Characterization of Toxicokinetics and Toxicodynamics with Linear Systems Theory: Application to Lead-Associated Cognitive Decline

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We present a theoretical approach to analysis of toxicokinetics and toxicodynamics using linear systems theory. In our approach, we define two impulse response functions that characterize the kinetic behavior of an environmental agent in the body and the dynamic time-course behavior of its effect on the body. This approach provides a formalism for understanding the relation among exposure, dose, and cumulative biologically effective dose and for understanding the implications of an effect time-course on cross-sectional and longitudinal data analyses. We use lead-associated cognitive decline as a specific example where the approach may be applied. *Key words*: bone lead, exposure assessment, linear systems model, toxicodynamics, toxicokinetics. *Environ Health Perspect* 109:361–368 (2001). [Online 16 March 2001]
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Environmental and occupational epidemiologic research involves the identification of relations between past exposures to putative toxicants and subsequent adverse health effects in individuals within study populations. Such relations are often hard to fully characterize because of difficulties in accurately quantifying exposure, dose, and effect. Meaningful quantification is particularly difficult because the exposure–effect relation arises from a multistage process, often referred to as the toxicologic paradigm. As a result, the use of biomarkers in molecular epidemiology research has gained widespread attention (*1*).

In using biomarkers in environmental epidemiology, it is critical to consider the actual process or parameter a given biomarker reflects. For example, the concepts of exposure, internal dose, and biologically effective dose are often blurred in practice, and the loss of these distinctions can influence interpretation of data. Measures of exposure or internal dose are often assumed to be surrogates of the biologically effective dose; their use implies a set of assumptions that is not usually fully articulated or considered. These assumptions involve considerations of the toxicokinetics of the agent and "exposure" biomarker, which are influenced by varying exposure intensity and duration, the residence time of the active form of the agent at the sensitive target, saturation effects, and release from body stores. Similarly, measures of health effects depend on the time course of response to a given exposure (the toxicodynamics of the agent and "response" biomarker) and are influenced by varying response magnitude and duration, dose–dependent repair mechanisms, and multiagent synergistic effects.

In practice, simplifying assumptions are usually made to directly relate exposure to biologically effective dose and to relate this surrogate of dose to health effect. These simplifying assumptions generally overlook the potentially important influence of bidirectional transfer of the agent from one anatomic or physiologic compartment to another. Less frequently, more sophisticated approaches to relating exposure to internal dose and internal dose to biologically effective dose at the sensitive target have been used. In general, these approaches are based on multicompartmental pharmacokinetic modeling (*2–4*). Even with sophisticated approaches to characterizing the toxicokinetics of a given agent–effect paradigm, simplified models of response are usually used in epidemiologic investigations (e.g., the assumption of an irreversible, static response to a given exposure). To help understand these issues, we present a conceptual framework based on linear systems theory and its application to the analysis of lead-associated neurocognitive decline. We specifically consider issues of residence time of the agent at the sensitive target, later release of the agent from body stores (with corresponding re-residence at the sensitive target), and the time course of response.

Theory

A fundamental assumption in dose–response studies of toxic agents is that the active form of the agent at the sensitive target site causes the effect (*4*). Thus, to characterize the relation between exposure to an environmental agent and subsequent development of an adverse health effect, two types of processes must be modeled: the toxicokinetics that describe the relation between environmental exposure and ultimate cumulative dose at the sensitive target, and the toxicodynamics that describe the relation between this cumulative dose at the sensitive target and the adverse effects. In practice, it is frequently assumed that the total adverse effect is proportional to the area under the curve (AUC_T) of the time–concentration relation for the active form of the agent at the target site, (*4*):

$$AUC_T = \int C_T(t)dt \qquad [1]$$

Thus, it is highly desirable in environmental epidemiologic investigations to be able to estimate AUC_T and to be able to characterize the relation between AUC_T and the observed effect at any given measurement time.

Toxicokinetics: Measures of AUC_T

A common surrogate for AUC_T is cumulative exposure, E, defined as the integral exposure to a certain time-dependent environmental concentration of a toxic agent, $C_E(t)$:

$$E = \int C_E(t)dt \qquad [2]$$

Another common surrogate for AUC_T is the cumulative dose, D, which is frequently based on the assumption of a linear relation between exposure and dose:

$$D(t) = kE(t)$$
$$D = k\int C_E(t)dt \qquad [3]$$

The use of either cumulative exposure or cumulative dose as a surrogate for AUC_T implies the assumption of a linear relation between AUC_T and either E or D, with y-intercept equal to zero:

$$AUC_T = k_E E = k_D D \qquad [4]$$

In other words, Equation 4 implies that the toxicokinetics are strictly linear; that is, the transfer of a toxic agent from the environment to the sensitive target (including bioactivation, if relevant) follows a linear

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dependence. Of perhaps greater importance, Equation 4 implies that the residence time of the agent at the sensitive target is relatively brief, and that, if the agent is concentrated in body stores, it is not subsequently released and made bioavailable to the sensitive target. This conclusion follows directly from characterization of the transfer of an environmental agent to the body [i.e., from exposure, $C_E(t)$, to internal dose, $C_D(t)$] and from the initial biodistribution in the body to the sensitive target [i.e., biologically effective dose, $C_T(t)$] with linear systems theory (*5*), as discussed below. If these assumptions are incorrect, $C_T(t)$ will be significantly different in shape than either $C_E(t)$ or $C_D(t)$ [i.e., $C_T(t)$ will be spread out compared with either $C_E(t)$ or $C_D(t)$], even in the case of a truly linear relation between exposure and internal dose, and the use of Equation 4 will lead to an underestimation of the actual AUC_T. Thus, the common use of the cumulative exposure index [i.e., integrated exposure over time (Equation 2)] (*4*) may underestimate the AUC_T.

For the sake of our discussion, a system is considered linear if the relation between the input [e.g., $C_E(t)$] and the output [e.g., $C_T(t)$] has the properties of additivity and scaling. A system has the property of additivity if the sum of the outputs from two independent inputs equals the unified output from the sum of the inputs. That is, if the function f that describes a system is linear, then:

$$f[x_1(t) + x_2(t)] = f[x_1(t)] + f[x_2(t)].$$

In our case, this implies that the sum of the two actual time–concentration curves at the sensitive target resulting from two separate exposures would be the same as the single time–concentration curve resulting from the sum of the exposures. That is, we assume that a single, complex time-varying exposure may be conceptualized as a series of intensity-scaled instantaneous exposures, and that the actual observed time–concentration curves resulting from the single, complex time-varying exposure can be modeled as the sum of individual time–concentration curves from the series of intensity-scaled instantaneous exposures.

A system has the property of scaling if the output is scalable by the input:

$$f[ax(t)] = af[x(t)].$$

In our case, this implies that the time–concentration curve at the sensitive target multiplicatively scales with a multiplicative change in exposure. For example, if the exposure intensity doubles, we assume that the time–concentration curve will double (i.e., the concentration will double, and the curve will retain the same shape).

Additivity and scaling are usually combined into the principle of superposition. In general, biological systems follow this superposition principle, up to the point of saturation or other mass effects at very high concentrations.

An equally important consideration for a linear system is time invariance. In a time-invariant system, a time shift or delay of the input produces a corresponding shift in the output, without any other change. In our case, this implies that the toxicokinetics do not change over time (i.e., the same exposure would always produce the same time–concentration curve). This assumption is frequently made in assessments of environmental concentrations of toxicants (*6*), but has not always explicitly been applied to toxicokinetic behavior. In practice, this assumption implies that ongoing exposure does not influence the toxicokinetics per se. Such is the case when the adverse effects (i.e., the toxicodynamics) do not influence physiologic processes that govern the toxicokinetics. For example, if lead does not influence blood flow or organ extraction from blood, chronic exposure does not change the biodistribution of an additional dose relative to the first dose. As a contrasting example, initial exposure to a respiratory irritant may influence airway caliber or mucociliary clearance, thus changing the toxicokinetics of subsequent exposures. For the example given below (lead-associated neurocognitive decline), we assume that the system is time invariant.

Given a linear, time-invariant system, the output [e.g., $C_T(t)$] for any given arbitrary input [e.g., $C_E(t)$] can be directly predicted from knowledge of the input and the system's impulse response function (IRF). This IRF characterizes the system's response (i.e., the output) to an infinitely short duration input (mathematically equivalent to a delta or Dirac function in time, denoted as δ) (*5*):

$$\delta \xrightarrow{transfer} \mathrm{IRF}(t) \qquad [5]$$

From a toxicokinetic perspective, IRF_{TK} is the $C_T(t)$ curve that would be observed from a single, infinitely short duration exposure. Because IRF_{TK} is a time-varying curve and is conceptually derived from a single pulse exposure, its use implies that the ongoing toxicokinetics of even a single molecule of a toxicant could be captured by this formalism. For example, IRF_{TK} could represent the behavior of a single lead molecule that enters the brain (and produces an effect), is cleared and stored in bone, is then released back to the blood, and reenters the brain (and produces a second effect).

Assuming that the toxicokinetics can be described by a linear, time-invariant system, the observed target site concentration–time curve from an arbitrary exposure time course is given by the mathematical convolution (denoted by $\otimes$) of the actual exposure time course with the target site IRF_{TK} (*5*):

$$C_T(t) = k_E C_E(t) \otimes \mathrm{IRF}_{TK}(t), \qquad [6]$$

where $C_E(t)$ is the exposure time–concentration curve, and k_E is a constant relating units of exposure to units of biologically effective dose. Equation 6 may be recast as the standard convolution integral:

$$C_T(t) = k_E \int C_E(\tau)\mathrm{IRF}_{TK}(t-\tau)d\tau, \qquad [7]$$

where τ is a dummy variable of integration. Of major importance, if either of the two terms in the convolution [e.g., $C_E(t)$ or IRF_{TK} in Equations 6 and 7] is a δ function, then that term drops out of the convolution, and the relation reduces to a straight equivalency. That is, if the residence time of a toxicant at the sensitive target is very short, and if there is no subsequent bioavailability due to release from body stores, then IRF_{TK} is essentially a δ function, and the assumptions implicit in Equation 4 are valid. On the other hand, if the residence time is significant, then IRF_{TK} is not a δ function, but a curve with some spread in time, and Equation 7 must be used (with Equation 1) instead of Equations 2 and 4 to determine AUC_T. In a similar fashion, if there is significant subsequent release from body stores, IRF_{TK} will be multi-peaked or have an initial peak (representing the initial transfer from the environment), followed by non-zero values over time (representing the release from body stores), and Equation 7 must again be used to compute AUC_T via Equation 1.

In essence, one extreme scenario for the IRF and Equations 6 and 7 is that IRF_{TK} is a δ function, implying that the residence time is essentially zero (i.e., the active agent rapidly transits through the sensitive target) and that no release from body stores occurs. The opposite scenario from a toxicokinetic perspective is that the active agent permanently resides in the sensitive target; that is, IRF_{TK} is a constant with time (either because the agent never clears from the sensitive target or because ongoing significant biorelease from body stores constantly replenishes that amount of agent cleared from the target). In general, Equations 1 and 7 can be combined, with AUC_T expressed as a double integral, with integration limits from time zero to the current observation time, T:

$$AUC_T = k_E \int_0^T \int_0^T \mathrm{IRF}_{TK}(T-t)C_E(t)dt\,dT \qquad [8]$$

If IRF_{TK} is a δ function, the inner integral drops out, and Equation 8 reduces to that relation implied by Equations 3 and 4:

$$\mathrm{AUC}_T = k_E \int_0^T C_E(t)dt \quad [9]$$

If IRF_{TK} is a constant function, it drops out of the inner integral in Equation 8, but the double integration remains:

$$\mathrm{AUC}_T = k_E \int_0^T \int_0^T C_E(t)dt\, dT \quad [10]$$

[Note that IRF_{TK} is causal, which means that it results from an event in real time; thus, IRF_{TK} $(t < 0) = 0$. This assumption facilitates the simplification of Equation 8 to either Equation 9 or Equation 10.]

Equations 9 and 10 define opposite scenarios. If the actual IRF_{TK} is closer to a δ function than a constant, the use of Equation 9 in environmental epidemiologic investigations should provide biologically effective dose estimates that are better predictors of health outcomes than would the use of Equation 10. If the actual IRF_{TK} is significantly spread, either because of significant residence time or significant release over time from body stores, then the use of Equation 10 should provide better biologically effective dose estimates, and thus stronger and more consistent associations with the health outcomes under study.

Toxicodynamics: Time-Dependent Measures of Response

In the previous section, we used linear systems theory to characterize the time course of the active form of the agent at the sensitive target, $C_T(t)$. We now want to characterize the time course of the health outcome or response, $R(t)$. As with $C_T(t)$, $R(t)$ can be conceptualized as a linear system characterized by an IRF. Since we designated the IRF for the toxicokinetic relation IRF_{TK}, a similar IRF, designated IRF_{TD}, can be used to characterize the toxicodynamics:

$$\begin{aligned} &C_E(t) \xrightarrow{\text{toxicokinetics}} \\ &C_T(t) \xrightarrow{\text{toxicodynamics}} R(t) \\ &R(t) = C_E(t) \otimes \mathrm{IRF}_{TK} \otimes \mathrm{IRF}_{TD} \end{aligned} \quad [11]$$

From a toxicodynamic perspective, IRF_{TD} is the $R(t)$ curve that would be observed from a single, infinitely short duration $C_T(t)$ curve [i.e., in the case where both $C_E(t)$ and IRF_{TK} are δ functions]. The shape of IRF_{TD} directly indicates reversible versus irreversible (persistent) versus progressive effects from a single exposure, as shown in Figure 1. A reversible effect would yield an IRF_{TD} that goes from zero at the point of exposure to some response value and back to zero. An irreversible effect would yield an IRF_{TD} that goes from zero at the point of exposure to some response value that persists, independent of time. A progressive (increasing) effect (from a previous exposure) would yield an IRF_{TD} that continuously increases with time, starting from zero at the point of exposure.

The utility of Equation 11 is that it identifies issues in the design of an epidemiologic study in which response is measured either cross-sectionally at some time after exposure ceases, or longitudinally. For example, a cross-sectional design uses a surrogate of cumulative exposure and a single measure of response at some later time. Equation 11 tells us that such a design is only applicable if IRF_{TK} is a δ function and if IRF_{TD} is a step function (i.e., the effect is persistent rather than either reversible or progressive). In a longitudinal study, it is imperative to consider possible shapes for IRF_{TD}, because the observed $R(t)$ implies underlying IRF_{TK} and IRF_{TD} functions that are usually not directly obtainable. For example, the observation of a progressive increase in effect with time could be the result of *a*) the ongoing presence of active agent at the sensitive target, due to either long residence time or ongoing release from body stores, either of which would cause AUC_T to continue to increase with time; *b*) progressive response from past exposures; or *c*) a combination of the two. Equation 11 suggests that the observed $R(t)$ could be the result of a significantly non-δ function IRF for either the toxicokinetic or toxicodynamic portion of the toxicologic paradigm. For example, from a purely mathematical point of view, the same $R(t)$ would be observed if either IRF_{TK} or IRF_{TD} were a δ function and the other were a step function.

Application to Longitudinal Data Analysis

Bandeen-Roche et al. (*7*) have previously described a general model for analysis of data from prospective observational studies with multiple outcome measures over time. Their model includes a family of exposure summaries whose mathematical formalism is a convolution integral similar to Equation 7, although they did not approach the overall model's conceptualization with linear systems. In their data analysis model, outcome or response is a function of exposure history plus a random error. With our notation:

$$R(t) = F[C_T(t)] + \varepsilon(t) \quad [12]$$

In their approach, $F[\cdot]$ represents a complex function representing the effects of the family of exposure summaries. They use this construct to create a generalized linear model that separates cross-sectional, longitudinal, historic, and regression-to-the-mean effects. Here, "cross-sectional," "longitudinal," and "regression-to-the-mean" have their conventional epidemiologic definitions, and "historic" addresses the influence of previous exposures on the toxicodynamics resulting from subsequent exposures. If we use separate β coefficients to designate cross-sectional (β_{cs}), longitudinal (β_l), historic (β_h), and regression-to-the-mean (β_{rm}) terms in the model, then initial (i.e., cross-sectional) and subsequent (i.e., longitudinal) relations

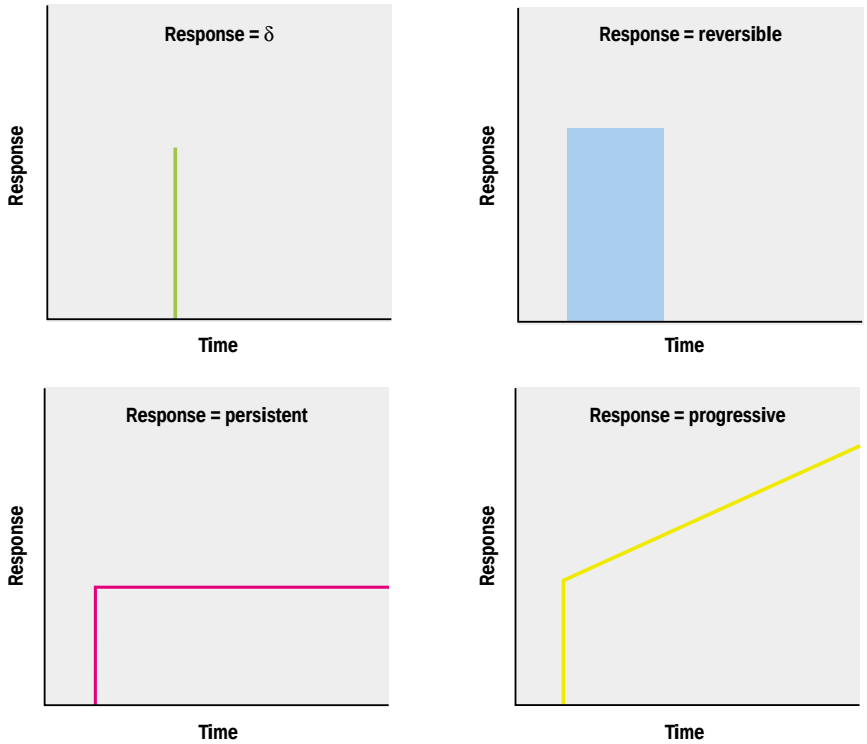

Figure 1. Possible IRF_{TD} curves.

can be defined (with our notation):

$$R_1 = \beta_0 + \beta_{cs}AUC_1 + \varepsilon_1$$
$$R_2 = \alpha_0 + \beta_{cs}AUC_1 + \beta_l(AUC_2 - AUC_1) + \beta_h AUC_1 + \beta_{rm}(\beta_0 + \beta_{cs}AUC_1 + \varepsilon_1) + \varepsilon_2 \quad [13]$$

It is common in data and statistical analyses of longitudinal data to use the difference between successive measurements. In such a case, the model above reduces to:

$$\Delta R = R_2 - R_1 = \beta^* + \beta_1(AUC_2 - AUC_1) + \beta_h AUC_1 + B_{rm}R_1 + \varepsilon^* \quad [14]$$

This equation is important in generalizing the use of the linear systems approach we propose in longitudinal data analysis. Such analyses go directly to the heart of the toxicokinetic and toxicodynamic implications of the IRF model we have invoked.

Empirical Validation: Cognitive Effects of Lead

Lead is widely recognized as a significant neurotoxicant, and the development of biomarkers of lead exposure has been vigorously pursued. X-ray fluorescence (XRF) measurement of lead in bone has been adopted as the method of choice to assess cumulative exposure (*8*,*9*) because lead in blood has a clearance half-time of 30 days, whereas lead in bone has a clearance half-time of 15–30 years. XRF measures of bone lead highly correlate with the integral of the time-course of blood lead concentration [also called the "cumulative blood lead index" (*10*,*11*)]. Independent data suggest an association between cumulative blood lead level and cumulative brain uptake (*12*–*13*). Because multiple blood-lead level measurements as a function of time, which are necessary to compute the cumulative index, are usually not available in epidemiologic studies and only rarely in occupational studies, single XRF measures of lead in bone are taken to represent "cumulative exposure" (via Equation 2) or "cumulative dose" (via Equation 3).

Hu et al. (*14*) considered two paradigms for the interpretation of skeletal lead, as measured by XRF: bone lead as an indicator of cumulative lead exposure, and bone lead as a source of body lead burden that can be mobilized into the circulation. The first paradigm considers bone lead as a surrogate marker for cumulative dose to sensitive targets, whereas the second considers bone lead as an important endogenous source of further exposure. With either paradigm, the dose–response relation could be linear or nonlinear and involve a threshold or not (*14*). In either case, XRF measures of bone lead could be predictive of a given health outcome (such as cognitive decline) as long as a strictly linear relation exists between the XRF measure and AUC_T. As discussed above, if either the residence time (assuming the first paradigm) or release from bone stores (assuming the second paradigm) is significant, then the XRF measure, although clearly better than a blood measure, would not correlate as highly with health outcome as a truer AUC_T metric. For example, a comparison of the strength of association with health outcomes between AUC_T estimated from Equation 9 versus Equation 10 would provide some evidence for which of the two scenarios (short residence time and no release versus long residence time and/or subsequent release) is more likely. This distinction is particularly useful in making hypotheses about whether an effect is likely to be transient or persistent or progressive.

Methods

To empirically investigate these scenarios, we reanalyzed data from a longitudinal study of 535 former organolead manufacturing workers, for whom we have already reported results (*15*–*19*). Informed consent was obtained before a subject was enrolled in the study. As reported in more detail elsewhere (*19*), a battery of 19 cognitive tests was obtained annually (Table 1). The results of this battery were compared with blood and bone lead measurements. Current tibial lead was measured via XRF and used to estimate the peak tibial lead value at the time of cessation of occupational exposure. To do so, the clearance of lead from bone was modeled with a mono-exponential function, as has previously been demonstrated to fit longitudinal bone lead data (*20*); the clearance half-time was assumed to be 27 years (*21*). The AUC_T was estimated in

Table 1. Generalized estimating equation linear modeling results identifying predictors of annual change in neurobehavioral test scores in 535 former organolead manufacturing workers comparing four different dose metrics, 1994–1998.

Neurobehavioral measure[a] (used in separate regression models of change)	Current PbB[b] β (SE β)	Current TL[c] β (SE β)	Peak TL[d] β (SE β)	AUC-lead[e] β (SE β)
Block design (Wechsler Adult Intelligence Scale)	0.161 (0.135)	–0.058 (0.160)	–0.223 (0.165)	–0.213 (0.173)
Digit symbol (Wechsler Adult Intelligence Scale, revised)	–0.187 (0.126)	–0.133 (0.135)	–0.038 (0.140)	0.058 (0.139)
Symbol digit	–0.012 (0.102)	–0.099 (0.099)	–0.206 (0.107)**	–0.225 (0.108)**
Serial digit learning	–0.160 (0.132)	–0.020 (0.153)	–0.104 (0.161)	–0.149 (0.165)
Rey complex figure, copy	–0.001 (0.097)	–0.030 (0.091)	–0.138 (0.094)	–0.170 (0.096)
Rey complex figure, delayed recall	–0.174 (0.088)**	–0.077 (0.090)	–0.174 (0.096)*	–0.196 (0.097)**
Rey auditory verbal learning test, immediate recall, 5 trials	0.049 (0.167)	–0.255 (0.175)	–0.571 (0.193)***	–0.671 (0.204)***
Rey auditory verbal learning test, delayed recall	–0.055 (0.055)	–0.128 (0.054)**	–0.149 (0.058)**	–0.134 (0.058)**
Rey auditory verbal learning test, recognition	–0.009 (0.053)	0.035 (0.062)	–0.028 (0.072)	–0.054 (0.078)
Trails A	–0.225 (0.252)	–0.586 (0.273)**	–0.503 (0.308)	–0.451 (0.309)
Trails B	–0.790 (0.629)	0.285 (0.740)	–0.061 (0.851)	–0.212 (0.881)
Finger tapping, dominant hand	–0.196 (0.124)	–0.260 (0.155)*	–0.170 (0.153)	–0.056 (0.150)
Finger tapping, nondominant hand	–0.137 (0.099)	–0.224 (0.128)*	–0.169 (0.135)	–0.094 (0.137)
Pegboard, dominant hand	0.045 (0.087)	–0.133 (0.092)	–0.138 (0.098)	–0.122 (0.098)
Pegboard, nondominant hand	–0.093 (0.087)	–0.254 (0.100)**	–0.250 (0.101)**	–0.182 (0.102)*
Pegboard, both hands	0.053 (0.074)	–0.048 (0.095)	–0.094 (0.087)	–0.117 (0.086)
Pegboard assembly	0.461 (0.235)**	–0.034 (0.329)	–0.320 (0.320)	–0.577 (0.301)*
Stroop (C form – A form)	–0.740 (0.389)*	–0.676 (0.544)	–1.122 (0.606)*	–1.366 (0.675)**
Choice reaction time average	–0.393 (2.541)	–0.298 (2.703)	–2.121 (2.776)	–2.968 (2.989)
SIGNS of β coefficients	14/19 negative	20/22 negative	22/22 negative	21/22 negative
Statistical significance	—	—	1 < 0.01	1 < 0.01
	2 < 0.05 (1+)	3 < 0.05	3 < 0.05	4 < 0.05
	1 < 0.01	2 < 0.10	2 < 0.10	2 < 0.10

[a]Adjusted for age, education, visit number, testing technician, and baseline score; tests were standardized for direction so that a negative coefficient indicates worsening performance with increasing blood or tibial lead. Beta coefficients are standardized so that they can be directly compared for each neurobehavioral test. The units of each β coefficient indicate change in neurobehavioral test score per SD unit increase in the lead biomarker. [b]Current PbB = current blood lead level. [c]Current TL = current tibial lead. [d]Peak TL = peak tibial lead, estimated from current tibial lead and years since last exposure, using an estimated half-time of lead in tibia of 27 years (see "Methods"). [e]AUC-lead = area under the curve of estimated tibial lead levels versus time (see "Methods"). *** *p*-value ≤ 0.01; ** *p*-value ≤ 0.05; * *p*-value ≤ 0.10.

three ways: *a*) by assuming that the current tibial lead was proportional to AUC_T, *b*) by assuming that the peak tibial lead was proportional to AUC_T, and *c*) by forming and integrating the estimated tibial lead time course (which we designate AUC´). To do the latter, we back-extrapolated from the current tibial lead value to the time at which exposure ended (i.e., the same process used to get the peak tibial lead value), and then assumed a straight line between that peak value and a value of zero at the start of occupational exposure some years earlier, as shown in Figure 2. (We knew the date of start of occupational exposure and cessation of exposure for each subject.)

If the XRF measurement can be conceptualized via Equation 2 (i.e., the XRF value at a given time T represents the integral exposure from time zero to that time T), then the AUC_T estimated by the third approach above (AUC´) represents

$$AUC' = \int_0^T XRF(t)dt = \int_0^T \int_0^T C_E(t)dt\,dT \quad [15]$$

Equation 15 is thus identical in form to Equation 10 and implies permanent residence of lead at the sensitive target (central nervous system receptors in the brain, in our case). This is also seen by comparison of Equation 15 with Equation 8; the only way that Equation 8 (which is the general form) could be identical to Equation 15 is if IRF_{TK} in Equation 8 is a constant.

In other words, we have four estimates of cumulative biologically effective dose or AUC_T:

1. Current blood-lead level, which reflects both past exposures with a 30-day clearance half-time (i.e., it is a poor index of cumulative exposure) and the reintroduction of lead into the circulation via release from bone stores. This metric assumes a short residence time in brain and is mainly reflective of present release from bone and other stores because our population is no longer occupationally exposed.
2. Current tibial lead level, which reflects cumulative exposure (with a 27-year clearance half-time) and the magnitude of bone stores potentially available for release. This metric assumes a short residence time in brain and allows for the possibility of some release from body stores; it better reflects cumulative exposure than blood lead because of the much slower clearance.
3. Peak tibial lead level, which reflects cumulative exposure (and is corrected for clearance) and the magnitude of bone stores potentially available for release. This metric also assumes a short residence time in brain and allows for the possibility of some release from body stores.
4. AUC´, which reflects cumulative biologically effective dose in the case of permanent residence (either because the initial residence time is long and/or there is significant ongoing release from bone stores).

Comparisons of the association of these four estimates of AUC_T with each of the 19 cognitive tests were based on linear regression using generalized estimating equations methodology. Beta coefficients for the four estimates of AUC_T, for each of the 19 cognitive tests, were obtained and assessed for statistical significance. The cognitive test outcomes were *z*-transformed before modeling so that the β coefficients could be directly compared. The linear regression models controlled for age, education, visit number, testing technician, and baseline score on each test.

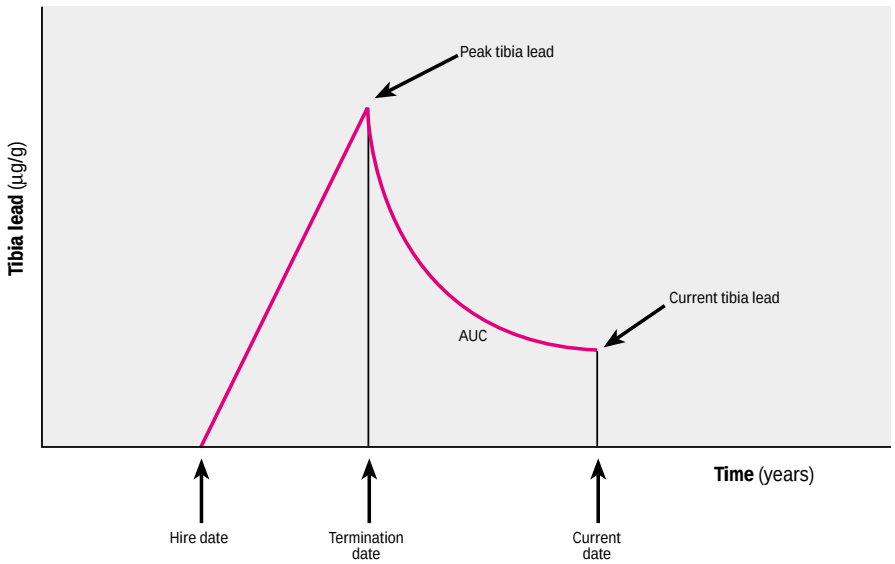

Figure 2. Calculation of AUC.

Two types of linear regression models were used. The first (R_1 in Equation 13) focused on cross-sectional data from the baseline measurements. Here, the baseline score on each of the 19 cognitive tests were the dependent variables in the linear regression models. The second focused on longitudinal data, starting with the baseline data, and adding three subsequent measurements, each 1 year apart, for each of the 19 cognitive tests. Here, the dependent variables were the annual change scores (via Equation 14), in practice defined as $(R_i - R_0)/\Delta$ time rather than $R_i - R_{i-1}$, and generalized estimating equations methods were used to examine associations of the lead measures with change in test scores over time.

Results

Cross-sectional analyses. We previously reported the associations of current and peak tibial lead levels with 19 neurobehavioral test scores (*16*); we now report associations for blood-lead level and AUC´. Taking a *p*-value < 0.05 as significant, current blood lead level was significantly associated with 4/19 tests, current tibial lead level with 9/19, peak tibial lead level with 11/19, and AUC´ with 14/19. All significant β coefficients indicated that increasing lead levels were associated with lower neurobehavioral test scores.

Longitudinal analyses. Table 1 shows the results of the linear regression analyses using the baseline values for blood lead, current tibial lead, peak tibial lead, and AUC´. Taking a *p*-value < 0.05 as significant, current blood lead level was associated with 2/19 neurobehavioral test change scores (although one of the two significant β coefficients was in the opposite direction than expected), current tibial lead level with 3/19, peak tibial lead level with 4/19, and AUC´ with 5/19. Of interest, current blood lead level had the highest β for 3/19 associations, current tibial lead level for 4/19 associations, peak tibial lead level for 3/19 associations, and AUC´ for 9/19 associations. There was only one instance where AUC´ did not have a significant β coefficient when one of the other estimates did; in this case, current tibial lead level produced the only β coefficient that achieved statistical significance.

Because the cognitive test outcomes were standardized, both the direction and magnitude of the associations (β coefficients) of each AUC_T lead measure with each neurobehavioral test change score could be directly compared. Current blood-lead level had only two significant β coefficients, one of which was positive (i.e., in the opposite direction than expected). Use of current blood level would thus lead to the conclusion of no association between lead and cognitive decline. In contrast, progressing from current tibial

lead level to peak tibial level to AUC´ increased the number of significant β coefficients, and AUC´ produced the largest β coefficient in more of the 19 tests than any of the other 3 measures.

Even though the β coefficients changed depending on which of the four estimates of AUC was used, some association between the four estimates was present. Pearson's correlations were significant ($p < 0.01$) between *a*) blood and current tibial lead ($r = 0.44$), peak tibial lead ($r = 0.26$), and AUC´ ($r = 0.18$); *b*) current tibial lead and peak tibial lead ($r = 0.86$), and AUC´ ($r = 0.70$); and *c*) peak tibial lead and AUC´ ($r = 0.94$).

A critical issue in the interpretation of the longitudinal test outcomes is whether the change in cognitive performance over time could be completely (and thus solely) explained by the increase in AUC´ with time (since the limit of integration for any AUC measure progressively increases with time). In other words, is any progressive cognitive decline simply the result of progressive cumulative dose? Accordingly, we also evaluated a generalized model in which δ-AUC´ (δ-AUC´ = AUC´ end-of-interval – AUC´ baseline) was used instead of baseline AUC´. With this model, only 2/19 tests had significant beta coefficients, and the change in cognitive test outcomes could not be explained by the change in AUC alone. This suggests that a progressive model for IRF_{TD} needs to be considered.

Discussion

In epidemiology, assessing the association between exposure to a putative toxicant and subsequent health outcome implies the existence of an underlying biologically based dose–response relation. The goal of exposure or internal dose assessment is thus to find an index that best represents the cumulative biologically effective dose of the active form of the toxicant at the sensitive target. In practice, this index should be proportional to the integral of the time course of the concentration of active agent at the sensitive target (i.e., Equation 1), designated AUC_T. The use of cumulative exposure or internal dose as a surrogate for this time–concentration integral will only prove useful if the toxicokinetics are approximately linear over the concentration range expected, and if the effects are approximately cumulative (*4*,*6*). This is simply a restatement of Haber's rule: tissue damage should be related to the product of the mean exposure intensity and time (*6*). Thus, a major issue in the development and use of biomarkers is the degree to which these assumptions hold. In other words, it is important to understand whether the toxicokinetics and toxicodynamics are linear and time-invariant mathematically, and whether the toxicodynamics represent a reversible, persistent, or progressive process.

In this report, we present a conceptual framework based on linear systems theory as an aid to identifying and considering these issues. With respect to toxicokinetics, we have attempted to relate different surrogates of cumulative biologically effective dose and to identify the conditions under which certain assumptions are implicitly invoked. We have introduced the common linear systems concept of an impulse response function, IRF_{TK}, to describe the toxicokinetics following an infinitely short duration exposure. Use of linear systems theory and this concept allows us to define a general relation between the exposure time-course and the time-concentration curve of the active form of the agent at the sensitive target, whose integral, AUC_T, is likely best correlated with response. This general relation (Equation 8) could be transformed into two more specific relations, one representing the case where the residence time in the sensitive target is infinitely short and no biorelease occurs (Equation 9), and another representing the case where the effective residence time is infinitely long (either because the agent never clears from the sensitive target or because ongoing significant biorelease from body stores constantly replenishes that amount of agent cleared from the target; Equation 10).

We also sought to use linear systems theory to conceptualize the time-course of response, which was particularly important for the longitudinal data presented here, in which we studied the association of four estimates of AUC_T with cognitive decline in an occupationally exposed cohort of 535 workers. We found that progressively more β coefficients were statistically significant as we moved from current blood lead level to current tibial lead level to peak tibial lead level to AUC´ in both cross-sectional and longitudinal analyses.

Conceptualizing AUC_T in this way and generating multiple estimates of AUC_T is helpful in several ways: it clarifies the distinctions between exposure, internal dose, and biologically effective dose; it guides the development of different estimates of AUC_T; and the results of a comparison of the association of these different estimates of AUC_T with health outcomes provide indirect evidence of the underlying biological phenomena. For example, for those health outcomes in which a measure of recent or current dose has the highest association, the health outcome is likely an acute, reversible process, whereas for those health outcomes in which a measure of cumulative dose has the highest association, the health outcome is likely a persistent or progressive accumulative process. For those health outcomes in which a measure that includes consideration of residence time has the highest association, the agent's toxicokinetics likely include significant residence in the sensitive target and/or significant release from body stores.

Significant successes and utility have been reported with the use of pharmacokinetic modeling in predicting the toxicokinetics of environmental agents such as lead (*4*,*22*). Our approach differs from these previous mathematical efforts in that it is "model free" (i.e., it does not assume a certain "topology" or relationship among a series of anatomic or physiologic compartments, as do classical pharmacokinetic modeling approaches). Rather, our approach makes use of linear systems theory to describe the IRF of the system nonparametrically. This nonparametric IRF exists independent of compartment-based descriptions. In other words, there are no predetermined parameters whose presence, number, and character are fixed by an *a priori* hypothetical model.

Having stated this, we emphasize that we are not asserting that our approach is intrinsically better than multicompartment modeling; rather, we view the two approaches as highly complementary. For example, both may lead to a useful prediction of AUC_T, but they require different assumptions and independent data. The choice of approach depends on prior knowledge and on the types of information and relations desired. If compartments and relations can be identified and appropriate quantitative rate constants determined, then the multi-compartment toxicokinetic approach yields accurate predictions (*4*,*22*). On the other hand, when less is known about the potential compartments, and particularly when little is known about rate constant values, the conceptualization of the toxicokinetics via a linear system with an IRF may prove useful. Such is the case when an empirical time course in an organ or structure of interest is already known (*23*). In this regard, the empirical time course need not come from a δ input function because deconvolution analysis can be used to obtain IRF_{TK} from the combination of any arbitrary but known input function and the empirical time course (*5*). Once IRF_{TK} is obtained in this way, the time-course for any other arbitrary but known input function can be predicted via Equation 6.

We also emphasize that our conceptual framework covers both toxicokinetics and toxicodynamics, whereas pharmacokinetic modeling only addresses predictions of toxicokinetics. In this regard, we highlight the complementary nature of pharmacokinetic modeling and linear systems analysis by suggesting that pharmacokinetic modeling can be used, when available, to predict IRF_{TK} for subsequent use in linear systems analysis of

the toxicodynamics. In essence, either pharmacokinetic modeling or our approach could be used to predict the needed kinetic time-course if the right data are in hand; it is important that the types of data are strikingly different. In practice, we used the toxicokinetic portion of our conceptual framework to create four different estimates of AUC_T; we do not claim that any of these estimates is more accurate than one obtained from pharmacokinetic modeling. Rather, the conceptual framework provides a different understanding of the meaning of each of these estimates than one derived from pharmacokinetic modeling, and this understanding helps in the interpretation of the actual data.

The conceptual framework as presented, embodied in specific equations, and used so far, requires that the toxicokinetics and toxicodynamics be linear and time invariant. With respect to toxicokinetics, when the active form of the agent is produced by metabolism, nonlinear effects, especially at high doses, are expected as the processes saturate. Nonlinear kinetics could also arise from changes in individual uptake or susceptibility with time, from synergistic or antagonistic effects related to concurrent mixed exposures to other toxicants, from allergic responses, from changes produced by the initial exposure to that agent (e.g., upregulation of cytochrome P450), or from dose-rate effects (*6*). With respect to toxicodynamics, for stochastic processes like carcinogenesis, dose–response relations may be linear or nonlinear. In the case of a direct genotoxic carcinogen, a linear or linear-quadratic relation between AUC_T and response is expected (*4*). For nonstochastic processes, a linear relation with a threshold is commonly observed. As with the toxicokinetics, up- or downregulation of receptors or tolerance effects can introduce nonlinearities. To the extent to which strict linearity is not present, the power of the conceptual framework and actual approach decreases; the degree of linearity may be different for the toxicokinetic and toxicodynamic portions of the analysis in a given application.

In contrast to the requirement for linearity, time invariance is not strictly required in our approach. For the sake of simplicity of presentation and implementation, we have invoked the assumption of time invariance; this assumption is what leads to the specific convolution integral given in Equation 7, and repeated below:

$$C_T(t) = k_E \int C_E(\tau)\mathrm{IRF}_{TK}(t-\tau)d\tau \qquad [7]$$

If IRF_{TK} is time varying, the convolution integral given in Equation 7 must be modified, as follows:

$$C_T(t) = k_E \int C_E(\tau)\mathrm{IRF}_{TK}(t;\tau)d\tau \qquad [7']$$

Equation 7′ differs in a subtle way from Equation 7: IRF_{TK} is now a function of both t and τ, not just $t - \tau$. An analogous situation holds for IRF_{TD}. This implies the need to obtain a family of IRFs (as a function of t) rather than just a single IRF. In practice, if the IRF changes only slowly, then it becomes possible to treat segments of time as being time invariant and to use a single IRF during that time period.

In essence, our approach has permitted initial comparisons of different assumptions about the residence time of lead in brain, release from bone stores, and the persistence or progression of lead-associated neurobehavioral effects. Using both cross-sectional and longitudinal data analysis, we have been able to show, in preliminary form, that *a*) a measure of cumulative lead dose (AUC′) that implies either long residence time of lead in brain or significant ongoing release of lead from body stores is the best predictor of both test scores at cross-section and test score declines over time; *b*) the change in this AUC metric over time is a poor predictor of longitudinal test score change; and *c*) the observed longitudinal change in test scores is consistent with a model of progressive neurobehavioral effect. Given our knowledge about the clearance half-time of lead in brain and the current blood and tibial lead levels in the former workers, we believe that the observed annual test score declines are likely due to a combination of newly induced effects from lead released from bony stores and, more significantly, progressive effects from past exposures to lead. In any event, the model requires that, at a minimum, effects persist for many years past the exposure that triggered the effect. These data thus support the hypothesis that this is not a transient neurochemical effect, that, by necessity, would depend on the continued presence of lead in brain to sustain the effect, but rather a persistent structural change (which may have been initially triggered by neurochemical events).

In general, elimination of the toxicant from the sensitive target site probably represents the rate-limiting step between exposure and response (*6*). For example, Rappaport (*6*) has alluded to the physiologic damping that "resulted from accumulation of lead over several months owing to the slow rate of elimination and distribution of this metal from the blood." If this elimination is slow, the accumulated burden is large relative to the amount of toxicant received (*6*). In such a case, knowledge of AUC_T itself (compared with either integrated exposure or internal dose) will better predict response because even large short-term fluctuations in exposure will not directly provide important information on biologically effective dose.

Ultimately, we are interested in the development, validation, and application of biomarkers whose behavior we understand vis-à-vis the toxicologic paradigm. In this regard, the use of the IRF has already been described in noninvasive imaging (*24*) and may provide a means to obtain impulse response functions for the toxicokinetics and toxicodynamics of environmental agents of interest, such as lead, which has been radiolabeled (*25*).

References and Notes

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