Supplementary Information for Synergy Theory Article (Dae Woong Ham et al.)

This supplement has 5 sections, as follows.

- A1 contains glossaries and figures explaining mathematical symbols, acronyms, and terminology.
- A2 compares the chromosome aberration models in the main text with models in [Cacao et al. 2016].
- A3 surveys the biomedical literature we consider most relevant to radiobiological synergy theory.
- A4 shows by an example that time-incremental synergy analysis can be applied to low dose-rate exposures, assuming linear-quadratic IDER and linear repair kinetics.
- A5 discusses the general form of the equation of incremental effect additivity.

Appendix A1. Brief Explanations

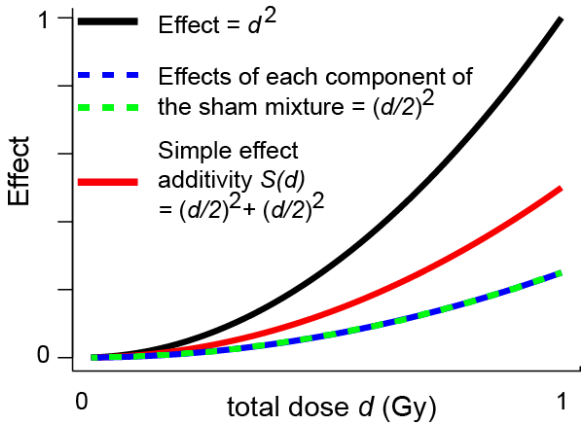
A1.1. Mathematical Symbols	
α_λ	Linear coefficient of the LQ equation for effects induced by gamma radiation
α	Usually the linear coefficient of LQ approximation
β^*	The velocity of an ion divided by the velocity of light
β	Usually the quadratic coefficient of LQ approximation
d_0	A nominal dose where the IDER slope due to NTE has already become substantially smaller than the initial slope at the origin. See section A2
$\mathrm{d}E_j/\mathrm{d}d_j$	The slope of an IDER
$D_j(E_j)$	The compositional inverse function of an IDER: $D_j(E_j(d_j))=d_j$
$d_j = r_j d$	dose of a mixture component as a fraction r_j of total mixture dose d.
$E_j(d_j)$	IDER for the jth component of a mixture.
$E(d)$	IDER
$G(E)$	An inverse function used in sub-section A3.4
$G(z)$	A slope extrapolator, described in sub-section A5.2
$I(d)$	Incremental effect additivity MIXDER and default hypothesis
r_j	Fixed ratio of component dose to total mixture dose, $r_j = d_j / d, 0 < r_j < 1.$
$S(d)$	Simple effect additivity MIXDER and default hypothesis
Z_{eff}	Effective ion charge, almost equal to the atomic number in our calculations

A1.2. Acronyms and Terminology	
AIC and BIC	Akaike and Bayesian information coefficients
AIDER	Analytically-defined IDER. See section A5
AIVP	Autonomous Initial Value Problem, used in section A5
Baseline MIXDER	A Mixture Dose-Effect Relationship which defines absence of synergy or antagonism.
CA	Chromosome Aberration(s)
Concave	The opposite of convex, with which it is frequently confused. See Fig. A1.2 below
Convex	A standard mathematical term that can be used to describe curve shapes. See Fig. A1.1
Default synergy hypothesis	A hypothesis consisting of a baseline no-synergy/no-antagonism MIXDER and also a way to calculate baseline mixture effect uncertainties when IDER uncertainties are known
Explicit function	Roughly, a function $f(x)$ built from the standard functions used in introductory calculus. Specifically, an "elementary" function, as defined in math texts
IDER	Individual Dose Effect Relationship
Incremental effect additivity	ODE method of calculating a MIXDER $I(d)$ from the component IDERs, with $I(d)$ then used as the definition of no synergy or antagonism
$I(d)$	Abbreviation both for the incremental effect additivity baseline MIXDER and the corresponding default hypothesis
IVP	Initial Value Problem, here for an ordinary differential equation
Inverse function	For a continuous monotonically increasing function $F(x)$, $D(F(x))=x$ for all x in the domain of F defines the inverse function D. For example exp and ln are inverses of each other
L	Stopping power, LET_∞
LNT	Linear-No-Threshold. A straight line through the origin dose=0, effect=0
LQ	Linear-Quadratic. In section A3 for acute dosing and in section A4 for protracted dosing
MIXDER	Mixture Dose-Effect Relationship
NTE	Non-Targeted Effects due to inter-cellular interactions. "Bystander" effects
NTE1, NTE2	IDERs used in [Cacao et al. 2016]
ODE	Ordinary Differential Equation(s)
Sham mixture	The mixture of an agent with itself. See Fig. A1.1 below
Slope extrapolator	A function of a complex variable that specifies a particular AIDER
TE	Targeted Effects. Due to a direct hit by a radiation track or a near miss by $\ll 1$ μm
Very low dose	$0 \leq$ dose < 5 mGy
Ultra-low dose	$0 \leq$ dose < 0.5 mGy
WGE	Whole Genome Equivalent. Used when part of a genome is painted in a CA experiment

A1.3. Figures Illustrating the Sham Mixture Principle, Convexity, and Concavity

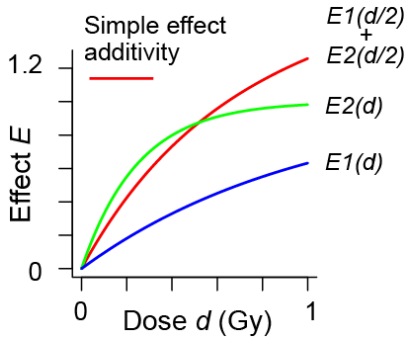
The Introduction section of the main text discussed some terminology that will frequently be used in this supplement. Similar figures that summarize the relevant terminology are repeated here for reference. The second derivative criteria given for concavity and convexity are adequate for our purposes, though not as general as the standard mathematical definitions.

Fig. A1.1. The sham mixture principle and convexity.



Consider a hypothetical case where a 1-ion beam has a pure quadratic IDER $E(d)=\beta d^2$ with β=1 Gy^{-2}. The second derivative d^2E/dd^2 is positive and the curve is convex. Regard the beam as a 50-50 mixture of two 1-ion beams, both of which happen to have exactly the same IDER as the original beam. Then for total mixture dose d, each of the two beams contributes dose $d/2$ and thus has effect $E/4$ (blue and green curves). Using simple effect additivity gives a baseline no-synergy/no-antagonism mixture dose-effect relation $E/2$ rather than the correct value E. It thus violates the sham mixture principle. Similar discrepancies arise whenever all mixture components have convex IDERs and the simple effect additivity theory $S(d)$ is used.

Fig. A.1.2. A mixture with concave IDERs.



The figure shows results for hypothetical 1-ion dose response curves $E_k(d_k)$=1-exp(-$\alpha_k d_k$) with k=(1,2), α_1=1 Gy^{-1}, and α_2=4 Gy^{-1}. Instead of being approximately the average of the two IDERs the simple effect additivity baseline dose effect relation for a 50-50 mixed ion beam of the two ions becomes larger than that for either ion by itself even though it is supposed to characterize absence of synergy. Similarly misleading synergy assertions arise whenever all components of a mixture have concave IDERs (i.e. IDERs with negative second derivatives) and the simple effect additivity theory is used.

Appendix A2. CA IDERs

We here review CA data considered in the Results section of the main text and compare our CA IDERs with some previous models. The source-codes used are available on GitHub.

The data was previously analyzed in [Cacao et al. 2016], with three kinds of models, Linear, NTE1, and NTE2. The Linear model does not incorporate NTE; the other two incorporate both TE and NTE. All three models modify Katz' parametric track structure approach which relates heavy ion action to the action of gamma rays via analyzing delta-ray tracks (reviewed and motivated, e.g., in [Katz 1988; Cucinotta et al. 1999; Goodhead 2006; Cucinotta, Kim, Chappell, et al. 2013; Hada et al. 2014; Cacao et al. 2016]). The models used the biophysical parameters in a table of the main text, repeated here as Table A2.1.

ion	**^{16}O**	**^{28}Si**	**^{48}Ti**	**^{56}Fe**		
Z	8	14	22	26		
E/u (MeV)	55	170	600	600	450	300
L (keV/μm)	75	100	125	175	195	240
Z_{eff}^2/β^{*2}	595	690	770	1075	1245	1585
dmax (Gy)	0.4	1.2	0.6	0.8	0.4	0.8

Table A2.1. Dose and Track Parameters. Z is atomic number. E/u is kinetic energy per atomic mass unit. L is stopping power LET_∞, Z_{eff} is the effective ion charge, almost equal to Z for the ions and E/u values shown. β^* is ion speed relative to the speed of light.

Fig. A2.1 shows how slopes behave in the NTE2 model.

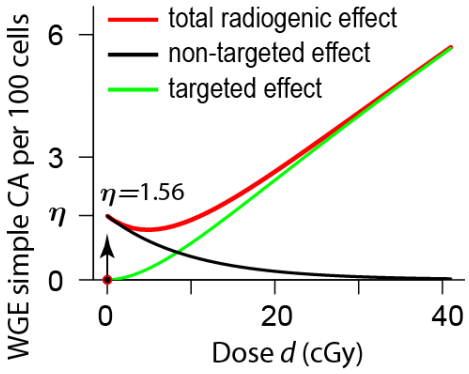


Fig. A2.1. Shape of a Typical NTE2 Model Curve. In the NTE2 model the total radiogenic effect is the sum of NTE and TE contributions (black and green curves). The two properties most relevant for the discussion of our CA IDERs in the main text are the following. First, NTE are modeled as jumping, at an ultra-low dose smaller than 1 mGy, from 0 to η with infinite slope (arrow) and then decaying back to 0 (black curve). As a result the total radiogenic effect is decreasing rather than increasing at doses between the ultra-low dose and ~5 cGy. The second relevant property is that at doses larger than about 10 cGy, where the NTE2 model predicts TE are starting to dominate NTE, the TE curve (green) has a positive slope that is almost constant.

Equations, illustrating synergy theories, in the Results section of the main text specify smooth monotonically increasing IDERs that used the same data. Regression gave the average values of the adjustable parameters and the significance level of their difference from zero that are shown in the IDER row of Table A2.2.

Table A2.2. Values and Statistical Significance Levels of the Parameters

Model	η_0	η_1 (μ/keV)	σ_0 (micron^2)	κ
IDER	1.5e-4±2.2e-5 (<1e-3)***	3.5e-3±9.0e-4 (<1e-3)***	4.2±1.4 (<0.01)**	469±247 (0.064)*
NTE1	1.1e-4±9.0e-5 (<0.299)	7.0e-3±5.6e-3 (<0.256)	6.12±1.66 (<0.021)*	796±287 (<1e-4)***
NTE2	4.7e-4±2.6e-4 (<0.152)	1.1e-2±3.5e-3 (<0.036)*	6.75±1.67 (<0.016)*	590±236 (<1e-4)***

In the table the following conventions and abbreviations are used. (a) Powers of 10 are indicated by 'e'; for example 1.5e-4=0.00015. (b) Standard errors are indicated by ±. (c) Asterisks indicate a statistically significant difference from 0 at respective levels: * ≤ 0.1; ** ≤0.01; *** ≤10^{-3}.

The two last rows of the table show used the models and parameters in [Cacao et al. 2016] but were re-calculated to take into account our way of calibrating models, emphasizing radiogenic IDERs, with background subtracted out.

A comparison with NTE2 models at ultra-low doses is shown in Fig A2.2

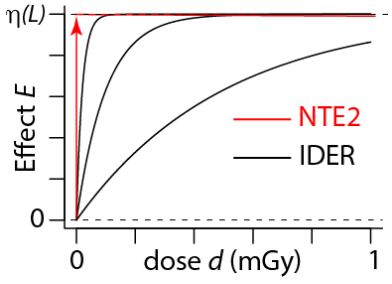


Fig. A2.2. Smooth Concavity vs. Infinite Slope. The figure shows modeled NTE contributions to total effect for Si ions (L=100). Red lines are for the NTE2 model used in [Cacao et al. 2016], where the slope at an ultra-low dose was taken to be infinite. The subsequent decrease at higher doses, shown in Fig. A2.1, is here barely visible at 1 mGy where the red line has dipped slightly below the horizontal line at height $\eta(L)$. The three black curves are for NTE contribution to our corresponding IDER in the main text, with the respective d_0 values, from left to right, of 0.02, 0.1, and 0.5 mGy. Our final results are essentially unchanged if any value of $d_0 \leq$0.1 mGy is used. The black curves are monotonic increasing at all doses; they approach $\eta(L)$ and zero slope as dose increases. The value for $\eta(L)$ after model parameter calibration is different for the red and black curves, but the y-axis has here been linearly re-scaled to facilitate comparison of the curve shapes.

In addition to thus replacing a discontinuous function by a smoother function, the monotonic increase in our model is based on the following reasoning. Even at doses above a few mGy intercellular signaling still occurs and produces some effect, which at doses where TE dominate is just a small perturbation that cannot be disentangled experimentally from the overall effect. The NTE1 model in [Cacao et al. 2016], which they consider their preferred model according to their information criteria, also does not assume a decrease in NTE as dose increases.

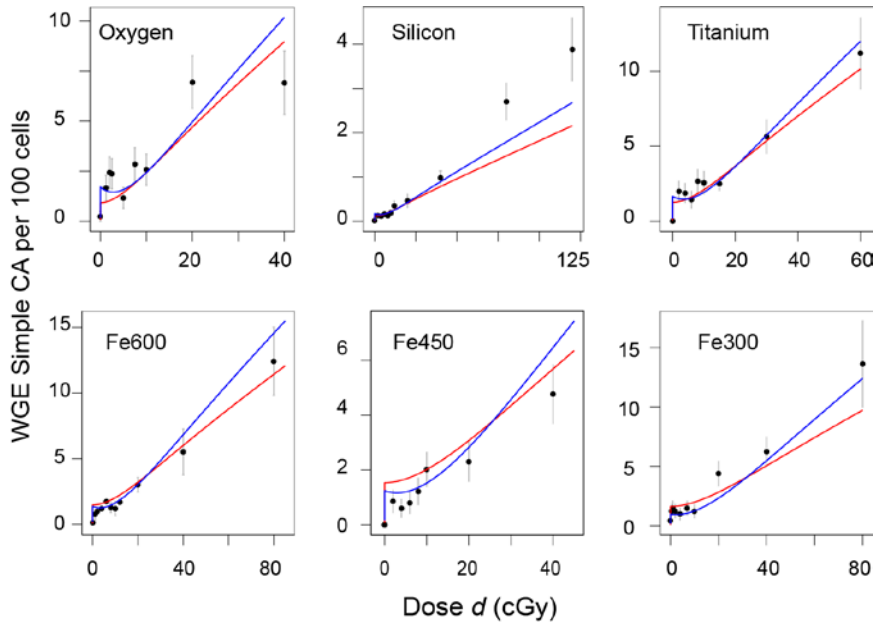
We calculated the Akaike information criterion (AIC) [Shuryak 2016] and the Bayesian information criterion (BIC) [Cacao et al. 2016] for our IDER. In Table A2.3, the results are compared with the AIC and BIC for the NTE1 and NTE2 models in [Cacao et al. 2016]. Values in the NTE1 and NTE2 rows used the models and parameters in [Cacao et al. 2016] but, as in our regression calculations, were re-calculated to take into account our way of calibrating models, emphasizing radiogenic IDERs, with background subtracted out.

Model\IC	AIC	BIC
NTE1	229.69	239.45
NTE2	277.24	287.00
IDER	200.99	210.75

Table A2.3. Akaike and Bayesian Information Criteria. Smaller values, here those in the last row, are preferred. Only differences between the rows are relevant. As in [Cacao et al. 2016], our calculations indicate that the NTE1 model is preferred over the NTE2 model. For details on the calculations see the scripts available on GitHub.

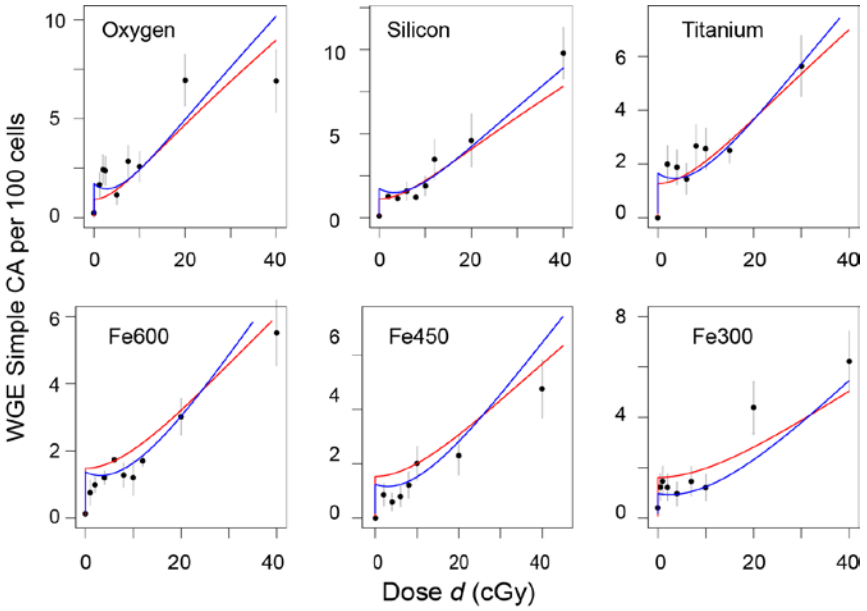
In order to check visually that our insistence on monotonic increasing IDERs is acceptable, we compared our calibrated IDERs graphically with corresponding NTE2 IDERs from [Cacao et al. 2016]. The results are shown in Figs. A2.3 and A2.4.

Fig. A2.3 Monotonically increasing IDERs vs. NTE2 model curves.



The figure compares our IDERs (red curves) with the NTE2 model curves of [Cacao et al. 2016] (blue curves), which are decreasing in the dose range from ultra-low doses to about 5 cGy. Visually, the monotonically increasing IDERs are not inferior, a result expected from Table A2.3.

Fig. A2.4. Zooming in on Smaller Doses



The figure zooms in on doses ≤ 40 cGy, so that the region between 0 and 5 cGy can be seen more clearly. It again compares our IDERs (red curves) with the NTE2 model curves of [Cacao et al. 2016] (blue curves).

Visual comparisons to the NTE1 model preferred in [Cacao et al. 2016] are shown in Fig. A2.5.

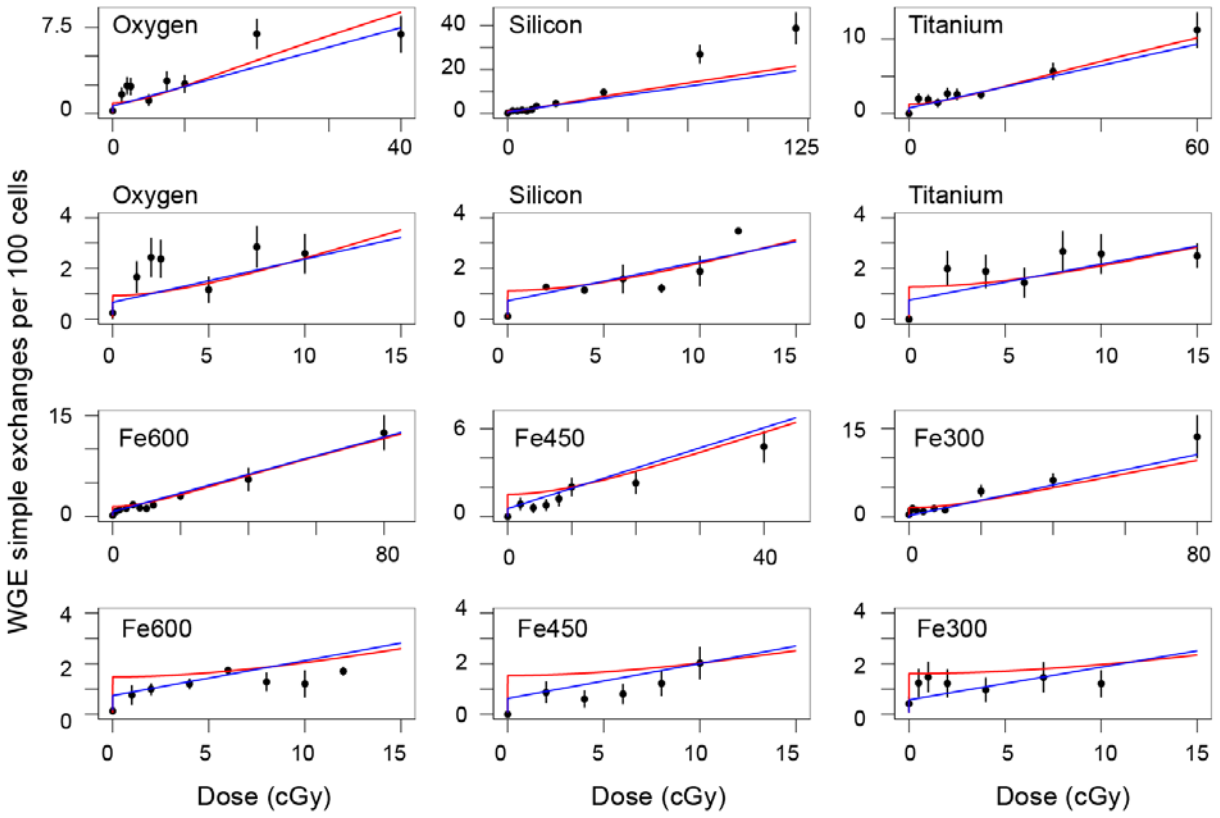


Fig. A2.5. Comparing with the NTE1 model.

The IDERs for our calibrated model (red curves) are compared with the IDERs (blue curves) that were found to result from the NTE1 model identified as the preferred NTE model in [Cacao et al. 2016]. Points are the observed values. Rows 2 and 4 zoom in on the low dose range, 0-15 cGy, of the rows above them. It is seen that overall the NTE1 model and our IDER model are comparable visually. The silicon data at doses of 1 Gy or more show evidence of convexity, presumably due to 2-track action, neglected in the IDER. Data at lower doses show no distinct upward curvature in any of the panels.

Appendix A3. Synergy Analysis for Monotonically Increasing IDERs

A3.1. Precise Quantification vs. Generality

As qualitative, general concepts “synergy” and “antagonism” are used heavily in radiobiology; this usage will no doubt continue for a long time to come. However, no quantitative, precisely defined synergy analysis theory applicable to a substantial fraction of situations where the vague qualitative concept of synergy is used are available or will become available in the foreseeable future [Ashford 1981; Geary 2013; Piggott et al. 2015]. For example, most current synergy analysis theories concern a scalar endpoint, not more complicated endpoints such as a dose-dependent function of time to tumor, so they only produce a baseline no-synergy-antagonism MIXDER, not more complicated outputs.

Even when only a scalar endpoint is involved, no specific synergy analysis theory sufficiently general to handle all mixed radiation fields of interest has been published. The simple effect additivity theory $S(d)$ cannot be used unless all the mixture components have IDERs that are at least approximately LNT. Published replacements for $S(d)$ have substantial limitations.

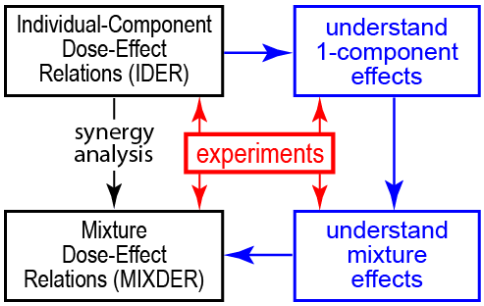
As far as we are aware, every previously published systematic replacement explicitly or implicitly assumes that mixture components have IDERs which are all monotonically increasing (or are all monotonically decreasing; this case is so similar to the case where all are monotonically increasing it need not be described separately). In addition to this monotonicity limitation, each replacement has additional limitations of its own.

In section A3 we now assume monotonicity; we discuss and compare these replacements. In section A5 we will show a way to improve on them substantially.

A3.2. Barenbaum's Approach

A famous pharmacology paper [Berenbaum 1989] reviews, extends, and advocates a general approach to synergy analysis theory applicable to mixtures each of whose IDER is monotonically increasing. The approach is illustrated in a main-text figure, here duplicated for reference as Fig. A3.1.

Fig. A3.1: a long hard road or a temporary shortcut.



Eventually, but almost certainly not soon, synergy analysis of mixed radiation field effects based solely on mathematical manipulations of IDERs (leftmost downward arrow), will be replaced by biophysically-based predictions that incorporate whatever synergy or antagonism actually occurs (blue path). For the time being, optimizing synergy theory, a much simpler, faster and cheaper shortcut, is important.

A3.2.1. Some Assumptions and Motivations of Barenbaum's General Approach

Assuming that each IDER for the components of a mixture is known and essentially no further information is available, one can define a reasonable baseline MIXDER, characterizing absence of synergy or antagonism. Such baseline MIXDERs are useful first steps in understanding mixture effects (Fig.A3.1). They are less reliable than predictions based on biophysical understanding of agent interactions but typically much faster, simpler, and cheaper [Berenbaum 1989; Greco et al. 1995; Geary 2013; Norbury et al. 2016].

The baseline MIXDERs, in the Berenbaum approach, are typically not mechanistic: they use mathematical manipulations of IDERs, not additional biophysical insights specific to the endpoint being analyzed [Berenbaum 1989; Greco et al. 1995]. The reasoning is that biophysical insights should be used in devising IDERs, but that subsequently intermingling the temporary synergy-analysis shortcut shown in Fig. A3.1 and the long biophysical-understanding path (Fig.

A3.1, blue arrows) would merely undermine the shortcut's practical advantages without adequately replacing the long path.

A3.2.2. Requiring Obedience to The Sham Mixture Principle

The sham mixture principle, that the mixture of an agent with itself always has as a baseline no synergy/antagonism MIXDER the agent's own IDER, is required in Barenbaum's general approach (other researchers disagree, as discussed in A3.3.4 below). Incremental effect additivity $I(d)$, as defined in the Methods section of the main text, does always obey the sham mixture principle, as proved at the end of sub-section A3.4.2 below. Simple effect additivity $S(d)$ does not obey the sham mixture principle (Fig. A1.1). Therefore, in the Barenbaum approach, $S(d)$ cannot be used for mathematical synergy analysis. An obvious exception occurs if, in some special situation, $S(d)$ gives essentially the same answer as using a synergy definition that obeys the principle. For example, when all IDERs for a mixture have the LNT form, $E_j(d_j)=A_j d_j$ with A_j a positive constant, all synergy definitions, including $S(d)$ and $I(d)$, have baseline MIXDERS $d\sum_{j=1}^{N} r_j A_j$ whether or not they violate the sham mixture principle when applied to curvilinear IDERs. When all theories give essentially the same answer, there can be no objection to using the simplest, namely $S(d)$. Less trivial examples were given in [Siranart et al. 2016]. These were mixtures with some component IDERs being concave and others being convex, where accidental cancellations between $S(d)$ under- and over-estimates resulted in $S(d)$ being almost equal to $I(d)$.

A3.3. Examples of Synergy/Antagonism Definitions

A3.3.1. Isobole Synergy Analysis

By far the most commonly used synergy analysis theory based on the ideas in A3.2 is the linear isobole method (reviewed in [Chou 2006; Brun and Greco 2010; Lee 2010; Tallarida 2012; Geary 2013; Foucquier and Guedj 2015]). This method requires monotonically increasing IDERs

for the components of a mixture. It computes from IDERs a default total mixture dose for any given mixture effect *E*. If a mixture experiment shows that using a dose smaller than the default dose is sufficient to produce *E* then there is by definition synergy. Intuitively the idea is that there must have been some kind of extra cooperation between components that enables a small dose to produce an unexpectedly large effect *E*. Similarly if a dose larger than the default dose is needed to produce *E* then by definition antagonism is present.

In the notation of the main text, the default total mixture dose *d* for a given effect *E* to be produced is calculated by the following equation:

(A3.1) $$\frac{1}{d} = \sum_{j=1}^{N} \frac{r_j}{D_j(E)} \text{ for } E > 0, \quad d(E = 0) = 0$$

For sufficiently small mixture effect *E* there is always a unique solution *d*(*E*) of Eq. (A3.1). The proof is the following. For *d* very small, the left hand side of Eq. 1 is larger than the sum on the right, which is independent of *d*. The right hand side is greater than zero; it remains fixed as *d* is gradually increased, while the left side decreases continuously and monotonically, eventually approaching 0. So there must be exactly one solution *d*.

An important “minimax” limitation of linear isobole synergy analysis theories occurs in the following kind of situation, which is common in practice. Suppose some of the IDERs have finite maxima which they can never exceed for any dose of interest. Among these IDERs there must be at least one, which we can take to be $E_N(d_N)$ without essential loss of generality, whose maximum is no larger than any of the other maxima. Call that minimum maximum $E_{minimax}$. Suppose there is at least one other IDER in the mixture which either has no maximum at all or has a maximum larger than $E_{minimax}$. Then values *E* of the total mixture effect greater than $E_{minimax}$ are of interest but $D_N(E)$ is undefined for such *E* and thus Eq. (A3.1) cannot be used to calculate *d*. The N^{th} IDER not only refuses to play linear isobole but also spoils the game for everybody

else. In practice this minimax limitations sometimes limits the range of *d* to such small values that linear isobole synergy analysis theory becomes virtually useless.

In addition to this practical limitation of the linear isobole default hypothesis there is a conceptual issue. Barenbaum attempted to prove Eq. (A3.1) from the sham mixture principle. However his proof is now known to be incorrect ([Grabovsky and Tallarida 2004; Bosgra et al. 2009], reviewed in [Tallarida 2012; Foucquier and Guedj 2015]).

Curvilinear isobole synergy analysis theories (reviewed in [Tallarida 2012]) take into account Berenbaum's error. However they also have drawbacks [Geary 2013], which preclude their application to complex mixtures of HZE ions [Siranart et al. 2016].

A3.3.2. Two Other Approaches to Synergy Analysis

In radiobiology synergy is often analyzed by dual radiation action theory [Zaider and Rossi 1980]. The theory uses linear-quadratic (LQ) dose-effect relations. For one acute dose an LQ IDER is the sum of a term linear in dose and another quadratic in dose:

(A3.2) $$E(d) = \alpha d + \beta d^2,$$

where α and β are non-negative constants at least one of which is non-zero. A generalization of Eq. (A3.2) to protracted dosing, such as fractionation or chronic low dose rate exposures, is discussed in section A4 below but for the time being we consider only the acute dosing case.

The dual radiation action theory applies only to mixtures each of whose components has an LQ IDER. For such mixtures it predicts the baseline MIXDER is LQ. The theory differs from Barenbaum's general approach, described in sub-section A2.2 above, in many ways, including the following:

(a) Mechanistic biophysical arguments are used to obtain the parameters α and β for the baseline no-synergy/no-antagonism LQ MIXDER.

(b) This baseline MIXDER does not obey the sham mixture principle unless β=0.

(c) It is assumed, explicitly or implicitly, that synergy or antagonism must always be defined as a deviation from the simple effect additivity baseline $I(d)$ that Barenbaum deprecates.

(d) The terminology used differs strongly from that of Barenbaum. For example, a single radiation field having an LQ IDER with β>0 is said to be synergistic with itself whereas Barenbaum's definitions imply self-synergy can never occur. The various terminological discrepancies are merely verbal differences arising from comparing different disciplines such as radiobiology and pharmacology. They are not in themselves actual scientific disagreements; but they are extraordinarily confusing.

LQ IDERs do not incorporate NTE, so the dual radiation action synergy analysis theory was not used in the main text and there are many other situations where restricting to LQ IDERs is inappropriate. Details on and examples of the theory are given, e.g., in [Zaider and Rossi 1980; Bird et al. 1983; Zaider 1990; Berenbaum 1991; Furusawa et al. 2002; Suzuki et al. 2002].

Independent action [Lam 1994] is another theory that also allows self-synergy. In general its usefulness is restricted not only by the condition of IDER monotonic increase but also by the minimax restriction: if one mixture component, acting on its own, can never exceed some finite upper limit no matter how large the dose, then the same must hold for all the other components, with the same upper limit.

A3.3.3. Asymmetrical Synergy Analysis Theories

A number of synergy analysis theories, including some variants of curved isobole theories, are asymmetrical: the baseline MIXDER for combining r_1 of agent 1 with r_2 of agent 2 can be different than that for combining r_2 of agent 2 with r_1 of agent 1. This makes sense when one of the two agents is somehow clearly subordinate to the other agent, as can occur in adjuvant

therapy. However, asymmetrical theories cannot be used for mixed radiation fields such as GCR fields, actual or simulated. These have many different components, none of which are somehow clearly subordinate to others since all act via ionizing radiation exposure.

A3.4. Some Properties of Incremental Effect Additivity $I(d)$

In this sub-section we discuss the earlier version of $I(d)$, given in the Methods section of the main text, not the general version given in the Results section of the main text. The latter is discussed below, in section A5. Incremental effect additivity $I(d)$ was originally suggested by, and designed to improve on, the linear isobole theory of sub-section A3.3.1 above. Essentially, $I(d)$, like the linear isobole theory, considers effect, rather than dose, as the basic independent variable. This switch in perspective often also occurs [Durante 2014a], for different reasons and with different equations, in radiobiological discussions of relative biological effectiveness (RBE).

Unlike most other replacements for $S(d)$, $I(d)$ is applicable to mixtures with very heterogeneous IDERs. For example, one can in principle use $I(d)$ for a single mixture where some IDERs of each of three qualitatively different shapes are involved.

a) IDERs having a concave shape, as shown in Fig. A1.2.
b) LQ IDERs, Eq. (A3.2), where the quadratic component obeys $\beta>0$. These IDERs are convex.
c) Hill function IDERs with Hill coefficient <1 [Greco et al. 1995; Chou 2006; Foucquier and Guedj 2015]. These functions are sigmoidal (“S-shaped”). They are often used for agent mixtures in pharmacometrics, toxicology, evolutionary ecology and other fields

We will now discuss some mathematical properties of incremental effect additivity. We start with an existence and uniqueness theorem for $I(d)$. Then we review mixtures whose component

IDERs are so similar that the ODE initial value problem (IVP) for $I(d)$ can be solved without resort to computer numerical integration. Then we prove some results comparing $I(d)$ with simple effect additivity $S(d)$.

A3.4.1. Baseline MIXDERs I(d): Existence, Uniqueness and Properties

The right hand side of the ODE IVP for $I(d)$ is the sum of a finite number N of terms. Each term comes from one component of the mixture. In the relevant computations, each component of the mixture has an IDER that is standard, i.e. obeys the standard conditions defined in the Methods section of the main text as regards smoothness, finiteness of slope, and monotonic increase.

Theorems due to Picard and others (summarized in [Coddington and Levinson 1955]) guarantee existence and uniqueness for the $I(d)$ IVP. Specifically there is some number A>0 such that in the half-open interval [0,A) there is one and only one solution and that solution is smooth. In our cases, the interval can be extended to an interval $[0, E_{minimax})$, where $E_{minimax}$ is defined in sub-sections A3.3.1 and A3.3.2.

A3.4.2. Mixtures Whose Component IDERs are "Similar", and the Sham Mixture Principle

Two agents with respective IDERs $E_1(d_1)$ and $E_2(d_2)$ are said to have "constant relative potency" (or said to be "similar") if there is some relative potency constant $P>0$ such that $E_2(d_2) = E_1(Pd_2)$. Then $E_1(d_1)= E_2(P^{-1}d_1)$. The intuitive interpretation is that for all relevant doses, agent 2 is P times as potent in producing the effect as agent 1. For example if both IDERs are LQ, they have constant potency ratio if $\alpha_2 d_2+\beta_2 d_2^2= \alpha_1 P d_2+\beta_1 P^2 d_2^2$ for all d_2, which implies $\alpha_2 =P\alpha_1$ and $\beta_2= P^2\beta_1$. For mathematical, historical, and practical reasons IDERs with constant relative potency are important in synergy (and other) analyses [Berenbaum 1989]. Mathematically, the case of a mixture all of whose IDERs have constant relative potency covers almost all situations

where incremental effect additivity $I(d)$ can be evaluated as an explicit function of mixture dose d instead of being evaluated numerically by ODE integration using computer programs.

Specifically, suppose the following: g(d) is a smooth function for $0 \leq d < \infty$ with $g(0)=0$; $g(d)$ has limit Emax for d approaching infinity, where $0 < \text{Emax} \leq \infty$; and the derivative g′ is greater than 0 for all d. Suppose in a mixture of N components each IDER obeys $E_j(d) = g(P_j d)$ for some "potency constant" $P_j > 0$. Then all the IDERs are pairwise similar and the following holds.

Theorem. $I(d)=g(w)$, where $w = [\Sigma r_j Pj]d$

Interpretation. The intuitive interpretation is that $P=\Sigma r_j Pj$ can be regarded as an average potency using the discrete probability distribution r_j, and thus the dose w to be used in evaluating the baseline MIXDER $I(d)$ is average potency times total mixture dose.

Proof of the Theorem. All pairs of IDERs are similar since $E_j(d) = g(P_j d) = g((P_j d/P_i)P_i) = E_i(P_j d/P_i)$. The compositional inverse $G(E)$ of g is defined for any E in the interval [0,Emax) and the compositional inverse D_j of E_j is given by $D_j(E) = (1/P_j)G(E)$. For $I(d)$ we therefore have, denoting the derivative function for E_j by E_j',

(A3.3) $$E_j'(d) = P_j g'(P_j d) \Rightarrow E_j'(D_j(I)) = P_j g'(P_j D_j(I)) = P_j g'(G(I)).$$

Using the ODE for $I(d)$ given in the Methods section of the main text now gives

(A3.4) $$dI / dd = g'(G(I))\sum_{j=1}^{N} r_j P_j \Rightarrow dI / dw = g'(G(I)) \Rightarrow I(d) = g(w).$$

Here the last implication follows from Picard's theorem on the uniqueness of the solution of the IVP for an ordinary differential equation [Coddington and Levinson 1955]. The reader may wish to trace the steps of the theorem's proof using toy examples so simple that all the individual steps can also be carried out explicitly, such as $g=x^2$, $g=2x+x^2$, $g=x/(1+x)$, or $g=ln(x+1)$.

For linear isobole synergy analysis a corresponding theorem has been known for a long time. It is discussed, e.g., in [Berenbaum 1989], which gives more details on the interpretation of the average mixture potency $P=\Sigma r_j Pj$.

Any sham mixture, where all the IDERs are identical, is an example of constant relative potency, with $P_j=1=P$. Therefore the theorem above implies that $I(d)$, like the linear isobole theory, always obeys the sham mixture principle.

A3.4.3. Simple Effect Additivity Defined Incrementally

Suppose we have a mixture each of whose components has an IDER $E_j(d_j)$ that is "standard" as defined in the main text: it is zero at dose=0, has continuous first and second derivatives, and has positive slope for some half open dose interval $[0, A_j)$ with $A_j>0$. Recall that the simple effect additivity baseline MIXDER is defined by

$$\text{(A3.5)} \qquad S(d) \equiv \sum_{j=1}^{N} E_j(d_j); \text{ where } d_j = r_j d; \quad r_j > 0; \quad \sum_{j=1}^{N} r_j = 1.$$

We can also define $S(d)$ by a slope equation equivalent to (A3.5) in some half open dose interval $[0, A)$ with $A>0$, and this slope equation can then be compared to the baseline incremental effect additivity $I(d)$ MIXDER defined in the Methods section of the main text by

$$\text{(A3.6)} \qquad dI/dd = \sum_{j=1}^{N} r_j \left[dE_j/dd_j \right]_{d_j = D_j(I)}; \quad d = 0 \Leftrightarrow I = 0.$$

In fact, differentiating Eq. (A3.5), using $E_j(0)=0$, using $d_j=r_j d$ and using the inverse function definition $d_j=D_j(E_j)$ gives

$$\text{(A3.7)} \qquad dS/dd = \sum_{j=1}^{N} r_j \left[dE_j/dd_j \right]_{d_j = D_j(E_j)}; \quad d = 0 \Leftrightarrow S = 0.$$

Eq. (A3.7) is equivalent to Eq. (A3.5) on some interval $[0, A)$ with $A>0$ by Picard's theorem and the fact that the number of summands is finite. In Eq. (A3.7) the subscript on the square brackets could have been omitted, since the derivative function is by definition a function of d_j but the

comparison of Eq. (A3.6) and (A3.7) pinpoints the fact that the sole difference between $S(d)$ and $I(d)$ comes from the fact that for $I(d)$ the incremental contributions are determined by the biophysical system variable I, rather than by dose control variables d_j which the system has no way to sense directly.

A3.5. The Mixtures of Mixtures Principle

A3.5.1. Mixtures of Mixtures

An advantage of incremental effect additivity theory is that it obeys what we shall call the mixture of mixtures principle. In radiobiology, mixed beams whose components are themselves mixtures by the time they hit the biological target are important; they are especially important in studying galactic cosmic rays (reviewed in [Norbury et al. 2016]).

Pharmacological practice indicates that synergy analysis theory is then still applicable. A drug whose active ingredient is a single chemically pure compound can nonetheless act in complicated ways: on various organs in various locations at various concentrations by various mechanisms after transformation in the body to various other compounds [Ashford 1981]. So it is essentially already a mixture as far as its physiological effects are concerned. But if an IDER for such a chemically pure compound is known, and the compound is one component of a mixture, then pharmacometric mathematical synergy analyses routinely treat the component on the same footing as a different compound which has essentially just one simple physiological effect; indeed in many cases one does not even know if the action of a chemically pure compound is very complex or very simple.

So conversely, if a drug is some standard mixture of various active compounds and its IDER is known the drug can also be considered as single agent which can become one component of a bigger mixture. This then implies a constraint on synergy theories. For a single acute treatment, a

mixture of agents, some of which are themselves mixtures, must have the same baseline MIXDER defining absence of synergy and antagonism, as any other regrouping of the same components in the same amounts. As with the sham mixture principle, imposing the mixture of mixture principle excludes a number of synergy theories in the literature. Some theories always obey the mixture of mixtures principle. We will show that $I(d)$ is an example. Other theories sometimes violate the mixture of mixtures principle. We will show that simple effect additivity is an example.

A3.5.2. I(d) Always Obeys the Mixture of Mixtures Principle.

Note from Eq. (A3.6) that the slope of the baseline MIXDER $I(d)$ is just a sum

(A3.8) $$\sum_{j=1}^{N}\left[r_j F_j(E_j)\right]_{E_j=I},$$

which can be grouped, regrouped and shuffled like any other sum. This remark and Picard's existence/uniqueness theorem for ODE IVPs are enough to show that $I(d)$ baseline MIXDERs are well defined in some, perhaps small, half open dose interval including the origin, and to show that such MIXDERs obey the mixture of mixtures principle for appropriately restricted dose and effect intervals.

A3.5.3. Simple Effect Additivity: An Example of a Mixture Theory Failing to Obey the Principle

In this section we will show by an example that using the baseline simple effect additivity MIXDER $S(d)$ can violate the mixture of mixtures principle. Consider two agents with respective IDERs defined as follows:

(A3.9) $$E_1 = e^{d_1} - 1,\ E_2 = d_2.$$

Suppose we have 3 mixtures M_j whose respective dose fractions r_{jk} of total mixture dose have the following patterns. For M_1, r_{1k} = (0.4, 0.6), i.e. M_1 is a 40-60 mixture of agent 1 and agent 2. For

M_2, r_{2k} = (0.6, 0.4). For M_3, r_{3k} = (0.5, 0.5). Then a 50-50 mixture M_4 of mixtures M_1 and M_2 is clearly just M_3 in disguise. But $S(d)$ does not match since $\frac{1}{2}[\exp(0.4d) + \exp(0.6d)] \neq \exp(0.5d)$ except at d=0. Thus simple effect additivity sometimes violates the mixture of mixtures principle. Additional calculations, not shown here, lead to two further conclusions: the violation of the principle by simple effect additivity is the generic case for that synergy definition; and there are other synergy analysis theories in use that also typically violate the principle. As far as we know, all synergy analysis theories that obey the sham mixture principle also obey the mixture of mixtures principle and vice-versa. Whether there is a theorem that states this equivalence must always hold, or instead there is a counter example, is an open question.

Appendix A4. Synergy Analysis for Chronic Low Dose Rate Radiation Fields

A4.1. General Comments.

Dose protraction consists of a series of acute dose fractions, or of a chronic non-zero low dose rate which need not be constant in time, or of any combination of the two. In interplanetary space astronauts will experience chronic GCR irradiation protracted over several years (reviewed, e.g., in [Durante 2014b; Kim et al. 2015; Norbury et al. 2016]), whereas the acute irradiation considered up to this point is so rapid compared to other relevant processes, such as radiation damage repair, that it can be considered instantaneous. The chronic GCR dose rate in the absence of shielding and excluding solar particle events is, very roughly, 0.2 Gy per year, but this number depends on the solar cycle stage and other factors; current shielding configurations have a drastic effect on the HZE charge and energy spectra but are not effective in reducing estimated carcinogenesis risks from HZE.

Thus radiobiological synergy analyses will eventually have to be extended to mixtures whose components have IDERs appropriate for highly protracted dosing. We are a long way from understanding such IDERs for GCR radiations. We do not know the relevant radiation target sizes or relaxation times or the importance of NTE. We do not even know if we should consider IDERs or consider instead a function of dose and dose rate as co-equal variables, as can occur in a dynamic steady state [Lubin et al. 1995]. We do not know if protracting a given dose over a long time will decrease damage, as is often found in radiobiology, or actually increase it, as is sometimes found, especially for high LET radiations (reviewed, e.g., in [Stevens et al. 2014]). There are also discrepancies between accelerator experiments and interplanetary exposures. Exposure above low earth orbit is chronic in the absence of a solar particle event; experiments often involve fractionation instead of chronic irradiation and even the dose rate averaged over the

entire experiment may be much higher than the chronic interplanetary GCR dose rates. Until these factors are better understood we do not know what kind of IDERs to assign to mixture components in a protracted dosing situation, let alone what default hypotheses should be used in mathematical synergy analyses.

In section A4 we will now give one proof of principle example to show that synergy analysis can sometimes be carried out for protracted dosing mixtures. The example does not attempt to answer the above questions. It assumes an answer to the questions and shows how synergy analysis works under that assumption. We will review known IDERs that assume protraction decreases effects in a specific way and discuss the mathematical properties of these IDERs. Then we will specialize to the case of a constant dose rate. We will conclude by giving examples of baseline no-synergy/no-antagonism MIXDERs for a mixture whose components have such IDERs.

A4.2. LQ IDERs with Generalized Lea-Catcheside Dependence on Dose Timing

A4.2.1. The G function

Radiobiologists often use a standard dose-protraction LQ (linear-quadratic) formula for the effect accumulated by time $t>0$ due to an irradiation that started at time $t=0$. The formula, which can be used to incorporate the influence of repair and of damage-damage interactions during dose protraction, is the following:

(A4.1) $E(t) = \alpha d(t) + G\beta d^2(t)$, where $G(t) = \frac{2}{d^2(t)} \int_{w=0}^{t} R(w)dw \int_{s=0}^{w} \exp\left[-\lambda(w-s)\right] R(s)ds.$

Here α, β, and λ are non-negative constants with all 3 positive unless explicitly stated to the contrary. $R(t)$ is the dose-rate at time t and

(A4.2) $d(t) = \int_0^t R(s)ds$

is the dose accumulated by time t. G is the generalized Lea-Catcheside functional. This functional G, and various special cases of G, have been introduced by many different research groups using many different arguments (reviewed, e.g., in [Sachs et al. 1997]).

A4.2.2. Intuitive Interpretations of the Generalized Lea-Catcheside Functional

A sometimes useful intuitive interpretation of G in Eq. (A4.1) in terms of dual radiation action is the following. Assume the part of the total effect contributed by the β term comes from potentially damaging lesions that are, in competing processes, repaired or interact bilinearly to make irreparable lesions. We have three different times: $t > w > s > 0$. The intuitive interpretation of the double integral is the following, reading from right to left. A small increment $R(s)ds$ of dose arrives at an early time s. This increment $R(s)ds$ makes some potentially damaging lesions. The increment of these potentially damaging lesions is proportional to $R(s)ds$ with a fixed proportionality constant, say K. Some of these potentially damaging lesions are repaired during the time between s and w, so that only a fraction $\exp[-\lambda(w-s)]$ remains at later time w; here the factor $\exp[-\lambda(w-s)]$ results from a simple repair model called linear repair with per-capita rate constant λ. The potentially damaging lesions remaining at time w can then interact bilinearly with potentially damaging lesions due to the later arriving dose increment $R(w)dw$ to make an irreparable effect. Adding all the contributions from all intermediate times s and w by double integration we get a value proportional to irreparable effect added by time t. The constant β is given by $\beta=K^2B$, where B is the proportionality factor for the production of irreparably harmful lesions per pair of potentially harmful ones.

A4.2.3. Properties of G

G in Eq. (A4.1) does not depend on the magnitude of the dose, just its time course. Specifically, if $R^*(t)=CR(t)$ for some constant scaling factor $C>0$, then $d^*(t)=Cd(t)$ but a short calculation

shows $G^*(t)=G(t)$. For a single acute exposure the term $\exp[-\lambda(w-s)]$ in Eq. (A4.1) is $\exp[-\lambda(w-s)]=1$ since $t > w > s > 0$. Integration then shows $G=1$ so $E(t)$ has the LQ form (A3.2) with step-function time dependence for the dose $d(t)$:

(A4.3) $$E(t) = \alpha d(t) + \beta d^2(t).$$

For finite dose rate instead of the Dirac delta function dose rate corresponding to a single acute dose, it can be shown that G obeys $0< G <1$, so that spreading a given dose over a finite time interval always does decrease the effect, as expected from the intuitive interpretation of the previous sub-section, rather than increasing the effect.

A4.3. Constant Chronic Dose Rate

For time $T>0$, consider irradiation with a constant dose rate $R>0$ during the interval $(0,T)$, with $R=0$ otherwise. Thus T could represent the duration of a space voyage beyond low earth orbit, where GCR dose rate is approximately constant during transit, or represent irradiation time during a chronic dosing experiment. In the case of HZE irradiation during a prolonged interplanetary space voyage, one might have $T= 1$ yr. $T\lambda$ is a "dimensionless duration"; it is $ln2$ times the duration T of irradiation divided by the half-life of repairable lesions subjected to linear repair.

In the case of constant dose rate R the LQ equation with dose protraction functional G, Eq. (A4.1), simplifies to

(A4.4) $$d(t) = Rt,$$

so going back and forth between dose and time is very easy here. Moreover, the double integral for G can be carried out explicitly to get results we will need for synergy analyses, as follows:

$$E(t) = \alpha Rt + 2R^2\beta\int_{w=0}^{t} dw\int_{s=0}^{w} \exp\left[-\lambda(w-s)\right]ds = \alpha Rt + 2R^2\beta\int_{w=0}^{t} \exp(-\lambda w)dw\int_{s=0}^{w} \exp(\lambda s)ds$$
$$= \alpha Rt + (2R^2\beta/\lambda)\int_{w=0}^{t} \exp(-\lambda w)dw[\exp(\lambda w)-1] = \alpha Rt + (2R^2\beta/\lambda^2)\left[\lambda t - 1 + \exp(-\lambda t)\right];$$

which implies

(A4.5) $$E(d) = \alpha d + (2R^2\beta / \lambda^2)\left[\lambda d / R - 1 + \exp(-\lambda d / R)\right].$$

Also, taking the time derivative of $E(t)$ in Eq. (A4.5) gives

(A4.6) $$\frac{dE(t)}{dt} = \alpha R + (2\beta R^2 / \lambda)[1 - \exp(-\lambda t)].$$

For $t \ll 1/\lambda$, Eq. (A4.6) appropriately reduces to the acute irradiation limit, namely

(A4.7) $$\frac{dE(t)}{dt} = \alpha R + 2\beta R^2 t = \frac{d}{dt}(\alpha d + \beta d^2).$$

Eq. (A4.6) shows that dE/dt is positive everywhere in the interval $(0,T)$, so $E(t)$ increases monotonically, which implies there must exist some inverse function τ such that $t = \tau(E)$. The inverse function $\tau(E)$ has a vivid intuitive interpretation as a biodosimetry function which estimates how long an astronaut has been in orbit by counting his or her chromosome aberrations [Sigurdson et al. 2008; Maalouf et al. 2011; Beinke et al. 2013; George et al. 2013].

A4.4. Dose Rate Sparing

We next give a graph, Fig. A4.1 that summarizes some implications of the results in sub-section A4.3 above. Like the lung cancer relative risk graphs in [Lubin et al. 1995] for underground miners exposed for years to chronic levels of radiation from radon daughters, our graph here shows the effect E for the entire irradiation duration T at various total dose levels $d=RT$. It takes advantage of the fact that in Eq. (A4.5) with d fixed, $T=d/R$ and λ do not appear separately, only their product, the dimensionless duration $T\lambda$.

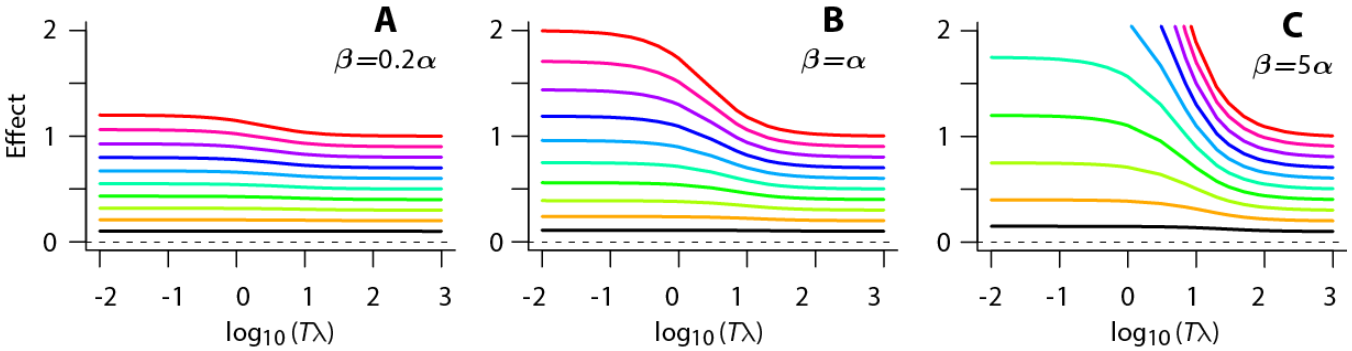


Fig. A4.1. Dose rate sparing. The panels show total effect for total doses that run from d=0.1 Gy (black lowest curve in each panel) in equal steps to 1 Gy (red uppermost curve). The scale of the vertical axis is governed by the value of α and the curves shown assume that for this endpoint α=1 per Gy.

The following properties of Eqs. (A4.1) and (A4.5) can be seen from the graphs.

- For $T\lambda \leq 10^{-2}$ the slope is almost zero and the effect is at its largest for that dose and that parameter set. This is the acute (short duration) limit. Since the Lea-Catcheside G factor approaches 1 in that limit, one must have $E=\alpha d+\beta d^2$. For example if $\alpha=1$, $\beta=1$, $d=1$ (red topmost line in panel B) one sees that to good approximation the height is $\alpha d+\beta d^2=2$.
- For $T\lambda \geq 10^{3}$, the curves are decreasing toward a lower limit of about αd. Manipulation of Eq. (A4.3.5) shows that in fact this is the exact value for $T\lambda$ approaching infinity. In terms of section A4.2, the intuitive explanation is that if the dose rate is small enough a repairable lesions is always repaired before it can interact with a later-arriving repairable lesion to make irreparable damage, so the entire β term in the LQ equation drops out leaving only the dose-rate independent term αd. The decrease, when going from left to right along a curve, from $E=\alpha d+\beta d^2$ to $E=\alpha d$ is called "dose rate sparing" or "the direct dose-rate factor".

Frequently used standard LQ models for many endpoints, when applied to HZE radiations, often give results similar to panel A in Fig. A4.1: the curves are so nearly horizontal one may as well ignore the beta term entirely. However, for low LET radiation induction of leukemias one can instead have, in the dose range 0-2 Gy, $\alpha/\beta \ll 1$ Gy [Little 2009] so that dose rate sparing is pronounced, as in panel C.

In contrast to the dose rate sparing there is theoretical and experimental evidence (reviewed, e.g. in [Lubin et al. 1995; Brenner and Sachs 2003; Stevens et al. 2014]) that HZE action may

(for many endpoints, dose rates and doses of interest) give qualitatively different results whereby effect increases as radiation duration increases for a given fixed dose, corresponding to an "inverse" dose-rate factor instead of a direct one. In such cases Eq. (A4.1), for LQ IDERs with generalized Lea-Catcheside *G* factor, cannot be used. And mathematical models quite different from LQ models, e.g. models incorporating NTE such as those in [Cacao et al. 2016] and [Shuryak et al. 2017], are often now being used . Here our interest is mainly in producing proof of principle calculations illustrating how synergy can be analyzed even when dosing is protracted and dose-effect relations are highly curvilinear. These calculations are given in the next sub-section, assuming Eq. (A4.1).

A4.5. Examples of Baseline no-Synergy/no-Antagonism MIXDERs

We now can calculate and compare baseline MIXDERs for mixed radiation fields each of whose components has an IDER of the form (A4.5). For the incremental approach we assume that for a small increment of time *dt*, each component contributes an incremental effect $dE_j=r_j(dE_j/dt)dt$, where dE_j/dt is given by Eq. (A4.6) evaluated at that time when that component acting by itself would have produced the total effect *I* that both components acting together have already produced. This definition just transfers the arguments used to motivate incremental effect additivity synergy analyses to the case where small increments of time, rather than small increments of total mixture dose are considered. Formally the compositional inverse functions τ_j discussed below Eq. (A4.6) are used to find the appropriate time for each component. No explicit forms for τ_j are available. However, in computer calculations a 1-dimensional root finder can readily give high precision numerical versions of τ_j: the complications sometimes attendant on finding roots are not present because $E(t)$ and thus its inverse $\tau(E)$ are always monotonically increasing in our case. We will assume the repair rates of the two radiations are the same, mainly

for simplicity but also on the grounds that probably these rates are primarily a property of the biological target.

As an example, suppose in an NSRL experiment we have a mixture of low LET ion radiation 1 (e.g. high energy protons) with high LET radiation 2 and the endpoint is a surrogate for radiogenic excess relative risk for leukemia. As in mixture guidelines for recent NASA calls we will take 80% of the total dose to be due to the low LET radiation, i.e. $r_1=0.8$ so that $r_2=0.2$.We will assume chronic irradiation at constant dose rate for one week. For the dose rate R we will assume 0.5 Gy per week, i.e. 40 cGy total of low LET radiation mixed with 10 cGy total of high LET radiation over the course of the week.

For the low LET radiation a possible choice of α and β in Eq. (A4.7), judging from leukemias in the atom-bomb victims, is $\alpha_1=0$, $\beta_1=2$ Gy^{-2} [Little 2009; Hsu et al. 2013]. The scale of the vertical axes in the figures we shall show is essentially arbitrary. One might calculate effect, or effect in %, or effect divided by some reference effect so a uniform re-scaling of all four LQ coefficients ($\alpha_1, \beta_1, \alpha_2, \beta_2$) makes no essential difference to the baseline MIXDERs or their relation to the IDERs in the figures we shall show. Thus a numerical value of one non-zero LQ coefficient, e.g. β_1 here can be chosen arbitrarily without essential loss of generality.

For the high LET radiation, on the usual argument that for such radiation irreparable one-track action probably dominates, we will choose $\beta_2=0$; for α_2 we choose a nominal value $\alpha_2=5$ Gy^{-1}; then the values of λ_2 and R_2 become irrelevant and need not be specified. Fig. A4.2 shows baseline no-synergy/no-antagonism curves for this mixture assuming various values of the repair rate $\lambda = \lambda_1$.

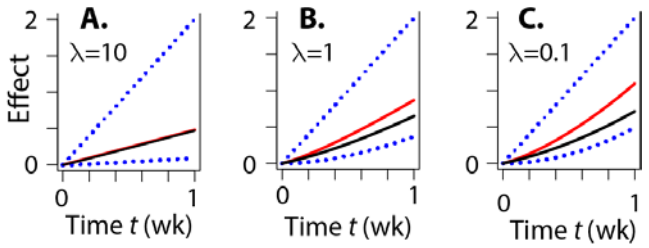


Fig. A4.2. Baseline MIXDERs. The figure shows incremental effect additivity curves (red lines) and simple effect additivity curves (black lines) for a mixture of a high LET ion component and a low LET component. The IDERs, i.e. the curves that would have resulted were the total dose given entirely to one or the other component, are shown as dotted blue lines.

Even for this very simple choice of the two IDERs, no explicit analytic expression is available, so the calculation was done with the customized script Lea.R that is available on GitHub. Panel A shows a typical result for a repair rate that is not much smaller than those normally assumed. It is trivial from the point of view of illustrating differences between $I(d)$ and $S(d)$ because both mixture components are effectively LNT: the high LET component IDER is exactly LNT and the low LET IDER might as well be LNT because the dose rate is so low compared to the repair rate that the quadratic term is negligible. Panels B and C show cases of unusually slow repair rate, as could perhaps occur due to some cause – e.g. a state of chronic irritation by ROS or RNS [Cucinotta, Kim and Chappell 2013], reviewed in [Shuryak 2017]. These panels show differences between $I(d)$ and $S(d)$.

In trying to decide what further figures to use to illustrate our mixture results we again found too many possibilities. Even when concentrating on 2-component mixtures to avoid the previously discussed confounding factor of having too many possible sets r_j for dividing total mixture dose d into component doses $d_j=r_j d$, the fact that there are essentially 4 other relevant parameters ($\alpha_1, \beta_1, \lambda_1=\lambda_2, \alpha_2,$ and β_2, where only 3 of the 4 LQ coefficients count because of possible y-axis rescaling mentioned above) prevented any systematic choice of a few "representative" mixtures. Indeed, even for acute dosing, trying to compare $S(d)$ and $I(d)$ over the entire relevant parameter space leads to unsolved mathematical/computational problems [Siranart et al. 2016]. The script Lea.R therefore did not attempt to consider the even more

numerous possibilities that would arise for mixtures with more than 2 different ions entering the beam upstream of shielding.

Fig. A4.3 shows two more examples; its caption gives some intuitive interpretations.

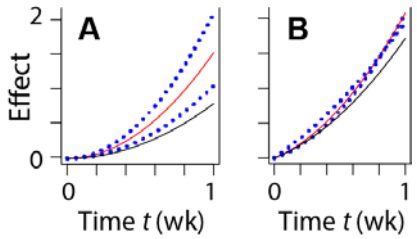


Fig. A4.3. Other Mixtures. The figure gives results for two components having the parameters shown in the table: It shows the time-incremental effect additivity baseline no-synergy/no-antagonism MIXDERs (red lines) and the simple effect additivity no-synergy/no-antagonism MIXDER (black lines). The IDERs, i.e. the curves that would have resulted were the total dose given entirely to one or the other component, are shown as dotted blue lines.

panel	ion	α	β	λ	r_j
		Gy^{-1}	Gy^{-2}	$week^{-1}$	
A	1	0	8.4	0.05	0.5
	2	0	4.2	0.05	0.5
B	1	1.2	6	0.05	0.8
	2	2.6	2.6	0.05	0.2

The fact that the simple effect additivity baseline MIXDER falls below both component IDERs is due to the fact that when all mixture components are convex, as is the case for the mixture here, the simple effect additivity baseline is an underestimate. Fig. A1.1 shows that such underestimates can be traced back to the failure of simple effect additivity to obey the sham mixture principle. Panel B shows a case where the two component IDERs cross.

A4.6 Summary of Low Dose-Rate, Time-Incremental Effect Additivity Synergy Analysis

We have given explicit, reasonably realistic examples of how one can do synergy analyses for protracted mixture exposures. Our examples only apply to one very specific kind of component IDERs, which all had to be LQ with linear repair of potentially damaging lesions. We emphasized chronic irradiation at constant dose rate. Our treatment also involved a number of implicit assumptions. For example the time constant for resolution of potentially damaging lesions by either repair or transformation into irreparable lesions was assumed to be so short that one such lesion does not ever suffer a second hit. Conceptually it is clear that it should be possible to generalize the LQ example at least somewhat, but that has not been done and thus may involve unexpected difficulties.

Appendix A5. Incremental Effect Additivity Synergy Theory

A5.1. The General Equation for $I(d)$

Prior to the availability of computers which can rapidly provide accurate numerical solutions to non-linear ODE, it was natural to specify IDERs by giving effect as an appropriate explicit function $E=E(d)$ of dose, as in [Cacao et al. 2016], in [Cucinotta and Cacao 2017], and in very many other papers. We suggest that nowadays it is often preferable to specify IDERs via their slope as a function of E itself, by solving an ODE IVP of the form given in the main text, namely

$$\text{(A5.1)} \quad \text{(A)} \quad dE/dd = F(E); \quad \text{(B)} \quad E = 0 \text{ when } d = 0,$$

with the slope function $F(E)$ sufficiently well behaved that there is one and only one solution for all sufficiently small non-negative doses. Additional requirements on $F(E)$, e.g. the requirement that the solution not approach ± infinity as dose approaches some finite value from below, will be analyzed in subsequent sub-sections. Eq. (A5.1A) is what is called an "autonomous" ODE, referring to the fact that $F(E)$ depends only on E, with no explicit dose dependence, and Eq. (A5.1) is called an autonomous IVP (AIVP).

Some motivations for taking F as a function of E rather than a function of d are similar to some of the motivations for using incremental effect additivity $I(d)$. E, unlike d, is a state variable, determined by the changing biophysical state of the target system as dose and effect accumulate [Lam 1994]. Moreover, mechanistically analyzing how a small increment of effect interacts with effects caused by earlier dose increments is sometimes easier than mechanistically analyzing the entire effect of the entire dose [Lam 1987].

Consider a mixture consisting of $N \geq 0$ agents whose IDERs are in the usual explicit form $E_j(d_j)$ together with $K \geq 0$ agents whose IDERs are defined by Eq. (A5.1), where $N+K \geq 2$. Let

$r_1, r_2, ..., r_{N+K}$ be the corresponding proportions. The general equation of incremental effect additivity for $I(d)$,

$$\text{(A5.2)} \qquad dI / dd = \sum_{j=1}^{N} r_j \left[dE_j / dd_j \right]_{d_j = D_j(I)} + \sum_{j=N+1}^{N+K} r_j F_j(I); \quad d = 0 \Leftrightarrow I = 0,$$

was given in the main text. Importantly, a monotonic $I(d)$ baseline MIXDER can often be calculated with Eq. (5.2) for mixtures some of whose IDERs have $F(E) < 0$ while others have $F(E) > 0$. This surprising relaxation of the exasperating restriction that all IDERs be monotonic in the same direction is a far-reaching generalization whose possible applications in radiobiology are just starting to be explored.

Thus, in general, incremental effect additivity synergy theory consists of analyzing the solution of Eq. (A5.2). The corresponding default assumption, again denoted by $I(d)$ for brevity, consists of this synergy analysis plus carrying out uncertainty analyses.

A5.2. IDERs Defined by Solving Autonomous IVPs (AIVPs)

Allowing IDERs that are defined by Eq. (A5.1) instead of being given as functions of dose is essential for using the general equation, Eq. (A5.2), to calculate $I(d)$. However this approach is unfamiliar in radiobiology. This sub-section, A5.2, describes some differences in the two approaches, shows that there are many functions $F(E)$ in Eq. (5.1) which allow explicit calculation of the corresponding $E(d)$, shows that not all functions $F(E)$ lead to suitable $E(d)$, and suggests an approach, using functions of a complex variable, to trying to find simple necessary and sufficient conditions on $F(E)$ for $E(d)$ to be suitable.

A5.2.1. Slope Addition vs. Function Addition

The IDERs we used in the main text to reanalyze the CA data set involved adding two terms, for NTE and TE respectively. The corresponding approach when using AIVPs, to add two slopes, gives somewhat different results. Specifically, suppose the slope $F(E)$ in Eq. (A5.1) is modeled as a sum of two terms:

(A5.3) $dE / dd = F_1(E) + F_2(E).$

Then $E(d)$ is in general not merely the sum of the two AIVPs defined by

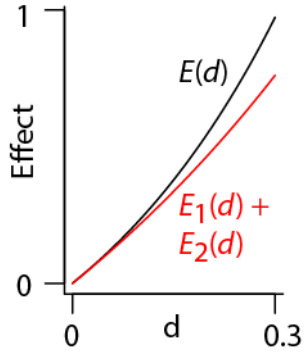
(A5.4) $dE_1 / dd = F_1(E),\ E_1(0) = 0;\quad dE_2 / dd = F_2(E),\ F_2(0) = 0.$

For example consider the following, with $F_1(E)$ and $F_2(E)$ both chosen to be linear so that solving all three AIVPs given by Eqs. (A5.3) and (A5.4) is simple

(A5.5)…. $F_1(E) = 1 + E,$ and $F_2(E) = 1 + 2E \Rightarrow F(E) = 2 + 3E.$

Integrating each of the three AIVPs explicitly gives

(A5.6) $E_1 = \exp(d) - 1;\quad E_2 = (1/2)[\exp(2d) - 1];\quad E = (2/3)\left[\exp(3d) - 1\right].$



So $E(d) > E_1(d)+E_2(d)$, as shown in the Figure. In fact, this inequality holds whenever both $F_1(E)$ and $F_2(E)$ are positive monotonic increasing functions for all relevant E, with all three IDERs involved then necessarily being convex.

The result $E(d)>E_1(d)+E_2(d)$ contrasts with the result where a slope is determined by functions of dose. For any integrable functions $F_1(d)$ and $F_2(d)$ we have

$$dE / dd = F_1(d) + F_2(d) \Rightarrow E = \int F_1 + \int F_2 + cons. \Rightarrow E(d) = E_1(d) + E_2(d).$$

Here the second implication follows from the fact that all 3 effects are 0 at dose zero.

A5.2.2. Examples of Explicit IDERs Defined by Eq. (A5.1).

Over the years, radiobiologists have developed IDER equations given by explicit equations to fit various biophysically motivated and/or experimentally observed curve shapes. Examples include multi-target, multi-hit equations, amorphous track structure equations, LQ equations, many generalizations of LQ equations, equations incorporating NTE, etc. In Eq. (A5.1) one instead starts with the slope $F(E)$. Often no explicit equation for $E(d)$ itself can be found. Finding $E(d)$

then involves using a standard ODE integrator such as the function ode() in the package deSolve of the computer language R and results in a numerical version of $E(d)$. Subsequent calculations then either just use this numerical form to get further numerical results or use the qualitative theory of ODE [Brauer and Nohel 1989], which involves analyzing slopes to determine solution properties without attempting to actually integrate an ODE.

However we will now show by examples that there are many cases where the IVP (A5.1) can be solved explicitly. Such explicit IDERs, and methods for generating them from Eq. (A5.1), are often useful, in helping understand numerical IDERs, when debugging customized software, and to supplement results obtained from the qualitative theory of ODE.

Suppose we have N+1 real numbers: $c > 0$; and $a_k \neq 0$, with k=1, 2, …, N. Suppose no two a_k are equal. In Eq. (A5.1) suppose

(A5.7) $$F(E) = c\prod_{k=1}^{N}(a_k + E).$$

Thus $F(E)$ is an N^{th} degree polynomial with non-zero, distinct real roots $-a_k$. In this case one can always use the method of partial fractions to integrate the ODE (A5.1) and obtain d as a smooth monotonically increasing or monotonically decreasing function of E on some half-open interval [0,A). Sometimes the inverse function $E(d)$ can be expressed explicitly. For example when N=1 and a>0 the solution $E(d)$ obtained by integrating and using an inverse function involves an exponential:

(A5.8) $$F(E) = c(a+E) \Leftrightarrow d = \frac{ln(a+E)}{c} \Leftrightarrow E(d) = a\exp(cd) - a \Rightarrow E'(0) = ca,\ E''(0) = c^2 a.$$

$E(d)$ is then similar to an LQ curve with both α and β positive in the following respects: for doses so small terms cubic or higher in dose can be neglected, $E(d)$ is LQ with $\alpha=ca$ and $\beta=(c/2)\alpha$; $E(d)$ is strictly convex, with positive second derivative, for all doses (Fig. A5.1 below); $E(d)$ does not

approach ∞ as some finite value is approached by d; and $E(d)$ is unbounded, approaching ∞ as d approaches ∞.

As another example, for N=2 with a, b, c>0 one has:

(A5.9) $$F(E) = c(a+E)(b-E) \Leftrightarrow E = \frac{a\exp[(a+b)cd] - a}{1+(a/b)\exp[(a+b)cd]}.$$

In this case $E(d)$ approaches b as d approaches ∞ and, depending on the choice of parameters, the curve can be concave or sigmoidal (Fig. A5.1).

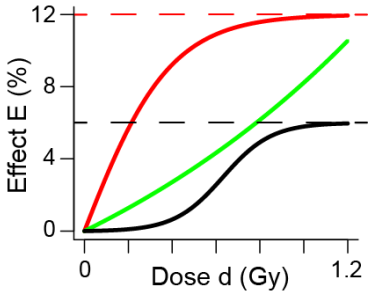


Fig. A5.1. Curve shapes. All three curves are monotonically increasing with finite positive slope at all doses. The green curve is described explicitly by Eq. (A5.8) with a=10 and c=0.6. It has properties similar to an LQ curve; at low doses it is LQ, with α= 6% per Gy and α/β=10/3 Gy. The black curve and red curves are described by Eq. (A5.9) with upper limits b=6% or 12% respectively. The black curve has a=0.02 and has c=1.5; it is sigmoidal, with a point of inflection. The red curve has a=13 and has c=0.2. It is concave. The criterion for concavity vs. sigmoidicity is $a>b$ vs. $a<b$. It is seen that one can readily find AIDERs with explicit $E(d)$ functions and various qualitatively specified shapes.

A5.2.3. Unsuitable Slope Functions F(E)

Some solutions of Eq. (5.1) approach infinity as dose approaches some finite value from below. For example, with ξ a real constant > 0 suppose $F(E)$ in Eq. (A5.1) is $F=\xi(1+E^2)$ Gy^{-1}. Integrating $dE/(1+E^2)$ gives E=tangent(ξd). In the interval [0, $\pi/2\xi$) the IDER is smooth. However, as d approaches $\pi/2\xi$ from below, E approaches infinity, as shown in Fig. A5.2.

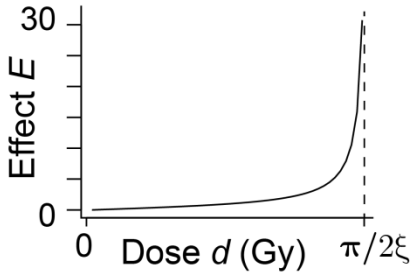


Fig. A5.2. The IDER E=tan(ξd).

An IDER that approaches ∞ at finite dose is not useful in any radiobiology analysis we know of, and attempts to use synergy theories on a mixture one of whose components has IDER $E(d)$=tan(ξd) give strange results, with that

component completely dominating mixture behavior. We therefore consider the IDER tan(ξd) unsuitable.

A5.2.4. Analytically Defined IDERs (AIDERs).

In practice unsuitable IDERs such as those in the preceding sub-section are easily avoided. However until/unless one finds simple necessary and sufficient conditions on $F(E)$ for the implied IDER to be suitable, incremental effect additivity must remain somewhat unsatisfactory as a mathematical theory. This sub-section discusses some aspects of that problem and suggests a possible solution.

To decide on candidate slope functions $F(E)$ for suitable IDERs we reasoned that the motivating example in the main text involved an extrapolation and, mathematically speaking, complex analysis encourages extrapolations. In Eq. (A5.1) $F(E)$ is a real function of a real variable, but instead of choosing $F(E)$ directly we can, and in this sub-section will, assume $F(E)$ is specified using a complex function G of a complex variable, with G chosen to approximate whatever is known or inferred about the IDER slope. We will call G a "slope extrapolator".

Specifically, we assume that $F(E)$ is the restriction of $G(z)$, with z the complex variable $z=E+iy$, to the non-negative E axis in the complex plane, where G is holomorphic in some open neighborhood of the origin $E=0=y$. For example any polynomial $F(E)$ is such a restriction of $G=F(E+iy)$, so all the examples in Fig. A5.1 above can be defined by such a G. In general we call an IDER defined by Eq. (A5.1) and a function $G(z)$ holomorphic in some neighborhood of $z=0$ an "Analytically-characterized" IDER (AIDER).

Suppose throughout the rest of this sub-section that the slope extrapolator $G(z)$ is a polynomial of (finite) degree M greater than 0 with real coefficients a_j (more sophisticated slope extrapolators will be discussed in sub-section A5.3 below). Thus $G(z)$ has no singularities in the

complex plane (i.e. on the finite part of the extended complex plane). Suppose first the only zeros of $G(z)$ lie on the imaginary axis. Then it follows that M is even, that $a_M>0$, and, since $a_M E^M$ increases at least as fast as E^2 for large E, that $E(d)$ is unsuitable because it approaches infinity for some finite d. On the other hand suppose all zeros of $G(z)$ lie on the real line with at least one zero for $E>0$. Then the qualitative theory of ODE [Brauer and Nohel 1989] shows that $E(d)$ approaches that zero on the positive real axis which is closest to the origin; Fig A5.1 shows specific examples; it happens that $E(d)$ can be found explicitly in these specific examples, but that is not a major consideration in the present argument. The AIDERs shown in Fig. A5.1 are suitable.

To summarize, by placing restrictions on the location in the complex plane of the slope extrapolator's zeros in a case where there are no singularities and a finite number of zeros, we have been able to generalize, obtaining examples of suitable and unsuitable AIDERs. The calculations were rendered mathematically very simple by starting with the assumption that G is a polynomial, an assumption far too strong to be used as a general restriction. We suspect that analogous restrictions on zeros and singular points, for meromorphic slope extrapolators rather than just polynomials, may turn out to be the necessary and sufficient suitability conditions needed to make our approach mathematically satisfying.

A5.3. The Broad Scope of the Incremental Effect Additivity Synergy Theory

We have already shown that Eq. (A5.2) for $I(d)$ can be used, and gives reasonable answers, in virtually all typical cases where any other published synergy theory replacement for $S(d)$ has been used. In this sub-section we show that $I(d)$ is useful in more general situations than those amenable to treatment by other replacements, and gives reasonable answers to some questions for which the published consensus has been that they are of interest but insoluble.

A5.3.1. Exemplifying Qualitative ODE Theory and also Sophisticated Slope-Extrapolators

As an example, let us analyze mixtures of three agents, as follows. Two of the agents have conventional IDERs of the form *E=f(d)*, with *f(d)* explicit, smooth and monotonically increasing; the third agent's IDER is an AIDER that has negative slope at large effect values. As shown in the main text, *I*(*d*) can then be calculated for large doses, even when the third agent starts to contribute negative slope, which tends to counterbalance (but cannot override) the other two agents' tendency to increase the effect.

In the example, all effects of interest are in the interval [0,1) so, as in the main text, we can and will define smooth monotonically increasing IDERs in terms of corresponding hazard functions $H_j(d_j)$, which are themselves smooth monotonically increasing IDERs, as follows:

(A5.10) $$E_j(d_j) = 1 - \exp[-H_j(d_j)] \Rightarrow H_j = -\ln(1 - E_j);\ \ j \in \{1,2,3\}.$$

Eq. (A5.10) implies slope equations, which will be needed to calculate *I(d)*:

(A5.11) $$dE_j / dd_j = (dH_j / dd_j)\exp[-H_j(d_j)] = (dH_j / dd_j)[1 - E_j(d_j)].$$

With α, β, and *k* adjustable constants greater than zero, the example uses two IDERs defined by Eq. (A.5.10) and the following explicit equations.

(A5.12) $$H_1(d_1) = \alpha d_1 + \beta d_1^2;\quad H_2(d_2) = \exp(kd_2) - 1.$$

The third IDER E_3 is an AIDER. With *c* and *a* real, positive numbers and with $z=E_3+iy$ it is defined by the following slope extrapolator, more sophisticated than just a polynomial:

(A5.13) $$G(z) = (1 - z)\big[c[a - \ln(1 - z)]\big][0.5 + \ln(1 - z)].$$

There is an open neighborhood of the origin on an appropriate Riemann sheet where *G*(*z*) is holomorphic and its restriction to the real axis is real and is positive for $E_3 \geq 0$.

The motivation for this choice of *G*(*z*) was to illustrate behavior similar to that discussed in the main text, by defining an AIDER which approaches but never quite reaches some level less than 1. This can be achieved by starting with the ODE IVP

(A5.14) $$\frac{dH_3}{dd_3} = c(a + H_3)(0.5 - H_3); \quad H_3(0) = 0.$$

The slope of H_3 is thus positive when $H_3 < 0.5$. The qualitative theory of ODE [Brauer and Nohel 1989] shows that H_3 approaches but never quite reaches 0.5 (its slope approaches 0 as H_3 approaches 0.5). The corresponding limit for E_3=1-exp(-H_3), shown as the dashed line in panel A of Fig. A5.3 below, is 1-exp(-0.5) ≈ 0.39347. Using Eq. (A5.10) to rewrite the slope in Eq. (A5.14) in terms of E_3 gives for the slope

(A5.15) $$(1 - E_3)\big[c[a - \ln(1 - E_3)]\big][0.5 + \ln(1 - E_3)].$$

Eq. (A5.13) extrapolates Eq. (A5.15) to a function of a complex variable and thereby extrapolates to values of E_3 where the slope becomes negative, namely values between 1-exp(-0.5) and 1.

Fig. A5.3 shows the result of calculating $I(d)$ for two different mixtures of the three agents. We used three parameters that differ between panels as shown in Table 5.1, $r_1 = r_2 = (1-r_3)/2$, a = 0.2 Gy^{-1}, c = 10 Gy^{-1}; and k = 4 Gy^{-1}.

Table A5.1. Three of the eight parameters used in Fig. A5.3.

Panel	parameter		
	r_3	α (Gy^{-1})	β (Gy^{-2})
A	0.35	1	2
B	0.50	3	0

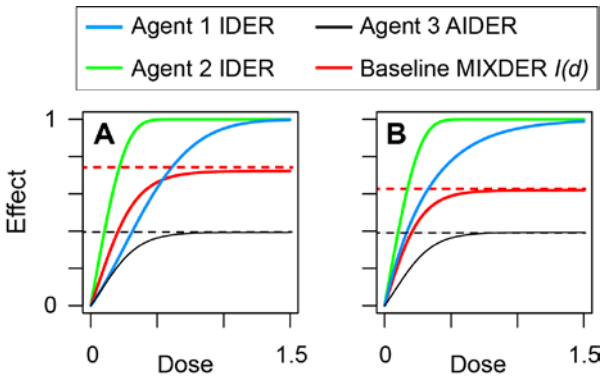


Fig. A5.3. $I(d)$ for two 3-component mixtures. Agent 3 acting on its own cannot produce effects greater than 1-exp(-0.5) as shown by the black dashed lines. Initially all three IDER slopes are positive so $I(d)$ increases. However, when the other two agents pull $I(d)$ above 1-exp(-0.5) by their positive slopes, the extrapolated negative slope of agent 3 starts a battle of slopes which results in I approaching a compromise asymptote at indefinitely large doses, shown as the dashed red lines. In panel A the initial slope of agent 1 is smaller than in panel B but the main difference between the two panels is that in panel B agent 3 contributes a larger fraction of the total dose so that it is able to pull the compromise asymptote down more effectively, as shown.

One interesting aspect is that to get the dashed red lines one need not first integrate the equation of incremental effect additivity for $I(d)$, then eyeball a line which $I(d)$ approaches. The qualitative theory of ODE, though mainly developed for systems of ODE rather just one ODE as here, states here that to find the asymptotic dashed red lines one only needs to find the first effect value I such that the sum of the three slopes =0. In panel B we used a 1-dimensional root finder to get the value Effect = 0.61936. Characteristically, the qualitative theory of ODE gives only partial information -- not the full red curve, just the existence and numerical value of the asymptote -- but involves far less calculation. For ODE systems the qualitative theory is quite powerful; even for a single ODE as here it is often useful.

There are routine extensions of the above results to more than two conventional explicit IDERs and/or to more than one AIDER and/or to IDERs given directly rather than in the hazard function form of Eq. (A4.33).

A5.3.2. A Mixture of an Effector and an Inhibitor

Consider some effect, e.g. an excess of reactive oxygen species, and two agents. Suppose both agents' IDERs are AIDERs, with respective slope generators

(A5.16) $\quad G_1 = c(1+z_1); \quad G_2 = -\lambda z_2.$

Here: $z_k = E_k + iy$, $k \in \{1,2\}$; c and λ are real and positive constants. Restricting G_k to the non-negative real axis gives

(A5.17) $\quad (A) \quad dE_1 / dd_1 = c(1+E_1); \quad (B) \quad dE_2 / dd_2 = -\lambda E_2.$

As for all IDER the initial conditions are $E_1(0)=0$ and $E_2(0)=0$. The IVPs are readily solved:

(A5.18) $\quad (A) \quad E_1 = \exp(cd_1) - 1; \quad (B) \quad E_2 = 0.$

Here Eq. (A5.18A) is fully equivalent to Eq. (A5.17A) with its implied initial condition, but Eq. (5.18B) contains no information on the strength λ of the inhibitor while Eq. (A5.17B) does.

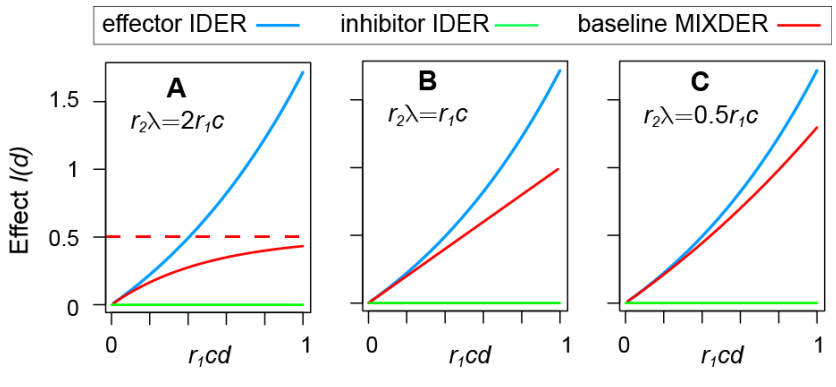
The interpretation of Eq. (A5.17A) is that a small increment in d_1 causes a positive effect increment proportional to $c(1+E_1)$. Thus agent 1 is an effector, e.g. an HZE beam. The interpretation of Eq. (A5.17B) is that if any positive effect is present a small increment in d_2 tends to cause a small decrease in effect. Thus agent 2 is an inhibitor, e.g. an anti-oxidant. The interpretation of Eq. (A5.18B) is that if no effect is present, a small increment in d_2 does nothing at all. This could be the case if background effect Y_0 is negligible, so that agent 2 acting by itself cannot drive total effect $Y_0 + E_2$ belowY_0. Not even simple effect additivity theory, let alone its published replacements, can provide no-synergy/antagonism for this situation.

We now consider a mixture of the two agents with proportions r_1 and r_2. The IVP for incremental effect additivity, (A5.2), reads

(A5.19) $$dI / dd = r_1 c(1+I) - r_2 \lambda I; \quad I(0) = 0.$$

Intuitively, this equation says that the effector tends to make more and more effect, and the inhibitor, as soon as it has some effect to inhibit, acts in the opposite direction. The qualitative theory of ODE [Brauer and Nohel 1989] shows that there are two main types of solutions of Eq. (A5.19), characterized respectively by $r_2\lambda/r_1c \geq 1$ (e.g. a large admixture of a strong inhibitor) and by $r_2\lambda/r_1c < 1$ (e.g. a small admixture of a weak inhibitor). Fig. A5.4 shows the pattern.

Fig A5.4. Stabilizing or Only Partial Inhibition.



In all 3 panels the inhibitor IDER is the horizontal straight line at effect 0, but the inhibitor AIDER, given by Eq. (A5.17B), is non-trivial. In panel A, inhibition is so strong the baseline no-synergy/antagonism MIXDER $I(d)$ approaches a finite limit

(dashed line) as dose increases. In panels B and C the baseline MIXDER grows indefinitely, at a slower rate than if no inhibitor were present.

If one has many effectors and many inhibitors $I(d)$ can be calculated similarly. In the equation of incremental effect additivity, each effector contributes a positive amount to the slope and each inhibitor a negative amount. Recently [Piggott et al. 2015] reviewed known attempts to determine whether, when many effectors and inhibitors are in a mixture, the observed MIXDER is higher than, lower than, or approximately equal to the MIXDER one would have expected from the component IDERs. It was concluded that no known synergy analysis theory was adequate to answer the question and that probably no acceptable systematic quantitative method could be devised. However, the incremental approach here in fact gives the baseline MIXDER quantitatively and systematically.

A5.3.3. A Breakthrough?

We have repeatedly emphasized that essentially all published replacements for simple effect additivity synergy theory $S(d)$ are tormented by the unwelcome restriction that for small doses all components of a mixture have monotonic increasing IDERs (or all have monotonic decreasing IDERs). But the preceding sub-section shows the torment may be avoidable: Fig. A5.4 made us (belatedly) realize that instead the key restriction is that incremental effect additivity $I(d)$ for a mixture be itself monotonic at low doses, and that can easily occur even if at low doses some component IDERs are monotonically increasing, others are identically zero like the inhibitor AIDER in Fig. A5.4, and some other IDERs are monotonically decreasing.

For example, suppose all components of a mixture have AIDERs given by slope extrapolators which are polynomials with real coefficients. Then one can integrate the equation of incremental additivity, Eq. (A5.2), and the slope of $I(d)$ will not change sign. Denote the zeroth order coefficient of the k^{th} AIDER polynomial by a_{0k}. Then $I(d)$ will be monotonic increasing,

identically zero, or monotonic decreasing respectively according as $\sum_k r_k a_{0k}$ is positive, 0, or negative respectively. One can mix monotonically increasing with monotonically decreasing AIDERs and $I(d)$ will nonetheless be well defined.

This is a major generalization. It is not fully satisfactory because: (a) one will sometimes need AIDERs more sophisticated than polynomials, e.g. the AIDER with slope generator given by Eq. (A5.13); and (b) we have not given simple necessary and sufficient conditions to exclude unsuitable behavior such as $I(d)$ shooting up or down to ± infinity at finite doses, as exemplified in Fig. A5.2. But ameliorating both these problems is quite clearly possible and perhaps both can be eliminated entirely to develop a synergy theory as general as simple effect additivity but capable of handling highly curvilinear IDERs sensibly.

A5.4. Evaluating Substitutes for Simple Effect Additivity $S(d)$

A5.4.1. Advantages of the Incremental Effect Additivity Synergy Theory I(d)

Simple effect additivity $S(d)$ is always available for synergy analyses; in practice it is always used to help plan for mixture experiments and to interpret mixture data. But the simple effect additivity synergy theory has important known limitations for analyzing mixtures whose component IDERs are highly curvilinear, so that a supplement or replacement for $S(d)$ is often needed. $I(d)$ appears preferable to other known alternatives. Some of the main reasons are the following.

- $I(d)$ can be used for mixtures whose component IDERs have quite heterogeneous shapes.
- $I(d)$ obeys the sham mixture principle and the mixture of mixtures principle.
- Eq. (A5.2) for $I(d)$ does not restrict AIDERs by monotonicity requirements, only those mixture component IDERs, if any, that are not AIDERs. It consequently applies to more

kinds of mixtures, and over larger dose or effect ranges, than other synergy theories can handle. This is a major generalization whose possibilities are just beginning to be explored.

- $I(d)$ emphasizes the possibility of using effect as predictor variable and dose as response variable instead of vice-versa. This emphasis makes sense: effect is a state variable, i.e. a property of the biological system. On the other hand a biological system can sense the various doses delivered by various agents in a mixture only indirectly by the effects the combined dosing induces.
- As the graphs in this paper attest, $I(d)$ tends to lie nested within the band formed by the various component IDERs of a mixture, as would be expected of a MIXDER that is supposed to indicate absence of synergy and absence of antagonism.
- Using $I(d)$ and AIDER together in incremental effect additivity synergy theory can incorporate additional biophysical information on the incremental action of an agent's incremental dose when an effect larger than the agent can induce when acting by itself has already been induced by other agents in a mixture.

A5.4.2. Problems with the Incremental Effect Additivity Synergy Theory

Although $I(d)$ is the preferred substitute for simple effect additivity its limitations should not be underestimated. We next review some of the main limitations.

First, $I(d)$ shares the limitations of all reasonably general mathematical synergy theories such as those summarized in Fig. A3.1 above. For example $I(d)$ emphasizes mathematical curve manipulation rather than biophysical insights, and it is usually just a real-valued function of dose, not a more complicated mathematical object that could take into account more complex endpoints such as probability distributions of time to tumor.

Second, calculating $I(d)$ usually involves heavy use of numerical methods, e.g. programs to solve ODEs. This emphasis on numerical methods instead of explicit functions is somewhat unfamiliar; it makes global results that hold for all relevant values of parameters hard to find. Finally, there is, at least as yet, no standardized, critically tested consensus protocol for using and interpreting $I(d)$. The advantages of $I(d)$ should be balanced against these problems.

The indicated strategy is as follows. Whenever at least one component of a mixture has a highly curvilinear IDER, the incremental effect additivity synergy theory should be used to supplement or replace the simple effect additivity theory. Some other synergy theory such as the LQ mixture equation should be used as an additional supplement when there are biophysical reasons for doing so. Though somewhat clumsy, this strategy does provide an improved, systematic, quantitative approach to many of the synergy questions that arise in radiobiology.

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