Non-monotonic dose-response relationships: plausibility and consequences for risk assessment

Lagarde F[1], Beausoleil C[1], Belzunces LP[2], Guerbet M[3], Prat O[4], Schroeder H[5] and Rousselle C[1]*
*Contact: christophe.rousselle@anses.fr

1 French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France;
2 INRA, UR 406 A&E, Laboratoire de Toxicologie Environnementale, CS 40509, 84914 Avignon cedex 9, France. Member of the Anses working group on « endocrine disruptors and reprotoxic substances »
3 Toxicology Laboratory, ABTE-EA 4651, Rouen University, 22 boulevard Gambetta, 76183 Rouen, France. President of the Anses committee « Assessment of the risks related to chemical substances »
4 CEA, IBEB, SBTN, F-30207 Bagnols-sur-Cèze, France. Member of the Anses committee « Assessment of the risks related to chemical substances »
5 URAFPA, INRA UC340, Université de Lorraine, Faculté des Sciences et Technologies, F-54500 Vandoeuvre les Nancy, France. Member of the Anses committee « Assessment of the risks related to chemical substances »

AIMS OF THE WORK

Dose-response relationship is defined as non monotonic when the slope of the curve changes sign within the range of examined doses. An increasing number of scientific data describes non-monotonic dose-response relationships (NMDRRs) but there is still some debate within the scientific community concerning their scientific validity and how to use them in a risk assessment context. The aim of this work is to provide a thorough analysis of numerous studies showing NMDRRs in the field of endocrine disruption (ED) and to propose a chart flow for the assessment of such relationships.

METHODS

1) Literature search in order to identify reported NMDRRs with EDs
2) Plausibility assessment of each reported NMDRR :
   a) Statistical plausibility : when no statistical test is done, criteria of analysis developed initially by Calabrese *et al*[6] to test hormesis relationships are applied. At least four tested doses (included control) are needed to apply these criteria.

Number of doses below ZEP[7] (excluding the control)	**Score A**
1	1
2	2
3	3
4	4
≥ 5	5
Experimental determination of ZEP	**Score B**
Yes	1
No	0

Number of statistically different doses	**Score C**
1	2
2	4
3	8
≥ 4	16
Reproducibility of the dose-response relationship	**Score D**
Yes	3
No	0

Magnitude of response (percentage control value)		**Score E ***
Inverted-U curve	**U curve**	
≥ 100 ≤ 125%	≤ 97 ≥ 92%	0,5
> 125 ≤ 150%	< 92 ≥ 84%	1
> 150 ≤ 200%	< 84 ≥ 68%	2
> 200 ≤ 400%	< 68 ≥ 5%	3
> 400%	< 5%	4

* The point value is multiplied by the number of experimental doses falling within the corresponding percentage range.

Total score (A+B+C+D+E)	**Plausibility of a non-monotonic dose-response relationship**
1-2	No
> 2-8	Very low
> 8-12	Low
> 12-16	Moderate
> 16-20	High
> 20	Very high

b) Biological plausibility : research of biological mechanisms involved in the observed NMDRR.

LITERATURE SEARCH

Fifty-one experimental studies (20 *in vitro*, 29 *in vivo*, 2 epidemiological) representing 170 NMDRRs, with many effects associated.

Compounds	**Number of NMDRRs**
Hormones	**42**
17β-estradiol	35
17α-estradiol	2
Ethinyl estradiol	2
Dihydrotestosterone	1
Pregnenolone	1
Dehydroepiandrosterone	1
Bisphenol A	**59**
Alkylphenols	**16**
Diethylhexylphthalate	**10**
Phyto-estrogens	**10**
Coumestrol	2
Daidzein	2
Genistein	3
Lavendustin	1
Resveratrol	2
Pesticides	**12**
Organochlorine	9
Methoxychlor	3
PCB	**9**
Diethylstilbestrol	**12**

STATISTICAL PLAUSIBILITY

- Criteria of analysis have been applied on 148 NMDRRs.
- Dose-response relationships with “moderate”, “high” and “very high” plausibility of being statistically non-monotonic represented 55% of the 148 listed NMDRRs.

Plausibility of NMDRRs	**n (*in vitro*)**	**n (*in vivo*)**	**n (total)**
No	0	0	0
Very low	9	29	38
Low	9	19	28
Moderate	9	17	26
High	6	10	16
Very high	20	20	40

BIOLOGICAL PLAUSIBILITY

- Among the 51 studies, mechanisms of action were discussed for 31 studies.
- Several mechanistic hypotheses could be mentionned in each study.

Mechanistic hypotheses	**Number of studies**
Existence of several molecular targets with different affinities and opposite effects	23
Negative feedback phenomenon	11
High-dose receptor desensitization	3
Dose-dependent metabolism modulation	2
High-dose toxicity	3
Dose-dependent protein ionisation	1

CONCLUSION

- NMDRRs have been described for some compounds with endocrine disrupting properties and are credible, based on a statistical and biological plausibility analysis.
- Statistical plausibility should be evaluated based on define specific criteria (reproducibility, number of statistically different doses, magnitude of responses…) and biological plausibilty before considering them in a risk assessment context.

6 Calabrese, E.J., Blain, R.B., 2011. The hormesis database: the occurrence of hormetic dose responses in the toxicological literature. Regul. Toxicol. Pharmacol. 61, 73-81.
7 ZEP = Zero Equivalent Point (i.e. the point where the response crosses the control value in a hormetic effect).