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In vitro *Interactions of Rubber Chemical Additives with Cells from Varying Lineages*

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In cytotoxicity study, it is possible that in vitro *cell responses will vary depending on the cell lineage. This consequently affects the toxicity ranking and the threshold levels considered safe for use. This study aims to examine the probable variations in the cytotoxic effects elicited by rubber chemical additives evaluated against three different cell lines, U937 (monocytic), HeLa (epithelial) and L929 (fibroblastic) cells. Additives tested were zinc diethyldithiocarbamate (ZDEC), zinc dibutyldithiocarbamate (ZBUD), zinc-2-mercaptobenzothiazole (ZMBT), zinc oxide (ZnO) and 2,2'-methylene-bis(4-methyl-6-*tert*-butylphenol) (2246). Cell response was quantified using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, which measures the cell metabolic activity* via *the reduction of MTT salt by dehydrogenase enzymes in living cells. Results show that ZDEC was least tolerated by all three cell lines, followed by ZBUD, while the toxicity ranking of ZMBT, ZnO and 2246 changes depending on the types of cell. The lethal chemical concentrations (LC_{50}) against the three cell lines were constant for ZDEC and ZMBT, but varied for ZBUD, ZnO and 2246. This indicates cell-dependent toxicity among some of the rubber chemical additives. Caution should therefore be exercised when using these toxicity threshold values to gauge the safety levels for use.*

Natural rubber latex (NRL) is used widely as a raw material in the manufacturing of medical devices, primarily gloves, condoms and catheters because of its excellent physical properties[1]. Recent studies have implicated that some chemical additives used both in the vulcanisation of natural and synthetic rubber latices, caused adverse reactions in living cells[2,3]. It should be noted that during manufacturing, NRL products are washed to remove residual chemicals, which otherwise may impair their physical properties[4]. Therefore, these products are not expected to contain residual chemicals of levels that are adverse to the users. Nevertheless, leaching of rubber chemical additives such as mercaptobenzothiazoles, thiurams, carbamates and zinc compounds from medical products was reportedly evident in some cases[5–7]. The cells and tissue responses in general, varied widely from non-detectable to severe damage[8]. This wide range of responses suggests that manufacturing processes may play a greater role than chemical additives alone in governing the biocompatibility of medical

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devices. 'Good Manufacturing Process (GMP)' has been reported to be accountable for the resolution of some cytotoxicity problems associated with urinary catheters[7].

In vitro cell culture methods have been widely used to examine and simulate the effects of chemical additives on the body system. Despite their undoubted practicality, it is important that findings from these methods are evaluated over a broader perspective, especially in cases where the type of cells used could influence the *in vitro* responses[9,10]. In the current study, several types of rubber chemical additives, particularly those used in the sulphur vulcanisation of both the synthetic and natural rubber latices, were assessed for their effects on varying cell lines. This is to gauge their tolerance thresholds and to identify possible dependence of cell responses on the types of cell being used.

MATERIALS AND METHODS

Chemical Additives

Chemical additives (*Table 1*) were obtained from the Malaysian Rubber Board (MRB), Kuala Lumpur, Malaysia and the Tun Abdul Razak Research Centre (TARRC), Hertford, United Kingdom. Stock solutions at 0.1 g ml^{-1} were prepared in dimethylsulphoxide (DMSO), aliquoted and stored at –70°C. Each aliquot was used once and discarded after each experiment.

Cell Culture

In vitro effects of the chemical additives were evaluated against three different cell lines namely U937, HeLa and L929 cells, which represent the monocytic, epithelial and connective tissue cells, respectively (*Table 2*). These are established cell lines obtained from the European Collection of Animal Cell Cultures (ECACC), Salisbury, United Kingdom.

All cell culture reagents used are products of Gibco BRL Company Limited, United Kingdom. L929 cells were maintained in Dulbecco medium (DMEM) while RPMI 1640 medium was used for U937 and HeLa cells. Both types of media were supplemented with 100 IU mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 10 μM of 2-mercaptoethanol and 10 % fetal bovine serum. An additional 2 mM of glutamine was added into

TABLE 1. CHEMICAL ADDITIVES AND THEIR ROLES IN THE VULCANISATION OF SYNTHETIC AND NATURAL RUBBER LATICES

Chemical additives	Role in vulcanisation
Zinc oxide (ZnO)	Activator
Zinc-2-mercaptobenzothiazole (ZMBT)	Accelerator
Zinc diethyldithiocarbamate (ZDEC)	Accelerator
Zinc dibutyldithiocarbamate (ZBUD)	Accelerator
2,2`-methylene-bis(4-methyl-6-*tert*-butylphenol) (2246)	Antioxidant

TABLE 2. THE THREE DIFFERENT CELL LINES USED TO DETERMINE THE TOLERANCE THRESHOLDS OF RUBBER CHEMICAL ADDITIVES

Cell lines	*Origin of cells and descriptions*	*ECACC No.*
U937	Malignant cells obtained from the pleural effusion of a 37-year-old Caucasian male with diffuse histiocytic lymphoma. Cells express monocytic-like characteristics exhibited by cells of histiocytes origin	87010802
HeLa	Aneuploid, epithelial-like cell line derived from human cervic carcinoma tissues	85060701
L929	Fibroblastic mouse connective tissue cells of unknown passage, a subclone of the parental strain L derived from normal subcutaneous areolar and adipose tissue of C34/An mouse	85011425

the supplemented RPMI 1640 medium. Cells were maintained in humidified atmosphere with 5% CO_2 at 37°C. The non-adherent U937 and HeLa cells were sub-cultured routinely by removing 2–3 parts of the cell suspension and substituting the removed volume with fresh supplemented RPMI medium. For the adherent L929 cells, confluent cells were detached from culture flask surface *via* trypsination. Growth medium was first removed from the culture flask followed by incubation of the confluent cells with Trypsin-Gibco Solution A 0.25% for 5–10 minutes. Cells were washed with supplemented DMEM medium and re-seeded at 5×10^3 to 2×10^4 cells per cm^2 area of culture flask.

Exposure of Cells to Chemical Additives

100 μL of complete culture medium was first added into a 96-well microplate. Stock chemical solutions (0.1 g mL^{-1}) were diluted with complete culture medium to appropriate working concentrations. 100 μL of the working solution was then added into each well, mixed and serially diluted to give final chemical concentrations ranging between 0.02–100 μg mL^{-1}. Complete culture medium with no added chemical, was used as the negative control. 50 μL of cell suspension at $6-8\times10^5$ cells/mL was next added into each well. Cells were incubated for 24 h in humidified 5% CO_2 at 37°C following which, the cell activity was quantified using the MTT assay described below.

Quantitation of Cell Response by MTT Assay

The MTT assay is a colorimetric method based on the reduction of tetrazolium salt by the dehydrogenase enzyme in the mitochondrias of living active cells to produce blue formazans[11]. When solubilised, these formazans absorb strongly at 600 nm wavelength, giving absorbance readings that relate proportionally to the number of living cells. A working solution of MTT salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (Sigma Chemicals Company Limited, United Kingdom) at 5 mg mL^{-1} was prepared in

phosphate buffer saline (0.1 M, pH 7.2). Solution was filtered through a 0.2 μm sterile cellulose acetate membrane disc filters (Anderman Company Limited, Surrey, United Kingdom) and stored at 4°C prior to use.

After 24-h exposure of cells to the chemical additives, 50 μL of the MTT working solution was added into each well and monitored for a period between 2 h–4 h for optimum formazan development. The resultant formazan crystals were then solubilised overnight with 50 μL per well of 20% sodium dodecyl sulphate solution (SDS) containing 20 mM L^{-1} of hydrochloric acid. Absorbance was measured at 600 nm using *BioRad Model 2550 EIA* Microplate Reader (BioRad, United Kingdom). Absorbance reading of the negative control (cells exposed to culture medium only) was regarded as representative of a 100 % MTT metabolic activity (*i.e.* no cell death). The absorbance readings of test samples were normalised against that of the negative control using the following equation:

$$\text{MTT metabolic activity of test sample (\%)} = \frac{A - B}{C} \times 100 \quad \ldots 1$$

where

- A is the absorbance reading of test sample (cells exposed to chemicals)
- B is the absorbance reading of blank (chemical solutions without cells)
- C is the absorbance reading of negative control (cells exposed to culture medium only).

Data Analysis

Descriptive statistics represented by the means and standard errors of mean (SE), was used to analyse data related to all test groups and controls. In cases where results were expressed as normalised data, these were obtained by dividing the test results with that of the appropriate control. Where applicable, inferential statistics by ANOVA (analysis of variance) and *Posthoc* Tukey-B test were used to determine the significance of difference between different groups of sample. All statistical analyses were carried out using the SPPS software (version 6.0 for Windows).

RESULTS

Some chemical additives used in this study were observed to react with tetrazolium salt of the MTT assay, generating blue artifacts. When solubilised, these artifacts absorbed at wavelength similar to that of the test measurements (*Figure 1*). These non-specific reactions were negligible at chemical concentrations below 10 μg mL^{-1} while beyond that, absorbance readings were found to increase exponentially. ZMBT and 2246 elicited strongest non-specific reactions followed by ZBUD. There were no distinct non-specific reactions by ZDEC (*inset*) within the concentration range similar to that used on the cells. No appreciable reaction was seen with ZnO at below 100 μg mL^{-1}. In view of this background interference, all absorbance readings associated with the MTT assay were corrected against appropriate blank controls. Blank controls comprised chemical additives that were reacted with MTT salt in the absence of cells. Experiments were carried out under conditions similar to those used in the actual study with cells.

Cell Responses at Varying Chemical Concentrations

Figure 2 depicts the effects of rubber chemical additives on three different cell lines, averaged from a minimal of three separate

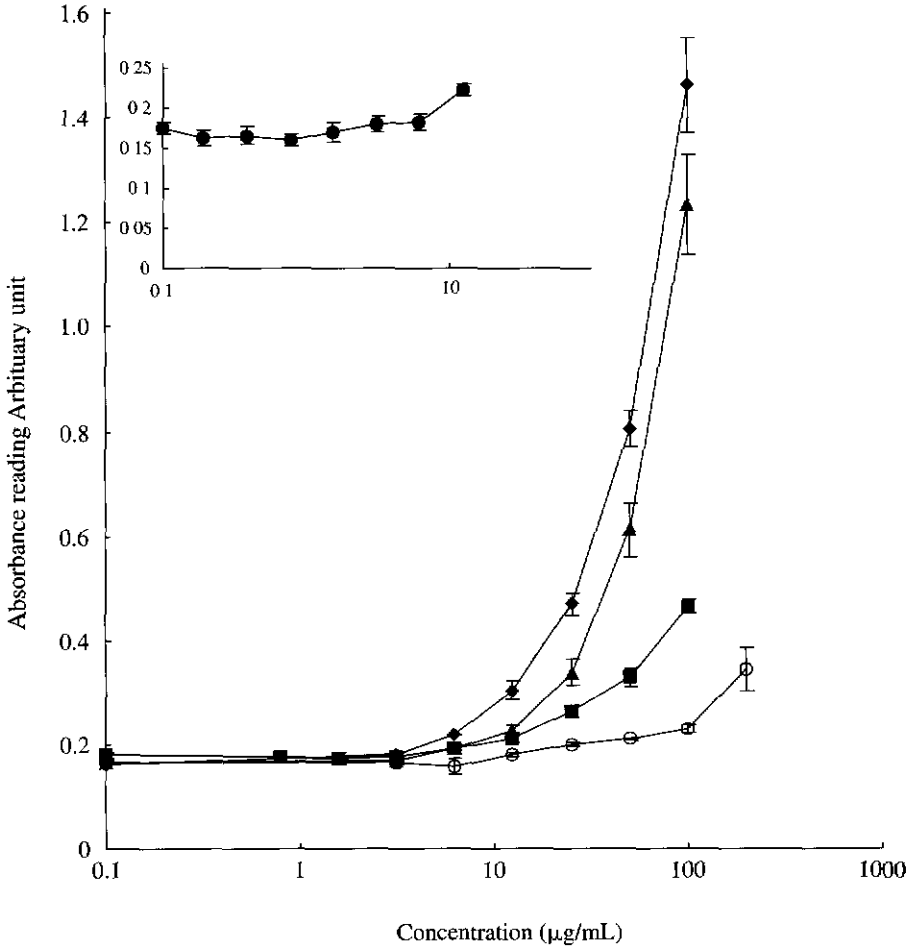

Figure 1. Non-specific reactions between chemical additives and MTT salt. Chemicals were reacted with MTT salt under experimental conditions that were identical to those used in the actual cytotoxicity evaluation by the MTT assay. Data are expressed as mean ± SE (n = 3). (● = ZDEC; ■ = ZBUD; ▲ = ZMBT; ○ = ZnO and ◆ = 2246.)

experiments. ZDEC initiated the earliest cell death on all three types of cell, characterised by the abrupt descent of its cytotoxicity curve at concentration approximately 1μg mL^{-1}. In the case of ZMBT, all cell lines showed relatively similar responses with cells being able to withstand chemical challenge of up to approximately 25 μg mL^{-1}.

Antioxidant 2246, ZnO and ZBUD appeared to elicit cell-specific toxicity. 2246 initiated killing of U937 and HeLa cells at chemical concentration of approximately 6 μg mL^{-1} whereas L929 cells were only affected at a much higher chemical concentration (above 25 μg mL^{-1}). U937 and HeLa cells were able to withstand ZnO challenge at up to approximately 25 μg mL^{-1} while L929

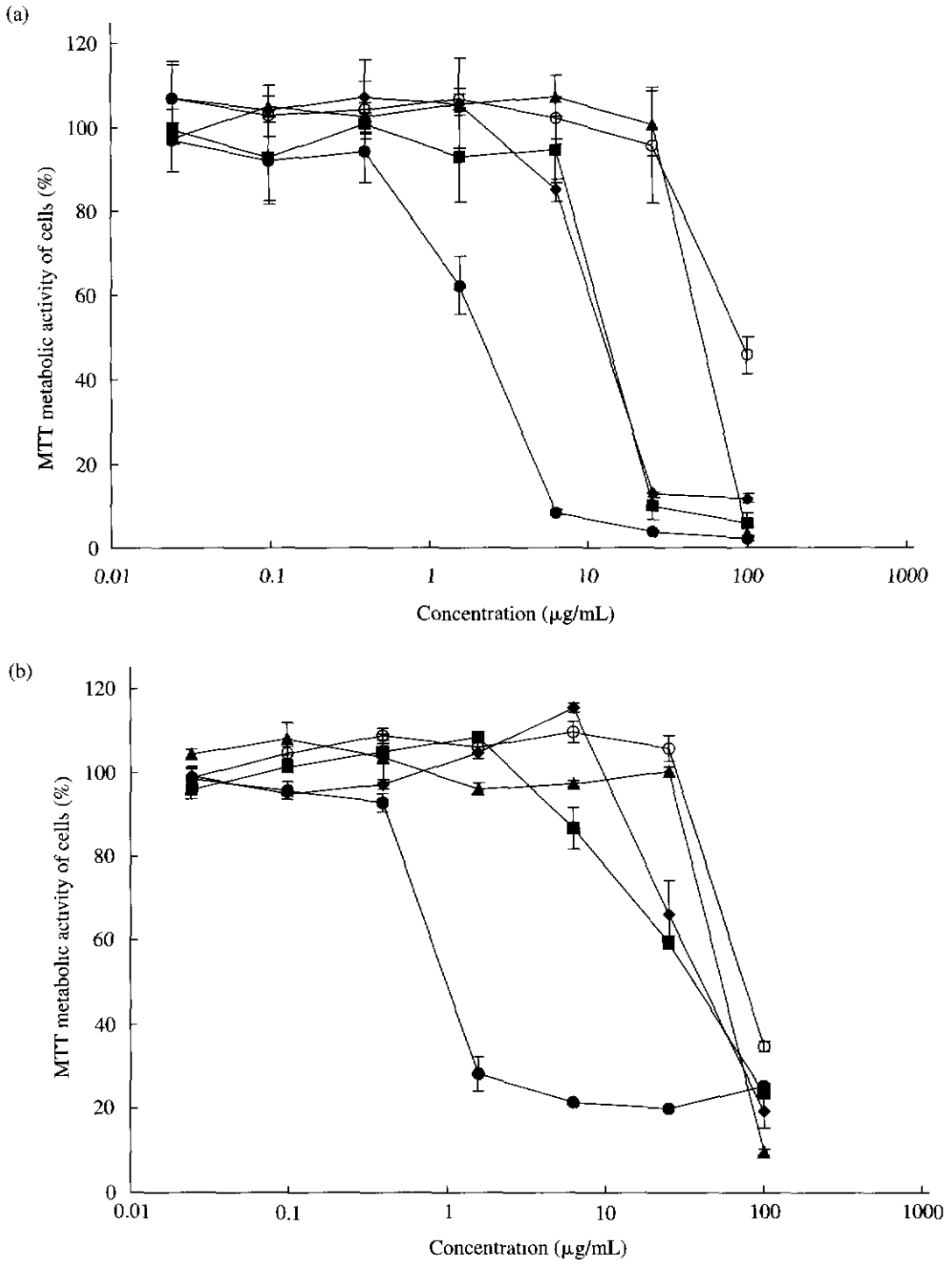

Figure 2. Cytotoxicity curves of chemical additives tested on (a) U937, (b) HeLa and (c) L929 cells. Each data point is expressed as mean ± SE ($n \geq 3$).
(● = ZDEC; ■ = ZBUD; ▲ = ZMBT; ○ = ZnO and ◆ = 2246.)

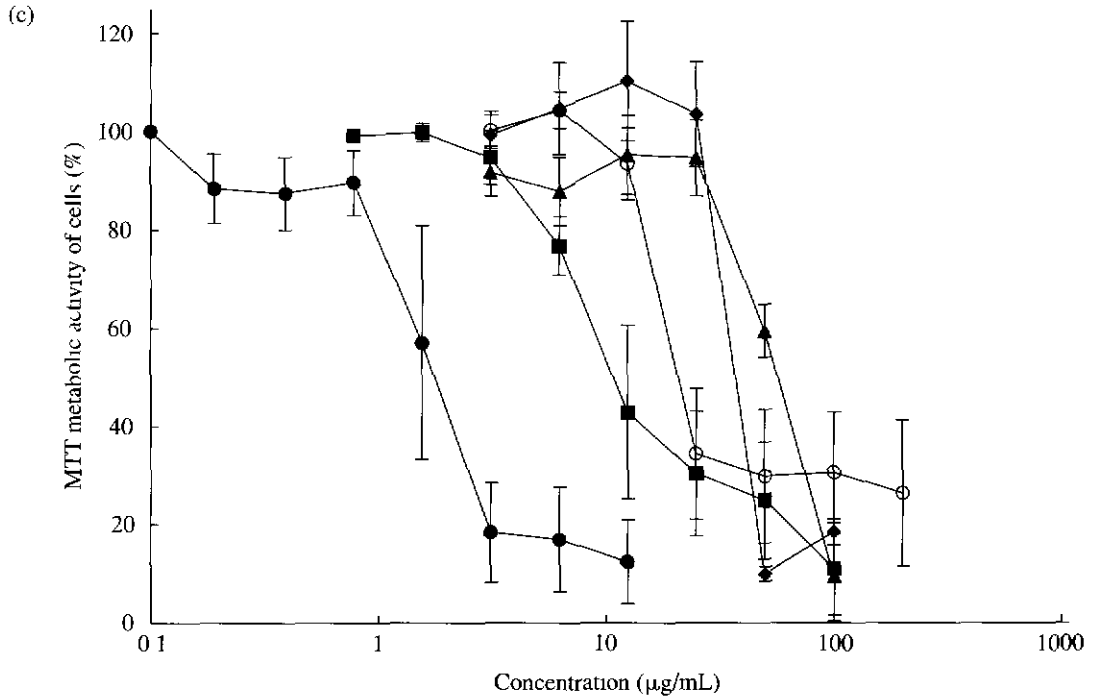

Figure 2 Cont Cytotoxicity curves of chemical additives tested on (c) L929 cells
Each data point is expressed as mean ± SE (n ≥ 3)
(● = ZDEC ■ = ZBUD ▲ = ZMBT, ○ = ZnO and ◆ = 2246)

cell death was already evident at chemical concentration of slightly above 15 μg mL ^{1} U937 cells tolerated ZBUD at up to approximately 6 μg mL ^{1} while HeLa and L929 cells showed tolerance of up to approximately 3 μg mL ^{1}

Comparison of the LC_{50} (Lethal Concentration) Values

Within the context of this study, the LC_{50} values are regarded as chemical concentrations that suppress MTT metabolic activity of cells to 50% of that expressed by the negative control (*i e* cell exposed to culture medium only). Cell response elicited by the negative control, was regarded as 100 % MTT metabolic activity

Comparison of the LC_{50} values of chemical additives tested on U937 indicates the following toxicity ranking namely ZDEC > ZBUD = 2246 > ZMBT > ZnO (*Figure 3*) The LC_{50} values varied widely between 0–100 μg mL^{-1} and showed significant difference in toxicity (ANOVA, $P < 0.001$) ZDEC was significantly more cytotoxic than all the other chemicals while ZBUD was only significantly more toxic than ZMBT and ZnO but showed no significant difference to 2246 (Tukey-B

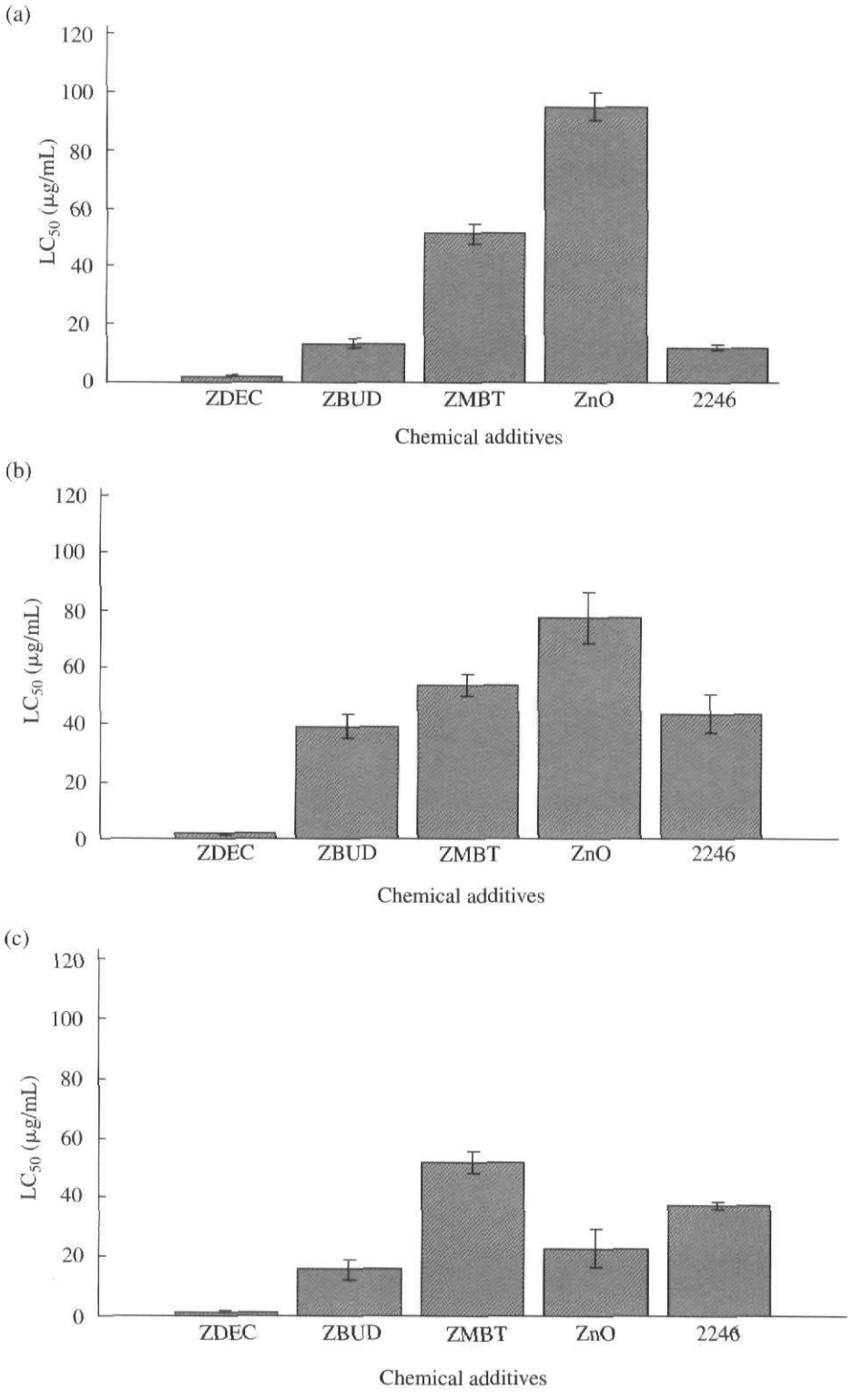

Figure 3. LC_{50} values of chemical additives tested on (a) U937 cells, (b) HeLa cells and (c) L929 cells. Values represent chemical concentrations that suppressed the MTT metabolic activity of cells to 50% of that expressed by the culture medium control. Each data point is expressed as mean ± SE ($n \geq 3$).

test at 0.05 significance level). ZnO was significantly the least toxic among the five chemicals tested (*Table 3a*).

Studies with HeLa cells elicited LC_{50} values ranging from 0–80 μg mL^{-1}, the order of toxicity being as follows; ZDEC > ZBUD = 2246 > ZMBT > ZnO (*Figure 3*). Similar to observations with U937 cells, ZDEC was significantly more cytotoxic than the other chemicals. Likewise, ZnO was found to be significantly least toxic (*Table 3b*). There was no significant difference in toxicity between ZMBT, ZBUD and 2246 (Tukey-B test at 0.05 significance level).

Experiment with L929 cells generated LC_{50} values ranging from 2–60 μg mL^{-1}, with the toxicity ranking being as follows; ZDEC > ZBUD > ZnO > 2246 > ZMBT (*Figure 3*). ZDEC was significantly more toxic than ZMBT, ZnO and 2246 (*Table 3c*). ZBUD was only significantly more toxic than ZMBT and 2246 but showed no significant difference

TABLE 3a. SIGNIFICANCE OF DIFFERENCE IN THE MEAN LC_{50} VALUES OF CHEMICALS TESTED ON U937 CELLS[a]

Chemical[b]	ZDEC	ZBUD	ZMBT	ZnO
ZBUD	S			
ZMBT	S	S		
ZnO	S	S	S	
2246	S	N	S	S

[a]Results were analysed using ANOVA and Tukey-B test at 0.05 significance level.
S = significant and N = not significant.
[b]P<0.001 (ANOVA)

TABLE 3b. SIGNIFICANCE OF DIFFERENCE IN THE MEAN LC_{50} VALUES OF CHEMICALS TESTED ON HeLa CELLS[a]

Chemical[b]	ZDEC	ZBUD	ZMBT	ZnO
ZBUD	S			
ZMBT	S	N		
ZnO	S	S	S	
2246	S	N	N	S

[a]Results were analysed using ANOVA and Tukey-B test at 0.05 significance level.
S = significant and N = not significant.
[b]P<0.001 (ANOVA)

to ZDEC and ZnO (Tukey-B test at 0.05 significance level). In contrast to the results for the U937 and HeLa cells, ZMBT rather than ZnO was found to be least cytotoxic.

In general, comparison of the LC_{50} values suggests that some of the chemical additives elicited toxicity that varied depending on the types of cell used (*Table 4*). ZDEC and ZMBT showed no significant difference in their LC_{50} values when these chemicals were tested against U937, HeLa and L929 cells. In contrast, ZBUD, ZnO and 2246 incurred significant difference in cell response (ANOVA, $P < 0.01$). ZBUD was significantly more toxic to U937 and L929 than to HeLa cells, the former two cell lines showed no significant difference in cell response. Likewise, ZnO was significantly more toxic to L929 than to U937 or HeLa cells; the latter two cell lines demonstrated no significant difference in cell response. 2246 was found to be most detrimental towards the U937 cells while both the HeLa and L929 cells showed no significant difference in cell response.

TABLE 3c. SIGNIFICANCE OF DIFFERENCE IN THE MEAN LC_{50} VALUES OF CHEMICALS TESTED ON L929 CELLS[a]

Chemical[b]	ZDEC	ZBUD	ZMBT	ZnO
ZBUD	N			
ZMBT	S	S		
ZnO	S	N	S	
2246	S	S	N	N

[a]Results were analysed using ANOVA and Tukey-B test at 0.05 significance level.
S = significant and N = not significant.
[b]$P<0.001$ (ANOVA)

TABLE 4. COMPARISON OF THE TOXICITY OF CHEMICAL ADDITIVES TOWARDS DIFFERENT CELL LINES[a]

	Chemical additives					
	ZBUD ($P<0.01$)		ZnO ($P<0.001$)		2246 ($P<0.01$)	
Cell line	U937	HeLa	U937	HeLa	U937	HeLa
HeLa	S		N		S	
L929	N	S	S	S	S	N

[a]Results were analysed using ANOVA and Tukey-B test at 0.05 significance level.
S = significant and N = not significant. The 'P' values were obtained from ANOVA.

DISCUSSION

Sulphur vulcanisation is by far, the most widely used method of crosslinking for NR latex. This is mainly because of the low cost, fast vulcanisation, excellent physical properties, minimal interference with other compounding chemicals and easy manipulation of the chemical recipe in order to change the crosslinking rate or to generate a product of required physical properties[12]. It is common to use supplementary chemicals to facilitate the sulphur-vulcanisation process, which accordingly also enhance the physical properties of the vulcanised material[4,12,13]. These include accelerators and activators for the main vulcanising mechanism, with additional chemicals such as antioxidants and colourants for specific purposes[4]. Examples of some common accelerators include dithiocarbamates, thiazoles, thiurams, thioureas and xanthates[13] while zinc oxide is the predominant activator. The common antioxidants used are the secondary amines and the phenolic compounds, the latter having the advantage of being non-staining[14].

In this study, chemical additives from the accelerator, activator and antioxidant groups showed dissimilar cytotoxic potentials when tested against three different cell lines, some of which exhibited apparent cell-type dependency. In the case of the dithiocarbamate accelerators, experiments with U937 and HeLa cells showed ZDEC to be significantly more cytotoxic than ZBUD ($P<0.05$). It is possible that the butyl group of ZBUD makes it less soluble in aqueous medium compared to the ethyl group of ZDEC, causing the former to be less toxic to cells. This observation was similarly reported in the *in vitro* cell culture studies by Nakamura *et al.*[3] who found that ZDEC and ZBUD were distinctly more cytotoxic among the wide spectrum of chemicals tested. One other commonly used non-dithiocarbamate accelerator is ZMBT, a chemical from the mercaptobenzothiazole group. ZMBT was significantly less cytotoxic than ZDEC and ZBUD when tested on the U937 and L929 cells. ZMBT was also comparatively less cytotoxic than ZBUD when tested on the HeLa cells although the difference did not reach a significance level. ZMBT was nevertheless, significantly less cytotoxic than ZDEC. This lower cytotoxicity of ZMBT relative to ZDEC and ZBUD also concurs with findings by Nakamura *et al.*[3] described earlier, who reported that the mercaptobenzothiazoles are markedly less cytotoxic than the dithiocarbamates.

Comparison of the toxicity ranking between ZnO activator and 2246 antioxidant varied depending on the cell line. Both U937 and HeLa cells showed ZnO to be less toxic than 2246. In contrast, experiments with L929 cells, indicated ZnO to be more toxic than 2246. These observations suggest the presence of cell-type related toxicity. It is noteworthy to mention that both U937 and HeLa cell lines were derived from human origin while L929 cells were obtained from murine species. It might be possible that cells from different species, express different responses to the same compounds. This could explain the differences in tolerance thresholds observed in this study. In one study on the Taxotere® anticancer agent[9], the *in vitro* cell growth of four different human colonic cell lines as determined by the MTT assay, was found to vary widely from 5% – 70%. This strongly suggests that different cell lines although from the same species, do express different reactivity towards the same chemical.

In general, U937 and HeLa cells showed similar chemical toxicity ranking in the following order; ZDEC > ZBUD = 2246 > ZMBT >ZnO. Toxicity ranking of L929 cells (ZDEC > ZBUD > ZnO > 2246 > ZMBT)

concurred only for ZDEC and ZBUD while ZMBT, ZnO and 2246 differed from those of the U937 and HeLa cells. Further examination of the individual LC_{50} value, suggests a cell-dependent toxicity. Interestingly, comparison of the cytotoxic responses among the U937, HeLa and L929 cells shows that ZDEC and ZMBT exerted similar LC_{50} values regardless of the types of cell line. In contrast, ZBUD exerted similar LC_{50} value in the U937 and L929 but not in HeLa cells. Likewise, ZnO incurred similar responses only in U937 and HeLa but not in L929 cells while 2246 showed similar responses in HeLa and L929 but not in U937 cells. LC_{50} values are useful for ranking the toxicity potentials of chemicals tested using the same cell line. Nevertheless, the present findings also suggest that caution should be reserved when using the LC_{50} values as a safety gauge, because of the possible variation in the cytotoxic potency with different cell lines.

In the present study, cytotoxicity of chemical additives was determined using the MTT assay described by Mosmann[11]. This assay was reported to be accurate, reproducible and sensitive even at a low cell density without interference from dead cells[15]. Interestingly, some chemical additives were found to react non-specifically with the MTT salt. The resulting colour development also absorbed strongly at the same test wavelength, attenuating the absorbance readings. This could indirectly imply that the chemical additives were well tolerated by cells since absorbance intensity of the MTT assay is related proportionally to the number of living cells[11]. It is apparent that some rubber chemical additives mimicked the conversion of MTT salt to similar blue formazans, thus masking the true nature of tetrazolium salt reduction by dehydrogenase enzymes in living cells. Microscopic examination showed the presence of blue formazans when the MTT salt was exposed to the chemical additives alone. However, these formazan displayed slightly amorphous structures compared to the crystal-like spikes elicited by living cells.

Among the chemical additives tested, ZMBT and 2246 elicited the strongest non-specific reactions, the intensity of which corresponded with their chemical concentrations. ZBUD reacted quite moderately at high concentrations (>10 $\mu g\ mL^{-1}$) whereas ZnO and ZDEC displayed very mild non-specific reactions. The reactivity of ZDEC was monitored under a smaller concentration range compared to the other chemicals because of the marked cell killing at high concentrations of ZDEC. Therefore, the non-specific reactivity of ZDEC as reported is only applicable within the chemical concentration range studied. No direct relationship was evident between intensity of the non-specific reactions and the cytotoxicity of these chemical additives. Precaution however, needs to be considered when the chemical concentration exceeds 10 $\mu g\ mL^{-1}$, particularly for ZMBT and 2246. It was not determined whether there exists a competition between chemical additives and cells for the MTT salt. However, non-specific reactions were only evident at high concentrations of chemicals. At these high concentrations, microscopic examination also revealed marked cell killing hence, there was unlikely to be any competition for the MTT salt by cells.

Although non-specific reactions may exist, in view of the many advantages of the MTT assay particularly in term of rapidity, precision and avoidance of radioisotopes, this method still offers immeasurable benefits in cytotoxicity studies. Nevertheless, in the application of MTT assay in cytotoxicity study, it is essential to employ appropriate blank controls to eliminate background interference attributed to possible non-specific reactions. In this study, blank

controls used consist of chemical solutions that were reacted with MTT salt in the absence of cells. Blank controls were employed in every experiment and this was found to be sufficient to account for the background interference. Cell tolerance towards test materials as deduced from spectroscopic measurements was found to support observations from microscopic examination of cells.

CONCLUSION

Among the five chemical additives, ZDEC and ZBUD were observed to be comparatively more cytotoxic when tested against three different cell lines. The toxicity rankings of ZDEC and ZBUD were not dependent on the type of cells used in the cytotoxicity assay, contrasting those observed with ZMBT, ZnO and 2246 chemicals which showed cell-dependent tendency. This study shows that LC_{50} values are useful in ranking the toxicity potentials of chemicals tested on the same cell line. Nevertheless, findings also suggest that caution should be reserved when using the LC_{50} values as a safety gauge, because of the possible variation in the cytotoxic potency with different cell lines.

Although the MTT assay is undoubtedly useful for cytotoxicity study, it is important to be aware of possible non-specific reactions between test materials and the tetrazolium reagent, which could generate false positive results. Background correction should be carried out simultaneously to eliminate such interferences.

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REFERENCES

1. ROBERTS, A. D. (1988) *Natural Rubber Science and Technology* . New York: Oxford University Press.

2. IKARASHI, Y., TOYODA, K., OHSAWA, N., UCHIMA, T.. TSUCHIYA, T., KANIWA, M., SATO, M., TAKAHASHI, M. AND NAKAMURA, A. (1992) Comparative Studies by Cell Culture and *in vivo* Implantation Test on the Toxicity of Natural Rubber Latex Materials. *Journal of Biomedical Materials Research*, **26**, 339–356.

3. NAKAMURA, A., IKARASHI, Y., TSUCHIYA, T., KANIWA, M. A., SATO, M., TOYODA, K., TAKAHASHI, M., OHSAWA, N. AND UCHIMA, T. (1990) Correlations among Chemical Constituents, Cytotoxicities and Tissue Responses: In the Case of Natural Rubber Latex Materials. *Biomaterials*, **11**, 92–94.

4. GAZELEY, K. F., GORTON, A. D. T. AND PENDLE, T. D. (1988) Technological Processing of Natural Rubber Latex. *Natural Rubber Science and Technology (Roberts, A. D. ed.)*, pp. 99–140. New York: Oxford University Press.

5. REEPMEYER, J. C. AND JUHL, Y. H. (1983) Contamination of Injectable Solutions with 2-mercaptobenzothiazole Leached from Rubber Closures. *Journal of Pharmaceutical Sciences*, **72**, 1302–1305.

6. KNUDSEN, B. B., LARSEN, E., EGSGAARD, H. AND MENNE, T. (1993) Release of Thiurams and Carbamates from Rubber Gloves. *Contact Dermatitis*, **28**, 63–69.

7. TALJA, M., SAARELA, K., RUUTU, M., ANDERSSON, L. C. AND ALFTHAN, O. (1993) Zinc Compounds in Urethral Catheters. A Possible Source of Toxicity? *Annales Chirurgiae et Gynaecologiae Supplementum*, **206**, 74–79

8. GRAHAM, D. T., MARK. G. E., POMEROY, A. R. AND MACARTHUR, E. B. (1984) *In vivo* Validation of a Cell Culture Test for Biocompatibility Testing of Urinary Catheters. *Journal of Biomedical Materials Research*. **18**, 1125–1135.

9. KARANTANIS, E., NICHOLSON, S. AND MORRIS. D. L. (1994) Taxotere Inhibits *in vitro* Growth of Human Colonic Cancer Cell Lines. *European Journal of Surgical Oncology*, **20**, 653–657.

10. REINECKE, P., CORVIN, J., GABBERT, H. E. AND GERHARZ. C. D. (1997) Anti-proliferative Effects of Paclitaxel (Taxol) on Human Renal Clear Cell Carcinomas *in vitro*. *European Journal of Cancer*, **33**, 1122–1129.

11. MOSMANN, T. (1983) Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *Journal of Immunological Methods*, **65**, 55–63.

12. CROWTHER, B. G., LEWIS, P. M. AND METHERELL, C. (1988) Compounding. *Natural Rubber Science and Technology* (*Roberts, A. D. ed.*), pp. 177–234. New York: Oxford University Press,

13. MAUSSER. R. F. (1987) Latex and Foam Rubber. *Rubber Technology* (*Morton, M. ed.*), pp. 518–560. New York: Van Nostrand Reinhold Company.

14. MORTON, M. (1987) Introduction to Polymer science. *Rubber Technology*, (*Morton, M. ed.*), pp. 1–19. New York: Van Nostrand Reinhold Company.

15. HUSSAIN, R. F., NOURI. A. M. AND OLIVER, R. T. (1993) A New Approach for Measurement of Cytotoxicity Using Colorimetric Assay. *Journal of Immunological Methods*, **160**, 89–96.