Ensayos *in vitro*

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Biological Systems and Stressors

"Toxicant Totality Tolerance Trajectory" - 4T's

Christian G. Daughton, Ph.D.
October 2002
http://www.epa.gov/nerlesd1/chemistry/pharma/index.h

Sources, Origins, Occurrence, Transport (Characterization of Stressors)

Chemical Universe

entry of chemicals to environment

Agriculture (pesticides)
PPCPs
Food
Household Chemicals
Combustion Products
Industrial Products
Industrial Wastes

Anthropogenic
Naturally Occurring
Known Chemicals
Unknowns
Toxicity

Important Factors: concentration (dosage, level), weathering, transformation, chemical 'species'

Transport, Distribution & Fate

Matrix:
Air
Water
Soil
Sediment

Metabolism & Excretion

Non-Chemical Universe

(those non-chemical stressors acting at the [sub]cellular level)

Pathogens (immune response; apoptosis/necrosis)
Physical Stress (stress protein response; hormonal modulation)
Temperature/ Humidity (heat balance; stress protein response)
Electromagnetic Radiation (ionizing damage; diurnal cycles)
Noise (hormonal modulation; chemical signal cascades)

Exposure Pathways

Important Factors: absorbed dose, amount available to receptors

Parenteral
Dermal
Ingestion/enteral
Respiratory: Pulmonary/Gills
Organism
Individual
Population
Community
Ecosystem

"Window of Vulnerability"

Timing: critical time for exposure; developmental/reproductive stage & health status can determine current/ future susceptibility to effects

Routes of Exposure

Past Exposure Trajectory (Prior Exposure History):

multidimensional time line; spatial/temporal function of countless variables, including exposure duration, timing, and complex interplay of genetics, nutrition, metabolic stress, activities, behaviors, and events

Long-term, continual, chronic exposure

Short-Term Exposure

(acute, sequential, intermittent, episodic)

Interactions/Potentiation

Additive, Synergistic, Antagonistic (can occur from interactions between chemical and non-chemical stressors and a vast, complex array of biological receptors; resulting from similar and dissimilar mechanisms of action)

molecular stressors
biochemical receptors

Dose-Response
response - effect
dose - concentration

Future Exposure Trajectory

February
September

Future
Prior
Health State

Phenotype

Changes: Overt and Subtle

Effects on health or populations = reactions to exposure
Alteration/disruption to function/processes, morbidity (including mutagenesis, carcinogenesis, teratogenesis), mortality, selective disadvantage, enhancement of function (selective advantage)
No effect = homeostasis (*status quo*)

The **4T's** account for an organism's complete exposure time line (a trajectory described by its prior multi-dimensional exposure history) and the fact that a major objective of all organisms is to maintain homeostasis (in the face of continual perturbation by stressors).

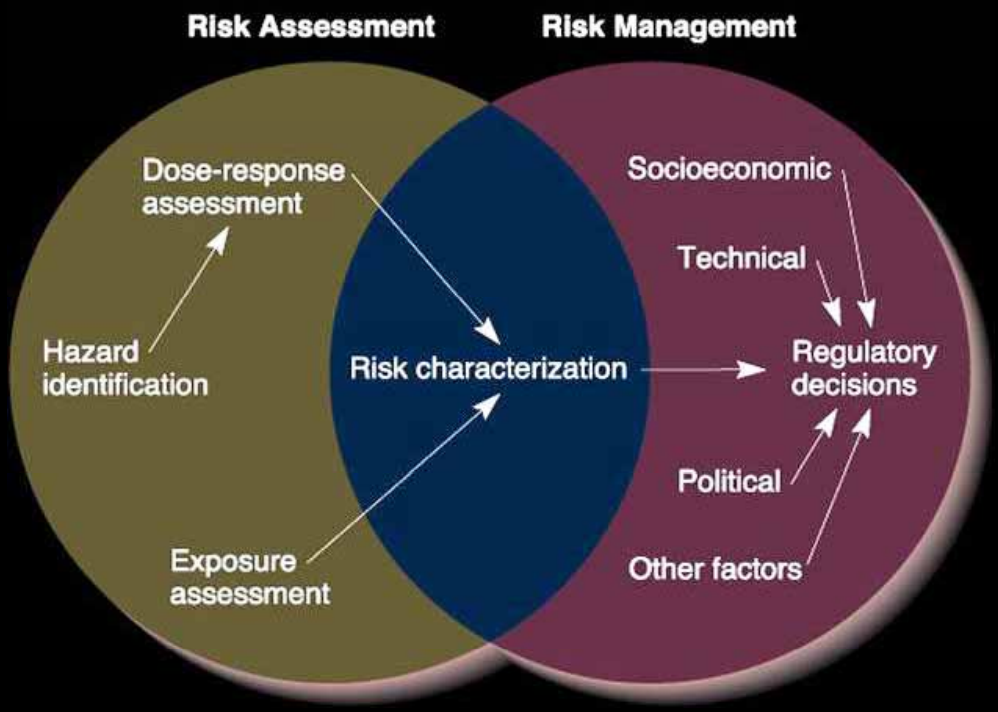
Toxicant: Toxic chemical (spanning the spectrum of proteinaceous toxins to man-made synthetics; excluding radionuclides).

Totality: Emphasizes that organisms can be exposed to a multitude of different members from the large universe of toxicants (not just to individual toxicants in isolation from others).

Tolerance: Ability to resist change (susceptibility or vulnerability to perturbing homeostasis; homeostasis can be maintained only within the tolerance bounds for an organism's biochemical defensive repertoire).

Trajectory: Individually unique spatial/temporal exposure route experienced by an organism (multidimensional function of its activities and behaviors, as well as isolated/sustained exposure events). Note that any exposure event (short-term, long-term) or exposure phenomenon (e.g., "Window of Vulnerability") may be applicable at any time in an organism's exposure trajectory (past or future).

For more information regarding the many facets of exposure and stressors, see:
http://www.epa.gov/nerlesd1/chemistry/ppcp/stressors.htm
http://www.epa.gov/nerlesd1/chemistry/pharma/critical.htm

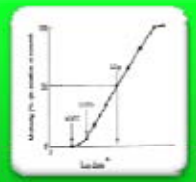


La evaluación y control del riesgo de productos químicos son procedimientos integrados.

✓ La caracterización del riesgo se define como la estimación cuantitativa y/o cualitativa de los efectos nocivos asociados a los agentes químicos.

Identificación del riesgo

- Capacidad inherente del compuesto para producir efectos nocivos.



Evaluación de toxicidad

- Efecto del compuesto en función de la concentración.

Valoración de la exposición

- Probabilidad de interacción con el compuesto.

Identificación del riesgo

Confirmación de que un agente químico es capaz de causar un efecto adverso bajo determinadas circunstancias.

¿ Cúales son los productos químicos presentes ?

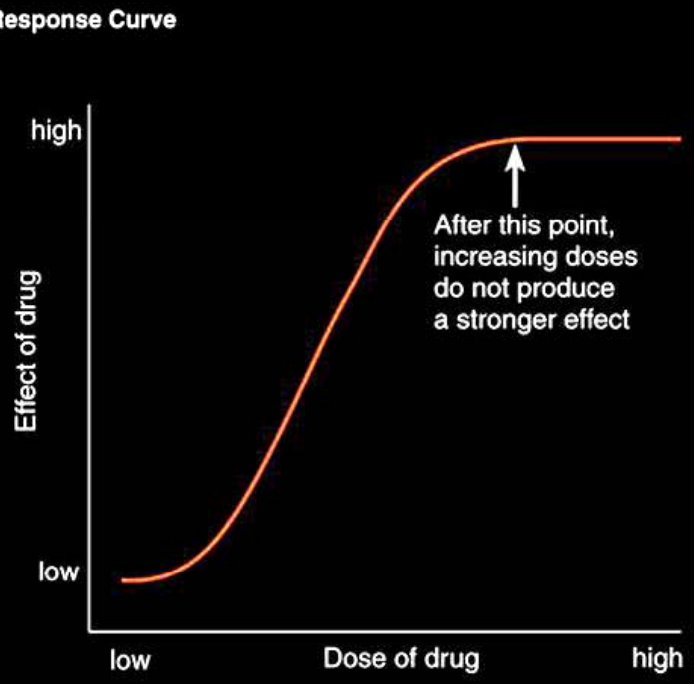
Recogida y análisis de datos sobre peligrosidad y toxicidad

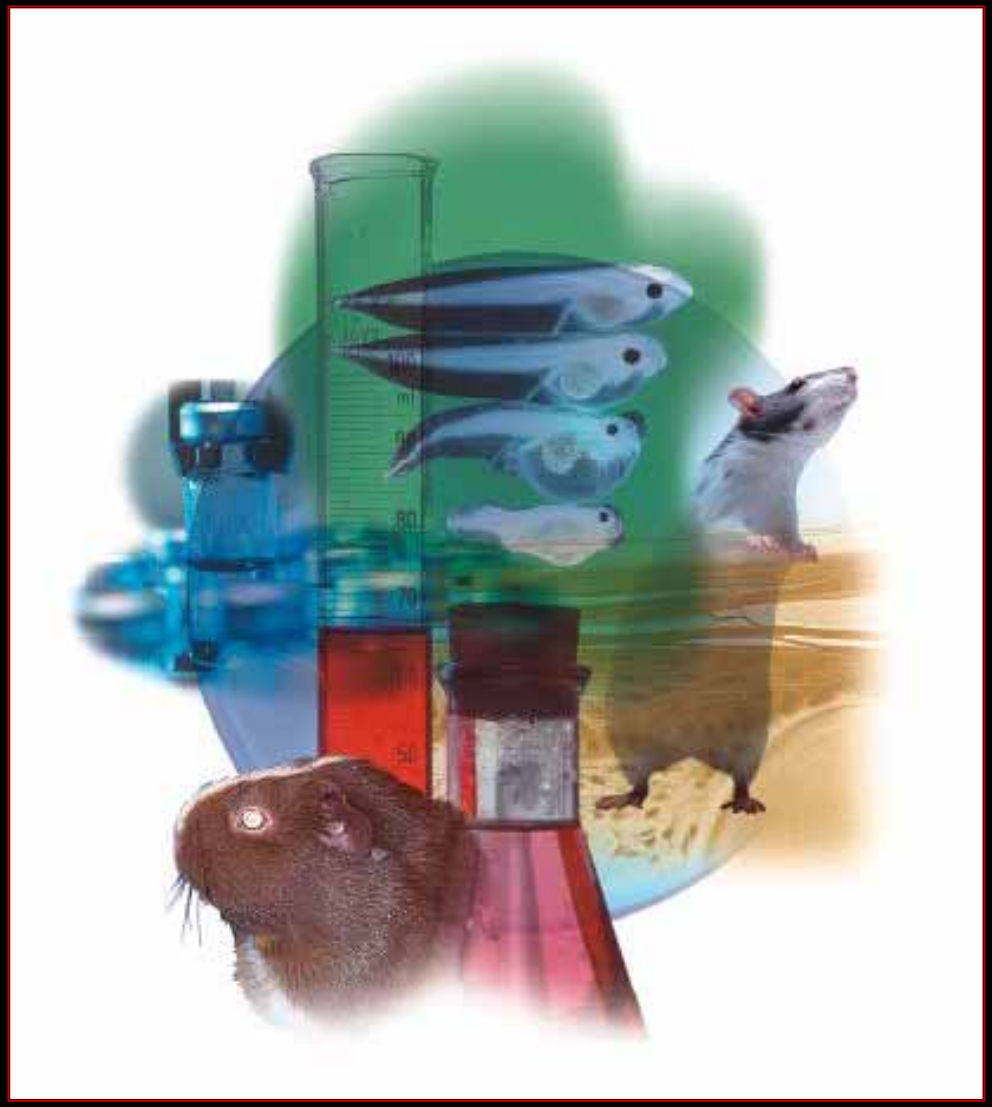
Información acerca de las posibles vías de transporte

Evaluación de toxicidad

Metodologías diversas cuyos resultados deben ser integrados para permitir la extrapolación a condiciones humanas.

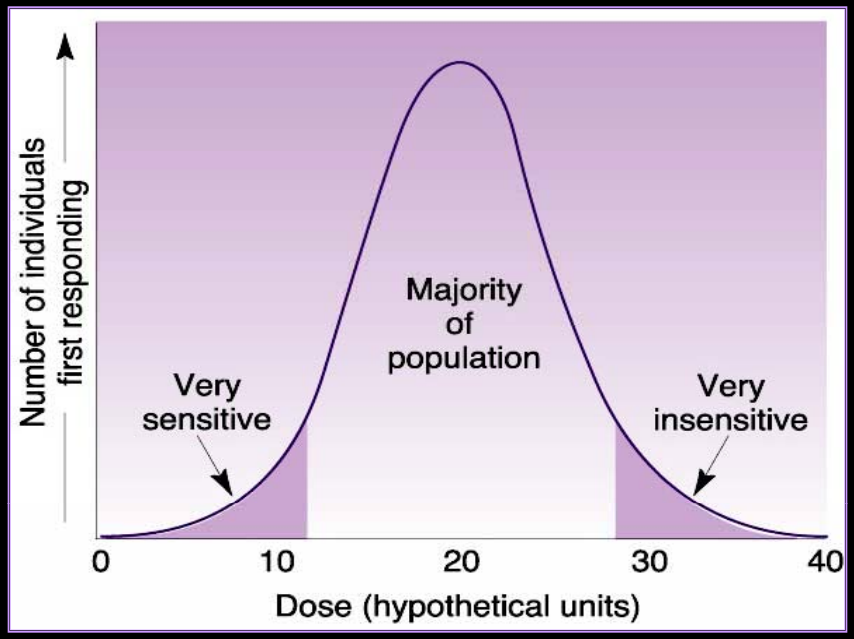
- Definir la toxicidad del compuesto
- Definir los niveles de exposición





NOEC LOEC EC50

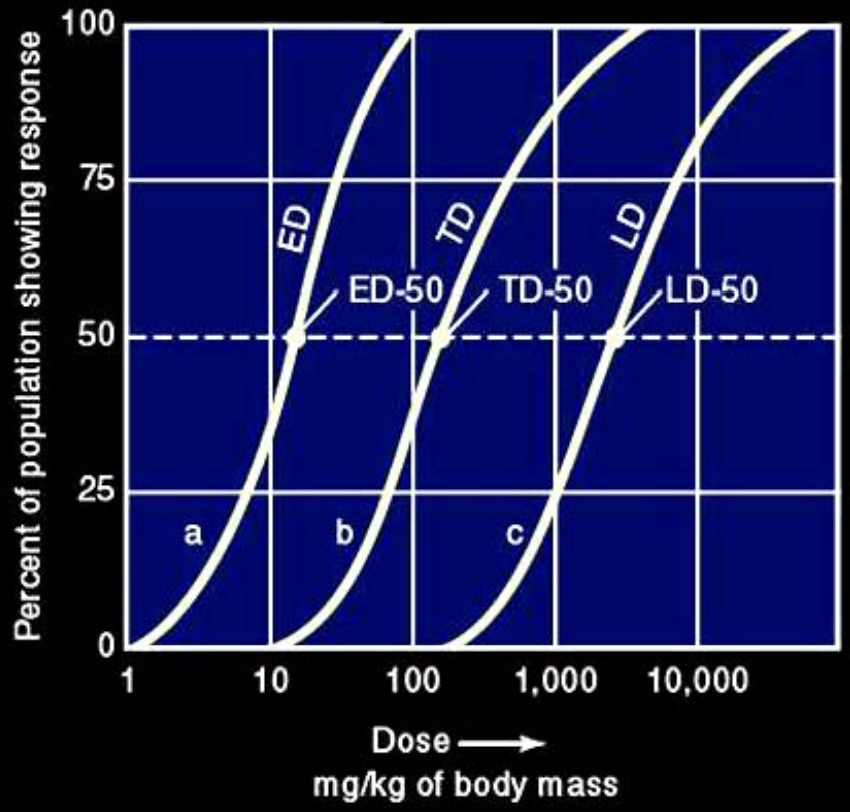
Valoración de la exposición

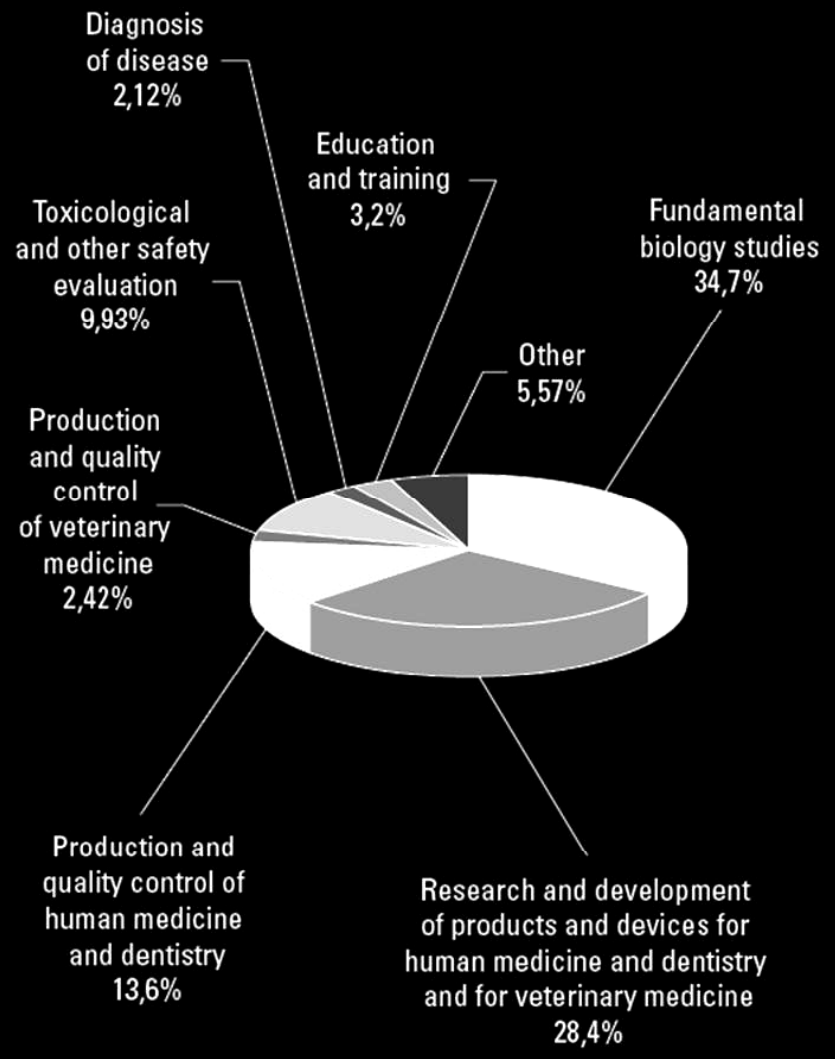


Factores de riesgo

- Vías de exposición
- Concentración del compuesto
- Duración de la exposición
- Poblaciones expuestas

Evaluación toxicológica





En el año 2005 se utilizaron en Europa 12.1 millones de vertebrados para experimentación.

Evaluación toxicológica

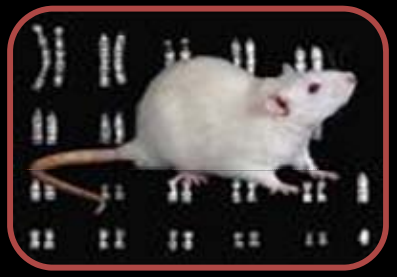
Toxicidad aguda y crónica

Toxicidad para la reproducción

Toxicidad para el desarrollo

Ensayos de irritación ocular

Ensayos de hipersensibilidad



Ensayos de genotoxicidad

Ensayos de carcinogenicidad

Ensayos de fototoxicidad

Estudios toxicocinéticos

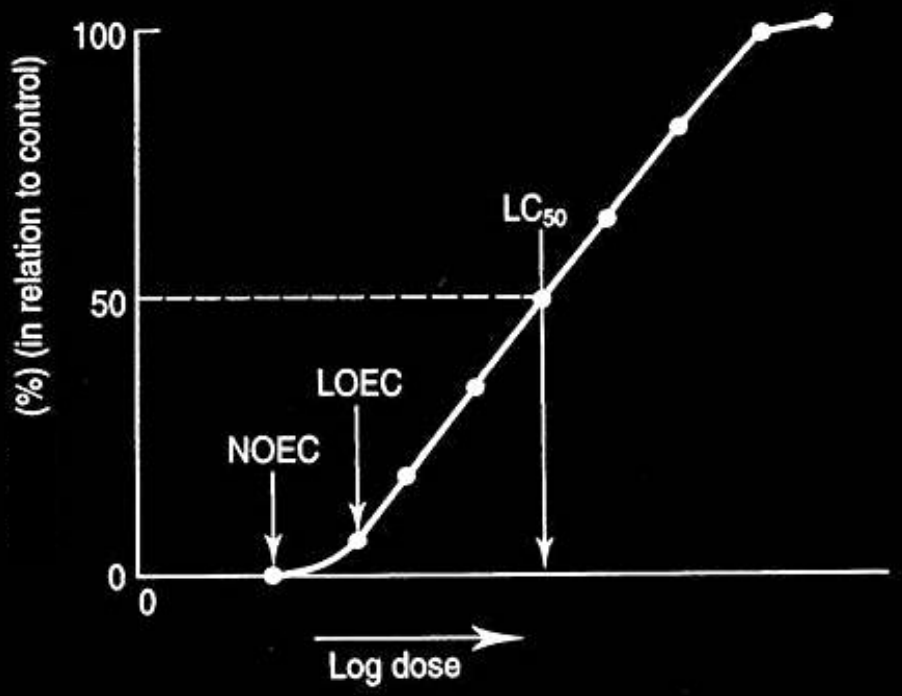
Estudios de comportamiento

Evaluación toxicológica

Toxicological endpoints		Type of animal test
Eye irritancy	Animal	Adult albino rabbit (3 animals)
	Test	Dose effect monitored (21 days). Irritancy is scored according to a standard.
	OECD	No 405: Acute eye irritation/Corrosion (updated 24 April 2002)
Skin irritancy and corrosion	Animal	Adult albino rabbit (3 animals)
	Test	Dose applied on a shaved area of the skin. Irritancy is scored by checking against control patch of skin.
	OECD	No 404: Acute Dermal Irritation/Corrosion (updated 24 April 2002) No 434: Acute Dermal Toxicity – Fixed Dose Procedure (updating guideline 404)
Skin allergy	Animal	Albino guinea pigs (17-30 per chemical)
	Test	Dose substance is delivered to the skin by surface application or injection. Multiple doses applied in order to cause any local reaction
	OECD	No 406: Skin Sensitisation (updated 27 July 1995)
Acute toxicity	Animal	Usually rats (15-30 per chemical).
	Test	Oral dosing of animals and observation for 14 days. All animals are autopsied at the end of the test period. Sex-specific responses are noted.
	OECD	No 401: Acute Oral Toxicity (deleted 20 December 2002) No 420: Acute Oral Toxicity - Fixed Dose Method (adopted 20 December 2001) No 423: Acute Oral Toxicity - Acute Toxic Class Method (adopted 20 December 2001) No 425: Acute Oral Toxicity: Up -and Down -Procedure (updated 23 March 2006)
Repeat dose toxicity	Animal	Usually rats (40-80 rats per chemical). Sometimes dogs are also used (32 dogs per chemical). Animal repeatedly dosed with a chemical for 28-90 days. Dose administered orally, dermally or inhaled.
	Test	Animals are then killed and their tissues examined pathologically and biochemically
	OECD	No 407: Repeated Dose 28-day Oral Toxicity Study in Rodents (updated 27 July 1995) No 408: Repeated Dose 90-day Oral Toxicity Study in Rodents (updated 21 September 1998) No 409: Repeated Dose 90-day Oral Toxicity Study in Non-Rodents (updated 21 September 1998) No 412: Repeated Dose Inhalation Toxicity (original 12th May 1981).
Carcinogenicity	Animal	Very young rats or mice (at least 400)
	Test	Doses are administered either orally, dermally or inhalation. Outcome of exposure is assessed by blood sampling, pathological appearance and tissue and organ examination to detect cancerous changes. Tests costs $2 million per chemical and take 5 years to complete.
	OECD	No 451: Carcinogenicity Studies (original 12th May 1981)
Chronic toxicity	Animal	Rats (400) or dogs as second species (32).
	Test	Oral and inhalation routes of administration. Length of study from 12 months to 2 years.
	OECD	No 452: Chronic Toxicity Studies (original 12th May 1981)
Teratogenicity	Animal	Pregnant rats (80) or rabbits (48)
	Test	Doses delivered by mouth and the embryos killed and examined for gross or more subtle changes.
	OECD	No 421: Reproduction/ Developmental Toxicity Screening Test (original 27th July 1995).
Mutagenicity	Animal	Rats, mice or Chinese hamsters (minimum 40)
	Test	Single or multiple orally/injected doses are administered into the body cavity. Two control groups are used (one receives no chemical and another receives a chemical known to cause an effect on genes). Tissue sampling is performed up to 48 hrs after dosing.
	OECD	No 478: Genetic Toxicology: Rodent Dominant Lethal Test (Updated guideline 4th April 1984).
Reproductive toxicity	Animal	Rats (100 females (80 pregnant) and 40 males)
	Test	Doses administered during reproductive cycle. Assessment is made on fertility, pregnancy. Tissue, brain or secondary sexual systems are studied for effects.
	OECD	No 421: Reproduction/ Developmental Toxicity Screening Test (original 27th July 1995).
Toxicokinetics	Animal	Rodents and sometimes dogs (8 of each species).
	Test	Doses are administered (oral, inhalation, via the skin). Animals are then killed and examined for accumulation of test substance in presumed target organs. Excretion and metabolism time courses are also followed.
	OECD	No 417: Toxicokinetics (updated 4th April 1984)
Endocrine disruptors		Currently no validated methods specific to endocrine disruptors. Both *in vivo* and *in vitro* are being developed.

(Table modified from BUAV report (2001) and OECD Guideline for Testing of Chemicals – Section 4)

Evaluación toxicológica



LD_{50} : estimated dose that causes 50% mortality of tested organisms

LC_{50} : estimated concentration that causes 50% mortality of tested organisms

EC_{50} : estimated concentration that causes an effect (reduced reproduction, growth, etc) on 50% of tested organisms

IC_{50} : estimated inhibitory concentration that reduces the normal response by 50%

NOEC : No Observed Effect Concentration

LOEC : Low Observed Effect Concentration

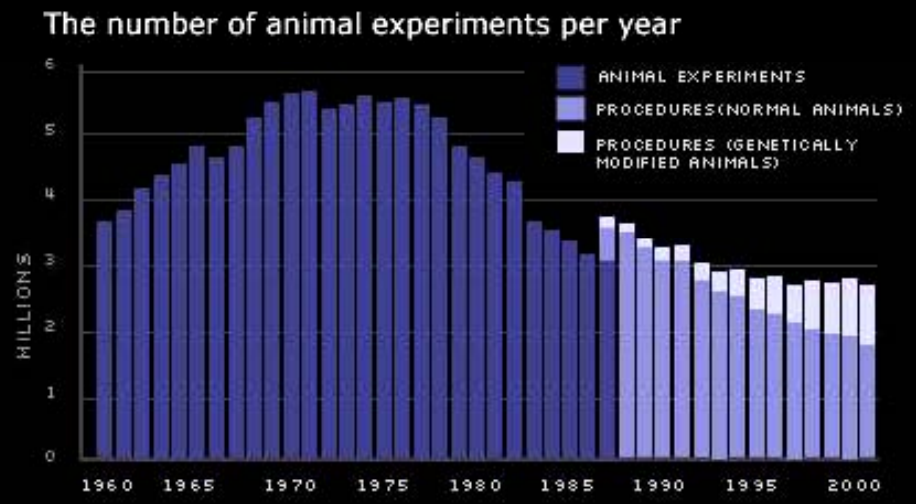
Others involving different percent change (10%, 20%, etc)

Evaluación toxicológica

El uso de animales de experimentación permite la caracterización del riesgo químico y es un paso imprescindible para cumplir con los requisitos legales.

Inconvenientes

Diferencias interespecíficas
Diferencias de exposición
Diferencias de dosis



Evaluación toxicológica

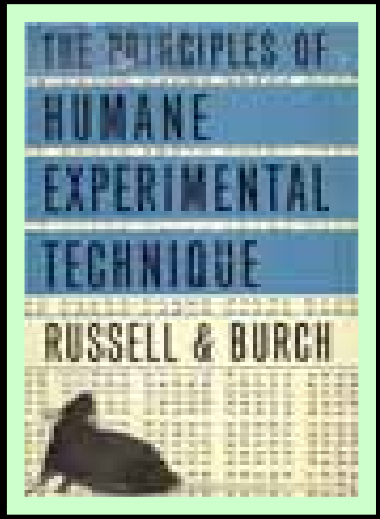
Directiva del Consejo 86-609/EEC de 24 de Noviembre de 1986 relativa a la aproximación de las disposiciones legales, reglamentarias y administrativas de los Estados miembros respecto a la protección de los animales utilizados para experimentación y otros fines científicos.

Métodos alternativos (1959)

R efinamiento de los métodos de experimentación animal.

R educción en el número de animales empleados.

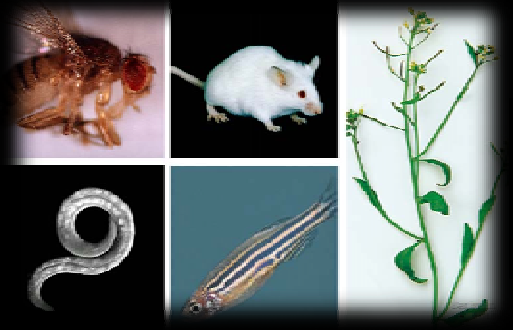
R eemplazo de las técnicas por otras que no utilicen animales.



Métodos alternativos

- Refinement

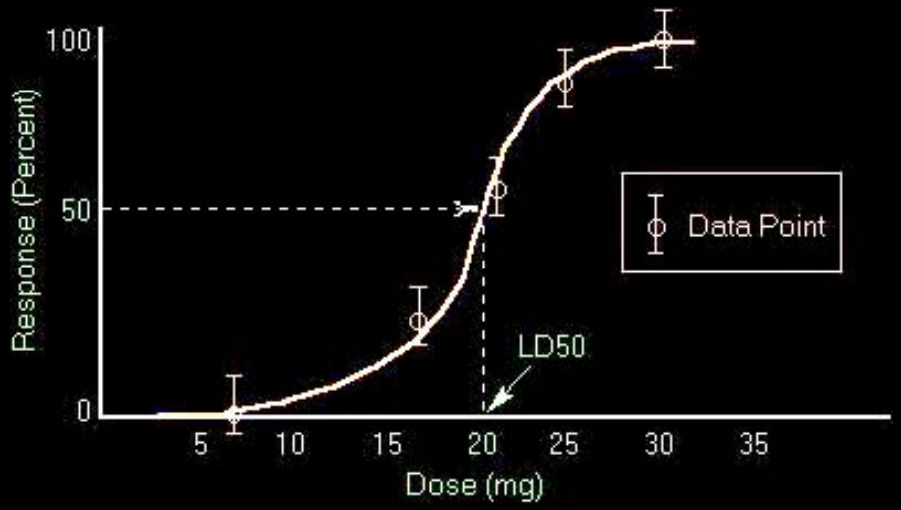
- Reduction



- Replacement

Métodos alternativos

OECD abolished the requirement for the oral test (LD50) in 2002 (Test Guideline 401).

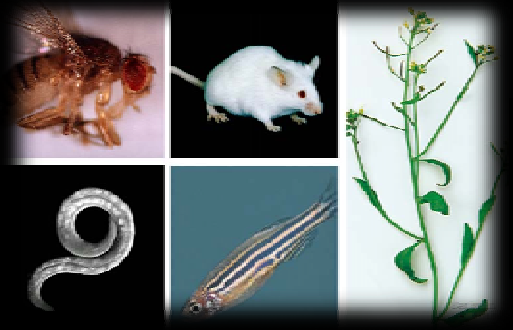


The dose of chemical calculated to be lethal to 50% of the organisms in a specific test situation.

It is expressed in weight of the chemical (mg) per unit of body weight (kg) of the test organism.

- Refinement

- Reduction



- Replacement

Normativa REACH

Registro

Evaluación

Autorización

Restricción

Table 2 Toxicological standard data requirements under REACH (waiving conditions not cited)

Toxicological endpoint	Production volume
8.1 Skin irritation, skin corrosion	≥1 t/a
8.2 Eye irritation	≥1 t/a
8.3 Dermal sensitisation	≥1 t/a
8.4 Mutagenicity	≥1 t/a
8.5.1 Acute oral toxicity	≥1 t/a
8.5.2 Acute inhalative toxicity	≥10 t/a (case by case)[a]
8.5.3 Acute dermal toxicity	≥10 t/a (case by case)[a]
8.6.1 Repeated dose toxicity (28 days)	≥10 t/a
8.6.2 Repeated dose toxicity (90 days)	≥100 t/a
	≥10 t/a (case by case)[b]
8.7.1 Reproductive toxicity screening	≥10 t/a
8.7.2 Developmental toxicity	≥100 t/a
8.7.3 Two-generation reproductive toxicity study	≥100 t/a (case by case)[b]
	≥1,000 t/a
8.8 Toxikokinetics	≥10 t/a (assessment on the basis of relevant information available)
8.9.1 Carcinogenicity study	≥1,000 t/a (case by case)[b]

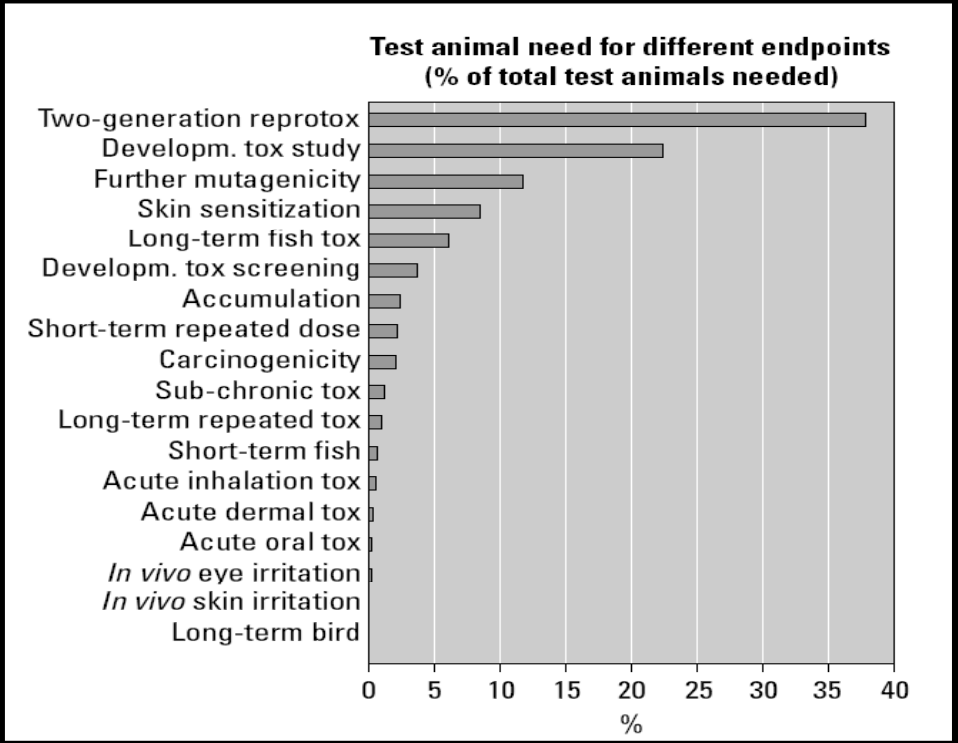
[a] Depending on the assumed route of exposure to humans

[b] For details see text

Article 23(1):

In order to avoid unnecessary animal testing, testing on vertebrate animals for the purposes of this Regulation shall be undertaken only as a last resort.

It is also necessary to take measures limiting unnecessary duplication of other tests.



Normativa REACH

Registro

Evaluación

Autorización

Restricción

Refinamiento

Reducción

Reemplazo

Aproximación por analogía (read-across)

Métodos *in silico* (QSAR)

Genómica, proteómica y metabonómica

Organismos con menor sensiblilidad

Embriones de vertebrados

Ensayos *in vitro*

Voluntarios y epidemiología

Article 12

Before new tests are carried out to determine the properties listed in this Annex, all available in vitro data, in vivo data, historical data, data from valid Q(SAR) and data from structurally related substances (read-across approach) shall be assessed first.

Normativa REACH

Registro

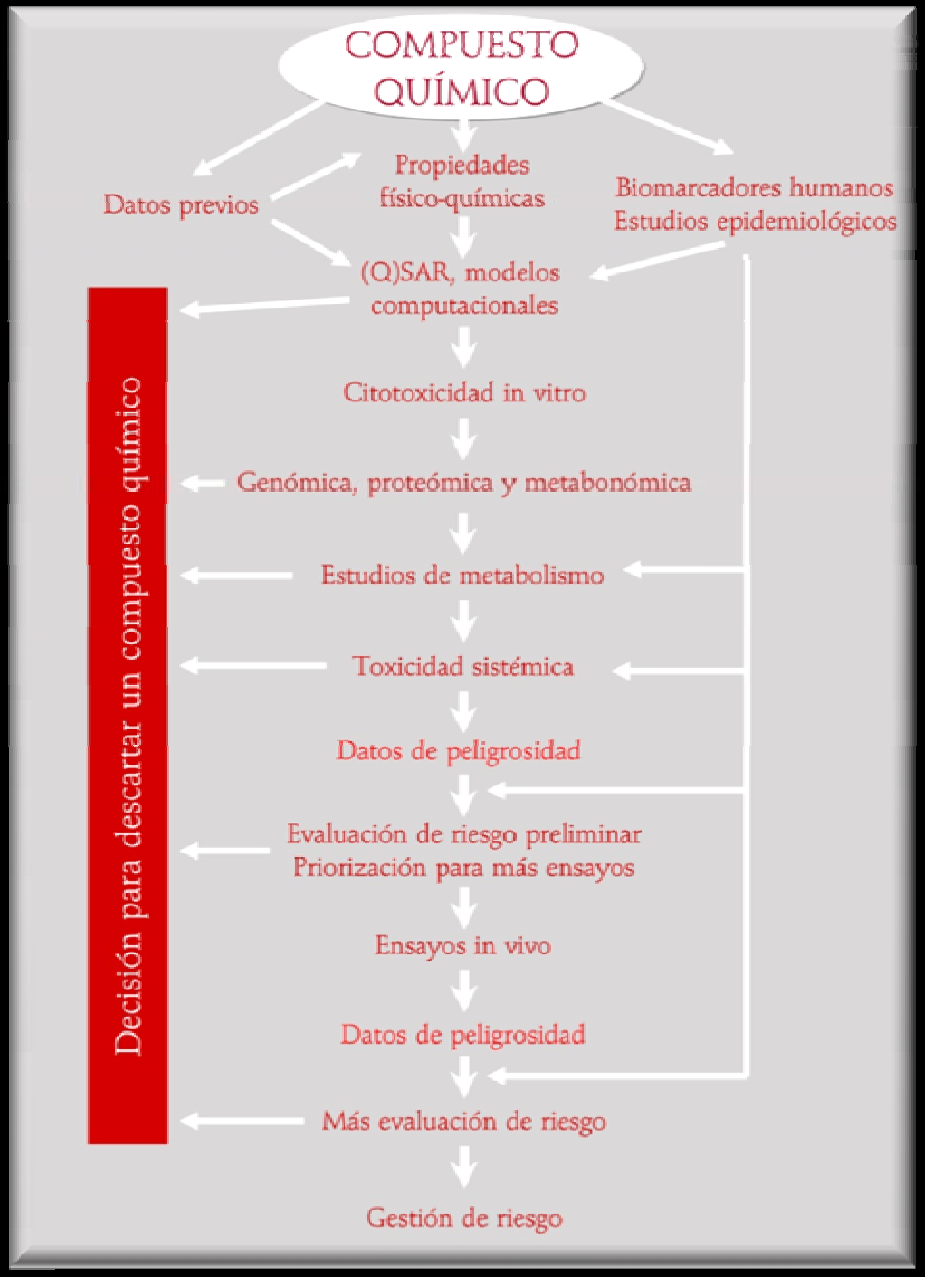
Evaluación

Autorización

Restricción

Annex V-VIII

Before new tests are carried out to determine the properties listed in this Annex, all available in vitro data, in vivo data, historical data, data from valid Q(SAR) and data from structurally related substances (read-across approach) shall be assessed first.



Esquema de una estrategia integrada de ensayo inteligente.
(Modificada de Boghal y col. 2005).

Ensayos *in vitro*

Refinamiento

Reducción

Reemplazo

Fracciones subcelulares
Láminas de tejidos
Órganos perfundidos
Explantes
Reagregados celulares
Cultivos organotípicos 3D
Cultivos primarios
Líneas celulares

VENTAJAS

Versatilidad del diseño experimental.
Resultados más rápidos y fiables
Menor coste económico.
Automatización y/o monitorización.
Utilización de material biológico humano.

LIMITACIONES

Simplicidad de los resultados
Complejidad de la interpretación
Dificultad de extrapolación
No sustituyen ensayos *in vivo*
Requieren validación

Ensayos *in vitro*

Registro

Evaluación

Autorización

Restricción

La normativa REACH requiere ensayos *in vitro* para todos los compuestos químicos (Anexo V) y además métodos alternativos y ensayos *in vivo* para sustancias que esten presentes en cantidades ≥ 10 Tm (Anexos VI-X).

Annexes V to VIII of the REACH proposal include lists of tests which will be involved in the chemical safety testing programme.

- **Annex V** includes standard information requirements for substances manufactured or imported in quantities of 1 tonne or more. In order to meet this requirement, data will generally be collected with non-animal tests, with Annex V listing *in vitro* skin and eye irritation, *in vitro* skin corrosivity, *in vitro* mutagenicity, and non-animal ecotoxicity. There is one animal test listed, the Local Lymph Node Assay for the determination of skin sensitisation. There is also an additional test proposed for acute toxicity; the vote will determine whether this will be an animal or non-animal test.

- **Annexes VI, VII and VIII** provide additional standard information requirements for substances manufactured or imported in quantities of 10, 100, and 1,000 tonnes or more respectively. They are mainly based on animal tests and do not include all validated non-animal tests currently available.

- **Annex X** (previously Annex V of the Dangerous Substances Directive (67/548/EC), which provides a list of test methods, does not include all *in vitro* and other non-animal tests currently available; Annex X details the animal test protocols.

Table 3 Overview of toxicological in vivo and in vitro testing methods accepted for regulatory purposes

Toxicological end point	In vivo methods (no. of OECD test guideline in brackets)	In vitro methods (no. of OECD test guideline in brackets, if applicable)
1. Acute Toxicity	Acute Toxicity	
	Oral [401, 420 (fixed dose procedure), 423 (acute toxic class method), 425 (up-and-down procedure)][f],	
	dermal (402, 434[a])[f] or	
	inhalation [403, 433[b] (fixed concentration procedure), 436[c] (acute toxic class (ATC) method)][f]	
2. Dermal resorption	Skin absorption (427)[f]	Skin absorption (428)[f]
3. Phototoxicity		3T3 NRU Phototoxicity test (432)[f]
4. Skin irritation and corrosion	Acute dermal irritation/corrosion (404)[f]	Transcutaneous electrical resistance test (TER) (430)[f]
		Skin corrosion: human skin model test (431)[f]
		Membrane barrier test method for skin corrosion (435)[f]
5. Eye irritation and corrosion	Acute eye irritation/corrosion (405)[f]	Embryonated chicken egg (HET-CAM)[g]
		Isolated bovine cornea (BCOP)[g]
		Isolated chicken eye (CEET)[g]
		Isolated rabbit eye (IRE)[g]
6. Sensitation	Skin Sensitisation (406)[f]	
	Local lymph node assay (LLNA) (429)[f]	
7. Hepatotoxicity 8. Nephrotoxicity 9. Hematotoxicity 10. Cardiotoxicity	Repeated dose toxicity study in rodents oral (28 day) (407)[f], dermal (21/28–day) (410)[f] or inhalation (28-day or 14-day study) (412)[f] Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (422)[f] Repeated dose 90-day toxicity study oral in rodents (408)[f] or non-rodents (409)[f], dermal (411)[f] or inhalation (413)[f] Chronic toxicity studies (452)[f]	

Lilienblum et al. (2008). Arch Toxicol 82: 211-236

Table 3 Overview of toxicological in vivo and in vitro testing methods accepted for regulatory purposes

Toxicological end point	In vivo methods (no. of OECD test guideline in brackets)	In vitro methods (no. of OECD test guideline in brackets, if applicable)
11. Endocrine effects	Two-generation reproduction toxicity study (416)[f]	
	Repeated dose 28-day oral toxicity study in rodents; updated with parameters for endocrine effects (May 2007 version) (407[d])[f]	
12. Neurotoxicity	Delayed neurotoxicity of organophosphorus substances following acute exposure (418)[f]	
	Delayed neurotoxicity of organophosphorus substances, 28-day repeated dose study (419)[f]	
	Neurotoxicity study in rodents (424)[f]	
13. Immunotoxicity		
14. Prenatal development	Prenatal developmental toxicity study (414)[f]	Whole embryo culture (WEC)[g]
		Micromass Test (MM)[g]
		Embryonic stem cell test (EST)[g]
Pre- and postnatal development	Two-generation reproduction toxicity study (416)[f]	
	Developmental neurotoxicity study (426)[f]	
15. Fertility	Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (422)[f]	
	Reproduction/developmental toxicity screening test (421)[f]	
	Repeated dose 28-day toxicity study (407, 410, 412)[f]	
	Repeated dose 90-day toxicity study (408, 409, 411, 413)[f]	
	One-generation reproduction toxicity study (415)[f]	
	Two-generation reproduction toxicity study (416)[f]	

Lilienblum et al. (2008). Arch Toxicol 82: 211-236

Table 3 Overview of toxicological in vivo and in vitro testing methods accepted for regulatory purposes

Toxicological end point	In vivo methods (no. of OECD test guideline in brackets)	In vitro methods (no. of OECD test guideline in brackets, if applicable)
16. Genotoxicity, mutagenicity	Mammalian erythrocyte micronucleus test (474)[f]	Bacterial reverse mutation test (471)[f]
	Mammalian bone marrow chromosomal aberration test (475)[f]	*Saccharomyces cerevisiae*, gene mutation assay (480)[f]
	Sex-linked recessive lethal test in *Drosophila melanogaster* (477)[f]	*Saccharomyces cerevisiae*, mitotic recombination assay (481)[f]
	Rodent dominant lethal test (478)[f]	Mammalian chromosome aberration test (473)[f]
	Mammalian spermatogonial chromosome aberration test (483)[f]	Mammalian cell gene mutation test (476)[f]
	Mouse spot test (484)[f]	Micronucleus test (487[e])[f]
	Mouse heritable translocation assay (485)[f]	Sister chromatid exchange assay in mammalian cells (479)[f]
	Unscheduled DNA synthesis (UDS) test with mammalian liver cells (486)[f]	DNA damage and repair, UDS in mammalian cells (482)[f]
16. Carcinogenicity	Carcinogenicity studies (451)[f]	Cell transformation assays (SHE, Balb/c 3T3, C3H10T)[g]
	Combined chronic toxicity/carcinogenicity Studies (453)[f]	

[a] Draft Test Guideline 434 Acute Dermal toxicity-Fixed Dose Procedure, May 2004 Version (http://www.oecd.org/searchResult/0,3400,en_2649_34377_1_1_1_1_1,00.html)

[b] Draft Test Guideline 433: acute inhalation toxicity—fixed concentration procedure, June 2004 Version (http://www.oecd.org/searchResult/0,3400,en_2649_34377_1_1_1_1_1,00.html)

[c] Draft New Test Guideline 436: acute inhalation toxicity—acute toxic class (ATC) Method, December 2004 Version (http://www.oecd.org/searchResult/0,3400,en_2649_34377_1_1_1_1_1,00.html)

[d] Draft Updated Test Guideline 407: repeated dose 28-day oral toxicity study in rodents; Updated with parameters for endocrine effects, May 2007 version (http://www.oecd.org/document/55/0,3343,en_2649_34377_2349687_1_1_1_1,00.html)

[e] Draft Proposal for a New Guideline 487: in vitro micronucleus test, December 2006 Version (http://www.oecd.org/document/55/0,3343,en_2649_34377_2349687_1_1_1_1,00.html)

[f] OECD Guidelines for the testing of chemicals (http://oberon.sourceoecd.org/vl=5003995/cl=13/nw=1/rpsv/cw/vhosts/oecdjournals/1607310x/v1n4/contp1-1.htm);

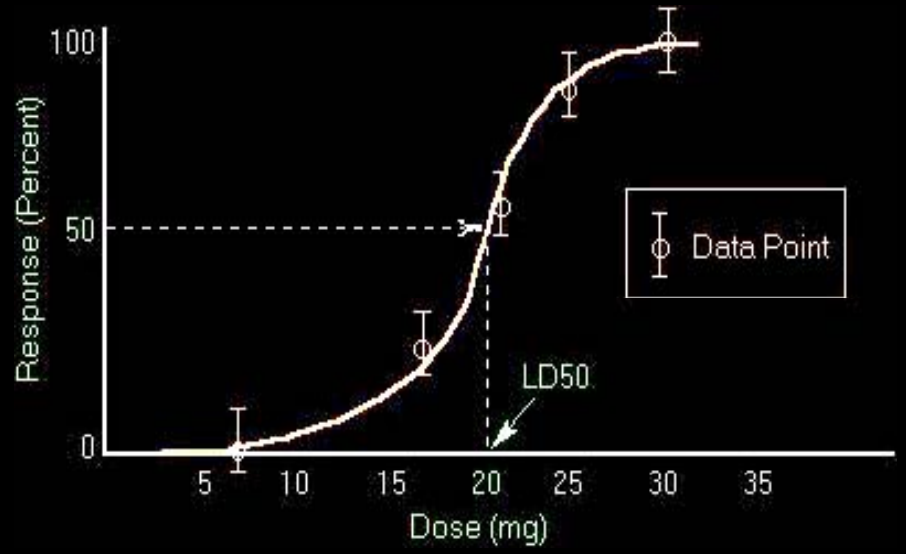
[g] Tests listed only in Annex V of Directive 67/548/EEC or accepted by regulatory authorities of some EU Member States

Lilienblum et al. (2008). Arch Toxicol 82: 211-236

Ensayos *in vitro*

Annex V (≥ 1 tonne)

- Skin irritation/corrosion (*in vitro*)
- Eye irritation (*in vitro)*
- Skin sensitization (LLNA)
- Mutagenicity (*in vitro*)
- Acute toxicity test (*in vivo / in vitro)*



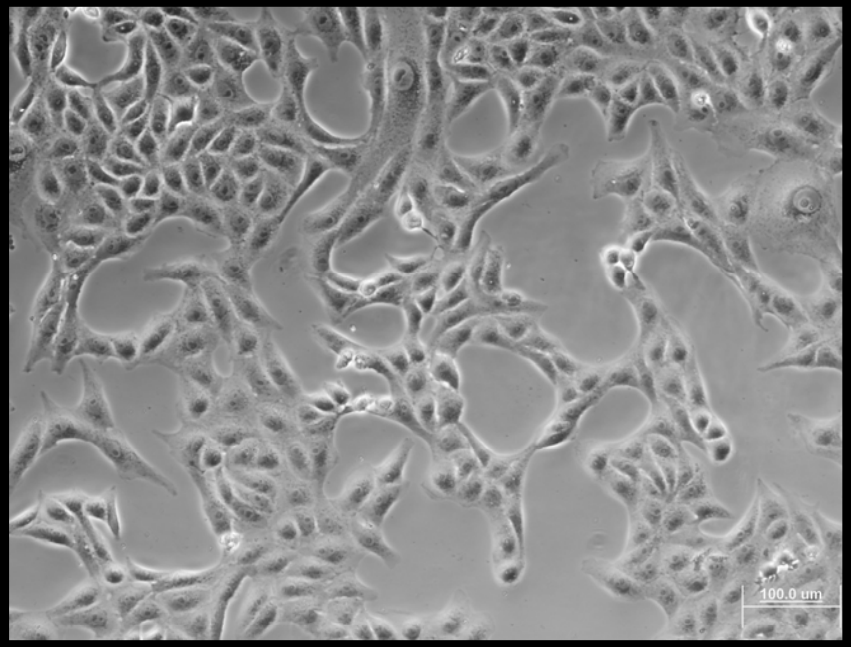
The principles of the three alternative methods

	420 (Fixed Dose)	423 (Acute Toxic Class)	425 (Up and Down)
Methodology	Single bolus dose. Young adult rats (one sex). Oral gavage with constant volume or concentration, clinical observations, bodyweight, mortality over 14 days. Necropsy at termination.		
Sighting study	Yes	No	No
Dose levels	Fixed doses of 5, 50, 300, 2000 (5000) mg/kg 5 rats per dose level	Fixed doses of 5, 50, 300, 2000 (5000) mg/kg 3 rats per dose level	Starting at best estimate of LD50 (or 175 mg/kg) and using dose progression factor of 3.2, single animals dosed until one of three stopping criteria met
Aim	Identify lowest fixed dose causing **evident toxicity**	Identify lowest fixed dose causing **mortality**	Estimate LD50
Output	Range estimate of LD50 Signs of acute toxicity. Target organ(s)	Range estimate of LD50 Signs of acute toxicity. Target organ(s)	Point estimate of LD50 with confidence intervals. Signs of acute toxicity. Target organ (s)

Ensayos *in vitro*

Annex V (≥ 1 tonne)

Skin irritation/corrosion (*in vitro*)
Eye irritation (*in vitro)*
Skin sensitization (LLNA)
Mutagenicity (*in vitro*)
Acute toxicity test (*in vivo / in vitro)*



Refinamiento

Reducción

Reemplazo

Órganos perfundidos
Explantes
Láminas de tejidos
Cultivos organotípicos 3D
Reagregados celulares
Cultivos primarios
Líneas celulares
Fracciones subcelulares

Ensayos *in vitro*

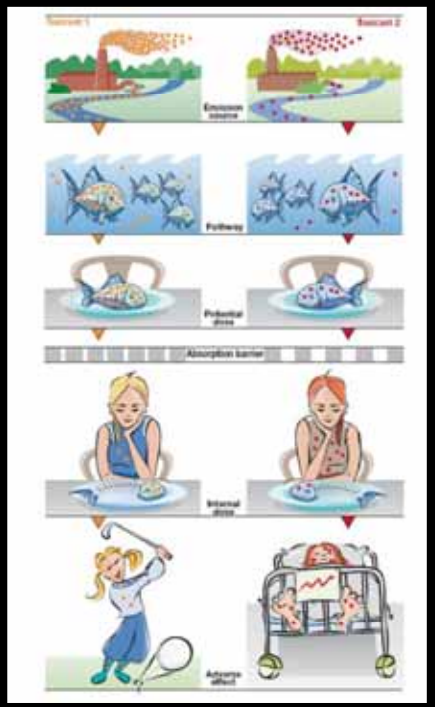
Annex V (≥ 1 tonne)

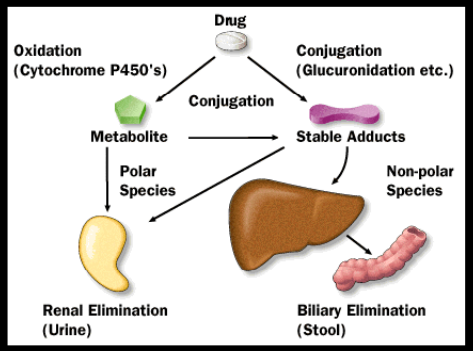
- Skin irritation/corrosion (*in vitro*)
- Eye irritation (*in vitro)*
- Skin sensitization (LLNA)
- Mutagenicity (*in vitro*)
- Acute toxicity test (*in vivo / in vitro)*

CITOTOXICIDAD

Efectos adversos o interferencias con estructuras y/o propiedades esenciales para la supervivencia, proliferación y/o función celular

Efectos tóxicos → Alteración funcional



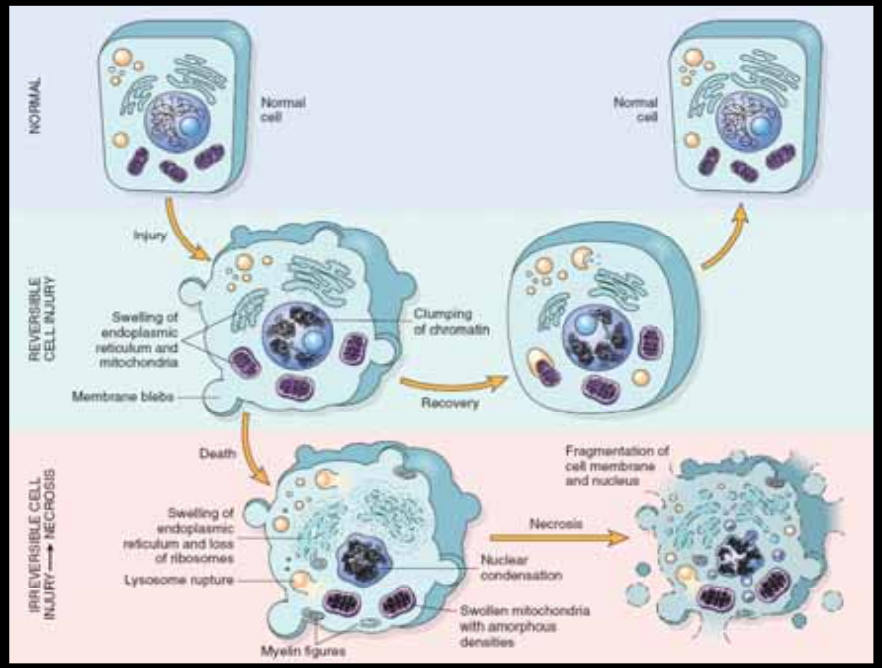


Dosis efectiva

☺ Metabolito inocuo y eliminación.

☹ Eliminación incompleta del tóxico y sus metabolitos.

☠ Metabolito activo más tóxico que el compuesto original.



Ensayos *in vitro*

Annex V (≥ 1 tonne)

- Skin irritation/corrosion (*in vitro*)
- Eye irritation (*in vitro)*
- Skin sensitization (LLNA)
- Mutagenicity (*in vitro*)
- Acute toxicity test (*in vivo / in vitro)*

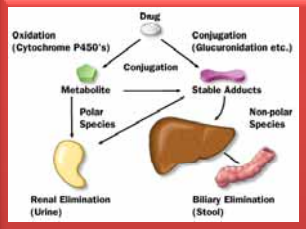
CITOTOXICIDAD
Efectos adversos o interferencias con estructuras y/o propiedades esenciales para la supervivencia, proliferación y/o función celular

Efectos tóxicos → Alteración funcional

Citotoxicidad basal

Implica la alteración de al menos un proceso o estructura celular.

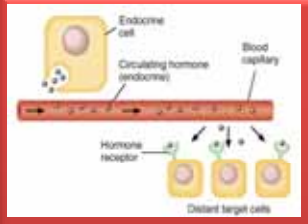
Sensibilidad es similar en tipos celulares de distinto origen.



Citotoxicidad selectiva

Refleja daños órgano-específicos característicos.

Diferente sensibilidad en tipos celulares de distinto origen.



Citotoxicidad específica

Afecta a funciones intercelulares vitales para el organismo.

Diferente sensibilidad en tipos celulares de distinto origen.

Ensayos *in vitro*

Annex V (≥ 1 tonne)

- Skin irritation/corrosion (*in vitro*)
- Eye irritation (*in vitro)*
- Skin sensitization (LLNA)
- Mutagenicity (*in vitro*)
- Acute toxicity test (*in vivo / in vitro)*

CITOTOXICIDAD

Efectos adversos o interferencias con estructuras y/o propiedades esenciales para la supervivencia, proliferación y/o función celular

Efectos tóxicos → Alteración funcional

Citotoxicidad basal

Implica la alteración de al menos un proceso o estructura celular.

Sensibilidad es similar en tipos celulares de distinto origen.

La mayoría de compuestos químicos reflejan su toxicidad a nivel basal.

Un reducido número de sustancias son tóxicas por interferencia con funciones organo-especificas o extracelulares.

Ensayos *in vitro*

Annex V (≥ 1 tonne)

- Skin irritation/corrosion (*in vitro*)
- Eye irritation (*in vitro)*
- Skin sensitization (LLNA)
- Mutagenicity (*in vitro*)
- Acute toxicity test (*in vivo / in vitro)*

CITOTOXICIDAD
Efectos adversos o interferencias con estructuras y/o propiedades esenciales para la supervivencia, proliferación y/o función celular

Efectos tóxicos → Alteración funcional

Citotoxicidad basal

Implica la alteración de al menos un proceso o estructura celular.

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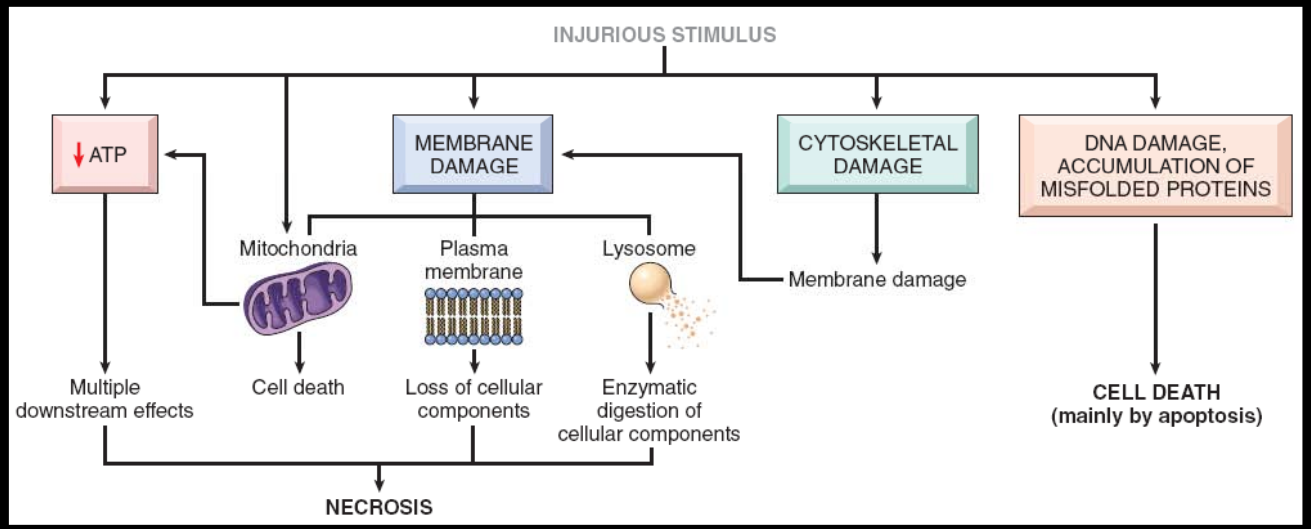
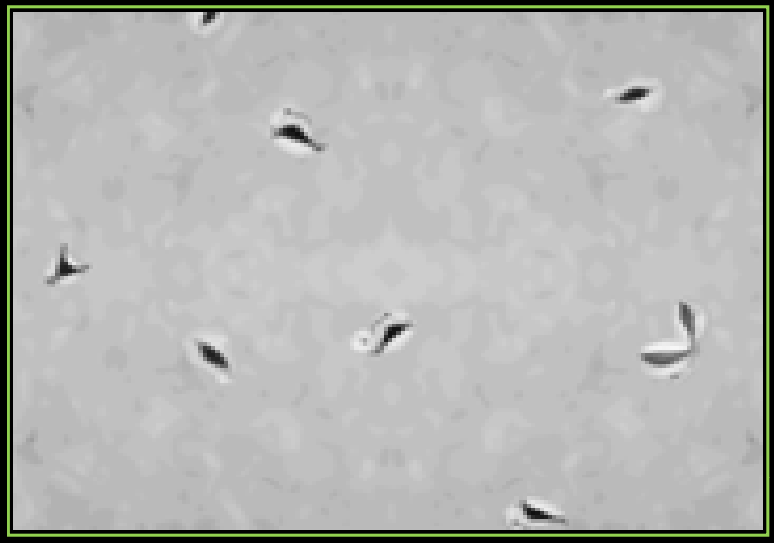


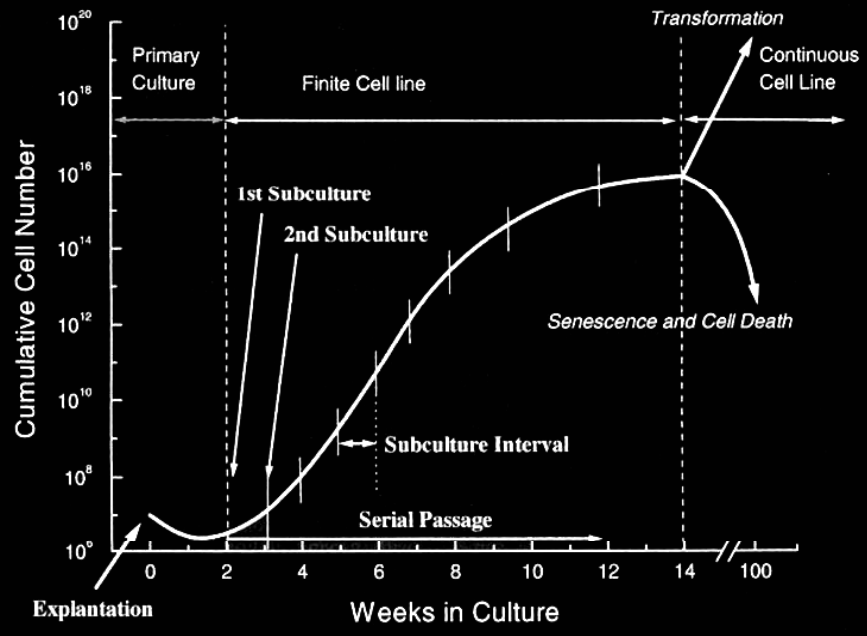
Toxicidad aguda *in vitro*

La calidad y especificidad de los datos generados por los ensayos *in vitro* dependen de varios factores:

- Correcta elección del modelo biológico.
- Selección de los parámetros adecuados
- Valor predictivo del modelo.



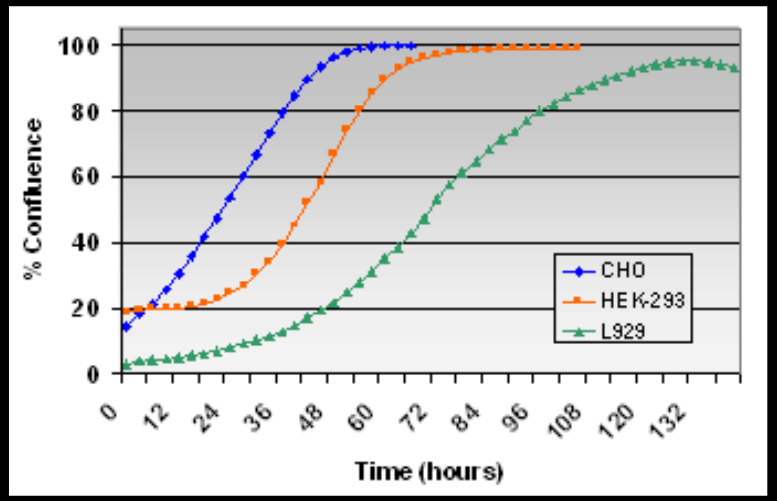
	C. primarios	Líneas
Ploidia	Diploide / Euploide	Aneuploide
Transformación	Normal	Transformada
Inhibición por contacto	Si	No
Mantenimiento	Cíclico	Posible quiescencia
Requerimientos de suero	Elevados	Bajos
Eficiencia de clonaje	Baja	Elevada
Marcadores	Pueden expresar marcadores especificos	Se suelen perder
Funciones especializadas	Se mantienen	Se suelen perder
Tasa de crecimiento	Baja (24 a 96 h tiempo de replicación)	Rápida (12 a 24 h)



Modelos celulares

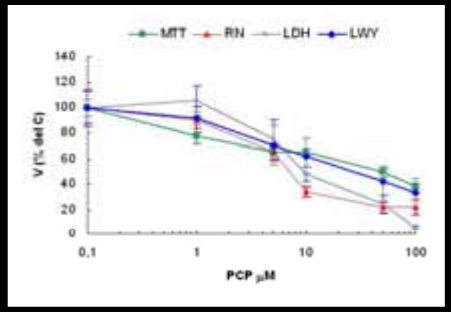
Table 1 | **Popular mammalian culture cells**

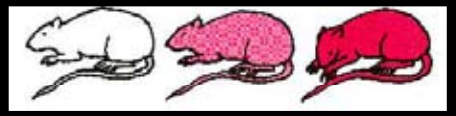
Cell name	Origin	Characteristics	Reference
HeLa	Human cervical carcinoma	Epithelial-like, aneuploid	124
293	Human embryonic kidney	Epithelial, aneuploid	125
MCF-7	Human breast	Epithelial, aneuploid	126
Saos-2	Human osteosarcoma	Epithelial, aneuploid	127
PC-3	Human prostrate carcinoma	Epithelial, aneuploid	128
Jurkat	Human T-cell leukaemia	Lymphoblasts, pseudodiploid	129
Cos-7	Monkey kidney	Fibroblasts, aneuploid	130
Vero	African green monkey	Fibroblasts, aneuploid	131
CHO	Chinese hamster ovary	Epithelial-like, pseudodiploid	132
L-929	Mouse connective tissue	Fibroblasts, aneuploid	133
3T3	Mouse connective tissue	Fibroblasts, aneuploid	134
BHK-21	Syrian baby hamster kidney	Fibroblasts, diploid	135
HUVEC	Human umbilical-vein endothelial cells	Epithelial, diploid	136
ES-D3	Mouse embryonic stem cells	Epithelial, diploid	137



Las líneas celulares de mamífero son las más empleadas.

- Facilidad de propagación.
- Obtención de resultados reproducibles y relevantes.

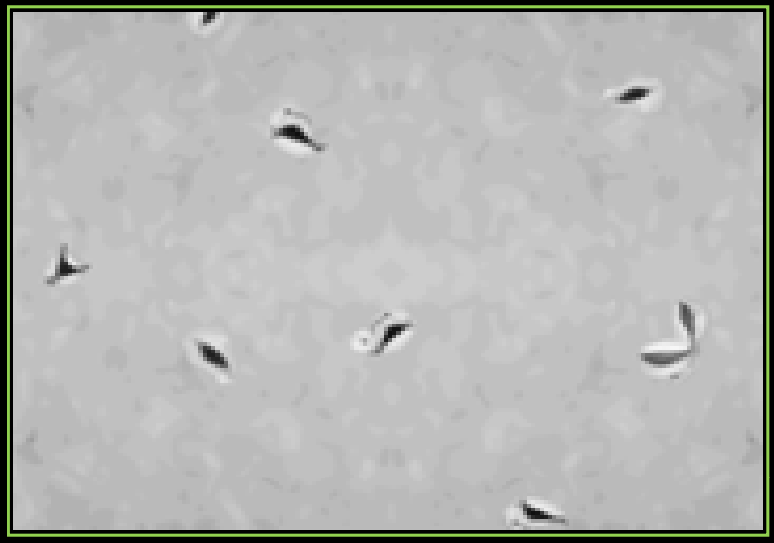


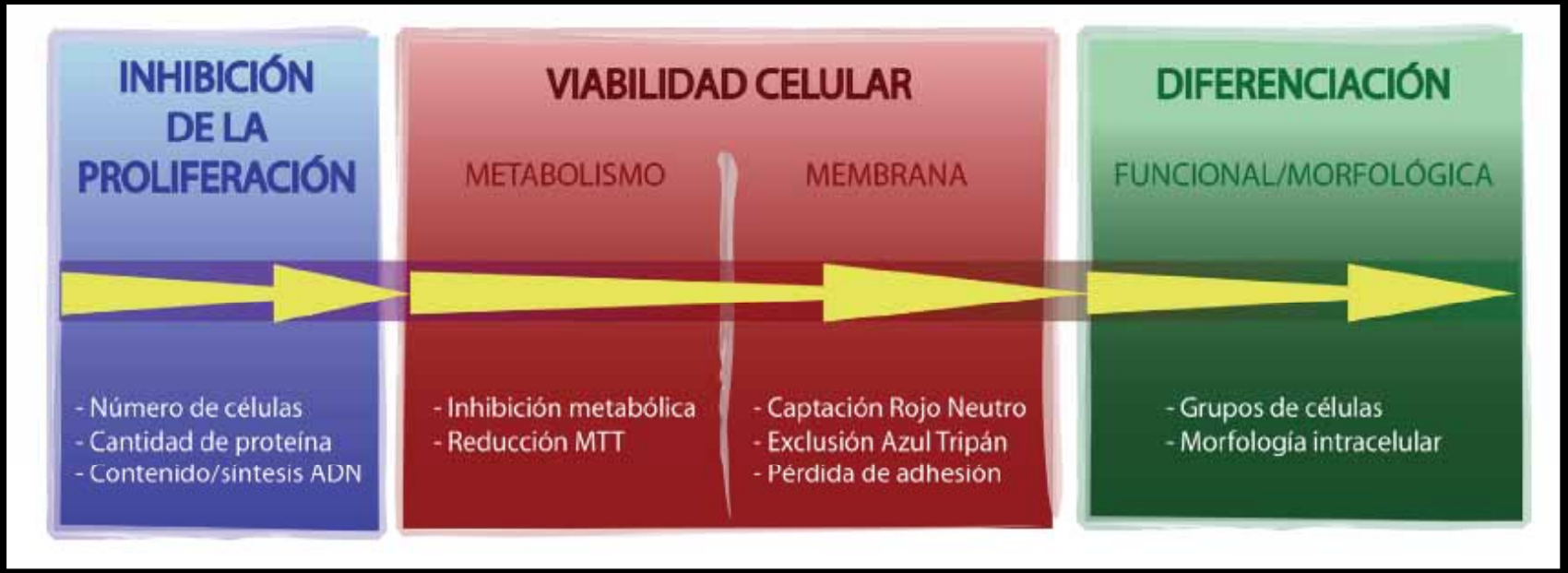


Toxicidad aguda *in vitro*

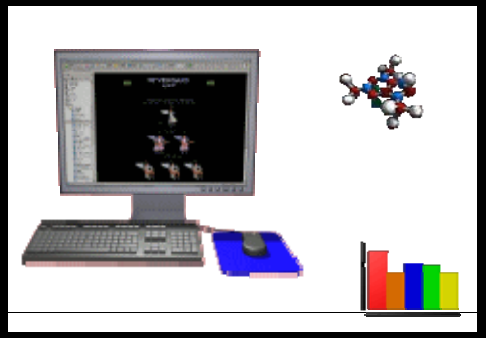
La calidad y especificidad de los datos generados por los ensayos *in vitro* dependen de varios factores:

- Correcta elección del modelo biológico.
- Selección de los parámetros adecuados
- Valor predictivo del modelo.



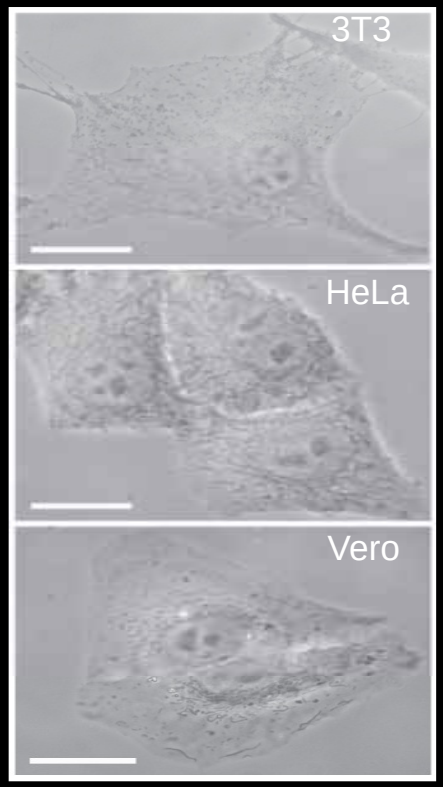


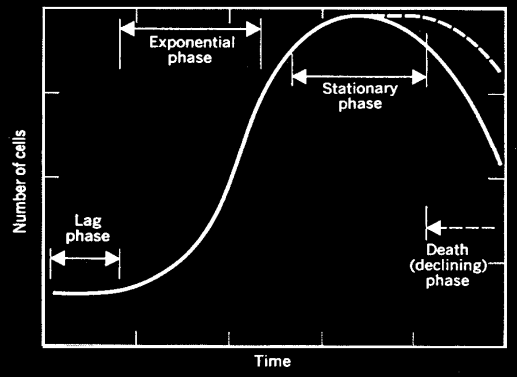
Toxicidad aguda *in vitro*



Recopilación de información

- Propiedades fisico-químicas
- Presencia en el medio
- Estudios epidemiológicos
- Datos toxicológicos previos *in vivo* e *in vitro*





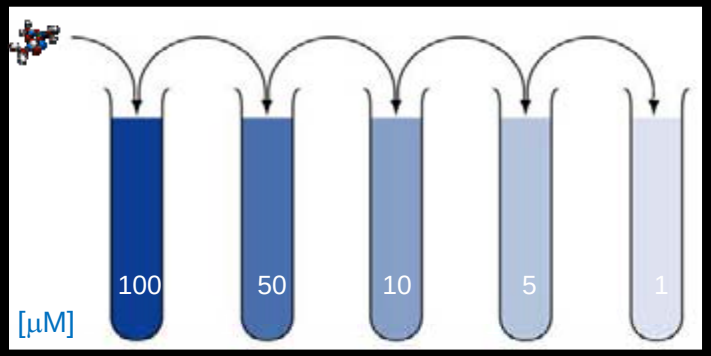
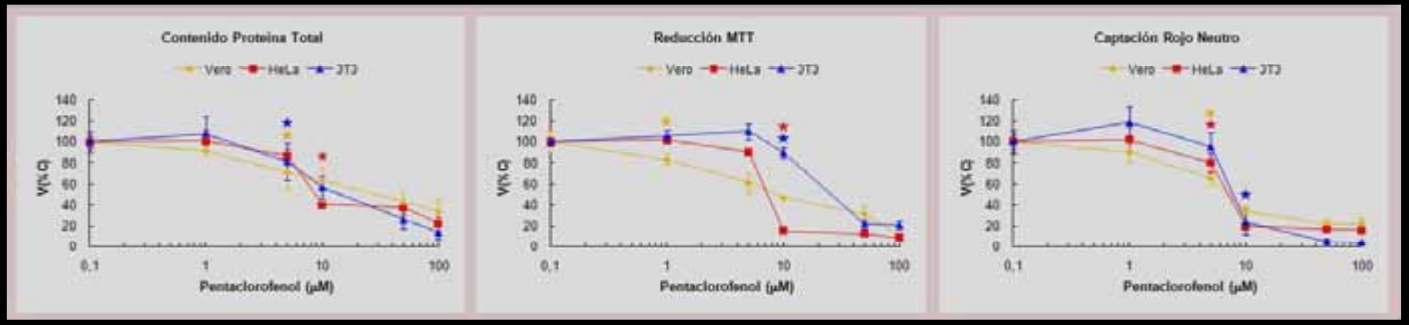


Figura 13

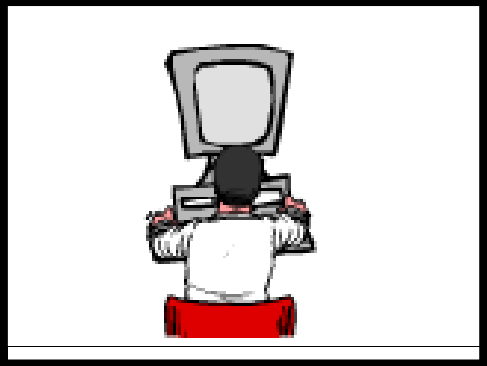
Esquema temporal general que indica el desarrollo de los tratamientos de los cultivos con los compuestos de referencia.

Toxicidad aguda *in vitro*

Lectura espectrofotométrica



Parámetros clásicos de citotoxicidad basal



Análisis de datos

Morfología general

Retículo mitocondrial

Compartimento endosomal

Citoesqueleto

- Microfilamentos
- Microtúbulos

Toxicidad aguda *in vitro*


Toxicology in Vitro xxx (2008) xxx–xxx

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

In vitro assessment of the cytotoxic and mutagenic potential of perfluorooctanoic acid

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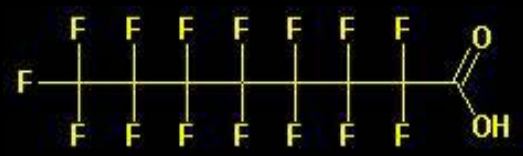
[b] Grupo de Mutagénesis Ambiental, Centro de Ciencias Medioambientales CSIC, 28006 Madrid, Spain



CAS 335-67-1

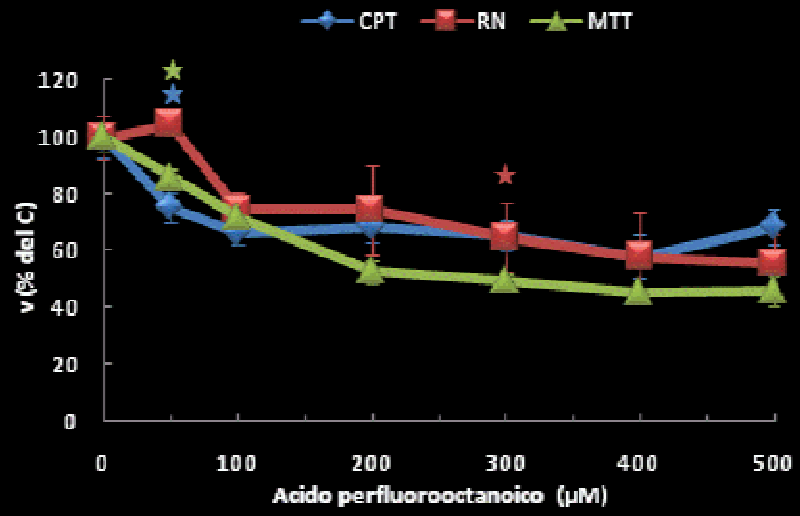
Estudios recientes de biomonitorización revelan el predominio de los niveles de PFCs, particularmente PFOA, en tejidos de la fauna de todo el mundo así como en el suero de las poblaciones generales humanas .

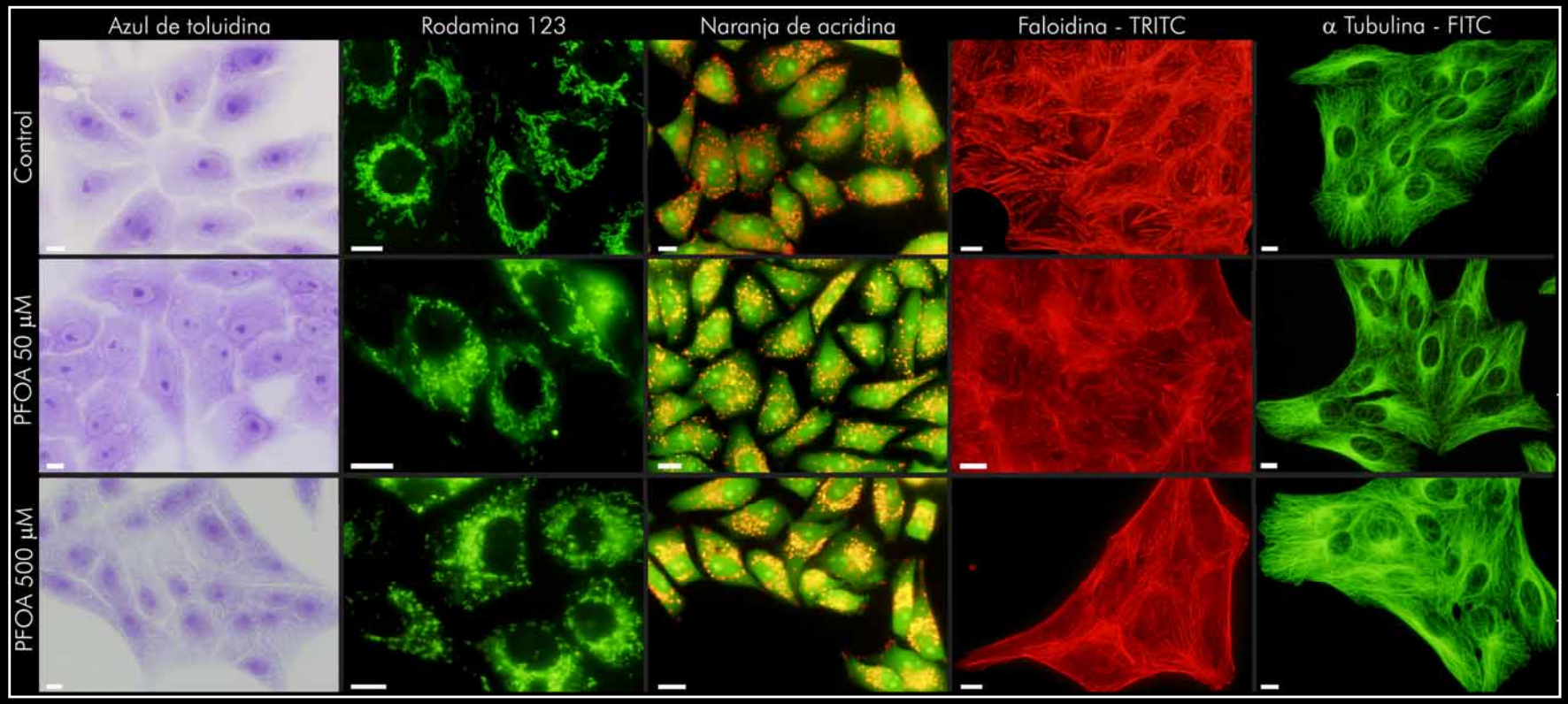
Las investigaciones no sugieren una asociación consistente entre la exposición al PFOA y problemas de salud significativos aunque el compuesto es considerado como un “probable” carcinógeno para los seres humanos (EPA, 2002).

Toxicidad aguda *in vitro*

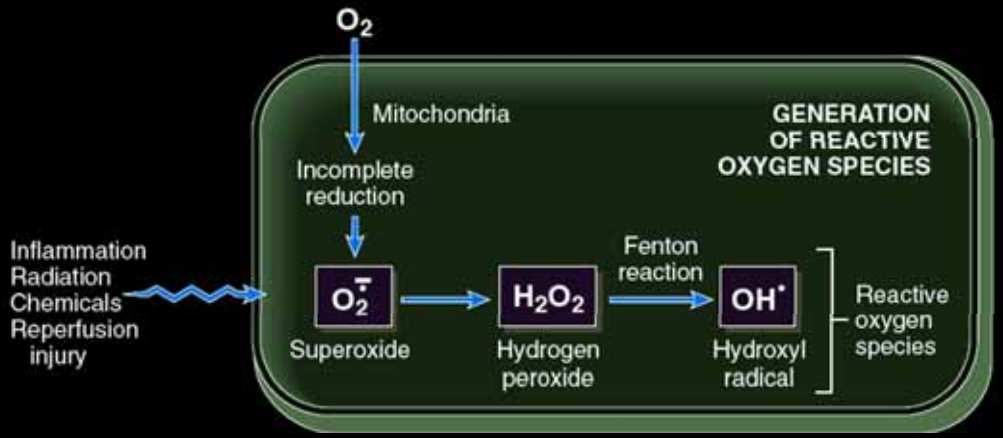
Los ensayos de citotoxicidad basal muestran que las mitocondrias son la principal diana celular del PFOA.

Estrés oxidativo
Proliferación
Muerte celular





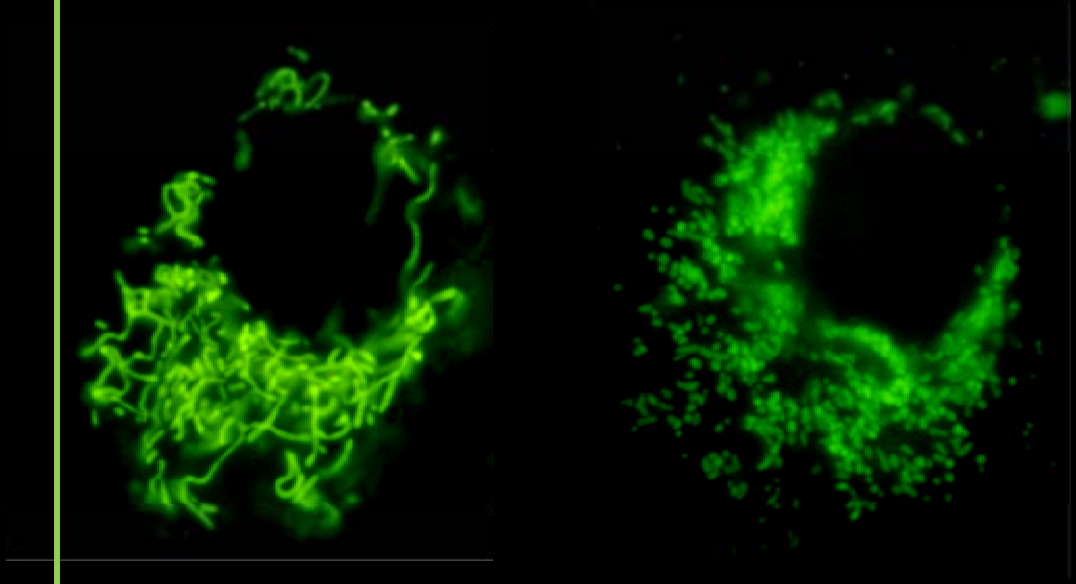
Toxicidad aguda *in vitro*

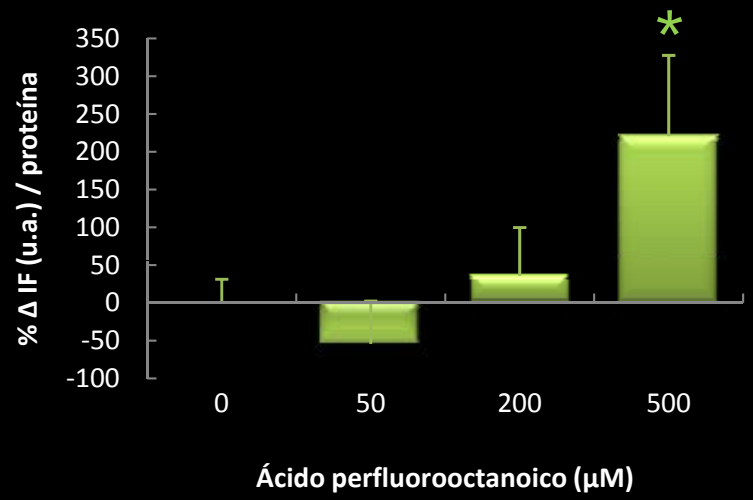


Disfunción mitocondrial

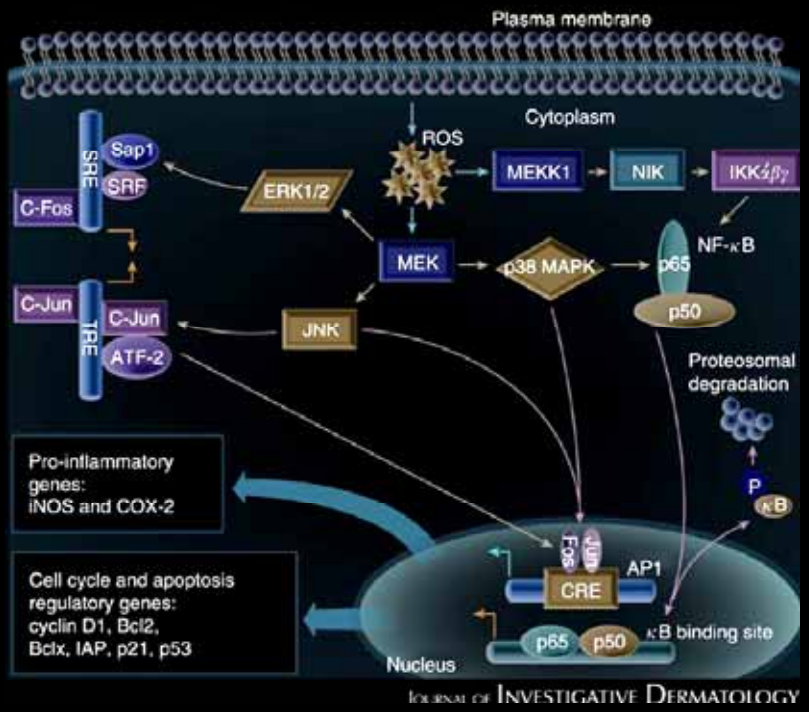
- Generación de radicales libres de oxígeno mediante cuantificación de la fluorescencia intracelular de diclorofluoresceína.

ESTRÉS OXIDATIVO





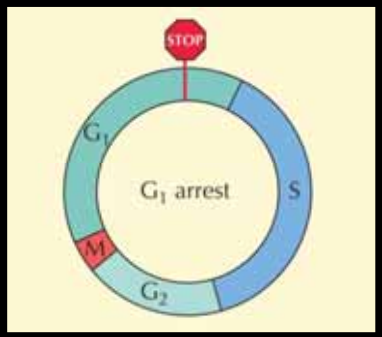
Toxicidad aguda *in vitro*

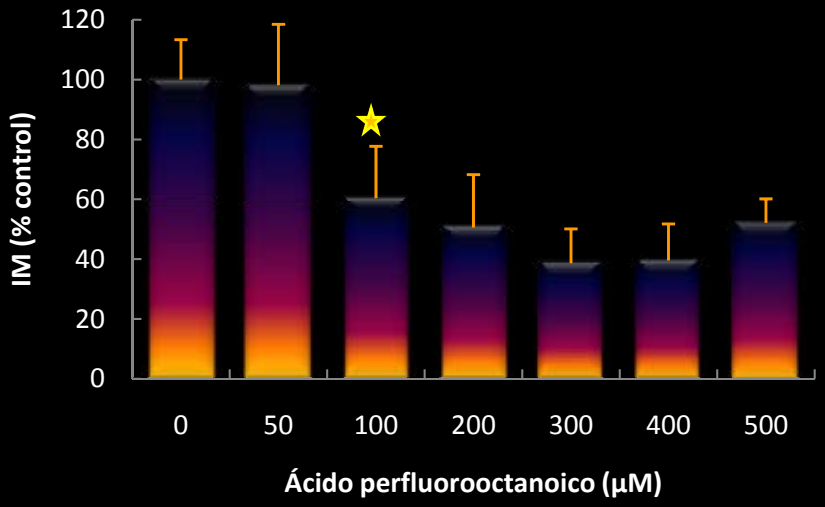


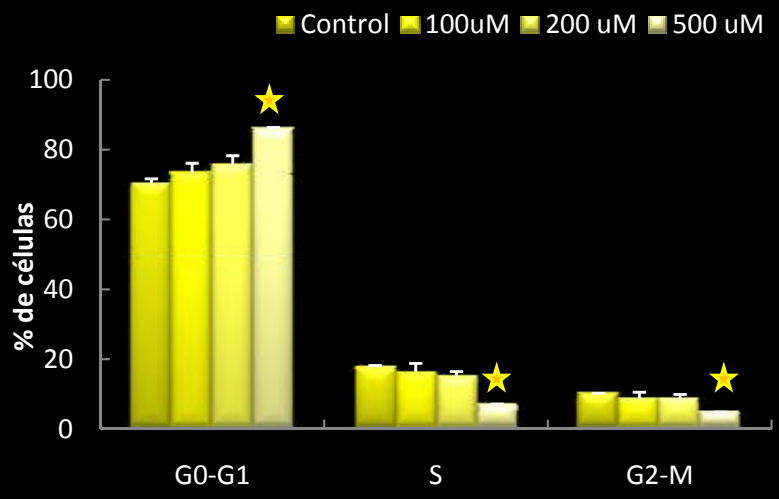
Proliferación celular

- Recuento de índices mitóticos
- Citometría de flujo

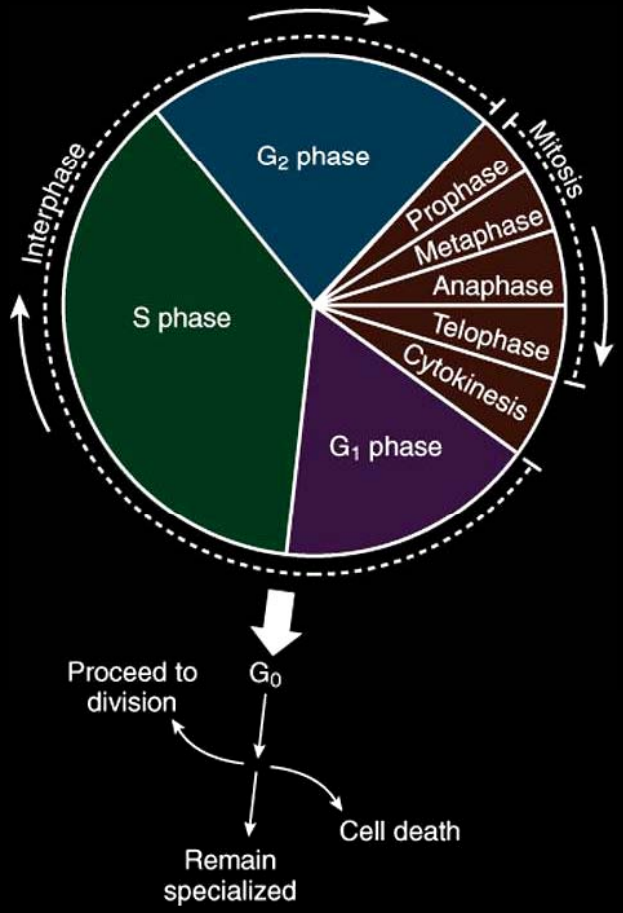
Parada de ciclo en fase G0 /G1





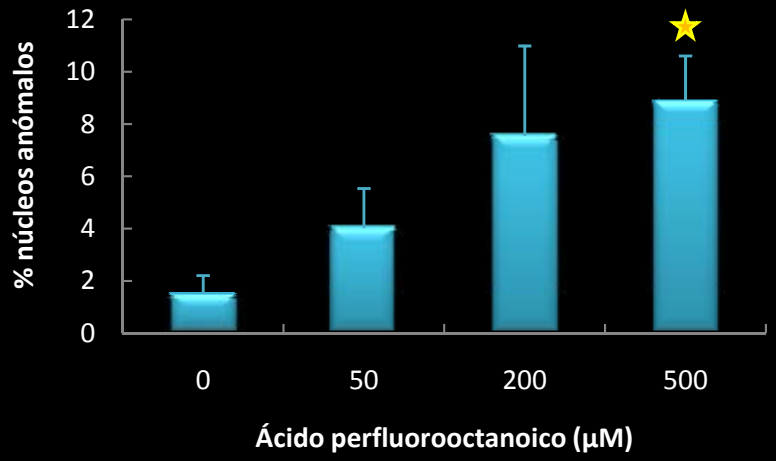


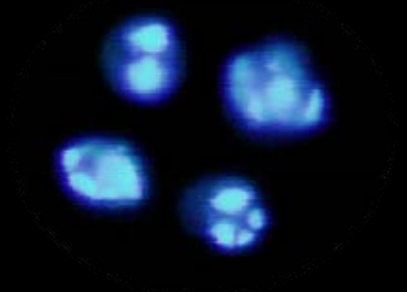
Toxicidad aguda *in vitro*



Muerte celular

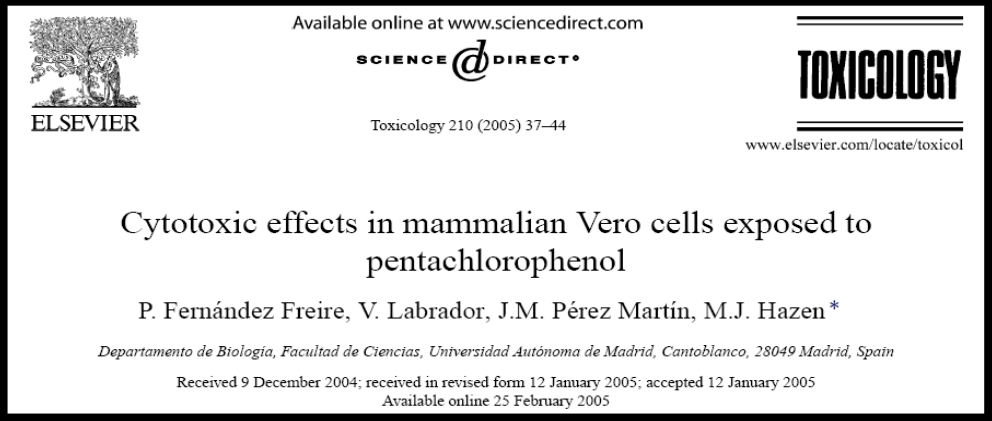
- Observación y recuento de morfologías nucleares.





NÚCLEOS APOPTÓTICOS

Toxicidad aguda *in vitro*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Toxicology 210 (2005) 37–44
TOXICOLOGY
www.elsevier.com/locate/toxicol

Cytotoxic effects in mammalian Vero cells exposed to pentachlorophenol

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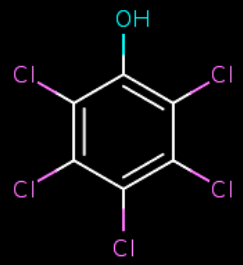
Received 9 December 2004; received in revised form 12 January 2005; accepted 12 January 2005
Available online 25 February 2005



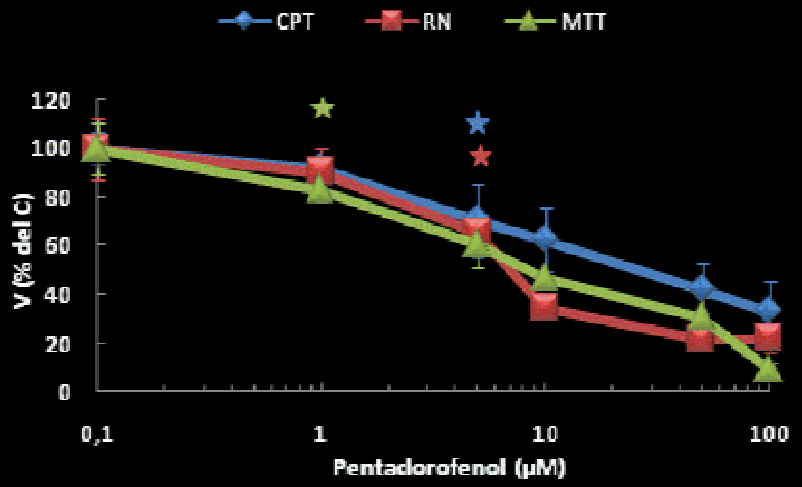
CAS 87-86-5

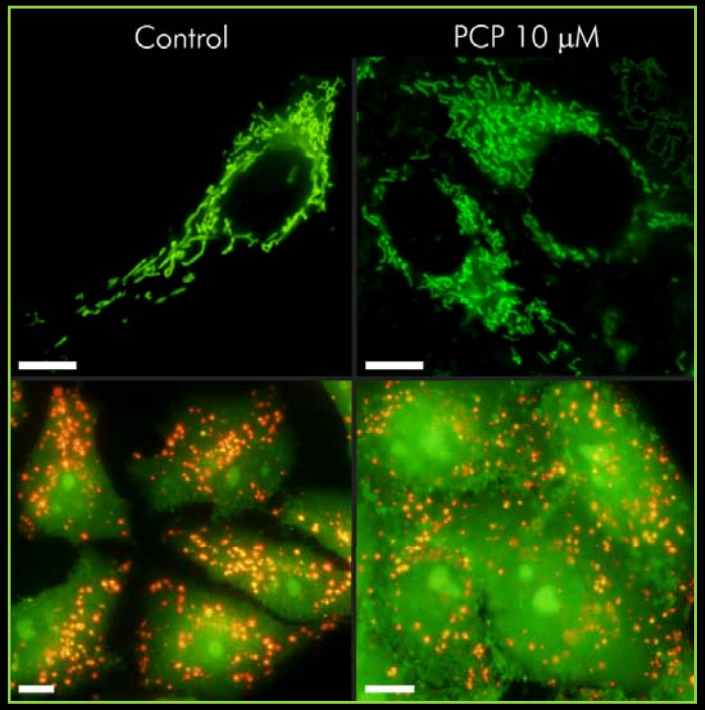
A pesar de que el uso está restringido en E.E.U.U. y Europa, se han detectado niveles de PCP en tejidos de la fauna de todo el mundo así como en el suero de las poblaciones generales humanas.

Es considerado un contaminante prioritario debido a su lenta e incompleta biodegradación y puede producir problemas de salud significativos al ser considerado como disruptor endocrino y “probable” carcinógeno en humanos (ATSDR, 2001).

Toxicidad aguda *in vitro*

Los ensayos de citotoxicidad basal muestran que las mitocondrias y los lisosomas son dianas intracelulares celulares del PCP.





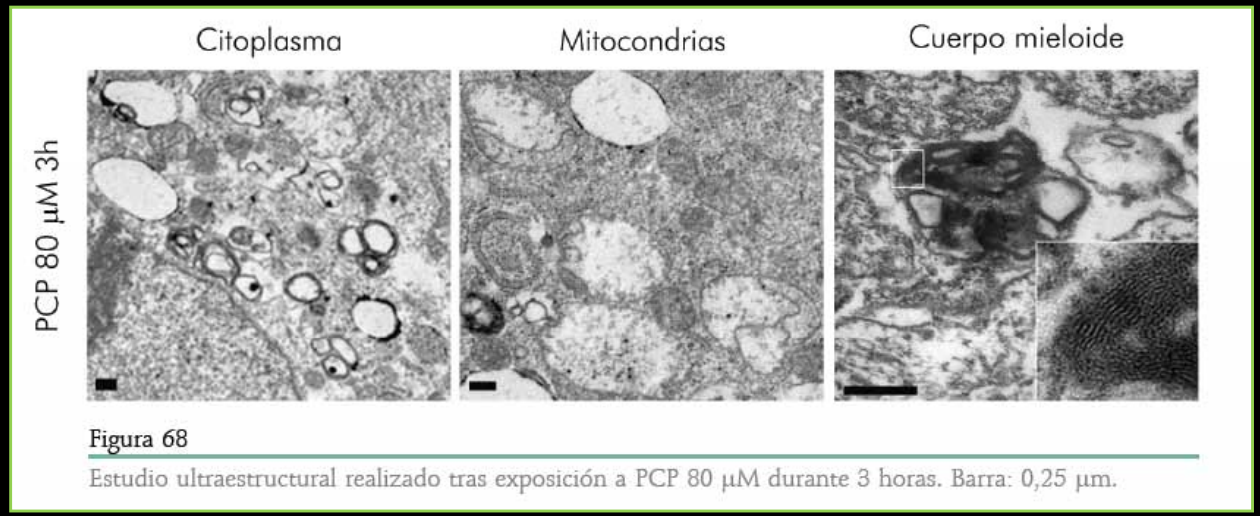


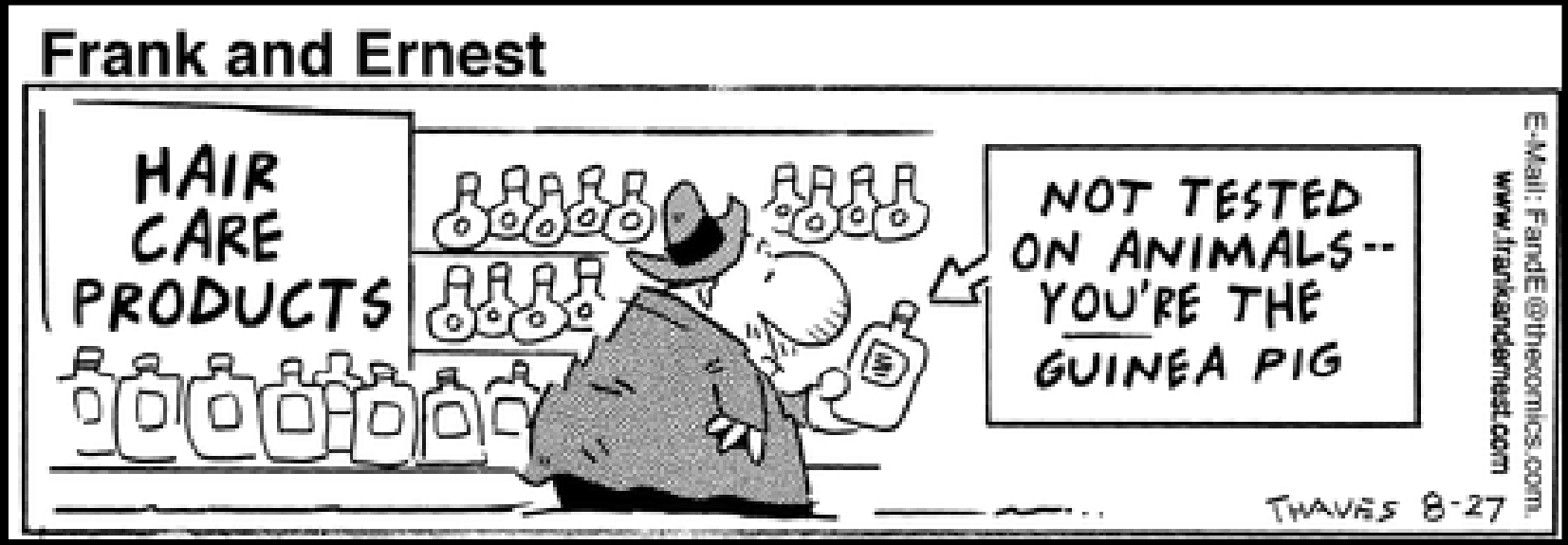
Figura 68

Estudio ultraestructural realizado tras exposición a PCP 80 µM durante 3 horas. Barra: 0,25 µm.

Mitocondrias y compartimento endosomal afectados

Lisosomas primera diana subcelular

Toxicidad aguda *in vitro*



Ensayos *in vitro*

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