Synergy Theory in Biology
Simulating Radiation Damage During Interplanetary Voyages as an Example

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1. Abstract

Synergy analysis uses the dose-effect relations (DERs) of mixture components to define a baseline mixture DER characterizing absence of synergy/antagonism.

Pharmacologists have known for a very long time that the "obvious" method of simply adding component effects is wrong if the component DERs are highly curvilinear, so many replacements for the simple effect additivity baseline are in use [1-8]. We here discuss a recently introduced replacement [9], incremental effect additivity, which has fewer drawbacks than previous replacements. We exemplify incremental effect additivity by discussing the mixed radiation field above low earth orbit, whose components are believed to have highly curvilinear DERs for carcinogenesis.

2. Space Radiations May Have Very Curvilinear Dose-Effect Relations (DERs)

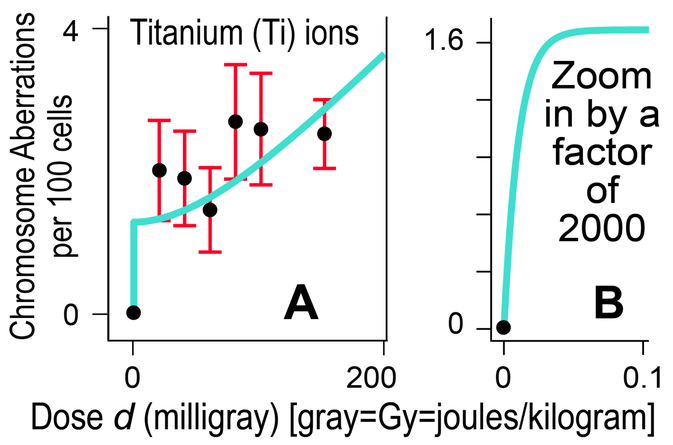
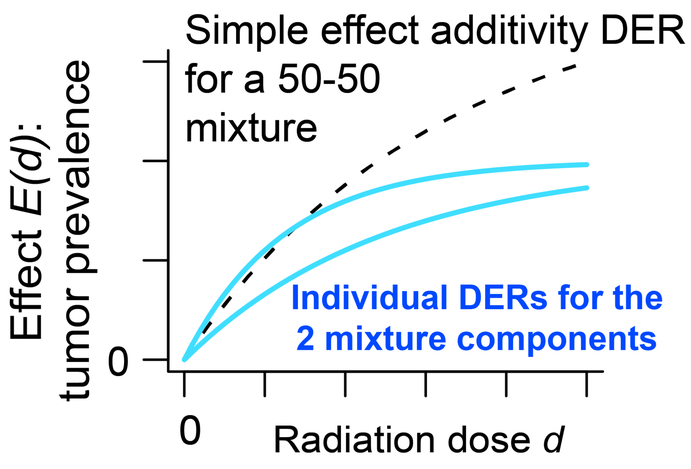


Fig. shows effects measured in a NASA experiment [10] exposing cells to Ti atoms moving at 87% of light speed -- so fast all electrons are stripped off leaving only the highly charged nucleus. Such exotic radiations almost never penetrate to ground level but at present cannot feasibly be shielded against in space because collisions would produce a shower of secondary radiations more dangerous than the original. Probably Ti and similar particles give highly curvilinear DERs, very concave at very low doses, as shown in the figure. Panel B shows the apparent kink in panel A is instead smooth and highly curvilinear. Theoretical arguments suggest intercellular interactions can produce damage when even one cell in a multi-cellular niche is hit [11]. This can lead to high DER concavity. At higher doses only comparatively minor curvilinearity (here convexity) is typically measured.

3. Simple Effect Additivity is Wrong if Dose-Effect Relations (DERs) are Very Curvilinear



If all components of a mixture have strictly concave DERs as shown, the simple effect additivity mixture DER is always an over-estimate. Here, it is larger than the DER average, and since it becomes larger than either component DER, it can't reasonably define absence of synergy. If instead all mixture components have strictly convex IDERs, the simple effect additivity mixture DER is an under-estimate.

Famously, curvilinearity makes simple effect additivity fail to obey what is called the sham mixture principle [4]. For example, suppose an agent has DER $E=d^2$. Consider the agent as a 50-50 mixture of two components each of which happens to have the same IDER. Then simple effect additivity gives mixture DER $E_M = 2(d/2)^2 = \frac{1}{2} d^2$. But of course one cannot cut an agent's damage in half just by thinking about it as a 50-50 mixture.

4. The Equation of Incremental Effect Additivity

• *Basic Ideas.* (a) Linear superposition of effect derivatives makes more sense than linear superposition of effect values. (b) Mixture effect is a property of the biological system and is thus a more informative control variable than dose.

• *Notation.* Mixture of $N \geq 1$ components. j^{th} component has DER (Dose-Effect Relation) $E_j(d_j)$, contributes dose $d_j = r_j d$ to mixture dose $d = \sum_{j=1}^{N} d_j$, where r_j is a positive constant. $\sum_{j=1}^{N} r_j = 1$.

• *Special Case.* $\forall j$, $E_j(d_j)$ is smooth and monotonically increasing for all non-negative doses. Thus each E_j has an inverse function D_j, defined on $[0, a_j)$ with $0 < a_j \leq \infty$. Consider the equation

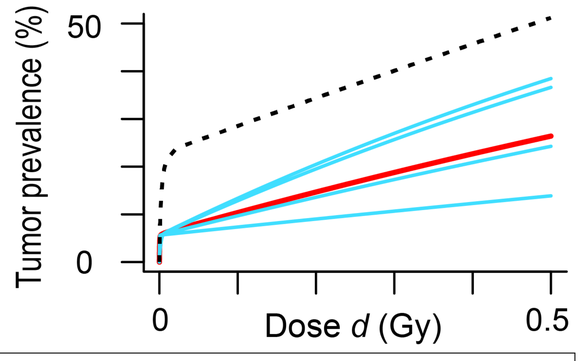
$$(1) \qquad dI/dd = \sum_{j=1}^{N} r_j \left[dE_j/dd_j \right]_{d_j = D_j(I)}; \quad d = 0 \Leftrightarrow I = 0.$$

(1) is the equation of incremental effect additivity [9]. It determines a baseline no-synergy/antagonism mixture dose-effect relation $I(d)$ on some interval $[0,a)$. The key step is using $d_j = D_j(I)$ instead of the seemingly more natural $d_j = D_j(E_j)$.

Eq. (1) can be interpreted as follows. As the total mixture dose d increases slightly, every individual component dose d_j has a slight proportional increase since $dd_j/dd = r_j > 0$. Therefore every mixture component contributes some incremental effect. The size of the incremental effect is determined by the state of the biological target, specifically by the total effect already contributed by all the components collectively (and <u>not</u> by the dose the individual component has already contributed). Different components thereby appropriately track changes of slope both in their own DER and in the other DERs. Using $I(d)$ would have been impractical before computers became adept at solving non-linear ODE. Importantly, Eq. (1) can be generalized to cases where the component DERs are much less restricted than assumed above.

5. Incremental Effect Additivity is Appropriate Even for Mixtures with Highly Curvilinear Component DERs

Figure for a mixture of 4 different 1-ion beams, each contributing 1/4 of the mixture dose



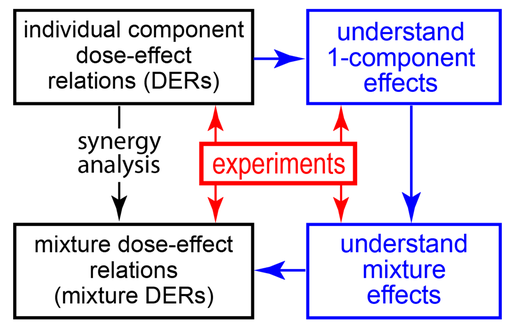
The figure shows Dose-Effect Relations (DERs) inferred from NASA experiments on 1-ion beams inducing murine Harderian gland tumors [13, 14]. DERs that would result if mixture dose were all contributed by one of the 4 components are shown in blue. The incremental effect additivity baseline no-synergy/antagonism DER $I(d)$ compromises among the 4 curves. Simple effect additivity gives nonsense, due to pronounced component DER concavity at very low doses.

6. Advantages of Incremental Effect Additivity DER $I(d)$

In contrast to simple effect additivity and many of its replacements, incremental effect additvity $I(d)$:

- Always obeys the sham mixture principle defined in panel 3.
- Always obeys a mixture of mixtures principle: if some mixture components are themselves mixtures, the obvious consistency condition holds. For example, space radiation mixture components are typically mixtures at the biological target because intervening shielding leads to secondary radiations.
- Has a much larger domain of applicability.

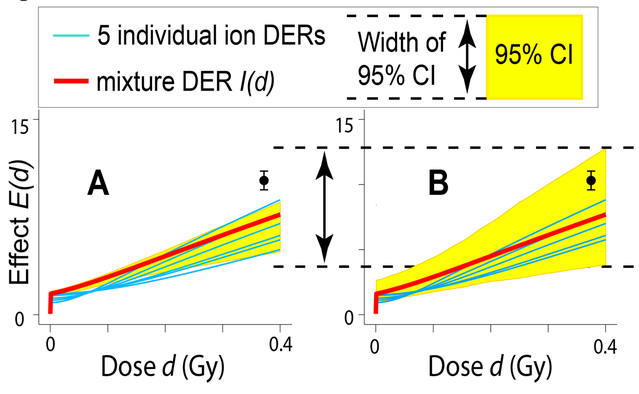
7. All Synergy Theories Have Questionable Aspects



• The figure illustrates one drawback, out of many. Eventually, but probably not soon, space radiation mixture DERs based merely on mathematical manipulations of component DERs (leftmost arrow) will be replaced by understanding whether, how, and why radiations interact (long blue road). The far simpler and more practical synergy analysis is important in the meantime, but only until it helps make itself obsolete.

8. Many Problems Remain

Mathematical Problems, e.g. Obedience to the mixture of mixtures principle implies obedience to the sham mixture principle. Can "implies" here be replaced by "iff"?
Statistical Problems, e.g. NASA safety requirements and synergy significance estimates both emphasize 95% confidence intervals (CI). Our calculations of $I(d)$ show that for DERs highly concave at low doses, taking correlations of regression-calibrated parameters into account (Panel A) gives tighter CI than unrealistically neglecting them (Panel B). How general is this behavior?



Biophysical Problems, e.g. In space, radiation mixtures occur at very low chronic dose rates [11]. Very little relevant mathematical synergy analysis has been done, despite the key role of dose rate in radiobiology. The only exception is the special case of constant dose rate, where there is now the outline of a theory and, exceptionally, there are considerable relevant data, e.g. [15].

References. 1. Fraser, T.R., Lecture on the Antagonism between the Actions of Active Substances. Br Med J, 1872. **2**(618): p. 485-7. 2. Loewe, S. and H. Muischnek, Ueber Kombinationswirkungen. I. Mitteilung Hilfsmittel der Fragestellung. Archiv for Experimentelle Pathologie und Pharmakologie, 1926. **114**: p. 313-326. 3. Zaider, M. and H.H. Rossi, The synergistic effects of different radiations. Radiat Res, 1980. **83**(3): p. 732-9. 4. Berenbaum, M.C., What is synergy? Pharmacol Rev, 1989. **41**(2): p. 93-141. 5. Geary, N., Understanding synergy. Am J Physiol Endocrinol Metab, 2013. **304**(3): p. E237-53. 6. Foucquier, J. and M. Guedj, Analysis of drug combinations: current methodological landscape. Pharmacol Res Perspect, 2015. **3**(3): p. e00149. 7. Piggott, J.J., et al., Reconceptualizing synergism and antagonism among multiple stressors. Ecology and Evolution:, 2015. 5(7): p. 1538–1547. 8. Tang, J., et al., What is synergy? The Saariselkä agreement revisited. Frontiers in Pharmacology, 2015. 6: p. 181. 9. Siranart, N., E.A. Blakely et al., Mixed Beam Murine Harderian Gland Tumorigenesis: Predicted Dose-Effect Relationships if neither Synergism nor Antagonism Occurs. Radiat Res, 2016. 186(6): p. 577-591. 10. Cacao, E., M. Hada, et al., Relative Biological Effectiveness of HZE Particles for Chromosomal Exchanges and Other Surrogate Cancer Risk Endpoints. PLoS One, 2016. 11(4): p. e0153998. 11. Norbury JW, W Schimmerling, TC Slaba, . . ., CJ Zeitlin. Galactic cosmic ray simulation at the NASA Space Radiation Laboratory. Life Sci Space Res (Amst) 8: 38-51. (2016). 12. Hatzi, V.I., et al., Non-targeted radiation effects in vivo: a critical glance of the future in radiobiology. Cancer Lett, 2015. 356(1): p. 34-42. 13. Chang, P.Y., E.A. Blakely et al., Harderian Gland Tumorigenesis: Low-Dose and LET Response. Radiat Res, 2016. 185(5): p. 449-60. 14. Cucinotta, F.A. and E. Cacao, Non-Targeted Effects Models Predict Significantly Higher Mars Mission Cancer Risk than Targeted Effects Models. Sci Rep, 2017. 7(1): p. 1832. 15. Lubin, J.H., et al., Radon-exposed underground miners and inverse dose-rate (protraction enhancement) effects. Health Phys, 1995. 69(4): p. 494-500.

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