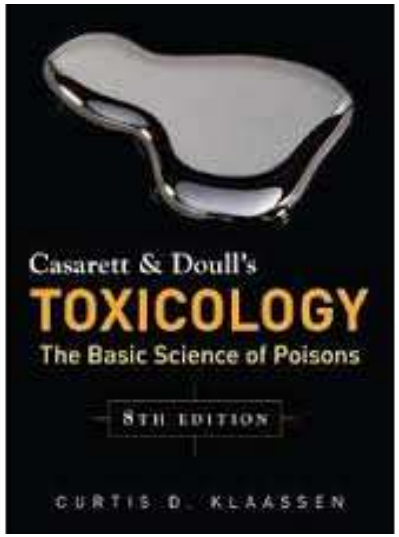
VALUTAZIONE DEI DATI DI TOSSICITA' AI FINI DELLA VALUTAZIONE del PDE



Dr. Antonio Conto

ERT® (European Registered Toxicologist)

CHEMSAFE Srl

CONTENUTI

- *I programmi sperimentali tossicologici*
- *I metodi alternativi*
- *Come ricercare e valutare i dati tossicologici*
- *Quali criteri di valutazione utilizzare per gli studi sperimentali*
- *Come si definisce l'indice di affidabilità dei risultati secondo i criteri europei*
- *Assegnazione degli Assessment Factors per il calcolo PDE in base ai risultati sperimentali disponibili*
- *Gli end-points "in silico"*
- *Il risk assessment Report*

PROGRAMMI SPERIMENTALI TOSSICOLOGICI ed ECO-TOSSICOLOGICI

Vengono impostati in base alla finalità che si vuole raggiungere

- programmi di registrazione (regolatori) sostanze chimiche
- programmi di registrazione di novel drugs (EMA), pre-clinica
- scheda di sicurezza
- registrazione di un fitosanitario
- registrazione di un biocida
- classificazione ambientale
- ERA (Environmental Risk Assessment) of medicines (EMA)
- valutazione di un cosmetico (no test su animali) e/o suoi ingredienti
- test chimico-fisici singoli (es. Proprietà esplosive, Infiammabilità)
- classificazione del trasporto (GHS)
- valutazione dei livelli di esposizione occupazionale (OEL, OEB, ASL) nell'ambiente di lavoro
- valutazione del PDE in shared facilities (EMA) nel processi di cleaning validation

LA TOSSICOLOGIA SPERIMENTALE

NON E' VIVISEZIONE !!!!

- **si differenzia dalla tossicologia classica (avvelenamenti)**
- **è di tipo regolatorio e non ha carattere di ricerca di base**
- **è regolata da linee guida, metodi, leggi**
- **è standardizzata in modo da essere riproducibile**
- **soggiace alle regole della Buona Prassi di Laboratorio (BPL-GLP)**
- **si fonda su studi su animali da laboratorio ma non solo**
- **permette di ottenere risultati che necessitano di adattatamentoall'uomo tramite inserimento fattori di sicurezza (UF= Uncertainty Factor)**
- **si fonda essenzialmente sulla ricerca della dose che provoca un effetto**
- **(curva dose-risposta).**

Viene effettuata da Centri specializzati che operano secondo le GLP e sottostanno ad una autorizzazione Ministeriale per :

- **utilizzo dell'animale e salute dell'animale da laboratorio durante la sperimentazione (L. 116/86 e successive)**
- **esecuzione di determinati test secondo le GLP**
- **scadenza ogni 2 anni**

Consiste in uno studio "*in vivo*" con valutazioni cliniche dello stato di salute (Clinical observation) seguito da una valutazione dopo il sacrificio (Post mortem observation) per la valutazione istopatologica delle eventuali lesioni microscopiche.

Le osservazioni vengono riportate in una relazione finale di lavoro.

Metodi alternativi

Principio delle "tre R" per la riduzione degli animali nella sperimentazione

- *Refinement* (ridefinizione) = portare al minimo la sofferenza nell'animale durante gli studi

- *Reduction* (riduzione) = diminuire il numero degli animali ottenendo lo stesso (o maggiore) numero di informazioni (es LLNA)

- *Replacement* (sostituzione) = utilizzare metodi che non prevedono l'uso dell'animale o comunque non l'intero animale (es. cellule, tessuti) e che possano offrire lo stesso numero di informazioni (problema!)

Allo stato attuale

- Test di citotossicità (usati per cosmetici)

- Test di irritazione cutanea (validati ed accettati per screening)

- Test di irritazione oculare (BCO-P = Bovine corneal opacity)

In sviluppo :

- colture cellulari di vari tessuti per sostituire le prove di tossicità acuta

- Metodi computerizzati : QSAR (Quantitative Structure-Activity Relationship)

- Approccio per famiglie di sostanze e Read across

ALTERNATIVE METHODS IN REACH Regulation

(REACH Art. 13 & 28)

Information on intrinsic properties may be generated by means other than vertebrate animal testing with :

a) “in vitro methods”

b) Qualitative or Quantitative Structure Activity Relationship (QSAR)

c) Structurally related substances (grouping or read across)

d) Intelligent Testing with suitable justification for certain end- points (i.e. repro toxicity)

TARGET = reduce the number of testing vertebrate animals

“IN VITRO” TESTING
(REACH Annex XI)

Test shall be suitable

SUITABLE means “sufficiently well developed according to the international agreed test development criteria” (by ECVAM)

In vitro test may be carried out and accepted but :

- **scientific validity has been established by a validation study**
- **results are adequate for the purpose of C&L and RA**
- **adequate and reliable test documentation is provided**

In case of negative results, the relevant test shall be nevertheless carried out.

QSAR APPROACH

(REACH Annex XI)

Means “from structure to activity”

Models may predict the absence or the presence of certain dangerous properties (targeted models and domains)

May be accepted instead of testing if :

- the QSAR model whose scientific validity has been established

- the substance fall within the domain of applicability of the QSAR model

- results are adequate for the purpose of C&L and RA

- adequate and reliable documentation is provided

GROUPING and READ ACROSS APPROACHES

Substance with similar safety properties may be considered as a group or "category"

"Group concept" means the possibility to predict hazard properties from data for reference substance within the group by interpolation to other substances in the group (read-across approach)

TARGET = Avoid test on every substance for every end-point

Similarities may be based on :

- a common functional group;
- a common precursor or breakdown products which results in structurally similar chemicals;
- a constant patter of changing of the potency of the properties across the category

INTELLIGENT TESTING

Taking into consideration :

- Epidemiological data (Historical human data)

- proper selection of the exposed group
- adequate characterisation of exposure
- sufficient length for disease occurrence
- valid method for observing an effect
- proper consideration of bias and confounding effects
- reasonable statistical reliability to justify the conclusion

- Weight of evidence that a substance

- is not particularly dangerous on the whole
- give negative results from test not included in the validated ones

- Stepwise procedure for toxicological evaluation

- avoid test on the basis of results of previous studies

Premessa in *EMA/CHMP/CVMP/SWP/169430/2012*

The derivation of a PDE or TTC should be the results of a structured scientific evaluation of all available pharmacological and toxicological data including both non clinical and clinical data.

Substance- based evaluation

Le azioni da effettuare

Steps

- Safety Data search

- Safety Data evaluation

- Selection of the reference NOAEL, NOEL or LOAEL

- Introduction of suitable UF (*Uncertainty Factors*)

- Calculation of PDE

- Calculation of final OEL, if requested

Needs backgroud in human toxicology to evaluate the Data and studies available

LA RICERCA DEI DATI di SICUREZZA

Si effettua su:

- **banche dati specializzate**
- **Informazioni dell'originator**
- **SDS dell'originator**
- **altre SDS (attenzione!!)**

Ex-European Chemical Bureau

http://ecb.jrc.ec.europa.eu

American Chemical Society (CAS)

http://www.cas.org/expertise/cascontent/regulated/substance.html

eChemPortal

http://webnet3.oecd.org/echemportal

Nordic Council of Ministers in collaboration with ECB

http://apps.kemi.se/nclass/SubstanceInfo.asp?id=27771

NIOSH

http://www.cdc.gov/niosh/ipcsnitl/nitlcas.htm

OSHA/EPA Occupational Chemical Database

http://www.osha.gov/web/dep/chemicaldata/default.asp#target

Sigma Aldrich

http://www.sigmaaldrich.com/catalog/AdvancedSearchPage.do

STN – a pagamento

http://stneasy.fiz-karlsruhe.de/html/english/login1.html

USA National Toxicology Program

http://ntp.niehs.nih.gov/index.cfm

ChemExpert

http://www.chemexper.com/

ChemBioFinder

http://chemfinder.cambridgesoft.com/chembiofinder/SimpleSearch.aspx

ChemIDplus Lite

http://chem.sis.nlm.nih.gov/chemidplus/chemidlite.jsp

DrugBank

http://www.drugbank.ca

FDA

http://www.fda.gov

Medline Plus Health

http://medlineplus.gov

USP

http://www.usp.org/products/referenceStandards/catalog.html

Banca dati tossicologica

http://bdt.regione.puglia.it/pquery.php

OSHA PEL and TWA

http://www.ilpi.com/msds/ref/pel.html

Integrated Risk Information System (IRIS)

http://www.epa.gov/ncea/iris/index.html

HSDB

http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB

CCRIS

http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS

RTECS

http://accelrys.com/products/databases/bioactivity/rtecs.html

GENETOX

http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?GENETOX

Drug Portal

http://druginfo.nlm.nih.gov/drugportal

EDQM

https://www.edqm.eu/en/edqm-databases-10.html

ITER

http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?iter

LactMed

http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?LACT

ISS- centro nazionale. Sostanze chimiche

http://www.iss.it/cnsc

ChemSpider

http://www.chemspider.com/Search.aspx

QUALI DATI RICERCARE

Dati di tipo quantitativo

NOEL = No Observed Effect level

NOAEL = No Observed Adverse Effect Level

LOAEL = Low Observed Adverse Effect Level

LD_{50} (orale, dermale, inalatoria)

Dati di tipo qualitativo

Potere irritante sensibilizzante

Potere cancerogeno, mutageno

Proprietà di tossicità della riproduzione

(anche NOEL, NOAEL, LOAEL riproduttivi)

Altri dati utili

Dati epidemiologici

OEL, OEB, ASL, TLA, TWA già disponibili

Effetti attesi del farmaco nell'uomo (indesiderati)

CRITERI DI QUALITA' del DATO

Klimish Rating

The Klimisch score is a method of assessing the reliability of the toxicological studies, mainly for regulatory purposes, that was proposed by H.J. Klimisch, M. Andreae and U. Tillmann in 1997 in a paper entitled *A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data* which was published in Regulatory Toxicology and Pharmacology (Vol 25 pp. 1-5).

*The scoring system is the **standard method** used in both the EU regulatory schemes (e.g. REACH). Generally, only Klimisch scores of **1** or **2** can be used by themselves to cover a safety endpoint.*
*However, Klimisch score 3 and 4 data can still be used as supporting studies or as part of a **weight of evidence approach**.*
The Klimisch score can be found as a standard field within the IUCLID database

CRITERI DI QUALITA’ del DATO

Klimish Rating

Prevede 4 classi di assegnazione

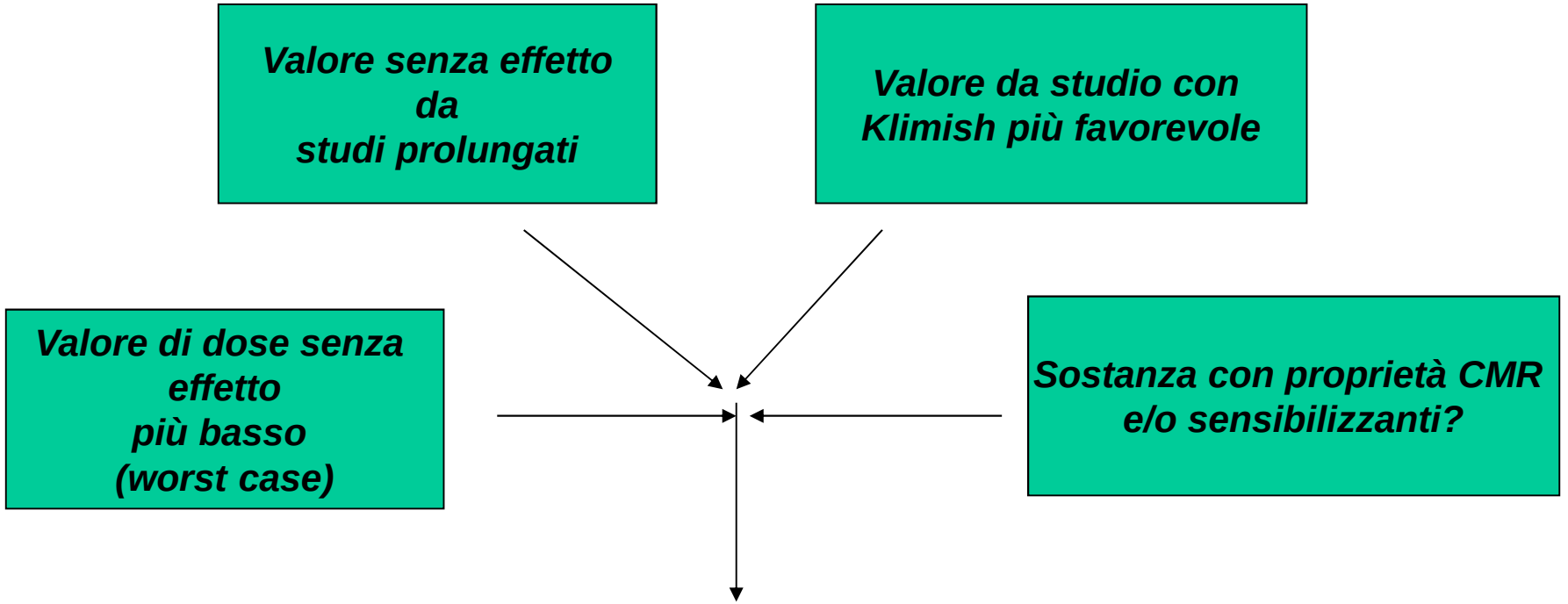
1) *Reliable without restriction*
2) *Reliable with restriction*
3) *Not reliable*
4) *Not assignable*

Le classi 3 e 4 sono comunque utilizzabili nel weigh of evidence approach.

Score	Description
1	This includes studies or data from the literature or reports which were carried out or generated according to generally **valid and/or internationally accepted testing guidelines** (preferably performed according to **GLP**) or in which the test parameters documented are based on a specific (national) testing guideline (preferably performed according to GLP) or in which all parameters described are closely related/comparable to a guideline method
2	This includes studies or data from the literature, reports (mostly not performed according to GLP), in which the test parameters documented **do not totally comply** with the specific testing guideline, but are **sufficient** to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.
3	This includes studies or data from the literature/reports in which there are interferences between the measuring system and the test substance or in which organisms/test systems were used which **are not relevant** in relation to the exposure (e.g., unphysiologic pathways of application) or which were carried out or generated according to a **method which is not acceptable**, the documentation of which is not sufficient for an assessment and which is not convincing for an expert judgment."
4	This includes studies or data from the literature, which **do not give sufficient experimental details** and which are only listed in short abstracts or secondary literature (books, reviews, etc.).

SELEZIONE DEL DATO da utilizzare nell'approccio quantitativo

CRITERI



CALCOLO OEL (Occupational Exposure Level) (m^3)

$$PDE = \frac{NOEL \times BW}{SFn \times \alpha} \qquad OEL = \frac{PDE}{V}$$

Dove:

BW = 60-70 Body weight

α = ***Absorption Factor which account for differences in absorption by route of NOEL to absorption by inhalation. In absence of quantitative data, it is assumed to be 100%.***

V = Volume of air inhaled during an 8-hour workday, typically assumed by default for a 70-kg adult worker to be 10 m^3 (worst case)

SFn = number of safety factors which consider uncertainties in the data.
Si compone dei seguenti:

SF1 for extrapolation from animals to humans

SF1 = 5 ratto – uomo

SF1 = 12 topo-uomo

SF1 = 2 cane-uomo

SF1 = 2.5 coniglio – uomo

SF1 = 3 scimmia – uomo

SF1 = 10 altri animali - uomo

SF2 to account for variability between individuals

SF2 = 10

SF3 to account for study duration

SF3 = 1 per studi della durata di almeno mezza lifetime (un anno per roditori o lagomorfi, 7 anni per cani e scimmie)

SF3 = 1 per studi di tossicità riproduttiva nei quali l'intero periodo di organogenesi è coperto dal trattamento

SF3 = 2 per studi di 6 mesi nel roditore, 3,5 anni nel non roditore

SF3 = 5 per studi di 3 mesi nel roditore, 2 anni nel non roditore

SF3 = 10 per studi di durata più corta

SF4 to account for teratogenic effects with maternal toxicity

SF4 = 1 in presenza di tossicità fetale con tossicità materna

SF4 = 5 in presenza di tossicità fetale senza tossicità materna

SF4 = 5 in presenza di effetti di teratogenesi con tossicità materna

SF4 = 10 in presenza di effetti di teratogenesi senza tossicità metarna

SF5 in absence of a clear NO EFFECT LEVEL (es solo LOAEL)

SF5 = 1-10

CALCOLO ASL (Acceptable Surface Limits) (cm^2)

to establish a quantitative measure for the potential risk from exposure by dermal contact

$$ASL = \frac{NOEL \times BW}{SFn \times \alpha \times SA}$$

Dove:

BW = 60-70 Body weight

α = ***Absorption Factor which account for differences in absorption by route of NOEL to absorption by dermal contact . In absence of quantitative data, it is assumed to be 100%.***

SA = Surface area potentially contacted, typically assumed to be 100 – 200 cm^2 for the average adult (approximately the surface of 1-2 palms).

Applicazione dei metodi *“in silico”*

Quando non ho dati sufficienti per effettuare una congrua valutazione (es. intermedi)

Per ragioni economiche (costo di sperimentazione troppo elevato)

Per effettuare approcci di screening e selezionare la sperimentazione più corretta

In un *Weight of Evidence Approach* (WoE)

“in silico” evaluation

Soprattutto per sostanze intermedie (carenza di dati)

LOCAL EFFECTS

End-point	***Predittore***
Skin irritation	ACD percepta/Toxtree
Skin corrosion	Toxtree
Eye irritation	ACD percepta/Toxtree
Eye corrosion	Toxtree
Skin sensitisation, LLNA	Toxtree, Vega

GENOTOXICITY

End-point	*Predittore*
Microbial in vitro salmonella	ACD percepta/Toxtree, Leadscope, Vega
Microbial in vitro E. Coli	ACD percepta, Leadscope
Mammalian in vitro Mouse Lymphoma	ACD percepta, Leadscope
Mammalian in vitro CHO V79 hgprt	ACD percepta/Toxtree
Mammalian in vivo rodent mutation	Leadscope
Chromosome aberrations in vitro composite	ACD percepta, Leadscope
Chromosome aberration in vitro CHO	Leadscope
Chromosome aberrations in vitro HL	Leascope
Chromosome aberration in vitro other rodents	Leadscope
Chromosome aberration in vivo rat	Leadscope
Chromosome aberration in vivo composite	ACD percepta, Leadscope
Micronucleus in vivo mouse	Leadscope
Micronucleus in vivo rodent	Leadscope, Toxtree, ACD Percepta

CARCINOGENESIS

End-point	*Predittore*
Carcinogenicity rodent (composite)	ACD Percepta, Leadscope
Carcinogenicity mouse (composite)	ACD Percepta, Leadscope
Carcinogenicity mouse male	ACD Percepta, Leadscope
Carcinogenicity mouse female	ACD Percepta, Leadscope
Carcinogenicity rat (composite)	ACD Percepta, Leadscope
Carcinogenicity rat male	ACD Percepta, Leadscope
Carcinogenicity rat female	ACD Percepta, Leadscope
Carcinogenicity	Toxtree, Vega, OECD QSAR Tollbox

ACUTE EFFECTS

End-point	*Predittore*
Acute toxicity mouse oral	ACD Percepta
Acute toxicity rat oral	ACD Percepta

REPRODUCTIVE TOXICITY

End-point	***Predittore***
Reproductive toxicity mouse female	Leadscope
Reproductive toxicity mouse male	Leadscope
Reproductive toxicity rat female	Leadscope
Reproductive toxicity rat male	Leadscope
Reproductive toxicity rodent female	Leadscope
Reproductive toxicity rodent male	Leadscope
Develpmental toxicity binary FDA class.	Leadscope
Structural dysmorphogenensis rabbit	Leadscope
Structural dysmorphogenensis rat	Leadscope
Structural dysmorphogenensis rodent	Leadscope
Structural dysmorphogenensis mouse	Leadscope

RISK ASSESSMENT REPORT

Deve contenere

- **Indicazione delle ricerche di letteratura (metodo e sorgenti)**
- **Discussione critica sulla scelta degli end-point e della dose diriferimento (NOEL, NOAEl etc.)**
- **Chiara descrizione degli studi (pivotal studies) utilizzati per la derivazione del PDE**
- **Discussione sulla qualità degli studi utilizzati (vedi Klimish rating)**
- **Un chiaro razionale per la scelta dei fattori di sicurezza**
- **Il summary**
- **La conclusione**
- **Firma e CV dell'esperto**

GRAZIE!

Chemsafe Srl

Via Ribes 5

10010 Colleretto Giacosa (TO), ITALY

E-mail: a.conto@chemsafe-consulting.com