

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*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 | 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 |
|------------------------------|------------------|
| 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 (<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.