Nature of an effect: adverse or non-adverse?

EFSA Scientific Colloquium N°17 on low dose response in toxicology and risk assessment

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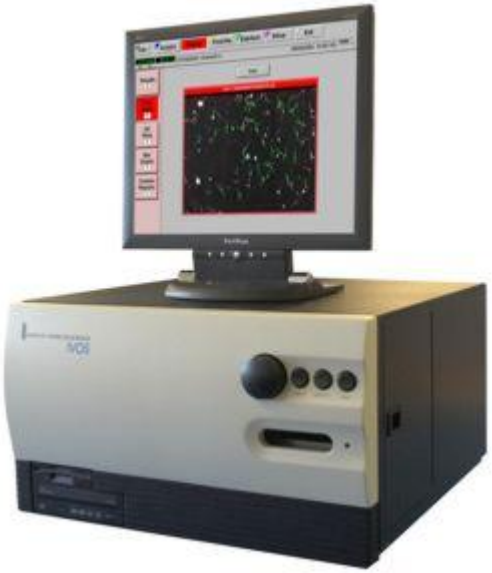
Overview

- Effects and experiments
  - The importance of the experimental setting
- Interpreting effects
  - Considerations to aid in interpretation

Effects and experiments

The primacy of experimental context

A crystal ball



A sperm analysis machine

Good Laboratory Practice

- REACH Article 14(4)
  - Ecotoxicological and toxicological tests and analyses shall be carried out in compliance with the principles of good laboratory practice provided for in Directive 2004/10/EC or other international standards recognised as being equivalent by the Commission or the Agency and with the provisions of Directive 86/609/EEC, if applicable.

- GLP is very important
  - Assuring the quality of scientific studies
  - Trained personnel, validated standard operating procedures, …
  - "inspect the final reports to confirm that the methods, procedures, and observations are accurately and completely described, and that the reported results accurately and completely reflect the raw data of the studies"

Robust experimental design

- REACH Article 13(3)
  - Where tests on substances are required to generate information on intrinsic properties of substances, they shall be conducted in accordance with the test methods laid down in a Commission Regulation or in accordance with other international test methods recognised by the Commission or the Agency as being appropriate. The Commission shall adopt that Regulation, designed to amend the non-essential elements of this Regulation by supplementing it, in accordance with the procedure referred to in Article 133(4).
  - Information on intrinsic properties of substances may be generated in accordance with other test methods provided that the conditions set out in Annex XI are met.

Test guidelines

- The test protocol is thoroughly evaluated
  - By multiple stakeholders
- Output is understood
- Methodology is reliable
- Use of multiple exposure levels
  - Is an effect caused by the substance ?
  - Enables evaluation of the relationship between dose and effect

Study reliability

- Is a study reliable ?
- May be measured using the Klimisch score
  - H.J. Klimisch, M. Andreae and U. Tillmann (1997) A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data Regulatory Toxicology and Pharmacology Vol 25 pp 1-5
  - In IUCLID 5.x, the reliability field must be filled in for key studies

Score	Description
1	Reliable without restriction
2	Reliable with restriction
3	Not reliable
4	Not assignable

ECHA
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Why the emphasis on study reliability ?

Computational Design of a Biologically Active Enzyme

Mary A. Dwyer,[1] Loren L. Looger,[1]* Homme W. Hellinga[1]†

Rational design of enzymes is a stringent test of our understanding of protein chemistry and has numerous potential applications. Here, we present and experimentally validate the computational design of enzyme activity in proteins of known structure. We have predicted mutations that introduce triose phosphate isomerase activity into ribose-binding protein, a receptor that normally lacks enzyme activity. The resulting designs contain 18 to 22 mutations, exhibit 10^5- to 10^6-fold rate enhancements over the uncatalyzed reaction, and are biologically active, in that they support the growth of *Escherichia coli* under gluconeogenic conditions. The inherent generality of the design method suggests that many enzymes can be designed by this approach.

Enzymes are among the most proficient catalysts known (*1*), and they catalyze a wide variety of reactions in aqueous solutions under ambient conditions with exquisite selectivity and stereospecificity (*2*, *3*). The rational design of enzymes has tremendous practical potential for developing novel synthetic biochemical pathways (*4*, *5*), but presents a formidable challenge and is one of the m
stringent tests for understanding prot
chemistry. Here, we present structure-bas
computational design techniques (*6*, *7*) t
predict mutations for the construction of c
alytically active sites in proteins of kno
structure. Using these methods, we conver
ribose-binding protein (*8*) into analo
(NovoTims) of the glycolytic enzyme trio
phosphate isomerase (*9*). Several NovoTi
exhibit rate enhancements of about 10^5 to
and are biologically active, as seen in th
support of the growth of *Escherichia c*
under gluconeogenic conditions.

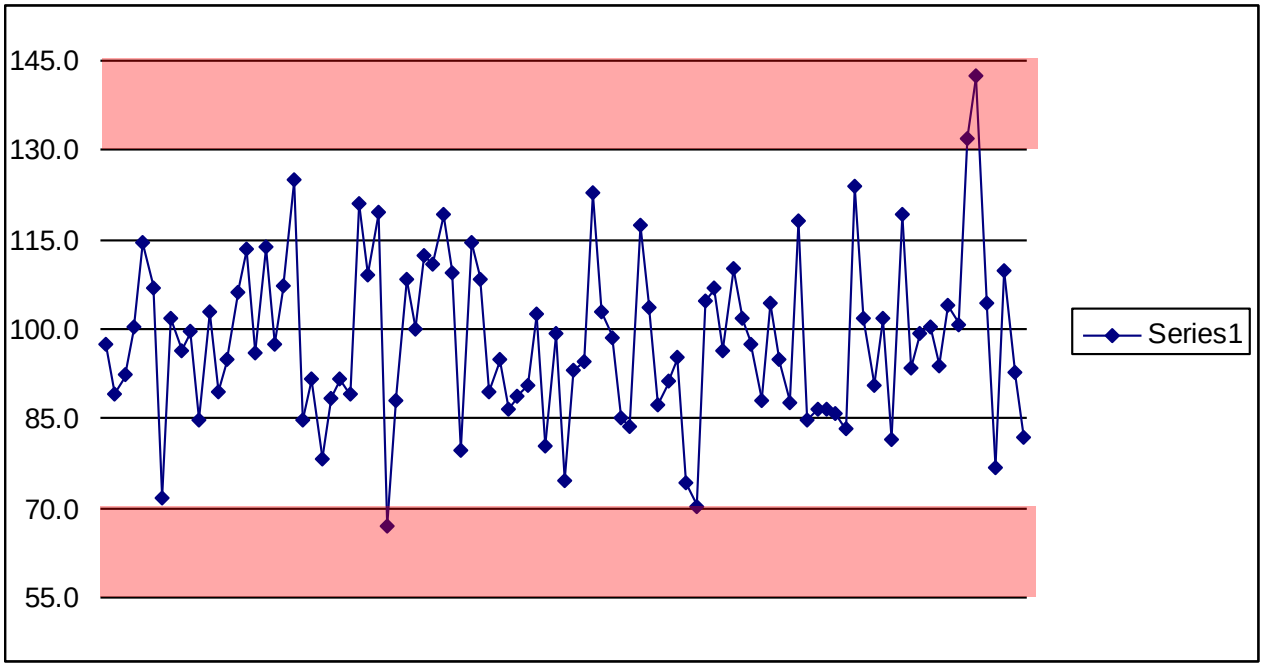
Triose phosphate isomerase (TIM) is an
sential component of the Embden-Meyerh
pathway (*10*), interconverting dihydroxyaceto
phosphate (DHAP) and glyceraldehyde
phosphate (GAP) (Fig. 1A). In glycolysis, T
channels these two triose phosphate produ
of aldolase into pyruvate; in gluconeogene

Interpreting effects

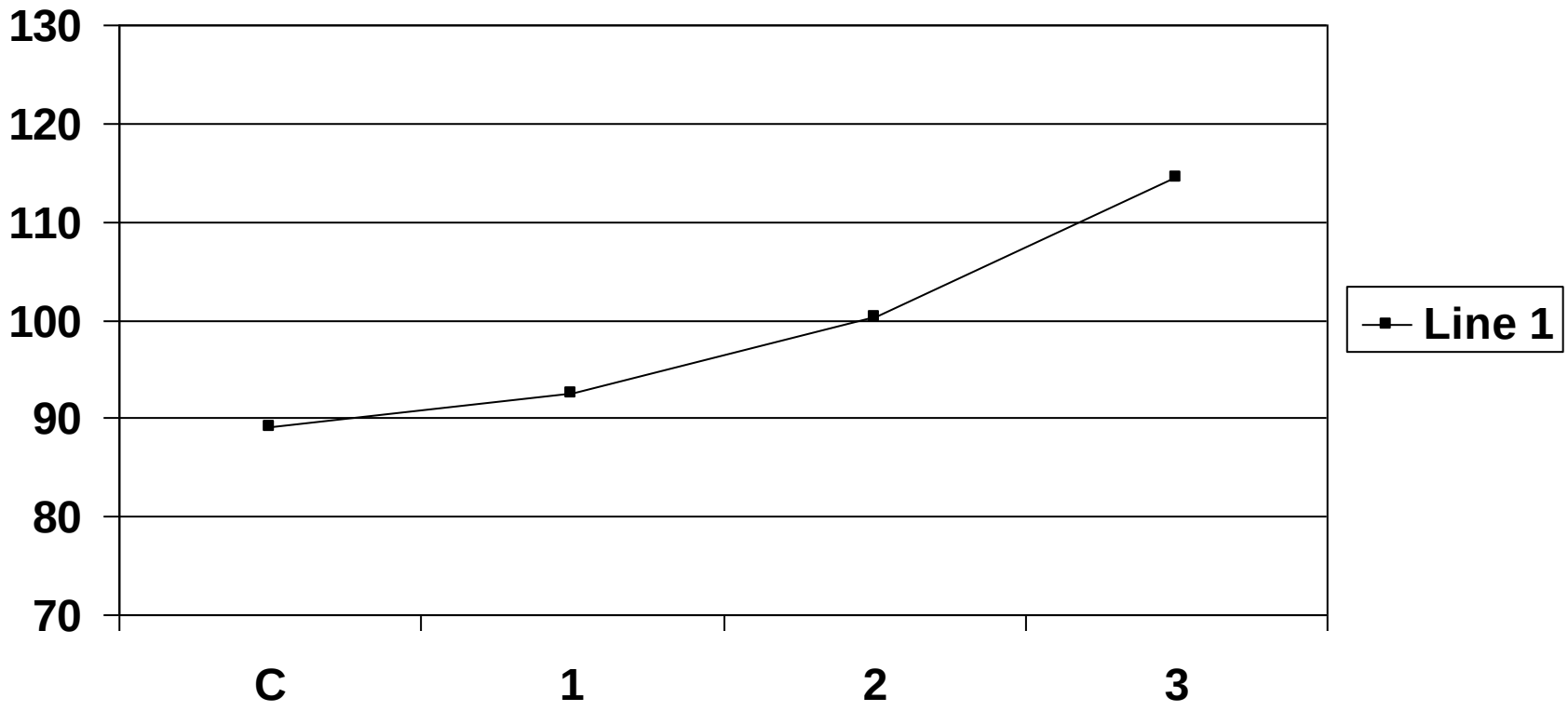
- Substance-related effect
- Adversity

Substance-related effect

- Statistics used to evaluate whether something is different from control
  - At an α=0.05, one in 20 comparisons will give a 'statistically-significant' result



Substance-related effect



Looks like a dose-related, and significant, effect

It is just random noise from the previous slide

Substance-related effect

- Consider
  - Dose-response
  - Precision of the measurement in question
  - Range of natural variation
  - Effect size
  - Relationship to other effects
  - Biological plausibility

  - Weight of evidence from multiple studies

Adversity

- No definition in REACH

- CLP guidance for reproductive toxicity
  - "Any effect of substances that has the potential to interfere with sexual function and fertility."
  - "Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation."

Adversity

- "Change in the morphology, physiology, growth, development, reproduction, or life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences."
  - International Programme on Chemical Safety. IPCS risk assessment terminology. http://www.inchem.org/documents/harmproj/harmproj/harmproj1.pdf

Considerations for adversity

- Function of organism/ organ/ tissue impaired
- Morphological change
- Adaptive response
- Transient response
- Effect severity/ size
- Biological plausibility

Considerations…

- For example
  - Transient effects can be adverse
  - Transient anoxia for a couple of minutes

- Biological plausibility is key
  - Biochemical or transcriptional responses often not adverse in themselves
  - But there may be sufficient knowledge to conclude that such measurements are adverse

Conclusions

- Experimental context is important
  - GLP, Guideline studies
- Substance-related effect
- Consider relevant factors
- Biological plausibility
  - Importance of interpretation

Thank You.

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