

Case Study 3: Use of Mechanistic Data for Evidence Integration across PCBs as a Chemical Class

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**IARC Monographs on the Carcinogenic Risk to Humans,
Volume 107: Polychlorinated biphenyls and polybrominated biphenyls
IARC, Lyon, France, 12-19 February 2013**



The IARC Evaluation stands on 4 legs

- 1) Exposure
- 2) Cancer in Humans
- 3) Cancer in Animals
- 4) Mechanistic Data

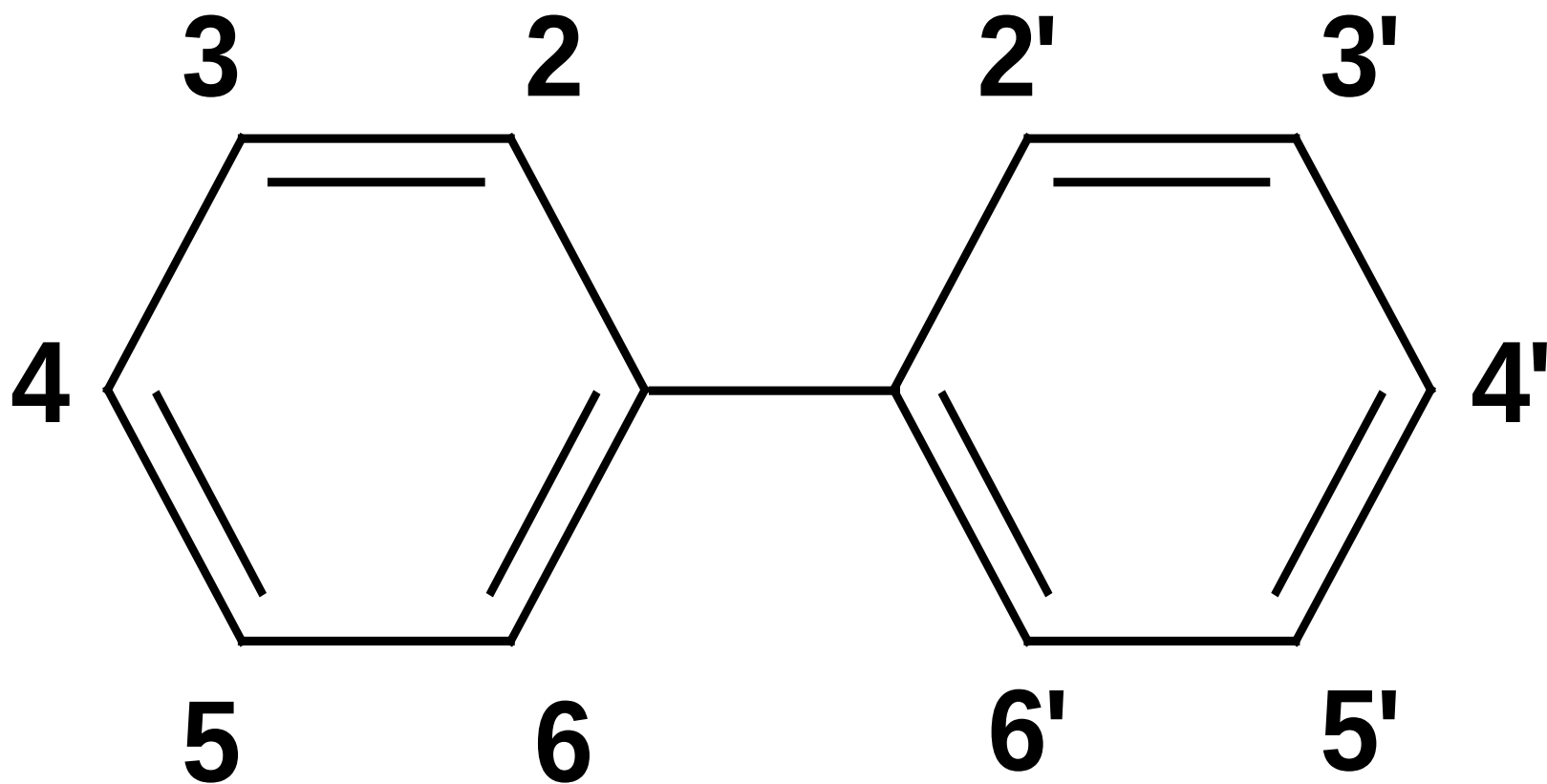
Each area is important and necessary

IARC's Classification of each agent

Evidence was deemed sufficient, limited, inadequate

- Carcinogenic to humans (group 1)
- Probably carcinogenic to humans (group 2A)
- Possibly carcinogenic to humans (group 2B)
- Not classifiable (group 3)
- Probably not carcinogenic to humans (group 4)

Biphenyl

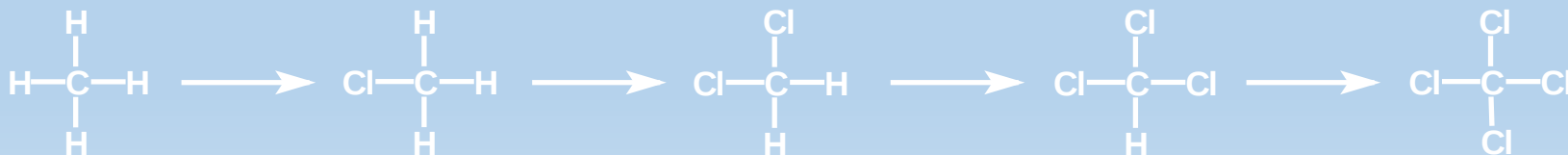


HALOGENATION CONFERS UPON ORGANIC MOLECULES:

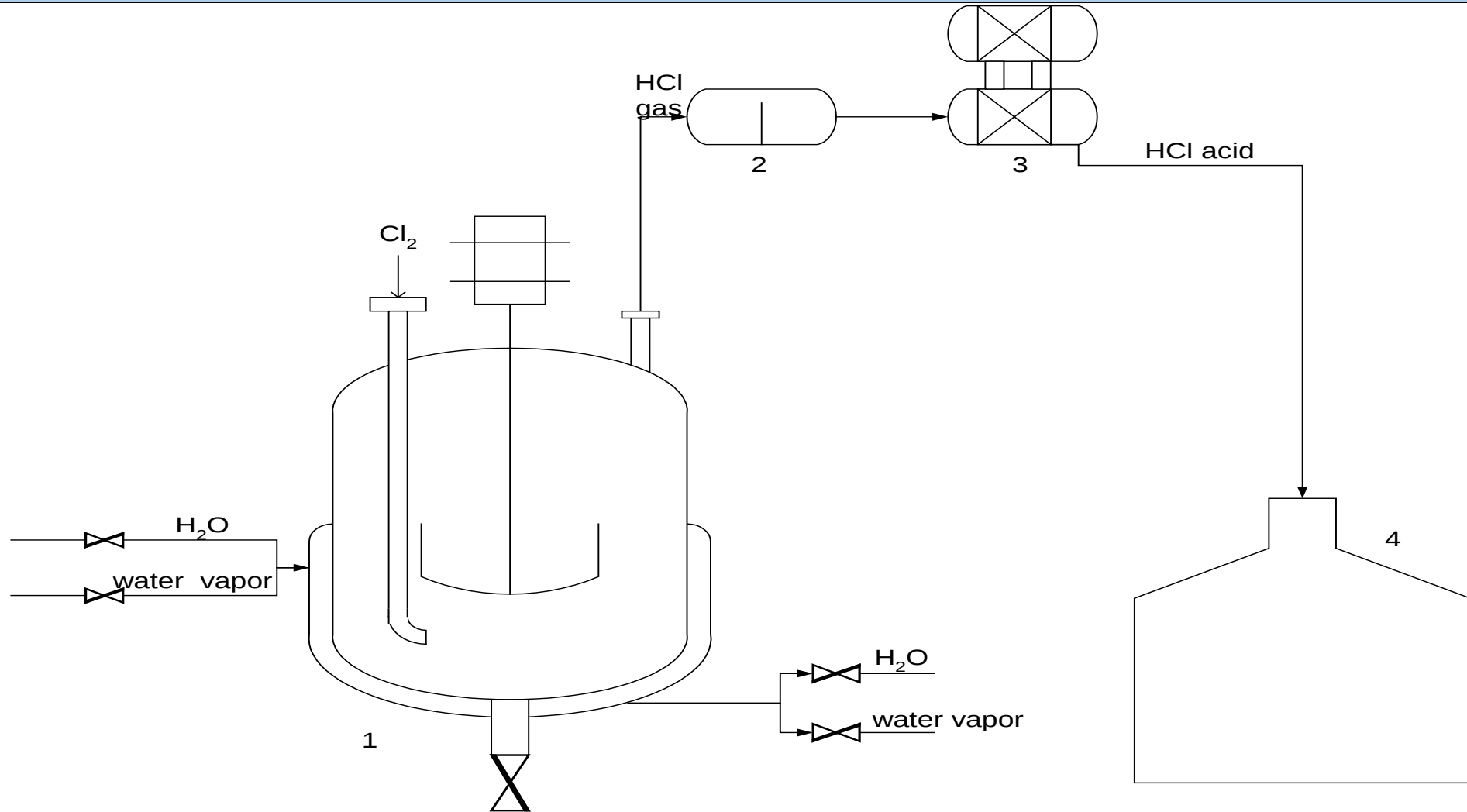
Stability against chemical and biological oxidation, including diminished flammability.

Increased melting points, diminished volatility, diminished water solubility.

Halogenated compounds may persist in the environment and, due to their lipophilic nature, bioconcentrate, bioaccumulate in fatty tissues.



PCB Batch Production



(1) chlorinator (2) sublimate (3) absorber (4) tank for hydrochloric acid

Commercial PCB Mixtures

	Aroclor 1242	Aroclor 1248	Aroclor 1254	Aroclor 1260	Clophen A 30	Clophen A 60
% of chlorine	40-42	48	52-54	60	40-42	60
average Cl per molecule	3.4	4.1	5.1	6.3	3.1	6.2
average molecular weight	261	288	327	372	261	372
monochlorobiphenyl	1	-	-	-	-	-
dichlorobiphenyl	13	1	-	-	20	-
trichlorobiphenyl	45	21	1	-	52	-
tetrachlorobiphenyl	31	49	15	-	22	1
pentachlorobiphenyl	10	27	53	12	3	16
hexachlorobiphenyl	-	2	26	42	1	51
heptachlorobiphenyl	-	-	4	38	-	28
octachlorobiphenyl	-	-	-	7	-	4
nonachlorobiphenyl	-	-	-	1	-	-
decachlorobiphenyl	-	-	-	-	-	-

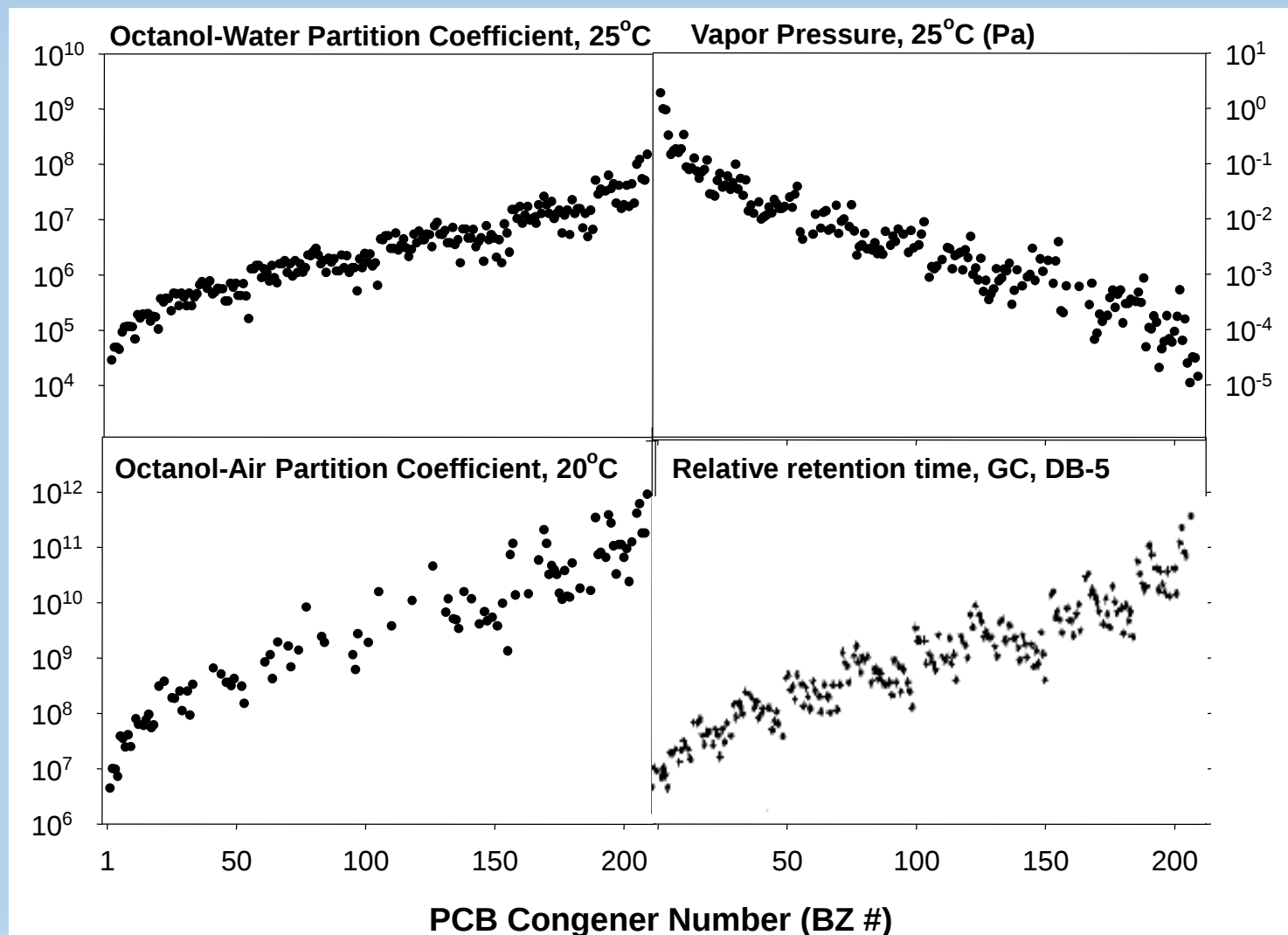
Modified from Crit. Rev. Toxicol. 20, 439-496 (1990)

Numbering System for PCBs

(Ballschmitter and Zell, 1980)

- 1 Chlorine (Mono-) PCBs 1 - 3
- 2 Chlorines (Di-) PCBs 4 - 15
- 3 Chlorines (Tri-) PCBs 16 - 39
- 4 Chlorines (Tetra-) PCBs 40 – 81
- 5 Chlorines (Penta-) PCBs 82 – 127
- etc.

Physical Properties

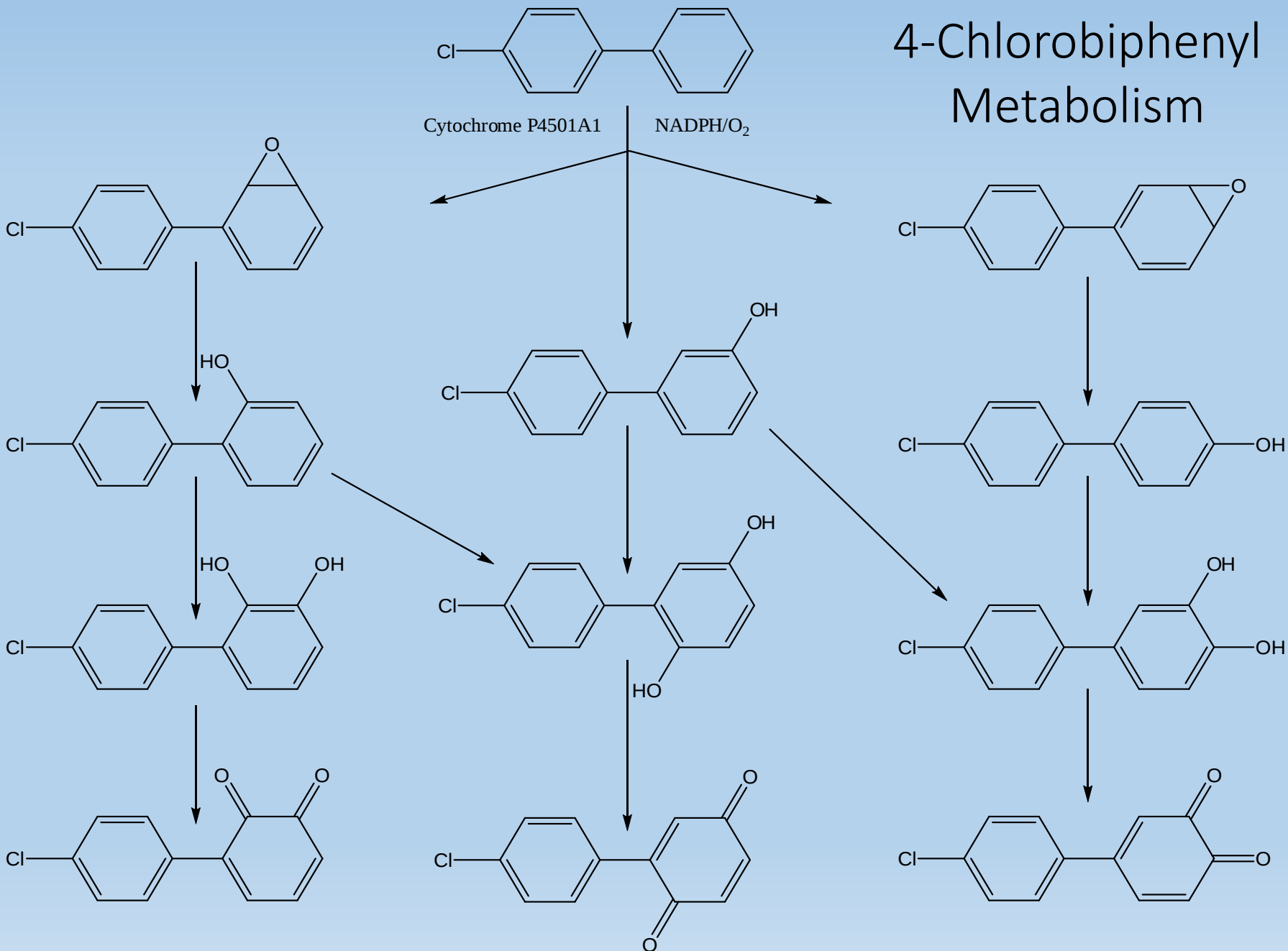


Edited by Luthe, G., from Hornbuckle, K.; Carlson, D.; Swackhamer, D.; Baker, J.; Eisenreich, S. "Polychlorinated Biphenyls in the Great Lakes", In *The Handbook of Environmental Chemistry; Persistent Organic Pollutants in the Great Lakes*. Hites, R. A., Ed.; Springer-Verlag: Heidelberg, 2006, pp 13-70 and from EST 18, 468 (1984).

PCBs in Two Groups: Lower Chlorinated (Episodic, Airborne) PCBs or Higher Chlorinated PCBs

PCBs may be **lower chlorinated biphenyls** –fewer chlorines, more susceptible to environmental breakdown and biologic attack, more volatile, “Episodic”

Higher chlorinated biphenyls, traditionally stable, **food chain PCBs**, found in fish, are considered food chain contaminants – **body burdens increase with age**.



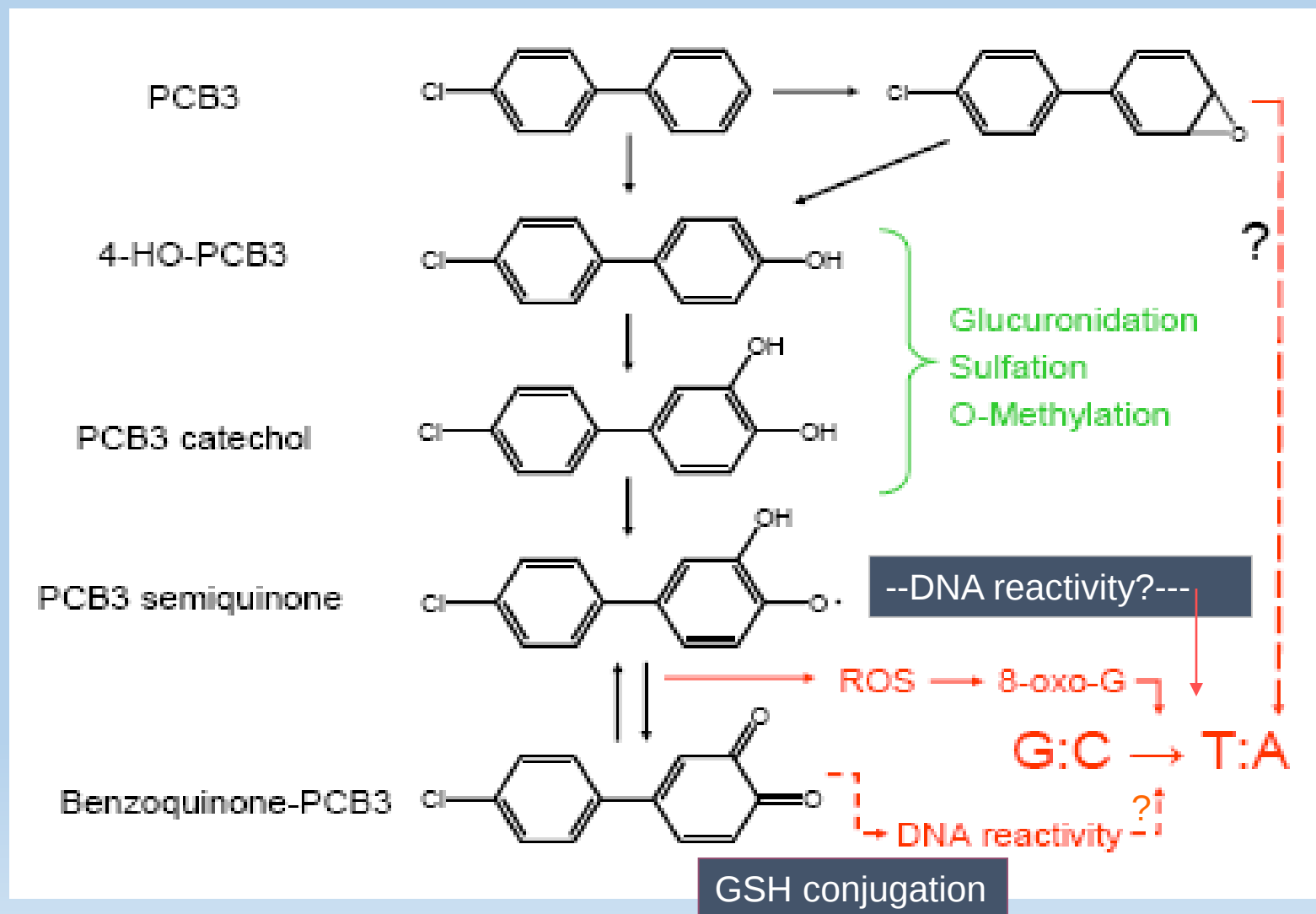
Chem. Res. Toxicol. 9, 158-164 (1996)

Genotoxicity profile of PCB3 and its metabolites in vitro

V79 cells, lowest effective concentration, uM

Compound	TG-R mutat.	MN – breaks	MN – chromos. loss	SCE, Poly- ploidy	COMETS & Others (HL-60, Jurkat)
PCB3	-	-	-	-	
2-OH-	-	-	50		
3-OH-	-	-	100		
4-OH-	-	75	75		
3,4-Cat	-	25	15	5 (SCE)	
3,4-oQ	0.6	15	5	-	
2,5-HQ	-	5	2.5	7.5 (PP)	COMET 37C, not 6C, HL, not J, 0.1 (ROS)
2,5-pQ	0.5	1	2.5	-	COMET 37C & 6C HL & J, 0.1 (ROS) 2.5 (GSH↓)

What is the Mechanisms of Mutagenesis?



PCBs in Two Groups: Lower Chlorinated (Episodic, Airborne) PCBs or Higher Chlorinated PCBs

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Receptors Activated by PCBs

P-450 Subfamily	Receptor	Receptor Ligand	Coreceptor	Coreceptor Ligand
CYP1A	AhR	TCDD, PAHs, β-NF, PCBs	Arnt	none
CYP2B	CAR	PB, Androstanol, PCBs	RXR	9-cis-retinoic acid
CYP3A	PXR	PCN, Rifampin, PCBs	RXR	9-cis-retinoic acid
CYP4A	PPAR α	Peroxisome prolif.	RXR	9-cis-retinoic acid

RXR-related Nuclear Receptors - Nuclear Receptor Family 1 (NR1)

Higher Chlorinated PCBs

Potent and efficacious inducers of drug metabolism.

Up and down regulate the expression of numerous genes.

Promote hepatocarcinogenesis.

Support fatty liver formation (alter expression of numerous genes involved in carbohydrate and lipid metabolism).

And many others.

Mayes et al, Comparative carcinogenicity in Sprague-Dawley rats of the polychlorinated biphenyl mixtures Aroclors 1016, 1242, 1254, and 1260. Toxicol Sci. 1998 Jan;41(1):62-76.

A comprehensive chronic toxicity and carcinogenicity study was conducted on a series of Aroclors (1016, 1242, 1254, and 1260). Each Aroclor was assessed at multiple dietary concentrations, ranging from 25 to 200 ppm, for 24 months in male and female Sprague-Dawley rats.

Hepatocellular carcinomas		
Aroclor	Male S-D Rats	Female S-D Rats
1016	--	+
1242	--	+
1254	--	+
1260	+	+

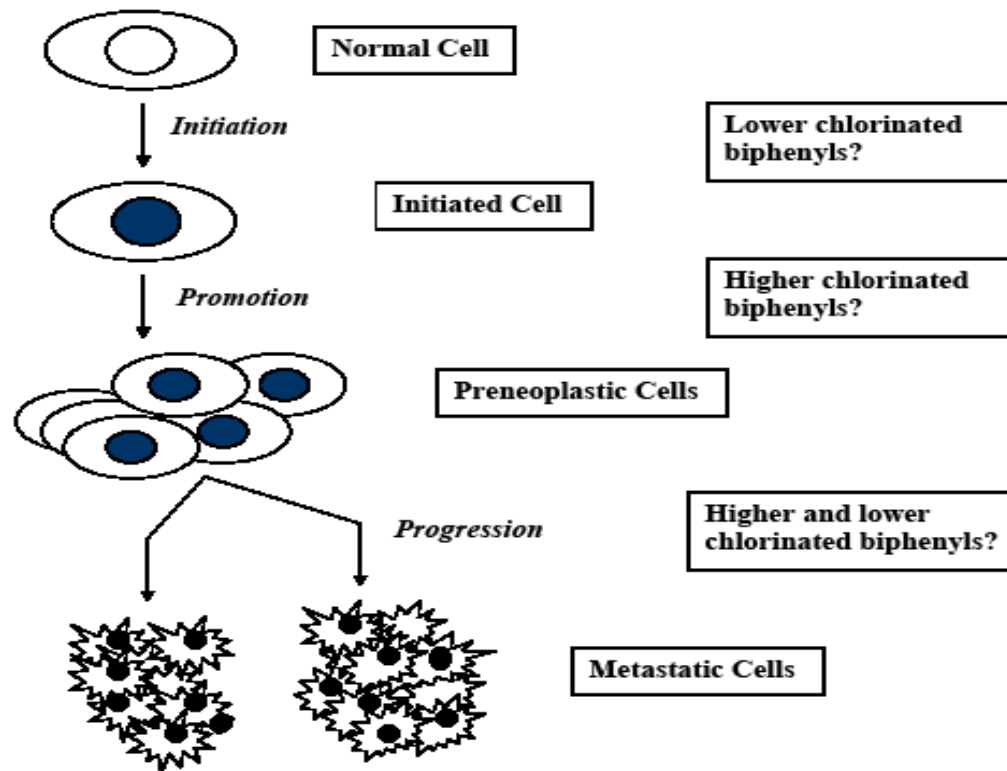
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Pathway from Normal to Malignant Cell

Proposed Role of PCBs





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IARC Evaluation Process

Human Studies

- Sufficient evidence
- Limited evidence
- Inadequate evidence
- Lack of evidence

Animals Studies

- Sufficient evidence
- Limited evidence
- Inadequate evidence
- Lack of evidence

Mechanistic Studies

- Identify established mechanisms
- Identify likely mechanisms
- Determine likelihood of mechanism in humans



Overall evaluation

Group 1 Carcinogenic to humans

Group 2A Probably carcinogenic in humans

Group 2B Possibly carcinogenic in humans

Group 3 not classifiable in humans

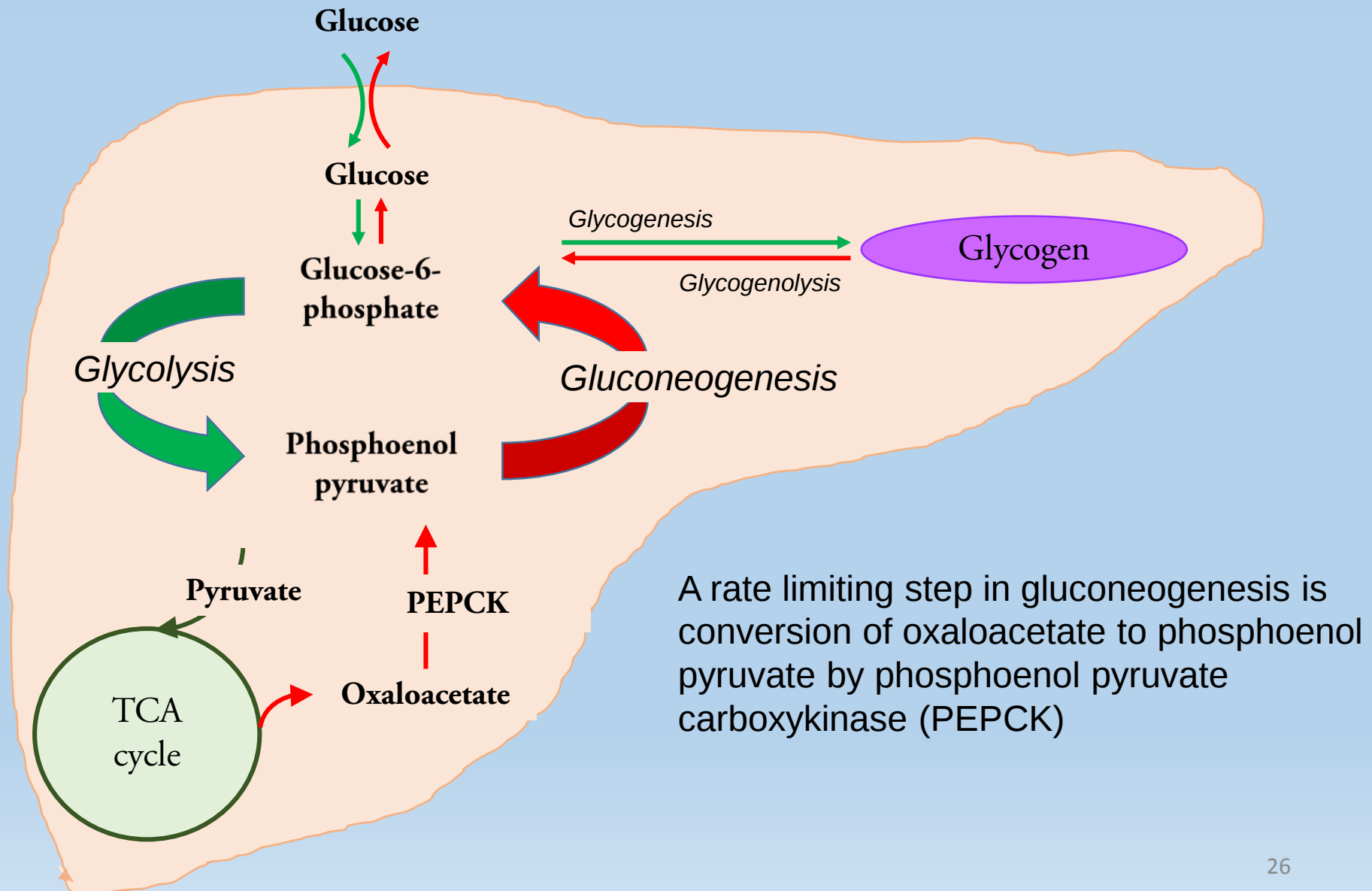
Group 4 Probably not carcinogenic in humans

The IARC Committee found for PCBs:
Excess risk for melanoma,
increased risks for non-Hodgkin lymphoma
and breast cancer,
classified PCBs as carcinogenic to humans
(Group 1)

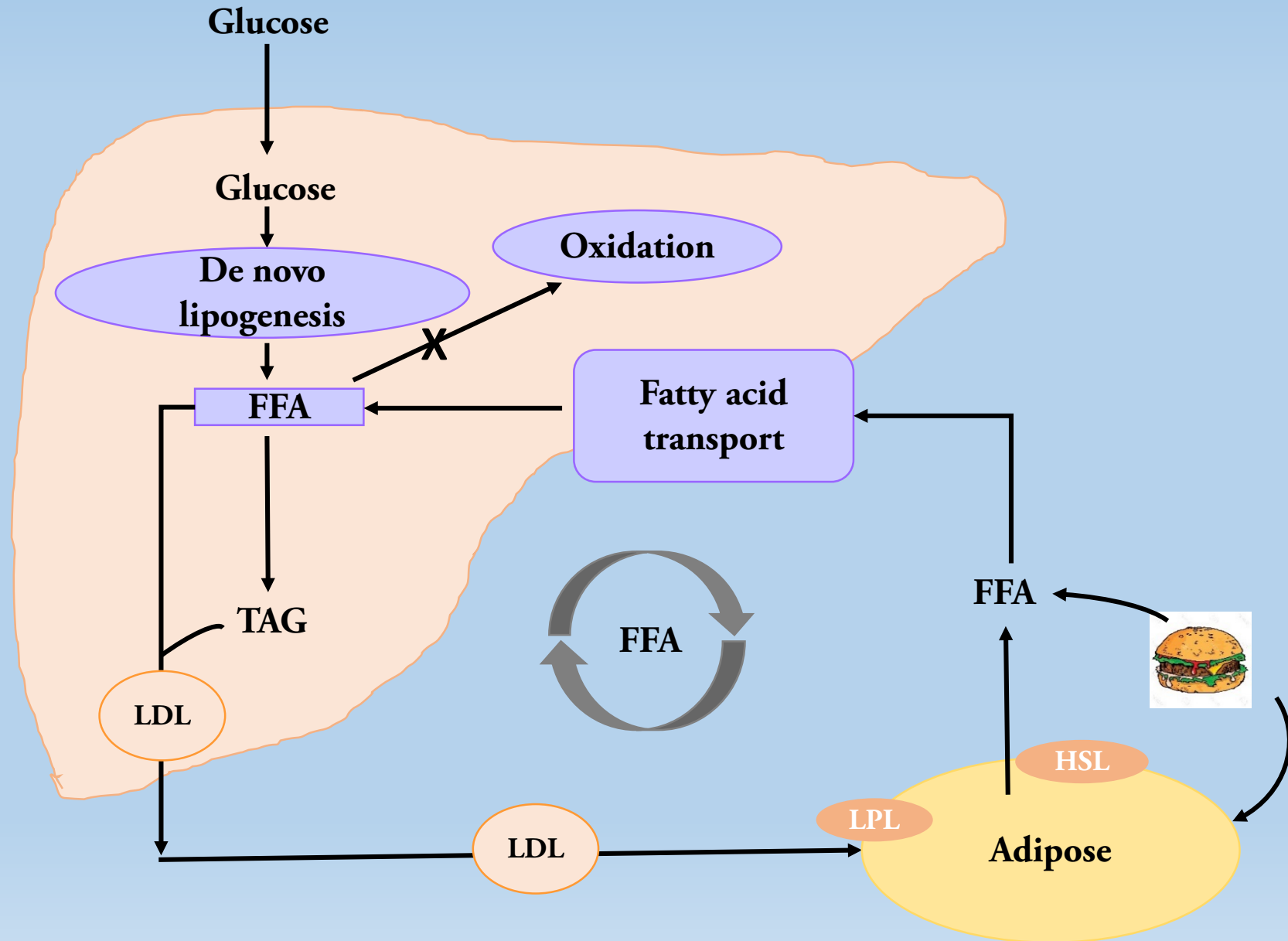
The IARC Committee found for PBBs:
Clear evidence for PBBs as animal carcinogens,
inadequate evidence for carcinogenicity for PBBs
in humans,
PBBs were upgraded to Group 2A, probably
carcinogenic to humans.

Discussion

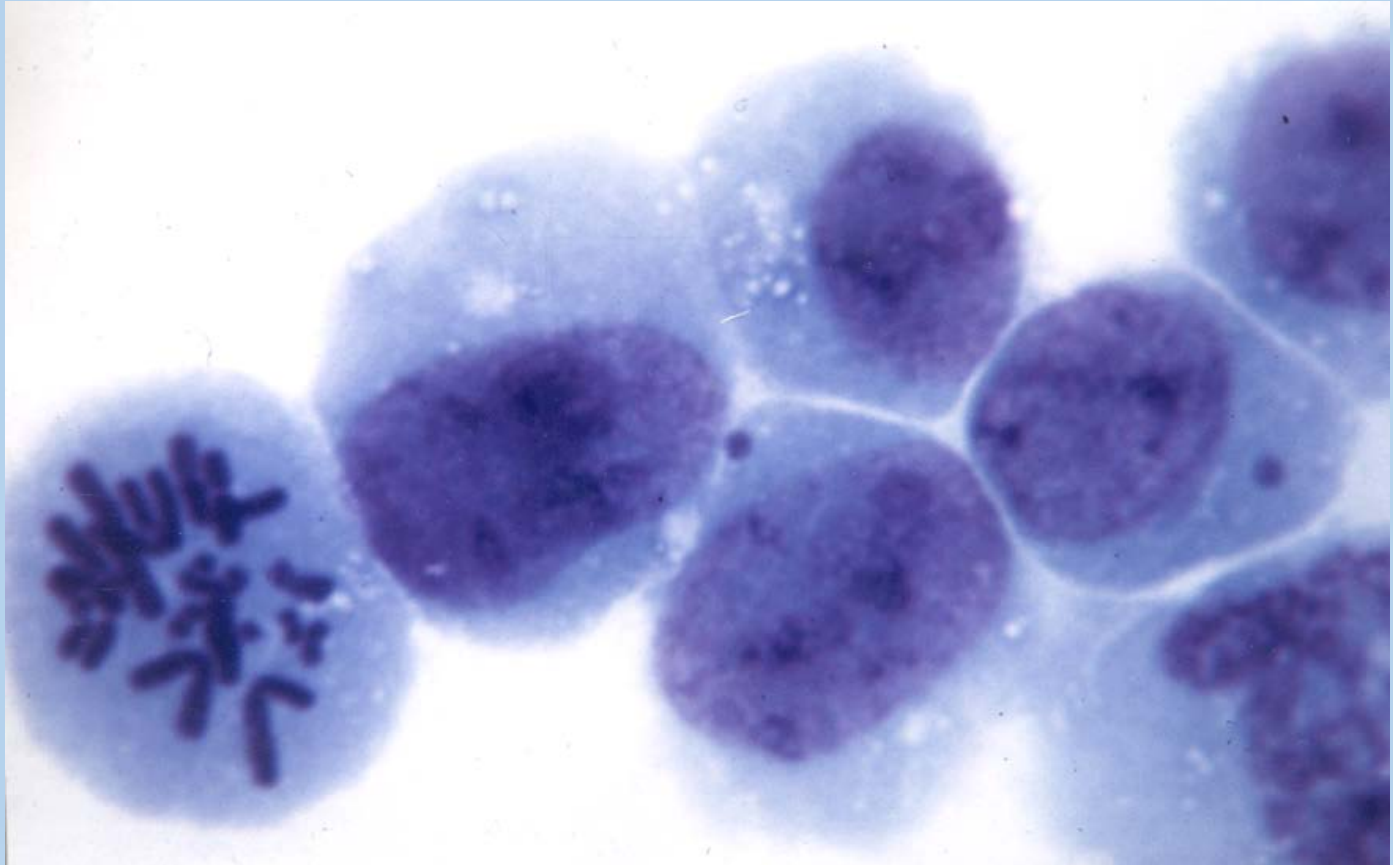
Liver plays an important role in metabolic and energy homeostasis



Mechanisms for lipid accumulation in the liver



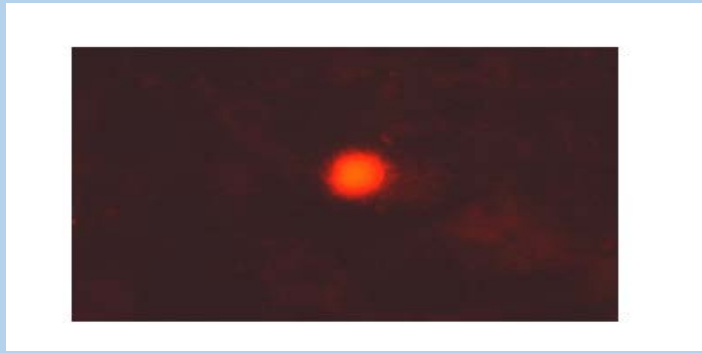
Micronuclei in V79 cells
exposed to 10 μ M Hydroquinone (24 hrs)



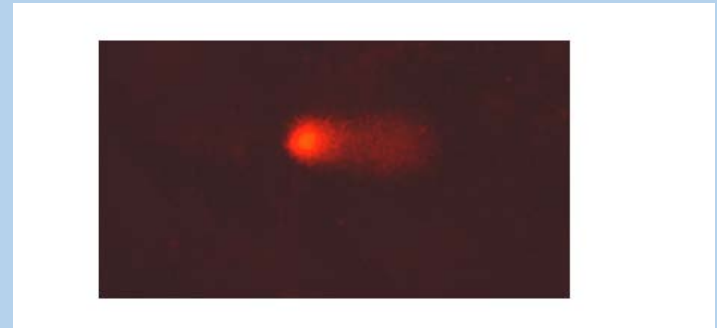
SCE in V79 cells exposed to 10 μ M Benzene-*anti*-diol-epoxide (24 hrs)



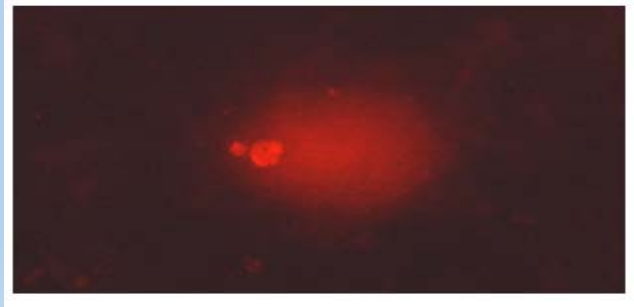
Types of DNA damaged visualized in the “comet assay”



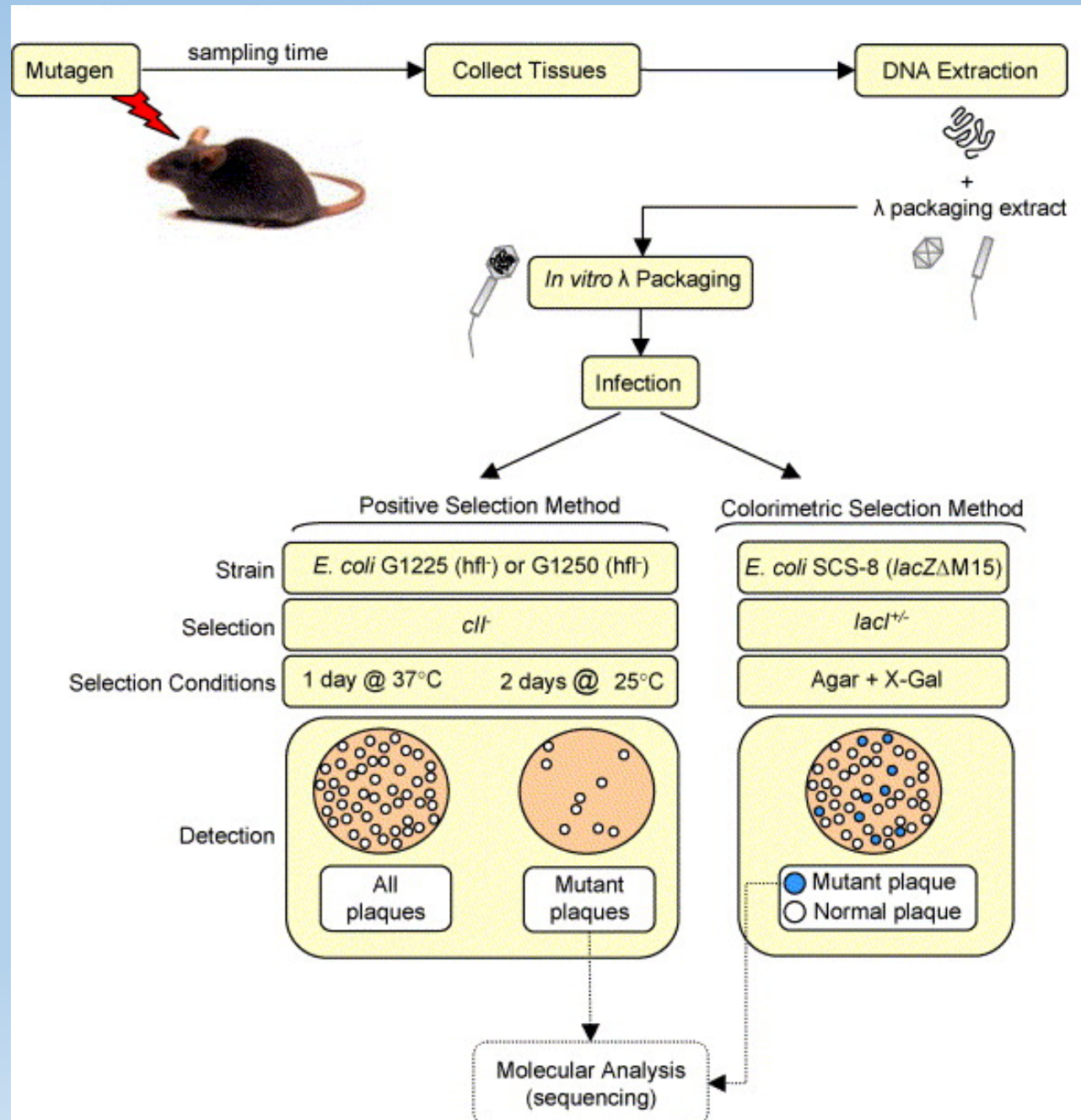
Undamaged nucleus



Moderately damaged nucleus



Highly damaged nucleus



<http://www.ratsareus.com>

Type of Mutations

Type of mutation	% of total mutations			
	Control	3-MC	PCB3	4-HO-PCB3
Transitions (total)	63	30*	30*	56
G:C → A:T	56	27	30	48
(at CpG sites	22	58	43	23)
A:T → G:C	6	2		7
Transversions (total)	19	43	43	22
G:C → T:A	6	27	30	15
G:C → C:G	13	9		4
A:T → C:G		5	9	4
A:T → T:A		2	4	
Frameshifts (total)	19	27	26	22
-1 Frameshift		14	13	4
+1 Frameshift	6	2	4	4
Insertions	6	7	4	11
Deletions	6	5	4	4

PCBs were Produced as Mixtures

	Aroclor 1242	Aroclor 1248	Aroclor 1254	Aroclor 1260	Clophen A 30	Clophen A 60
% of chlorine	40-42	48	52-54	60	40-42	60
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octachlorobiphenyl	-	-	-	7	-	4
nonachlorobiphenyl	-	-	-	1	-	-
decachlorobiphenyl	-	-	-	-	-	-

Modified from Silberhorn et al, 1990