Prediction of the reduced glutathione (GSH) reactivity of dental methacrylate monomers using NMR spectra — Relationship between toxicity and GSH reactivity

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It has been established that the toxicity of acrylate and methacrylate monomers is driven by their reactivity towards glutathione (GSH). With this relationship, the objective of this study was to predict the GSH reactivity of dental methacrylate monomers, and hence their toxicity, using the ¹³C-NMR chemical shifts of β -carbon ($\delta_{C\beta}$) and the ¹H-NMR shifts of the protons attached to β -carbon (δ_{Ha} , δ_{Hb}). The different nucleophiles were chosen to compare the different nucleophilic reactions involving acrylate and methacrylate monomers. In conjunction with the use of literature data for monomer/GSH reactivity, significant linear relationships between GSH reactivity (log K) and $\delta_{C\beta}$ or δ_{Ha} were observed ($p < 0.001$). As for the oral LD₅₀ values of some dental dimethacrylates in mice, they were estimated using linear regression curve fitting of GSH reactivity-toxicity response data. Results revealed an acceptable correlation between the oral LD₅₀ values of acrylates and methacrylates and GSH reactivity ($p < 0.05$, outlier: HEMA). In conclusion, the present findings suggested that NMR spectra might be useful for predicting the toxicity of dental methacrylates.

Keywords: Dental methacrylates, NMR spectra, Structure-activity relationships

Received Jun 9, 2009; Accepted Jul 24, 2009

INTRODUCTION

Methacrylate monomers are widely used in dentistry as denture base materials, lining materials, restorative resins, and bonding agents. However, methacrylate monomers generally do not polymerize completely in air because oxygen acts as a biradical and suppresses polymerization, leaving unreacted methacrylate groups on the resin surface¹. Consequently, unpolymerized methacrylates in dental materials play an inevitable role in causing irritation and allergic reactions in the oral cavity^{2,3}. Depending on the lipophilicity (octanol-water partition coefficient, log P) of the monomers, they predominantly reside in the cell membranes of oral tissues. Apart from the lipophilicity factor, α , β -unsaturated methacrylate esters used as resin monomers may exhibit toxicity through reaction with tissue nucleophiles *via* Michael addition⁴ (Fig. 1).

Acrylates are known to have higher electrophilic reactivity than methacrylates, and have been previously reported to have high toxicity^{5–7}. The thiol group of glutathione (GSH) is often used as a model nucleophile, whereby the reaction rates of GSH (log K) have been used to investigate the quantitative structure-activity relationships (QSAR) of acrylates and methacrylates. This is because the reactivity of acrylates and methacrylates with sulfhydryl groups such as GSH and cysteine-containing polypeptides is closely related to their toxicity^{5,6}. Besides, methacrylates with α , β -unsaturated carbonyl structures have been reported to possess allergenic properties due to their depletion of GSH⁸. In dentistry, the toxicity of triethylene glycol dimethacrylate (TEGDMA) has been shown to be related to its

reactivity with GSH^{4,9}, resulting in almost total depletion of intracellular GSH *via* Michael addition predominantly⁹.

According to spectral data, the reactivity of methacrylate monomers in polymerization and copolymerization reactions usually depends on the π -electron density on the double bond, particularly on the β -carbon. The higher the π -electron density of the β -carbon, the higher is the magnetic field where the NMR peak is observed, thus leading to a reduction in NMR chemical shift. On this premise, it becomes possible to correlate the magnitude of an NMR chemical shift with the reactivity of monomers *via* reactions such as Michael addition¹⁰. Coupled with the availability of quantitative reactivity data for GSH with α , β -unsaturated carbonyl compounds such as acrylates and methacrylates^{5–7}, the magnitudes of the NMR chemical shifts of β -carbons for methacrylate monomers may thus be used to estimate their GSH reactivity.

To clarify the mechanism of methacrylate-induced toxicity, a study¹¹ was undertaken to investigate the QSARs between the toxicity of acrylates and methacrylates against electronegativity (χ term), NMR chemical shifts of β -carbons ($\delta_{C\beta}$), and log P. Findings of that study demonstrated acceptable QSARs for both χ and log P terms¹¹. However, the QSAR between GSH reactivity and NMR spectra for dental methacrylate monomers has not been investigated.

Against this backdrop of information scarcity, this study was undertaken to predict the GSH reactivity of dental methacrylate monomers using the ¹³C-NMR chemical shifts of β -carbon ($\delta_{C\beta}$) and the ¹H-NMR chemical shifts of the protons attached to β -carbon (δ_{Ha} and δ_{Hb}). In addition, we investigated the QSAR

R₁ : H for acrylates; CH₃ for methacrylates

R₂: alcohol moiety

Fig. 1 Structures of acrylates and methacrylates.

between GSH reactivity and acute toxicity exhibited by dental methacrylate monomers. The mechanisms underlying the toxicity of dental methacrylates were then discussed in light of this study's findings.

MATERIALS AND METHODS

Monomers

The monomers used in this study were methyl acrylate (MAA), ethyl acrylate (EAA), *n*-butyl acrylate (nBAA), isobutyl acrylate (iBAA), hexyl acrylate (HAA), 2-ethylhexyl acrylate (2-EHAA), methyl methacrylate (MMA), ethyl methacrylate (EMA), isopropyl methacrylate (iPMA), *n*-butyl methacrylate (nBMA), isobutyl methacrylate (iBMA), *t*-butyl methacrylate (tBMA), allyl methacrylate (AllylMA), 2-hydroxyethyl methacrylate (HEMA), benzyl methacrylate (BZMA), ethylene glycol dimethacrylate (EGDMA), triethylene glycol dimethacrylate (TEGDMA), tetraethylene glycol dimethacrylate (tetraEGDMA), and 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl]propane (bis-GMA).

NMR spectra

The ¹³C-NMR and ¹H-NMR chemical shift data for the different acrylate and methacrylate monomers in chloroform-*d* (CDCl₃) were adopted from Hatada *et al.*¹⁰. Briefly, the chemical shifts of the selected monomers were measured in chloroform-*d* at 35°C at 125 and/or 500 MHz, using tetramethylsilane (TMS) as an internal standard, as reported. Besides, the ¹³C-NMR chemical shifts of EGDMA, TEGDMA, and bis-GMA were determined in CDCl₃ using a JEOL Alpha-500 NMR spectrometer^{11,12}, while the ¹H-NMR chemical shift data for TEGDMA and bis-GMA were adopted from Vankerckhoven *et al.*¹³.

GSH reactivity

Three sets of data for the GSH reactivity of acrylates and methacrylates under cell-free conditions were taken from Tanii and Hashimoto¹⁴, Freidig *et al.*⁶, and McCarthy *et al.*⁵. Briefly, the GSH solution was brought to 37°C and mixed with an equal volume of selected acrylates, methacrylates, or dimethacrylate also at 37°C. Optical density was measured immediately at 412 nm using a spectrophotometer. From the loss of absorbance over time, the second-order rate constants (K) were calculated⁶.

Animal testing

LD₅₀ (50% lethal dose) data for intraperitoneal injection of mice with acrylate and methacrylate monomers were taken from Lawrence *et al.*¹⁵. Briefly, male albino ICR mice weighing 25±5 g were used as test animals, and the LD₅₀ dose for each compound was calculated for 7-day mortality. Data for the acute oral LD₅₀ in male ddY mice (24–27 g) were taken from Weil¹⁶. For the LD₅₀ data of EAA¹⁷, MMA¹⁸, BZMA¹⁹, AllylMA¹⁷, nBMA²⁰, TEGDMA²¹, and bis-GMA²² to be orally administered into rats, they were taken from the material safety data sheets of the respective agents.

Multiple regression analysis

The regression equations were derived using a StatMate III software program (ATMS Co. Ltd., Tokyo, Japan).

RESULTS

Prediction of GSH reactivity using ¹³C-NMR spectra

The GSH reactivity values of acrylates and methacrylates as measured in previous studies^{5,6,14}, as well as the calculated data (in the form of log K_{1–4}), are summarized in Tables 1 to 3. For each acrylate or methacrylate monomer, their calculated data of log K₁, log K₂, log K₃, and log K₄ were obtained using Eq. (1), Eq. (2), Eq. (3), and Eq. (4) respectively (Table 4). As for Eq. (1), Eq. (2), Eq. (3), and Eq. (4), they were derived using the K values of monomers observed in previous studies^{5,6,14}.

Michael addition has been proposed as the mechanism for the reaction of thiols with the unsaturated carboxylic esters of (meth)acrylates⁷, and that the unsaturated β-carbon atom was most probably the site of attack. On this premise, the ¹³C-NMR chemical shift of β-carbon atom, δ_{Cβ}, was used as a descriptor to predict the GSH reactivity of (meth)acrylates. Table 1 shows the ¹³C-NMR chemical shifts of β-carbon and α-carbon for 18 (meth)acrylates. Further, Fig. 1 shows the structure of (meth)acrylates for the assignment of ¹H- and ¹³C-NMR spectra.

The QSARs according to the published data of GSH reactivity in terms of δ_{Cβ} were investigated, whereby the results are shown in Eqs. 1–3 (Table 4). The ¹³C-NMR chemical shifts of δ_{Cβ} and δ_{Cα} for (meth)acrylates, particularly the former, were significantly correlated with their GSH reactivity (log K_{1–3}) (*p*<0.001). An

Table 1 ^{13}C -NMR chemical shifts of beta carbon ($\delta_{\text{C}\beta}$), alpha carbon ($\delta_{\text{C}\alpha}$), and the rate constants for the reaction with reduced glutathione (log K) for (meth)acrylates

Compounds	Chemical shifts ^{a)}		log K ($\text{M}^{-1}\text{s}^{-1}$)					
	$\delta_{\text{C}\beta}$	$\delta_{\text{C}\alpha}$	log K ₁		log K ₂		log K ₃	
	ppm	ppm	obsd ^A	pred ^B	obsd ^C	pred ^D	obsd ^E	pred ^F
MAA	130.56	128.15	1.6	1.0	—	1.1	1.7	1.9
EAA	130.24	128.59	1.4	0.9	1.6	0.9	1.4	1.8
nBAA	130.21	128.61	1.4	0.8	—	0.9	1.6	1.8
iBAA	130.23	128.60	1.4	0.9	1.5	0.9	—	1.8
HAA	130.23	128.63	—	0.9	1.3	0.9	—	1.8
2-EHAA	130.17	128.67	—	0.8	—	0.9	—	1.8
MMA	125.2	136.2	−0.7	−1.0	−0.7	−1.1	−0.5	−0.3
EMA	124.97	136.51	−0.4	−1.1	—	−1.2	−0.9	−0.4
iPMA	124.95	136.52	−0.9	−1.2	—	−1.2	—	−0.4
nBMA	124.70	136.41	−0.8	−1.2	−0.7	−1.3	—	−0.6
iBMA	124.98	136.52	−0.6	−1.1	—	−1.2	—	−0.4
tBMA	124.09	137.96	−0.6	−1.5	—	−1.5	—	−0.8
HEMA	125.89	135.96	−0.3	−0.8	—	−0.8	—	−0.1
AllylMA	125.46	136.23	—	−1.0	−0.3	−0.9	—	−0.2
BZMA	125.66	136.21	—	−0.9	−0.5	−0.9	—	−0.1
EGDMA	125.9	136.1	—	−0.8	—	−0.8	−0.1	0.0
TEGDMA	125.4	136.3	—	−1.0	—	−1.0	—	0.0
tetraEGDMA	—	—	—	—	—	—	0.2	—
tetraEG*	—	—	—	—	—	—	2.2	—
bis-GMA	126.2	135.9	—	−0.7	—	−0.7	—	0.1

*: Tetraethyleneglycol; *: From Refs 10 and 11; ^A: From Ref.14; ^B: Log K₁ was calculated using Eq. (1) derived from 11 compounds shown in obst^A; ^C: From Ref. 6; ^D: Log K₂ was calculated using Eq. (2) derived from 7 compounds shown in obst^C; ^E: From Ref. 5; ^F: Log K₃ was calculated using Eq. (3) derived from 6 compounds shown in obst^E.
See Table 4 for details on each equation.

acceptable dependence on both $\delta_{\text{C}\beta}$ and $\delta_{\text{C}\alpha}$ was observed, indicating that GSH reactivity might be associated with the α, β -unsaturated carboxylic esters of (meth)acrylates. As a high correlation coefficient was observed for Eq. (1), namely $r^2=0.998$, $\delta_{\text{C}\beta}$ was used to predict K₂ and K₃. The predicted log K_{1–3} values for (meth)acrylates are shown in Table 1. The high correlation coefficients suggested that it was thus feasible to predict GSH reactivity with methacrylates using NMR chemical shifts. Using Eqs (1) – (3), the GSH reactivity obtained in this study declined in the following order: acrylates (MAA, EAA, nBAA, iBAA, HAA, 2-EHAA) >> methacrylates with a functional group (HEMA, AllylMA, BZMA), dimethacrylates (EGDMA, TEGDMA, bis-GMA) > methacrylates (MMA, EMA, iPMA, nBMA, iBMA, tBMA). Acrylates were ranked as having the highest GSH reactivity, hence

clearly suggesting the strong effect of the substitution pattern (H) on the α -carbon as compared to that (CH_3) on the α -carbon for methacrylates (Fig.1).

Prediction of GSH reactivity using ^1H -NMR spectra

^1H -NMR chemical shifts are also affected by the π -electron density of the attached carbon. On this premise, we speculated that ^1H -NMR chemical shifts could also be correlated with the reactivities of monomers, and hence could likewise serve as an important tool in the investigation of GSH reactivity with monomers. Although the chemical shift range for ^1H -NMR is much smaller than that of ^{13}C -NMR, determination of ^1H -NMR chemical shifts could be easier, more time-saving, and more convenient than with ^{13}C -NMR. On this premise, we investigated the QSARs using ^1H -NMR chemical shifts.

Table 2 ^1H -NMR chemical shifts (δ_{Ha} , δ_{Hb}), chemical shift differences between δ_{Ha} and δ_{Hb} ($\Delta|\delta_{\text{Ha}} - \delta_{\text{Hb}}|$), and rate constants for the reaction with reduced glutathione ($\log K_4$) for (meth)acrylates

Compounds	NMR chemical shifts (ppm) ^{a)}			$\log K_4$ ($\text{mol}^{-1}\text{min}^{-1}$)	
	δ_{Ha}	δ_{Hb}	$\Delta \delta_{\text{Ha}} - \delta_{\text{Hb}} $	obsd ^A	pred ^B
MAA	5.825	6.406	0.581	1.60	1.52
EAA	5.807	6.395	0.588	1.43	1.39
nBAA	5.805	6.391	0.586	1.40	1.37
iBAA	5.813	6.400	0.587	1.62	1.45
HAA	5.804	6.391	0.587	1.31 ^{d)}	1.36
2-EHAA	5.806	6.385	0.579	–	1.40
MMA	5.550	6.100	0.545	–0.68	–0.60
EMA	5.541	6.096	0.555	–0.40	–0.66
nBMA	5.532	6.091	0.559	–0.77	–0.75
iBMA	5.543	6.108	0.565	–0.63	–0.65
HEMA	5.601	6.149	0.548	–0.27	–0.20
AllylMA	5.574	6.138	0.564	–0.29 ^{d)}	–0.41
BZMA	5.572	6.153	0.581	–0.49 ^{d)}	–0.42
TEGDMA ^{b)}	5.56	6.10	0.54	–	–0.52
bis-GMA ^{b)}	5.56	6.10	0.54	–	–0.52
bis-GMA ^{c)}	5.60	6.14	0.54	–	–0.21
TEGDMA ^{e)}	5.56	6.13	0.57		

^a: From Ref. 10; ^b: From Ref. 13; ^c: From Ref. 12; ^d: From Ref. 6; ^e: Measured in this study; ^A: From Ref. 14; ^B: Calculated using Eq. (4) derived from 12 compounds shown in obst^A.

For details on Eq. (4), see Table 4.

As shown in Fig. 1, H_a and H_b represent the protons *trans* and *cis* respectively as the substituents on the ester moiety. The chemical shifts of H_a (δ_{Ha}) and H_b (δ_{Hb}), and the shift difference between δ_{Ha} and δ_{Hb} for the selected (meth)acrylates are shown in Table 2. Further in Table 4, Eq. (4) demonstrated that an acceptable QSAR was found for GSH reactivity ($\log K_4$) and δ_{Ha} . The correlation coefficient for δ_{Ha} was similar to that for δ_{Hb} (data not shown). Regarding the correlation coefficient for the shift difference between δ_{Ha} and δ_{Hb} , it was smaller than that for δ_{Ha} or δ_{Hb} . Owing to the high correlation coefficient observed for Eq. (4), the latter was used to predict the GSH reactivity ($\log K_4$) for 16 selected monomers and their data are shown in Table 2.

The predicted $\log K_4$ values for (meth)acrylates were shown to decline in the following order: acrylates (MAA, EAA, nBAA, iBAA, HAA, 2-EHAA) >> methacrylates with a functional group (HEMA, AllylMA, BZMA), dimethacrylates (EGDMA, TEGDMA, bis-GMA) > methacrylates (MMA, EMA, nBMA, iBMA). The order of reactivity was similar to that obtained using ^{13}C -NMR spectra. In other words, apart from ^{13}C -NMR chemical shifts, ^1H -NMR chemical shifts are similarly useful for the prediction of GSH

reactivity.

QSAR between acute toxicity and GSH reactivity

Detoxication of acrylates and methacrylates involves conjugation with GSH, whereby chemical reactivity leading to GSH depletion has been reported as a cause for the acute toxicity of acrylates and methacrylates^{5,6)}. Using five acrylates and five methacrylates, we investigated the relationship between LD₅₀ in mice and GSH reactivity ($\log K_{1-4}$). The results shown in Table 3 indicated that the acute toxicity exhibited by (meth)acrylates in ICR mice was about 10-fold greater than that in ddY mice. This vast disparity might be caused by the different toxicity testing methods employed in this study: the LD₅₀ values in ICR mice were obtained by intraperitoneal injection, whereas it was by oral administration for ddY mice.

Table 4 shows the QSARs between GSH reactivity ($\log K_1$, $\log K_4$) and acute toxicity (LD₅₀) in mice. Except for HEMA, the intraperitoneal LD₅₀ values in ICR mice were highly correlated with $\log K_1$ or K_4 ($r^2 \approx 0.8$, $p < 0.01$). In contrast, the oral LD₅₀ values in ddY mice were slightly but nonetheless significantly correlated with $\log K_1$ or K_4 ($r^2 \approx 0.5$, $p < 0.05$). On the different toxicity testing methods used, a linear

Table 3 The observed and predicted values for 50% lethal dose (LD₅₀), and log P (octanol-water partition coefficient) for (meth)acrylates

Compounds	Log P ^{a)}	LD ₅₀ ^{b)}		LD ₅₀ ^{a)}		Oral rat LD ₅₀ ^{c)}
		Intraperitoneal ICR mice		Oral ddY mice		
		Obsd	Pred ^A	Obsd	Pred ^B	
		mmol/kg	mmol/kg	mmol/kg	mmol/kg	
MAA	0.80	2.95	5.5	9.60	29.4	—
EAA	1.33	5.99	5.8	17.97	51.4	<50 ^{e)}
nBAA	2.36	6.65	5.8	58.98	85.5	—
iBAA	2.22	5.93	5.7	47.63	85.1	—
2-EHAA	3.72 ^{b)}	7.20	5.8	—	—	—
MMA	1.38	11.22	10.3	51.97	69.4	79.9 ^{d)}
EMA	1.94	10.90	10.4	68.64	107.3	—
nBMA	2.88	10.48	10.6	142.70	144.6	113 ^{f)}
iBMA	2.66	8.94	10.4	83.14	134.6	—
HEMA	0.47	4.06	9.4	45.24	—	—
AllylMA	1.57	—	9.8	—	90.3	2.8-5.1 ^{e)}
BZMA	2.53	—	9.9	—	125.4	28.4 ^{g)}
TEGDMA	1.55 ^{h)}	—	11.2	—	89.7	37.9 ⁱ⁾ , 37.6 (mice) ^{j)}
bis-GMA	5.07 ^{h)}	—	10.1	—	218.2	>9.3 ^{j)}

^A: Predicted using Eq. (9) derived from the observed values (Obsd) for 9 compounds except HEMA; ^B: Predicted using Eq. (13) derived from the observed value for 8 compounds except HEMA; ^a: From Ref. 14; ^b: From Ref. 15; ^c: Literature values; ^d: From Ref. 18; ^e: From Ref. 17; ^f: From Ref. 20; ^g: From Ref. 19; ^h: From Ref. 11; ⁱ: From Ref. 21; ^j: From Ref. 22.

Table 4 Quantitative structure-activity relationships (QSARs) between: (A) reduced glutathione GSH reactivity (log K₁, K₂, K₃, or K₄) and NMR-chemical shifts (δ_{CB} , δ_{CA} , δ_{HA} , δ_{HB} or $\Delta|\delta_{HA}-\delta_{HB}|$); and (B) between LD₅₀ (50% lethal dose) and descriptors (log K₁, log K₄, log P) for (meth)acrylates

A)		
log K ₁ =	$-48.6 (\pm 0.2) + 0.4 (\pm 0.0)\delta_{CB} (n=11, r^2=0.998, p<0.001)$	Eq. (1)
log K ₂ =	$-51.2 (\pm 0.1) + 0.4 (\pm 0.0)\delta_{CB} (n=7, r^2=0.99, p<0.001)$	Eq. (2)
log K ₃ =	$-52.9 (\pm 0.2) + 0.4 (\pm 0.0)\delta_{CB} (n=6, r^2=0.85, p<0.001)$	Eq. (3)
log K ₄ =	$-43.5 (\pm 0.1) + 7.7 (\pm 0.3)\delta_{HA} (n=12, r^2=0.99, p<0.001)$	Eq. (4)
log K ₄ =	$-27.5 (\pm 0.6) + 48.6 (\pm 12.5) \delta_{HA}-\delta_{HB} (n=12, r^2=0.63, p<0.01)$	Eq. (5)
B)		
LD ₅₀ [*] =	$9.1 (\pm 2.2) - 1.9 (\pm 0.7) \log K_1 (n=10, r^2=0.48, p<0.05)$	Eq. (6)
LD ₅₀ [*] =	$9.9 (\pm 2.1) - 1.7 (\pm 0.7) \log K_4 (n=10, r^2=0.48, p<0.05)$	Eq. (7)
LD ₅₀ [*] =	$10.4 (\pm 1.3) - 2.2 (\pm 0.5) \log K_1$ (outlier: HEMA, $n=9, r^2=0.80, p<0.01$)	Eq. (8)
LD ₅₀ [*] =	$8.9 (\pm 2.1) - 2.3 (\pm 0.7) \log K_4$ (outlier: HEMA, $n=9, r^2=0.8, p<0.01$)	Eq. (9)
LD ₅₀ [#] =	$60 (\pm 30.9) - 27.7 (\pm 10.6) \log K_1$ (outlier: HEMA, $n=8, r^2=0.52, p<0.05$)	Eq. (10)
LD ₅₀ [#] =	$-4.8 (\pm 27.1) + 435.5 (\pm 11.5) \log P (n=9, r^2=0.58, p<0.05)$	Eq. (11)
LD ₅₀ [#] =	$70.2 (\pm 31.4) - 26.1 (\pm 10.6) \log K_4$ (outlier: HEMA, $n=8, r^2=0.55, p<0.05$)	Eq. (12)
LD ₅₀ [#] =	$15.1 (\pm 17.3) + 37.9 (10.1) \log P - 16.4 (\pm 6.7) \log K_1$ (outlier: HEMA, $n=8, r^2=0.88, p<0.01$)	Eq. (13)

For more details on log K_{1–4} and LD₅₀, see Tables 1–3, respectively.
LD₅₀^{*} in intraperitoneal ICR mice. LD₅₀[#] in oral ddY mice.

relationship was observed between the two sets of LD₅₀ values (intraperitoneal *versus* oral), but with a poor correlation ($n=9$, $r^2 = 0.457$, $p<0.05$).

In general, the acute toxicity of (meth)acrylates is correlated with their log P term^{14,15}. In the present study, oral LD₅₀ was significantly correlated with the log P term as shown in Eq. (11), whereas there was no correlation between intraperitoneal LD₅₀ and the log P term. This contrast in toxic response (LD₅₀) might be caused by discrepancies in the log P function of the monomers under different toxicity testing methods (intraperitoneal *versus* oral). The log K term of a monomer is related to the latter's interaction with the relevant receptor(s), whereas the log P term is related to a monomer's penetration into the site of action^{11,14,15}.

Interestingly, oral LD₅₀ in ddY mice showed a more acceptable QSAR in terms of both log K₁ and log P in Eq. (13) ($r^2 = 0.88$, $p<0.01$, outlier: HEMA), as compared with only log K₁ in Eq. (10) and log P in Eq. (11). On this premise, Eq. (13) was used to predict the oral LD₅₀ values for the (meth)acrylates and the data are shown in Table 3. In addition, the predicted LD₅₀ values in mice for EAA, MMA, nBMA, BZMA, AllylMA, TEGDMA, and bis-GMA were compared against those reported in the literature for rats *via* the oral route (Table 3). Upon comparison, it was found that the oral LD₅₀ values for EAA, MMA, and TEGDMA in mice were similar to those in rats (Table 3). However, the predicted oral LD₅₀ values for AllylMA and bis-GMA in rats were about 20-fold greater than those in mice. Similarly, the oral LD₅₀ values for nBMA and BZMA in rats were about 4-fold greater than those in mice. The high toxicity levels of AllylMA and bis-GMA might be related to their hydrolysis products and/or metabolized intermediates in addition to differences stemming from the animal species and toxicity testing methods employed.

DISCUSSION

Based on the results obtained in this study, it was shown that GSH reactivity with acrylates, methacrylates, and dimethacrylates was significantly correlated with their NMR chemical shifts. Although the chemical shift range of ¹H-NMR was smaller than that of ¹³C-NMR, the spectra of both ¹³C- and ¹H-NMR were used as descriptors in this study to predict the GSH reactivity of monomers. Results revealed that GSH reactivity declined in the following order: acrylates >> methacrylates with a functional group: hydroxyl, benzyl, or allyl substituent (ester group) > dimethacrylates > methacrylates with a short-chain aliphatic substituent (ester group). In previous studies^{6,23}, acrylates such as EAA and iBAA were reported to exhibit markedly greater toxicity than methacrylates such as MMA and iPMA in fathead minnow or mice. Taking into consideration the results of the present study and those of published literature^{6,23}, it could be claimed that acrylates with a high GSH reactivity showed higher toxicity than

methacrylates with a low GSH reactivity.

On the other hand, AllylMA showed lower GSH reactivity than EAA and iBAA although it exhibited higher toxicity than the latter acrylates²². In a previous study²⁴, this anomaly was attributed to the hydrolysis products and/or metabolized intermediates of AllylMA. The hydrolysis of AllylMA produced allyl alcohol, and that toxicity was caused by the oxidation/metabolism of allyl alcohol by alcohol dehydrogenase in the liver to the highly reactive acrolein. In the present study, the predicted LD₅₀ for AllylMA was similar to that for BAA, further confirming that the strong toxicity of AllylMA in animal tests could be related to its hydrolysis products.

For HEMA bearing a hydroxyl substituent, its intraperitoneal LD₅₀ value in mice was higher than those for nBAA and iBAA¹³, although the GSH reactivity of the former was lower than the latter two. Similarly, this could be attributed to a hydrolysis product of HEMA, namely ethylene glycol. Ethylene glycol is then metabolized by liver alcohol dehydrogenase to glycolaldehyde, which in turn is rapidly metabolized to glycolate — a toxic metabolite. The toxicity of ethylene glycol is complex and not fully understood, chiefly due to the severe metabolic acidosis caused by glycolate²⁵.

For the methacrylate monomers investigated in this study, the results obtained suggested that their toxicity might be influenced by the hydrolysis and/or metabolic products derived from the original parent monomers. For example, the relatively high acute toxicity of EGDMA (oral LD₅₀ in mice: about 10 mmol/kg²⁶) could be related to its hydrolysis products — namely, methacrylic acid, HEMA, and ethylene glycol. On the same note, the GSH reactivity of BZMA, an aromatic methacrylate, was greater than that of aliphatic methacrylates. This could be chiefly due to a hydrolysis product derived from BZMA, benzyl alcohol, an alcohol moiety which was reported to be of toxicological importance because benzyl alcohols seemed to exhibit toxicity *via* a radical mechanism²⁷. Further, while similar LD₅₀ values were predicted for BZMA and BMA in the present study, disparity was found between the predicted and experimental data of BZMA. The latter finding could be attributed to the hydrolysis of BZMA, because its hydrolysis products were not taken into account when the toxicity of this compound was predicted using QSAR.

For the dimethacrylates EGDMA and TEGDMA, their GSH reactivity was similar to that of HEMA. It has been found in previous studies that TEGDMA depletes intracellular GSH and interferes with cellular metabolism^{9,28}. This is chiefly because the hydrolysis of TEGDMA produces triethylene glycol and methacrylic acid, and triethylene glycol might have exhibited reactivity toward GSH. As shown in Table 1, tetraethylene glycol exhibited markedly higher GSH reactivity than tetraethylene glycol dimethacrylate (tetraEGDMA)⁵. This suggested that the ethylene glycol oligomers — namely, diethylene glycol,

triethylene glycol, and tetraethylene glycol — derived from the hydrolysis of the corresponding dimethacrylates might have played a larger and more active role in acute toxicity than the original dimethacrylates bearing ethylene glycol groups.

For bis-GMA, it is a reaction product of methacrylic acid and diglycidyl ether of bisphenol A. Upon enzymatic or chemical-induced hydrolysis, it yields the following products^{29,30}: methacrylic acid, (2-[4-(2,3-dihydroxypropoxy)phenyl]-2-[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane), 2,2'-bis[4-(2,3-dihydroxypropoxy)phenyl]propane (BHP), and 2-(4-hydroxyphenyl)-2-[4-(2,3-dihydroxypropoxy)phenyl]propane. In the present study, bis-GMA was predicted to have a low toxicity at 218 mmol/kg (Table 3). However, animal testing *via* intraperitoneal injection yielded a relatively high toxicity at about 10 mmol/kg²². Therefore, while it was reported on one hand that apoptosis or cell death was induced by the metabolic oxidation of a single terminal hydroxyl group of the parent glycol², the relatively high toxicity of bis-GMA was probably attributed to its hydrolytic metabolites.

In light of the findings of the present study, acceptable relationships were found for the NMR chemical shifts ($\delta_{C\beta}$ and δ_{Ha}) and GSH reactivity (log K) of (meth)acrylates. MMA-induced depletion of GSH and oxidized glutathione (GSSG) in rat liver and kidney has been previously reported³¹. In this study, the probability of causable relationships between acute toxicity and the GSH reactivity (log K) of (meth)acrylates^{5,6,14} further suggested that the detoxication of (meth)acrylates might be related to *in vivo* GSH and GSSG depletion. In other words, the NMR chemical shifts of the α , β -unsaturated carboxylic esters of methacrylate monomers could be used as a means to measure and predict their acute toxicity in addition to the log P term.

CONCLUSIONS

Based on the published data for GSH reactivity, acute toxicity, and NMR spectra of acrylate and methacrylate monomers, QSAR studies were carried out. It was found that the GSH reactivity of (meth)acrylates was significantly related to their ¹³C-NMR spectra ($\delta_{C\beta}$) and ¹H-NMR spectra (δ_{Ha} and δ_{Hb}). This meant that the NMR spectra of (meth)acrylates could be used to predict their toxicity because there was an acceptable correlation between GSH reactivity and acute toxicity. However, there were discrepancies between the predicted and experimental data, hence suggesting that metabolic alterations of the original monomers might affect *in vivo* toxicity.

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