

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

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## 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*<sup>6</sup> to test hormesis relationships are applied. At least four tested doses (included control) are needed to apply these criteria.

Number of doses below ZEP <sup>7</sup> (excluding the control)	Score A	Number of statistically different doses	Score C	Magnitude of response (percentage control value)		Score E *	Total score (A+B+C+D+E)	Plausibility of a non-monotonic dose-response relationship	
				Inverted-U curve	U curve				
1	1	1	2				1-2	No	
2	2	2	4	≥ 100 ≤ 125%	≤ 97 ≥ 92%	0,5	> 2-8	Very low	
3	3	3	8	> 125 ≤ 150%	< 92 ≥ 84%	1	> 8-12	Low	
4	4	≥ 4	16	> 150 ≤ 200%	< 84 ≥ 68%	2	> 12-16	Moderate	
≥ 5	5	Reproducibility of the dose-response relationship	Score D	> 200 ≤ 400%	< 68 ≥ 5%	3	> 16-20	High	
Experimental determination of ZEP	Score B			> 400%	< 5%	4	> 20	Very high	
Yes	1			Yes	3				
No	0			No	0				

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

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

## 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 ( <i>in vitro</i> )	n ( <i>in vivo</i> )	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 mentioned 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 plausibility before considering them in a risk assessment context.