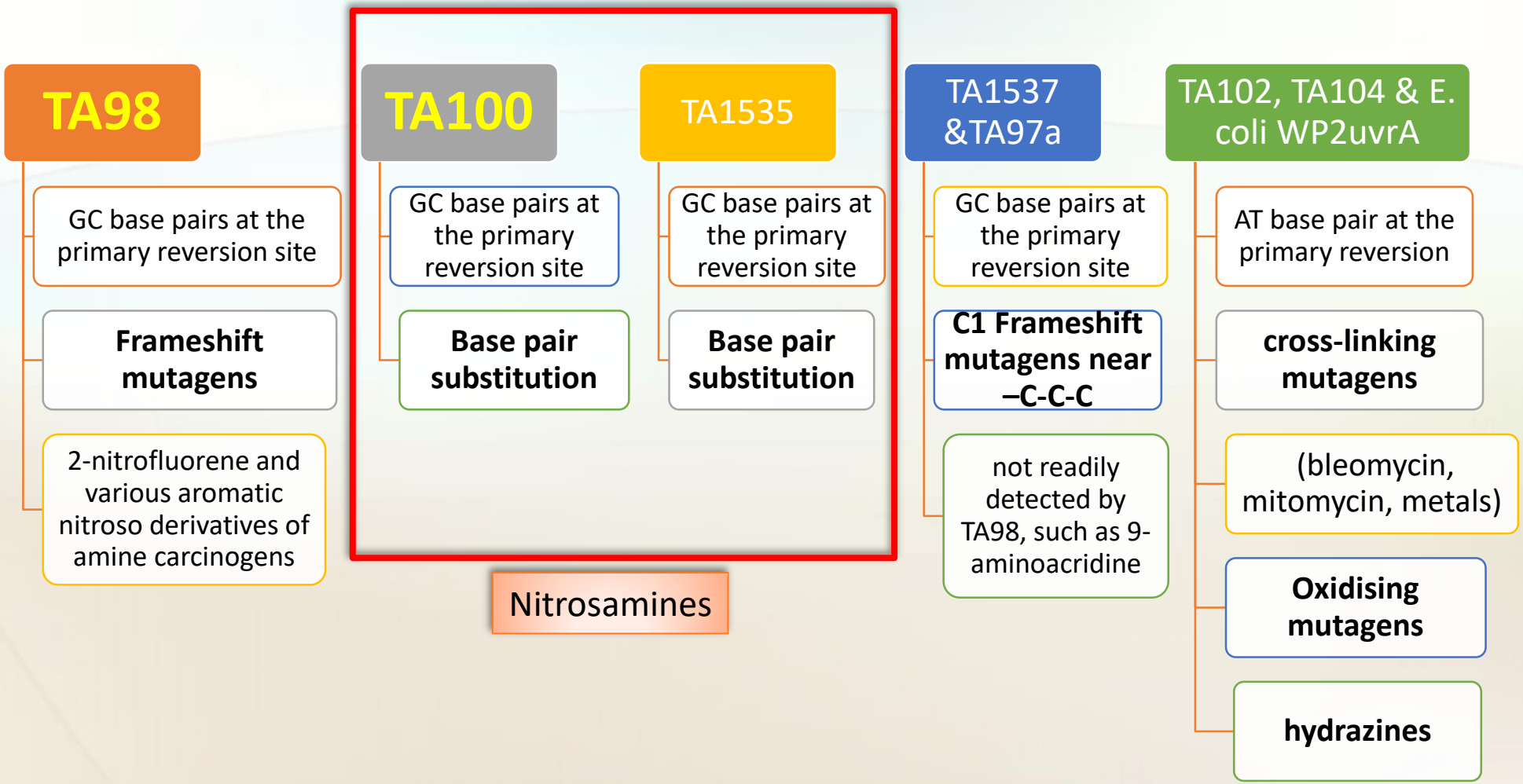
## METABOLISATION: MOLECULES REACTIVES




Assessment report EMA/217823/2019

Metabolic activation of NDMA formation of the DNA-reactive methyldiazoniumion from NDMA (adapted from Sulc et al., 2010)

# Test d'Ames: Les principales souches utilisées



# Généalogie des souches TA100, TA1535 sont issues de la même souche TA1530

Nitrosamines

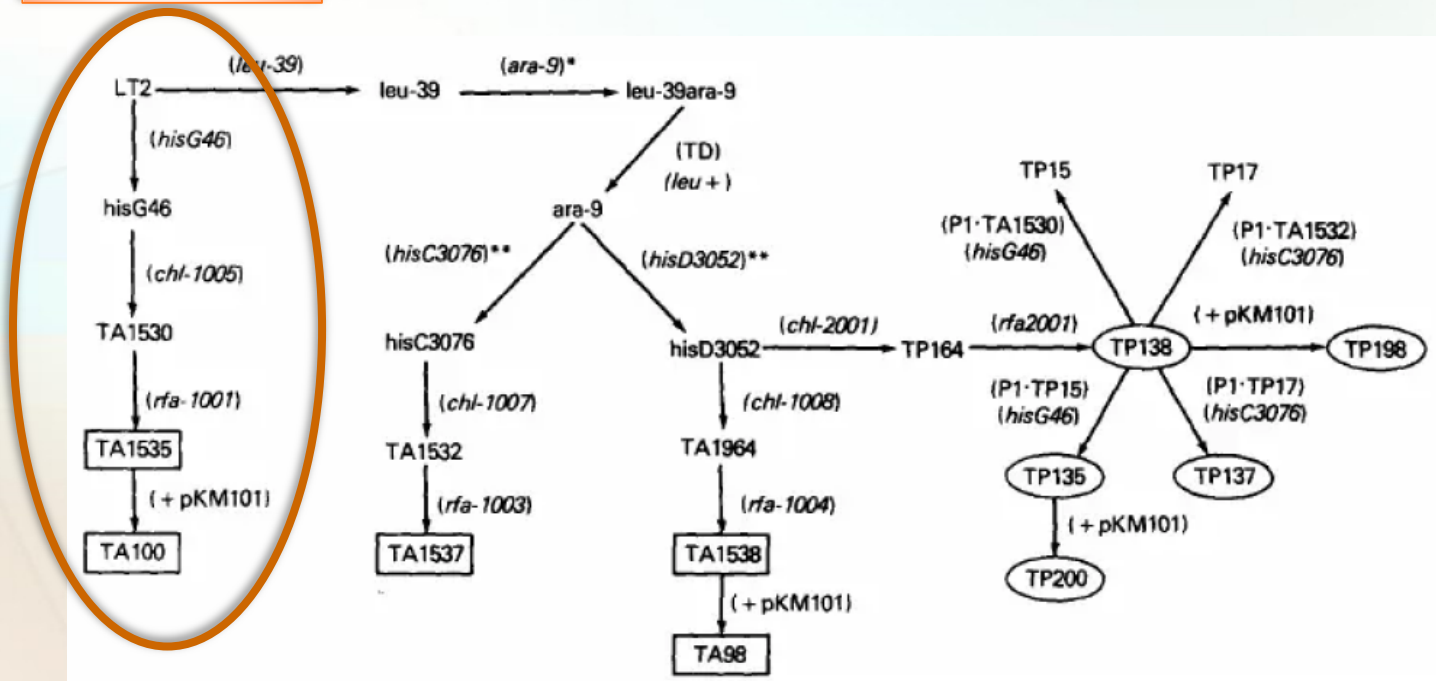



Fig. 1. Genealogy of Ames and isogenic tester strains. Ames tester strains in boxes; isogenic tester strains in ovals. Complete genotypes of all strains shown in Table 1. The allele or plasmid added at each step is shown in parentheses. * Indicates mutagenesis with ultraviolet light; ** indicates treatment with frameshift mutagen ICR364-OH. (TD) indicates that the leu$^+$ allele was introduced by transduction (phage unspecified); (P1) indicates that allele was introduced by transduction with bacteriophage P1cmCl·100. The donor strain for the P1cmCl·100 transductions is shown after the "P1·". The allele transferred in each transduction is also indicated. All alleles not obtained by an indicated mutagenesis or transduction were derived by spontaneous mutation. (+pKM101) indicates that plasmid pKM101 was introduced by conjugation. Figure modeled after that published by Busch et al. (1986).

*Mutation Research*, 224 (1989) 453–464
Elsevier

MUTGEN 01487

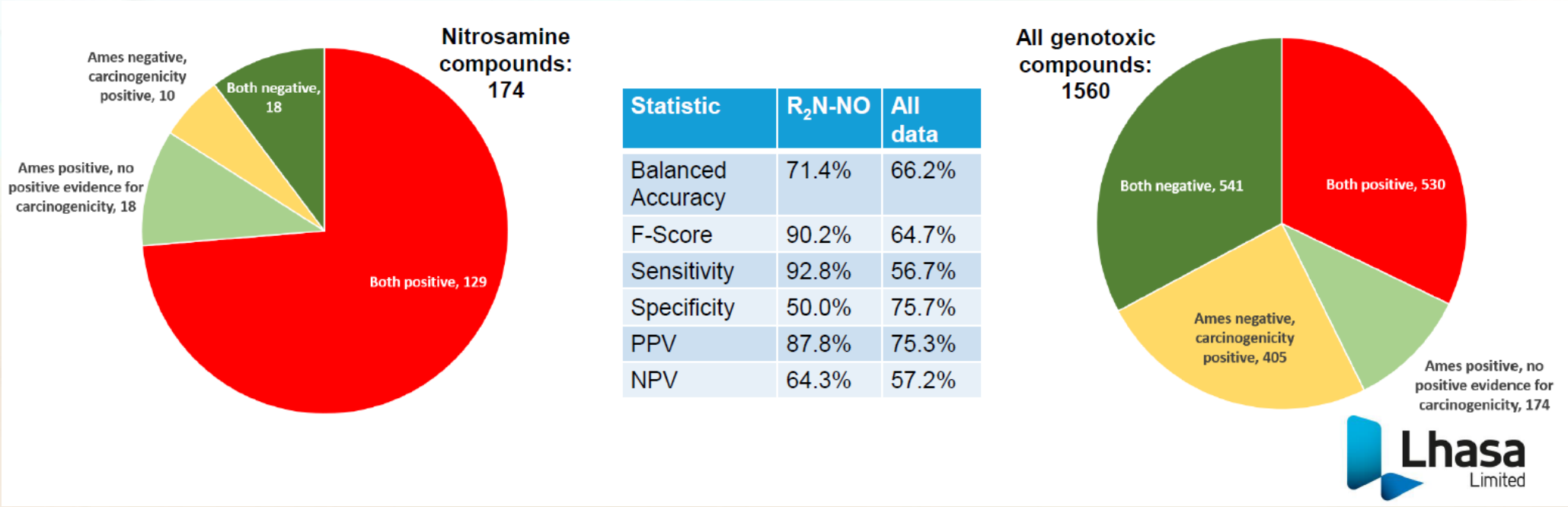


Isolation and characterization of an isogenic set of *Salmonella typhimurium* strains analogous to the "Ames" tester strains

David J. Popkin, Valerie M. Davis and Michael J. Prival
*Genetic Toxicology Branch, Food and Drug Administration, Washington, DC 20204 (U.S.A.)*

(Received 21 November 1988)
(Revision received 15 May 1989)
(Accepted 6 June 1989)

# Environ ¾ des nitrosamines sont + dans le test d'Ames




| Statistic | $R_2N$-NO | All data |
|---|---|---|
| Balanced Accuracy | 71.4% | 66.2% |
| F-Score | 90.2% | 64.7% |
| Sensitivity | 92.8% | 56.7% |
| Specificity | 50.0% | 75.7% |
| PPV | 87.8% | 75.3% |
| NPV | 64.3% | 57.2% |




Lhasa Limited

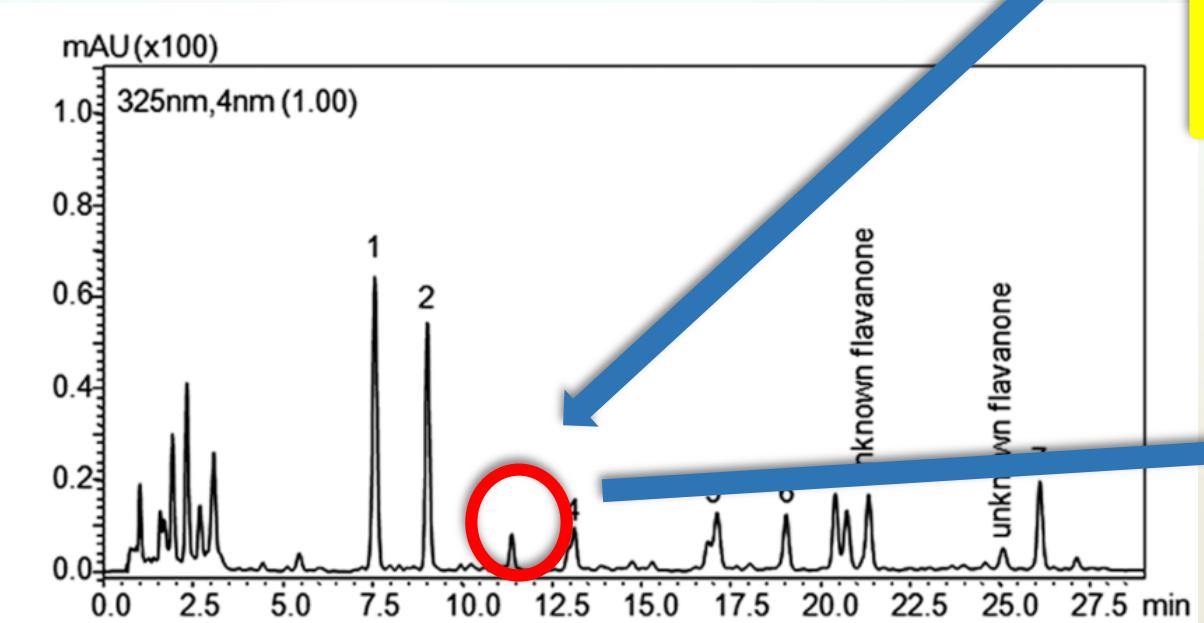
# Cependant……

- Génotoxicité
  - Il n’y a pas de test unique qui détecte tous les produits génotoxiques
    - Mutation, clastogène, aneugène : *in vitro* et *in vivo*.
      - *In vitro* plus rapide et moins couteux que les tests *in vivo* (ex test de cancérogenèse 2 ans et plusieurs millions d’euros).
      - Test suivants les lignes directrices OCDE complétées par les lignes directrices ICH-M7 TTC 1,5 µg/jour inférieur à  la durée de vie  (pharma) ,  EFSA (alimentaire) . SCCS (cosmétiques), ECHA (REACH annexe VII (1 à 10 tonnes)).
      - EFSA et OMS: Le seuil de préoccupation toxicologique (TTC) pour les produits chimiques génotoxiques est 0.0025 µg/kg pc/jour sauf pour les produits hautement cancérogènes comme les nitrosamines.
  - **Produit pur** versus **mélange complexe ?**

# Impureté inattendue dans un produit pur, mélange d'impuretés,

# Impureté inattendue

Supposons un pic de dégradation, impureté
⇒ Identification
⇒ Caractérisation *in silico* (QSAR…)
⇒ Synthèse mg to g
⇒ Test …




**Solution:**
- **Collecter le pic et le tester directement comme un échantillon**
- **Mais quantité limité de produit (< mg, = µg)**

# Les hautes performances de l'Analyse chromatographique 1/3

FOOD ADDITIVES & CONTAMINANTS: PART A
https://doi.org/10.1080/19440049.2019.1664772

Taylor & Francis
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**Value and limitation of *in vitro* bioassays to support the application of the threshold of toxicological concern to prioritise unidentified chemicals in food contact materials**

Benoit Schilter[a], Karin Burnett[b], Chantra Eskes[c], Lucie Geurts[d], Mélanie Jacquet[e], Christian Kirchnawy[f], Peter Oldring[g], Gabriele Pieper[h], Elisabeth Pinter[i], Manfred Tacker[i], Heinz Traussnig[j], Peter Van Herwijnen[k] and Alan Boobis[l]

La forêt de pics est un mélange de plusieurs dizaines milliers de molécules/impuretés inconnues, dont la réactivité, la stabilité et l'innocuité sont inconnues:
**comment les caractériser ?**

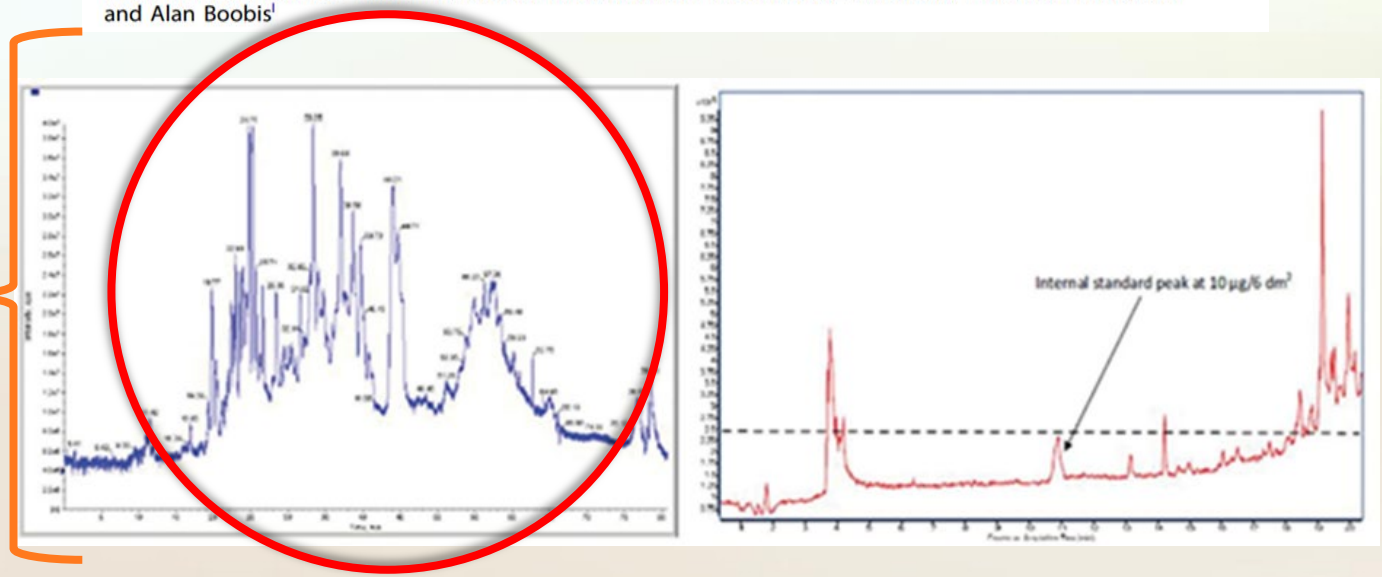



Figure 1. Illustrative examples of forests of peaks. Total ion chromatograms of extracts from FCMs are presented, along with a line(dashed) representing a 10 µg/6 dm² standard (right-hand panel). Such profiles are typical from many different FCM extracts or migrates.

# Definitions 2/3

**Box 1.** Terminology associated with the concept of limit of detection (LOD) as used in the present manuscript.

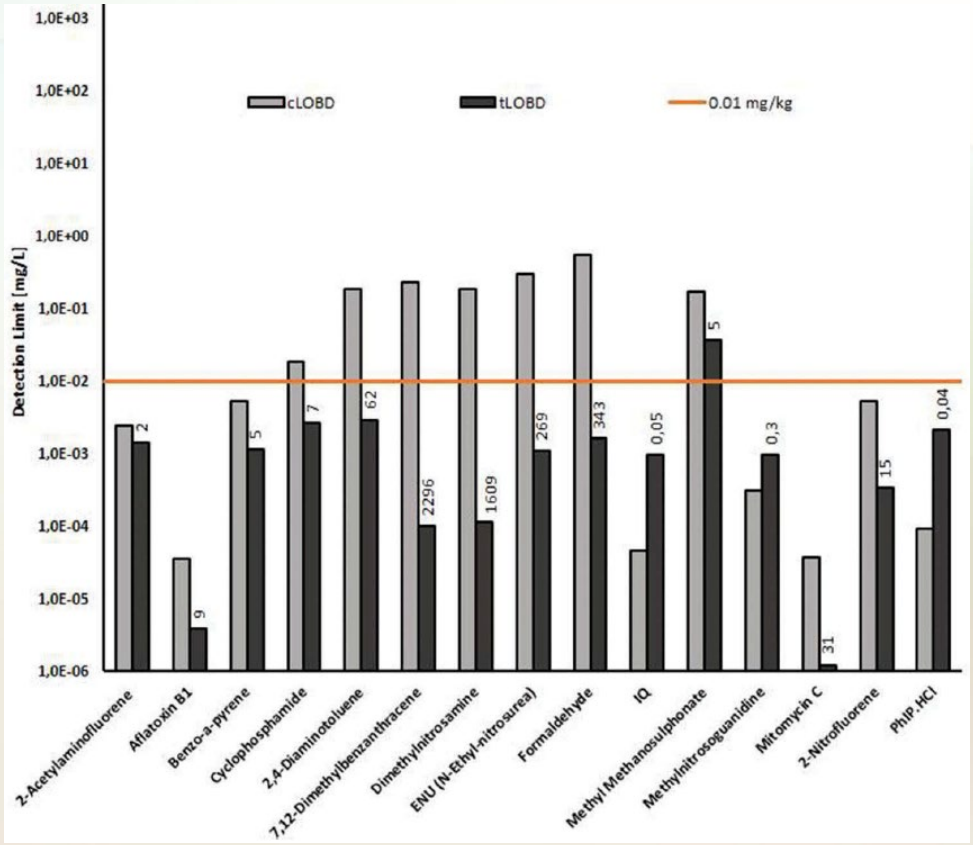
| | |
|---|---|
| **LOD** | **Limit of detection (LOD)**. In analytical chemistry, LOD is defined as the lowest concentration of an analyte in a sample that the analytical process can reliably detect, meaning that the signal of the analyte is statistically different from that of a blank. |
| **LOCD** | **Limit of chemical detection (LOCD)**. Used to describe the LOD for analytical chemistry methods. |
| **LOBD** | **Limit of biological detection (LOBD)**. Used to describe the LOD for bioassay-based methods. |
| **LEC** | **Lowest Effective Concentration (LEC)**. Defined as the lowest concentration that gave a measurable positive response in the bioassay. For a pure chemical, the LEC is obtained by establishing a concentration-response curve. |
| **cLOBD** | **Calculated limit of biological detection (cLOBD)**. LOBD in packaging migrate based on the LEC and accounting for the sample preparation procedure (including migration study protocol, migrate concentration, transfer into bioassay compatible solvent and then application in the bioassay). Concentration and dilution factors specific for each relevant sample preparation step are integrated into a factor which is applied to the LEC. |
| **tLOBD** | **Target limit of biological detection (tLOBD)**. LOBD required to comply with a predefined level of health (carcinogenic) risk. In practice, in the present paper, the total daily dose calculated to produce an excess carcinogenic risk of 1 in $10^5$ (derived from a tumorigenic dose causing a 50% response in an animal cancer bioassay, TD50) as applied to 1 kg of packaged food for a 60 kg adult. |

# Test biologique versus analyse 3/3

**Table 2.** Calculated limits of biological detection (cLOBDs) for selected genotoxic chemicals in Ames plate incorporation test as compared to required target LOBDs (tLOBD) based on carcinogenic potency (TD50). For comparison, data derived from mammalian MLA hprp test are also provided.

| | Ames test | | | | | | *Mammalian (MLA hprp)* | | |
|---|---|---|---|---|---|---|---|---|---|
| Chemicals | $LEC_{plate}$[1] (µg/plate) | $LEC_L$[2] (mg/L) | **cLOBD[3] (mg/L)** | TD50[4] (mg/kg/d) | **tLOBD[5] (mg/L)** | **cLOBD/tLOBD[6]** | ***LEC[7] (mg/L)*** | ***cLOBD[8] (mg/L)*** | ***cLOBD/tLOBD[6]*** |
| Aflatoxin B1<br>1162-65-8 | 0.0038 | 0.0014 | **0.000035** | 0.0032 | **0.0000039** | **9.2** | 0.1 | **0.008** | **2083** |
| Mitomycin C<br>50-07-7 | na | 0.0015 | **0.00004** | 0.00102 | **0.00000122** | **30.6** | 0.075 | **0.0075** | **6127** |
| IQ (2-amino-3-methylimidazol [4,5-f]quinolone)<br>76180-96-6 | 0.005 | 0.0019 | **0.00005** | 0.812 | **0.00097** | **0.05** | na | **na** | **na** |
| PhIP.HCl (2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine<br>105650-23-5 | 0.01 | 0.0037 | **0.00009** | 1.78 | **0.002** | **0.04** | na | **na** | **na** |
| Methylnitrosoguanidine<br>70-25-7 | 0.034 | 0.013 | **0.00033** | 0.803 | **0.0009** | **0.3** | 0.025 | **0.0025** | **3** |
| 2-Acetylaminofluorene<br>53-96-3 | 0.266 | 0.100 | **0.0025** | 1.22 | **0.0015** | **2** | 50 | **5** | **3415** |
| 2-Nitrofluorene<br>607-57-8 | 0.57 | 0.21 | **0.005** | 0.285 | **0.00034** | **15** | na | **na** | **na** |
| Benzo(a)pyrene<br>50-32-8 | 0.57 | 0.21 | **0.005** | 0.956 | **0.0011** | **5** | 0.1 | **0.01** | **9** |
| Cyclophosphamide<br>6055-19-2 | 2 | 0.7 | **0.02** | 2.21 | **0.003** | **7** | 1 | **01** | **38** |
| Methyl methanesulfonate<br>66-27-3 | 19 | 7.4 | **0.175** | 31.8 | **0.039** | **5** | 5.5 | **0.55** | **14** |
| 2,4 Diaminotoluene<br>95-80-7 | 20 | 7 | **0.2** | 2.47 | **0.003** | **62** | na | **na** | **na** |
| Dimethylnitrosamine<br>62-75-9 | 20 | 7 | **0.2** | 0.0959 | **0.0001** | **1609** | 50 | **5** | **43448** |
| 7,12-Dimethylbenzanthracene<br>57-97-6 | 25 | 9 | **0.2** | 0.084 | **0.0001** | **2296** | 1 | **0.1** | **992** |
| N-Ethyl-nitrourea<br>759-73-9 | 33 | 12 | **0.3** | 0.948 | **0.001** | **269** | 0.4 | **0.04** | **35** |
| Formaldehyde<br>50-00-0 | 60 | 22 | **0.6** | 1.35 | **0.002** | **343** | negative | **na** | **na** |

***1:*** *$LEC_{plate}$: Lowest Effective Concentrations (LEC) retrieved from (*Duffy et al. 2006*), expressed in mass/plate.*
*2: $LEC_L$ = $LEC_{plate}$ expressed in mass/L. For comparison with concentrations in migrates, $LEC_{plate}$ in mass/plate were converted in mass/L by dividing by a factor of 2.7 (to account for the 2.7 ml of top agar plated) and then multiplied by 1000.*
*3: cLOBDs: calculated LOBDs. LOBDs calculated from the $LEC_L$, accounting for the various concentration/dilution steps in sample preparation, leading to a final concentration factor of 40. cLOBD = $LEC_L$/40.*
*4: TD50 = dose in animal carcinogenicity bioassay estimated to induce tumours in 50% of treated animals (from carcinogenic potency database (Gold)).*
*5: tLOBD: Target Limit of Detection based on an excess risk of $10^{-5}$. tLOBD = ($TD_{50}$ x 60)/50000.*
*6: ratio cLOBDs/tLOBD. A ratio <1 indicates that the theoretical LOBD derived from an available LEC (cLOBD) is sufficiently low to detect a level in the migrate corresponding to an excess risk < $10^{-5}$.*
*7: LEC obtained in mammalian cells (MLA* hprt*); Lowest Effective Concentrations (LEC), expressed in mass/L from (*Green et al. 1976; Couch et al. 1978; Clive et al. 1979; Kaden et al. 1979; Phillips et al. 1980; Gupta and Singh 1982; *O'Neill et al. 1982*; Connor et al. 1983; Thompson et al. 1983; Zamora et al. 1983; Oberly et al. 1984, 1993; Wangenheim and Bolcsfoldi 1988; Eliopoulos et al. 1995; Speit and Hartmann 1995; Hakura et al. 2003; Valentin-Severin et al. 2003; Kawaguchi et al. 2010; Smith et al. 2013).*
*8: cLOBDs from mammalian-cell-based assays (MLA* hprt*). The cLOBDs were calculated from the LECs[7] (in mg/L) divided by 10 (assuming a total theoretical concentration factor of 10).*
***na***: not available.



# Constat….

- Dans l'état actuel des connaissances les essais biologiques *in vitro* peuvent fournir des informations supplémentaires importantes pour l'évaluation de l'innocuité des MCF. Toutefois , pour détecter de façon fiable les substances génotoxiques en deçà d'un seuil de 0,00015 µ/mL (TTC),
- **il faut encore les améliorer.**
- *Néanmoins, les méthodes chimiques actuelles utilisées pour l'analyse des Emballages en contact avec les aliments migrent utilisent une limite de détection de 0,01 µg/mL et sont donc également notables pour exclure la présence de toute substance génotoxique considérée par le TTC. Finalement la conduite des deux méthodes peut fournir des informations toxicologiques importantes sur une FCM pour rendre possible une évaluation de l'innocuité des substances inconnues.*

# NANOAMES™ TEST : METHOD

Solution of test article 5 concentrations

5 Strains, culture 12 hours at 37°C

Metabolic activation mix S-9 mix

DAY 1

DAY 2

Over night GenCar medium Exposure for tester Strains at 37°C

DAY 3

Molten agar less hist/biotine for each well

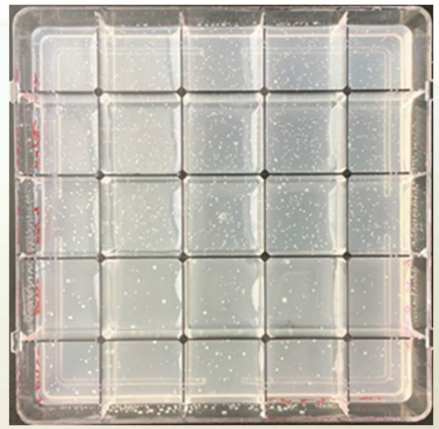
+

Culture 48 h at 37°C
25 well Agar plate

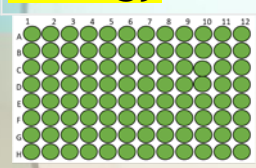
+

DAY 5

Scoring each well



Molecular Biology



Biomnigene

for PCR

XENOMETRIX
Swiss Commitment for Bioassays

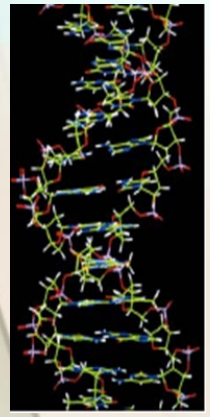
# Test d'Ames ultra miniaturisé

NANO AMES

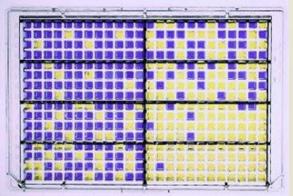
INNOVATION

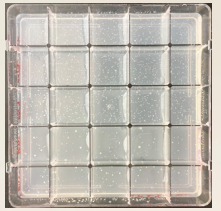
Il existe différentes methodes pour le test d'Ames.

Dans le cadre de la caractérisation d'impureté en très petite quantité, GenEvolutioN a ultra miniaturisé le test d 'Ames « NanoAmes ™ »



| Test de mutagenèse | Méthodes | Test item Quantité | Commentaires |
|---|---|---|---|
| Ames test | Standard | 250 mg/essai | 5 souches |
| Effet nourricier<br>Produit cytotox | « Treat and Wash »<br>« Treat and plate » | 250 mg/essai | 5 souches |
| Screening/ impureté | MPF (fluctuation) | 40 mg | 5 souches |
| Screening/ | Miniaturisé | 35 mg | 4 souches |
| Impureté non caractérisée | NanoAmes ™ | **70 µg/2 souches** | 5 souches |
| | | | |



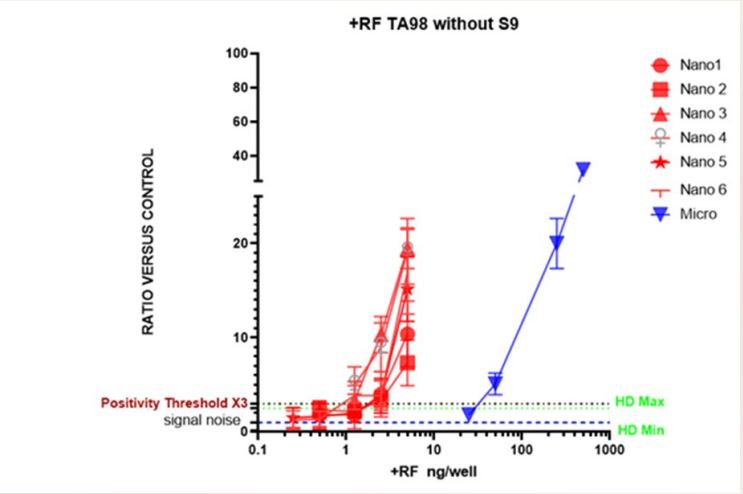


# Test d'Ames Ultra-miniaturisé versus mini

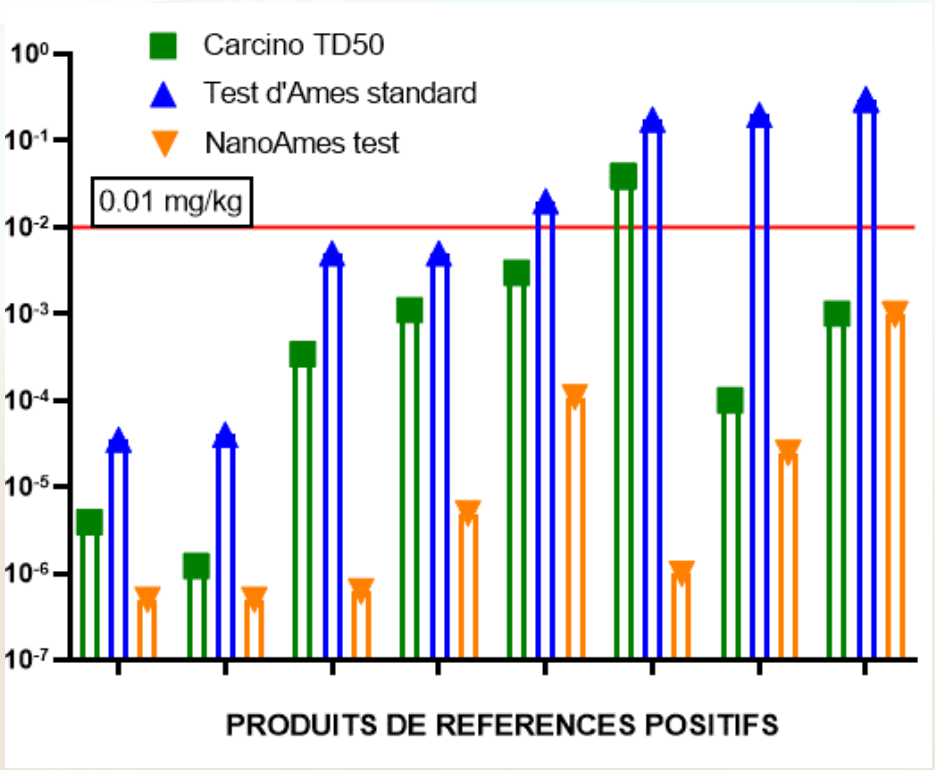
- 14 produits de référence et deux produits négatifs ont été testés en utilisant la méthode ultra miniaturisée en pré-incubation sur les 6 souches TA98, TA100, TA97a, TA1535, TA102 et E.Coli, avec et sans activation métabolique.

Bonne correspondance


À des doses plus faibles



# Conséquences: comparaison Ultra miniaturisation versus Ames standard




| NanoAmes | Ames standard |
|---|---|
| cLOBD/tLOBD | 'cLOBD/tLOBD" paper |
| 0,126 | 9,2 |
| 0,404 | 30,6 |
| 0,0018 | 15 |
| 0,0045 | 5 |
| 0,036 | 7 |
| 2,52644E-05 | 5 |
| 0,246 | 2296 |
| 0,985 | 269 |

FOOD ADDITIVES & CONTAMINANTS: PART A
https://doi.org/10.1080/19440049.2019.1664772

# Conclusion

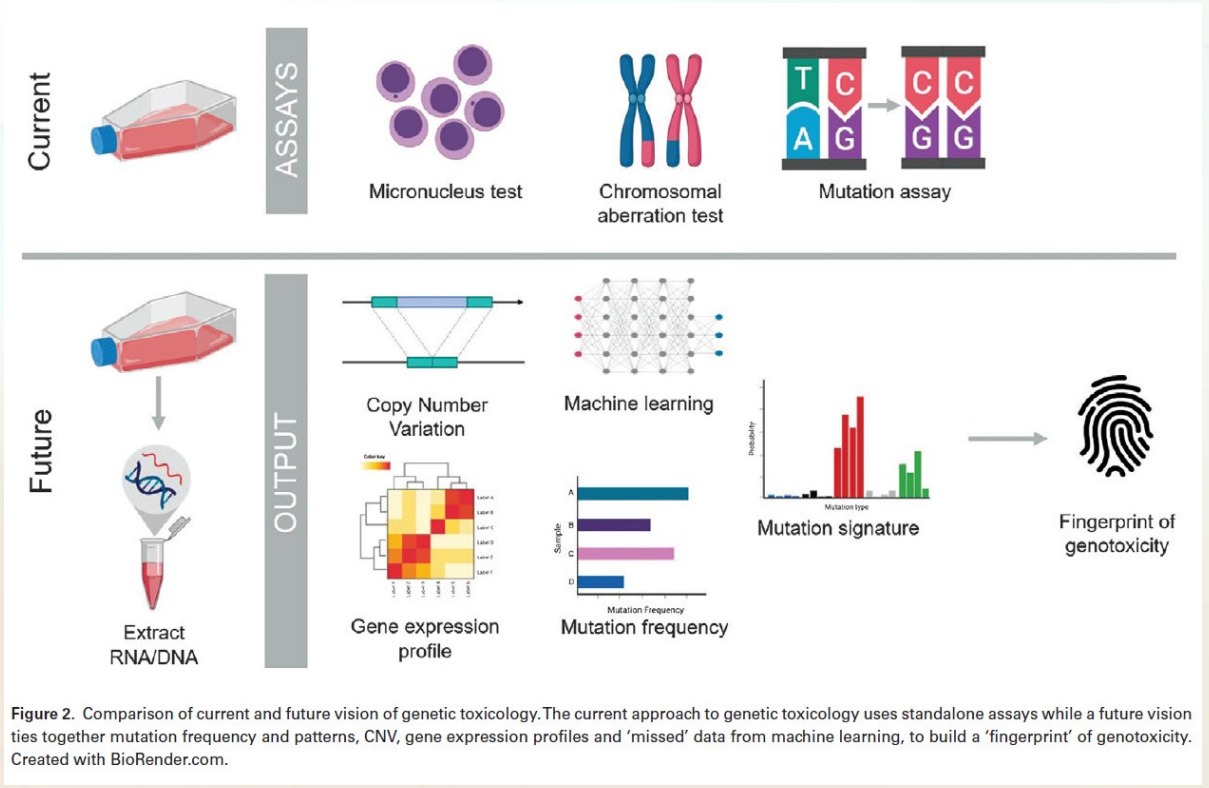



Figure 2. Comparison of current and future vision of genetic toxicology. The current approach to genetic toxicology uses standalone assays while a future vision ties together mutation frequency and patterns, CNV, gene expression profiles and 'missed' data from machine learning, to build a 'fingerprint' of genotoxicity. Created with BioRender.com.

**The promise of toxicogenomics for genetic toxicology: past, present and future**

**Rhiannon David***

Clinical Pharmacology and Safety Sciences, R&D, AstraZeneca, Cambridge, UK

* To whom correspondence should be addressed. Email: rhiannon.david@astrazeneca.com

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Automatisation des techniques

ARN/ADN expression

In silico => AI = « machine learning »

# Integrated solution

As part of the Seqens'Lab ecosystem, we provide you with a suitable solution to investigate the mutagenic potential of impurities and secure your development according to ICH M7 guidelines

**Identification of impurities along the synthesis of APIs**

SEQENS 'Lab

https://www.seqens.com

**_in silico_ analysis from chemical structure of impurities**

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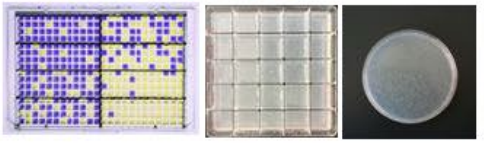
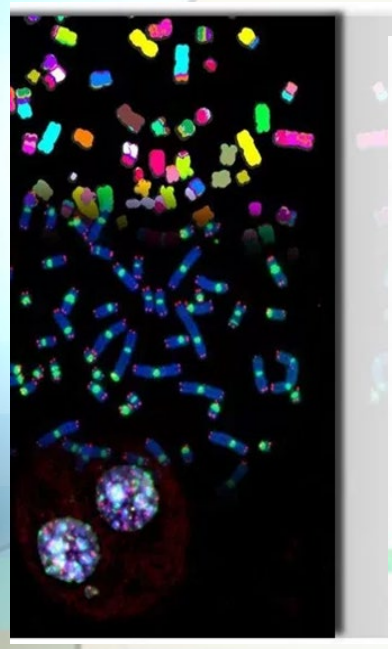
**_In vitro_ Ames test : Ames/NanoAMES – non GLP or GLP compliant**

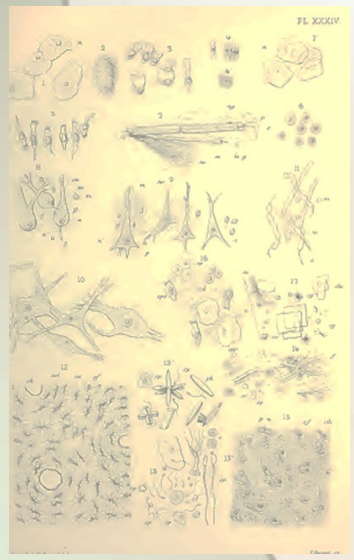
GENEVOLUTION



www.genevolution.fr

HARMONIC PHARMA
SAFETY BY DESIGN



- **Nestlé:** Maricel Marin-Kuan, Gabriele Scholz et Benoît Schilter
- **Servier**: Véronique Gervais
- **XenometriX** : Dimitrios Spiliotopoulos et Nicole Weilad-Jaegi
- **Biomnigene**: Alexandre Douablin
- **GenEvolutioN**: Olivier Cariou, Nina Coulange, Isabelle Mouche Laure Malésic, Françoise Gigan et Michèle Loeffler

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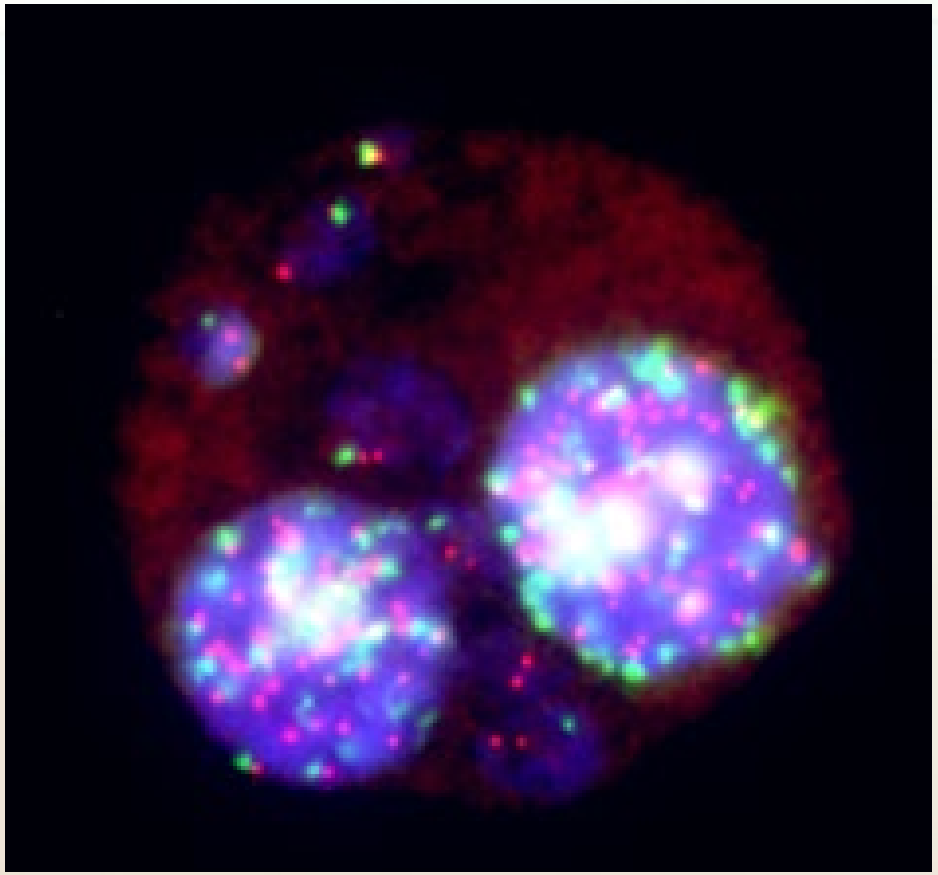
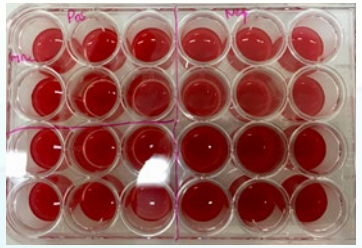
ANNEXE

# Cytogenetic tests
# Micronucleus OECD 487
# GLP

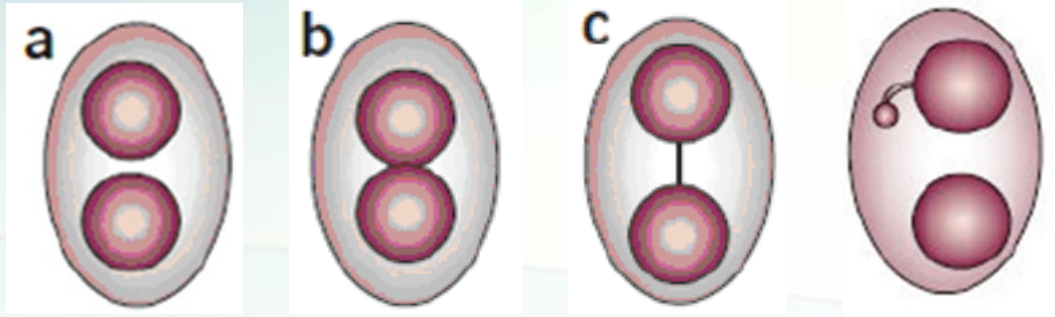


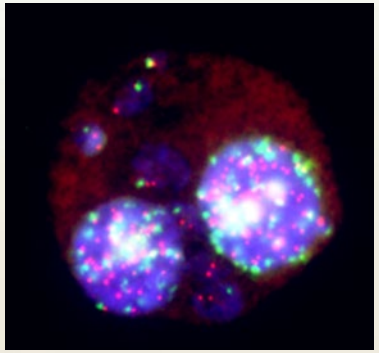
# *in vitro* Micronucleus test (OECD 487)

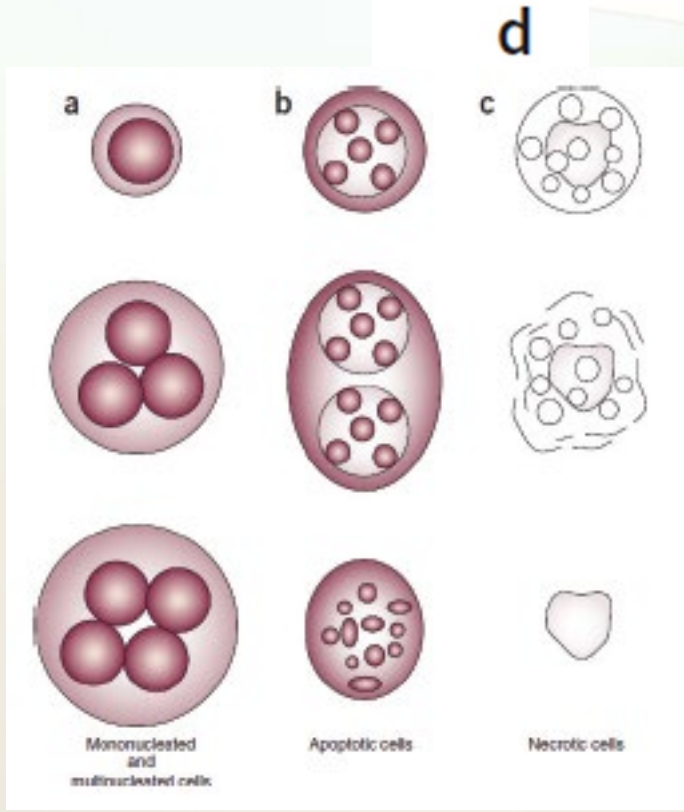
**3- hour short treatment with and without metabolic activation, then a long treatment 24-hour in the absence of metabolic activation. In case of cycle delay, a long alternative treatment could be performed :**



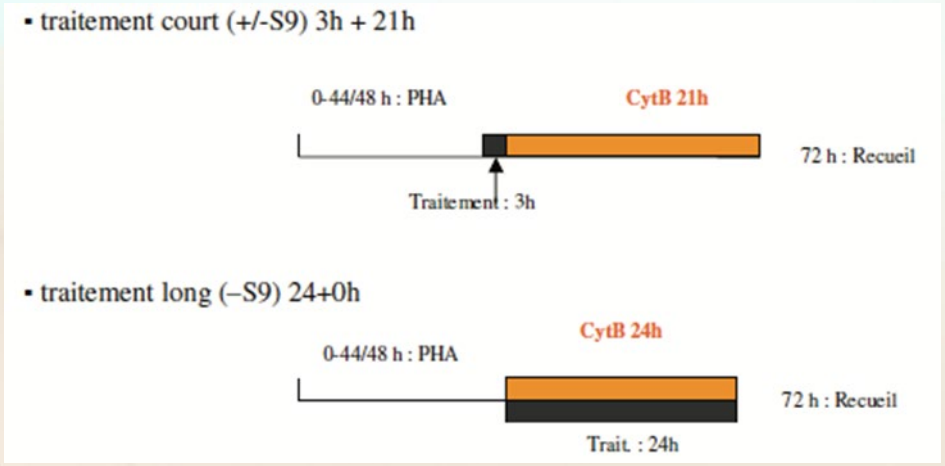
**In presence of phytohemagglutinine , Blocked by cytochalasine B**



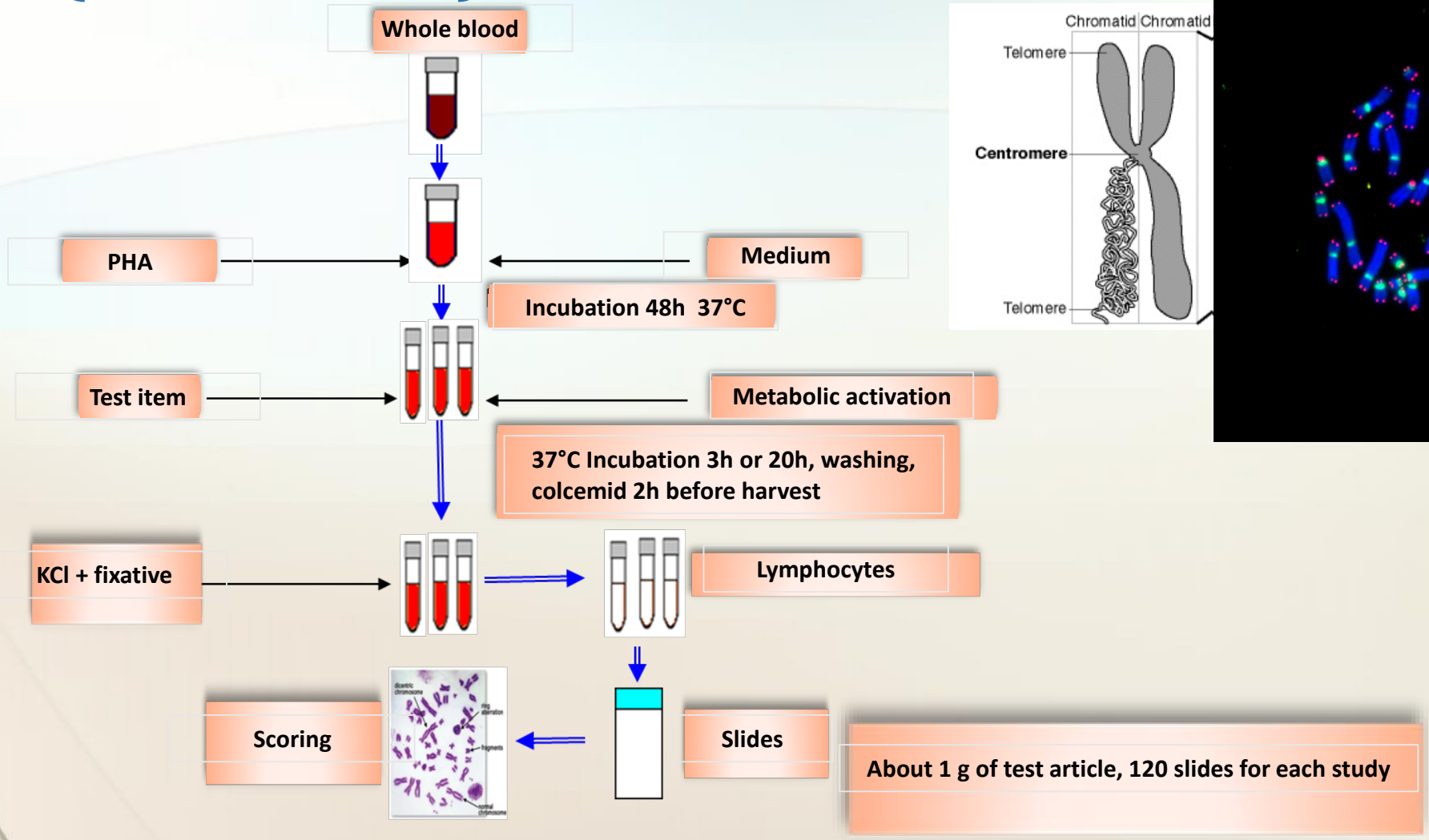






Double staining : show aneugen, or Clastogen (m-Fish)

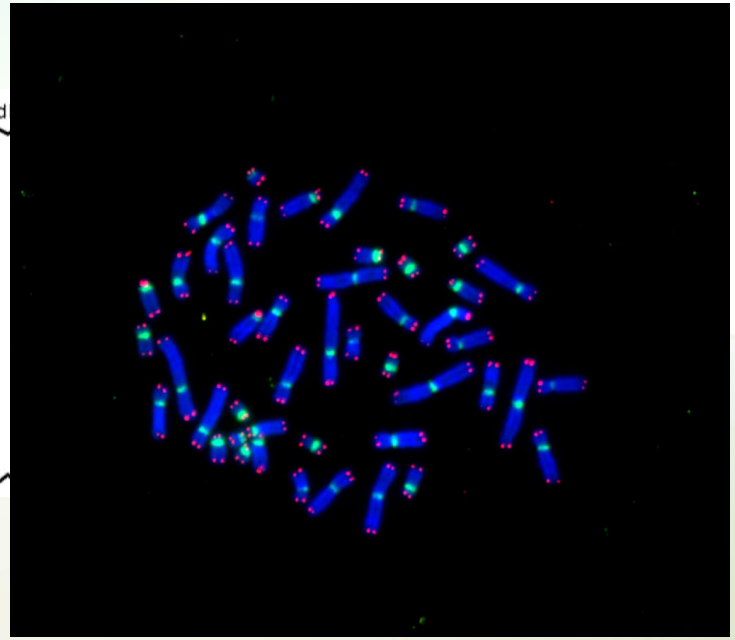



# Chromosomal aberration test: Method (OECD 473)









**Un CRO avec une expertise en genotoxicité**

# INNOVATION

GenEvolutioN Prospective Division (Février 2017)

Activité de Recherche Transfert de technologie

GenEvolutioN Holding SAS (Février 2018)

JEI

# Règlementaire BPL

ANSM
GIPC COFRAC

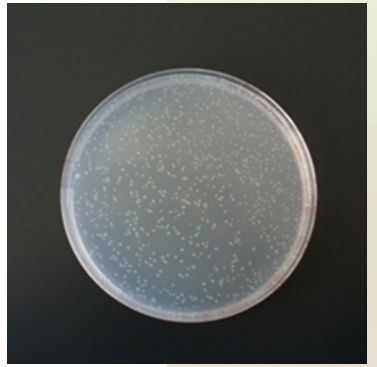
GenEvolutioN Testing Division (Juin 2017)

Tests Réglementaires

# OECD 471 guideline -bacteria

13. At least five strains of bacteria should be used. These should include four strains of *S. typhimurium* (TA1535; TA1537 or TA97a or TA97; TA98; and TA100) that have been shown to be reliable and reproducibly responsive between laboratories. These four *S. typhimurium* strains have GC base pairs at the primary reversion site and it is known that they may not detect certain oxidising mutagens, cross-linking agents and hydrazines. Such substances may be detected by *E.coli* WP2 strains or *S. typhimurium* TA102 (19) which have an AT base pair at the primary reversion site. Therefore the recommended combination of strains is:

1. *S. typhimurium* TA1535, and
2. *S. typhimurium* TA1537 or TA97 or TA97a, and
3. *S.* typhimurium TA98, and
4. *S.* typhimurium TA100, and
5. *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102.



In order to detect cross-linking mutagens it may be preferable to include TA102 or to add a DNA repair-proficient strain of *E.coli* [e.g. *E.coli* WP2 or *E.coli* WP2 (pKM101).]