The Mutagenic Potential of Chloroform, Orange Oil, Eucalyptus Oil and Halothane by *Salmonella*/Microsome Assay

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Summary

The aim of this study was to examine mutagenic activity of four commercially available gutta-percha solvents by means of the Salmonella*/microsome assay. The examined solvents were: chloroform, orange oil, eucalyptus oil and halothane in amounts of 10 µl, 30 µl, 50 µl, 100 µl and 200 µl. Standard plate incorporation Ames test was preformed by using two tester strains of* Salmonella typhimurium*, TA 98 and TA 100, with metabolic activation of S9. The results showed toxicity of eucalyptus oil in all aliquots, orange oil in aliquots of 50 µl and above and chloroform in aliquots of 100 µl and 200 µl, but all four substances responded negative to the Ames test. These results indicate that the tested solvents do not possess mutagenic activity toward the* Salmonella *strains used.*

Key words: *Ames test, Chloroform, Orange oil, Eucalyptus oil, Halothane.*

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Introduction

Retreatment of gutta-percha filled root canals could be achieved by rotary, manual or thermal techniques with or without addition of various solvents (1, 2), and laser (3, 4). The role of a solvent is not only in dissolving the gutta-percha but also in lubrication of instruments thus diminishing the possibility of instrument breakage, root perforation and canal straightening (2).

The most popular and efficient solvent, chloroform is classed as possibly carcinogen to humans (5, 6). Consequently, many other chemicals are tested for effectiveness in the search for a possible substitution in retreatment procedures (7). Among them halothane and eucalyptus oil have demonstrated many desirable working qualities (2, 8). Orange oil is also proposed as an effective gutta-percha solvent at 37ºC (2).

As can be concluded from the literature there is great concern about the mutagenic and carcinogenic properties of any material or chemical that is used in contact with the human body, such as root canal filling materials (9). Dental materials, drugs and solutions not only influence the patient they are used on, but furthermore, daily and repeated contact of dental staff with it could lead to severe occupational disorders (10). One of the tests recommended as

a screening mutagenesis test for chemicals and environmental samples is the short-term Ames test because of its extensive data base and good correlation with carcinogenicity (9). Cultured *Salmonella* strains have mutilated histidine gene and cannot grow on media that do not contain histidine. If the tested chemical is mutagenic, causes base pair substitution at GC and AT, and frame shift mutations. These mutations will replace the abnormal histidine gene and it will become functional again. Bacteria will now grow on the histidine-free medium. The increased number of bacterial colonies in comparison with the number of spontaneous revertants, that are not under influence of extrinsic factors, gives information on the mutagenic potential of tested chemicals (11). Although the mechanism involved in mutations are complex and vary between the species, the results of the bacterial Ames test are valuable because of the similar constitution of DNA in all organisms(12, 13) .

The aim of this study was to examine mutagenic activity of four gutta-percha solvents by means of the *Salmonella*/microsome assay.

Materials and methods

Tested solvents were Orange oil (Aromara, d.o.o., Zagreb, Croatia), Chloroform (Kemika, d.o.o., Zagreb, Croatia), Eucalypti aetherolum (Kemig, d.o.o. Zagreb, Croatia), and Fluothane (halothane) (Zeneca Ltd, MacClesfield, Chesire, UK).

Mutagenicity tests were carried out by the standard plate incorporation test as previously described by Maron and Ames (14). Two tester strains of *Salmonella typhimurium* TA 98 and TA 100, kindly provided by B. N. Ames, University of California, Berkley, USA, were used to detect frame-shift and base-pair mutation, respectively. Tested substances of 10 µl, 30 µl, 50 µl, 100 µl and 200 µl were plated into Wogel-Bonner's basal agar plate with 2 ml of soft agar. The amount of 0.5 mM L-histidine - 0.5 mM biotin solution had been previously added. Overnight culture of *Salmonella typhimurium* TA 98 or TA 100 (0.1 ml) with metabolic activation (0.5 ml of +S9 mixture) was added to the plate.

The S9 mixture contained 50 µl of hepatic S9 prepared from male Wistar rats pretreated with intraperitoneal injection containing Aroclor 1254 (500 mg/kg) dissolved in corn oil. Immediately before mutagenicity testing, the S9 fraction was passed sequentially through Millipore membrane filters (0.45 µm and 0.22µ m filter units) to remove any contaminating microorganism. Each sample was plated in triplicate, and its revertants were scored after 48 h incubation at 37ºC. As a positive control for this assay, 2-aminofluorene (2-AF) at a concentration of 25 µg/plate was used to monitor the sensitivity of bacterial strains and the activity of the rat-liver S9. The mutagenicity of gutta-percha solvents was expressed as the number of revertants per plate per µl of solvent.

Results

The results presented in Table 1 are the mean values of triplicate for certain tester strain and test substance aliquot (SD < 10%). All tested substances resulted in negative response to the Ames test both on TA98 and TA100, in relation to positive control 2-aminofluorene (diagnostic mutagen). Eucalyptus oil in all quantities (10 µl/plate, 30 µl/plate, 50 µl/plate, 100 µl/plate and 200 µl/plate) was toxic. The number of revertants per plate was the same as in negative control plates (the number of spontaneous revertants) for Orange oil in aliquots of 10 an 30 µl/plate, and Chloroform in aliquots of 10, 30 and 50 µl/plate. Higher concentrations of Orange oil and Eucalyptus oil showed toxic effects. Response of Fluothane in all aliquots was negative, i.e. without toxic effect.

Discussion

The need for testing biological qualities of gutta-percha solvents arises from the great number of retreatment procedures that are taken in everyday endodontic practice. The Ames test has been chosen for screening the mutagenic potential of four gutta-percha solvents because of its validity, acceptable cost and accessibility (13).

Although chloroform is classified as a possible carcinogen (15), disagreement still exists about its carcinogenicity. Although induction of carcinomas after oral administration of chloroform has been proved in laboratory animals, its carcinogenicity in

humans has not been proven (16). Testing of chloroform as a drinking water disinfection agent by Ames test on TA 100 and TA 98 strains of *Salmonella typhimurium* did not show mutagenic properties of chloroform (17), and not association between colorectal carcinoma and drinking water contaminated with chloroform (18).

Analyzing the air from the breathing zone of the dental team Allard and Andersson (10) found that concentration of chloroform during the root filling procedure using five percent rosin in chloroform, is within safe limits if care is taken and the liquids were administrated using special tubes. Nevertheless, as there is doubt about the carcinogenetic properties of chloroform and its possible metabolisation to highly hepatotoxic phosgene by cytochrome P-450 reductase, it should be avoided (19).

Orange oil is one of proposed substitutes for chloroform. In study by Hansen (20) there was no significant difference between orange oil and other solvents in its ability to dissolve gutta-percha. The findings of suppression of pulmonary adenoma formation by diet, of which one of the components was "terpeneless" orange oil, have been published recently (21). The negative Ames test confirms its non-mutagenic properties, although it showed some toxic effect.

Eucalyptus oil is not considered carcinogenic by the Public Health Service (PHS) (6). In addition, many investigations of its efficiency have confirmed its ability to dissolve gutta-percha (7, 8). Although eucalyptol in this study did not show mutagenicity, toxic effect was observed on *Salmonella typhimurium*.

Halothane (Fluothane) showed the most neutral, neither mutagenic or toxic effect on strains of *Salmonella typhimurium* used in this study. Results of the present study, in combination with those published by Wourms et al. (2) who found that halothane dissolved the gutta-percha samples about twice as fast as eucalyptus oil, indicate that this solvent could be the most suitable, with minimal side effects and relative efficiency. However, care must be taken to minimize staff and patient exposure because of possible respiratory depression.

In this study 2 of 5 bacterial strains recommended by Maron and Ames (14) were used, which is in accordance with the investigation of Örstavik et al. (9). Ames test showed that the tested solvents do not possess mutagenic activity, which should be confirmed by further tests.