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Relationship between in vitro *Cell Culture Cytotoxicity and Sweat-extractable Dithiocarbamates in Natural Rubber Latex Gloves*

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Cytotoxicity of some of the commercially available natural rubber latex gloves was evaluated by in vitro *cell culture test using L929 mouse fibroblast cell lines. The cytotoxic potential ranged from non-cytotoxic to severely cytotoxic for the different brands tested. Since the cytotoxicity of latex products could be related to the release of leachable and toxic residual chemicals, the different brands of gloves were extracted in a physiologically simulated medium, namely, artificial sweat. The results revealed that a rubber accelerator, zinc diethyldithiocarbamate (ZDEC), was present in the gloves as residues and released into the artificial sweat. The amount of ZDEC released into the artificial sweat (sweat-extractable ZDEC) was estimated using high performance liquid chromatography. It was observed that there existed a linear relationship between the amount of sweat-extractable ZDEC and the cytotoxic potential of gloves represented in grades ranging from 0 to 3 as per* ISO 10993-5 (1999) *standard. This preliminary investigation showed that the amount of sweat-extractable ZDEC is a good indicator of the cytotoxicity potential of gloves and hence, the artificial sweat extraction may be further developed as a reliable test method to screen new latex medical products/formulations.*

Key words: artificial sweat; cytotoxicity; natural rubber; latex; surgical; gloves; HPLC; sweat-extractable ZDEC; *in vitro*; cell culture

Over the past two decades, exposure to natural rubber latex medical products caused a multitude of adverse allergic reactions among both the healthcare personnel and the general public[1]. The extensive research that followed attributed the cause of these allergic reactions to proteins and residual chemicals present in finished latex products. The synthetic latex products also pose a similar risk of allergy to residual chemicals[2–3]. Addition of chemicals such as vulcanising agents, accelerators, activators, stabilizers and antioxidants into the latex is indispensable as they impart good mechanical properties and shelf life required for finished latex goods. Some of these chemicals, if available in the biological system through

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contact with skin or mucous membrane, may lead to the development of serious allergic and toxicity related reactions. The offending chemicals include thiurams, dithiocarbamates, thiazoles, antioxidants, and their decomposition products. The dithiocarbamates are reported to induce the highest cell toxicity and allergenicity among the different classes of compounds used in the latex industry[4,5]. Certain natural rubber formulations compounded with dithiocarbamates are even recommended as positive controls for cytotoxicity tests in some of the testing standards[6,7]. In fact, a wealth of literature is available on the incidence of contact dermatitis of rubber products containing these chemicals[8–15]. Other toxicological effects include goitrogenicity[16,17], production of toxic by-products from mercaptobenzothiazole during ethylene oxide sterilisation[18,19], and tumor promotion[20]. The presence of residual and leachable chemicals in urinary catheters is found to be responsible for urethritis and urethral strictures in patients[21,22]. Apart from these, the nitrosamines formed by the decomposition of dithiocarbamates and thiurams are carcinogenic to animals and possibly in humans[23,24].

Occupational exposure to gloves has caused an increase in the incidence of contact dermatitis[25–28]. The prevalence of contact dermatitis is much greater compared to Type I allergy caused by latex proteins. A recent study in an Italian hospital indicated that about 93% of the health care workers had contact dermatitis to gloves[26]. Even though, the contact dermatitis is rarely severe compared to the latex protein allergy (Type I hypersensitivity), it is quite unpleasant to those who suffer from it. An accurate estimation of the sensitization and elicitation thresholds for many of the rubber chemicals are not known[29]. However, Knudsen and Menne reported a likely threshold level of less than 0.001 mg/cm^2 for leachable thiurams in rubber gloves by patch test[30].

As a result of the widespread complaints associated with the use of gloves, an *in vitro* cell culture test was carried out to assess the toxicity potential of some of the brands available in the local market. Since the cytotoxicity to latex gloves is mainly due to the presence of leachable and toxic chemicals, an attempt was made to estimate the amount of chemicals that released into the cell culture medium by extraction with dichloromethane. However, it was observed that an effective separation was not obtained between the two phases. In such situations, the estimation of leachable chemicals is better carried out using an extraction medium like artificial sweat that closely mimics the clinical use conditions of latex gloves. The use of artificial sweat as a medium to study the release and elicitation threshold of residual chemicals such as thiurams, mercaptobenzothiazole, carbamates *etc*. from rubber products has been reported earlier[30–32].

In the present study, the cytotoxicity potential of some of the commercially available natural rubber latex gloves was evaluated by *in vitro* cell culture method according to ISO test methods. The gloves were then extracted in artificial sweat to evaluate the extent of release of residual chemicals into the sweat solution (sweat-extractable chemicals). The identification and estimation of the sweat extractable chemicals were performed using simple analytical techniques. In addition, this work was also aimed to find a possible relationship between the artificial sweat extractable dithiocarbamates and the cytotoxicity grade of latex gloves.

MATERIALS AND METHODS

Eleven brands of surgical/examination gloves (pre-powdered and powder-free) made of natural rubber latex were collected from local sources

and were code named with English alphabets. For the *in vitro* cell culture cytotoxicity test, L929 mouse fibroblast cell lines procured from the National Centre for Cell Science (NCCS, Pune, India) was used. All the test samples were sterilised by ethylene oxide gas before cell culture cytotoxicity test. Samples were de-gassed as per a validated procedure, which ensure the complete removal of residual ethylene oxide. Silica gel 60 plates for thin layer chromatography were procured from Merck, Germany and used without activation. Chemicals such as dichloromethane, acetone, hexane, ether, sodium chloride and urea were obtained from local sources. Lactic acid was procured from Sigma Aldrich, U.S.A. Copper (II) sulphate pentahydrate was obtained from Merck, Germany. Zinc diethyldithiocarbamate (ZDEC) and zinc dibutyldithiocarbamate (ZDBC) were supplied by National Organic Chemical Industries Limited (NOCIL, Mumbai, India) and zinc dibenzyldithiocarbamate (ZBEC) was obtained from Bayer AG, Germany. The antioxidant, Wingstay L® (reaction product of Butylated p-cresol and Cyclopentadiene) was purchased from Plasti Chem Pvt India, India.

In vitro Cell Culture Cytotoxicity Test

The gloves were tested by both direct contact and test on extract assays between L929 fibroblasts and either the materials or extracts from these materials. Both the tests were performed according to *ISO 10993-5 (1999)* standard[33]. The L929 cell lines were maintained in RPMI-1640 culture medium (Hi Media, Pune, India) supplemented with 10% fetal bovine serum (Sigma, U. S. A), 100 IU/mL penicillin and 100 µg/mL medical grade streptomycin. The cells were seeded and incubated at 37 ± 2ºC in 95% humid and 5% carbon dioxide atmosphere till 80% confluency was attained. The starting cell density was 1×10^3 cells/mL for direct contact method and 1×10^2 cells/100 µL for test on extract method.

In the direct contact assay, round-shaped samples of the glove material of 4 mm diameter was placed in contact with the cells in a 24-well dish and incubated at 37 ± 2ºC for 24 h. The material was glued to the bottom of the well with a silastic glue to prevent from floating. The test sample was placed at the centre of the well so that one-tenth of the cell layer surface was covered. Copper and tissue culture grade polystyrene (TCPS) discs were used as the positive and negative controls, respectively. The degree of cytotoxicity was determined qualitatively by viewing the cells around the material under an inverted phase contrast microscope (Leica, DMIL, Germany), and the material was graded according to a cytotoxicity scale ranging from 0–3 as per a validated test procedure based on *ISO 10993-5*. If the confluency of healthy cells is greater than 80%, it is graded as '0' (non-cytotoxic); if it is between 60%–80%, the grade given is '1' (mildly cytotoxic); if it is 40%–60%, the material is graded as '2' (moderately cytotoxic) and if it is less than 40%, a grading of '3' (severely cytotoxic) was given. Besides, the general morphology, vacuolisation, detachment of cells and cell lyses were also looked at. Three replicates were used for both the test material and the controls.

The test on extract assay assesses the toxicity of the glove extract. The glove extract was prepared at 37 ± 2ºC by incubating 0.1 g of the glove in 1 mL of the culture medium supplemented with foetal bovine serum for 24 h. A monolayer of cells grown in a 96 well dish containing the cell culture medium was taken and the medium was drained off completely. The glove extract was added carefully to the multi-well plate without disturbing the cell

monolayer. This was then incubated at 37 ± 2ºC for 24 h. The cell response was compared and graded in the cytotoxicity scale as described above. Diluted phenol (1.3 mg/mL) and extract of TCPS were used as the positive and negative controls, respectively. Six replicates were used for both the test material and the controls.

Extraction of Gloves in Artificial Sweat

Artificial sweat was prepared according to the composition given in the European Standard *EN1811:1998(E)* (*Table 1*).

The pH of the artificial sweat solution was adjusted to 6.5 ± 0.1 by adding 1% aqueous ammonia solution. The glove (2 g) was cut into pieces of approximately 2 × 0.5 cm^2 size and added to 100 mL of artificial sweat solution. The extraction was carried out at 37 ± 2ºC for a period of 24 h with constant mechanical shaking. After extraction, the sweat solution was extracted five times, each with 40 mL dichloromethane. The solvent fraction in each extraction was collected and distilled off using a flash evaporator at 60ºC to recover the residue. This was then re-dissolved in dichloromethane and subjected to ultraviolet-visible (UV-VIS) spectroscopy, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) for the identification and the estimation of sweat-extractable chemicals.

Identification and Estimation of Sweat-extractable Chemicals

UV-VIS Spectroscopy. A preliminary idea about the nature of the extractables in sweat solution was obtained from UV-VIS spectroscopic analysis. The dichloromethane fraction after sweat extraction was scanned in the range 200 nm – 500 nm using a UV spectrophotometer (Model UV 1601, Shimadzu, Japan). The absorption spectra of the sweat extract of the gloves were then compared with the standard solutions of the commonly used zinc salts of dithiocarbamates namely, ZDEC, ZDBC and ZBEC and a phenolic antioxidant, Wingstay L®. These solutions were prepared as 0.1% solution in dichloromethane. The sweat extract of the gloves showed absorption peaks very close to the absorption wavelengths of dithiocarbamates. Hence, further analysis (TLC and HPLC) was performed to identify and estimate the sweat extractable dithiocarbamates.

Thin Layer Chromatography

TLC was used to determine if the residues obtained after sweat extraction of gloves contained single or mixture of dithiocarbamates and, if present, to find the respective R_f values. Since the separation of zinc salts of dithiocarbamates was very poor on the TLC plate, they were converted into their respective copper complexes. The residue obtained after sweat extraction of gloves was re-dissolved in 10 mL of dichloromethane. From this solution, 5 mL was pipetted out and mixed with 1.7 mL of 0.01 M aqueous ammoniacal copper sulphate solution. The mixture was then vortexed for 2 min to obtain the copper-dithiocarbamate complex quantitatively[34,35]. The dichloromethane layer was separated out and the copper-dithiocarbamate complex was recovered by evaporating the solvent out. This residue was re-dissolved in 5 mL of HPLC grade acetone for analysis by HPLC. This procedure was repeated for all the different brands of gloves. Known quantities of ZDEC, ZDBC and ZBEC were taken and converted them into respective copper complexes, which served as standards.

TABLE 1. COMPOSITION OF ARTIFICIAL SWEAT

Constituent	Weight (%)
Sodium chloride	0.5
Lactic acid	0.1
Urea	0.1
Deionised water	To make to 100

A solution of hexane and ether in the ratio 90:10 (v/v) was used as the mobile phase for TLC analysis. Adequate time was given for the chamber to get saturated with solvent vapours. A 5 µL each of the above test samples and standards were applied on the TLC plate using a micro-syringe. The plate was dried and kept in the rectangular chamber containing the mobile phase. When the solvent front had moved about 13 cm above the application line, the plate was removed and dried. They were then kept in another chamber saturated with iodine vapours for colour development. The distance moved by the sample spot and the solvent front from the application line was noted. The respective R_f values (*i.e.*, the ratio of the distance moved by the sample spot to that of the solvent front) were determined and compared with the standard.

High Performance Liquid Chromatography

Various chromatographic techniques have been used for the quantification of allergologically relevant accelerators present in rubber products[14,30,32,36,37]. Since the zinc salts of dithiocarbamates were reported to undergo substitution reaction with the metal parts of the HPLC column[38], it was necessary to convert zinc dithiocarbamates into their copper complexes. The procedure used for this was already detailed in the previous section. The HPLC system consisted of a Waters 510 pump, C18 column and 7725 Rheodyne injector (Waters Inc., U.S.A). The presence of copper-dithiocarbamates complex was detected using a variable-wavelength UV detector 486 set at 435 nm. Separation was achieved in a column (4.6 × 75 mm) packed with C18 material (3.5 µm). The mobile phase used was acetone-water (90:10, v/v). A flow rate of 1.0 mL/min was used. Known quantity of ZDEC was converted into respective copper complex, which served as standard. The amount of sweat-extractable ZDEC from gloves was determined quantitatively by comparison with the standard.

Statistical Analysis

The cytotoxicity grade obtained in direct contact and test on extract assays was related to the amount of sweat-extractable ZDEC of some brands of gloves. Statistical analysis of the data was carried out using Spearman's rank correlation test[39].

RESULTS AND DISCUSSION

***In vitro* Cell Culture Cytotoxicity Test**

Of the eleven brands of gloves tested, eight were of surgical type and three were of examination type. The cell responses to the different brands of gloves are given in *Table 2*.

TABLE 2. CELL RESPONSES TO DIFFERENT BRANDS OF LATEX GLOVES

Sl. No.	Sample codes	Glove type	Direct contact	Test on extract
1	A	Surgical, pre-powdered	1	0
2	B	Surgical, pre-powdered	3	0
3	C	Surgical, pre-powdered	1	1
4	D	Surgical, pre-powdered	2	a
5	E	Surgical, pre-powdered	0	0
6	F	Surgical, pre-powdered	0	0
7	G	Surgical, pre-powdered	0	3
8	H	Surgical, pre-powdered	3	3
9	I	Examination, powder-free	0	0
10	J	Examination, powdered	3	3
11	K	Examination, powder-free	0	0

0: Non-cytotoxic; 1: Mildly cytotoxic; 2: Moderately cytotoxic; 3: Severely cytotoxic; *a*: Tests not conducted

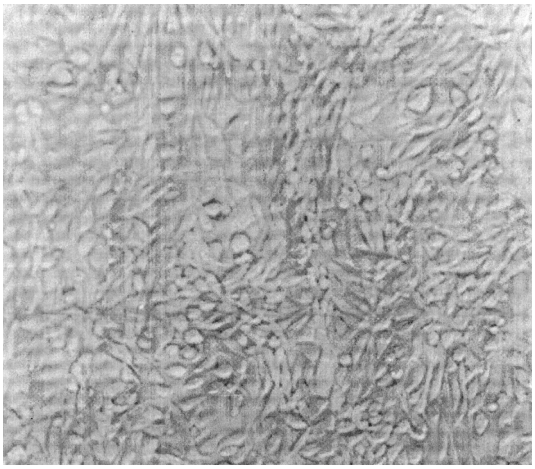
The cell responses varied from non-cytotoxic (grade '0') to severely cytotoxic (grade '3') for the different brands of gloves. The direct contact test showed that nearly 55% of the gloves selected were cytotoxic to L929 cells. However, the test on extract assay showed that only about 35% of the glove brands exhibited cytotoxicity. This may be due to the difference in the amount of residual chemicals in the gloves that is available to the cells in the two types of *in vitro* tests. *Figure 1* shows typical cell responses to the natural rubber latex gloves.

In the case of non-cytotoxic gloves, the L929 cells retained their morphology and were spindle shaped [*Figure 1(a)*]. On the other hand, exposure to the toxic gloves caused changes in the cell morphology and the damaged cells appeared nearly spherical in shape [*Figure 1(b)*]. The cytotoxic potential was determined from the morphological change and cytopathic or cell death exhibited by the cell line in comparison with the negative and the positive control. The *in vitro* cell culture cytotoxicity of gloves indicated that the residual chemicals were released into the culture medium inducing toxicity to the surrounding cells.

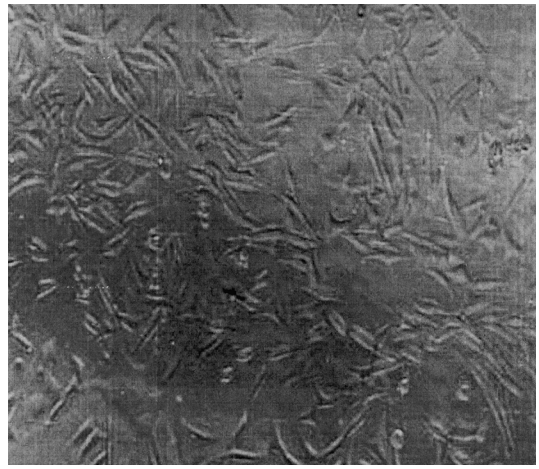
Identification and Estimation of Sweat-extractable Chemicals

Preliminary investigation using UV-VIS spectroscopy indicated that dithiocarbamates was present in the sweat extract. *Figure 2* gives a typical UV spectrum (brand 'E') obtained along with the control ZDEC sample.

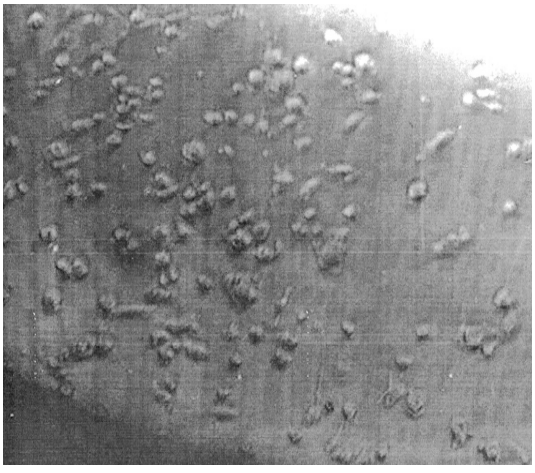
It was found that both the control and the brand 'E' showed an absorption band at 274 nm characteristic of the dithiocarbamate entity. This observation indicated that the



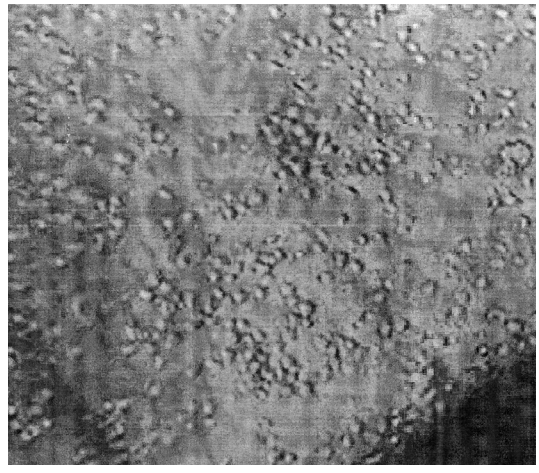
A



B



C



D

Figure 1. Cytotoxic responses of latex gloves. A: Non-cytotoxic; B: Mildly cytotoxic; C: Moderately cytotoxic; D: Severely cytotoxic.

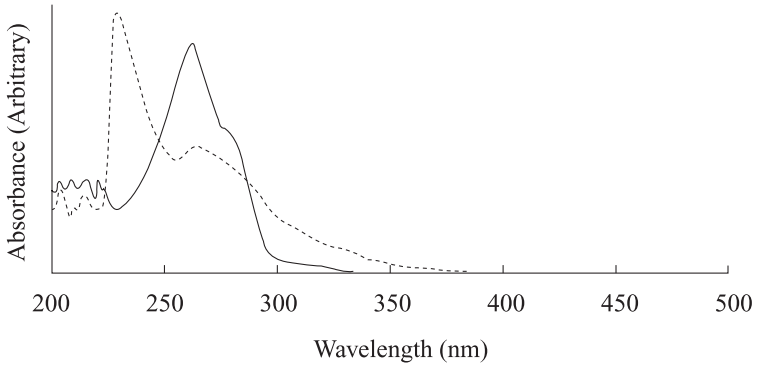


Figure 2. UV spectrum of ZDEC (——) and brand 'E' (- - - -).

dithiocarbamates accelerators present in the gloves were released into the artificial sweat solution. However, as the UV spectrumof the commonly used dithiocarbamates gave an absorption maxima in the range 265 nm – 270 nm, it was difficult to determine the presence of a single or a mixture of dithiocarbamates by UV spectroscopy. An additional peak at 228 nm in the spectrum of brand 'E' was attributed to lactic acid. The lactic acid, owing to its solubility in dichloromethane, migrated from artificial sweat into dichloromethane fraction during the recovery of ZDEC from artificial sweat solution.

It was anticipated that the zinc salts of dithiocarbamates would show different R_f values in TLC analysis and hence can be used to identify different dithiocarbamates present in the glove brands. However, the commonly used zinc salts of dithiocarbamates, namely, ZDEC, ZDBC and ZBEC when subjected to TLC showed very poor separation on the TLC plate. On the contrary, their respective copper salts exhibited excellent separation on the TLC plate. The R_f values of the copper-dithiocarbamate complexes obtained from standard ZDEC, ZDBC, ZBEC compounds and the sweat extracts of commercial gloves are given in *Table 3*.

It was observed that except for brands 'I' and 'K', the chromatograms of all other sweat extracts contained a common spot with an R_f value of 0.41 corresponding to ZDEC. It has already been established that ZDEC was the most cytotoxic compound among the commonly used dithiocarbamates (ZDEC, ZDBC and ZBEC) and antioxidants in the latex industry[4]. This indicated that the major contributing factor towards cell toxicity is the presence and availability of the residual ZDEC in the gloves. This is further supported by the observation that the brands 'I' and 'K' with no detectable ZDEC exhibited a non-cytotoxic response to L929 cell lines. Since the brands 'I' and 'K' did not contain any detectable ZDEC, they were excluded from further analysis. As all the remaining nine brands of gloves contained ZDEC, only five brands of gloves that differed in their cytotoxic response was selected for sweat extraction studies. Prior to the quantification of sweat-extractable ZDEC using HPLC, the UV spectrum of copper dithiocarbamate complex of standard ZDEC and the sweat extract of a commercial

TABLE 3. R_f VALUES OF COPPER-DITHIOCARBAMATE COMPLEXES OF STANDARD DITHIOCARBAMATES AND DITHIOCARBAMATES RELEASED FROM COMMERCIAL GLOVES

Type	Sample codes	R_f value of standard dithiocarbamates	R_f value of sweat extractable dithiocarbamates
Control	CDEC	0.41	–
	CDBC	0.86	–
	CBEC	0.70	–
Test samples	A*	–	0.40
	B*	–	0.41
	C*	–	0.41
	D*	–	0.41
	E*	–	0.41
	F*	–	0.41
	G*	–	0.41
	H*	–	0.41
	I*	–	–
	J*	–	0.42
	K*	–	–

CDEC: Copper diethyldithiocarbamate; CDBC: Copper dibutyldithiocarbamate; CBEC: Copper dibenzyldithiocarbamate

*Copper-Dithiocarbamate complex of the sweat extract.

glove (brand 'E') was recorded and is given in *Figure 3*.

The spectrum contained a characteristic absorption maximum at 435 nm for both the sample and the standard. Hence, this particular wavelength was selected as the detection wavelength in HPLC. A typical chromatogram of copper-dithiocarbamate complex of standard ZDEC and the sweat extract of a commercial glove (brand 'E') is given in *Figure 4*.

The presence of ZDEC in the sweat extract was confirmed from the retention time of the copper complexes of the sweat extract. *Figure 4* showed that the retention time of copper complex of both ZDEC and sweat extract of brand 'E' was 3.15 min, a value quite different from that of ZDBC (5.7 min) and ZBEC (4.5 min), indicating that the residual ZDEC was released into the artificial sweat solution from the gloves. Thus HPLC facilitated the identification of the type of dithiocarbamate present in glove brands using their respective retention times. *Table 4* gives the amount of sweat-extractable ZDEC of the different brands of gloves.

The artificial sweat extraction of the gloves showed that the amount of sweat-extractable ZDEC varied from brand to brand. The amount of sweat-extractable ZDEC ranged

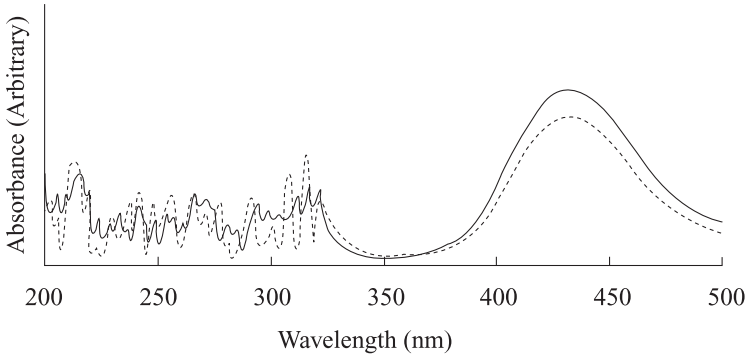


Figure 3. UV spectrum of copper complex of ZDEC (——) and brand 'E' (- - - -).

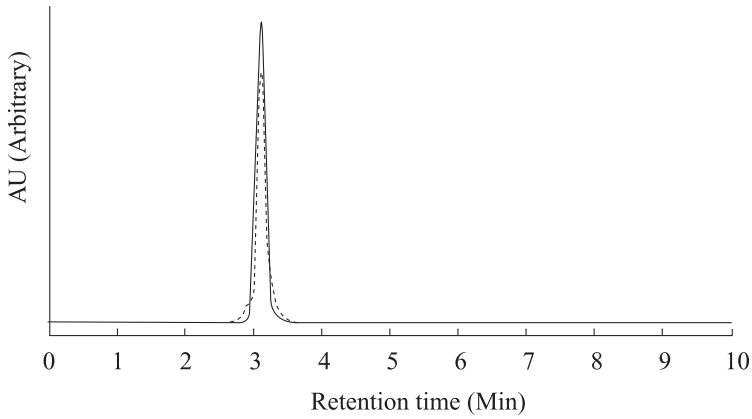


Figure 4. Chromatogram of copper complex of ZDEC (——) and brand 'E' (- - - -).

TABLE 4. AMOUNT OF SWEAT-EXTRACTABLE ZDEC IN COMMERCIAL GLOVES

Sample codes	Sweat-extractable ZDEC (μg/g)
A	94
F	39
C	78
E	61
J	173

from 39 μg/g (brand 'F') to as high as 173 μg/g (brand 'J'). Knudsen *et al*. found that gloves releasing higher amounts of thiurams and/or dithiocarbamates in synthetic sweat elicited more number of positive reactions during patch testing of gloves[31]. This follows that the allergenicity of brand 'J' would be more compared to that of brand 'F'.

Relationship between Cytotoxicity Grade and Sweat-extractable Dithiocarbamates

The occurrence of strictures associated with the use of urinary catheters in patients is found to be related to the leachable chemicals and a good agreement between *in vitro* cell culture and rabbit implantation test of catheters has been obtained[21]. This prompted the authors to explore the possibility of a relationship between the sweat-extractable ZDEC and the cytotoxicity response. The rationale for this study was based on the fact that the residual dithiocarbamates in gloves contribute to the cell toxicities rather than antioxidants[4]. The colony assay using V79 cells showed that the dithiocarbamates are highly toxic and ZDEC was the second highest cytotoxic chemical[40]. Apart from this, it was also shown that among ZDEC, ZDBC and ZBEC accelerators, ZDEC was the highest migrating accelerator into artificial sweat[41]. The above mentioned factors indicated that the major contributing factor towards cell toxicity is the presence and availability of the residual ZDEC in the gloves.

Figures 5a and *5b* give the relationship between the sweat-extractable ZDEC and cytotoxicity response of latex gloves in the direct contact assay and test on extract assay, respectively.

Figures 5a and *5b* indicated a linear relationship existing between the sweat extractable ZDEC and the cytotoxicity grade in the two types of *in vitro* cell culture methods. This follows that the cytotoxic potential of the gloves increased with an increase in the amount of sweat-extractable ZDEC. The Spearman's rank correlation coefficients between the sweat extractable ZDEC and cytotoxicity grade were 0.95 and 0.67 for direct contact assay and test on extract assay, respectively. The effect of the amount of leachable and toxic ZDEC on the cytotoxic potential of the latex gloves was statistically significant ($p = 0.01$) in the case of direct contact assay in contrast to test on extract assay ($p = 0.21$). *Figure 5a* showed that a non-cytotoxic response was evident in gloves having extractable dithiocarbamates equal to or less than 61 μg/g of glove. A mildly cytotoxic behaviour was observed when the amount of sweat-extractable ZDEC was in the range 78 μg/g – 94 μg/g of glove. The material was severely cytotoxic when the sweat-extractable ZDEC rose to about 173 μg/g of glove.

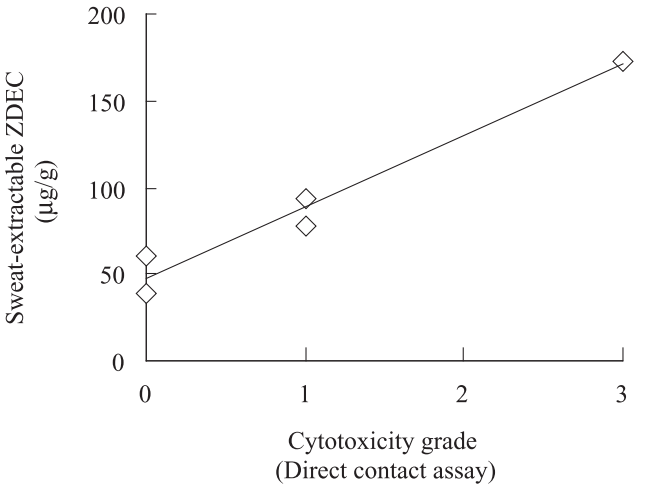


Figure 5a. Plot of cytotoxicity grade vs. *sweat-extractable ZDEC.*

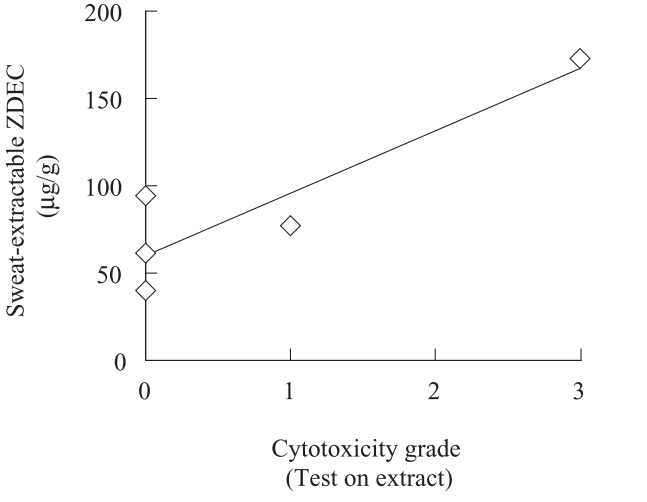


Figure 5b. Plot of cytotoxicity grade vs. *sweat-extractable ZDEC.*

In an earlier study by Ikarashi *et al*. it was showed that the thickness of the inflammatory layer in rabbit implantation test could be correlated with the amount of leachable chemicals from rubber gloves[40]. Their study indicated that the degree of inflammatory response is attributed to the amount and toxic potential of residual chemicals as well as their rate of leaching into the tissue and the correlation coefficient obtained was 0.87.

It is interesting to note that the glove brand 'A' showed a difference in cytotoxicity response in the two types of *in vitro* tests conducted. In the direct contact test, brand 'A' showed a mild cytotoxicity response while in test on extract assay a non-toxic response was observed. This may be due to the different rate of contribution of ZDEC to the cytotoxicity in the two types of *in vitro* tests. As a result, a lower correlation was observed between the sweat-extractable ZDEC and the cytotoxicity grade in the test on extract assay (*Figure 5b*). An earlier study by Nakamura *et al*. indicated that a different rate of contribution of dithiocarbamates towards cytotoxicity was found between colony assay which uses material extract and agar diffusion assay and the cause was attributed to solubility of dithiocarbamates and hydrophobicity of the cell culture medium[4].

It may be also noted that the sweat extractable ZDEC of brand 'C' (78 µg/g) is lower than that of brand 'A' but its cytotoxicity grade is '1'. The cytotoxicity response in the test on extract is dependant on the nature of leachable chemicals. Manufacturers adopted different ingredients as stabilizers, vulcanising agents and antioxidants as well as different online processing techniques for the production of gloves. This means that the gloves may contain residual chemicals that differ in their extractability into the cell culture medium. As a result, the exact cause of this discrepancy in the cytotoxic response of brands 'C' and 'A' is not known.

The present study demonstrated that the residual dithiocarbamates from the latex gloves was released into the artificial sweat. Graham *et al*. had successfully related latex catheter toxicity to the tissue damage occurred in a sheep model with the cytotoxicity ranking in an *in vitro* cell culture test[42]. They also suggested that the cell culture test could be employed as a routine safety tests for urinary catheters, however, is expensive. It was found that the cytotoxic potential of the gloves increased with an increase in the amount of the sweat extractable ZDEC. As a preliminary study, it is of potential to screen the new latex products containing dithiocarbamates using artificial sweat-extraction before subjecting them to expensive *in vitro* and *in vivo* biological tests. However, further studies with significantly higher number of latex gloves/latex formulations are needed to establish a threshold limit for sweat-extractable ZDEC that exhibit toxicity in *in vitro* cell culture evaluation.

CONCLUSIONS

The primary cause of the cytotoxicity was the chemicals, which were added into the latex to improve the mechanical performance of the gloves. The *in vitro* cell culture test indicated that the residual chemicals present in the finished latex gloves released into the culture medium and caused cell injury. The release of residual chemicals from the gloves was further established by extracting them in artificial sweat. The TLC analysis indicated that the three copper-dithiocarbamate complexes differed significantly in their respective R_f values, which in turn could effectively be used as an identification test for their presence in natural rubber or synthetic latex dipped goods.

Alternatively, HPLC could also be used for the identification of the dithiocarbamates in gloves from their retention times. The amount of sweat-extractable ZDEC showed a linear relationship with cytotoxicity grade indicating that the cytotoxic potential of gloves increased with an increase in the amount of sweat-extractable ZDEC.

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