Comparison of Points of Departure for Health Risk Assessment Based on High-Throughput Screening Data

Salomon Sand,1,2 Fred Parham,3 Christopher J. Portier,4 Raymond R. Tice,3 and Daniel Krewski2,5

1Department of Risk Benefit Assessment, National Food Agency, Uppsala, Sweden; 2McLaughlin Centre for Population Health Risk Assessment, University of Ottawa, Ottawa, Ontario, Canada; 3Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA; 4Department of Toxicogenomics, Maastricht University, Maastricht, Netherlands; 5Risk Sciences International, Ottawa, Ontario, Canada

BACKGROUND: The National Research Council’s vision for toxicity testing in the 21st century anticipates that points of departure (PODs) for establishing human exposure guidelines in future risk assessments will increasingly be based on in vitro high-throughput screening (HTS) data.

OBJECTIVES: The aim of this study was to compare different PODs for HTS data. Specifically, benchmark doses (BMDs) were compared to the signal-to-noise crossover dose (SNCD), which has been suggested as the lowest dose applicable as a POD.

METHODS: Hill models were fit to > 10,000 in vitro concentration–response curves, obtained for > 1,400 chemicals tested as part of the U.S. Tox21 Phase I effort. BMDs and lower confidence limits on the BMDs (BMLs) corresponding to extra effects (i.e., changes in response relative to the maximum response) of 5%, 10%, 20%, 30%, and 40% were estimated for > 8,000 curves, along with BMDs and BMLs corresponding to additional effects (i.e., absolute changes in response) of 5%, 10%, 15%, 20%, and 25%. The SNCD, defined as the dose where the ratio between the additional effect and the difference between the upper and lower bounds of the two-sided 90% confidence interval on absolute effect was 1, 0.67, and 0.5, respectively, was also calculated and compared with the BMDs.

RESULTS: The BMDL0, BMDL25, and BMDL15, defined in terms of extra effect, corresponded to the SNCD0.67, SNCD0.5, and SNCD0.35, respectively, at the median. Similarly, the BMDL25, BMDL50, and BMDL75, defined in terms of additional effect, corresponded to the SNCD1.0, SNCD0.67, and SNCD0.5, respectively, at the median.

CONCLUSIONS: The SNCD may serve as a reference level that guides the determination of standardized BMDs for risk assessment based on HTS concentration–response data. The SNCD may also have application as a POD for low-dose extrapolation.

CITATION: Sand S, Parham F, Portier CJ, Tice RR, Krewski D. 2017. Comparison of points of departure for health risk assessment based on high-throughput screening data. Environ Health Perspect 125:623–633; http://dx.doi.org/10.1289/EHP408

Introduction

The establishment of health-based guidance values is a key outcome of assessing the risk of chemical agents. The determination of such values includes the derivation of a point of departure (POD) from dose–response modeling or, more traditionally, use of the no-observed-adverse-effect-level (NOAEL). Dose–response modeling approaches, specifically the benchmark dose (BMD) method, are generally regarded by many international health organizations as the method of choice for derivation of the POD [Davis et al. 2011; European Food Safety Authority (EFSA) 2009].

For nongenotoxic agents, uncertainty factors accounting for inter- and intra-species differences are applied to the POD derived from the critical effect observed in animals or humans (Dourson et al. 1996). This results in a health-based guidance value, such as a tolerable daily intake (TDI), an acceptable daily intake (ADI), a reference dose (RfD), or a reference concentration (RfC). Although the exact formulation of the TDI/ADI [World Health Organization/International Programme on Chemical Safety (WHO/IPCS) 2004] differs to some extent from that for the RfD/RfC, these quantities are derived in essentially the same manner and can thus be interpreted similarly. The TDI/ADI/RfD is generally set for dietary exposure, whereas the RfC is generally set for occupational exposures occurring via inhalation; an extensive discussion of occupational exposure limits can be found in Deveau et al. (2015).

In the case of a genotoxic agent, the U.S. EPA risk-assessment guidelines recommend low-dose linear extrapolation when a) there are data to indicate that the dose–response curve has a linear component below the POD, or b) as a default for a tumor site where the mode of action is not established (U.S. EPA 2005). Linear extrapolation to low doses permits upper-bound estimates of risk at exposure levels of interest as well as estimation of “risk-specific doses” associated with specific (upper-bound) risk levels; the typical U.S. EPA target range for risk management is a 1/1,000,000 to 1/10,000 increased lifetime risk (U.S. EPA 2005). In contrast, both the European Food Safety Authority (EFSA) and the Joint FAO (Food and Agriculture Organization of the United Nations)/WHO Expert Committee on Food Additives (JECFA) have recommended a margin of exposure (MOE) approach rather than low-dose linear extrapolation for evaluating compounds that are both genotoxic and carcinogenic. EFSA and the JECFA considered that the MOE had the potential to help risk managers to distinguish between large, intermediate, and low health concerns, and thus to provide guidance for setting priorities for risk management actions (Barlow et al. 2006). The MOE is also cited in the U.S. EPA guidelines but is positioned as a quantity that provides an indication of the extent of extrapolation of risk estimates from the observed data to the exposure levels of interest in practice (U.S. EPA 2005).

Traditional approaches to risk assessment, including the establishment of health-based guidance values based on the results of mammalian toxicology tests, have been challenged by the U.S. National Research Council (NRC) in its report, Toxicity Testing in the 21st Century: A Vision and a Strategy (NRC 2007). This report envisages that future toxicity tests will be conducted largely in human cells or cell lines in vitro by evaluating cellular responses in a suite of toxicity pathway assays using high-throughput tests. Risk assessments would be performed based on the results of such tests, and the equivalents of today’s health-based guidance values would aim, according to the NRC,

Address correspondence to S. Sand, National Food Agency, P.O. Box 622, SE-751 26 Uppsala, Sweden. Phone: 46-18-17-5335. E-mail: Salomon.Sand@sv.se

Supplemental Material is available online (http://dx.doi.org/10.1289/EHP408).

This research was conducted in part while S.S. was a Visiting Scientist at the McLaughlin Centre for Population Health Risk Assessment at the University of Ottawa in 2014 and 2015. The work of F.P. and R.R.T. was supported in part by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences. D.K. is employed by the University of Ottawa and by Risk Sciences International, Ottawa, Canada.

The authors declare they have no actual or potential competing financial interests.

Received: 21 January 2016; Revised: 25 April 2016; Accepted: 13 June 2016; Published: 6 July 2016.

Note to readers with disabilities: EHP strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in EHP articles may not conform to 508 standards due to the complexity of the information being presented. If you need assistance accessing journal content, please contact nihdis@nih.gov.

Our staff will work with you to assess and meet your accessibility needs within 3 working days.
at representing dose levels that avoid significant perturbations of the toxicity pathways in exposed human populations. In vitro to in vivo extrapolations would rely on pharmacokinetic models to predict human blood and tissue concentrations under specific exposure conditions (Andersen and Krewski 2009; Krewski et al. 2009, 2011; NRC 2007). The NRC vision for the future of toxicity testing has recently been incorporated into the U.S. EPA’s framework for the next generation of risk science (Krewski et al. 2014).

In line with this vision, Judson et al. (2011) presented a framework for estimating the human dose at which a chemical significantly alters biological pathways in vitro, making use of in vivo assay data and an in vitro–derived pharmacokinetic model, along with information on population variability and uncertainty. Judson et al. (2011) calculated a “biological pathway altering dose” (BPAD), which they regarded as conceptually analogous to current risk-assessment metrics in that it combines dose–response data with analysis of uncertainty and population variability to arrive at conservative human exposure limits. Further discussion is needed on how a “biological significant perturbation,” and hence the BPAD, or related metric, should be defined. At a general level, in response to the NRC (2007), Crump et al. (2010) considered four possible definitions that were all regarded to incorporate the notion of an exposure threshold for apical response. At a more detailed level, this problem formulation may also concern the technical definition of the POD from a statistical standpoint, which is the focus of the present paper.

Historically, several approaches have been presented in the scientific literature on how to define the BMD and its lower confidence limit (BMDL) (Crump 1984; Murrell et al. 1998; Sand et al. 2006, 2008, 2011; Slob and Pieters 1998). In their opinion on the BMD, EFSA recommended a default setting for implementation of the BMD approach: in the case of quantal data, they recommended that the BMD by default be defined as the dose corresponding to an extra risk of 10%, and for continuous (experimental) data, they recommended that the BMD by default be defined as corresponding to a 5% change in response relative to the mean background response (EFSA 2009). The guidance provided by the U.S. EPA is similar to that issued by EFSA for quantal data, but the default approaches for continuous data differ between the two agencies (Davis et al. 2011).

Sand et al. (2011) introduced the concept of the signal-to-noise crossover dose (SNCD) as an objective approach to determine the lowest dose applicable as a POD, such that its corresponding effect is not overwhelmed by biological noise or uncertainty in the data. Specifically, the SNCD is defined as the dose at which the ratio between the additional effect (the “signal”) and the difference between the upper and lower bounds of the two-sided 90% confidence interval on absolute effect (the “noise”) correspond to some critical value (critical signal-to-noise ratios of 1, 0.67, and 0.5 are used in the present study). Sand et al. (2011) compared BMDLs and NOAELs to the SNCD, using values derived from fitting concentration–response data from the U.S. National Toxicology Program (NTP) carcinogenesis bioassay database. The NTP cancer studies represent one of the types of toxicity data that are currently used as a basis for risk assessment. Motivated by the anticipated shift towards the use of in vitro rather than whole-animal bioassay data as the basis for risk assessment, the present study extended the comparison of different BMDLs with the SNCD to the case of high-throughput in vitro screening data. Using the SNCD as a statistical reference point, this study aimed to provide insights into how low response levels in general may be associated with BMDs based on HTS data; the role of the SNCD as a starting point for low-dose extrapolation is also discussed. The analysis performed was based on >10,000 in vitro concentration–response curves generated on >1,400 compounds as part of the U.S. Tox21 Phase I effort (Tice et al. 2013).

Materials and Methods

Dose–Response Data

The Tox21 program (Tice et al. 2013) is a collaboration between U.S. federal health research agencies for the purpose of developing and applying new methods for chemical toxicity testing. Phase I of the Tox21 program tested ~2,800 chemicals, half of which were chosen by the NTP and half of which were chosen by the U.S. EPA. The chemicals were tested in >50 high-throughput screening assays. Data from the Tox21 Phase I assays include replicated data for some of the study chemicals. The present analysis in this paper does not take replication into account, that is to say, replicates were considered as separate concentration–response curves; however, an extended analysis focusing on NTP duplicates was also performed. The number of concentration–response curves used from each data set is given in Table 1.

Dose–Response Modeling and Estimation of PODs

Dose–response modeling was performed using the Hill model fit to the data by maximum likelihood, with a parametric bootstrap approach for obtaining confidence limits on the PODs derived from the fitted model. The 11,240 concentration–response curves included as a starting point in the analysis were modeled using an automated protocol developed in Matlab (The MathWorks, Inc.). The details associated with the model-fitting approach and POD estimation can be found...
is defined as a percent change in response relative to the estimated range of response. A subscript “e” is used to denote these BMDs (e.g., BMD_e, BMDL_e, BMD10_e, BMDL10_e).

• The BMD, with a two-sided 90% confidence interval, corresponding to additional effects of 5%, 10%, 15%, 20%, and 25%. The additional effect is defined as an absolute change in response compared to the estimated background response. A subscript “a” is used to denote these BMDs (e.g., BMD_a, BMDL_a, BMD10_a, BMDL10_a).

• The SNCD corresponding to signal-to-noise ratios of 1.0, 0.67, and 0.5, denoted

### Table 1. Data sets used in the analysis.

| Assay | Chemical source | Number of concentration–response curves in Classes 1 and 2* |
|-------|-----------------|---------------------------------------------------------|
| Human estrogen receptor antagonist | EPA 289 | 144 |
| Human estrogen receptor agonist | EPA 230 | 290 |
| Human estrogen receptor agonist | EPA 199 | 159 |
| Human estrogen receptor agonist | EPA 154 | 206 |
| Human estrogen receptor agonist | EPA 106 | 106 |
| Human estrogen receptor agonist | EPA 159 | 159 |
| Human estrogen receptor agonist | EPA 337 | 337 |
| Human estrogen receptor agonist | EPA 245 | 245 |
| Human estrogen receptor agonist | EPA 41 | 41 |
| Human estrogen receptor agonist | EPA 98 | 98 |
| Human estrogen receptor agonist | EPA 24 | 24 |
| Human estrogen receptor agonist | EPA 120 | 120 |
| Human estrogen receptor agonist | EPA 146 | 146 |
| Human estrogen receptor agonist | EPA 367 | 367 |
| Human estrogen receptor agonist | EPA 86 | 86 |
| Human estrogen receptor agonist | EPA 157 | 157 |
| Human estrogen receptor agonist | EPA 139 | 139 |
| Human estrogen receptor agonist | EPA 19 | 19 |
| Human estrogen receptor agonist | EPA 211 | 211 |
| Human estrogen receptor agonist | EPA 14 | 14 |
| Human estrogen receptor agonist | EPA 189 | 189 |
| Human estrogen receptor agonist | EPA 13 | 13 |
| Human estrogen receptor agonist | EPA 227 | 227 |
| Human estrogen receptor agonist | EPA 237 | 237 |
| Human estrogen receptor agonist | EPA 16 | 16 |
| Human estrogen receptor agonist | EPA 31 | 31 |
| Human estrogen receptor agonist | EPA 77 | 77 |
| Human estrogen receptor agonist | EPA 232 | 232 |
| Human estrogen receptor agonist | EPA 110 | 110 |
| Human estrogen receptor agonist | EPA 246 | 246 |
| Human estrogen receptor agonist | EPA 192 | 192 |

Notes: EPA, U.S. Environmental Protection Agency; NA, not available on PubChem; NTP, National Toxicology Program.

*Each concentration–response curve has a curve classification, based on the fit of a Hill equation to the curve (Xia et al. 2011; Huang et al. 2011). For this analysis, only curves in classes 1 and 2 ("complete response curve" and "incomplete curve," respectively) were used because the other curve classes indicate the lack of a concentration response or show significant activity only at the highest concentration and are therefore problematic for the purpose of fitting a sigmoidal (four-parameter) model such as the Hill model.
by SNCD$_{1.0}$, SNCD$_{0.67}$, and SNCD$_{0.5}$, respectively. The point estimate, as well as
the upper 95th confidence bound, for the
effect (under both the additional and extra
effect definitions) at concentrations corre-
sponding to each of the three SNCDs was
also derived.

The three types of POD approaches
(BMD$_e$, BMD$_a$, and SNCD) are illustrated
in Figure 1. Additionally, a discussion of
the BMD and SNCD definitions, including
why the applied BMD definitions were preferred
over the definition suggested for contin-
uous data by EFSA (2009), is provided in
"Definition of the SNCD and the BMD" in
the Supplemental Material.

Comparison of PODs

BMDLs were compared to the SNCD
(specifically, SNCD$_{1.0}$, SNCD$_{0.67}$, and
SNCD$_{0.5}$). These comparisons were based
on curves for which all estimated BMDs
and SNCDs (in total, 10 BMDs and 3
SNCDs) were within the experimental
concentration range ($n = 8,961$). In addition,
results associated with nonsignificant
concentration–response curves ($n = 192$) and
curves for which the estimated maximum
response was $> 150$ or $< -150$ ($n = 313 addi-
tional curves) were excluded. These combined
criteria reduced the 11,240 curves by 25%
to 8,456 curves for inclusion in the present
study. As noted previously, details of the
model-fitting approach and POD estimation
can be found in “Concentration–response
modeling and estimation of PODs” in the
Supplemental Material.

Results

**BMDLs Based on Extra Effect versus the SNCD**

Considering all curves selected for inclu-
sion ($n = 8,456$), the BMDL$_{10e}$
calibrated
to the SNCD$_{1.0}$ at the median (Figure 2A).
A concentration between the BMDL$_{20e}$
and the BMDL$_{30e}$ corresponded to the SNCD$_{1.0}$
for stress response assays; the BMDL$_{30e}$
calibrated
to the SNCD$_{1.0}$ for cytotoxicity assays;
and all BMDLs were below the SNCD$_{1.0}$
at the median for nuclear receptor assays
(Figure 2A).

A concentration level between the
BMDL$_{20e}$ and the BMDL$_{30e}$ corresponded
to the SNCD$_{0.67}$ at the median, across all
$n = 8,456$ curves (Figure 2B). A concentra-
tion between the BMDL$_{10e}$ and the BMDL$_{20e}$
corresponded to the SNCD$_{0.67}$ for stress
response assays; the BMDL$_{20e}$ calibrated
to the SNCD$_{0.67}$ for cytotoxicity assays; and
a concentration between the BMDL$_{30e}$ and the
BMDL$_{40e}$ corresponded to the SNCD$_{0.67}$
for nuclear receptor assays (Figure 2B).

Histograms for the ratios BMDL:SNCD$_{0.67}$

Figure 1. Illustration of the three types of point-of-departure (POD) approaches considered in the study.
Nuclear receptor assay concentration response data on pimozide is used as an example (solid circles).
The Hill model has been fitted to the data: in all three cases, the solid curves that describe the mean
response are the same, but the two-sided 90% confidence intervals around the mean response (the dotted
curves) depend on the POD approach considered. (A) The benchmark dose (BMD) associated with a 10% extra
effect (BMD$_{10e}$) is 0.24 units (solid red vertical line), and the lower 5th and upper 95th confidence
limits (vertical dotted lines) are 0.15 (BMDL$_{10e}$) and 0.37 units, respectively. (B) The BMD associated with a
10% additional effect (BMD$_{10a}$) is 0.28 units (solid red vertical line), and the lower 5th and upper 95th confi-
dence limits (vertical dotted lines) are 0.18 (BMDL$_{10a}$) and 0.42 units, respectively. (C) The SNCD$_{1.0}$ associated
with a signal-to-noise ratio (SNR) of 1.0 is 0.31 units (solid red vertical line). The difference between
the lower and upper bounds on absolute effect at the SNCD is $\approx 10.4 - (-1.2) = 11.6$ (difference between the
horizontal dotted lines). Because the SNR is 1.0, this approximates to the point estimate of additional
effect at the signal-to-noise crossover dose (SNCD), which is $\approx 4.6 - (-7.0) = 11.6$ (difference between the
horizontal solid line and the background response according to the fitted model). In this example, SNCD$_{1.0}$
is approximately twice the size of the BMDLs.
with medians closest to 1 are shown in Figure 3 (considering all \( n = 8,456 \) curves).

At the median, the BMDL_{20e} was closest to the SNCD_{0.5} when all 8,456 curves were considered (Figure 2C). The BMDL_{10e} calibrated to the SNCD_{0.5} for stress response assays; the BMDL_{10e} was closest to the SNCD_{0.5} for cytotoxicity assays; and a concentration between the BMDL_{20a} and the BMDL_{30a} corresponded to the SNCD_{0.5} for nuclear receptor assays (Figure 2C).

**BMDLs Based on Additional Effect versus the SNCD**

Considering all included curves (\( n = 8,456 \)), the BMDL_{15a} calibrated to the SNCD_{1.0} at the median (Figure 4A). The BMDL_{15a} calibrated to the SNCD_{1.0} for stress response assays; a concentration between the BMDL_{20a} and the BMDL_{25a} corresponded to the SNCD_{1.0} for cytotoxicity assays; and all BMDLs were below the SNCD_{1.0} at the median for nuclear receptor assays (Figure 4A).

At the median, the SNCD_{0.67} lay between the BMDL_{15a} and the BMDL_{20a} for all curves (\( n = 8,456 \)) (Figure 4B). The BMDL_{10a} was closest to the SNCD_{0.67} for stress response assays; the BMDL_{15a} calibrated to the SNCD_{0.67} for cytotoxicity assays; and a concentration between the BMDL_{20a} and the BMDL_{25a} corresponded to the SNCD_{0.67} for nuclear receptor assays (Figure 4B). Histograms for the ratios BMD:SNCD_{0.67} with medians closest to 1 are shown in Figure 5 (considering all \( n = 8,456 \) curves).

At the median, the SNCD_{0.5} lay between the BMDL_{10a} and the BMDL_{15a} when all curves (\( n = 8,456 \)) were considered (Figure 4C). The BMDL_{0.5a} was closest to the SNCD_{0.5} for stress response assays; the BMDL_{10a} approximated to the SNCD_{0.5} for cytotoxicity assays; and a concentration between the BMDL_{15a} and the BMDL_{20a} corresponded to the SNCD_{0.5} for nuclear receptor assays (Figure 4C).

**Effect at the SNCD**

Figures 6 and 7 show the medians, as well as the lower 5th and upper 95th percentiles, for the extra and additional effects at the SNCD, respectively, using all included curves (\( n = 8,456 \)) as the basis. These results indicate that the SNCD_{1.0}, SNCD_{0.67}, and SNCD_{0.5} corresponded to a median upper bound on the extra effect of 40% (corresponding to the BMDL_{40e}), 25% (corresponding to a concentration between the BMDL_{20e} and BMDL_{30e}), and 18% (corresponding approximately to the BMDL_{20e}) respectively (Figure 6). Similar results in Figure 7 show that the SNCD_{1.0}, SNCD_{0.67}, and SNCD_{0.5} corresponded to a median upper bound of the additional effect of 25% (corresponding to the BMDL_{25a}), 17% (corresponding to Figure 2. Ratios of the BMDL_e to the SNCD with BMDLs defined in terms of extra effects of 5%, 10%, 20%, 30%, and 40%. Ratios are given in terms of medians (solid circles) and intervals describing the lower 5th and upper 95th percentiles, based on different stratifications of the data. Red (large) circles correspond to results based on all selected curves (\( n = 8,456 \)); blue circles correspond to results based on cytotoxicity assays (\( n = 8,456 \)); green circles correspond to results based on nuclear receptor assays (\( n = 8,456 \)); and cyan circles are results based on stress response assays (\( n = 723 \)). (A) Ratios of the BMDL_e to the SNCD_{1.0}. (B) Ratios of the BMDL_e to the SNCD_{0.67}. (C) Ratios of the BMDL_e to the SNCD_{0.5}. BMDL, lower confidence limit of the benchmark dose; SNCD, signal-to-noise crossover dose.
a concentration between the BMDL$_{15a}$ and the BMDL$_{20a}$, and 13% (corresponding to a concentration between the BMDL$_{10a}$ and the BMDL$_{15a}$), respectively. The results illustrated in Figures 6 and 7 are consistent with those presented in Figures 2–5.

Analysis of NTP Duplicates

Chemicals tested in duplicate on the NTP assay plates were analyzed separately to investigate the stability of estimated quantities across duplicates, as well as the result of merging duplicates. Considering curves in classes 1 and 2 (“complete response curve” and “incomplete curve,” respectively), on which the overall analysis is based, 320 duplicates were identified (i.e., 640 individual curves). At the median, the BMDL differed between these duplicates by a factor of 1.6–2.2 for BMDLs defined in terms of extra effect and a factor of 1.6–2.0 for BMDLs defined in terms of additional effect: the differences decreased with increasing BMR (Table 2). At the median, the SNCD differed between duplicates by a factor of 1.7–1.8, depending on the SNR (Table 2). It may be noted that the upper 95th percentile of the BMDL ratio across duplicates was very high at low BMRs, ranging between 100 and 600 depending on the BMR. For other BMDLs, the upper 95th percentile of the ratio of difference between duplicates was large for a portion of the curves, particularly for BMDLs corresponding to low BMRs (see the upper 95th percentile of the difference between duplicates in Table 2). As shown by Sand et al. (2011), the SNCD decreases with increasing sample size because larger sample size permits the detection of smaller and smaller effects. This phenomenon was, however, not observed in the analysis of the NTP duplicates, possibly because the increase in sample size obtained by merging duplicates was too small (a factor of only 2). The dependence of the SNCD or the BMDL on sample size is typically evaluated theoretically assuming that no (or only a minimal) effect in the mean response occurs: the only effect considered is the effect of more or fewer data for a curve of the same mean response. The analyses in the present paper indicated that the difference between duplicates with respect to the mean response curve appeared to be larger, by a factor in the range of 2, than the change in SNCD that was obtained by merging duplicates: the SNCD based on the analysis of merged duplicates approximated

Discussion

In this article, we compared two points of departure—the traditional BMDL and the recently proposed SNCD—applied to > 8,000 high-throughput experimental concentration–response curves generated during Tox21 Phase I (Tice et al. 2013). The results from these comparisons showed that the BMDL$_{40}$, BMDL$_{25}$, and BMDL$_{18}$, defined in terms of extra effect, correspond to the SNCD$_{1.0}$, SNCD$_{0.67}$, and SNCD$_{0.5}$, respectively, at the median (Figure 6). Similarly, the BMDL$_{25}$, BMDL$_{17}$, and BMDL$_{13}$, defined in terms of additional effect, correspond to the SNCD$_{1.0}$, SNCD$_{0.67}$, and SNCD$_{0.5}$, respectively, at the median (Figure 7).

Separate analysis of NTP duplicates showed that the difference in BMDLs and SNCDs between duplicates was generally within a factor of 2 at the median (Table 2). However, the difference between duplicates was large for a portion of the curves, particularly for BMDLs corresponding to low BMRs (see the upper 95th percentile of the difference between duplicates in Table 2). As shown by Sand et al. (2011), the SNCD decreases with increasing sample size because larger sample size permits the detection of smaller and smaller effects. This phenomenon was, however, not observed in the analysis of the NTP duplicates, possibly because the increase in sample size obtained by merging duplicates was too small (a factor of only 2). The dependence of the SNCD or the BMDL on sample size is typically evaluated theoretically assuming that no (or only a minimal) effect in the mean response occurs: the only effect considered is the effect of more or fewer data for a curve of the same mean response. The analyses in the present paper indicated that the difference between duplicates with respect to the mean response curve appeared to be larger, by a factor in the range of 2, than the change in SNCD that was obtained by merging duplicates: the SNCD based on the analysis of merged duplicates approximated
the geometric mean of the SNCD associated with separate analysis of duplicates (Table 2).

The findings in this paper depended on the study designs used in the database, which comprised 13–16 concentrations (sometimes fewer after removing outliers) with one observation at each concentration level. SNCDs corresponding to three different SNRs (1, 0.67, and 0.5) were considered. How stringent to be with regard to the selection of the critical SNR that defines the SNCD is a point for discussion even though a critical SNR = 1 may intuitively appear to be most straightforward (“signal” equals “noise”). However, even using the least-stringent criteria (in terms of level of “noise” allowed) corresponding to an SNR of 0.5, BMDLs corresponding to responses in the range of 10% or below appear to be associated with high uncertainty using the SNCD as a reference (Figures 6 and 7). Similarly, in Figures 2 and 4, it can be noted that the BMDL_{10} is generally below the SNCDs at the median. The analysis of NTP duplicates from Tox21 Phase I also indicated that at least these HTS data could be very uncertain with respect to estimation of BMDLs corresponding to BRMs of 10% or below because such quantities could differ substantially between individual duplicates (Table 2).

For the NTP cancer bioassay data analyzed by Sand et al. (2011), the BMDL_{18} and BMDL_{7.3}, defined in terms of extra risk, corresponded to the SNCD_{1.0} and SNCD_{0.67}, respectively, at the median. The corresponding BMDLs in the present study would be the BMDL_{40} and BMDL_{25}, based on the extra-effect definition of the BMDL. There are several factors that may explain why the SNCD corresponded to higher BMDLs in the present study than those in the study by Sand et al. (2011). First, the data used in the present analysis were continuous in nature, complicating the ability to make a direct comparison between the two studies. In addition, a four-parameter model was used in the present study, whereas three- and two-parameter Hill models were used by Sand et al. (2011). The higher level of complexity of the four-parameter Hill model would be expected to result in wider confidence intervals, pushing the SNCD upwards. Furthermore, the SNCD is affected by sample size: whereas the NTP curves evaluated by Sand et al. (2011) typically included 200 observations (four dose groups, including the control, with 50 animals per group), the curves in the present analysis typically included only 13–16 observations (based on 1 observation per concentration). Moreover, a bootstrap approach was used in the present study for confidence interval estimation, whereas the profile likelihood method was used by Sand et al. (2011). In contrast to the analysis by Sand et al. (2011), the present analysis adjusted the estimate of variance (the likelihood

![Figure 4](image-url)

**Figure 4.** Ratios of the BMDL to the SNCD with BMDLs defined in terms of additional effects of 5%, 10%, 15%, 20%, and 25%. Ratios are given in terms of medians (solid circles) and intervals describing the lower 5th and upper 95th percentiles, based on different stratifications of the data. Red (large) circles correspond to results based on all selected curves (n = 8,456); blue circles correspond to results based on cytotoxicity assays (n = 3,130); yellow circles correspond to results based on nuclear receptor assays (n = 4,803); and cyan circles are results based on stress response assays (n = 723). (A) Ratios of the BMDL to the SNCD_{1.0}. (B) Ratios of the BMDL to the SNCD_{0.67}. (C) Ratios of the BMDL to the SNCD_{0.5}. BMDL, lower confidence limit of the benchmark dose; SNCD, signal-to-noise crossover dose.
estimator of the variance) to an unbiased estimator (see “Concentration–response modeling and estimation of PODs” in the Supplemental Material) in the process of confidence interval estimation. This adjustment increased the variance (sometimes marginal, depending on the sample size), which increased the SNCD. Additionally, for these reasons, the BMDL:SNCD ratio may be smaller under the applied bootstrap approach than under the profile likelihood method. Further analysis is needed to investigate the impact of model dependence (with respect to the mean response model) of the results associated with this analysis. The relatively large number of concentration levels (generally 13–16) will, however, constrain dose–response models such that they may not assume very different shapes (in the observable region of response). Using normalized data will tend to decrease the variance and therefore decrease the SNCD.

As an example of the use of the SNCD in a risk-assessment context, Sand et al. (2011) illustrated how an SNCD-based exposure guideline based on low-dose linear extrapolation, using the upper bound on extra risk at the SNCD as a starting point, might be calculated. The SNCD appears consistent with the definition of a POD given in the U.S. EPA (2005) cancer guidelines, which state that a POD “marks the beginning of extrapolation to lower doses.” Burgoon and Zacharewski (2008) described a POD in a way that conceptually resembles the SNCD: their POD was defined “as the point at which the upper 95% confidence limit for the vehicle response intersects the lower 95% confidence limit for the treated response based on parametric assumptions.”

The description of the SNCD and the illustration of its potential uses given by Sand et al. (2011) are statistical in nature. However, it has also been suggested that a POD derived from dose–response modeling should include a toxicological interpretation. For example, EFSA’s opinion on the BMD states that the response (benchmark response, BMR) associated with the BMD should be in the range of the data to avoid having to estimate a BMD by extrapolation. EFSA also notes that their default recommendations, which are based on calibration to the NOAEL approach, may be modified based on statistical or toxicological considerations (EFSA 2009).

Figure 6. Extra effect at the SNCD. Medians (solid circles) and intervals describing the lower 5th and upper 95th percentiles are shown based on all included curves (n = 8,456). Red circles correspond to the upper bound of the effect, and cyan circles correspond to the point estimate of the effect. SNCD, signal-to-noise crossover dose.

Figure 5. Histograms of the ratios BMDLx:SNCDx (BMDLs are based on additional effect) with medians closest to 1 based on all included curves (n = 8,456). Red circles correspond to the upper bound of the effect, and cyan circles correspond to the point estimate of the effect. BMDL, lower confidence limit of the benchmark dose; SNCD, signal-to-noise crossover dose.
Considering both statistical and biological aspects of the POD, Chiu et al. (2012) and Sand et al. (2012a) argued that the SNCD may represent a starting point for low-dose extrapolation when the upper bound on the risk (or effect) at the SNCD is greater than a “target effect level” (or BMR) established based on biological (Chiu et al. 2012; Sand et al. 2012a) or risk-management (Sand et al. 2012a) considerations. In case the SNCD is below the target effect level, the dose associated with that effect may be directly used as a POD (Chiu et al. 2012).

According to the NRC (2007) vision for the future of toxicity testing, increasing attention will be redirected towards determining exposure levels that avoid significant perturbations in toxicity pathways. Judson et al. (2011) introduced the concept of biological pathway activating dose (BPAD) and, as a starting point for the establishment of the BPAD, used the ToxCast™ AC_{50} values (the concentration at 50% of maximum activity) as PODs in their illustration of the BPAD concept. AC_{50} values have also been considered in other analyses of in vitro data (Burgoon and Zacharewski 2008; Thomas et al. 2012; Wetmore et al. 2012). As an alternative to using the AC_{50}, Sand et al. (2012b) suggested that the dose at which the slope of the S-shaped dose–response curve changes the most per unit log-dose, denoted BMD_{T}, may serve as a standardized reference point in the low-dose–region for in vitro data. The BMD_{T}/BMDL_{T}, which approximates the BMD_{20}/BMDL_{20} using the extra effect definition under the Hill model, was introduced by Sand et al. (2006) and was suggested as a mathematical definition of a dose within a “transition dose range,” as discussed by Slikker et al. (2004). Derivation of PODs like the BMD_{T} as well as the AC_{50} requires adequate characterization of the S-shaped concentration–response curve (including the asymptotes).

As noted in “Methods,” only curves in classes 1 and 2 were considered in this work to support modeling of the full S-shaped curve. Consequently, results from this analysis are limited in this context and do not address the issue of POD derivation for concentration–response curves that are poorly characterized. Shockley (2015) concluded that to improve nonlinear parameter estimation, optimal study designs should be developed, or alternative approaches with reliable performance characteristics should be used to describe concentration–response curves; suggestions that address the latter issue have also been proposed (Hsieh et al. 2015).

It may be questioned whether derivation of PODs for in vitro data should involve biological, policy, or risk-management considerations regarding the effect level associated with the POD. At this point, it is unclear if avoiding “significant perturbations in toxicity pathways” would imply that some (presumably small) changes in response might be allowed with regard to the suite of critical in vitro end points that would be needed to be evaluated in a future risk-assessment framework (Krewski et al. 2014). Although conceptually reasonable, the determination of BMRs representing “nonadverse” response levels, or similar, for various end points is a major challenge within the current risk-assessment approach, and, if applicable, such may also be the case for in vitro data. An even more complex issue is determination of which changes in biological effect parameters are acceptable in the case of end points that are not adverse and are not the critical effect or its known and immediate precursor. Issues related to this point have also been discussed by Crump et al. (2010) and Sand et al. (2012b).

![Figure 7. Additional effect at the SNCD. Medians (solid circles) and intervals describing the lower 5th and upper 95th percentiles are based on all included curves (n = 8,456). Red circles correspond to the upper bound of the effect, and cyan circles correspond to the point estimate of the effect. BMDL, lower confidence limit of the benchmark dose; SNCD, signal-to-noise crossover dose.](image-url)

### Table 2. Comparison of BMDLs and SNCDs for NTP duplicates.

| Type of comparison | Quantity | Median | 5th percentile | 95th percentile | \( \chi^2 \) |
|--------------------|----------|--------|----------------|----------------|----------|
| BMDL ratio between duplicates | BMDL_{0.5e} | 2.2 | 1.0 | 625 | — |
| (extra effect) | BMDL_{0.1e} | 1.9 | 1.0 | 140 | — |
| BMDL_{0.2e} | 1.7 | 1.0 | 43 | — |
| BMDL_{0.3e} | 1.6 | 1.0 | 26 | — |
| BMDL_{0.4e} | 1.6 | 1.0 | 17 | — |
| BMDL ratio between duplicates | BMDL_{0.5a} | 2.0 | 1.0 | 455 | — |
| (additional effect) | BMDL_{1.0a} | 1.7 | 1.0 | 104 | — |
| BMDL_{1.5a} | 1.6 | 1.0 | 51 | — |
| BMDL_{2.0a} | 1.6 | 1.0 | 32 | — |
| BMDL_{2.5a} | 1.6 | 1.0 | 29 | — |
| BMDL_{3.0a} | 1.6 | 1.0 | 26 | — |
| BMDL_{3.5a} | 1.6 | 1.0 | 17 | — |
| BMDL_{4.0a} | 1.6 | 1.0 | 14 | — |

| SNCD ratio between duplicates | SNCD_{1.0} | 1.7 | 1.0 | 29 | — |
| SNCD_{0.67} | 1.7 | 1.0 | 28 | — |
| SNCD_{0.5} | 1.8 | 1.0 | 35 | — |

| SNCD_{0.0} | 1.0 | 0.45 | 3.1 | 0.58 |
| SNCD_{0.67} | 1.1 | 0.47 | 3.0 | 0.62 |
| SNCD_{0.5} | 1.1 | 0.44 | 3.1 | 0.63 |

Notes: BMDL, lower confidence limit of the benchmark dose; GM, geometric mean; NTP, National Toxicology Program; POD, point of departure; SNCD, signal-to-noise crossover dose.

The analysis is based on 307 duplicates (614 individual curves). There are a total of 320 NTP duplicates with curves in classes 1 and 2; that is, 320–307 = 13 curves have been excluded from this analysis because they did not show a concentration–response trend according to criteria described in “Concentration–response modeling and estimation of PODs” in the Supplemental Material. The BMDL ratios have been calculated such that they are always > 1 (max value/ min value).

\(^a\)Ratio of extra effect BMDLs between duplicates.

\(^b\)Ratio of additional effect BMDLs between duplicates.

\(^c\)Ratio of SNCDs between duplicates.

\(^d\)Ratio of the geometric mean of the SNCD between duplicates (SNCD_{duplicate GM}) and the corresponding SNCD resulting from analysis of merged duplicates (SNCD_{merged}).

\(^e\)Fraction of curves for which the ratio is > 1.
It is likely that derivation of PODs from in vitro high-throughput screening data will need to rely on standardized approaches, at least as a starting point. Because the use of in vitro data significantly increases the amount of concentration–response data that needs to be processed, the use of standardized modeling protocols, including standardized PODs, may be of importance, at least from a practical point of view. Wignall et al. (2014) recently discussed the use of a standardized protocol for BMD analysis that was argued to provide greater transparency and efficiency than current approaches. Their approach was illustrated for traditional animal toxicity data, but the relevance of this type of approach was also suggested to be of particular value in the case of high-throughput in vitro testing (Wignall et al. 2014). Thomas et al. (2013) noted that more efficient risk-assessment approaches are needed owing to the fact that the number of chemicals without toxicity reference values combined with the rate of new chemical development is overwhelming the capacity of the traditional risk-assessment approach. Interestingly, the results of their studies of comparing transcriptional BMD values for the most sensitive pathway with BMD values for the noncancer and cancer apical end points showed a high degree of correlation, suggesting that (for their studied chemicals) transcriptional perturbation did not occur at significantly lower doses than apical responses (Thomas et al. 2013).

The SNCD may provide a reference level for determining how low a standardized BMD, BMDL, or similar (potency-based) quantity may be selected. For example, in risk-assessment applications where BMDs are derived for several chemicals or end points, a default or screening POD may be chosen such that it is generally not below the SNCD. Based on the present analysis, such a screening level may be lower than the commonly used AC50 discussed above, because the AC50 (i.e., the BMDL50) is higher than all SNCDs at the median (Figures 6 and 7). Considering the range of SNCDs evaluated, the BMDL20 may be more appropriate as a standardized POD in this context (in terms of extra effect, the BMDL20 corresponds to a concentration between the SNCD0.5 and the SNCD0.67 at the median; in terms of additional effect, the BMDL20 corresponds to a concentration between SNCD0.67 and SNCD0.5 at the median) (Figures 6 and 7). As noted previously, BMDLs associated with BMRs < 10% generally appear to not be supported from a statistical point of view when using the SNCD as a reference (Figures 6 and 7). BMRs < 10% may, however, be supported for individual curves when using the SNCD as a reference. The SNCD concept may also be used as a starting point for low-dose extrapolation in establishing exposure guidelines corresponding to a given target risk (Chiu et al. 2012; Sand et al. 2011, 2012a) using empirical models of a linear or nonlinear nature. This approach may also be viewed as the application of a curve–specific uncertainty factor to the SNCD, which depends on the risk/effect at the SNCD and the empirical extrapolation model used (Sand et al. 2011). It may be noted that, if the dose–response is sublinear, the risk estimate by the SNCD generally decreases as the sample size increases, as discussed by Sand et al. (2011). Increasing sample size lowers the SNCD, and under a linear extrapolation approach (drawing a straight line between the upper bound of risk/effect at the SNCD and the background response), the dose corresponding to a given target risk/effect then increases (less conservative) because the slope of the linear model becomes smaller. Although this approach may be appropriate for severe apical end points, the circumstances under which an approach involving low-dose extrapolation would be required in risk assessments based on in vitro data remain to be seen.

Conclusion

The NRC vision for the future of toxicity testing suggests that PODs for risk assessments may be increasingly based on in vitro HTS data, a notion that has been incorporated into the U.S. EPA’s framework for the next generation of risk science. The technical definition of a POD derived from dose–response modeling has stimulated significant discussion within the current risk-assessment paradigm; the present study has extended this discussion to the case of HTS data using a large database comprising HTS experimental concentration–response curves generated during Tox21 Phase I. How the POD for HTS data should be designed to support future risk-assessment applications warrants further discussion. Although end point–specific definitions of the BMD, based on judgment applied on a case-by-case basis, are conceptually appropriate, they may be problematic in practice given the vast amount of data that will be generated through the greatly expanded application of robotically mediated high-throughput in vitro testing. Such rich data may require the use of standardized procedures and PODs for practical application and meaningful interpretation. The SNCD may provide a reference level that guides the determination of standardized BMDs, or similar potency-based measures, such that they are not subject to excessive uncertainty. Based on the present database, comprising > 8,000 HTS curves, such BMDs and BMDLs may need to be associated with a response higher than the standard responses of 5% or 10%. The SNCD may also be of potential use as a starting point for low-dose extrapolation in the process of establishing safe exposure limits.

References

Andersen ME, Krewski D. 2009. Toxicity testing in the 21st century: bringing the vision to life. Toxicol Sci 107:324–330.

Barlow S, Renwick AG, Kleiner J, Bridges JW, Busk L, Dybing E, et al. 2006. Risk assessment of substances that are both genotoxic and carcinogenic report of an international conference organized by EFSA and WHO with support of ILSI Europe. Food Chem Toxicol 44:1636–1650.

Burgoo LD, Zacharewski TR. 2008. Automated quantitative dose–response modeling and point of departure determination for large toxicogenomic and high-throughput screening data sets. Toxicol Sci 104:412–421.

Chiu WA, Guyton KZ, Hogan K, Jinot J. 2012. Approaches to human health risk assessment based on the signal-to-noise crossover dose. Environ Health Perspect 120:A264, doi: 10.1289/ehp.1205212.

Crump KS. 1984. A new method for determining allowable daily intakes. Fundam Appl Toxicol 4:854–871.

Crump KS, Chen C, Louis TA. 2010. The future use of in vitro data in risk assessment to set human exposure standards: challenging problems and familiar solutions. Environ Health Perspect 118:1350–1354, doi: 10.1289/ehp.1001931.

Davis JA, Gift JS, Zhao QJ. 2011. Introduction to benchmark dose methods and U.S. EPA’s bench-mark dose software (BMDS) version 2.1.1. Toxicol Appl Pharmacol 254:181–191.

Deveau M, Chen CP, Johanson G, Krewski D, Maier A, Niven KJ, et al. 2015. The global landscape of occupational exposure limits—implementation of harmonization principles to guide limit selection. J Occup Environ Hyg 12(1):S127–S144.

Dourson M, Felter S, Robinson D. 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. Regul Toxicol Pharmacol 24(2 pt 1):108–120.

EFSA (European Food Safety Authority). 2009. Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. EFSA J 1150:1–72.

Hsieh JH, Sedykh A, Huang R, Xia M, Tice RR. 2015. A data analysis pipeline accounting for artifacts in Tox21 quantitative high-throughput screening assays. J Biomol Screen 20:867–897.

Huang R, Xia M, Cha MJ, Guanamura S, Shinn P, Houck KA, et al. 2011. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect 119:1142–1148, doi: 10.1289/ehp.1002952.

Inglese J, Auld DS, Jadhav A, Johnson RL, Simeonov A, Yasar A, et al. 2006. Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries. Proc Natl Acad Sci U S A 103:11473–11478.

Judson RS, Kavlock RJ, Setzer RW, Hubal EA, Martin MT, Knudsen TB, et al. 2011. Estimating toxicity-related biological pathway altering doses for high-throughput chemical risk assessment. Chem Res Toxicol 24:451–462.

Krewski D, Andersen ME, Manton E, Zeise L. 2009. Toxicity Testing in the 21st Century: implications for human health risk assessment. Risk Anal 29:474–479.

Krewski D, Westphal M, Al-Zoughool M, Croteau MC, Andersen ME. 2011. New directions in toxicity testing. Annu Rev Public Health 32:161–178.

Krewski D, Westphal M, Andersen ME, Paoli GM, Chiu W, Al-Zoughool H, et al. 2014. A framework for the next generation of risk science. Environ...
Comparison of points of departure

Murrell JA, Portier CJ, Morris RW. 1998. Characterizing dose-response I: critical assessment of the benchmark dose concept. Risk Anal 18:13–26.

NRC (National Research Council). 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. Washington, DC: National Academies Press.

Sand S, Portier CJ, Krewski D. 2011. A signal-to-noise crossover dose as the point of departure for health risk assessment. Environ Health Perspect 119:1766–1774, doi: 10.1289/ehp.1003327.

Sand S, Portier CJ, Krewski D. 2012a. Signal-to-noise crossover dose: Sand et al. respond. Environ Health Perspect 120:A264–A265, doi: 10.1289/ehp.1205212R.

Sand S, Ringblom J, Håkansson H, Öberg M. 2012b. The point of transition on the dose-effect curve as a reference point in the evaluation of in vitro toxicity data. J Appl Toxicol 32:843–849.

Sand S, Victorin K, Filipsson AF. 2008. The current state of knowledge on the use of the benchmark dose concept in risk assessment. J Appl Toxicol 28:405–421.

Sand S, von Rosen D, Victorin K, Filipsson AF. 2006. Identification of a critical dose level for risk assessment: developments in benchmark dose analysis of continuous endpoints. Toxicol Sci 90:241–251.

Shockley KR. 2015. Quantitative high-throughput screening data analysis: challenges and recent advances. Drug Discov Today 20(3):296–300.

Slikker W, Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, et al. 2004. Dose-dependent transitions in mechanisms of toxicity. Toxicol Appl Pharmacol 201:203–225.

Slob W, Pieters MN. 1998. A probabilistic approach for deriving acceptable human intake limits and human health risks from toxicological studies: general framework. Risk Anal 18:787–798.

Thomas RS, Black MB, Li L, Healy E, Chu TM, Bao W, et al. 2012. A comprehensive statistical analysis of predicting in vivo hazard using high-throughput in vitro screening. Toxicol Sci 128:398–417.

Tice RR, Austin CP, Kavlock RJ, Bucher JR. 2013. Improving the human hazard characterization of chemicals: a Tox21 update. Environ Health Perspect 121:756–765, doi: 10.1289/ehp.1205764.

U.S. EPA (U.S. Environmental Protection Agency). 2005. Guidelines for Carcinogen Risk Assessment. Final Report. EPA/630/P-03/001F. Washington, DC: U.S. EPA, Risk Assessment Forum.

Wang Y, Xiao J, Suzek TO, Zhang J, Wang J, Zhou Z, et al. 2012. PubChem’s BioAssay Database. Nucleic Acids Res 40(database issue):D400–D412.

Wetmore BA, Wambaugh JF, Ferguson SS, Sochaski MA, Rotroff DM, Freeman K, et al. 2012. Integration of dosimetry, exposure and high-throughput screening data in chemical toxicity assessment. Toxicol Sci 125:157–174.

Wignall JA, Shapiro AJ, Wright FA, Woodruff TJ, Chiu WA, Guyton KZ, et al. 2014. Standardizing benchmark dose calculations to improve science-based decisions in human health assessments. Environ Health Perspect 122:499–505, doi: 10.1289/ehp.1307539.

WHO/IPCS (World Health Organization, International Programme on Chemical Safety). 2004. Harmonization Document No. 1. IPCS Risk Assessment Terminology. Geneva:WHO.

Xia M, Shahane S, Huang R, Titus SA, Shum E, Zhao Y, et al. 2011. Identification of quaternary ammonium compounds as potent inhibitors of hERG potassium channels. Toxicol Appl Pharmacol 252:250–258.