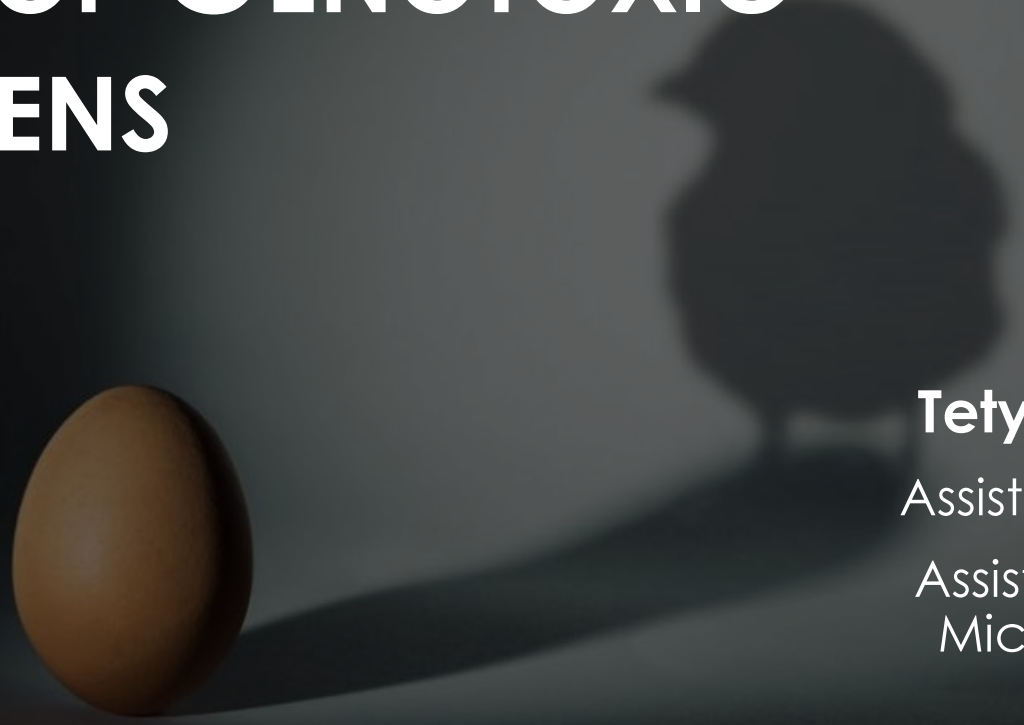
The Chicken Egg Model: an Alternative Model for Detection of Genotoxic Carcinogens



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1. Method Description

Historical Significance of Chicken Egg Model

Field	Time	Discovery
Development	1400 BCE	Egyptians are the first time artificially incubate chicken eggs, during the 18th dynasty.
	350 BCE	Aristotle begins work with chick embryos to study development (leads to major principles and mistakes) and is the first to actually dissect the embryo.
	1400s	Albert Magnus composes treatises on chick embryology that serve as the filler between Aristotle and the Renaissance.
	1567	Volcher Coiter publishes work on the development of the chick embryo, and compares this development to that of reptiles, humans, and other birds. This makes the field of comparative anatomy take off.
	1570	Volcher Coiter identifies the blastoderm using chick embryos.
	1628	William Harvey discribes the formation of blood islands and circulation, including functional differences between veins and arteries in chick embryos. He studies heart formation and commencement of beating in ovo using a magnifying lens. Previous to this, it was thought that the heart did not beat until birth/hatching.
	1651	William Harvey publishes findings that the generation of a chick is the result of epigenesis not metamorphosis. Rebukes Aristotles belief that chick eggs can grow without male fertilization.
	1671	Malpighi, through his studies of frogs and chicks, publishes work describing the role of capillaries.
	1672-1675	Malpighi discovers function of neural tubes and somites through the study of chick embryos. He describes the chick blastoderm, neural genesis and early heart development.
	1749	Beguelin perfects the window in the shell technique for chick observation as the embryo develops.
	1759	Casper Friedrich Wolff publishes "The Theory of Generation". His paper indicates that body organs develop in the embryo through a series of steps and challenged contemporary thought that organisms were preformed. His arguments sparked new interest in embryogenesis.
	1817-1828	Heinz Christian Pander, a follower of Wolff, and Karl Ernst von Baer discover and identify germ layers in the forming chick embryo.
	1826	Karl Ernst von Baer is the first to identify the mammalian ovum and notochord. He used the light microscope to extend Pander and von Baer's germ layer discovery, showing that it is universally present in vertebrates. Before him, it was suspected that changes between species in the stages of development represented progressive evolution. His findings supposedly influenced Darwin's thinking.
	1859	Darwin's publishes 'On the Origin of Species' and demonstrating correlations between organisms.

Field	Time	Discovery
Immunology and Cancer	1906	Levaditi introduces the chick embryo as a model to study infection.
	1907-1913	Goldman and Murphy graft human tumors onto the CAM and recognize the vascular response necessary for successful engraftment.
	1911	Peyton Rous identifies the retrovirus Rous Sarcoma virus (RSV) in chicken embryos. He won the Nobel prize for his work in 1966.
	1931	Francis Ernest Goodpasture and Alice Woodruff publish their groundbreaking paper on their cultivation of viruses on the chick embryo, using the chick embryo for the cultivation of viruses becomes a common method.
	1932	Waddington developes a procedure to remove the chick blastoderm and culture it ex ovo. This technique is improved by New (1955) and becomes a valuable experimental model for development.
Genetics	1936	Frederick Hutt publishes the first genetic map of the chicken.
Cancer	1945-1955	Dagg, Karnofsky and Toolan perform routine serial transplantation of human tumors and initiat therapeutic trials on tumor bearing chicks.
Neurology	1952	Rita Levi-Montalcini – nobel prize winner for discovering nerve growth factors. Most of her defining work involved nerve development in the chick.
	1967	Michel Abercrombie discovers the cellular process of contact inhibition through his studies on the chick embryo, this process is now used to distinguish between normal and cancerous cells.
Cancer	1974	Folkman publishes CAM assay as a model to study vascularization.
	1983	Schwartz,Tizard and Gilbert determine the 9312 nucleotide sequence for the Rous sarcoma virus (RSV).
		Bishop reviews 25 known oncogenes. Nine are from domestic fowl.
		Ossowski, Chambers, and Quigley establish the chick as a model for metastasis.
genetic model for human disease	1991	Tiersch and Wachtel discover that the genome of birds, specifically gallus gallus, is one third the size of mammals, indicating the chickas a simple genetic model.
	2004	Avain flu moves from chicken to human infection (starting in Vietnam and Thailand) causes a world-wide focus on avian biology and disease.
		Richard Wilson's group (Washington University) publish a full avian genome sequence.
Intravital imaging model	1996	Chambers monitors single-cell behavior in the CAM using In vivo video
	2006	Lewis implements viral nanoparticles to image CAM and tumor vasculature intravitally.
	2008	Zijlstra uses intravital imaging to demonstrate correlation between cell migration in the primary tumor and metastasis to distant organs.

Kain K.H., et al., (2014). Dev Dyn. 243(2):216-228.

> Arch Toxicol. 2002 Oct;76(10):606-12. doi: 10.1007/s00204-002-0380-4. Epub 2002 Aug 10.

In ovo carcinogenicity assay (IOCA): evaluation of mannitol, caprolactam and nitrosoproline

Klaus D Brunnemann [1], Harald G Enzmann, Carmen E Perrone, Michael J Iatropoulos, Gary M Williams

Review > Front Biosci. 1997 Dec 15:2:c30-9. doi: 10.2741/a168.

The in ovo carcinogenicity assay (IOCA): a review of an experimental approach for research on carcinogenesis and carcinogenicity testing

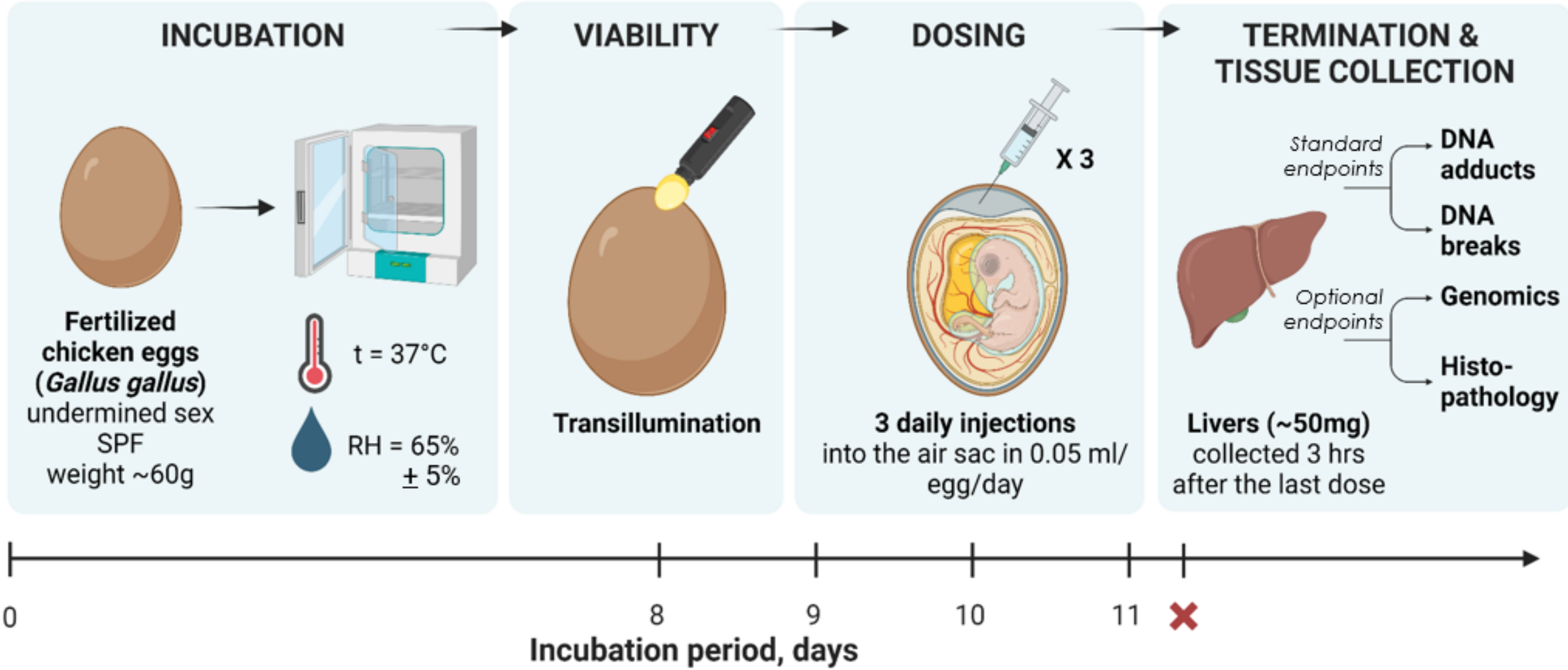
H Enzmann [1], K D Brunnemann

Comparative Study > Exp Toxicol Pathol. 2013 Sep;65(6):729-35. doi: 10.1016/j.etp.2012.09.007. Epub 2012 Oct 31.

Inter-laboratory comparison of turkey in ovo carcinogenicity assessment (IOCA) of hepatocarcinogens

H Enzmann [1], K Brunnemann, M Iatropoulos, S Shpyleva, N Lukyanova, I Todor, M Moore, K Spicher, V Chekhun, H Tsuda, G Williams

Chicken Egg Model (CEM)



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Chicken Egg Model (CEM)

- Vehicles used:
  - Deionized Water (hydrophilic compounds)
  - 20% Kolliphor Oil / Solutol HS15 (lipophilic compounds)
  - 20% Tween 20
- Positive control:
  - Quinoline
- Doses are selected based on Oral LD_{50} in rodents, solubility, or toxicity
- ~2 compounds / experiment, 3 dose levels each + controls
- At least 3 biological replicas per group per endpoint

TYPES OF DNA DAMAGE ASSESSED

STRUCTURAL DISTORTIONS

- bulky DNA adducts
- crosslinks / dimerization

DNA BACKBONE DAMAGE

- DNA strand breaks
  - single

SINGLE BASE CHANGE

- oxidative DNA damage

^{32}P-Nucleotide Postlabeling Assay

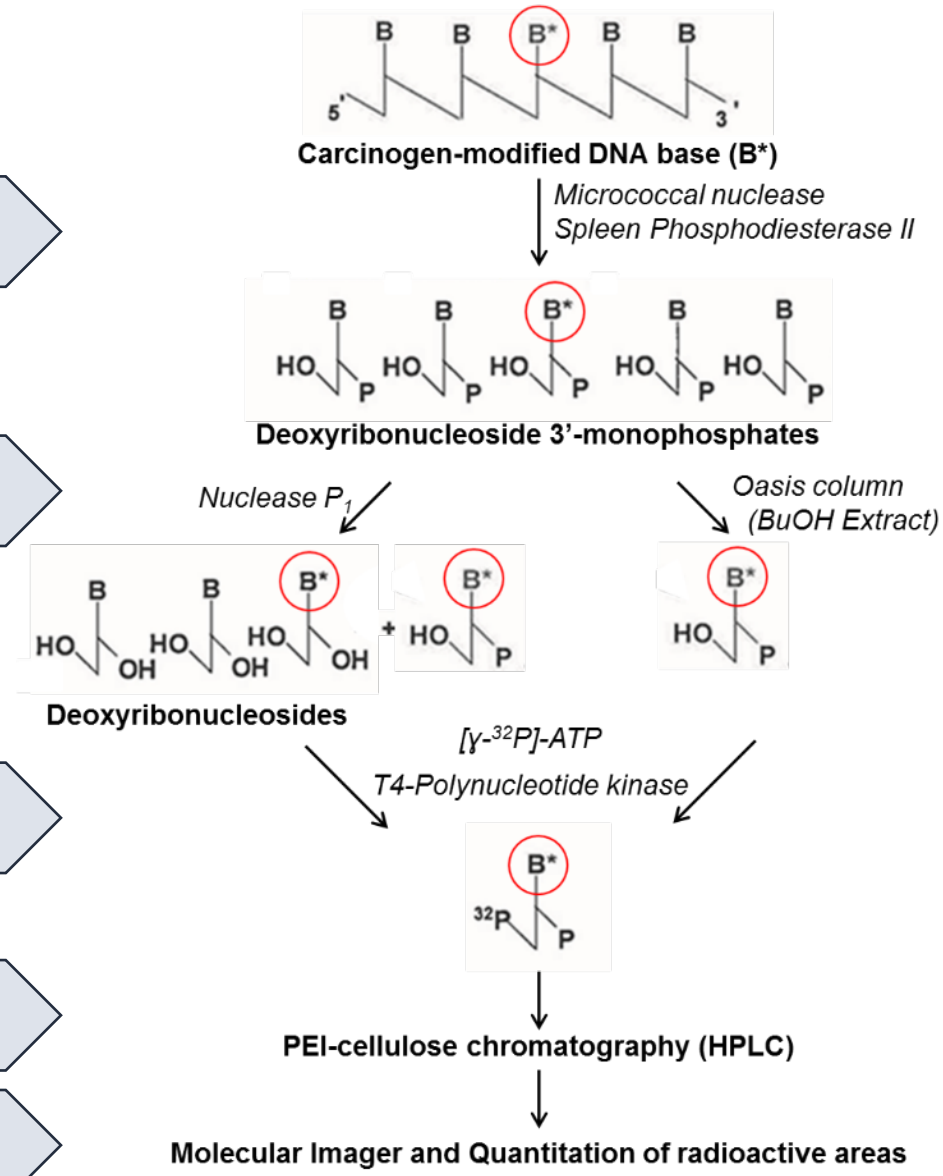
Enzymatic hydrolysis to 3'-mononucleotides

Adduct enrichment (selective removal of normal nucleotides)

Labeling of nucleotides with adducts with ^{32}P radiolabel

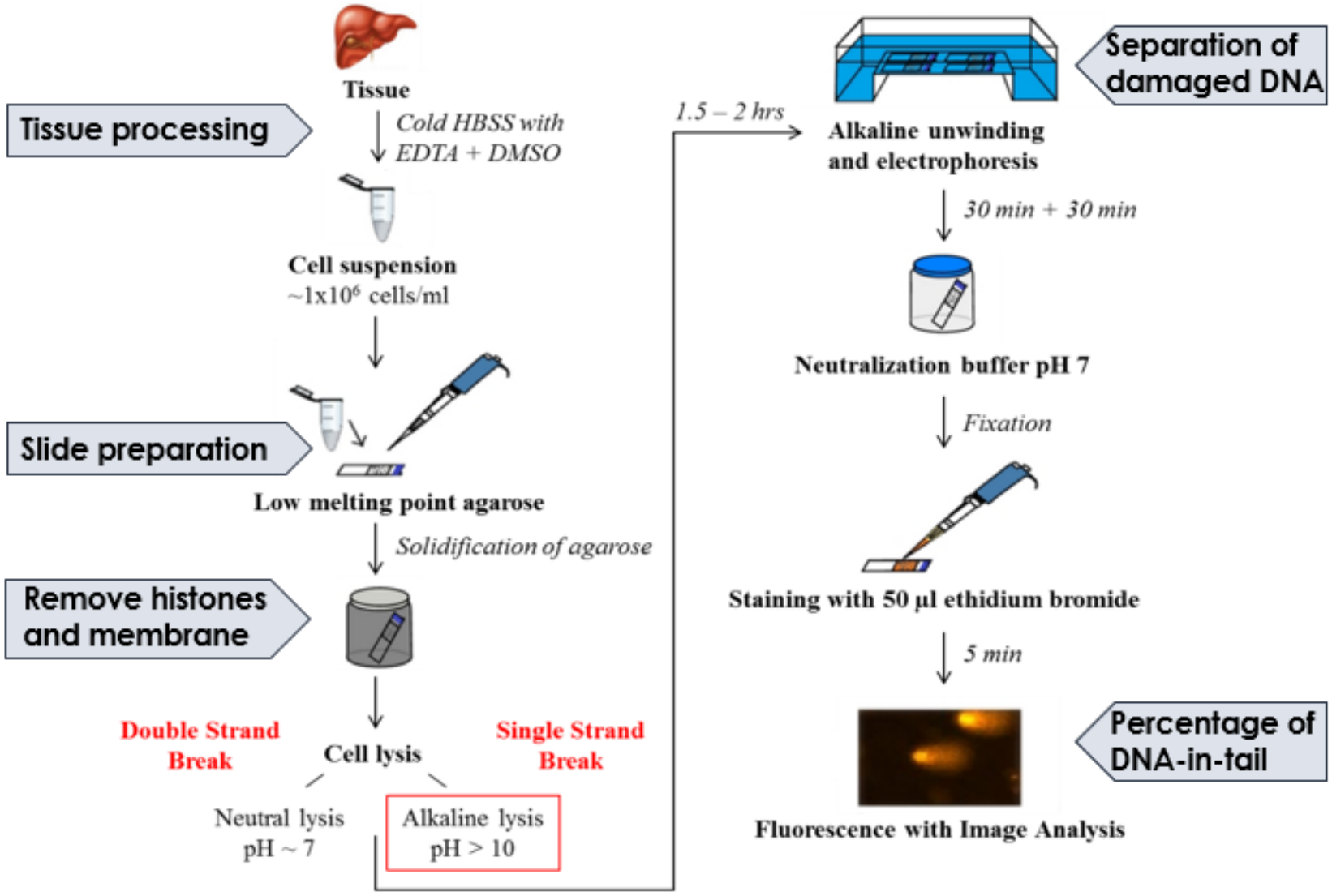
Adducts are separated using HPLC or TLC

Qualitative and quantitative assessment



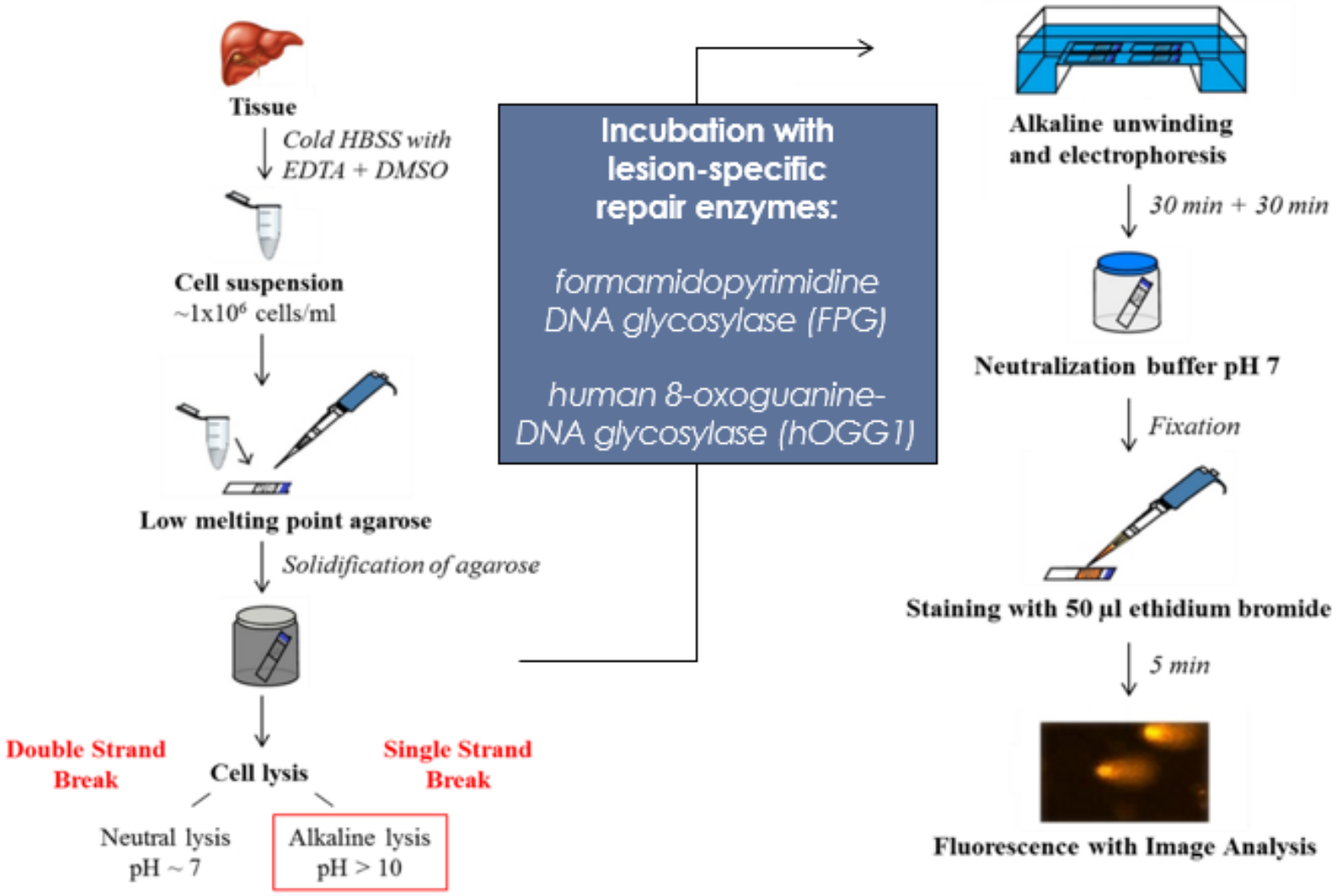
modified from Duan and Williams, Toxicol Open Access. 2016. 2

Comet Assay



modified from https://www.researchgate.net/figure/269993727_fig1_Schematic-representation-of-comet-assay-protocol

ENHANCED (MODIFIED) COMET ASSAY



modified from https://www.researchgate.net/figure/269993727_fig1_Schematic-representation-of-comet-assay-protocol

Chicken Egg Model (CEM)

ADVANTAGES

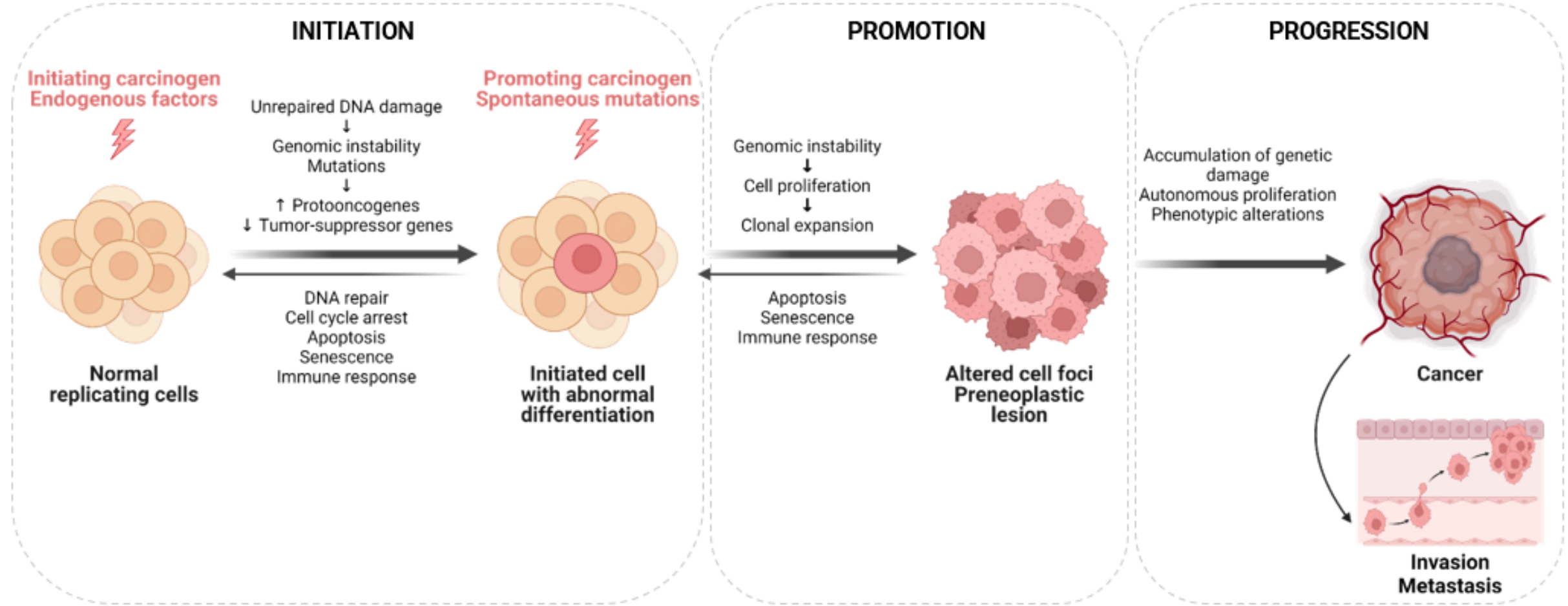
- Intact organisms, resembles in vivo conditions, but not an animal
- Large number of tested eggs per experiment
- Facile delivery of the test substance (lipo- and hydrophilic)
- Intrinsic metabolic activation / detoxication
- Specific pathogen free
- Rigorous environmental control
- Evaluation of multiple critical endpoints
- Elucidation of mechanism of action

POTENTIAL LIMITATIONS

- Developing organism
- Metabolic differences
- Route of exposure
- Undetermined sex
- Species difference

2. Context of Use

Chemical Carcinogenesis



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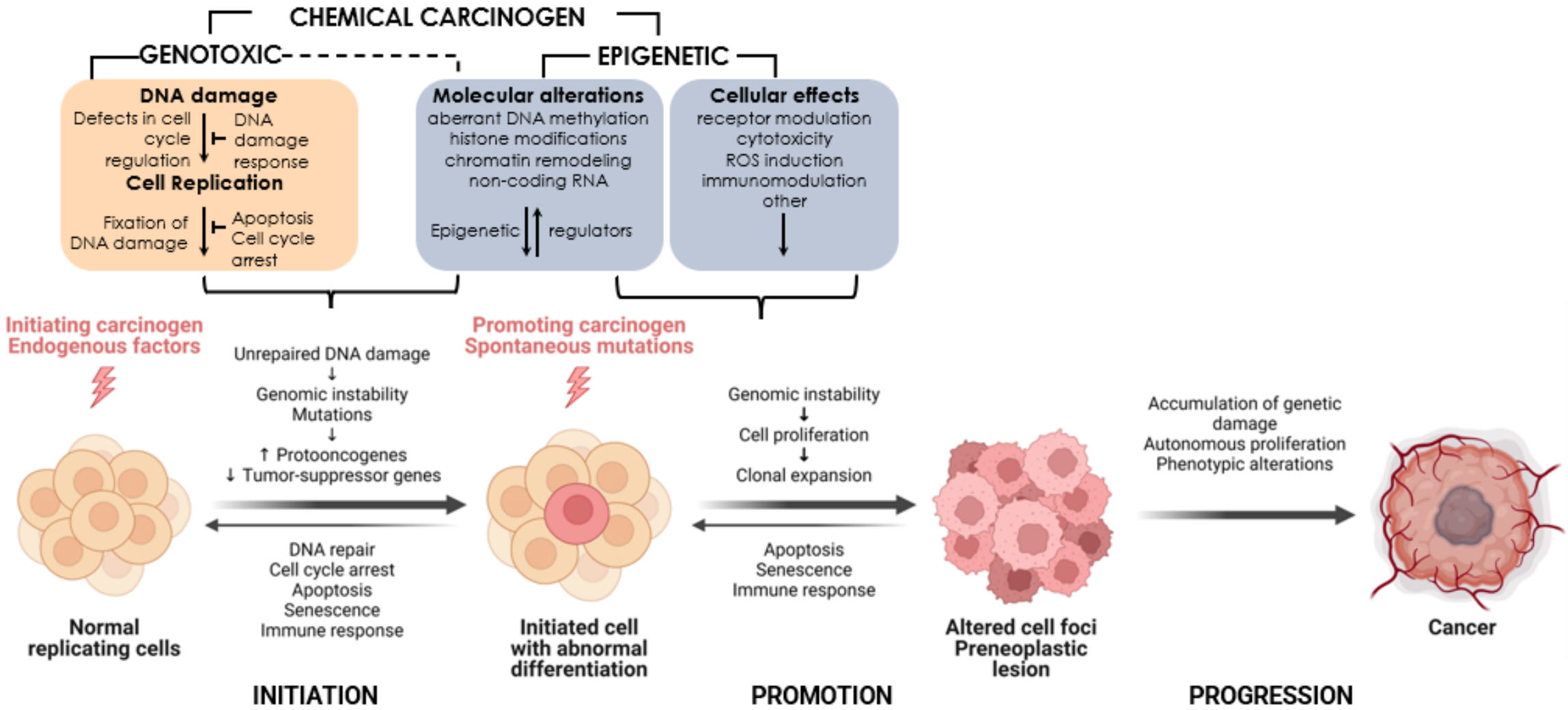
CHEMICAL CARCINOGENESIS



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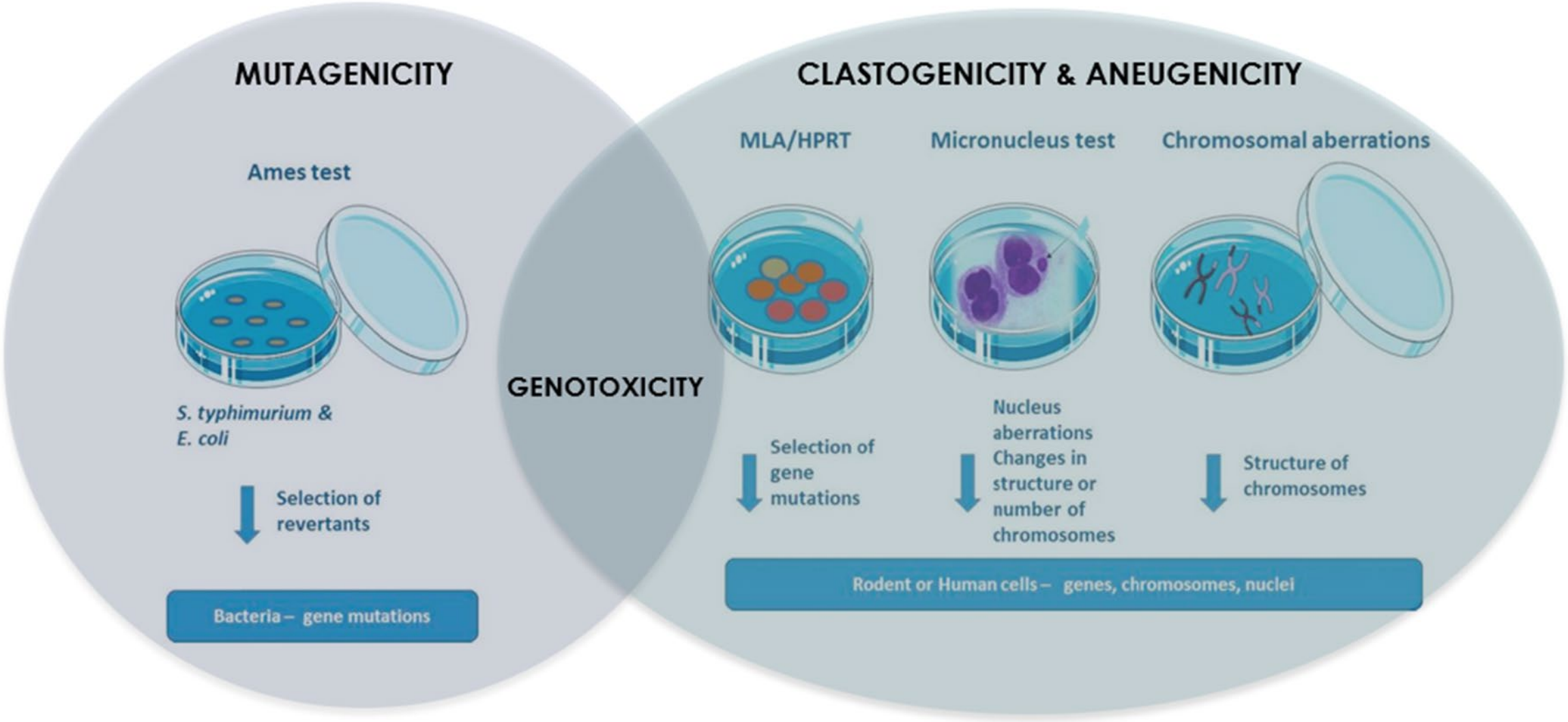
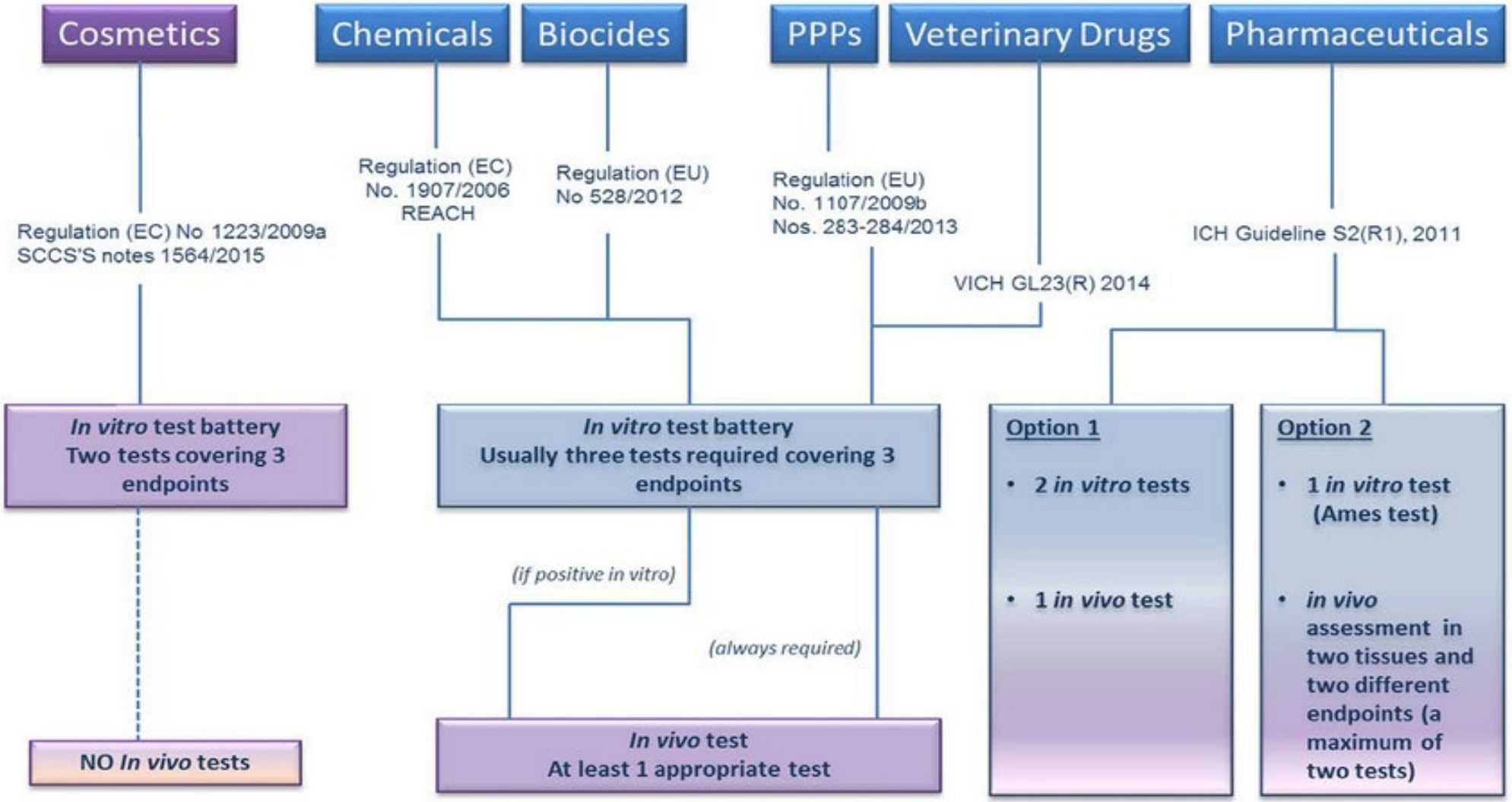
GENOTOXICITY ASSESSMENT



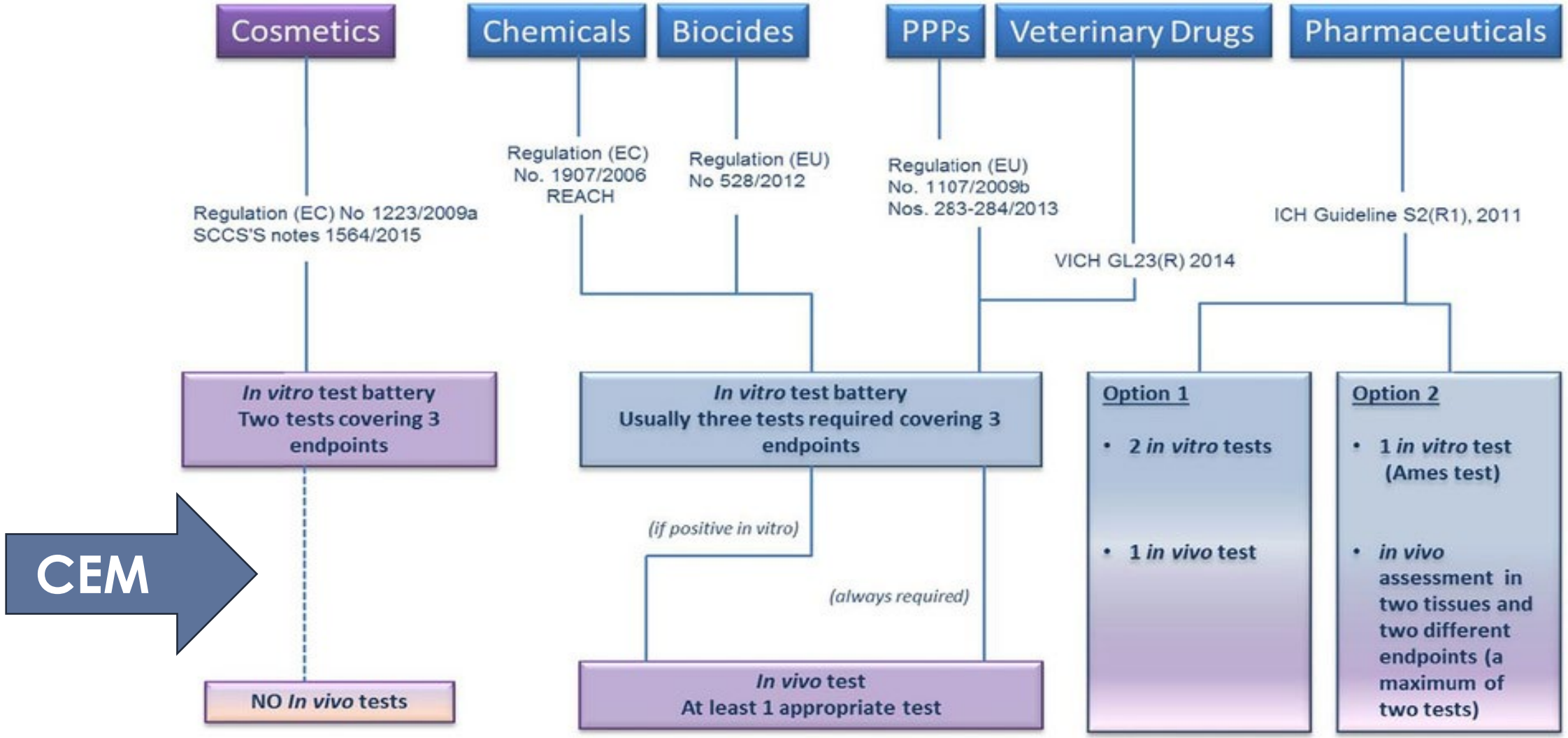
Modified from Corvi R, Madia F. (2017). Food Chem Toxicol. 106(Pt B):600-608

GENOTOXICITY ASSESSMENT



Corvi R, Madia F. (2017). Food Chem Toxicol. 106(Pt B):600-608

GENOTOXICITY ASSESSMENT



Corvi R, Madia F. (2017). Food Chem Toxicol. 106(Pt B):600-608

Context of Use

A. How is your method intended to be used?

   - **chemical screening, hazard identification, potency evaluation**

B. What regulatory testing need does your method address?

   - **in vitro follow-up**, **minimizing use of animal assays**, **targeted endpoint evaluation**

C. What regulatory space does your method address?

   - **cosmetics, industrial chemicals, agrochemicals, food/food additives, pharmaceuticals**

D. Has data generated by your method been used for regulatory submissions?

   - **not yet**

3. Biological Relevance

CEM Evaluation

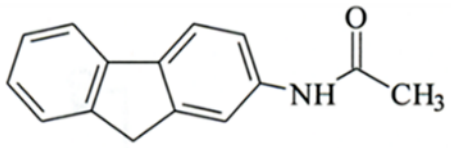
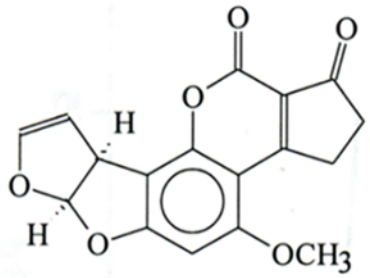
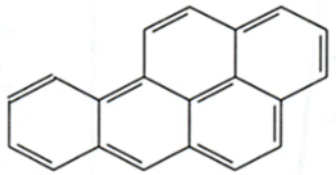
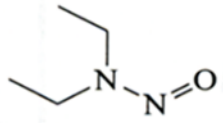
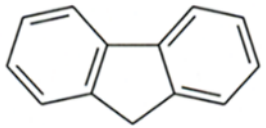
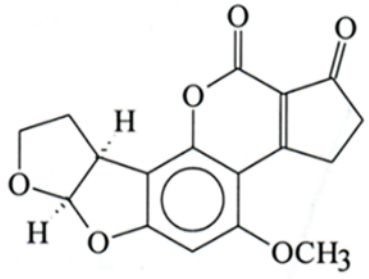
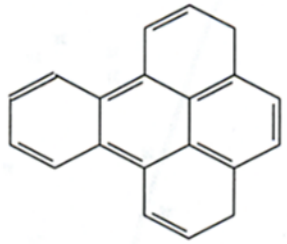
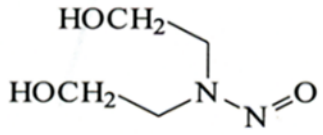
Toxicol Sci. 2014 Sep;141(1):18-28. doi: 10.1093/toxsci/kfu123. Epub 2014 Jun 27.

Chicken fetal liver DNA damage and adduct formation by activation-dependent DNA-reactive carcinogens and related compounds of several structural classes.

Williams GM[1], Duan JD[2], Brunnemann KD[2], Iatropoulos MJ[2], Vock E[3], Deschl U[3].

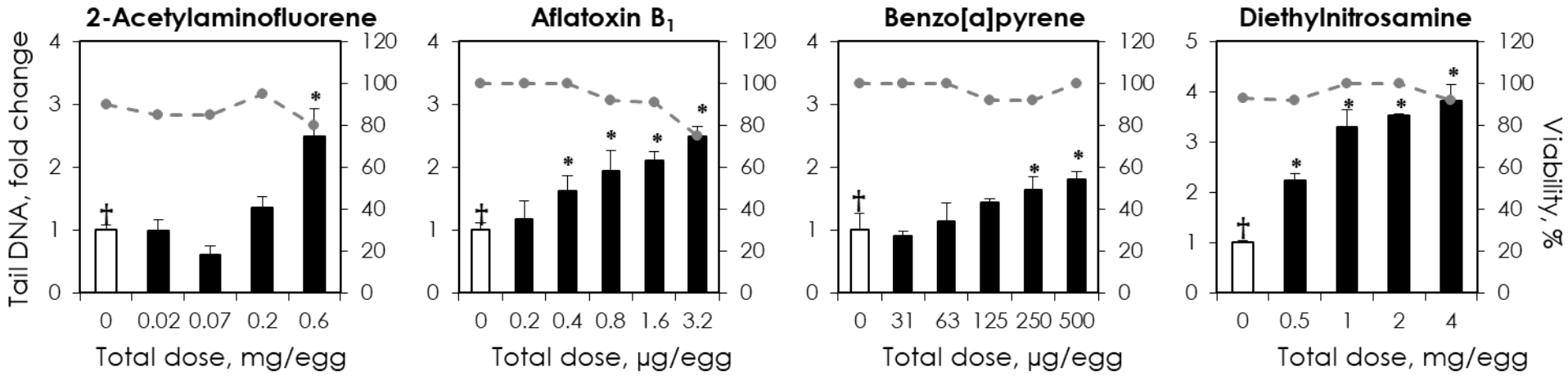
Genotoxic Carcinogens

Non/weak-genotoxic/ carcinogens

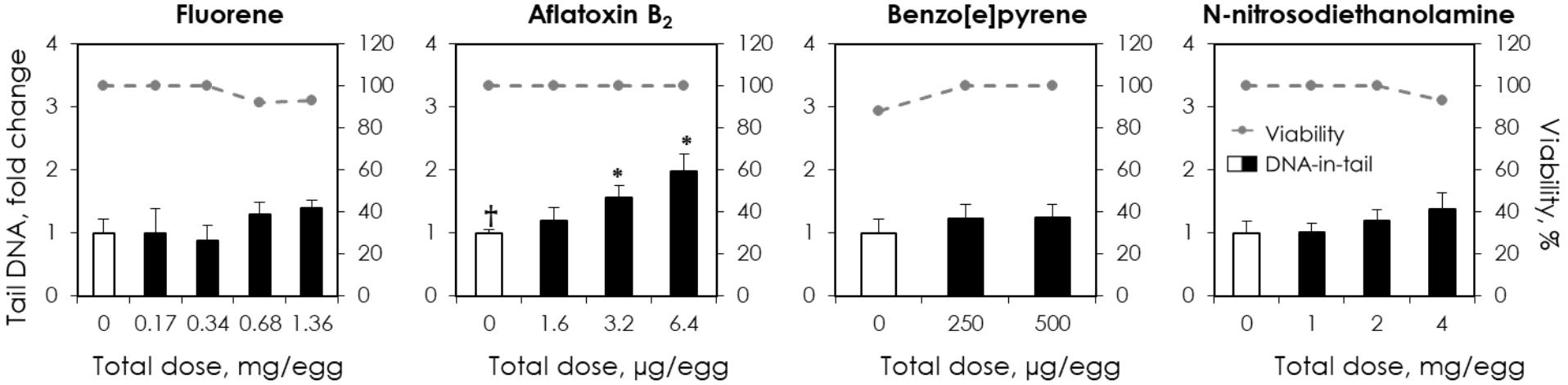
PAIR 1	PAIR 2	PAIR 3	PAIR 4
N-Hydroxylation	*Furan Ring Epoxidation*	*Ring Epoxidation*	*Alkyl Oxidation*
			
2-Acetylaminofluorene	**Aflatoxin B_1**	**Benzo[a]pyrene**	**Diethylnitrosamine**
			
Fluorene	**Aflatoxin B_2**	**Benzo[e]pyrene**	**N-nitrosodiethanolamine**

CEM Evaluation: Genotoxicity

Genotoxic Carcinogens



Non/weak-genotoxic/ carcinogens

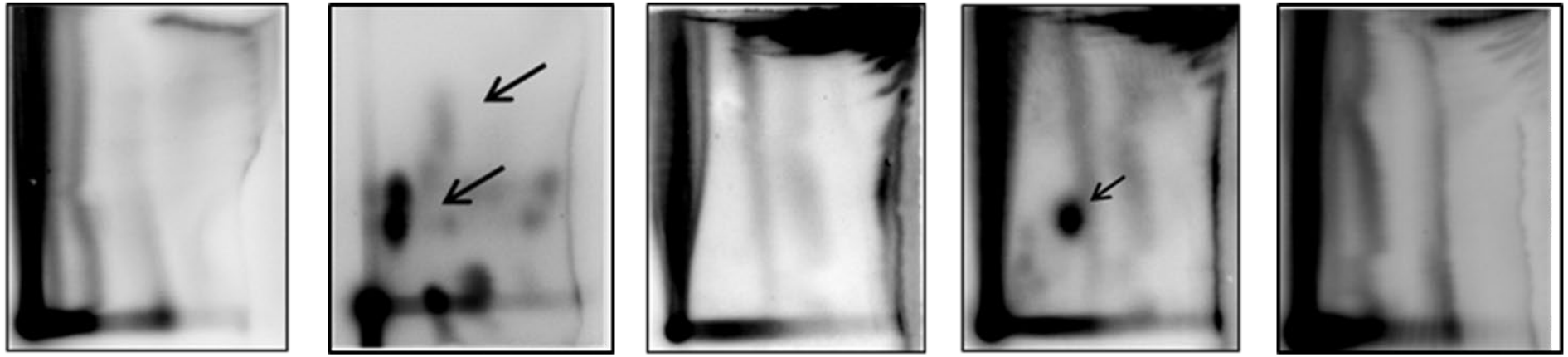


*, denotes significant ($p < 0.05$) difference from control group; †, denotes significant ($p < 0.05$) trend

Williams et al., Toxicol. Sci. 2014. 141: 18-28

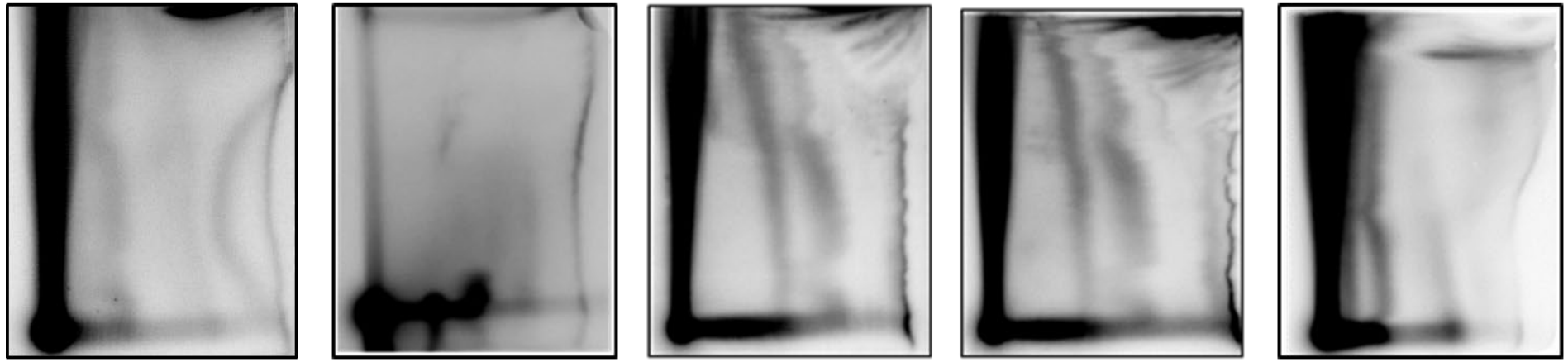
CEM Evaluation: Genotoxicity

Genotoxic Carcinogens



Vehicle (20% HS15) | 2-Acetylaminofluorene 0.6 mg | Aflatoxin B_1 3.2 µg | Benzo[a]pyrene 500 µg | Diethylnitrosamine 2 mg

Non/weak-genotoxic/ carcinogens



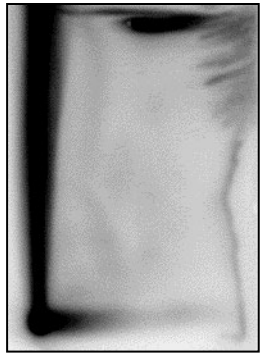
Vehicle (dd H_2O) | Fluorene 1.36 mg | Aflatoxin B_2 6.4 µg | Benzo[e]pyrene 500 µg | N-nitrosodiethanolamine 4 mg

Williams et al., Toxicol. Sci. 2014. 141: 18-28

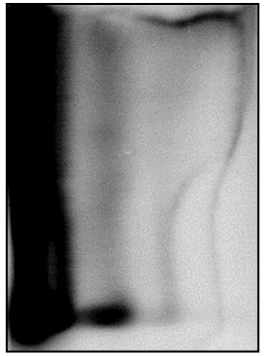
CEM Evaluation: Genotoxicity

CLOFIBRIC ACID

NPL Assay

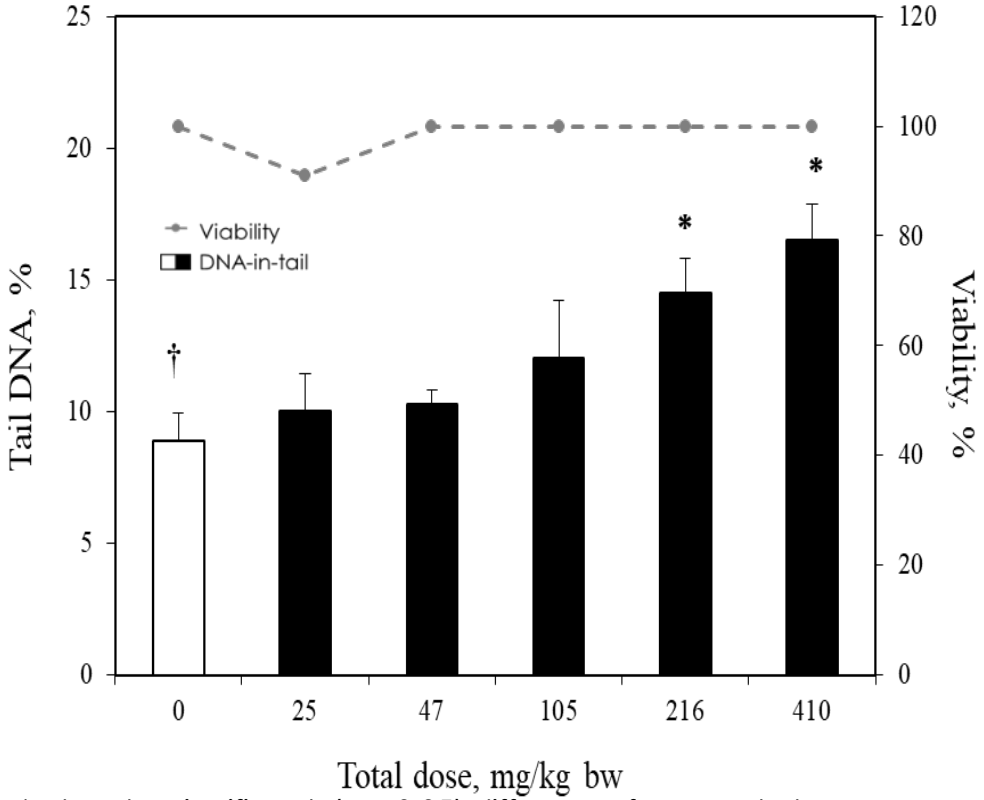


Vehicle, HS 15



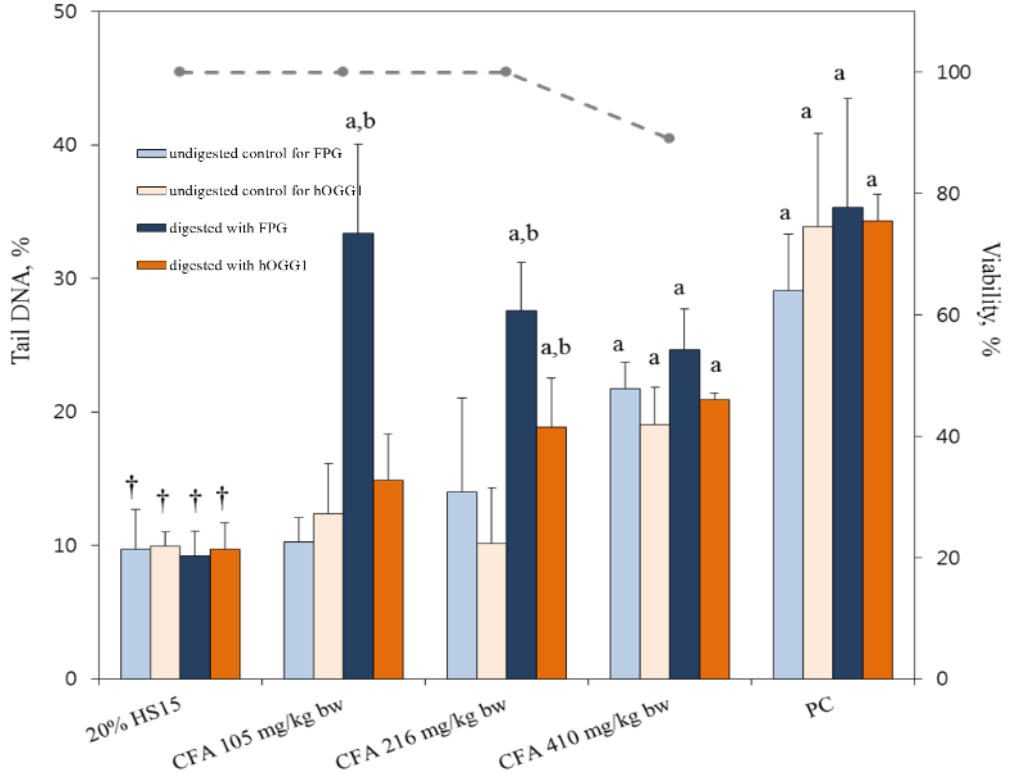
410 mg/kg bw

Comet Assay



*, denotes significant ($p < 0.05$) difference from control group; †, denotes significant ($p < 0.05$) trend

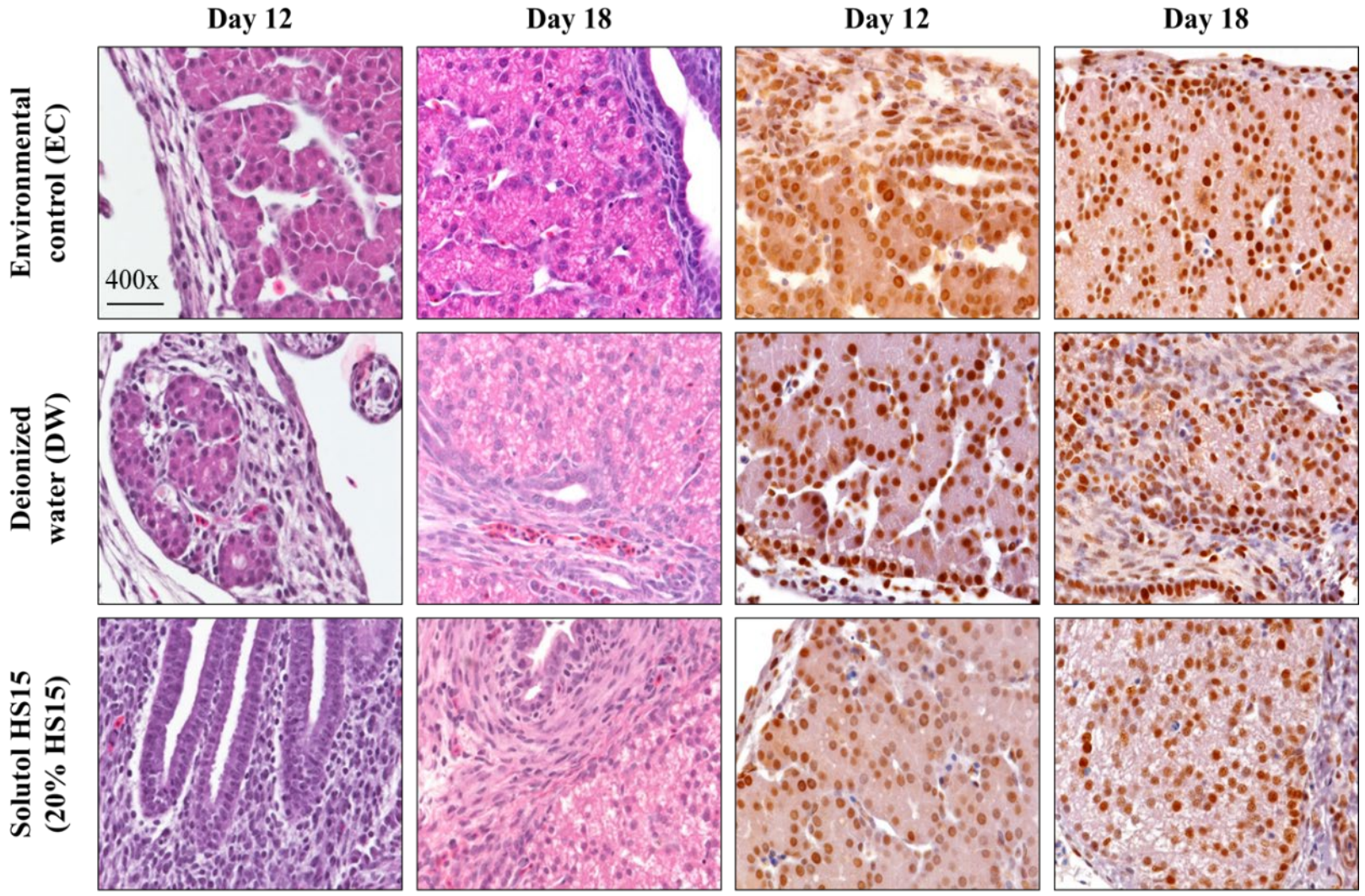
Enhanced Comet Assay



a, denotes significant ($p < 0.05$) difference from corresponding control group; b, denotes significant ($p < 0.05$) difference from corresponding undigested group; †, denotes significant ($p < 0.05$) trend; PC, positive control (H_2O_2)

Iatropoulos et al., Exper Tox Path. 2017

CEM Evaluation: Histopathology



Modified from Iatropoulos et al., Exper Tox Path. 2017

CEM Evaluation: Histopathology

Compound, dose	Termination	Distorted Hepatocellular Pattern	Hepatocellular Dysplasia	Cholangiocellular Dysplasia
Deionized water	day 12	–	–	–
	day 18	–	–	–
HS15 control	day 12	–	–	–
	day 18	–	–	–
2-Acetylaminofluorene,	day 12	+	+	–
75 mg/kg bw	day 18	+	+	+
2-Acetylaminofluorene,	day 12	+	+	–
135 mg/kg bw	day 18	++	++	+
Fluorene,	day 12	–	–	–
300 mg/kg bw	day 18	–	–	–
Aflatoxin B_1,	day 12	+	+	–
0.35 mg/kg bw	day 18	++	++	+
Aflatoxin B_2,	day 12	–	–	–
1.3 mg/kg bw	day 18	–	–	–
Benzo[a]pyrene,	day 12	+	+	+
100 mg/kg bw	day 18	++	++	+++
Benzo[e]pyrene,	day 12	–	–	–
120 mg/kg bw	day 18	–	–	–
Diethylnitrosamine,	day 12	++	++	++
180 mg/kg bw	day 18	+++	+++	+++
Diethylnitrosamine,	day 12	+++	+++	+++
360 mg/kg bw	day 18	++++	++++	++++
N-nitrosodiethanolamine,	day 12	–	–	–
1080 mg/kg bw	day 18	–	–	–
Clofibric acid,	day 12	–	–	–
410 mg/kg bw	day 18	–	–	–
Phenobarbital,	day 12	+	–	–
3500 mg/kg bw	day 18	+	–	++
D-mannitol,	day 12	–	–	–
11800 mg/kg bw	day 18	–	–	–

Severity scale: - absent; + mild; ++ moderate; +++ severe; ++++ extensive;

Iatropoulos et al., Exper Tox Path. 2017

CEM Evaluation: Genomics

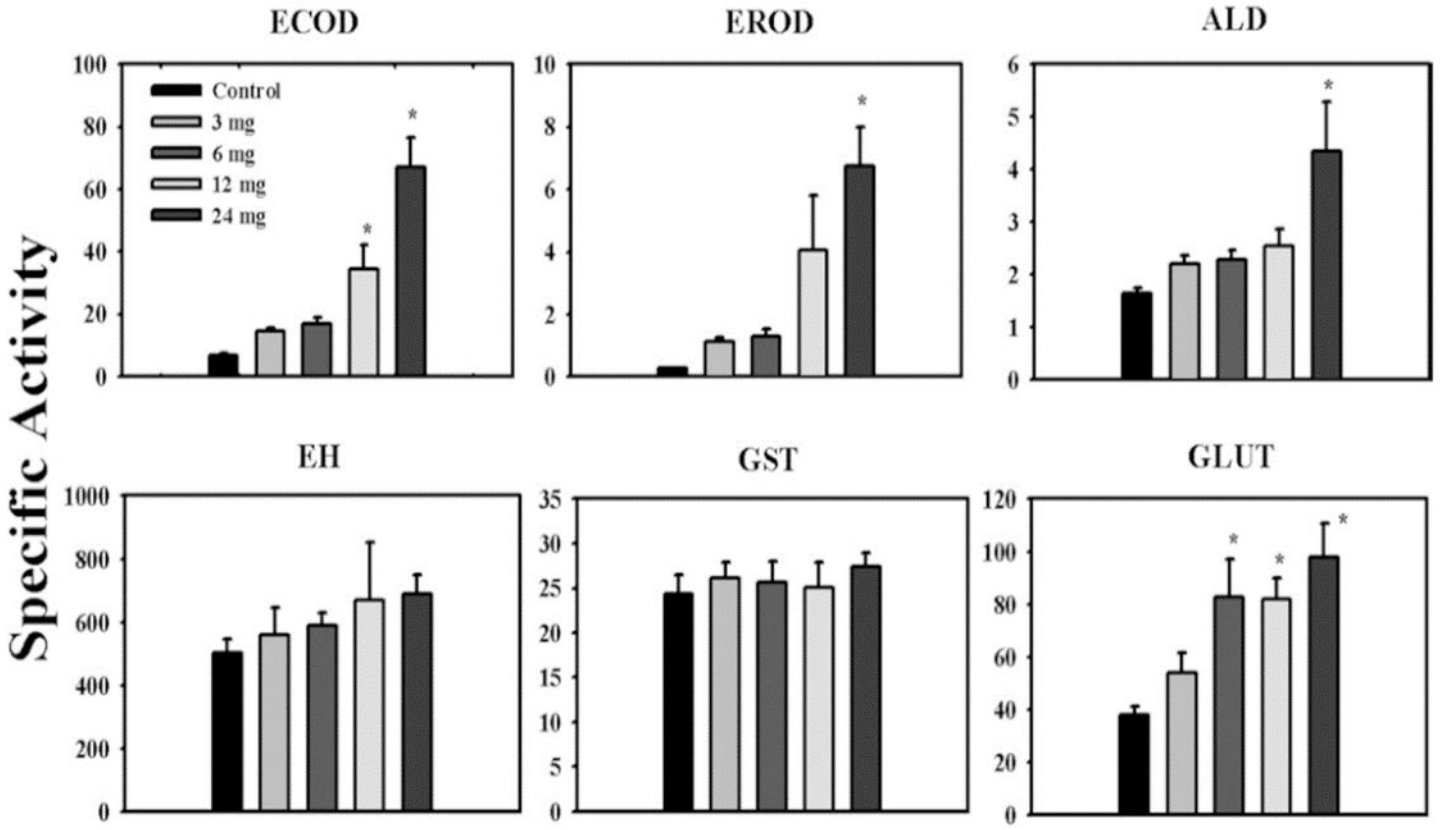
Deregulation of Biological Functions in Fetal Chicken Livers Dosed with Diethylnitrosamine

Pathways	Upregulated genes		Downregulated genes	
	# of genes	*p*-values*	# of genes	*p*-values*
METABOLISM				
Carbohydrate metabolism	10	1.47E-02	17	2.18E-02
Energy metabolism	2	3.59E-02	3	3.04E-02
Lipid metabolism	4	1.41E-02	21	2.34E-02
Nucleotide metabolism	3	7.06E-03	13	1.25E-02
Amino acid metabolism	7	2.41E-02	20	2.10E-02
Glycan biosynthesis and metabolism	4	1.99E-03	15	2.04E-02
Metabolism of cofactors and vitamins	2	1.90E-02	11	2.30E-02
Xenobiotics biodegradation and metabolism	4	2.21E-02	1	1.66E-03
GENETIC INFORMATION PROCESSING				
Transcription	3	1.48E-02	5	3.01E-02
Translation	2	2.65E-02	10	1.35E-02
Folding, sorting and degradation	8	1.81E-02	27	2.13E-02
Replication and repair	7	2.85E-02	2	2.08E-02
ENVIRONMENTAL INFORMATION PROCESSING				
Membrane transport	2	7.98E-03	1	9.55E-03
Signal transduction	16	1.98E-02	51	1.72E-02
Signaling molecules and interaction	1	1.72E-03	16	1.60E-02
CELLULAR PROCESSES				
Transport and catabolism	5	1.65E-02	24	1.71E-02
Cell motility			4	1.69E-02
Cell growth and cell death	10	1.96E-02	14	2.08E-02
Cellular community	3	1.83E-02	16	1.61E-02

*, *p*-values presented as average

METABOLIC CAPACITY

Activities of Phase I and Phase II Metabolic Enzymes in Fetal Turkey Liver



Control and phenobarbital-dosed groups

Liver enzyme	Turkey embryo*	Adult rat*
EROD	0.27	0.39
ECOD	6.70	2.90
ALD	1.64	20.4
EH	502	223
GST	24,400	83,400
GLUT	38	164

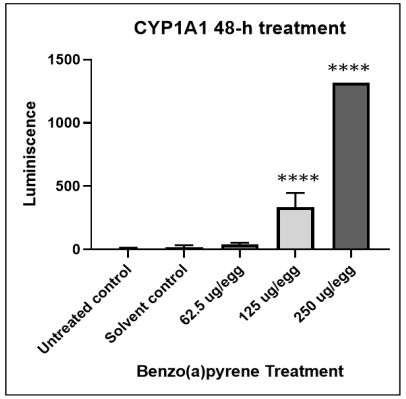
* Enzyme activity (nmol/gram*minute)

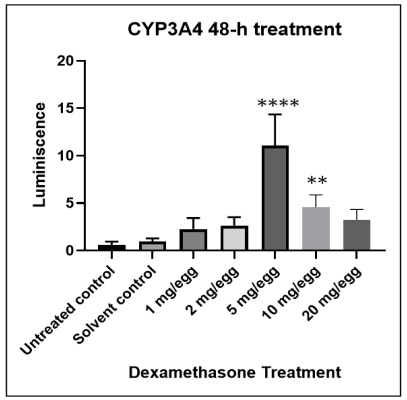
ECOD, 7-ethoxycoumarin de-ethylase; EROD, 7-ethoxyresorufin de-ethylase; ALD, aldrin epoxidase; EH, epoxide hydrolase; GST, glutathione S-transferase; GLUT, UDP-glucuronyltransferase

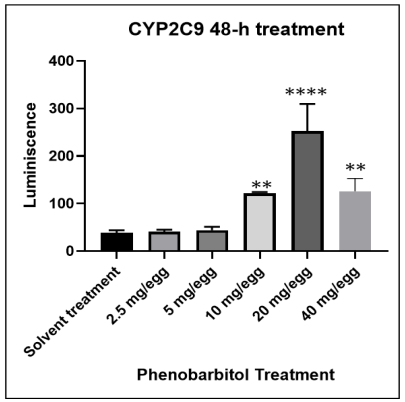
Perrone et al., Arch. Toxicol. 2004. 78

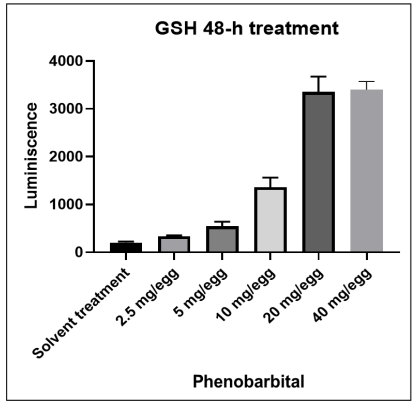
METABOLIC CAPACITY

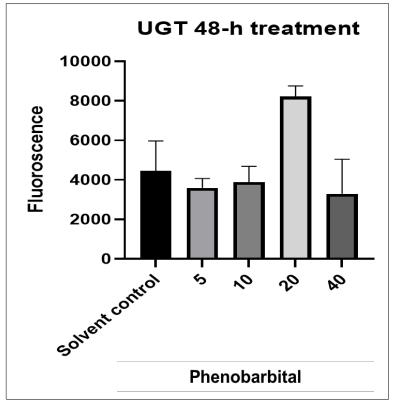
Activities of Phase I and Phase II Metabolic Enzymes in Fetal Chicken Liver











Sex	AND	AHH (nmoles/g liver/hr)	ECOD (nmoles/g liver/hr)	CYP-450 (nmoles/mg)
Male	571 ± 67	586 ± 77	3411 ± 305	0.26
	223-1107	227-1262	1896-5659	
Female	760 ± 150	461 ± 52	3333 ± 113	0.28
	237-1990	205-811	2656-3876	

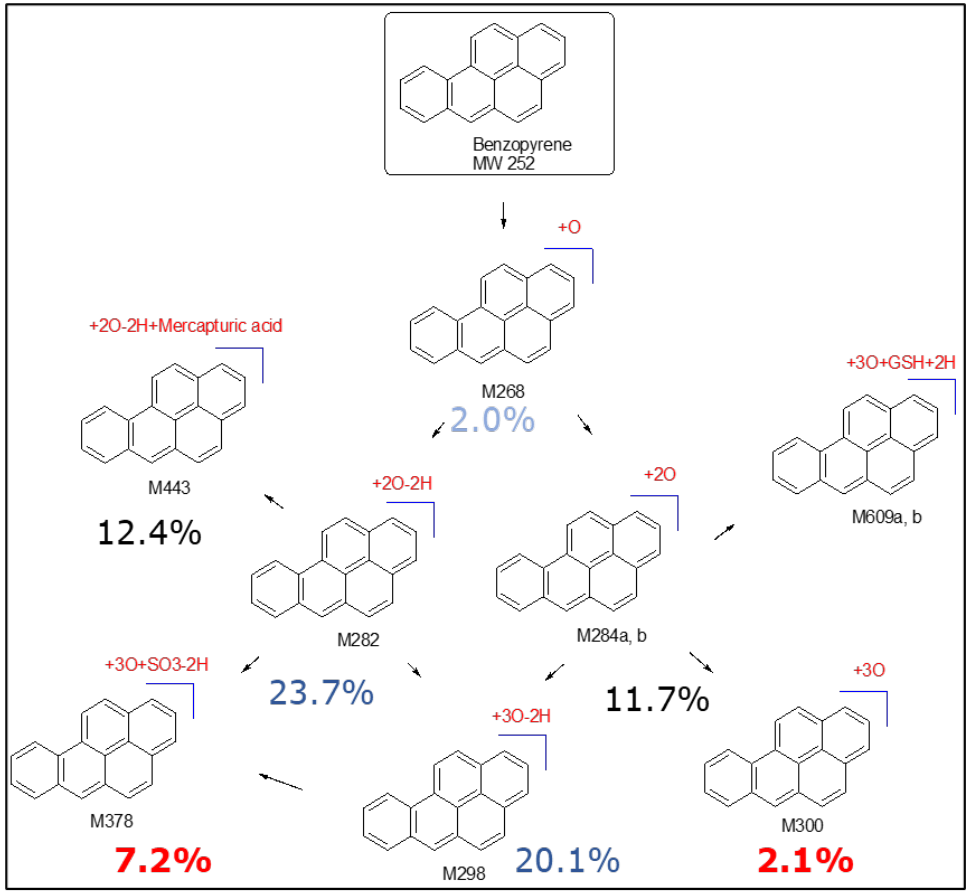
AHH, aryl hydrocarbon hydroxylase; AND, aminopyrine N-demethylase; ECOD, 7-ethoxycoumarin de-ethylase

Unpublished data

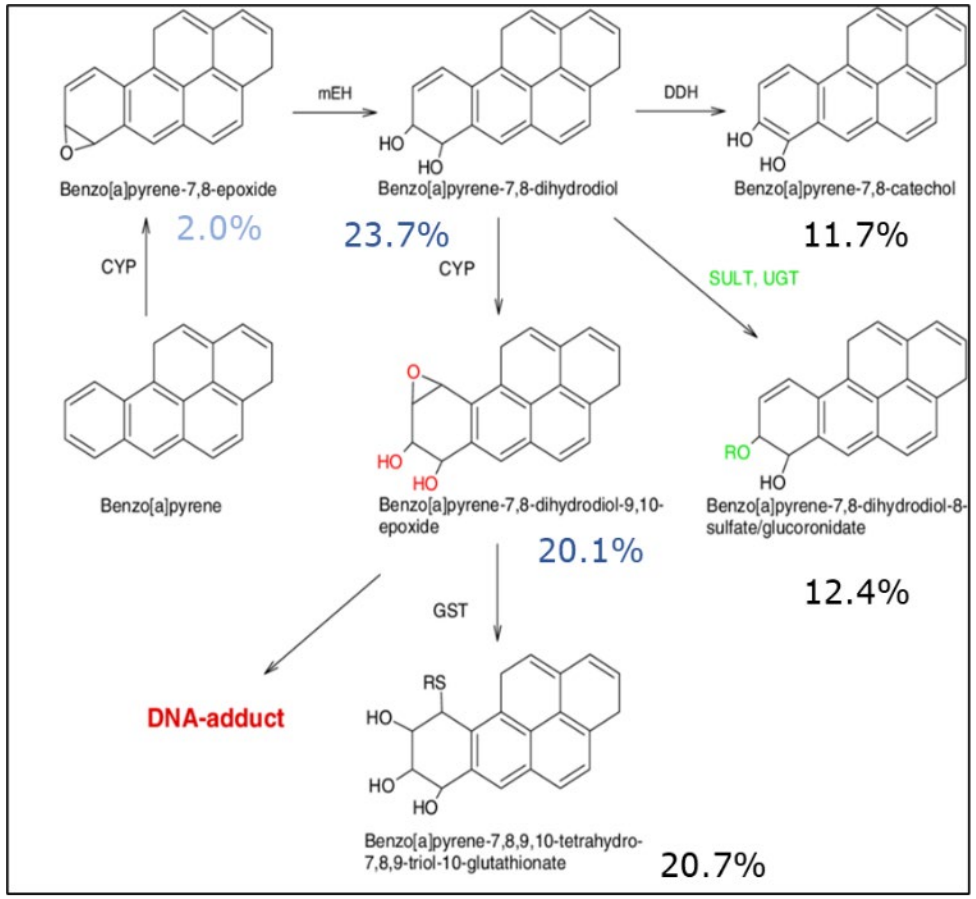
Rifkind et al., Biochem. Pharmacol. 1979. 28

Metabolic Capacity

B[a]P Metabolite Profiling in Fetal Chicken Livers

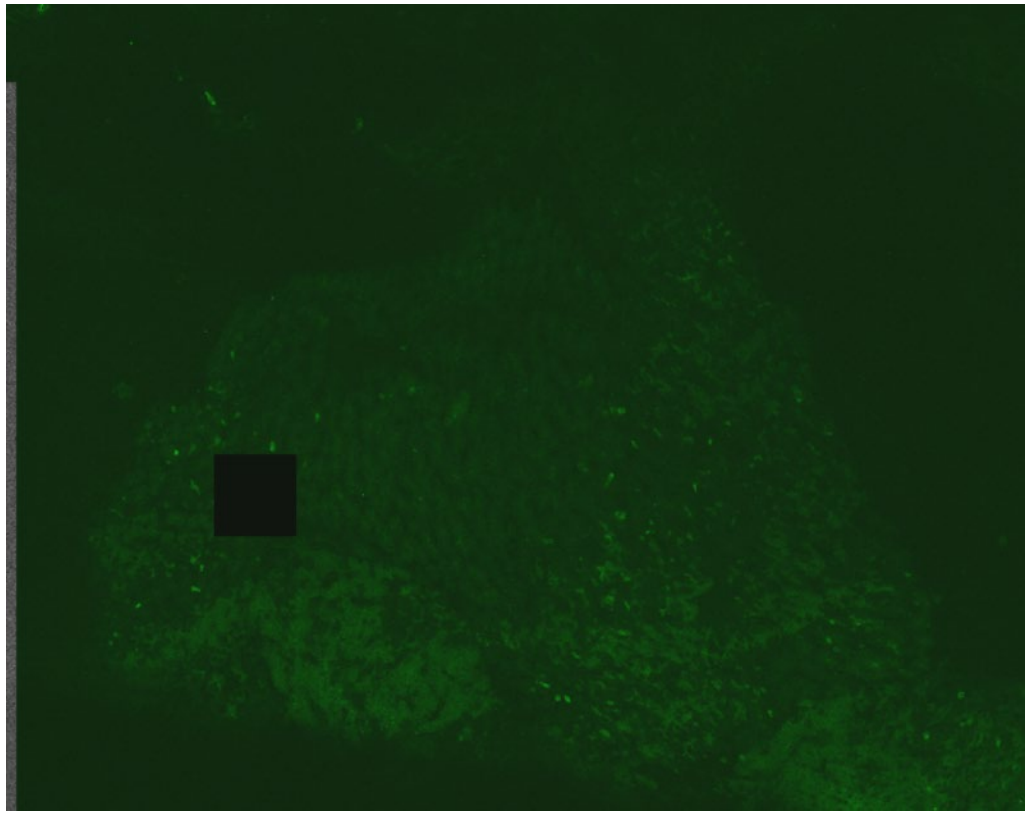


B[a]P Established Metabolic Pathways

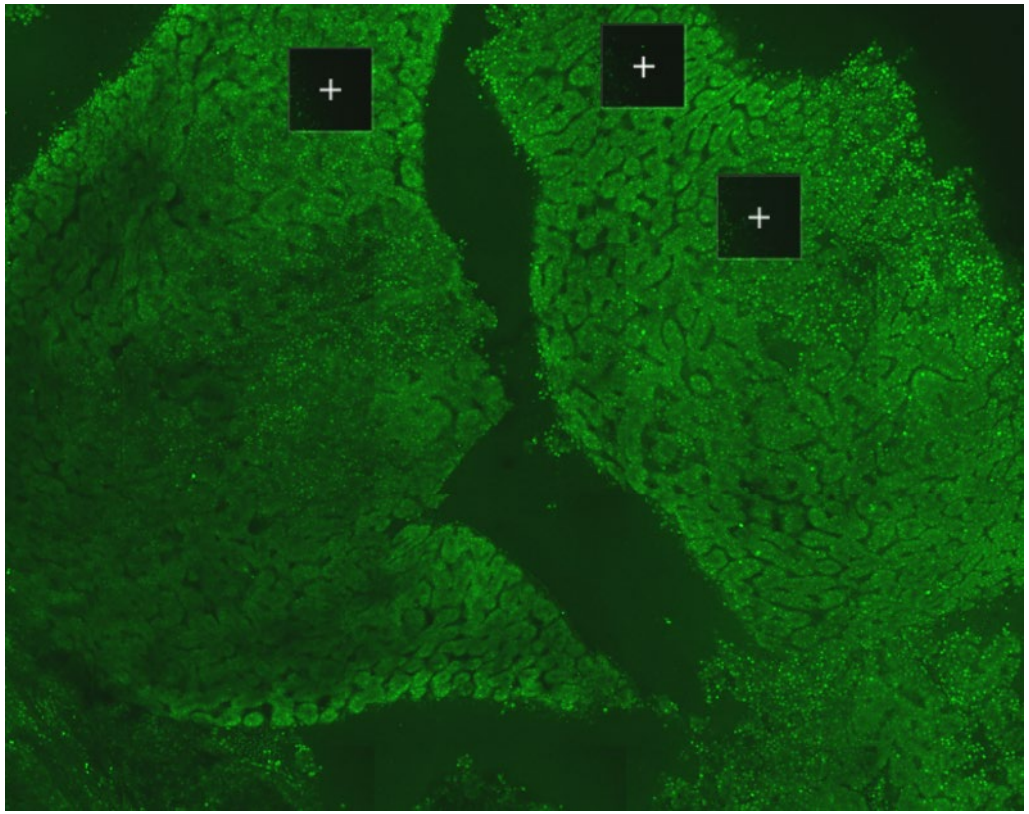


TARGET TISSUE EXPOSURE

Water control



Acridine orange



> Toxicol Sci. 2016 Apr;150(2):301-11. doi: 10.1093/toxsci/kfv322. Epub 2015 Dec 29.

Structure-Activity Relationships for DNA Damage by Alkenylbenzenes in Turkey Egg Fetal Liver

Tetyana Kobets [1], Jian-Dong Duan [2], Klaus D Brunnemann [2], Sylvain Etter [3], Benjamin Smith [4], Gary M Williams [2]

> Int J Toxicol. 2022 Aug;41(4):297-311. doi: 10.1177/10915818221093583. Epub 2022 Jun 4.

Evaluation of Pharmaceuticals for DNA Damage in the Chicken Egg Genotoxicity Assay (CEGA)

Tetyana Kobets [1], Jian-Dong Duan [1], Esther Vock [2], Ulrich Deschl [2], Gary M Williams [1]

Comparative Study > Food Chem Toxicol. 2018 May:115:228-243. doi: 10.1016/j.fct.2018.03.015. Epub 2018 Mar 13.

In ovo testing of flavor and fragrance materials in Turkey Egg Genotoxicity Assay (TEGA), comparison of results to in vitro and in vivo data

Tetyana Kobets [1], Jian-Dong Duan [2], Klaus D Brunnemann [3], Michael J Iatropoulos [4], Sylvain Etter [5], Christina Hickey [6], Benjamin Smith [7], Gary M Williams [8]

> Food Chem Toxicol. 2019 Jul:129:424-433. doi: 10.1016/j.fct.2019.05.010. Epub 2019 May 8.

Assessment and characterization of DNA adducts produced by alkenylbenzenes in fetal turkey and chicken livers

Tetyana Kobets [1], Alexander T Cartus [2], Julia A Fuhlbrueck [2], Alexander Brengel [2], Simone Stegmüller [2], Jian-Dong Duan [3], Klaus D Brunnemann [3], Gary M Williams [3]

Comparative Study > Mutat Res Genet Toxicol Environ Mutagen. 2019 Aug:844:10-24. doi: 10.1016/j.mrgentox.2019.06.004. Epub 2019 Jun 14.

DNA-damaging activities of twenty-four structurally diverse unsubstituted and substituted cyclic compounds in embryo-fetal chicken livers

Tetyana Kobets [1], Jian-Dong Duan [2], Klaus D Brunnemann [3], Esther Vock [4], Ulrich Deschl [5], Gary M Williams [6]

> Toxicology. 2024 Jan:501:153714. doi: 10.1016/j.tox.2023.153714. Epub 2023 Dec 22.

Assessment of no-observed-effect-levels for DNA adducts formation by genotoxic carcinogens in fetal turkey livers

Tetyana Kobets [1], Christina Hickey [2], George Johnson [3], Jian-Dong Duan [4], Sylvain Etter [5], Benjamin Smith [6], Gary M Williams [4]

In Ovo vs In Vitro & In Vivo

In ovo	In vitro GTX: POS	In vitro GTX: NEG	In vitro GTX: TOTAL
POS	39	8	47
NEG	27	9	36
TOTAL	66	53	**83**
Sensitivity: **59%**		Specificity: **53%**	
PPV: **83%**		NPV: **25%**	
Accuracy: **58%**		FDR: **17%**	

In ovo	In vivo GTX: POS	In vivo GTX: NEG	In vivo GTX: TOTAL
POS	37	6	43
NEG	10	22	32
TOTAL	47	28	**75**
Sensitivity: **79%**		Specificity: **79%**	
PPV: **86%**		NPV: **69%**	
Accuracy: **79%**		FDR: **14%**	

In ovo	Carcinogenicity: POS	Carcinogenicity: NEG	Carcinogenicity: TOTAL
POS	38	1	39
NEG	17	9	26
TOTAL	55	10	**65**
Sensitivity: **69%**		Specificity: **90%**	
PPV: **97%**		NPV: **35%**	
Accuracy: **72%**		FDR: **3%**	

FDR; false discovery rate; GTX, genotoxicity assays; NEG, negative outcome; NPV, negative predictive value; POS, positive outcome; PPV, positive predictive value; for the purposes of calculations, equivocal outcomes were considered to be positive

BIOLOGICAL RELEVANCE

A. Mechanistic understanding: How does the information provided by your method support known mechanistic knowledge of the carcinogenesis process

   - **elucidation of mechanism of action, carcinogenicity AOP**

B. Reference compounds: What are well-characterized and understood compounds that can be used or were used to assess the scientific validity or transferability of your method?

   - over 80 compounds (aromatic amines, pharmaceuticals, phytochemicals, flavor and fragrance materials) have been evaluated in the model

C. Comparison to existing laboratory animal methods: How does your method provide information that is equivalent or better than that from existing methods used for regulatory purposes?

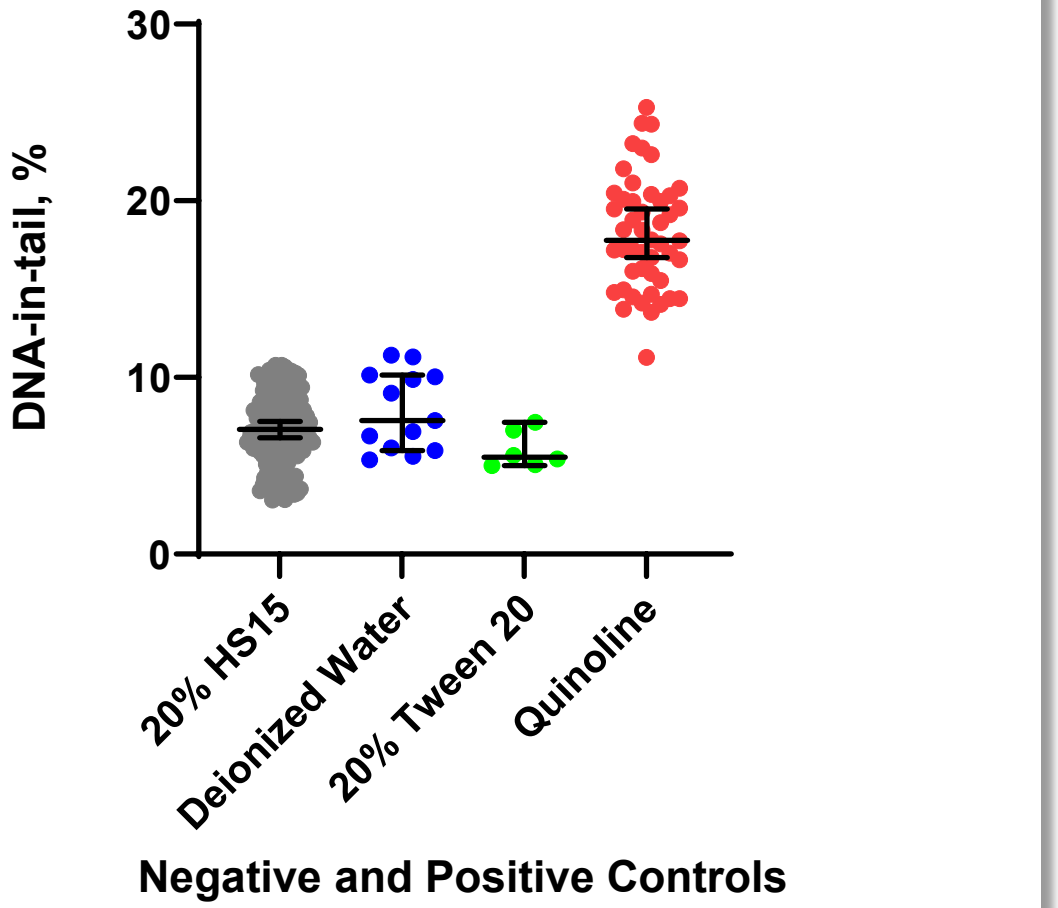
   - the model has higher accuracy, sensitivity, and specificity for the outcomes of in vivo genotoxicity and carcinogenicity testing compared to in vitro tests

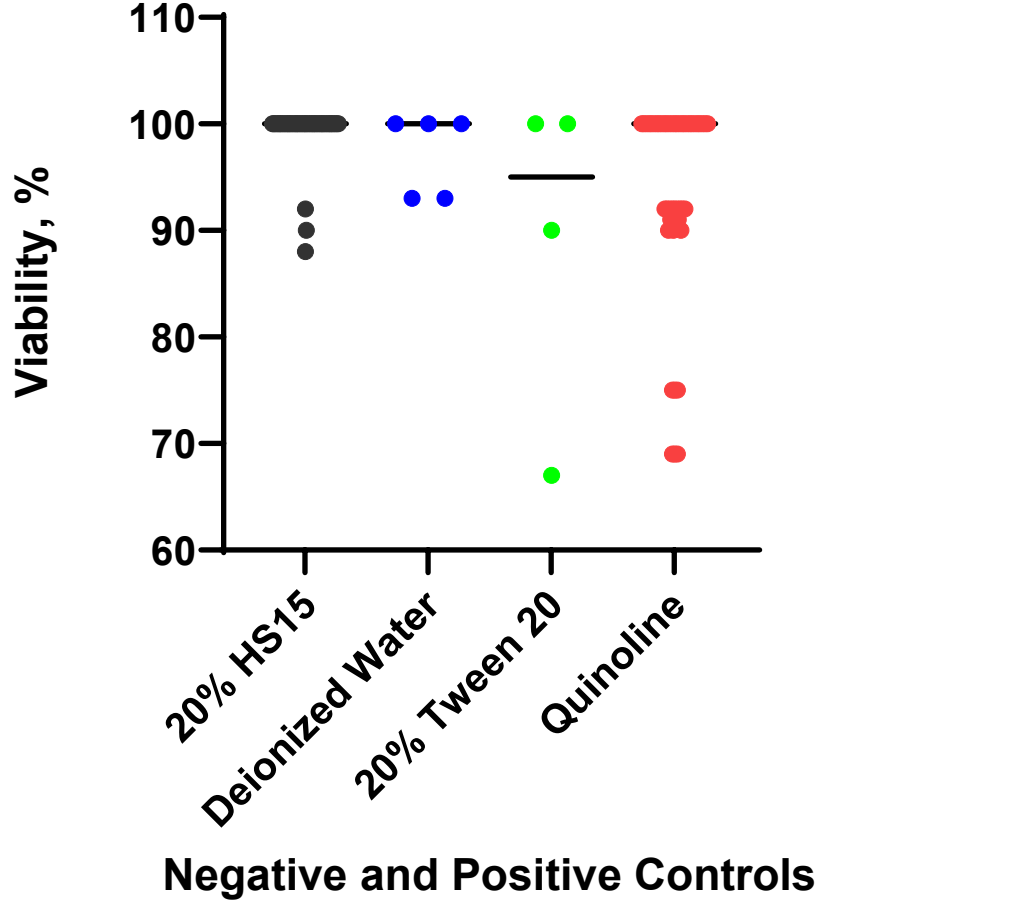
D. How does your method contribute to the reduction, refinement, or replacement of animal assays, and what complementary method development might be needed to comprehensively address carcinogenesis?

   - **potentially replace in vivo genotoxicity assays used to investigate the genotoxic or carcinogenic potential of chemicals which tested positive in genotoxicity assays in vitro**

4. Technical Characterization

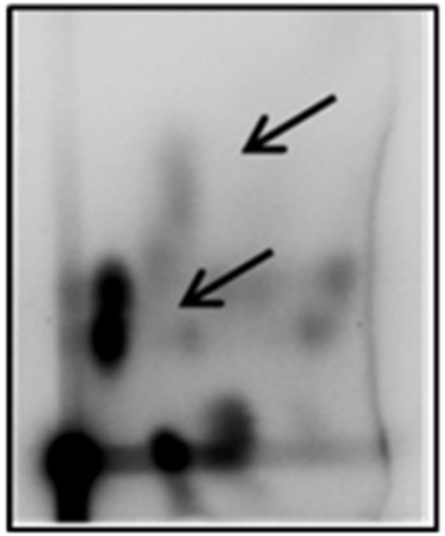
CONTROLS


DNA-in-tail, %
30
20
10
0
20% HS15
Deionized Water
20% Tween 20
Quinoline
Negative and Positive Controls

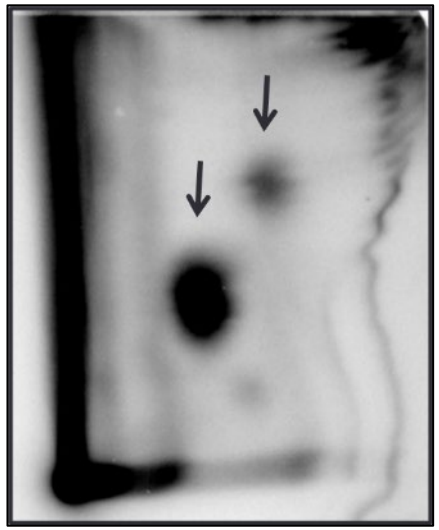

Viability, %
110
100
90
80
70
60
20% HS15
Deionized Water
20% Tween 20
Quinoline
Negative and Positive Controls

In Ovo vs In Vivo

DNA adducts formed in avian fetal livers *in ovo*



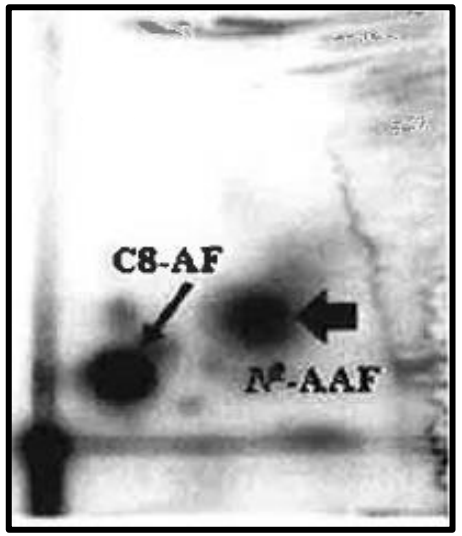
2- AAF, 0.6 mg/egg
~170 mg/kg bw



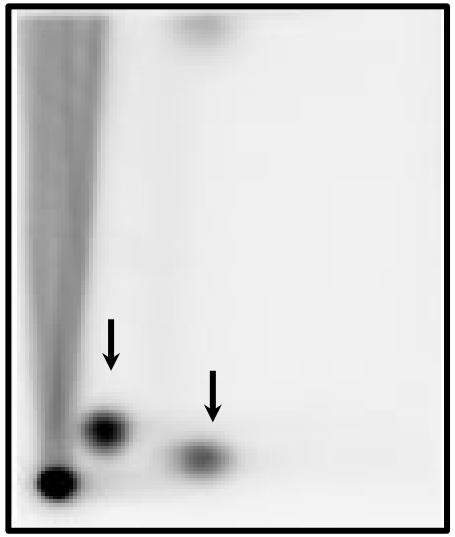
MEU, 4 mg/egg
1140 mg/kg bw

Williams et al., (2014) Toxicol. Sci. 141
Kobets et al., (2016). Toxicol Sci. 150: 301-311

DNA adducts formed in the livers of F344 male rats in vivo



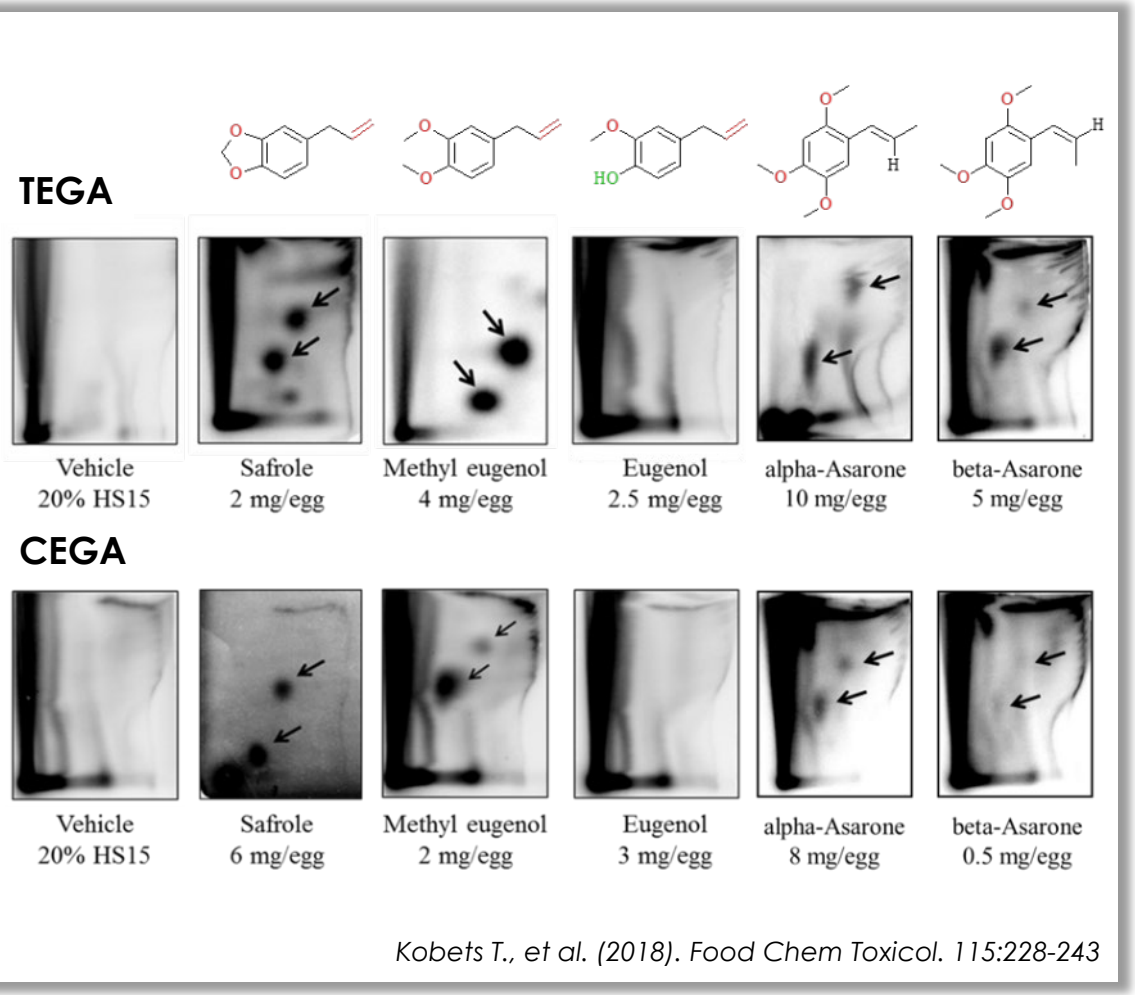
2-AAF, 2.24 mg/kg bw
4 weeks



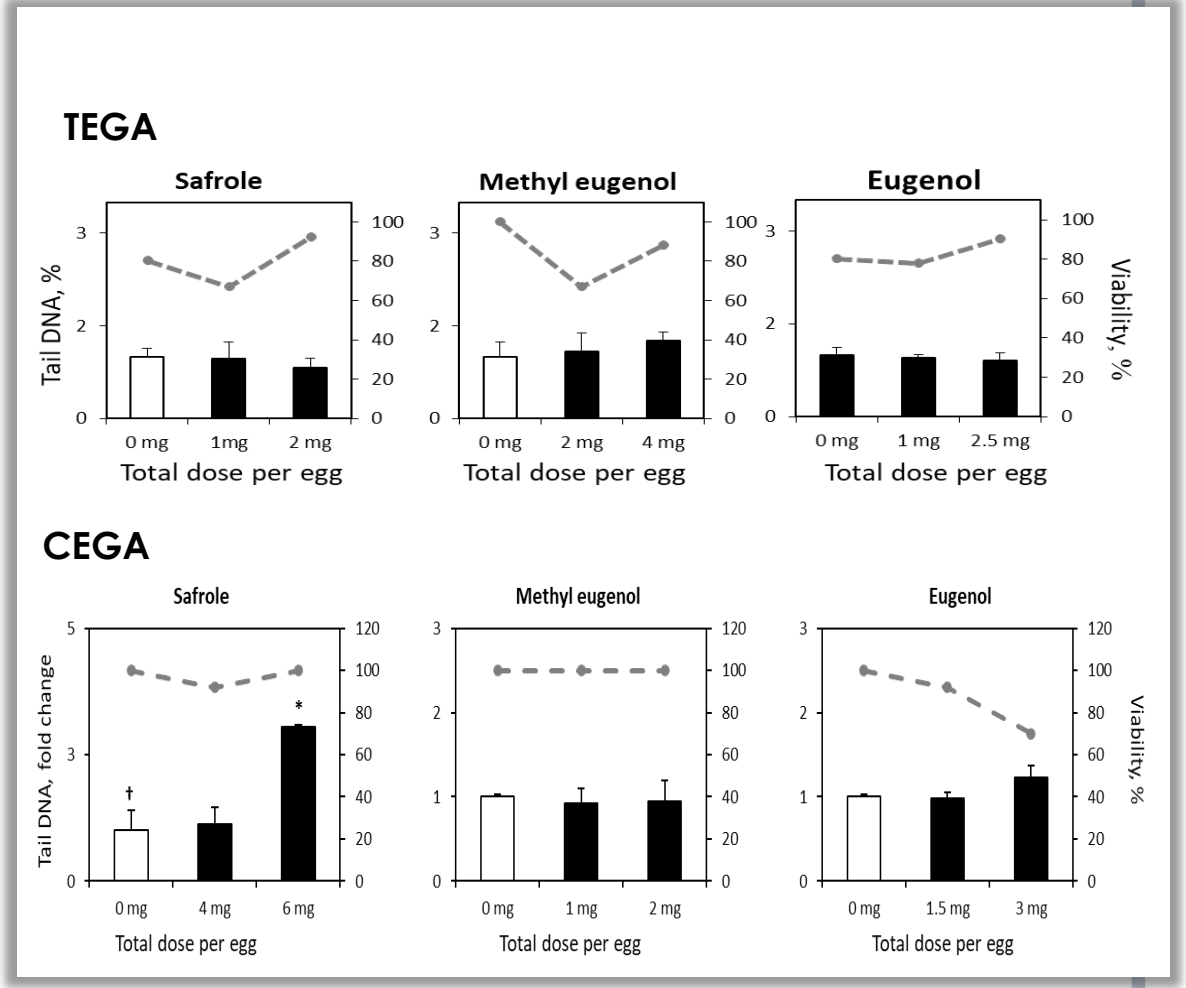
MEU, 3000 mg/kg bw
8 weeks

Williams G.M., et al. (2013). Food Chem Toxicol. 53
Williams G.M., et al. (2015). Tox Res 4: 233

ALKENYLBENZENES DNA ADDUCTS *IN OVO*

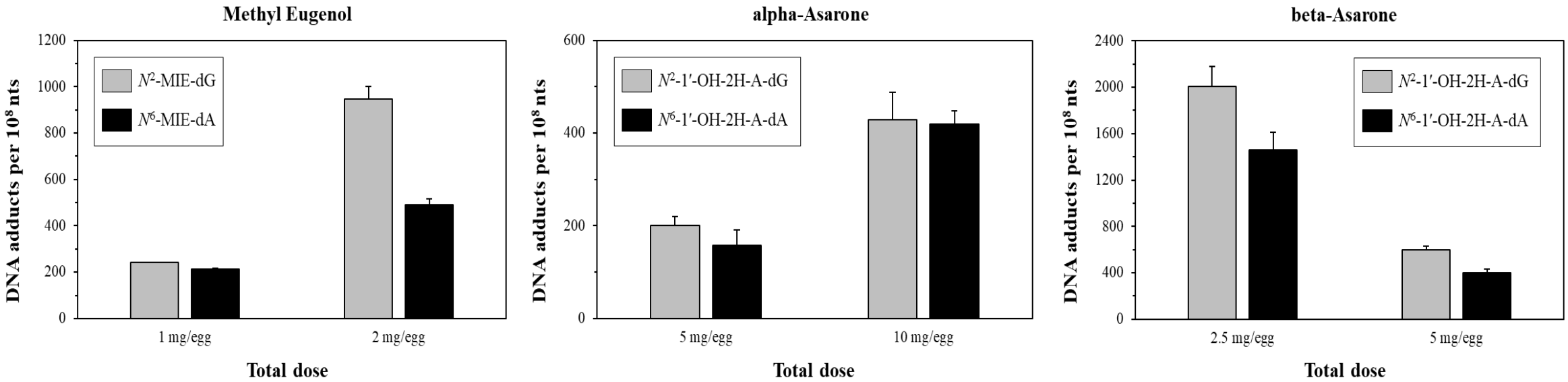


Kobets T., et al. (2018). Food Chem Toxicol. 115:228-243



ALKENYLBENZENES DNA ADDUCTS *IN OVO*

Ultra high-performance liquid chromatography electrospray ionization tandem mass spectrometry



Kobets T., et al. (2018). Food Chem Toxicol. 115:228-243

TECHNICAL CHARACTERIZATION

A. How have the sources of variability (e.g., interference, culture conditions, technique, contaminants) been evaluated?

   - the protocol allows to avoid environmental variability

B. How has robustness (i.e., the ability of the method to be reproduced under different conditions or circumstances, without the occurrence of unexpected differences in the obtained results) been evaluated?

   - several compounds were evaluated at different timepoints of termination or under similar conditions in a turkey egg model with a similar outcomes

C. How has intra-laboratory reproducibility (i.e., the consistency of individual test results obtained within a laboratory using the same test protocol and test samples) been evaluated?

   - yes, the results in the model are reproducible

D. How has transferability (i.e., the ability of the method to be accurately and reliably performed in different, competent laboratories) been evaluated (if relevant)?

   - IN DEVELOPMENT, open to collaborations

CONCLUSIONS

- CEM is a reliable alternative model for the evaluation of chemical-induced genotoxic and related events
- The model exhibits high sensitivity and specificity for genotoxic and non-genotoxic compounds
- Findings in the model are congruent with findings in other species
- The assay allows demonstration of the biological consequences of chemical genotoxicity and elucidation of chemical mode of action
- The use of mechanistic dose-effect studies for genotoxic endpoints can provide critical information for prioritization of concerns for risk assessment
- Avian models offer a potentially more acceptable alternative to current animal models for follow-up of in vitro positives in genotoxic assays

ACKNOWLEDGEMENTS

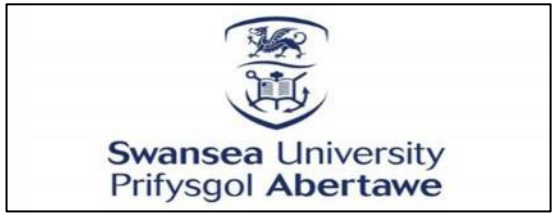
Gary M. Williams

Jian-Dong Duan

Klaus D. Brunnemann

Michael Iatropoulos

Yax Thakkar



George Johnson

Benjamin Smith

Sylvain Etter

Christina Hickey

Ulrich Deschl

Esther Vock

Alexander T. Cartus

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New York Medical College

BSB, 425

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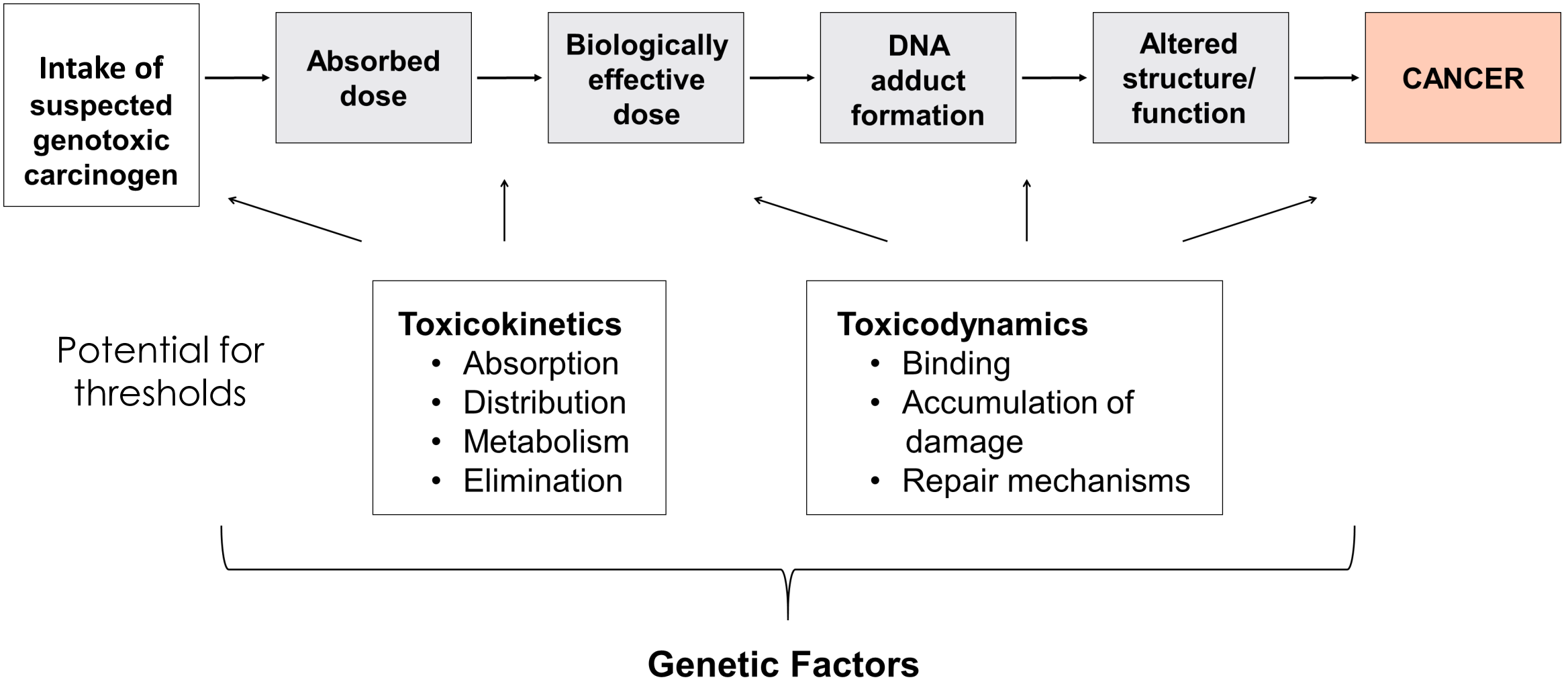
Tetyana_Kobets@nymc.edu

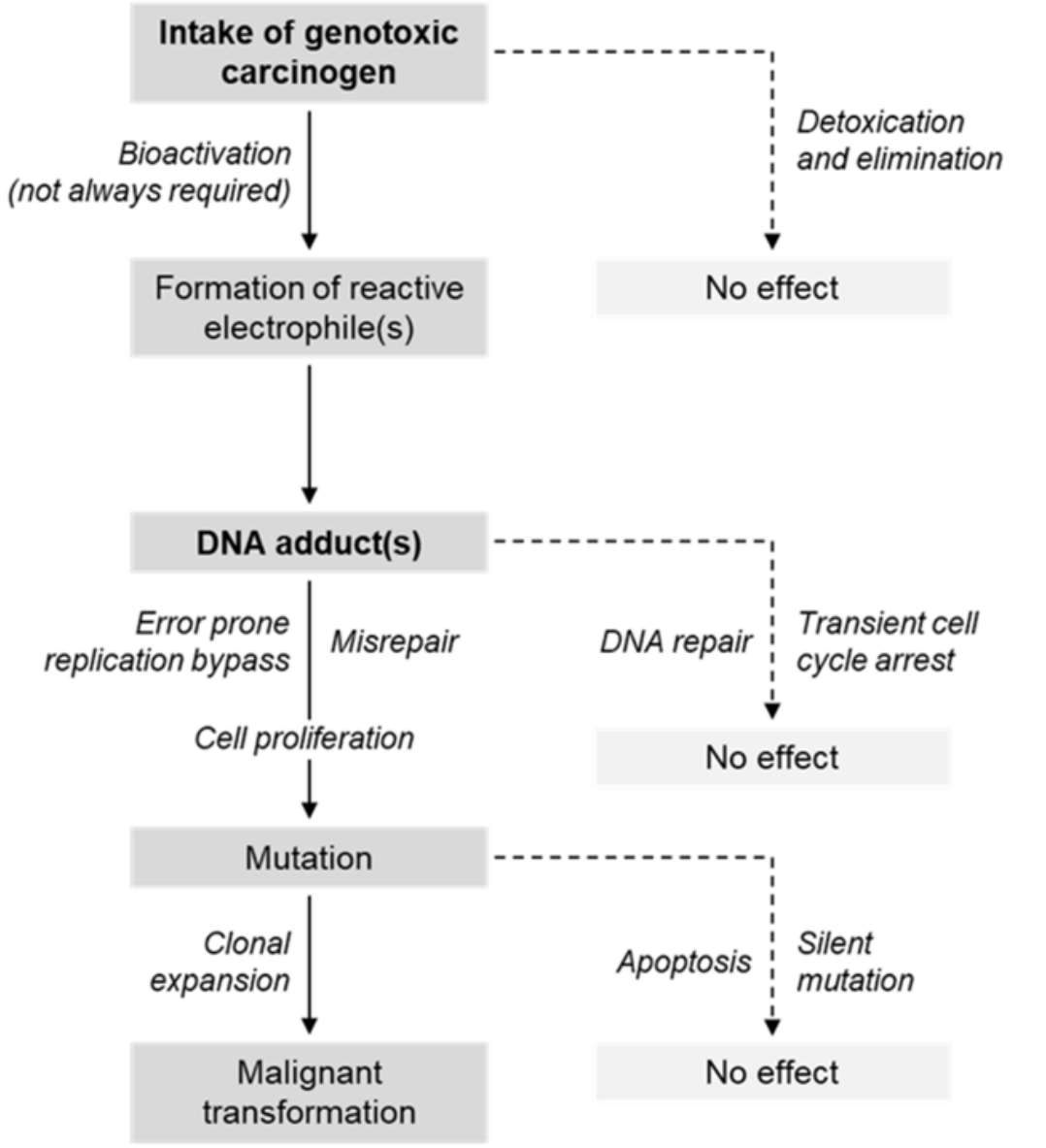
THANK YOU!

5. Additional Slides

In Ovo Mechanistic Dose-effect Studies



DNA Adducts as Biomarkers



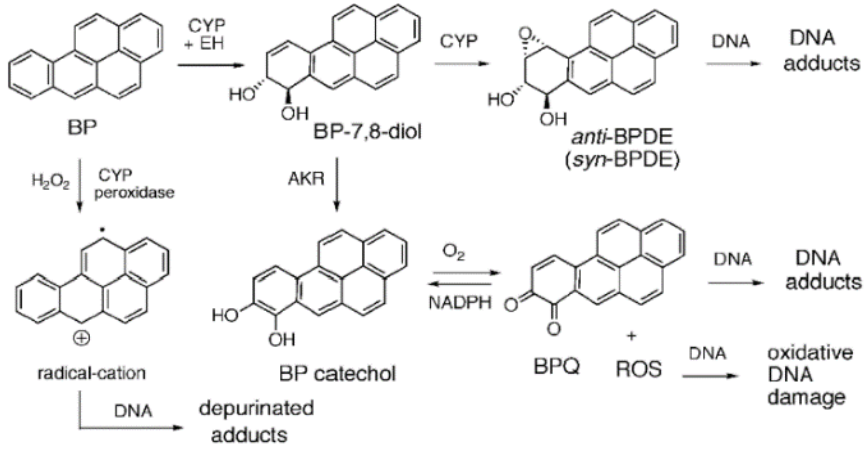
Williams et al. (2023). Hayes' Principle and Methods of Toxicology, 7ed. CRC Press

NOELs for DNA Adducts

- Thresholds exist for key steps in the multistep process of chemical carcinogenesis
- Adduct formation is a key event along the Adverse Outcome Pathway to cancer induced by DNA-reactive chemicals and can be treated as indicator assay or key initiating event assay
- Adduct NOELs are therefore expected to be at lower doses than cancer NOELs
- Safe levels of exposure can be delineated using the lowest threshold and safety factors
- Adducts in vivo are not considered to be suitable proxy for cancer bioassay for risk assessment yet, more a biomarker of exposure, however, they are chemical specific
- The conventional chronic bioassay can be replaced with alternatives

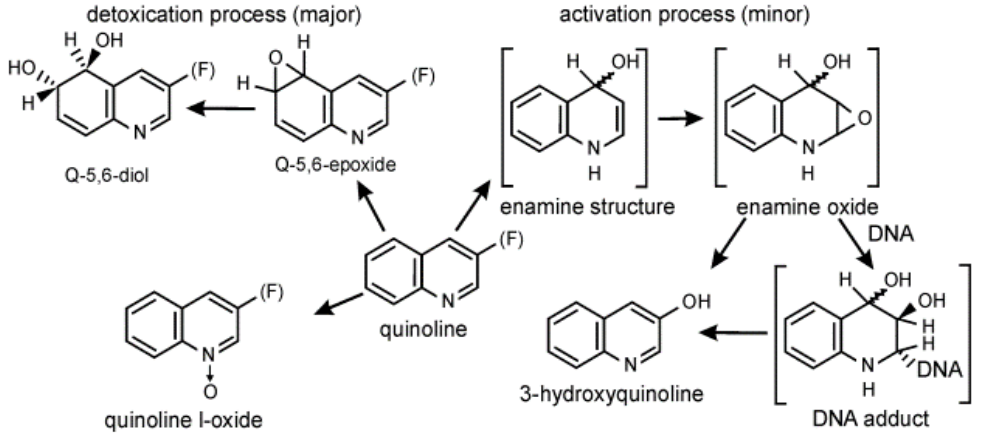
NOELs for Genotoxic Carcinogens *in Ovo*

Benzo[a]pyrene



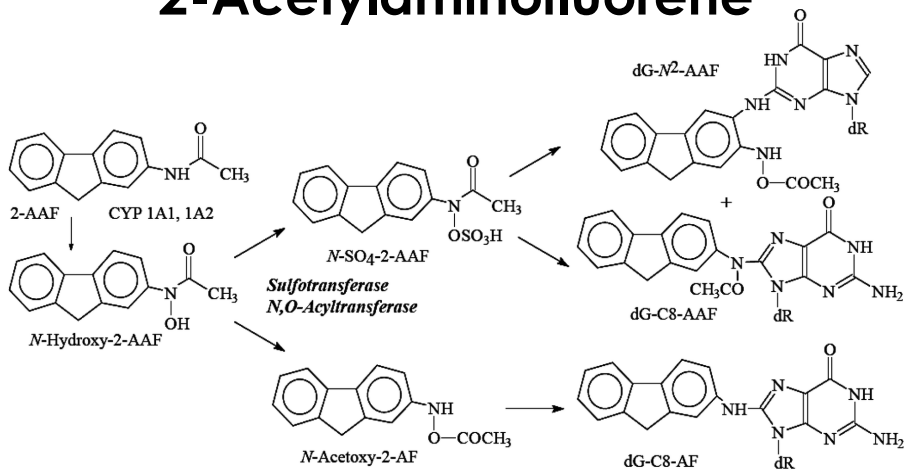
Hartwig A., et al. (2020). Arch Toxicol. 94(6):1787-1877

Qinoline



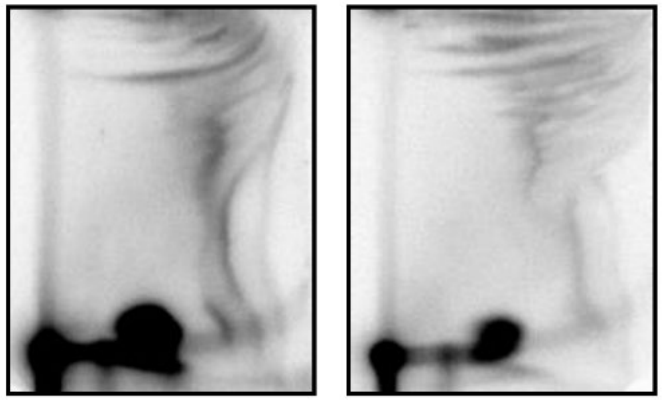
https://iris.epa.gov/static/pdfs/1004tr.pdf

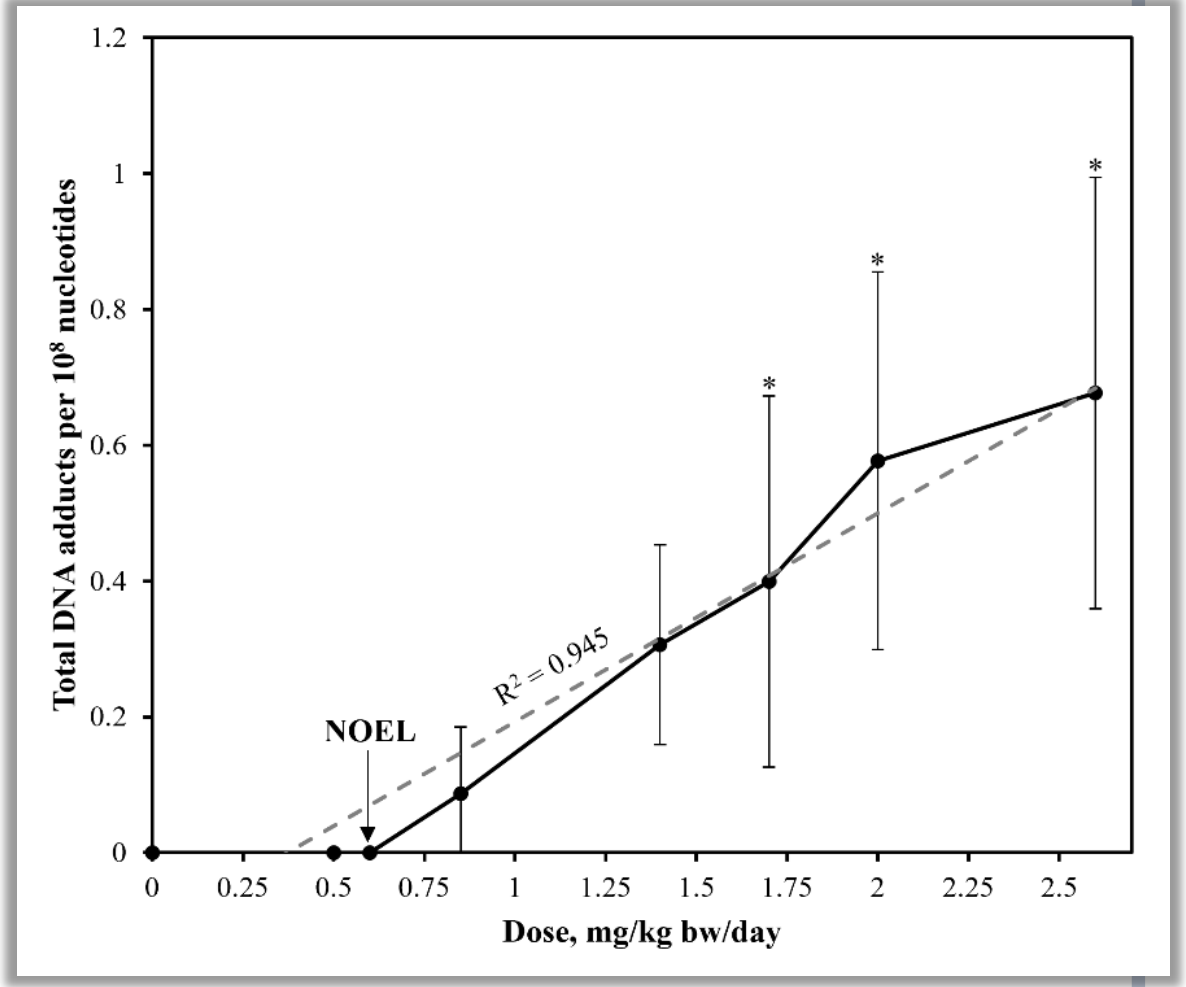
2-Acetylaminofluorene



https://pubs.rsc.org/

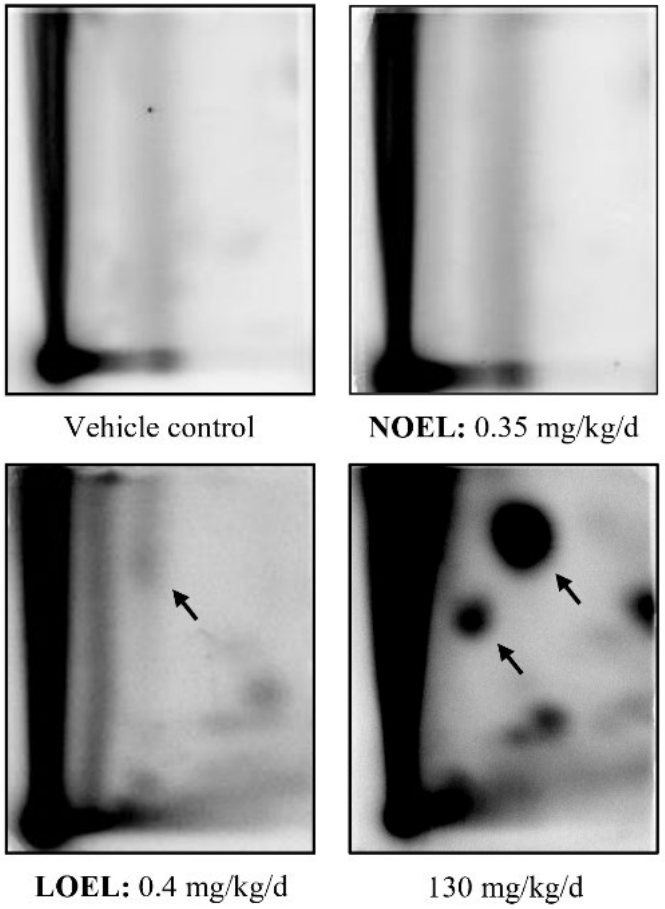
BENZO[A]PYRENE

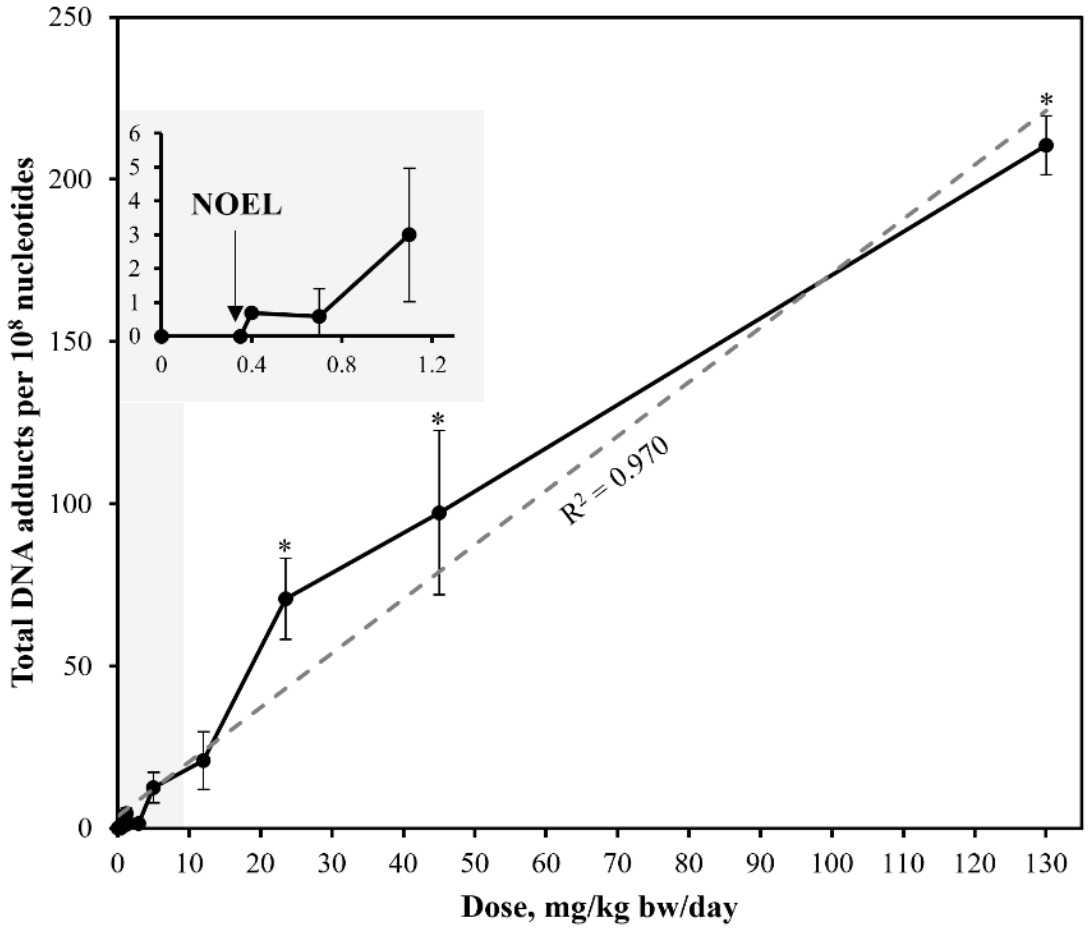




Kobets et al., (2024). Toxicology. 153711

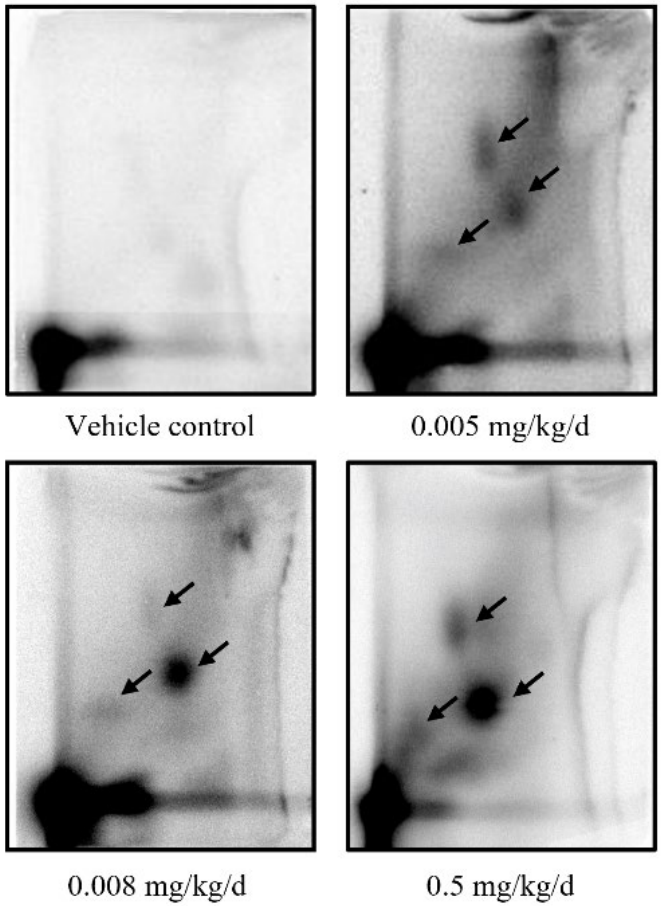
QUINOLINE

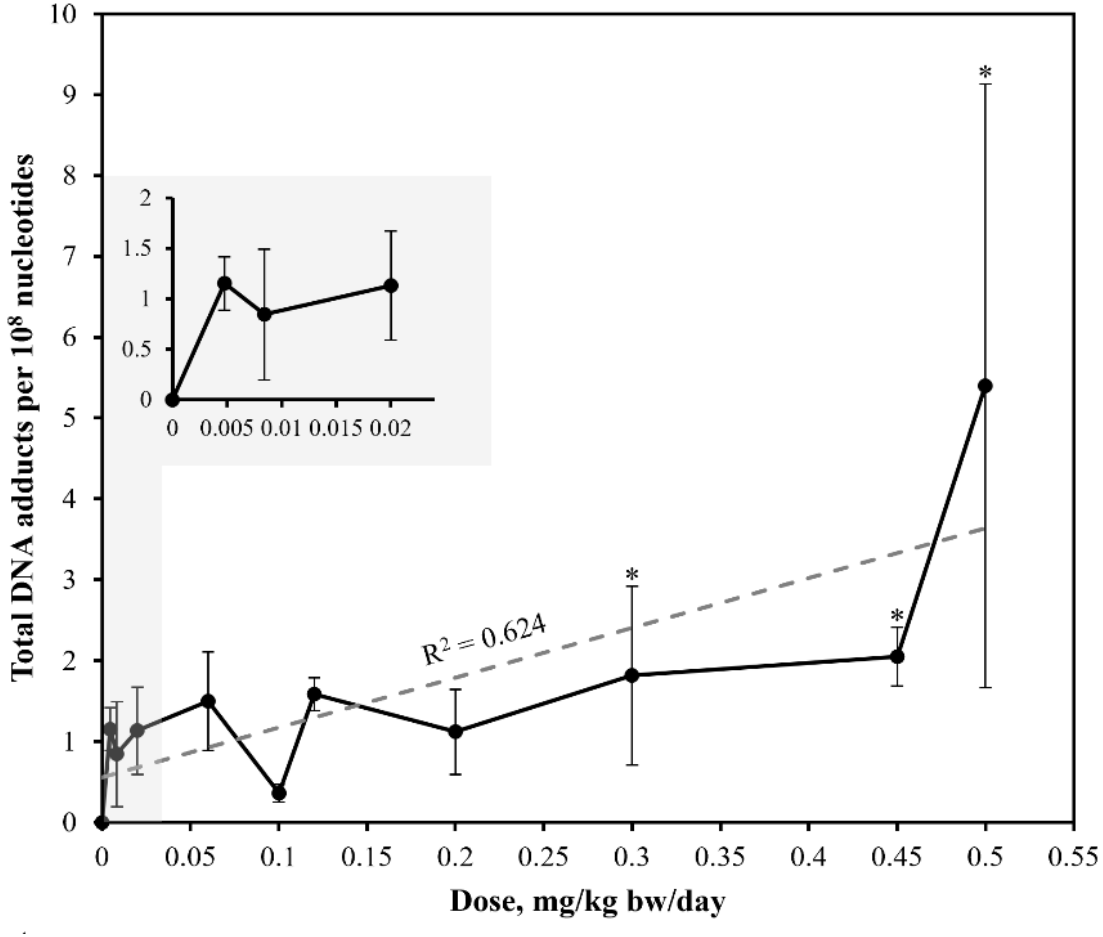




Kobets et al., (2024). Toxicology. 153711

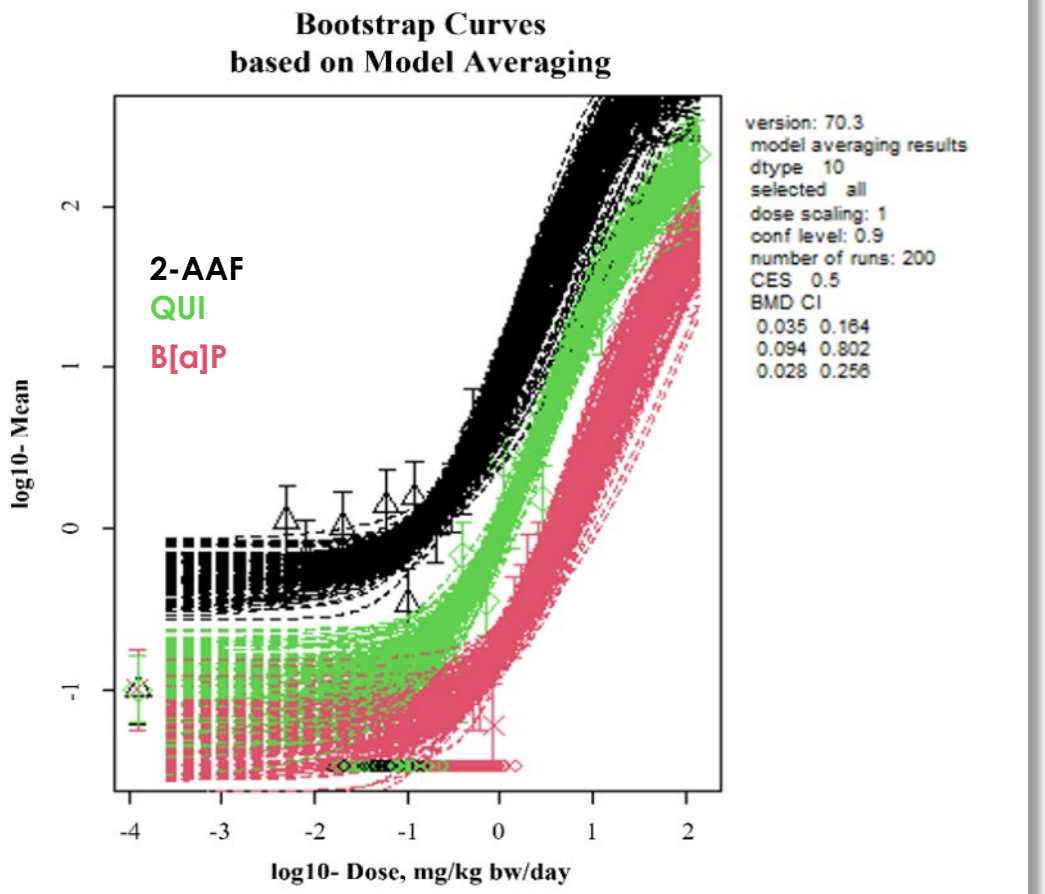
2-ACETYLAMINOFLUORENE

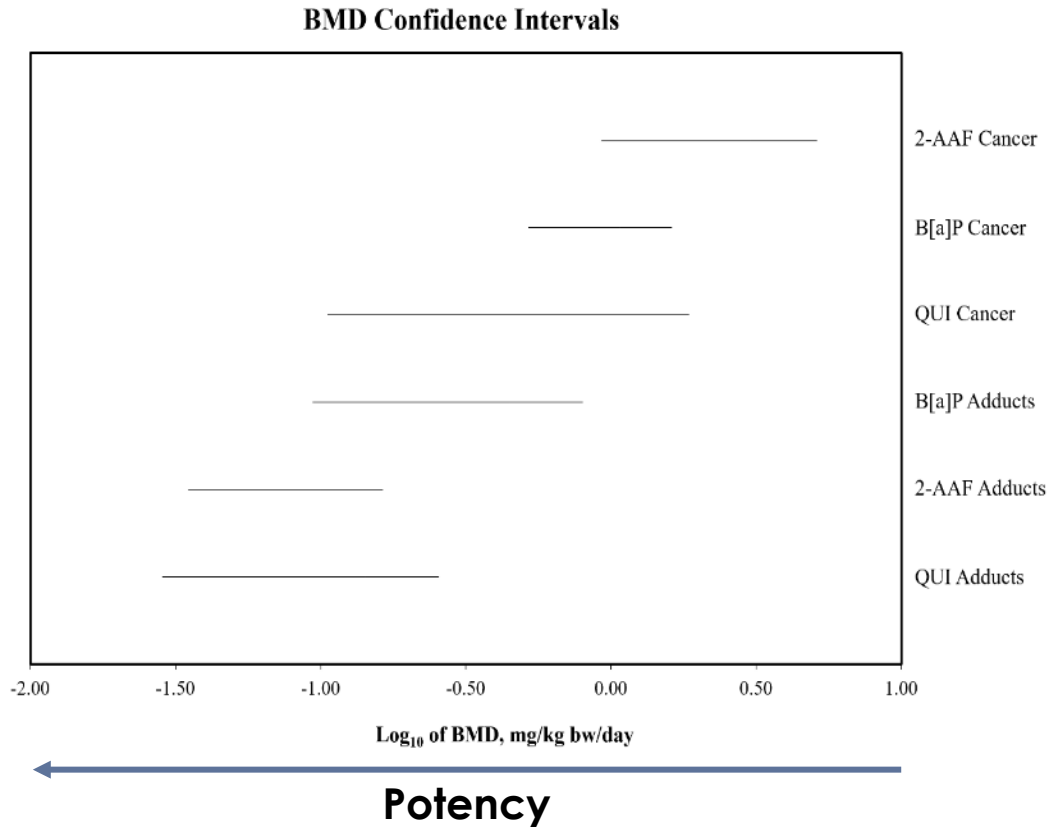




Kobets et al., (2024). Toxicology. 153711

BMD and Potency Ranking





Kobets et al., (2024). Toxicology. 153711

Summary of Dose-Response Findings

Compound	DNA adducts NOEL, mg/kg bw/d	Adducts BMD_{50}, mg/kg bw/d	Carc.$BMDL_{10}$, mg/kg bw	EDI, mg/day
Benzo[a]pyrene	0.65	0.09 – 0.8	0.5 – 1.6 (♀ mice)	4e-6
Quinoline	0.35	0.02 – 0.2	0.1 – 1.9 (rats)	0.02
2-Acetylaminofluorene	N/D	0.035 – 0.6	0.9 – 5.1 (mice)	N/A

Kobets et al., (2024). Toxicology. 153711