Received: 11 July 2022 | Revised: 28 September 2022 | Accepted: 1 November 2022

DOI: 10.1002/em.22517

Environmental and Molecular Mutagenesis | Environmental Mutagenesis and Genomics Society | WILEY

RESEARCH ARTICLE

Establishing a quantitative framework for regulatory interpretation of genetic toxicity dose–response data: Margin of exposure case study of 48 compounds with both in vivo mutagenicity and carcinogenicity dose–response data

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Funding information
Government of Canada's Chemicals Management Plan

Accepted by: S. Smith-Roe

Abstract

Quantitative relationships between carcinogenic potency and mutagenic potency have been previously examined using a benchmark dose (BMD)-based approach. We extended those analyses by using human exposure data for 48 compounds to calculate carcinogenicity-derived and genotoxicity-derived margin of exposure values (MOEs) that can be used to prioritize substances for risk management. MOEs for 16 of the 48 compounds were below 10,000, and consequently highlighted for regulatory concern. Of these, 15 were highlighted using genotoxicity-derived (micronucleus [MN] dose–response data) MOEs. A total of 13 compounds were highlighted using carcinogenicity-derived MOEs; 12 compounds were overlapping. MOEs were also calculated using transgenic rodent (TGR) mutagenicity data. For 10 of the 12 compounds examined using TGR data, the results similarly revealed that mutagenicity-derived MOEs yield regulatory decisions that correspond with those based on carcinogenicity-derived MOEs. The effect of benchmark response (BMR) on MOE determination was also examined. Reinterpretation of the analyses using a BMR of 50% indicated that four out of 15 compounds prioritized using MN-derived MOEs based on a default BMR of 5% would have been missed. The results indicate that regulatory decisions based on in vivo genotoxicity dose–response data would be consistent with those based on carcinogenicity dose–response data; in some cases, genotoxicity-based decisions would be more conservative. Going forward, and in the absence of carcinogenicity data, in vivo genotoxicity assays (MN and TGR) can be used to effectively prioritize substances for regulatory action. Routine use of the MOE approach necessitates the availability of reliable human exposure estimates, and consensus regarding appropriate BMRs for genotoxicity endpoints.

KEYWORDS
benchmark dose modeling, dose–response, genotoxicity, mutagenicity, risk assessment

1 | INTRODUCTION

Several studies have used in vivo genotoxicity BMD (benchmark dose) values derived from animal experiments to calculate a health-based guidance value (HBGV), that is, human exposure limits such as the permitted daily exposure or tolerable daily intake (Elder & Snodin, 2009; European Chemicals Agency [ECHA] Committee for Risk Assessment, 2018; Johnson et al., 2021; Muller & Gocke, 2009). The BMD, which is the interpolated dose that elicits a set fractional increase in response over background, has been described as the most robust and pragmatic PoD (point of departure) metric for risk assessment (EFSA Scientific Committee et al., 2017; Johnson et al., 2014; White et al., 2020). Use of the BMD to calculate an HBGV involves dividing a body-size adjusted BMD by the product of several UFs (uncertainty factors). Collectively, the UFs, which are also referred to as adjustment or safety factors, aim to account for animal-to-human extrapolation (i.e., interspecies adjustment), human differences in sensitivity (i.e., intraspecies adjustment), study duration, and effect severity (White et al., 2020). Other factors are sometimes used to account for, for example, database insufficiency and likely exposure of vulnerable individuals (e.g., children or elderly). As an alternative to the use of a BMD approach for calculation of an HBGV, the MOE (margin of exposure) approach has been developed. If human exposure data are available, the exposure and BMD values can be used to calculate an MOE, that is, the ratio of the BMD to known or estimated human exposure.

With respect to the BMD modeling approach more specifically, the set fractional increase above background is referred to as the benchmark response (BMR); depending on the software used, it is also known as the critical effect size. Although there is no consensus on the BMR values for in vivo genotoxicity endpoints, White et al. (2020) and Zeller et al. (2017) suggest values in the range of 34%–76%; the data presented in Slob (2017) correspond to a BMR of 71%–79% for the in vivo MN assay. These suggestions were based on analysis of historical control variability (Zeller et al., 2017) or within-group variance (i.e., variance values across all dose groups) (Slob, 2017; White et al., 2020).

The history of the MOE approach can be traced back to 1958 when the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2005), and the Food and Agriculture Organization of the United Nations, advocated use of a 100-fold "safety factor," that is, for adequate protection of public health, the ratio of a body weight (bw)-adjusted PoD (e.g., BMD) to human exposure should not be <100. The 100-fold margin of safety, later referred to as the MOE, was selected to reflect the product of 10-fold for interspecies adjustment and 10-fold for interindividual (i.e., intraspecies) adjustment. To account for inter-individual human variability in DNA repair and cell cycle control, and for dose–response extrapolation below a reference point (e.g., BMD), the composite safety factor for MOE calculation has been revised to incorporate an additional factor of 100 (European Food Safety Authority [EFSA], 2005) for substances that are both genotoxic and carcinogenic. Thus, the limit advocated for regulatory interpretation of MOEs is 10,000 (Benford, 2016; EFSA, 2005). As the second of the aforementioned safety factors of 100 can be argued to be actually covered by the first safety factor of 100 already, the basis and interpretation for the numerical value of 10,000 have not been fully agreed upon, only the numerical value itself (Barlow et al., 2006). For example, when using a $BMDL_{10}$ derived from an animal carcinogenicity assay dose–response relationship (i.e., lower confidence limit of the BMD corresponding to 10% extra risk compared with control), an MOE that is >10,000 is deemed to be "of low concern from a public health point of view and might reasonably be considered as a low priority for risk management actions" (EFSA, 2005). The MOE approach, which has been highlighted as particularly suitable for expeditious risk management, is most prominently advocated by the EFSA, and used for compounds in foods that are both genotoxic and carcinogenic. It has also been used by other regulatory authorities, for example, by the Environmental Protection Agency (U.S. EPA) for risk assessments pertaining to the herbicide glyphosate (U.S. EPA, 1997) and by WHO/FAO (e.g., JECFA, 2005). Health Canada and Environment Canada have used the MOE concept as the basis for regulatory decision-making in screening health assessments mandated by the Canadian Environmental Protection Act (Health Canada, 2008). Given the aforementioned recent developments in the field of quantitative genetic toxicology, and the fact that many chemicals of regulatory interest lack cancer bioassay data, the utility of MOE derivation from genetic toxicity dose–response data merits further investigation. Advocacy for the use of genetic toxicity data to determine an MOE for such purposes occurred as early as 1998 (Dearfield, 1998).

Recent studies by Hernandez et al. (2011) and Soeteman-Hernandez et al. (2016) examined the relationship between the $BMDL_{10}$ derived from carcinogenicity assay dose–response data and the $BMDL_{05}$ derived from the dose–response data generated using the in vivo micronucleus (MN), comet, and TGR (transgenic rodent) genotoxicity assays. More specifically, dose–response modeling of the data for 48 chemicals revealed correlations between the BMD values; indicating that in vivo MN assay-based BMDs could be used to estimate carcinogenic potency with an uncertainty factor of about 100. Although the established BMD relationship can be used to estimate the carcinogenic potency of high-priority chemicals that have not been previously examined, exploration of its regulatory utility has just started. For example, using the approach outlined in Soeteman-Hernandez et al. (2016), genetic toxicity (i.e., in vivo MN assay) dose–response data was used by the Dutch National Institute for Public Health and the Environment (RIVM, 2014) to predict the carcinogenic potency of 3-amino-2-oxazolidinone. Given that cancer bioassay data are often unavailable for compounds of potential regulatory interest, more thorough investigation regarding the use of genetic toxicity dose–response data for regulatory purposes is warranted.

The work presented here, which was conducted under the auspices of the HESI-GTTC (Health and Environmental Science Institute - Genetic Toxicology Technical Committee), builds upon Hernandez et al. (2011) and Soeteman-Hernandez et al. (2016) studies by supplementing their published BMD values with predicted exposure values, and calculating MOEs based on both carcinogenicity and genotoxicity (e.g., in vivo MN) assay BMD values. More specifically, we use human exposure estimations to calculate genotoxicity-derived and carcinogenicity-derived MOE values; and subsequently, using the HESI Risk21 tool (http://www.risk21.org; Wolf et al., 2016) interpret the MOE values in a regulatory context. This approach effectively uses both in vivo genotoxicity and carcinogenicity data to identify substances that might be deemed priorities for regulatory action and/or risk management, that is, circumstances where MOE < 10,000. The analyses were extended via the additional examination of in vivo TGR mutagenicity data; BMDs and corresponding MOE values were determined and subsequently interpreted using the Risk21 approach. The implications of the results, and opportunities to advance the development of a BMD-based framework for interpretation of in vivo genotoxicity dose–response data, are discussed.

2 | MATERIALS AND METHODS

2.1 | Categorization of compounds and BMD modeling of dose–response data

The categorization of the substances examined followed the categories from Wambaugh et al. (2014), with minor modifications based on data mining for missing compounds. For example, the designation "drug" adopted here is "drug product or related to the manufacturing of drugs" (U.S. EPA Chemicals Dashboard); compounds that belonged to this category include, for example, cyclophosphamide, chloroform, and theophylline. All BMD_{10} values, along with their lower and upper confidence limits (i.e., BMDL and BMDU) for murine carcinogenicity data, as well as those parameters for the BMD_{05} values of the MN data were obtained from the literature (Soeteman-Hernandez et al., 2016) where they were estimated using PROAST software and dose–response data from the U.S. National Toxicology Program (NTP, 2016, 2018) and the Carcinogenic Potency Database (CPDB). The subscript in BMD_{05} denotes a BMR of 5%, that is, 5% increase in MN frequency over background. The subscript in BMD_{10} denotes 10% extra cancer risk. Therefore, to study the effect of BMR on MOE, and the concomitant consequences for regulatory decision-making, a pragmatic value of 50% was also employed, in addition to 5% that is typically used as a default BMR value for continuous endpoints (EFSA Scientific Committee et al., 2017) and was thus also used previously (Soeteman-Hernandez et al., 2016), to calculate BMDs for the MN and TGR data.

In addition, BMD_{50} values for the exact same dose–response sets of MN data (see Table S1 for details); kindly provided by Dr. Wout Slob (RIVM, Bilthoven, The Netherlands, Retired), and BMD_{05} and BMD_{50} values for TGR data (described below) were derived de novo, as follows. The MN data were grouped in four batches by route of exposure (Table S1) and analyzed using combined-covariate BMD modeling (Wills et al., 2017) using the web-version of PROAST v.67.0 (https://proastweb.rivm.nl/). Analyzing these data in exposure-aligned batches avoided exposure-related BMD differences; moreover, it avoided modeling failure due to excessive computational requirements. Data sets were analyzed using both the exponential and the Hill model families, as recommended by the European Food Safety Authority (EFSA) for the analyses of continuous data (EFSA 2009). No model averaging was used. The combined-covariate analyses assumed that model parameters "c"' and "*d*" (i.e., maximum response and log-steepness after axis scaling) were constant across covariate subgroups. Data sets differing in any of the following parameters: study, compound, sex, transgene, route, duration of exposure, species, tissue, and sampling time, were labeled with a unique identifier "data set" and that was used as a covariate for parameters *a* (background response), *b* (potency), and *var* (within-group variance), as analyzed previously (Soeteman-Hernandez et al., 2016). In cases where there were multiple data sets for a single compound, the lowest BMDL value was used for the MOE calculations (see below).

TGR dose–response data for a subset of 12 compounds were obtained from the Health Canada Transgenic Rodent Assay Information Database v7.0 (TRAiD; Lambert et al., 2005; Table S2); the data were analyzed to determine BMD_{05} and BMD_{50} values. The TGR data sets were analyzed exactly as described above for the de novo analysis of the MN data, and also in four batches, corresponding to oral, dermal, inhalational, and intraperitoneal injection (*ip*) routes of exposure (see Table S2 for details).

2.2 | Collection of exposure data

Exposure data were obtained from the U.S. EPA Chemicals Dashboard (Williams et al., 2017), available at https://comptox.epa.gov/dashboard. Dashboard human exposure values are determined using a reverse pharmacokinetics approach, and multiple exposure pathways are taken into account to predict total exposures for several demographic categories. Models employed are based on a training set of 114 chemicals for which human biomonitoring data are available, for example, urinary metabolites (Wambaugh et al., 2014). The approach assumes that the biomonitoring data reflect total absorbed dose from all routes, with 100% absorption; moreover, that exposure rate is constant and individuals are at steady state. Values used for MOE calculations represent the upper 95% confidence interval (95% CI) on the estimated mean exposure rate for the "most exposed" (i.e., with the highest exposure estimates) of the 11 demographic groups within the U.S. population. In most cases, the most group predicted to be most exposed was "children aged 6–11" (Table S3). The upper 95% CI on the mean of the "least exposed" group was used as an estimate of the lower exposure range for each chemical. For five chemicals (i.e., *coc*, *mbr*, *scd*, *sel*, *tac*; see Table 1 for full names), whose predictions were unavailable from the U.S. EPA database, a conservative exposure of 0.1 mg/kg bw/d was used. This conservative exposure assumption is consistent with Ring et al. (2019) who demonstrated that humans may be exposed to such high levels (i.e., 0.1 mg/kg bw/d) for only a small fraction of existing substances (e.g., ~0.4% of

TABLE 1 Chemicals examined, with their estimated total human exposure, toxicological potency (i.e., BMD confidence intervals), and margins of exposure (MOE) for genotoxicity (i.e., MN induction) and carcinogenicity endpoints

								MOE$_{canc}$ range[c]				MOE$_{MN}$ range	
No.	Name	Abbreviations	CAS No.	Min exposure[a]	Max exposure[a]	BMDL$_{canc}$	BMDU$_{canc}$[b]	Min	Max	BMDL$_{MN}$	BMDU$_{MN}$[d]	Min	Max
1	2-acetylaminofluorene	aaf	53-96-3	9.22 E-06	1.73 E-05	2.70 E+00	3.40 E+00	1.56 E+05	3.69 E+05	1.19 E+00	4.37 E+00	6.88 E+04	4.74 E+05
2	5-Azacytidine	acd	320-67-2	8.34 E-06	2.02 E-05	2.00 E-01	4.80 E-01	**9.90 E+03**	5.76 E+04	1.01 E+01	6.05 E+01	5.00 E+05	7.25 E+06
3	Acetonitrile	ace	75-05-8	1.76 E-05	4.02 E-05	6.34 E+02	7.34 E+02	1.58 E+07	4.17 E+07	2.17 E+01	2.01 E+02	5.40 E+05	1.14 E+07
4	Allyl glycidyl ether	age	106-92-3	3.26 E-06	8.84 E-06	1.27 E+01	4.30 E+01	1.44 E+06	1.32 E+07	2.80 E+00	7.60 E+00	3.17 E+05	2.33 E+06
5	alpha-Methylstyrene	ams	98-83-9	3.01 E-04	1.53 E-03	9.88 E+01	5.05 E+02	6.46 E+04	1.68 E+06	1.04 E+02	3.03 E+02	6.82 E+04	1.01 E+06
6	3′-Azido-3′-deoxythymidine	azt	30,516-87-1	1.21 E-05	2.31 E-05	6.91 E+01	1.37 E+02	2.99 E+06	1.13 E+07	3.00 E-01	7.00 E-01	1.30 E+04	5.79 E+04
7	Benzo[*a*]pyrene	bap	50-32-8	5.12 E-05	1.26 E-04	9.00 E-01	1.30 E+00	**7.14 E+03**	2.54 E+04	1.40 E-01	3.60 E-01	**1.11 E+03**	**7.03 E+03**
8	Benzene	ben	71-43-2	3.26 E-04	2.12 E-03	1.24 E+01	1.81 E+01	**5.85 E+03**	5.55 E+04	7.00 E-02	1.80 E-01	**3.30 E+01**	**5.52 E+02**
9	1,3-Butadiene	but	106-99-0	8.00 E-07	3.51 E-04	2.33 E+01	8.05 E+01	6.63 E+04	1.01 E+08	3.64 E+00	1.18 E+01	1.04 E+04	1.48 E+07
10	Chlorambucil	cbc	305-03-3	1.02 E-05	3.19 E-05	8.00 E-02	2.50 E-01	**2.51 E+03**	2.45 E+04	5.00 E-02	1.40 E-01	**1.57 E+03**	1.37 E+04
11	Chloral hydrate	chl	302-17-0	1.41 E-06	2.80 E-06	4.97 E+01	1.98 E+02	1.78 E+07	1.41 E+08	3.81 E+01	1.07 E+02	1.36 E+07	7.60 E+07
12	Coconut oil acid diethanolamine condensate	coc	68,603-42-9	1.00 E-03	1.00 E-01	1.70 E+00	1.16 E+01	**1.70 E+01**	1.16 E+04	4.73 E+01	1.51 E+02	**4.73 E+02**	1.51 E+05
13	4-Chloro-o-phenylene-diamine	cop	95-83-0	1.46 E-06	2.80 E-06	5.22 E+02	9.60 E+02	1.86 E+08	6.58 E+08	4.24 E+00	1.32 E+01	1.51 E+06	9.05 E+06
14	Cyclophosphamide	cpa	50-18-0	4.29 E-09	1.07 E-07	3.00 E-01	1.10 E+00	2.80 E+06	2.56 E+08	2.30 E-01	8.90 E-01	2.15 E+06	2.07 E+08
15	p,p'-Dichlorodiphenyl sulfone	cps	80-07-9	4.01 E-05	1.72 E-04	3.04 E+01	6.60 E+01	1.77 E+05	1.65 E+06	1.78 E+01	8.54 E+01	1.04 E+05	2.13 E+06
16	1,2-Dibromo-3-chloropropane	dbcp	96-12-8	3.43 E-06	6.60 E-06	1.70 E+00	3.50 E+00	2.58 E+05	1.02 E+06	1.40 E+00	5.01 E+00	2.12 E+05	1.46 E+06
17	1,2-Dibromoethane	dbe	106-93-4	2.22 E-06	5.00 E-06	1.23 E+01	1.67 E+01	2.46 E+06	7.52 E+06	8.30 E-01	4.41 E+00	1.66 E+05	1.99 E+06
18	Decalin	dcn	91-17-8	2.10 E-06	4.20 E-06	3.81 E+02	1.15 E+03	9.06 E+07	5.46 E+08	4.87 E+00	2.77 E+01	1.16 E+06	1.32 E+07
19	Dimethyl hydrogen phosphite	dhp	868-85-9	1.11 E-06	3.44 E-06	1.78 E+02	2.43 E+02	5.18 E+07	2.19 E+08	2.80 E+01	1.25 E+02	8.14 E+06	1.13 E+08
20	Dimethylvinyl chloride	dmvc	513-37-1	3.72 E-06	1.24 E-05	1.49 E+01	2.21 E+01	1.20 E+06	5.94 E+06	3.01 E+00	8.88 E+00	2.43 E+05	2.39 E+06
21	1,1,1,2-Tetrachloroethane	eth	630-20-6	5.91 E-07	1.13 E-06	4.59 E+01	9.47 E+01	4.06 E+07	1.60 E+08	2.10 E+01	6.29 E+01	1.86 E+07	1.06 E+08
22	Chloroform	for	67-66-3	1.40 E-04	6.55 E-04	1.69 E+02	3.47 E+02	2.58 E+05	2.48 E+06	3.35 E+01	1.13 E+02	5.11 E+04	8.08 E+05
23	Glycidol	gly	556-52-5	3.65 E-06	7.90 E-06	2.15 E+01	3.25 E+01	2.72 E+06	8.90 E+06	1.29 E+00	5.08 E+00	1.63 E+05	1.39 E+06
24	4-Hexylresorcinol	hrc	136-77-6	7.59 E-06	1.35 E-05	7.11 E+01	1.87 E+02	5.27 E+06	2.47 E+07	1.32 E+01	2.75 E+02	9.78 E+05	3.62 E+07
25	Hydroquinone	hyd	123-31-9	1.60 E-03	3.83 E-03	1.94 E+01	6.31 E+01	**5.07 E+03**	3.94 E+04	2.80 E+00	7.40 E+00	**7.31 E+02**	**4.63 E+03**
26	Isoeugenol	ieg	97-54-1	8.27 E-05	2.83 E-04	7.10 E+00	3.01 E+01	2.51 E+04	3.64 E+05	2.20 E+00	3.86 E+01	**7.77 E+03**	4.67 E+05
27	Isobutyl nitrite	isn	542-56-3	8.47 E-07	2.40 E-06	3.91 E+01	1.79 E+02	1.63 E+07	2.11 E+08	5.06 E+01	1.54 E+02	2.11 E+07	1.82 E+08
28	Isoprene	iso	78-79-5	2.45 E-04	1.27 E-03	2.11 E+02	1.11 E+03	1.66 E+05	4.54 E+06	1.36 E+01	4.05 E+01	1.07 E+04	1.65 E+05
29	L-Ascorbic acid	las	50-81-7	9.20 E-04	2.09 E-03	8.16 E+05	8.16 E+05	3.90 E+08	8.87 E+08	6.97 E+01	2.05 E+02	3.33 E+04	2.23 E+05
30	Leucomalachite green	leu	129-73-7	1.29 E-05	3.45 E-05	2.56 E+01	1.21 E+02	7.42 E+05	9.38 E+06	3.88 E+01	2.74 E+02	1.12 E+06	2.12 E+07
31	Methyl bromide	mbr	74-83-9	1.00 E-03	1.00 E-01	3.18 E+02	4.18 E+02	**3.18 E+03**	4.18 E+05	1.03 E+01	3.32 E+01	**1.03 E+02**	3.32 E+04
32	Melphalan	mel	148-82-3	1.05 E-05	2.43 E-05	1.00 E-01	1.70 E+00	**4.12 E+03**	1.62 E+05	1.00 E-02	4.00 E-02	**4.12 E+02**	**3.81 E+03**
33	*N*-Nitroso-*N*-methylurea	mnu	684-93-5	3.78 E-06	8.80 E-06	2.60 E+00	6.30 E+00	2.95 E+05	1.67 E+06	1.00 E-01	3.00 E-01	1.14 E+04	7.94 E+04

TABLE 1 (Continued)

No.	Name	Abbreviations	CAS No.	Min exposure[a]	Max exposure[a]	$BMDL_{canc}$	$BMDU_{canc}$[b]	MOE_{canc} range[c]		$BMDL_{MN}$	$BMDU_{MN}$[d]	MOE_{MN} range	
								Min	Max			Min	Max
34	Monuron	mon	150-68-5	6.13 E-07	1.51 E-06	2.16 E+03	2.26 E+03	1.43 E+09	3.69 E+09	8.90 E+00	3.49 E+01	5.89 E+06	5.69 E+07
35	*N*-Nitrosodimethylamine	nda	62-75-9	1.55 E-05	3.61 E-05	1.80 E-02	4.80 E-02	**4.99 E+02**	**3.10 E+03**	1.00 E-01	4.00 E+00	**2.77 E+03**	2.58 E+05
36	4,4′-Oxydianiline	oxy	101-80-4	2.27 E-04	6.18 E-04	1.57 E+01	3.83 E+01	2.54 E+04	1.69 E+05	8.80 E+00	4.59 E+01	1.42 E+04	2.02 E+05
37	p-Chloraniline hydrochloride	pch	20,265-96-7	5.39 E-06	1.34 E-05	1.63 E+01	4.13 E+01	1.22 E+06	7.66 E+06	6.90 E-01	2.87 E+00	5.15 E+04	5.32 E+05
38	Propylene glycol mono-t-butyl ether	pge	57,018-52-7	1.20 E-04	3.36 E-04	1.10 E+03	3.17 E+03	3.28 E+06	2.64 E+07	1.39 E+02	5.38 E+02	4.15 E+05	4.49 E+06
39	Phenol	phe	108-95-2	3.66 E-03	1.25 E-02	1.09 E+05	1.09 E+05	8.70 E+06	2.97 E+07	1.33 E+01	4.08 E+01	**1.06 E+03**	1.11 E+04
40	Phenolphthalein	php	77-09-8	3.49 E-06	6.70 E-06	5.67 E+02	1.19 E+03	8.47 E+07	3.40 E+08	4.34 E+00	1.44 E+01	6.48 E+05	4.11 E+06
41	Resorcinol	rsc	108-46-3	2.26 E-03	3.77 E-03	1.77 E+03	1.87 E+03	4.71 E+05	8.29 E+05	1.21 E+01	4.88 E+01	**3.21 E+03**	2.16 E+04
42	Sodium dichromate dihydrate (VI)	scd	7789-12-0	1.00 E-03	1.00 E-01	3.40 E+00	6.10 E+00	**3.40 E+01**	**6.10 E+03**	5.00 E-01	1.40 E+00	**5.00 E+00**	**1.40 E+03**
43	Selenium sulfide	sel	7446-34-6	1.00 E-03	1.00 E-01	1.40 E+01	2.76 E+01	**1.40 E+02**	2.76 E+04	1.50 E+00	8.10 E+00	**1.50 E+01**	**8.10 E+03**
44	2,6-Toluene-diamine dihydrochloride	tac	15,481-70-6	1.00 E-03	1.00 E-01	1.18 E+01	1.15 E+02	**1.18 E+02**	1.15 E+05	3.50 E+00	9.40 E+00	**3.50 E+01**	**9.40 E+03**
45	Trichloroethylene	tce	79-01-6	3.49 E-04	1.51 E-03	2.31 E+02	3.78 E+02	1.53 E+05	1.08 E+06	2.91 E+02	2.60 E+03	1.93 E+05	7.45 E+06
46	Theophylline	teo	58-55-9	1.55 E-06	3.43 E-06	2.97 E+02	3.97 E+02	8.65 E+07	2.56 E+08	1.85 E+01	3.55 E+02	5.39 E+06	2.29 E+08
47	1,1,2,2-Tetrachloroethane	tet	79-34-5	1.67 E-03	4.03 E-03	2.59 E+01	3.90 E+01	**6.43 E+03**	2.34 E+04	1.06 E+01	2.57 E+01	**2.62 E+03**	1.54 E+04
48	Urethane	ure	51-79-6	3.48 E-06	6.60 E-06	7.50 E+00	1.52 E+01	1.14 E+06	4.37 E+06	2.80 E-01	7.30 E-01	4.24 E+04	2.10 E+05

Note: All BMD values are in mg/kg bw/d.

Abbreviations: BMD, benchmark dose; MOE, margin of exposurel; MN, micronucleus.

[a]Min and Max Exposures are estimated total human exposure for the demographic groups predicted to be exposed the most (Max—i.e., upper 95th percentile estimates for the most exposed group) or the least (Min—lower 95th percentile of the least exposed group). See Table S3 for specific information for each chemical.

[b]BMDU denotes upper confidence interval (CI) of the BMD. Where BMDU for carcinogenicity could not be defined (i.e., infinite), highest dose +100 units is indicated (i.e., ace, dhp, las, mon, phe, rsc, and tac). Where carcinogencity BMDU values exceeded top bioassay doses, BMDL +100 units was used in place of BMDU (i.e., mbr and teo).

[c]Min values for both carc and MN data were calculated as the ratios of BMDL to highest estimated exposure. Max values were calculated as the ratio of BMDU to lowest estimated exposure. MOE values <10,000 are shown in bold.

[d]In cases where BMDU values were undefined (i.e., infinite), the highest dose +100 dose units is indicated (i.e., for hrc, leu, and tce).

480,000 chemicals). The maximum exposure value for 1,3-butadiene was estimated using an inhalation exposure concentration of 1.0 μg/m^3 (i.e., 95th percentile population exposure level in Canada [Environment Canada & Health Canada, 2000]), and a human inhalation rate of 24.6 m^3/d (U.S. EPA, 2011). The minimum exposure value for 1,3-butadiene was set to the cumulative daily intake estimated by the FDA Office of Food Additive Safety (Williams et al., 2017). Predicted maximum human exposure for cyclophosphamide was from Environment Canada and Health Canada (2015); the lowest exposure value was estimated using the detection limit of 0.3 ng/ml from drinking water study of Zuccato et al. (2000), and a daily drinking water consumption value for a 70-kg individual of 1.5-L (Environment Canada & Health Canada, 2015).

2.3 | MOE calculation and interpretation

MOE values were calculated according to Equation (1):

$$\text{MOE} = \text{BMD}/\text{estimated human exposure}. \quad (1)$$

To estimate the lowest and highest MOE, Equations (2) and (3) we used, respectively:

$$\text{MOE} = \text{lowest compound} - \text{specific BMDL}/\text{highest estimated human exposure}; \quad (2)$$

$$\text{MOE} = \text{highest compound} - \text{specific BMDU}/\text{lowest estimated human exposure}. \quad (3)$$

The data were visualized using the HESI Risk21 Web tool. Both lower and upper confidence bounds on estimates of toxicity and exposure were calculated and displayed. For cases with an infinite upper bound confidence interval on the BMDU, the highest dose +100 was used. MOE values <10,000 would justify prioritization for public health concern and risk management. Conversely, values >10,000 would signify a lower level for public health concern.

3 | RESULTS

3.1 | Types of chemicals examined and predicted exposure levels

The 48 chemicals examined, with their abbreviations, are listed in Table 1, along with corresponding exposure, BMD, and MOE values. With respect to the types of compounds included, a large proportion of the substances (i.e., 26% out of 48% or 54%) are drugs; the remaining compounds appear in consumer products or are industrial chemicals and pesticides (Figure 1; Table S4). Not surprisingly, the members of the 48-chemical set include many well-studied carcinogens, such as benzene and benzo[*a*]pyrene. As indicated in Table 1 and Figure 1, the range of human exposure values used for the MOE calculations spans eight orders of magnitude from 6.43×10^{-8} to 0.1 mg/kg bw/d. These values are well-aligned with far larger data sets such as that described by Wambaugh et al. (2014; i.e., 8000 substances) and Ring et al. (2019; i.e., 480,000 substances). As noted, due to the absence of reliable exposure data, 5/48 chemicals examined here were assigned very conservative values of 0.1 mg/kg bw/d.

3.2 | Correspondence of MN-derived and carcinogenicity-derived MOE values

MOE values derived from carcinogenicity data for the 48 chemicals, along with their confidence limits, are shown in Table 1 and Figure 2. The MOE values for 13 substances are <10,000 (see Table 1). For two compounds, nitrosodimethylamine and sodium dichromate, the entire range of the MOE values is lower than 10,000. Table 1 also presents

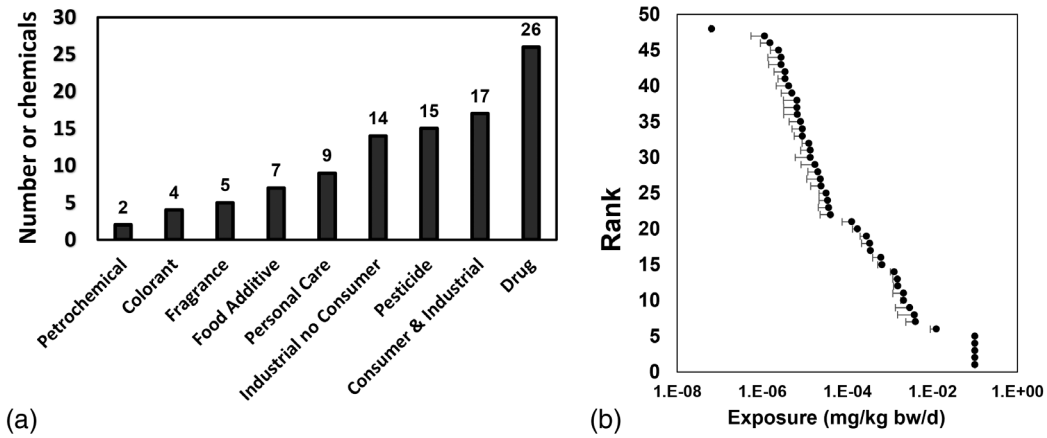

FIGURE 1 Categorization and range of estimated human exposure for the 48 substances examined. (A) substance categorization according to information from Wambaugh et al. (2014) and additional sources. A complete list of categories is presented in Table S4. (B) Ranking of estimated human exposure for the compounds examined. Each circle represents the upper 95th percentile estimates for the most exposed demographic group. Error bars represent the lower range of estimated human exposure. See text for details about compilation of estimated human exposure limits.

the genotoxicity-derived MOE values determined using the MN BMD_{05}; these results are graphically presented in Figure 3a. The MOE values for 15 chemicals (see Table 1) are <10,000, indicating greater public health concern. For seven compounds (i.e., benzo[*a*]pyrene, benzene, hydroquinone, melphalan, sodium dichromate, selenium sulfide, and 2,6-toluene-diamine dihydrochloride), the entire range of MOE values is below 10,000. The MOEs for the carcinogenicity and MN endpoints were largely overlapping (i.e., both were either below or above 10,000), except for phenol, isoeugenol, and resorcinol, for which the MOE_{MN} values are <10,000, whereas the corresponding MOE_{cancer} values are >10,000. The only compound that would be flagged for greater public health concern using the carcinogenicity-derived MOE, but not the MN-derived MOE, is 5-azacytidine, with a borderline MOE_{cancer} of 9901 and corresponding MOE_{MN} of 500,000. As a quality control step, it was determined that MOE values determined using our de novo BMD modeling with BMR of 5% (data not shown) did not differ from the values published by Soeteman-Hernandez et al. (2016); resulting MOE values were concordant.

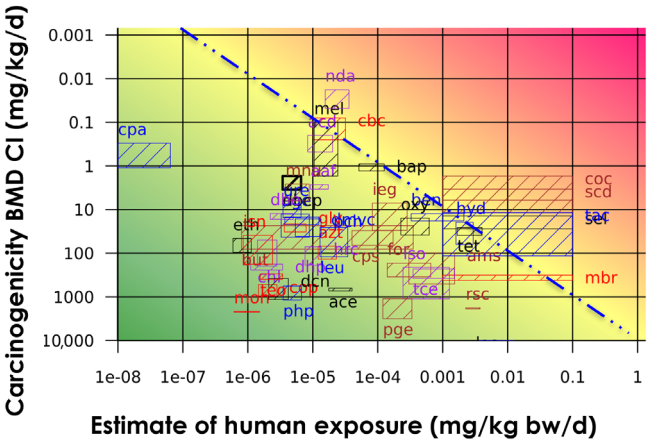

FIGURE 2 Margin of exposure values derived from carcinogenicity benchmark dose $(BMD)_{10}$ values and the corresponding human exposure estimates. The presentation graphic was prepared using the Risk21 webtool v.2.0 (http://risk21.org). The horizontal box limits (i.e., X-axis) represent the confidence intervals (CIs) of the carcinogenicity BMDs, that is, BMDL-BMDU. The vertical box limits (i.e., Y-axis) represent the lower and upper limits of the estimated human exposure. See text for details regarding the determination of BMDs and exposure values. The blue-dashed line represents an margin of exposure value of 10,000. Values <10,000 appear in the top-right red area, values >10,000 appear in the bottom-left green area. Boxes representing *phe* and *las* results are not shown; values were beyond the plot limits (see Table 1).

As mentioned above, there are currently no firmly established BMR values for in vivo genotoxicity endpoints. Although the default BMR value for continuous endpoints specified by some regulatory authorities (e.g., EFSA Scientific Committee et al., 2017) is 5%, several recent works recommend much higher values for genotoxicity endpoints. Therefore, we further compared MOE_{cancer} and MOE_{MN}, following BMD modeling of MN data using a BMR of 50% (Figure 3b). With respect to the BMD values based on a BMR of 50%, CIs for 31 chemicals did not overlap with corresponding CIs obtained using a BMR of 5% (Figure 3b and Table S5). Thus, the confidence limits of the MOE_{MN} values for five compounds (i.e., benzo[*a*]pyrene, chlorambucil, isoeugenol, nitrosodimethylamine, and tetrachloroethane) changed from <10,000 for a BMR of 5%, to >10,000 for a BMR of 50% (Table S5). More specifically, there was an increase in the MOE values, on average, by an order of magnitude (9.1-fold, SD = 7.9; see Table S5), when BMR of 50% was used instead of BMR of 5%.

3.3 | MOE values determined using TGR mutagenicity dose–response BMD values

We also investigated MOE values determined using dose–response data from the TGR mutagenicity assay. To this end, TRAID data for

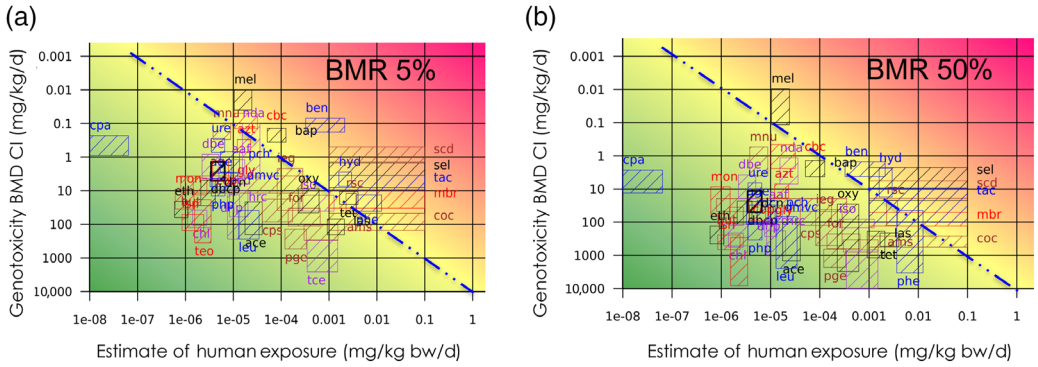

FIGURE 3 Margin of exposure values derived from genotoxicity (micronucleus) benchmark dose (BMD) values and the corresponding human exposure estimates. BMD confidence intervals (CIs) were computed for benchmark response (BMR) of 5% (a) or 50% (b). The horizontal box limits (i.e., X-axis) represent the confidence intervals (CIs) of the genotoxicity BMDs, that is, BMDL-BMDU. The vertical box limits (i.e., Y-axis) represent the lower and upper limits of the estimated human exposure. See text for details regarding the determination of BMD and exposure values. The blue-dashed line represents an margin of exposure value of 10,000. Values <10,000 appear in the top-right red area, values >10,000 appear in the bottom-left green area.

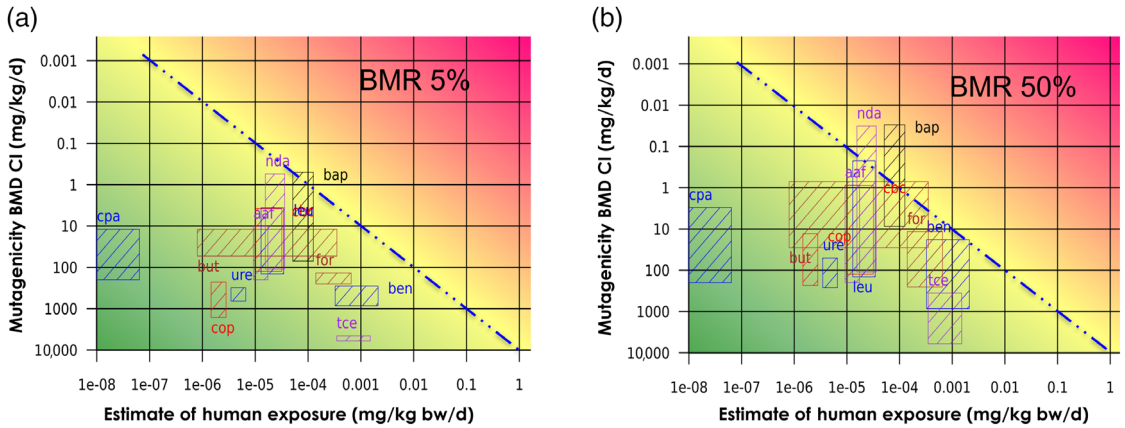

FIGURE 4 Margin of exposure values derived from mutagenicity (transgenic rodent) benchmark dose (BMD values) and the corresponding human exposure estimates. BMD CIs were computed for benchmark response (BMR) of 5% (a) or 50% (b). The horizontal box limits (i.e., X-axis) represent the confidence intervals (CIs) of the mutagenicity BMDs, that is, BMDL-BMDU. The vertical box limits (i.e., Y-axis) represent the lower and upper limits of the estimated human exposure. See text for details regarding the determination of BMD and exposure values. The blue-dashed line represents an margin of exposure value of 10,000. Values <10,000 appear in the top-right red area, values >10,000 appear in the bottom-left green area.

12 of the 48 chemicals examined were used for BMD modeling, using both the default BMR of 5%, and the alternative BMR of 50%. As shown in Figure 4a and Table S6, data analyses of the 12 chemicals examined yielded MOE values <10,000 for four chemicals (i.e., benzo[*a*]pyrene, benzene, 1,3-butadiene, nitrosodimethylamine) when a BMR of 5% was used. Similarly, four chemicals (i.e., benzo[*a*]pyrene, benzene, 1,3-butadiene, chlorambucil nitrosodimethylamine) also had MOE values <10,000 if carcinogenicity or MN data were used (Table S6). Three out of four chemicals (i.e., benzo[*a*]pyrene, benzene, 1,3-butadiene, nitrosodimethylamine) had MOEs <10,000 using all types of data (i.e., carcinogenicity, MN, and TGR). The discordant chemical, chlorambucil (*cbc*), was highlighted for regulatory concern using the MN-based and carcinogenicity-based MOEs, but not TGR-based MOE. More specifically, the carcinogenicity-based and MN-based MOE values for *cbc* were 2508 and 1567, respectively, whereas the TGR-based MOE was 28,000 (Table S6). It should be noted that the BMD modeling of TGR data for *cbc* revealed an extremely wide, essentially undefined, BMD CI (i.e., from zero to infinity; Table S7). In this particular case, the extremely wide CI may be related to a suboptimal experimental design with a single administration of several doses of *cbc* to transgenic mice via *ip* injection, followed by mutation scoring after 3, 7, or 10 days (Table S2). This design does not comply with the current test guideline (i.e., OECD TG 488), which prescribes 28-day repeat-dose treatment followed by a 3-day postexposure sampling time. As was the case for the MN data described above, increasing the BMR from 5% to 50% resulted in a significant drop in the number of compounds with an MOE < 10,000 (Figure 4b). More specifically, in the case of the TGR-derived MOE values, there were four chemicals with MOEs <10,000 when a BMR of 5% was used (i.e., benzo[*a*]pyrene, benzene, 1,3-butadiene, nitrosodimethylamine), and only one chemical, benzo[*a*]pyrene, yielded an MOE < 10,000 when a BMR of 50% was used (Table S6).

4 | DISCUSSION

The increasing recognition that genetic toxicity endpoints can be regarded as bona fide toxicological endpoints (Heflich et al., 2020) has opened the door for quantitative interpretation of genetic toxicity dose–response data for substance prioritization, risk assessment, and regulatory decision-making. For example, in the context of this work, MOE values can be determined via quantitative interpretation of genetic toxicity dose–response data to define PoD values (e.g., BMDs), and subsequent comparison of the PoDs with human exposure estimates. With respect to this work more specifically, the BMD values used by Soeteman-Hernandez et al. (2016) to quantitatively compare carcinogenic potency and genotoxic (MN) potency were combined with human exposure estimates to calculate genotoxicity-derived and carcinogenicity-derived MOE values for 48 chemicals. These analyses are a direct extension of Soeteman-Hernandez et al. (2016); permitting their work to be interpreted in a risk assessment context. Calculated MOE values were scrutinized to comparatively evaluate regulatory decisions that would be based on carcinogenicity data alone, or alternatively, on in vivo genotoxicity (MN) data alone. Moreover, we used the dose–response data underlying the Soeteman-Hernandez et al. (2016) analysis to calculate BMD values for a BMR that is now more generally regarded as appropriate for in vivo genotoxicity endpoints (i.e., 50%). We subsequently calculated and evaluated attendant MOE values. Last, we analyzed in vivo TGR mutagenicity dose–response data to comparatively evaluate TGR-derived and carcinogenicity-derived MOE values.

4.1 | Comparison of carcinogenicity-derived and genotoxicity-derived MOE values

Although the aforementioned work of Soeteman-Hernandez et al. (2016) already investigated the quantitative concordance between

MN-derived and carcinogenicity-derived potency values (i.e., BMDs), this work extended those analyses to permit interpretation of the relationship in a regulatory context. Comparisons investigated herein revealed that MOEs based on MN-derived BMDL values identified 15 compounds with MOE values <10,000; out of which 13 compounds with MOEs below 10,000 were identified using carcinogenicity-derived BMDL values. All compounds with MOE values below 10,000 would be flagged as priorities for risk management, for example, exposure restrictions to minimize the risk of adverse effect. As noted earlier, more careful examination of the results reveals lack of correspondence for only four substances. Of these, only three substances isoeugenol (*ieg*), phenol (*phe*), and resorcinol (*rsc*) would have been uniquely flagged using the MN-derived MOEs (i.e., <10,000); only one substance would have been uniquely flagged using the carcinogenicity-derived MOE, that is, 5-azacytidine (*acd*). With respect to *acd*, the result is consistent with its MOA (mode of action), which is *not* based on DNA damage, but rather inhibition of DNA methylation and aberrant gene expression (reviewed in NTP, 2016). This nongenotoxic MOA contributed to a high MN-derived BMDL, and a consequently high MOE. Apart from *acd*, all the compounds flagged using carcinogenicity-based MOE values would have also been flagged using MN-based MOE, if only the MN dose–response data were available.

With respect to the three compounds flagged as priorities using MN-derived MOEs, but not carcinogenicity-derived MOEs, that is, *ieg*, *phe*, and *rsc*, the following explanations can be provided. For *ieg*, the limits of the MN-derived MOE overlap with the limits of the carcinogenicity-derived MOE, that is, 7.7×10^3–4.7×10^5 and 2.5×10^4–3.6×10^5, respectively (Table 1). Therefore, the MOE values associated with the two endpoints cannot be statistically differentiated. Importantly, it should be noted that the administration routes employed for the MN and cancer studies that generated the dose–response data for BMD modeling (see Table 3 in Soeteman-Hernandez et al., 2016) were *ip* and oral, respectively. This discrepancy almost certainly influenced the outcome of the MOE comparisons. For example, the second discordant compound, *phe*, induced MN following *ip* injection, but not via oral administration, presumably due to detoxication in the gastrointestinal tract (reviewed in U.S. EPA, 2002). Thus, despite the fact that the MN-derived MOE for *phe* can be regarded as grounds for public health concern, it seems unlikely that analysis of dose–response data generated using oral administration would yield the same discordance with the carcinogenicity-derived MOE. Accordingly, although the regulatory action for *phe* based on the MN-derived MOE would be conservative, the comparisons of carcinogenicity-derived and MN-derived MOE conducted herein emphasizes the need to base comparative analyses on dose–response data generated using the same route of exposure, or alternatively, routes of exposure that are appropriately aligned with the MOA(s) underlying the adverse outcome under consideration (e.g., cancer, mutation, etc.). It appears that *rsc* behaves similarly to *phe* in the MN assay in rodents, yielding negative result when *rsc* was administered in rats by oral gavage (reviewed in EFSA, 2009), but positive following its *ip* injection in mice (NTP, 2018). With respect to MOA considerations more specifically, this would apply to a substance like *acd*, for which the aforementioned non-genotoxic MOA indicates that an MN-derived MOE might not be scientifically defensible. Going forward, it would be prudent to judiciously use MN-derived MOE values in situations where there are no carcinogenicity data; moreover, a paucity of information about MOA. With respect to tissue considerations, this could also be cause for concern, since the MN assay is routinely carried out using bone marrow or peripheral blood, whereas the carcinogenicity assay examines a wide range of solid tissues. Thus, although the MN-derived MOE values are well-aligned with carcinogenicity-derived MOEs, interpretation of the few observed discrepancies, some of which would lead to more conservative regulatory decisions (e.g., *ieg*, *rsc*, and *phe*), should be scrutinized by considering (a) route of exposure; (b) MOA, and/or (c) target tissue.

Although the MN assay can examine chromosomal damage in peripheral blood or bone marrow, the TGR assays can enumerate chemically induced transgene mutants in virtually any tissue, thus minimizing potential discordance with carcinogenicity test outcomes that can be related to tissue mismatch. Hence, in these instances, or when exposure to blood/bone marrow is limiting, it is reasonable to assert that the TGR mutagenicity endpoint might be more appropriate with respect to its utility for comparing genotoxicity-derived and carcinogenicity-derived MOE values in more highly exposed tissues (or sites of metabolism for protoxicants). Comparisons of MOEs derived using cancer, MN, and TGR data, for a limited subset of 12 compounds, show that TGR-derived MOE values are, for the most part, concordant with those determined using both carcinogenicity and MN dose–response data. The exceptions include the TGR-derived MOEs for chlorambucil (*cbc*) and 1,3-butadiene. With respect to the former, the TGR-derived MOE for *cbc* is 28,000, which, from a regulatory point of view, is large compared with 1567 and 2508 for the MN-derived and carcinogenicity-derived MOEs, respectively. With respect to the latter, the TGR-derived MOE for 1,3-butadiene (i.e., MOE of 2029; Table S6) is small compared with the corresponding carcinogenicity-derived and MN-derived MOEs. Thus, in comparison with the TGR assay, dose–response data in rodents for MN induction and carcinogenicity both failed to identify 1,3-butadiene, a known human carcinogen (reviewed in NTP, 2016), as a high priority for public health concern. As noted earlier, the discrepancy for *cbc* is likely related to problematic experimental design of the underlying TGR experiments, resulting in a very wide BMD CI spanning over two orders of magnitude. It could also be argued that this makes the *cbc* TGR data set unusable for regulatory decision-making, that is, as recommended in White et al., 2020 for cases where the BMDL-to-BMDU ratios are over 100. However, with respect to 1,3-butadiene, there is no obvious explanation regarding the relatively low TGR-derived MOE, which uniquely indicates a requirement for public health concern. This example clearly illustrates the utility of MOEs based on genetic toxicity endpoints, including TGR mutations, to prioritize compounds for further regulatory actions.

4.2 | Impact of BMR on calculated MOE values

The EFSA recommends a BMR of 5% as the default for quantitative interpretation of toxicological dose–response data (EFSA Scientific Committee et al., 2017); however, numerous recent works have

indicated that the value is far too low for interpretation of in vivo genotoxicity dose–response data (Slob, 2017; White et al., 2020; Zeller et al., 2017). In the light of the impact of BMR on BMD values, and the concomitant impact on MOE values, the choice of BMR is clearly important for regulatory decision-making (i.e., selection of substances for regulatory action). Although there is currently no uniform consensus on a BMR that is suitable for in vivo genotoxicity endpoints, some authors are recommending values in the 50% range (Marchetti et al., 2021; White et al., 2020). The results presented herein reveal that, with respect to MN-derived MOEs, only 10 of the 15 compounds identified as priorities using a BMR of 5% would be similarly highlighted using a BMR of 50% (i.e., MOE < 10,000). Thus, if a BMR of 50% were to be used, five compounds (i.e., *bap*, *cbc*, *ieg*, *nda*, and *tet*; see Table S5) would no longer be highlighted as priorities for regulatory action. Out of the five discordant compounds, *bap* had a borderline MOE of 11,000 when BMR of 50% was used, probably necessitating additional assessments if the MN data were the only type of data available. The discrepancy between carcinogenicity-based and MN-based MOEs for *ieg* was discussed above, that is, the compound would be missed as a target for regulatory action if only carcinogenicity data were available. The comparison of *ieg* MOE values determined using BMRs of 5% and 50% illustrates the fact that some substances may be unnecessarily prioritized if, for genotoxicity data, a BMR of 5% is used.

The validity of the BMR-related MOE discrepancies, and their regulatory importance, will depend on the suitability of the selected BMR (i.e., 50%) for the endpoint being considered (i.e., MN induction). Since there is currently no consensus regarding endpoint-specific BMR values for in vivo genotoxicity endpoints, the regulatory implications of the results discussed herein must be interpreted with caution. Research currently underway is employing large compilations of dose–response data to determine defensible, robust endpoint-specific BMR values; moreover, the work is examining the influence of experimental covariates such as route of administration, tissue, and treatment regime on the dose–response parameters employed to determine a BMR (e.g., within-group variance or *var*; see Slob, 2017).

4.3 | Potential influence of the estimates of human exposure on MOE

Since exposure estimates are critically required to calculate MOE values, it is important to consider the accuracy of the human exposure estimate values employed herein; moreover, the impact of exposure uncertainty on the regulatory concordance between calculated carcinogenicity-based and genotoxicity-based MOE values. Closer examination of the maximum exposure values presented in Table S8 reveals that exposure estimates for 14 of the 48 compounds can be considered atypically high (i.e., >1 μg/kg bw/d) relative to the estimated human exposure for the majority (95th percentile) of substances examined to date (i.e., <1 μg/kg bw/d for 480,000 substances, Ring et al., 2019). The MOE values for 10 of these 14 compounds were below 10,000 when based on MN dose–response data; 8 carcinogenicity-derived MOEs were below 10,000. Granted, for 5 of the 14 compounds where no exposure values were available, estimated exposures were arbitrarily high in order to be more conservative. Importantly, in the context of the current work, the use of a lower exposure estimate (e.g., 1 μg/kg bw/d as per; Ring et al., 2019) does not affect the overall conclusion that genotoxicity-based MOEs yield similar regulatory recommendations relative to carcinogenicity-based MOEs.

5 | CONCLUSIONS AND RECOMMENDATIONS

By documenting the regulatory alignment of MN-derived and carcinogenicity-derived MOEs, this study makes a strong case in support of using in vivo genetic toxicity dose–response data for regulatory decision-making, particularly when carcinogenicity data are lacking. With respect to the data analyzed and interpreted herein, carcinogenicity-derived MOE values would lead to regulatory prioritization of 13 compounds. The same substances, with the exception of *acd*, plus three more substances (*ieg*, *rsc*, and *phe*) yielding a total of 15, were flagged as priorities using MN-derived MOE values. Thus, if carcinogenicity dose–response data were unavailable, essentially the same set of compounds would have been prioritized, with the additional three compounds suggesting that regulatory actions based on MN-derived MOEs are conservative.

The study also accentuates the need to achieve consensus regarding BMD modeling of genetic toxicity data for regulatory decision-making. More specifically, if a BMR of 50% is employed, which is closer to the range of recently recommended values, several compounds would no longer be highlighted for regulatory action. That holds true for both MN-based and TGR-based MOE values. Once appropriate genetic toxicity BMRs have been established, analyses of genetic toxicity dose–response data could become instrumental for compound prioritization via MOE consideration.

MOE values based on TGR dose–response data for the subset of 12 compounds, revealed that TGR-based MOEs are in good agreement with carcinogenicity-based and MN-based MOEs for 10 compounds. Importantly, a TGR-based MOE would highlight 1,3-butadiene, a known human carcinogen that would not be prioritized if only rodent carcinogenicity-based and MN-based MOEs were considered. Conversely, unlike carcinogenicity-based and MN-based MOEs, a TGR-based MOE would not flag *cbc* as a priority for risk assessment. Overall, for the small subset of compounds examined, the results indicate that TGR-derived MOEs, and hence the attendant risk management concerns, are well aligned with those determined using carcinogenicity and MN dose–response data.

With respect to the regulatory use of genotoxicity-derived MOE values, that is, utilized in cases where carcinogenicity dose–response data are unavailable, the results presented herein support the use of MN-derived and/or TGR-derived MOEs to identify chemicals that should be prioritized for further scrutiny. Indeed, discrepancies between carcinogenicity-derived and genotoxicity-derived MOE

values were rare; they were attributed to differences in the route of exposure (e.g., *rsc* and *phe*), problematic experimental design (*cbc*), MOA (*acd*), or other unknown factors (1,3-butadiene). Importantly, caution is warranted with respect to selection of an appropriate, endpoint-specific BMR. Thus, to promote the use of genetic toxicity dose–response data for regulatory decision-making, it will be necessary to (a) standardize the design of in vivo genotoxicity studies, and appropriately consider the use of human-relevant route(s) of exposure, (b) establish endpoint-specific genotoxicity BMR values, and (c) conduct case studies like that described herein using data for other endpoints (e.g., *Pig-a* mutagenicity assay), and/or a wider array of compounds. Ultimately, what we demonstrate here is the practical application of the MOE approach using appropriate genetic toxicity data sets for risk assessment purposes. Broadly speaking, this illustrates how genotoxicity results can easily fit into the risk assessment paradigm and that the MOE approach is an important tool to achieve that.

AUTHOR CONTRIBUTIONS

Paul White conceived the project. Nikolai Chepelev extracted and analyzed the data and prepared the first draft of the article. Alexandra S. Long, Marc Beal, Tara Barton-Maclaren, George Johnson, Kerry L. Dearfield, Daniel J. Roberts, and Jan van Benthem provided substantial input for revision and preparation of the final version. All authors approved the final version of the article.

ACKNOWLEDGMENTS

The authors are grateful for helpful input from the Quantitative Analysis Working Group of HESI-GTTC (Health and Environmental Science Institute - Genetic Toxicology Technical Committee), Dr. Ella Atlas (Health Canada), Mr. Matthew Gagné (Health Canada), and three anonymous HESI reviewers. Mr. Matthew Gagné kindly provided guidance regarding the optimal source for predicted human exposure data. We also thank Dr. Wout Slob (RIVM) for providing MN dose–response data sets. The work was supported by Health Canada, and it was funded through the Government of Canada's Chemicals Management Plan.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Chepelev, N., Long, A.S., Beal, M., Barton-Maclaren, T., Johnson, G., Dearfield, K.L. et al. (2023) Establishing a quantitative framework for regulatory interpretation of genetic toxicity dose–response data: Margin of exposure case study of 48 compounds with both in vivo mutagenicity and carcinogenicity dose–response data. *Environmental and Molecular Mutagenesis*, 1–12. Available from: https://doi.org/10.1002/em.22517