Valutazione del rischio chimico

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Valutazione del rischio chimico

Processo chimico

(Emissioni)

(Dispersione
Trasferimenti di fase
trasformazioni ambientali)

Esposizione */ PEC*

Valutazione del rischio

*Valutazione degli **effetti** dell'esposizione a sostanze singole e a miscele / NOAEC /tossicologia*

Valutazione della tossicità per la valutazione del rischio per la salute umana (*RAoC cap.6*)

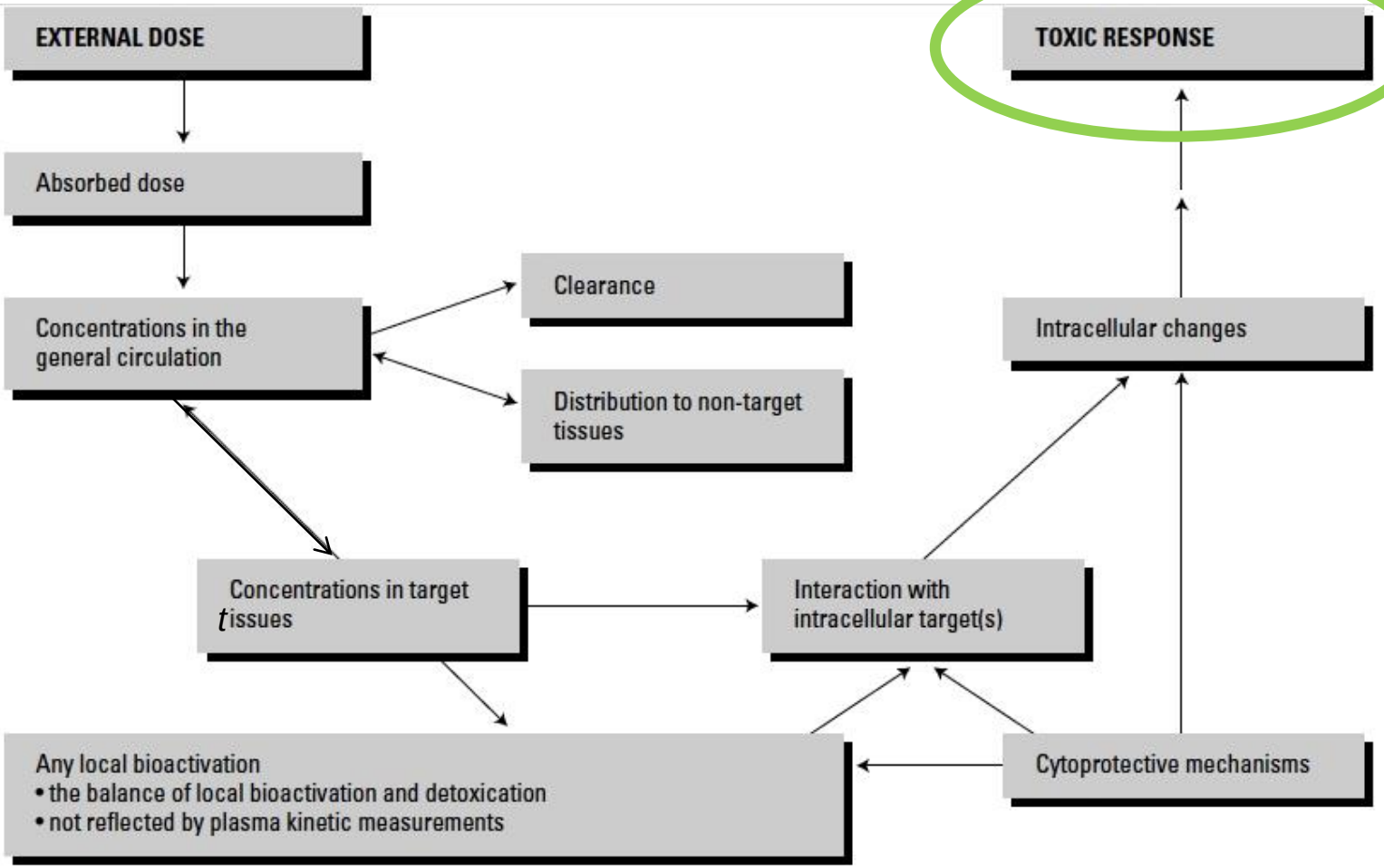


Figure 6.1. Processes leading to the generation of a toxic response [2].
Note:"Concentrations" refers to the relevant active form delivered by the general circulation and may be the parent compound or an active metabolite produced in another tissue and delivered to the target tissue or organ

Test di tossicità acuta:

singola dose o dosi ripetute nelle 24 ore
Esiti rilevati entro 14 giorni dall'esposizione
Richiesto nel REACh (esposizione orale se non sono disponibili dati di e. inalatoria) per sostanze con produzione importazione maggiore di 1 tonnellata/anno; per più di 10 t/y serve test su ulteriore via d'esposizione.
Si verifica in genere letalità

Test acuti locali

Irritazione e corrosione richiesti nel REACh per sostanze con t/y >10
"Irritant substances are non-corrosive substances which cause inflammation as evidenced by erythema and oedema of the skin and corneal opacity, iridal effects and conjunctival redness or swelling for the eye. Corrosive substances may destroy living tissues."
In vivo su conigli / no dose-risposta, no LOEL

Sensibilizzazione richiesto nel REACh per sostanze con t/y >1
Skin sensitization (-> allergic contact dermatitis) is a common form of allergy. Following skin exposure, it develops in 2 phases: induction (sensitization) and elicitation. 1) a primary immune response is triggered following a reaction between the chemical allergen and skin protein. 2)if the sensitized individual comes in contact with the same chemical allergen again in a later stage, a more pronounced secondary response is induced es Valutazione in silico http://www.caesar-project.eu/index.php?page=results&section=endpoint&ne=2

Test di tossicità acuta

Table 5.2. Acute toxicity tests

Conditions	chemical identification of substance, its purity and chemical characteristics
Route	oral, dermal, inhalatory
Experimental animals	rat, (mouse), rabbit, guinea pig, (dog)
Number of animals	5 of each sex per group
Dose levels	control and at least 3 or more if necessary for calculating LD50 or LC50[a]
Examinations	- clinical examination for signs of toxicity or death - gross examination - histopathological examination if indicated
Results	the LD50 or LC50 value for each sex at 95% confidence interval

[a] If a dose of 2000 mg/kg_{bw} does not cause acute toxicity and compound-related mortality, a full study may not be necessary.

Tossicità per dosi ripetute

Più che a dosi elevate a seguito di incidenti gli esseri umani possono essere esposti *ripetutamente a basse dosi* di contaminanti ambientali

Esempi di test con somministrazioni ripetute ad animali da esperimento sono la *prova sub-acuta di 28 giorni*, il *test subcronico di 90 giorni* e il *test cronico per la durata di vita*. Il requisito minimo di solito è la prova di 14 o 28 giorni con ratti.

In base al REACh, è richiesto uno studio di tossicità a dose ripetuta (almeno una prova di 28 giorni) per un livello annuale di produzione o di importazione di 10 tonnellate

Si ottengono informazioni su quelli che sono gli organi bersaglio, relazioni dose/risposta, NOAEL o BDC
Ratti e cani, usualmente giovani (più sensibili)

Table 5.5. Repeated dose studies (28 and 90 d)

Conditions	chemical identification of substance, its purity and chemical characteristics
Route	oral, dermal, inhalatory
Experimental animals	rat (mouse, dog)
Number of animals	5 to 10 of each sex per group[a]
Dose levels	control and at least 3 dose levels with an increment of 2 to 10 (sometimes satellite groups)
Examinations	- body weight, food consumption and water consumption - clinical examination. haematological parameters: haematocrit, haemoglobulin concentration, erythrocyte counts, total and differential leukocyte count, clotting potential; biochemical parameters: including organ function, parameters, electrolyte balance, carbohydrate metabolism, serum salts (Ca, P, Na, K, Cl), glucose, serum enzymes, urea nitrogen, albumen, serum protein, creatonin, bilirubin (lipids, hormones, methaemoglobin, choline-esterase activity), urine analysis - gross examination: daily observation and extensive examination at autopsy - organ weights - histopathological examination: of all preserved organs and tissues (30 or more) of highest dose and control. If indicated, intermediate and low dose groups
Results	information concerning effects of repeated dose exposure for parameters measured, target organ(s); if possible, mechanism of action and NOAEL

[a] For a range finding test 5 animals per sex per group may be sufficient for experiments with dogs, usually groups of 4 to 5 animals per sex are used.

Table 5.6. Chronic toxicity studies (6 to 24 months)

Conditions	chemical identification of substance, its purity and chemical characteristics
Route	oral, inhalatory
Experimental animals	rat (mouse, dog)
Number of animals	20 (dogs 4 to 5) per sex per group
Dose levels	control and at least 3 dose levels with an increment of 2 to 10 (sometimes satellite groups)
Examinations	- body weight, food consumption and water consumption during the first 13 weeks at weekly intervals and later at 4 week intervals (body weight) or 3 month intervals (food and water consumption) - clinical examination: haematological and biochemical examination and urine analysis (Table 5.5) at onset of study and at 6 month intervals - gross examination: daily observation and extensive examination at autopsy - organ weights - histopathological examination in full of all preserved organs and tissues of highest dose and controls. If indicated, of intermediate and lowest dose
Results	information concerning effects of repeated dose exposure on parameters studied, target organ(s); if possible, mechanism of toxicity and NOAEL

Genotossicità

La genotossicità si riferisce a effetti potenzialmente dannosi sul materiale genetico. Include la mutagenicità che può essere definita come l'induzione di cambiamenti trasmissibili permanenti nella quantità o struttura del materiale genetico.
I test di genotossicità forniscono anche indicazioni di altri danni al DNA attraverso *sintesi di DNA non programmato*, *scambio di cromatidi fratelli* (scambio di porzioni omologhe di DNA tra i due cromatidi costituenti un cromosoma), *rotture di filamento, formazione di addotti, ricombinazione mitotica e aberrazioni del numero di cromosomi* (aneuploidia - variazione nel numero dei cromosomi, rispetto a quello che normalmente caratterizza le cellule di un individuo).

I test di genotossicità sono molto utili come pre-screening di potenziale carcinogenicità genotossica

Table 5.8. Genotoxicity tests

Gene mutation assays	
Tests with prokaryotes	- *Salmonella typhimurium* reverse mutation assay (OECD Guideline 471)
	- *Escherichia coli* reverse mutation assay (OECD Guideline 472)
Tests with eukaryotes	- *Saccharomyces cerevisiae* gene mutation assay (OECD Guideline 480)
	- *in vitro* mammalian cell gene mutation assay (OECD Guideline 476)
	- *in vivo* sex linked recessive lethal test in *Drosophila melanogaster* (OECD Guideline 477)
Chromosomal damage assays	
In vitro tests	- mammalian cytogenic test (OECD Guideline 473)
	- chromatid exchange assay in mammalian cells (OECD Guideline 479)
In vivo tests	- mammalian bone marrow cytogenetic test for chromosomal analysis (OECD Guideline 475)
	- micronucleus test (OECD Guideline 474)
DNA damage/repair/adduct formation assays	
	- DNA adduct formation 32P-post coupling [43]
	- DNA repair synthesis in mammalian cells *in vitro* (OECD Guideline 482)
	- DNA repair test in primary liver cells [44]
	- DNA repair *in vivo* [44]

Biochimica della mutagenesi

La mutagenesi è il fenomeno per cui tratti ereditabili risultano da alterazioni del DNA

Mutazioni avvengono normalmente originando la diversità nelle specie, ma molte mutazioni sono dannose.

Specie tossiche che causano mutazioni son dette **mutagene** (spesso le stesse che causano tumori ed teratogenesi).

DNA contiene basi azotate *adenina*, *guanina citosina e timina*; l'ordine in cui si presentan nel DNA determina la struttura del RNA, sostanza prodotta per sintetizzare nuove proteine ed enzimi nelle cellule. *Cambiare, aggiungere o sottrarre una qualsiasi di queste basi azotate altera la natura del RNA prodotto e può cambiare processi biologici vitali*. Questo fenomeno, che può esser indotto da uno xenobiotico, è una mutazione che può esser passata alla progenie spesso con effetti deleteri.

Es. mutagenicità dell'acido nitrico nei batteri.

Tre basi azotate (A, G, C) contengono un amino gruppo $–NH_2$

l'acido nitrico rimpiazza amino- gruppi con atomi di ossigeno con legami doppi, poi inserisce un cheto gruppo (C=O) negli anelli delle basi azotate convertendole in altri composti (-> il DNA può non funzionare bene -> mutazione)

Alchilazioni possono inserire un piccolo gruppo alchilico (metile o etile) su di un N di basi azotate

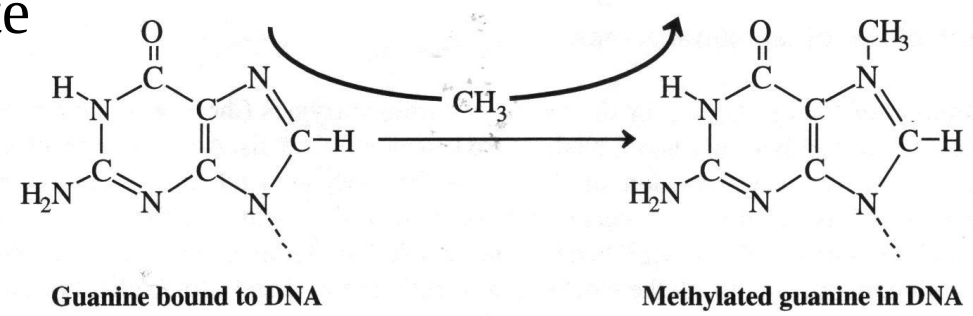


Figure 7.14 Alkylation of guanine in DNA.

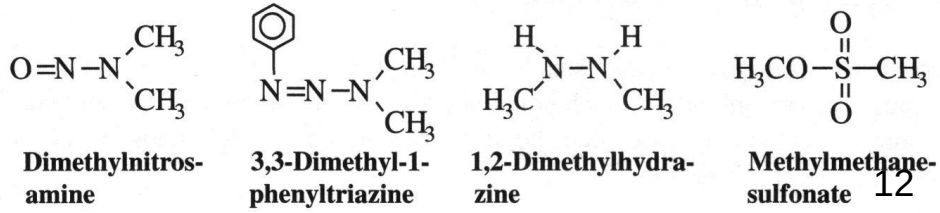
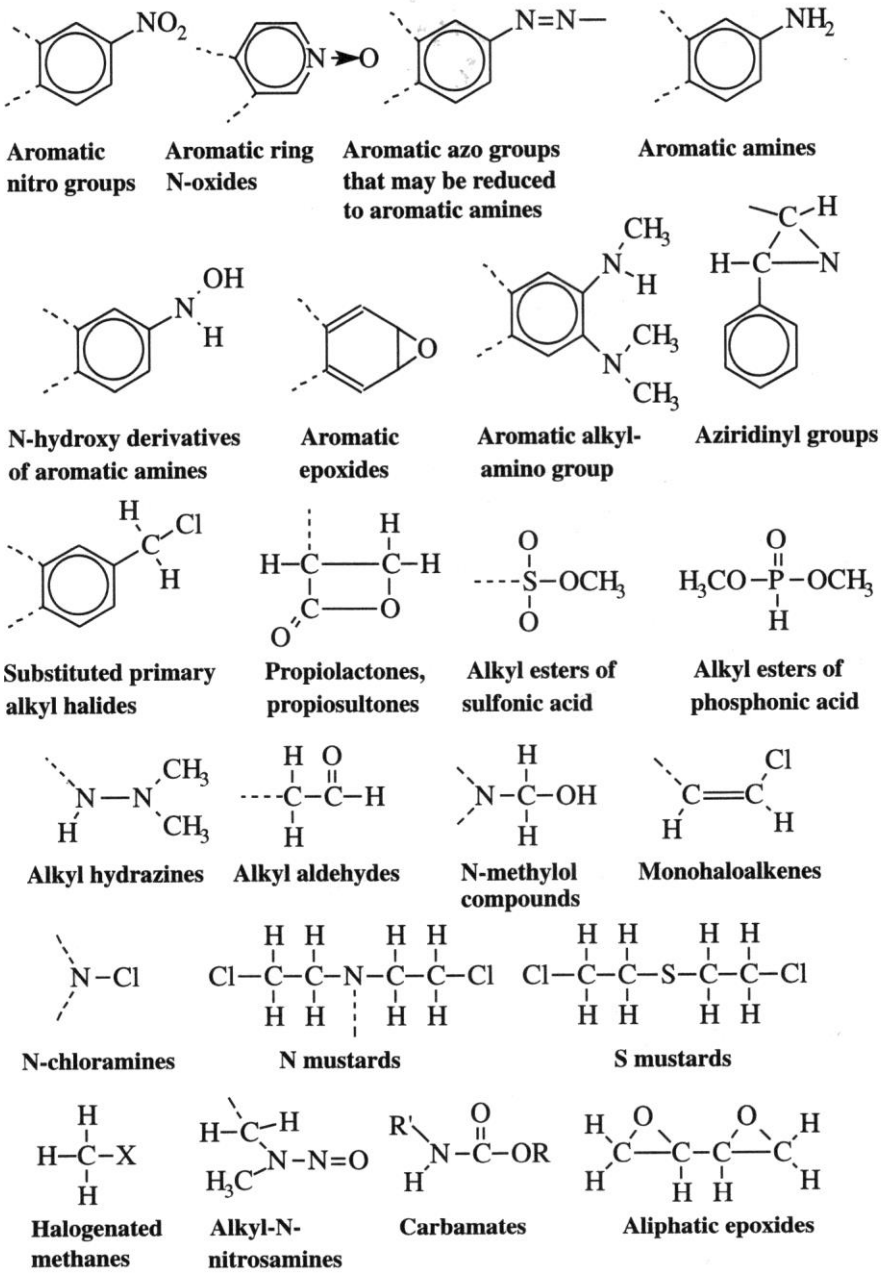
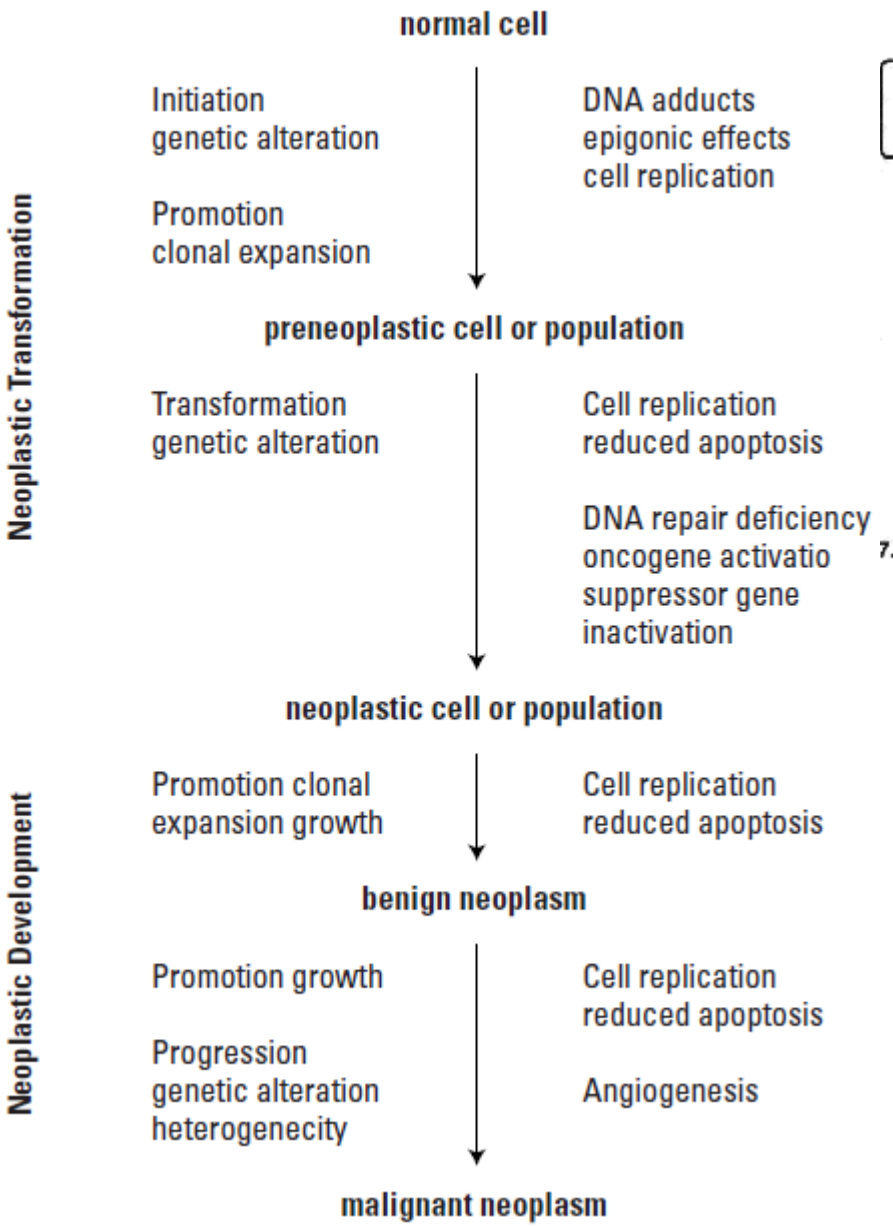


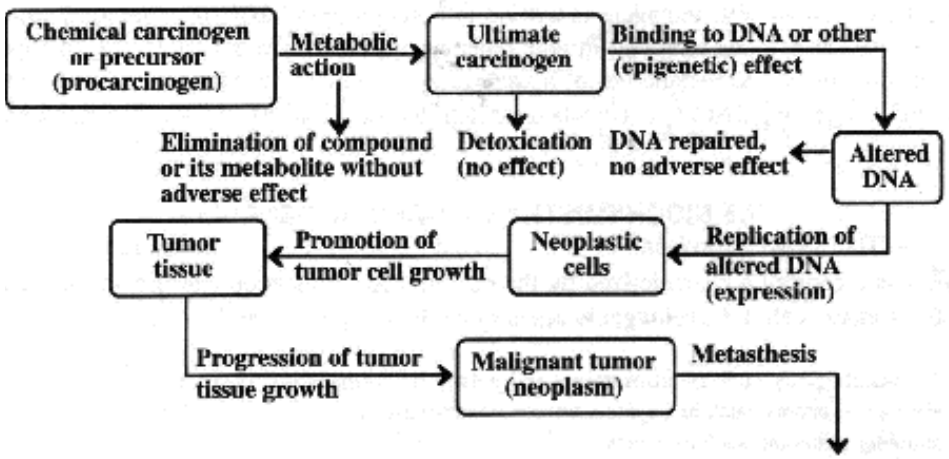
Figure 7.15 Examples of simple alkylating agents capable of causing mutations.



8.4 Functionalities commonly associated with genotoxicity and mutagenicity. These groups are used in structure-activity relationships to alert for possible carcinogenic substances.

Cancerogenesi chimica:





7.16 Outline of the carcinogenic process.

ROAC II ed. (2007)

Table 5.7. Carcinogenicity studies

Conditions	chemical identification of substance, its purity and chemical characteristics
Route	oral, inhalatory
Experimental animals	rat, mouse, (dog), (monkey)
Number of animals	50 per sex per group (sometimes satellite groups); dogs and monkeys usually not more than 7 to 10 per group
Dose levels	control and at least 3 dose groups, for proper quantitative risk assessment more dose groups
Examinations	- body weight, food consumption and water consumption at various intervals (see Table 5.6) - clinical examination at intervals (see Table 5.6) of 10 to 20 animals per sex per group - gross examination: daily observation and extensive gross examination on termination - histopathological examination in full of highest dose and controls where indicated, for other dose levels
Results	information on carcinogenic properties, tumour incidence in relation to dose, latency period, tumour multiplicity, potential for metastasis

Test sulla cancerogenicità

•studi epidemiologici

•test su animali, risultati estrapolati a esseri umani

•per screening: Ames test (mutagenicità - batteri di un ceppo di Salmonella, mutato geneticamente in modo che non sintetizzi l'aminoacido essenziale istidina, esposti a specie da testare; *se* si ha ritorno a specie originaria/naturale che sintetizza istidina *allora* in ambiente privo di i. verifico crescita di Salmonelle, *allora* la specie testata è mutagena)

Valutazione di pericolosità

Dati disponibili valutati per qualità e completezza, considerando studi su umani e non umani, in vitro e QSAR

Tra ***i primi risultati***: CLASSIFICAZIONE E ETICHETTATURA DELLA SOSTANZA

Successivamente: determinazione delle concentrazioni/dosi a cui gli esseri umani NON devono essere esposti - VALUTAZIONE DEGLI EFFETTI DOSE-RISPOSTA

Esempio: nell'ambito del regolamento EC 1907/2006

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

Es. da

W. Lilienblum · et alii «Alternative methods to safety studies in experimental animals: role in the risk assessment of chemicals under the new European Chemicals Legislation (REACH)» Archives of Toxicolology (2008)

https://www.researchgate.net/profile/Werner_Lilienblum/publication/5529029_Alternative_methods_to_safety_studies_in_experimental_animals_Role_in_the_risk_assessment_of_chemicals_under_the_new_European_Chemicals_Legislation_(REACH)/links/0fcfd511fc31eb48ae000000.pdf

Esempio: nell'ambito del regolamento EC 1907/2006

Quali i test?

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]	
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]	

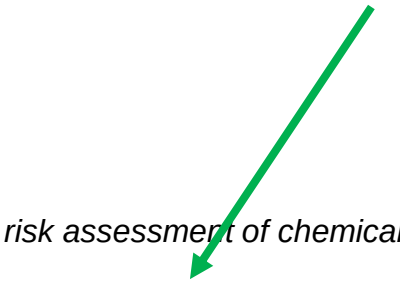


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Table 3 continued

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]	
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]	

Lista aggiornata a test alternativi validati a metodi «in vivo»

https://ec.europa.eu/jrc/en/eurl/ecvam/alternative-methods-toxicity-testing/validated-test-methods

Validated test methods

EURL ECVAM has contributed to the validation of the test methods listed below.

The validation of other test methods has been undertaken by ICATM (International Cooperation on Alternative Test Methods) partners.
More details are available here.

Acute toxicity

Acute systemic toxicity testing involves an assessment of the general toxic effects of a single dose or multiple doses of a chemical or product, within 24 hours by a particular route (oral, dermal, inhalation), and that occur during a subsequent 21-day observation period.
Read more here.

- Acute oral toxicity: the 3T3 Neutral Red Uptake (NRU) cytotoxicity assay

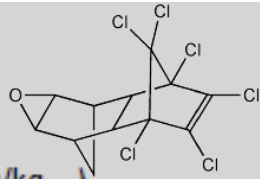
Aquatic toxicity

Classificazione e etichettatura

Obiettivo "*identificare tutte le proprietà fisico-chimiche, tossicologiche e ecotossicologiche di sostanze e preparati che possono costituire un rischio durante la normale manipolazione ed uso*. Identificate le proprietà pericolose, la *sostanza o preparato deve essere etichettata* per indicare i pericoli al fine di proteggere l'utilizzatore, il pubblico in genere, e l'ambiente" (EC, 2001)

- **EC** – simboli, frasi di rischio e frasi di sicurezza; direttiva 67/548 EEC superata da regolamento (EC) No 1272/2008
- **U.N. Global Harmonised System** for C. & L. (2002 e revisioni) simile a EC con estensioni; recepita nel regolamento REACH
- **IARC**: cancerogeni di gruppo 1 (cancerogeno per gli umani), 2 a (probabile cancerogeno per gli umani) e 2b (possibile cancerogeno per gli umani), 3 (non classificabile), 4 (probabilmente non cancerogeno)

Box 6.3. Example: classification and labelling of dieldrin (CAS: 60-57-1)



EC [54]: symbols T+ and N, risk phrases R25-27-40-48/25-50/53, meaning:

- T+, R27 = very toxic (skull and cross bones symbol) in contact with skin (LD50 dermal, rat or rabbit ≤ 50 mg/kg_{bw}).

T+

OLD

- T, R25 = toxic if swallowed (25 < LD50 or
- T, R48/25 = toxic with danger of serious ged oral exposure (serious damage to health to be caused at levels below 5 mg/kg_{bw}·d).
- R40, category 3 carcinogen = possible risk ible effects (concern to man owing to possible carcinogenic effects but for which the information available is not adequate for making a satisfactory assessment. There is some evidence from appropriate animal studies, but it is insufficient for a higher category).
- N, R50, R53 = dangerous to the environment (dead tree and fish symbol), very toxic to aquatic organisms (L(E)C50 fish or *Daphnia* or algae ≤ 1 mg/L), may cause long-term adverse effects in the aquatic environment (substance not be readily biodegradable or the log K_{ow} ≥ 3.0, unless the experimentally determined bioconcentration factor ≤ 100).

N

*The **CLP Regulation** (for "Classification, Labelling and Packaging") is a European Union regulation which **aligns the European Union system** of classification, labelling and packaging chemical substances and mixtures **to the Globally Harmonised System** (GHS). It is expected to **facilitate global trade** and the **harmonised communication of hazard information of chemicals** and to promote regulatory efficiency. It **complements** the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation (EC No 1907/2006) and **replaces** the current system contained in the Dangerous Substances Directive (67/548/EEC)and the Dangerous Preparations Directive (1999/45/EC).*

*The regulation incorporates the classification criteria and labelling rules agreed at UN level, the so called Globally Harmonised System of Classification and Labelling of Chemicals (GHS). It **introduces new classification criteria, hazard symbols (pictograms) and labelling phrases**, while taking account of elements which are part of the current EU legislation.*

*The regulation **requires companies to appropriately classify, label and package their substances and mixtures before placing them on the market**. It aims to **protect** workers, consumers and the environment by means of labelling which reflects possible hazardous effects of a particular chemical. It also **takes over provisions of the REACH Regulation** regarding the notification of classifications, the establishment of a list of harmonised classifications and the creation of a classification and labelling inventory.*

SIMBOLO DI PERICOLO (Direttiva 67/548)	PITTOGRAMMA e Categorie di pericolo associate (Regolamento 1272/2008)
 Esplosivo	 Esplosivi instabili; Esplosivi delle divisioni 1.1, 1.2, 1.3 e 1.4 Sostanze e miscele autoreattive, tipi A e B Perossidi organici, tipi A e B
 Facilmente infiammabile  Estremamente infiammabile	 Gas infiammabili, categoria di pericolo 1 Aerosol infiammabili, categorie di pericolo 1 e 2 Liquidi infiammabili, categorie di pericolo 1, 2 e 3 Solidi infiammabili, categorie di pericolo 1 e 2 Sostanze e miscele autoreattive, tipi B, C, D, E, F Liquidi piroforici, categoria di pericolo 1 Solidi piroforici, categoria di pericolo 1 Sostanze e miscele autoriscaldanti, categorie di pericolo 1 e Sostanze e miscele che a contatto con l'acqua emettono gas infiammabi pericolo 1, 2 e 3 Perossidi organici, tipi B, C, D, E, F
 Comburente	 Gas comburenti, categoria di pericolo 1 Liquidi comburenti, categorie di pericolo 1, 2 e 3 Solidi comburenti, categorie di pericolo 1, 2 e 3
	 Gas sotto pressione: Gas compressi; Gas liquefa refrigerati; Gas disciolti.
 Tossico  Molto tossico	 Tossicità acuta (per via orale, per via cutanea, per inalazione), categorie di pericolo 1, 2 e 3
	 Sensibilizzazione delle vie respiratorie, categoria di pericolo 1 Mutagenicità sulle cellule germinali, categorie di pericolo 1A, 1B e 2 Cancerogenicità, categorie di pericolo 1A, 1B, 2 Tossicità per la riproduzione, categorie di pericolo 1A, 1B e 2 Tossicità specifica per organi bersaglio – esposizione singola, categorie di pericolo 1 e 2 Tossicità specifica per organi bersaglio – esposizione ripetuta, categorie di pericolo 1 e 2
 Nocivo  Irritante	 Tossicità acuta (per via orale, per via cutanea, per inalazione), categoria di pericolo 4 Irritazione cutanea, categoria di pericolo 2 Irritazione oculare, categoria di pericolo 2 Sensibilizzazione cutanea, categoria di pericolo 1 Tossicità specifica per organi bersaglio – esposizione singola, categoria di pericolo 3 Irritazione delle vie respiratorie Narcosi
 Corrosivo	 Corrosivo per i metalli, categoria di pericolo 1 Corrosione cutanea, categorie di pericolo 1A, 1B e 1C Gravi lesioni oculari, categoria di pericolo 1
 Pericoloso per l'ambiente	 Pericoloso per l'ambiente acquatico – pericolo acuto, categoria 1 – pericolo cronico, categorie 1 e 2

Globally Harmonized System of Classification and Labelling of Chemicals (GHS, Rev.7)

Published: July 2017

The GHS addresses classification of chemicals by types of hazard and proposes harmonized hazard communication elements, including labels and safety data sheets. It aims at ensuring that information on physical hazards and toxicity from chemicals be available in order to enhance the protection of human health and the environment during the handling, transport and use of these chemicals.

The GHS also provides a basis for harmonization of rules and regulations on chemicals at national, regional and worldwide level.

This seventh revised edition of the GHS contains various new or revised provisions including, inter alia, revised criteria for categorisation of flammable gases within Category 1; miscellaneous amendments intended to clarify the definitions of some health hazard classes; additional guidance to extend the coverage of section 14 of the Safety Data Sheets to all bulk cargoes transported under instruments of the International Maritime Organisation (IMO), regardless of their physical state; revised and further rationalized precautionary statements in Annex 3; and a new example in Annex 7 addressing labelling of small packagings with fold-out labels.

http://www.unece.org/index.php?id=46260&L=0

https://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev07/English/ST_SG_AC10_30_Rev7e.pdf

Valutazione delle relazioni tra dose e risposta

Valutazione delle relazioni tra dose e risposta per effetti con soglia: l'approccio NOAEL

L'obiettivo è determinare una "dose" per cui non ci siano effetti significativamente rilevant

NOAEL = Not observable effect level
LOAEL = Lowest observable effect level
dipendono fortemente da progettazione del test (n° animali, dosi somministrate …)

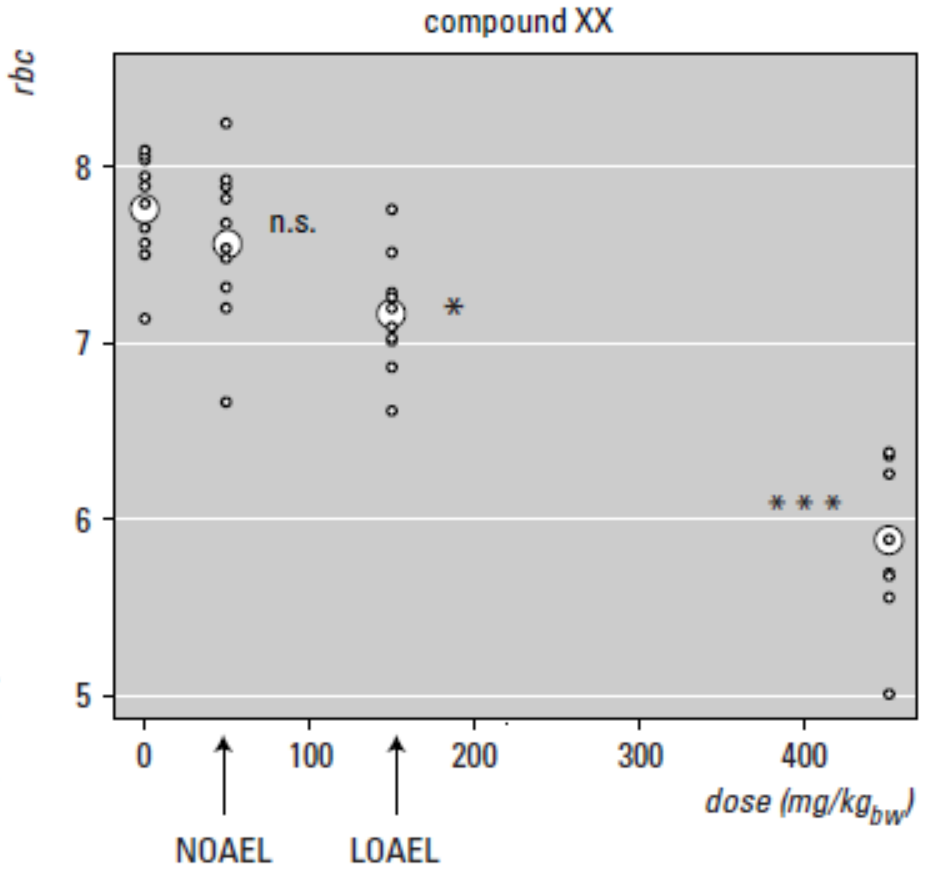


Figure 6.9. Illustration of the LOAEL and NOAEL for a decrease in red blood cell counts observed in an OECD toxicity study. The small marks indicate the observations in individual animals, the larger marks indicate the group means.
n.s.: not significantly different from the controls.
*: significantly different from the controls.
***: highly significantly different from the controls.

Valutazione delle relazioni tra dose e risposta

Valutazione delle relazioni tra dose e risposta per **effetti senza soglia**

Per le sostanze cancerogene, «anche singola molecola» ha piccola probabilità di generare un addotto con il DNA, e addotto ha piccola probabilità di causare una mutazione, in un gene potenzialmente correlato a un processo di carcinogenesi, aumentando la probabilità di generare una cellula maligna. Cancerogenesi è complessa, ma l'attivazione dei tumori sembra essere stocastica.

Diminuire le "dosi" porta sempre a diminuire la probabilità di tumori in una popolazione.

Manca quindi una soglia per le dosi d'esposizione → NOAEL non va bene

Una valutazione dei dati sull'incidenza dei tumori, può solo determinare una dose per la quale il rischio sia accettabilmente piccolo: **rischio *de minimis, es.* 10^{-6}, in una vita**

Nelle valutazioni sperimentali

Studi su animali effettuati su 50-100 individui per dose. Un rischio osservabile prevederebbe 1 caso su 10 (10^{-1}), quindi ci si trova in *condizioni di estrapolazione per basse dosi* o, meglio, *per bassi rischi*.

Alcune nazioni evitano valutazioni quantitative su dati di incidenza di tumori.

Altri applicano il principio *As Low as Reasonably Achievable (ALARA)* per cancerogeni genotossici. È approccio debole, tratta tutti i cancerogeni in modo eguale.

La tendenza attuale è verso l'**approccio BMD (*benchmark dose*)**

L'approccio BMD (*benchmark dose*)

Si costruisce/fitta un modello dose-risposta per i dati di incidenza dei tumori, e il modello è usato per stimare una dose che è associata con un livello di rischio che sta nell'intervallo osservabile (tipicamente il rischio del 10% = BMD_{10}, termine basso di confidenza è $BMDL_{10}$)).

Questo rischio di cancerogenesi non è accettabile, ed è considerato come punto di riferimento (RP) per successive valutazioni, come

Estrapolazione lineare

Margine di esposizione (l'esposizione stimata è divisa per il RP (=$BMDL_{10}$), e il rapporto risultante è l'intervallo tra l'esposizione umana e la dose con livello di rischio noto (EFSA: MOE>10000 basso livello di preoccupazione, non vi è consenso sul tema)

Valutazione delle relazioni tra dose e risposta

Valutazione delle relazioni tra dose e risposta: l'approccio BMD

benchmark dose approach

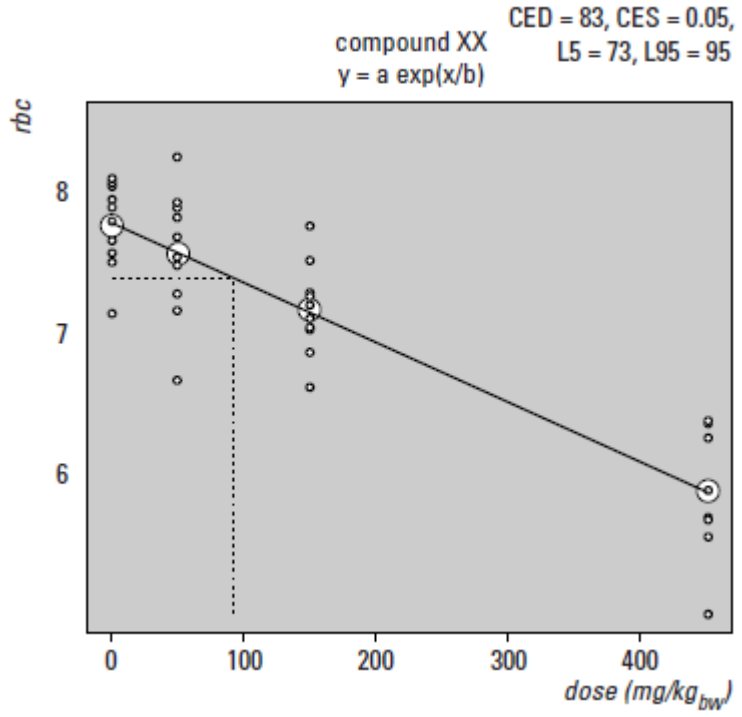


Figure 6.11. Illustration of the Benchmark Dose approach applied to the same data as in Figure 6.9. A curve, in this case an exponential function, is fitted to the data, and this curve is used to assess the CED (vertical dashed line) at a CES of 5% (horizontal dashed line). Next the confidence interval for the CED is calculated (see L-5 and L-95, denoting the lower and upper bound of the 90% confidence interval). The lower bound of this confidence interval (CEDL, or BMDL) is normally used as a RP (PoD) in risk assessment.

- BMR per endpoints quantizzati
- BMR per endpoints continui
- Selezione del modello

A toxicity test usually measures either the proportion of organisms affected *(quantal)*, or the degree of effect shown *(graded or quantitative)*, after exposure to specific levels of a stimulus (concentration or dose, or mixture of chemicals).

ROAC II ed. (2007)

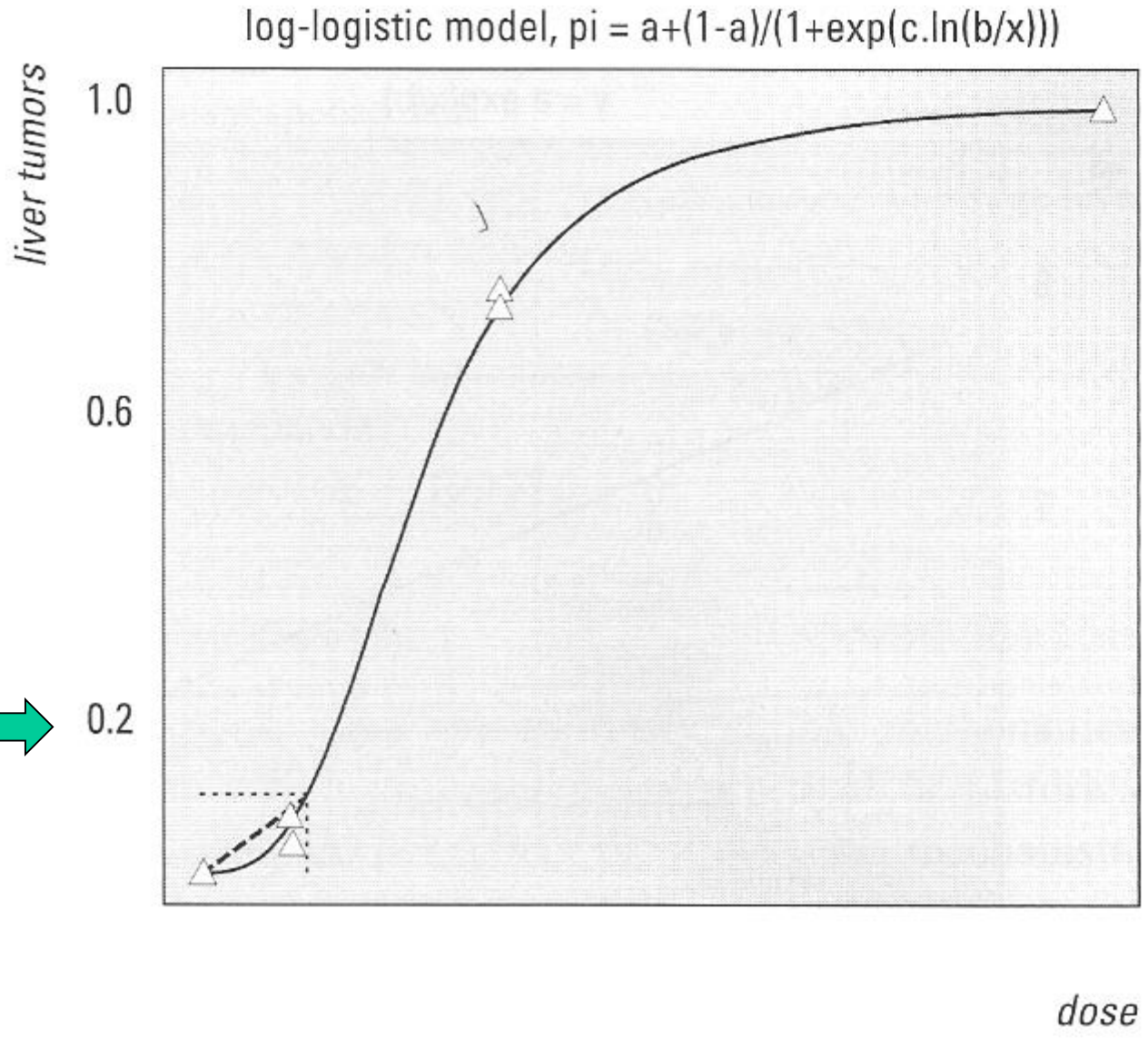


Figure 6.10. Sigmoidal dose-response relationship. Linear extrapolation from the BMD10 overestimates the risk.

http://www.efsa.europa.eu/it/topics/topic/marginofexposure

- Il calcolo del cosiddetto “margine di esposizione” (MOE in breve) è usato dai valutatori del rischio per analizzare possibili timori in termini di sicurezza connessi alla presenza di sostanze sia genotossiche (che possono cioè danneggiare il DNA) sia cancerogene in alimenti e mangimi.
- Il MOE rappresenta il rapporto tra due fattori che, in una data popolazione, valuta il quantitativo di sostanza alla quale un effetto avverso minimo ma misurabile viene osservato per la prima volta e il livello di esposizione alla sostanza in questione.

http://www.efsa.europa.eu/it/efsajournal/pub/282

…The Scientific Committee had serious reservations about extrapolating from animal tumour data at high doses using mathematical modelling in order to estimate risks to humans at low exposures from substances that are both genotoxic and carcinogenic…

…The Scientific Committee is of the view that in general a margin of exposure of 10,000 or higher, if it is based on the BMDL10 from an animal study, and taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view and might be reasonably considered as a low priority for risk management actions

Table 5.11. Estimated conversion factors between an LD50, the NOEL of a subchronic or chronic study and the acceptable daily intake (ADI)

LD50	NOEL subchronic	NOEL chronic	ADI
100,000 - 5,000,000	300 - 1000	100	1

Table 5.12. Actual intake and safety margin for a number of substances, adapted after Kroes and Feron [59]

Substances		Actual daily intake	Safety margin
Fat	(en %)[a]	40	<2
Sugar	(g)	100	2-3
Nicotin acid	(mg)	20[b]	50
Vitamin A	(IE)	5000[b]	18
Selenium	(mg)	1[b]	10
Tocoferol	(mg)	0.15-2	60-6
Vitamin D	(IE)	400[b]	5
Fluor	(mg)	1[b]	5
NaCl	(g)	9	<2
Mercury	(μg)	63	50
Sulphite	(mg)	3	1400
Bromide	(mg)	9.4	10
Solanin	(mg)	1	2
Dietary fibre	(g)	20-30[a]	2
Aspertame	(mg)	300	800
DDT	(μg)	6	5000
Dieldrin	(μg)	0.5	1200
Lindane	(μg)	2	30000
Dioxins	(pg)	135	500

[a] Energy percents.

[b] Recommended daily intake.

Dal NOAEL…
Fattori di valutazione di default

DNEL *derived no effect level* che tengano conto di:

- Differenze interspecie
- Differenze intraspecie
- Differenze nella durata dell'esposizione
- Aspetti associati alla dose-risposta
- Qualità della base di dati

Differenze **interspecie**

It has been demonstrated that generally equitoxic doses, expressed in mg per kg body weight (bw) per scale with body weight to the power of 0.75. This re in default allometric scaling factors for different an species when compared with humans.

$$\frac{bw_{human} / bw_{animal}}{(bw_{human} / bw_{animal})^{0.75}} = (bw_{human} / bw_{animal})^{0.25}$$

Table 6.10. Default assessment factors to cover toxicokinetic interspecies differences.

Species	Body weight (kg)	Allometric assessment factor
Mouse	0.03	7
Rat	0.25	4
Guinea pig	0.8	3
Rabbit	2	2.4
Monkey	4	2
Dog	18	1.4
Human	70	1

Differenze ***intraspecie***

Default assessment factor to cover intraspecies differences: 10.

Differenze nella ***durata*** dell'esposizione

Acute:	a single exposure (oral), or up to 24 h exposure (inhalation)
Sub-acute:	28 days of daily exposure
Semi-/sub-chronic:	90 days of daily exposure
Chronic:	1.5-2 years of daily exposure (for rodents)

The default AFs to extrapolate from short to long test periods are listed in Table 6.11. [125].

Estrapolazione	Fattori di valutazione
Semi/subcronico a cronico	2
Subacuto a cronico	6
Subacuto a Semi/subcronico	3
Acuto a subacuto/subcronico/cronico	impossibile

Box 6.4. Maximum Permissible Risk level

Examples of oral MPRs for non-carcinogenic substances are the ADI (acceptable daily intake, for substances deliberately added to food items) and TDI (tolerable daily intake, for substances unintentionally present in food items), both are expressed in mg/kg bw/day and defined as the daily intake of a chemical which, during the entire lifetime, appears to be without appreciable risk on the basis of all known facts at the time. The RfD (Reference Dose) is similar to the ADI/TDI, but is more strictly defined. Inhalation MPRs are defined in a similar way and expressed as concentrations in air. An example of another health-based limit value is the AOEL (acceptable operator exposure level): the level that has no harmful effects on the health of operators (people working with the substance).

MPRs for carcinogenic substances are usually defined as the daily dose, taken during the entire lifetime that will cause $1{:}10^4$, $1{:}10^5$ or $1{:}10^6$ additional cancer cases during the entire lifetime.

Tossicità di miscele

In generale l'informazione disponibile per sostanze testate
•in condizioni di laboratorio
•in studi di campo
si riferisce a singole sostanze

Ecosistemi terrestri e acquatici raramente (mai) inquinati da una singola sostanza

Necessario considerare possibili **interazioni tra specie chimiche** e **interazioni tra i loro effetti** sugli organismi.

E' importante considerare i "**modi di azione"** delle sostanze:
"A mode of action (MoA) describes a functional or anatomical change, at the cellular level, resulting from the exposure of a living organism to a substance."

Quattro tipi di azioni congiunte di specie chimiche secondo Plackett e Hewlett (J. Roy. Stat. Soc. B, **14**, 141 –163 (1952))

	Similar joint action	Dissimilar jont action
Interaction absent	simple similar action (concentration-addition)	independent action (response-addition)
Interaction present	complex similar action	dependent action

L'**azione congiunta** è definita
simile o **diversa** a seconda che i siti di azione primaria delle due specie chimiche considerate siano gli stessi o diversi,
interattiva o **non interattiva** a seconda che una specie chimica influenzi o meno l'azione biologica dell'altra

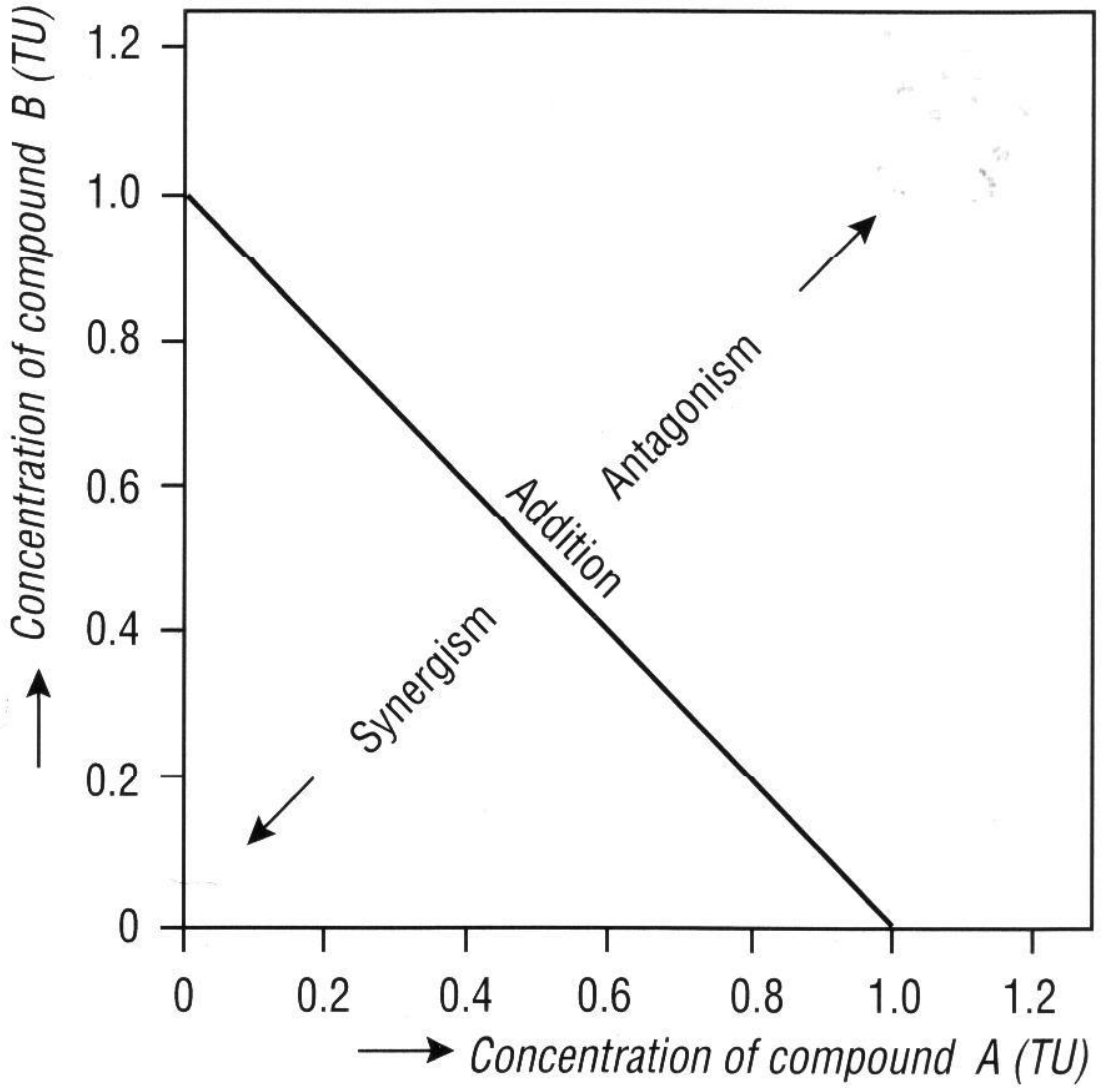


Figure 6.33. Possible toxicological interactions in a mixture of two chemicals.

https://ec.europa.eu/jrc/en/news/chemical-mixtures-safety

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Chemical mixtures: How to address the safety of combined exposures to multiple chemicals for people and the environment

JUN 28 2018

Every day we are exposed to low levels of hundreds of different manmade chemicals present for example in our food, consumer products and the air we breathe.

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Our environment too is exposed to a near-infinite number of chemical mixtures derived from numerous sources.

However, current safety assessment practice is primarily based on understanding the potential risk posed by single substances rather than their "real life" combinations, thus potential combination effects might be overlooked.

The JRC is investigating recent progress in considering combined exposures to multiple chemicals to help translate best science into best assessment practice. The latest policy brief, Something from nothing? Ensuring the safety of chemical mixtures, puts together issues around the topic, including the specific challenges that will further inform discussions of the working group of Commission services and EU agencies on the combination effects of chemicals.

Related Content

JRC policy brief: Something from nothing? Ensuring the safety of chemical mixtures

News: Towards improved safety assessment of combined exposure to chemicals

Report: Assessment of Mixtures - Review of Regulatory Requirements and Guidance

Report: Scientific methodologies for the assessment of combined effects of chemicals - a survey and literature review

Report: Review of case studies on the human and environmental risk assessment of chemical mixtures

Article: Mixtures of chemical pollutants at European legislation safety concentrations: how safe are they?

Article: Regulatory assessment of chemical mixtures: Requirements, current approaches and future perspectives

Tool: IPCHEM

https://ec.europa.eu/jrc/en/news/chemical-mixtures-safety

Per la descrizione matematica della tossicità congiunta di una miscela di n composti ($n \geq 2$), è (possibile in pochi casi in cui l'assenza di interazione sembra essere un prerequisito.) difficile.

http://ec.europa.eu/environment/chemicals/effects/effects_en.htm (2017)

http://ec.europa.eu/environment/chemicals/effects/pdf/report_mixture_toxicity.pdf (2009)

Relativamente a miscele di composti, gli effetti per esperimenti per valutare LC50 possono essere predetti per composti con azione simile semplice (addizione delle concentrazioni)

Azione simile semplice

Sostanze chimiche nella miscela agiscono nello stesso modo, attraverso gli stessi meccanismi, con diverse potenze. L'effetto additivo può essere descritto con la somma delle dosi di ciascun componente individuale, dopo la correzione per la differenza nelle potenze:

Fattori di tossicità equivalente (TEF) e equivalenti di tossicità (TEQs) si definiscono per diossine, furani, PCB *dioxin-like*, e altri per IPA

PCDXs e DL-PCBs What are TEFs and TEQs?

The complex nature of polychlorinated dibenzo-p-dioxin (PCDD), dibenzofuran (PCDF), and biphenyl (PCB) mixtures complicates the risk evaluation for humans. For this purpose the concept of **toxic equivalency factors (TEFs) has been developed and introduced to facilitate risk assessment and regulatory control of exposure to these mixtures.**

TEF values for individual congeners in combination with their chemical concentration can be used to calculate the total TCDD toxic equivalents concentration (TEQs) contributed by all dioxin-like congeners in the mixture using the following equation which assumes dose additivity:

$$TEQ = \sum (PCDD_i \times TEF_i) + \sum (PCDF_i \times TEF_i) + \sum (PCB_i \times TEF_i) + ..$$

The majority of studies have demonstrated that the interaction does not deviate significantly from dose additivity.

PCBs	TEF
2,3,7,8TCDD	1
PentaCB 126	0,1
HexaCB 156	0,0005
HexaCB 157	0,0005
TetraCB 77	0,0001
PentaCB 105	0,0001
PentaCB 118	0,0001
PentaCB 123	0,0001
HeptaCB 189	0,0001
HexaCB 167	0,00001

Martin Van den Berg et al. 1998 “Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for Humans and Wildlife” Environmental Health Perspectives Volume 106, 775-792,

Denison MS, Nagy SR (2003). "Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals". Annual Review of Pharmacology and Toxicology. 43: 309–34.

Le 17 “pecore nere” e la loro tossicità equivalente

Tabella 1: I fattori di tossicità equivalente secondo NATO e WHO

PCDD/F	I-TEFs (NATO/CCMS[14], 1988)	WHO-TEFs (Van den Berg *et al, 1998)*
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	0,5	1
1,2,3,4,7,8-HxCDD	0,1	0,1
1,2,3,6,7,8-HxCDD	0,1	0,1
1,2,3,7,8,9-HxCDD	0,1	0,1
1,2,3,4,6,7,8-HpCDD	0,01	0,01
OCDD	0,001	0,0001
2,3,7,8-TCDF	0,1	0,1
1,2,3,7,8-PeCDF	0,05	0,05
2,3,4,7,8-PeCDF	0,5	0,5
1,2,3,4,7,8-HxCDF	0,1	0,1
1,2,3,6,7,8-HxCDF	0,1	0,1
1,2,3,7,8,9-HxCDF	0,1	0,1
2,3,4,6,7,8-HxCDF	0,1	0,1
1,2,3,4,6,7,8-HpCDF	0,01	0,01
1,2,3,4,7,8,9-HpCDF	0,01	0,01
OCDF	0,001	0,0001

(T = tetra, Pe = penta, Hx = hexa, Hp = hepta, O = octa)

I 12 PCB, "parenti" pericolosi e la loro tossicità equivalente

Tabella 8: PCB dioxin-like e relativi fattori di tossicità equivalente

PCB_a (nome IUPAC)	PCB-TEF (Ahlborg et al., 1994)	WHO-TEF (Van den Berg *et al*, 1998)
3,3',4,4'-TCB (77)	0,0005	0,0001
3,4,4',5-TCB (81)	-	0,0001
3,3',4,4',5-PeCB (126)	0,1	0,1
3,3',4,4',5,5'-HxCB (169)	0,01	0,01
2,3,3',4,4'-PeCB (105)	0,0001	0,0001
2,3,4,4',5-PeCB (114)	0,0005	0,0005
2,3',4,4',5-PeCB (118)	0,0001	0,0001
2',3,4,4',5-PeCB (123)	0,0001	0,0001
2,3,3',4,4',5-HxCB (156)	0,0005	0,0005
2,3,3',4,4',5'-HxCB (157)	0,0005	0,0005
2,3',4,4',5,5'-HxCB (167)	0,00001	0,00001
2,3,3',4,4',5,5'-HpCB (189)	0,0001	0,0001

(T = tetra, Pe = penta, Hx = hexa, Hp = hepta)

IPA
Valutazione dell'equivalente internazionale di tossicità

$$I-TEQ_{B[a]P}=\sum_i [IPA]_i \cdot TEF_i$$

Table 13. Relative potency of individual PAHs compared with B[*a*]P (TEF values), according to different authors.[a]

Compound	Chu and Chen (1984) (cit. Nisbet and LaGoy 1992)	Clement (1986) (cit. Nisbet and LaGoy 1992); Krewski et al. (1989)	Nisbet and LaGoy (1992)	The Netherlands (RIVM 1989)	CARB (1994); Collins et al. (1998)	Health Canada (Meek et al. 1994)	Ontario (Muller 1997)	Larsen and Larsen (1998)
Anthracene			0.01	0				0.0005
Phenanthrene			0.001	0.01			0.00064	0.0005
Benz[*a*]anthracene	0.013	0.145	0.1	0–0.04	0.1		0.014	0.005
Benzo[*c*]phenanthrene							0.023	0.023
Chrysene	0.001	0.0044	0.01	0.05–0.89	0.01		0.026	0.03
Fluoranthene			0.001	0–0.06				0.05
Pyrene		0.081	0.001				0	0.001
B[*a*]P	1	1	1	1	1	1	1	1
Benzo[*e*]pyrene		0.004					0	0.002
Benzo[*b*]fluoranthene	0.08	0.14	0.1		0.1	0.06	0.11	0.1
Benzo[*j*]fluoranthene		0.061			0.1	0.05	0.045	0.05
Benzo[*k*]fluoranthene	0.04	0.066	0.1	0.03–0.09	0.1	0.04	0.037	0.05
Cyclopenta[*cd*]pyrene		0.023					0.012	0.02
Dibenzo[*a,h*]anthracene	0.69	1.11	5				0.89	1.1
Anthanthrene		0.32					0.28	0.3
Benzo[*ghi*]perylene		0.022	0.01	0.01–0.03			0.012	0.02
Dibenzo[*a,e*]pyrene					1		1.0[b]	0.2
Dibenzo[*a,h*]pyrene					10		1.2	1
Dibenzo[*a,i*]pyrene					10			0.1
Dibenzo[*a,l*]pyrene					10		100[b]	1
Indeno[1,2,3-*cd*]pyrene	0.017	0.232	0.1	0–0.08	0.1	0.12	0.067	0.1

[a]See comments in the text. [b]Data from Muller et al. (1995), cited in WHO/IPCS (1998).

Miscele con diversi modi d’azione → maggior complicazione.

Gli effetti possono essere predetti se e solo se i composti nella miscela agiscono in modo diverso e indipendentemente (**azione indipendente**).

Azione diversa semplice

(Azione semplice indipendente, azione indipendente congiunta o **addizione degli effetti** o delle risposte)

La natura, il meccanismo o il sito d'azione delle specie chimiche nella miscela sono diversi. Quindi ciascuna specie chimica esercita il suo effetto tossico individuale, e non altera l'effetto degli altri *chemicals* nella miscela.

Pericolosità di una miscela con azione diversa semplice ma correlazione positiva completa delle suscettibilità (risposte degli organismi ai tossici) è quella del componente più tossico della miscela.

Interazione

Effetti di due o più sostanze in una miscela, risultano in un effetto più forte della semplice somma di effetti delle sostanze individuali (sinergia, potenziamento, supra-additività).

Avviene per natura chimico-fisica

o nella fase tossicocinetica.

Supra-additività: possibile che

sostanze in conc. sotto livelli

di effetto avverso abbiano effetti

avversi

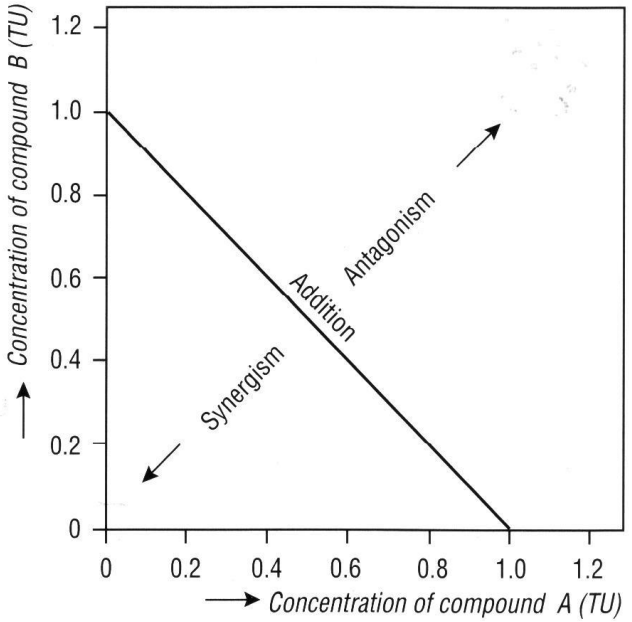


Figure 6.33. Possible toxicological interactions in a mixture of two chemicals.

Esposizione a miscele di sostanze genotossiche cancerogene

Sostanze senza soglia d'effetto: si assume additività delle risposte (nell'esprimere il rischio da sostanze cancerogene non si fa differenza tra i tipi di cancro)

$CR_{total} = \Sigma CR_i$

CR_i = rischio cancerogeno per l'i-mo componente nella miscela.

Scientific Committee on Health and Environmental Risks

Scientific Committee on Emergin and Newly Identified Health Risk

Scientific Committee on Consumer Safety

Dicembre 2011

ABSTRACT

The EU Chemicals legislation is based predominantly on assessments carried out on individual substances. Since humans and their environments are exposed to a wide variety of substances, there is increasing concern in the general public about the potential adverse effects of the interactions between those substances when present simultaneously in a mixture. Based on their analysis of the available scientific literature, the non-food Scientific Committees of the European Commission reached the following conclusions:

1. Under certain conditions, chemicals will act jointly in a way that the overall level of toxicity is affected.

2. Chemicals with common modes of action will act jointly to produce combination effects that are larger than the effects of each mixture component applied singly. These effects can be described by dose/concentration addition.

3. For chemicals with different modes of action (independently acting), no robust evidence is available that exposure to a mixture of such substances is of health or environmental concern if the individual chemicals are present at or below their zero-effect levels.

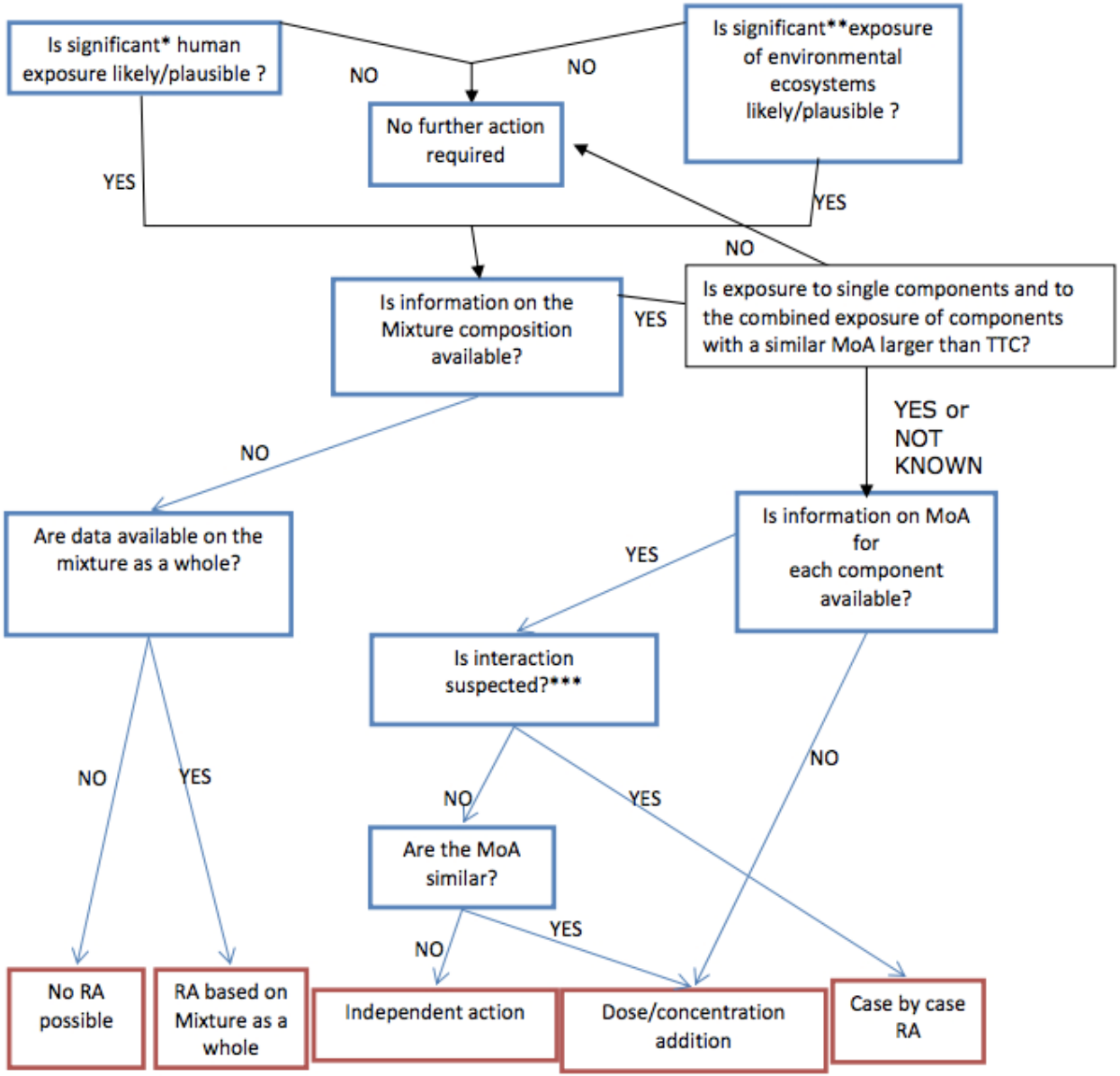
4. Interactions (including antagonism, potentiation, and synergies) usually occur at medium or high dose levels (relative to the lowest effect levels). At low exposure levels, they are either unlikely to occur or are toxicologically insignificant.

5. In view of the almost infinite number of possible combinations of chemicals to which humans and environmental species are exposed, some form of initial filter to allow a focus on mixtures of potential concern is necessary. Several criteria for such screening are offered.

6. With regard to the assessment of chemical mixtures, a major knowledge gap at the present time is the lack of exposure information and the rather limited number of chemicals for which there is sufficient information on their mode of action. Currently, there is neither an agreed inventory of mode of actions, nor a defined set of criteria how to characterise or predict a mode of action for data-poor chemicals.

7. If no mode of action information is available, the dose/concentration addition method should be preferred over the independent action approach. Prediction of possible interaction requires expert judgement and hence needs to be considered on a case-by-case basis.

Based upon these conclusions, a decision tree for evaluating the risk of chemical mixtures is proposed.


Is significant* human exposure likely/plausible ?
NO
NO
Is significant**exposure of environmental ecosystems likely/plausible ?
No further action required
YES
YES
NO
Is information on the Mixture composition available?
YES
Is exposure to single components and to the combined exposure of components with a similar MoA larger than TTC?
YES or NOT KNOWN
NO
Are data available on the mixture as a whole?
YES
Is information on MoA for each component available?
Is interaction suspected?***
NO
YES
NO
YES
NO
Are the MoA similar?
NO
YES
NO
YES
No RA possible
RA based on Mixture as a whole
Independent action
Dose/concentration addition
Case by case RA