MIGRATION OF STYRENE FROM POLYSTYRENE FOOD CONTAINERS INTO FOOD SIMULANTS: A META-ANALYSIS

by

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DEDICATION

I dedicate this research work to GOD.

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ABSTRACT

The use of polystyrene containers in this modern world has increased significantly over the years. As a result of our fast-paced environment, people want to go meals and drinks, hence the use of plastic take away containers. Polystyrene is suitable for a variety of food-contact applications as a result of its light weight, versatility and cost effectiveness. However, traces of residual styrene monomer remain in polystyrene containers after polymerization. The chronic effects of styrene monomer and metabolite of styrene are chromosomal aberrations in lymphocytes of humans and damage to the liver and nervous system. Since the human exposure to styrene is predominantly via inhalation, the lung represents one major organ of entry and first contact. Studies included in this meta-analysis were selected from various database. The amount of migrated styrene was obtained from the studies and the overall effect of styrene migration was determined. The result of the meta-analysis indicates that higher amount of residual styrene is present in polystyrene containers than the amount of styrene migrating from polystyrene containers into food simulants. Test for heterogeneity showed the presence of heterogeneity in the meta-analysis result. The presence of heterogeneity impacted the result obtained, by indicating that more migrated styrene was present in food simulants than residual styrene in polystyrene containers. The rate of styrene migration was observed to obey Fick's second law of diffusion with increasing amount of styrene from polystyrene containers into food simulants as the contact time increases.

LIST OF ABBREVIATIONS USED

.CSV	comma-separated-values
AHRQ	agency for healthcare research and quality
BASF	badische anilin & soda-fabrik
DNA	deoxyribonucleic acid
EDSTAC	endocrine disruptor screening and testing advisory committee
EPA	environmental protection agency
EU	European union
EPS	expandable polystyrene
FDA	food and drug administration
FAO	food and agriculture organization
GPPS	general purpose polystyrene
GRADE	grading of recommendations assessment, development and evaluation
GOSH	graphic display of heterogeneity
HIPS	high-impact polystyrene
IARC	International agency for research on cancer
PROSPERO	International prospective register of systematic reviews
LOD	limit of detection
MOOSE	meta-analysis of observational studies in epidemiology
PICOS	participant, interventions, comparations, outcomes and study design
PRISMA	preferred reporting items for systematic reviews and meta-analysis
QUOROM	quality of reporting of meta-analysis

SCF	scientific committee for food
SMD	standardized mean difference
SIRC	styrene information research center
TDIs	tolerable daily intakes
Tg	glass transition temperature
v/v	volume per volume
VOCs	volatile organic compounds
w/v	weight per volume
WHO	world health organization
wt/day	weight per day

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CHAPTER 1: INTRODUCTION

Eduard Simon, a German apothecary, discovered styrene first in 1839 [1]. He distilled the resin of the American sweetgum tree called storax, and obtained an oily substance called styrol (now styrene). Simon found that the styrol thickened into a rubber-like substance after some days upon exposure to heat, air or light. This was called styrol oxide, thinking that the material had oxidized [1]. Almost 80 years after, Herman Staudinger, a German organic chemist found that styrol, which comprised of long chains of styrene molecules, was polystyrene [2]. This led to the commercial manufacturing of polystyrene, which began in 1931 by scientists at Badische Anilin & Soda-Fabrik (BASF) [2]. Residual styrene monomer not chemically bonded to the polystyrene backbone during polymerization can easily diffuse into the food product. The goal of this research is to synthesize data from several studies and use meta-analysis to obtain a cohesive amount of styrene migrating from polystyrene food containers into food simulants.

1.1 Styrene

Styrene (Figure 1.1) can also be called styrol, ethenylbenzene, phenylethylene, vinylbenzene, cinnamene and styrene monomer. It has a chemical formula C_8H_8, and structural formula C_6H_5-CH=CH_2. It is identified by a sweet, pungent odor with colorless or light-yellow appearance. Styrene is a crosslinking agent that links one or more monomers together. It is an organic solvent and one of the most prolific industrial solvents worldwide. Traces of residual styrene remain in polystyrene containers after polymerization. This styrene that migrates into food has been known to have toxic effects, hence, the purpose of this study.

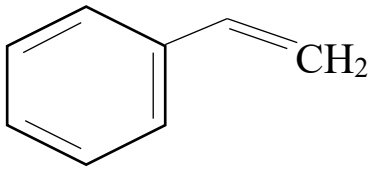

Figure 1.1: Styrene structural formula

Styrene can be found naturally in food. It is obtained from storax, the resin of Liquidambar orientalis trees. It can be produced during the biodegradation of a large mixture of naturally

occurring flavoring compounds with structures such as cinnamic acid, cinnamic aldehyde, cinnamyl acetate, cinnamyl alcohol, cinnamyl benzoate, cinnamyl cinnamate [2]. The occurrence of styrene in a variety of plants and foods has been researched. However, it is not clear if styrene is developed endogenously or because of environmental contamination. Trace amount of styrene has been found in many fruits and fruit products, vegetables, beans, eggs, fishes, cooked pork meat, fried chicken, cooked and roasted beef, guinea hen, mussels, turkey sausage, milk, cheese, olive oil, olives, honey, cocoa and coffee [3].

Likewise, styrene is generally present in the atmosphere. This is primarily as a result of emissions from the industrial production of styrene, polystyrene, and incineration of polystyrene garbage. Additional sources are emissions by coal-fired power stations, vehicle exhaust and cigarette smoke. The presence of styrene in the environment leads to direct and indirect exposure for humans. Inhalation represents the primary route of direct exposure to styrene. Consumption of food contaminated with styrene is an indirect exposure to styrene. Styrene has been found in various places of different countries, as well as forests and mountains, urban air, highway tunnels and sanitary landfills [4].

Styrene is produced primarily in the industry by the catalytic dehydrogenation of ethylbenzene. Ethylbenzene is obtained from the reaction of two petroleum derivatives- ethylene and benzene. The process of catalytic dehydrogenation involves superheated steam at high temperatures of 550 – 620^{o}C and the by-products from the reaction are benzene, toluene and hydrogen.

1.2 Polystyrene

Polystyrene (Figure 1.2) is produced by the polymerization of styrene molecules which are called monomers. Commercial polystyrene is primarily synthesized by bulk, suspension or solution polymerization of styrene. The most common polymerization reaction mechanism is free radical polymerization, employing benzoyl peroxide as an initiator. Other initiators like redox systems and azo compounds can be used to start the polymerization reaction as well. An initiator is a substance that reacts with a free radical to speed up radical reactions.

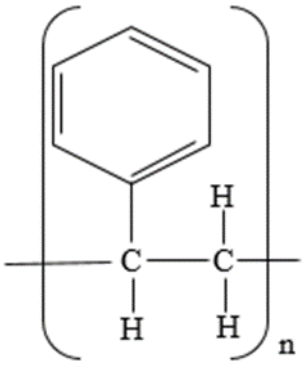

Figure 1.2: Polystyrene structural formula

Aside from free radical polymerization, other polymerization reaction mechanism includes cationic and anionic polymerization, as well as thermal and radiation polymerization. The relative ease of polymerization of styrene is because of the resonance stabilization of the growing polystyrene in its transition state, this indicates that the aromatic ring of the growth center delocalizes and evens out positive and negative charges including radicals. The study on the polymerization of styrene has been done extensively in comparison to other monomers, mostly as a result of its relatively reproducible and simple kinetics. The free radical mechanism for styrene involves (a) the formation of radicals along with some heat, followed by the radical's reaction with a styrene monomer, this is the Initiation step (b) the gradual addition of monomers to the growing polymer chain, the propagation step, and (c) a termination step, which is the destruction of the growth active center by the combination or coupling of two radicals (Figure 1.3) [3].

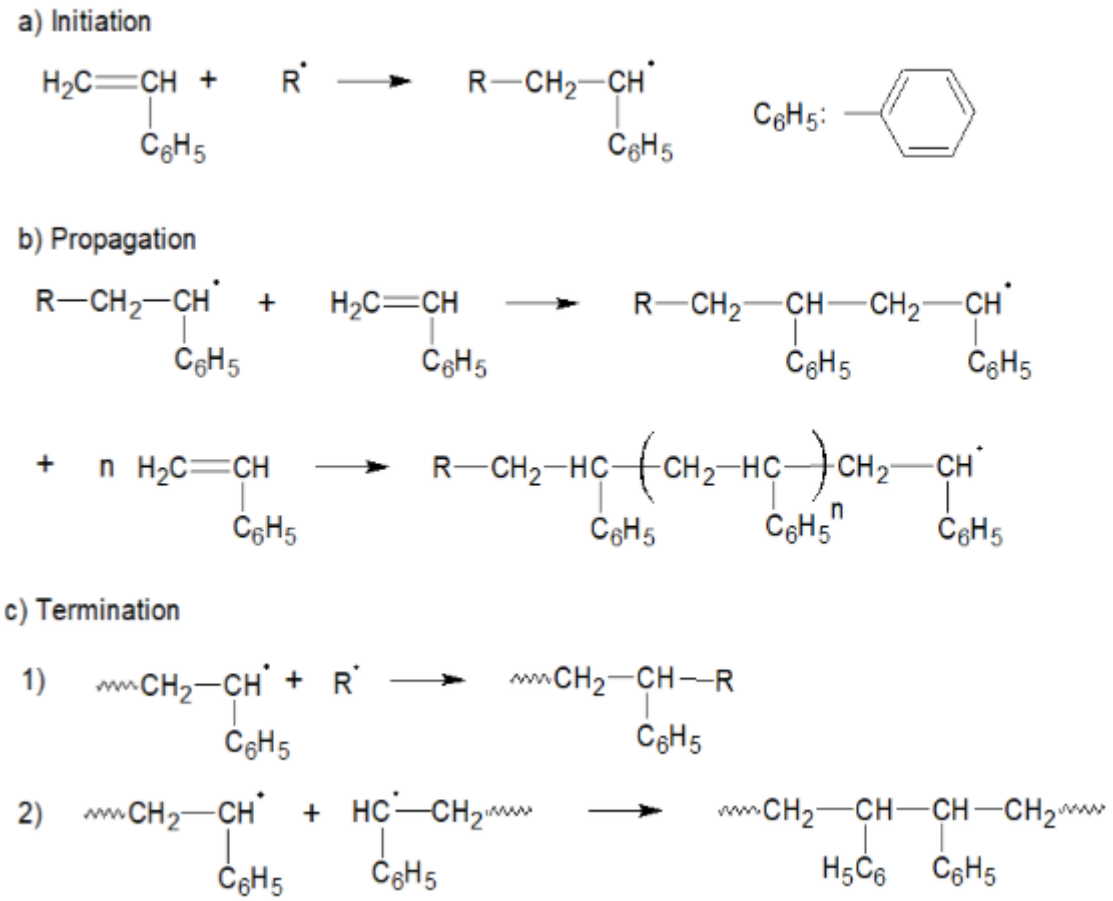

Figure 1.3: Free radical mechanism for styrene [3]

Additives are added to the production of polystyrene packaging to obtain the required physical properties. The additives added to styrene monomer during the production of polystyrene packaging materials are plasticizers, thermal stabilizers, slip additives, light stabilizers, anti-static agents, lubricants and antioxidants.

Plasticizers are a group of additives used to enhance the properties of polystyrene materials. The most common plasticizers with low toxicity are alkyl sebacates, acetyltributyl citrate, adipates and butyl stearate. There are restrictions on the use of phthalate plasticizers because of its potential carcinogenic and estrogenic effect. The European Commission Scientific Committee for Food (SCF) established tolerable daily intakes (TDIs) for phthalates after it was found to impair human fertility [4]. Thermal stabilizers are the most widely used additives, in addition to plasticizers. Largely, epoxidized seed and vegetable oils are extensively used in a range of food contact plastics heat stabilizers, lubricants and plasticizers

as well. Polystyrene, poly(vinylidene)chloride and poly (vinyl chloride) often contain epoxidized oils at levels ranging from 0.1 to 27% [5]. Their toxicity is influenced by their purity since the residual ethylene oxide is relatively toxic.

In pure epoxidized oils, the toxicity reduces with increasing molecular mass with decreasing solubility. Fatty acid amides are used as slip additives in a range of plastics used for packaging, such as polystyrene, polyvinyl chloride and polyolefins. Slip additives are added to plastic formulations, they gradually develop and tend to bloom to the surface imparting useful properties such as prevention of films sticking together, reduction of static charge and lubrication [6]. Light stabilizers are useful in enhancing the long-term weathering qualities of plastics, particularly polyolefins. Light stabilizers commonly used in polyolefins are polymeric hindered amines, including Chimasorb 944 and Tinuvin 622. Antioxidants are added to stabilize the plastics and slow down the oxidation process they undergo when exposed to light and air [7]. Most antioxidants have been observed to be nontoxic and have an acceptable stabilization effect.

Polystyrene comprises of a huge family of polymers and copolymers. The main members are general-purpose polystyrene (GPPS), and high-impact polystyrene (HIPS). GPPS in copolymerization with other monomers, particularly acrylonitrile and butadiene, produce oriented polystyrene sheet and polystyrene foam, as either extruded polystyrene foam sheet or expandable polystyrene (EPS). GPPS is an amorphous polymer; it is a molded and extrudable form of styrene homopolymer also known as ‘‘crystal’’ polystyrene. The amorphous nature and other properties, arising from the aromatic chemical structure and glass transition temperature (Tg) of about 100°C, vary significantly from the polyolefin plastics, such as polyethylene, which are aliphatic hydrocarbons and have below ambient glass transition temperatures [8]. This nature makes polystyrene an ideal plastic for injection moldings. The polystyrene film can be oriented bi-axially. This helps to maintain clarity and overcome some of the brittleness of un-stretched plastic. The stretching process also enhances the strength, even though crystals are not produced. To overcome the brittleness of non-orientated crystal polystyrene, butadiene synthetic rubbers (between 5% and 14%) are reacted with styrene during polymerization to manufacture HIPS plastics.

HIPS is an impact-modified graft copolymer, or rubber-containing blends with GPPS, also known as rubber-modified polystyrene. The strength of HIPS when compared to GPPS, is nullified by inferior clarity which makes them either translucent or opaque. HIPS plastics also

have lowered tensile strength, but there is a better resistance to stress cracking and to crazing caused by oils, fats and organic liquids. Recent development of special polymerization technology now allows crystal clear HIPS to be manufactured by anionic polymerization. As mentioned earlier, HIPS plastics are typically manufactured from blends of GPPS and styrene-butadiene copolymers. The ratios are chosen to achieve the required balance of physical properties for the different forms of packaging and the conversion process such as thermoforming or injection molding [8]. Containers for dairy products are manufactured either by the injection molding process or the thermoforming process. Vending cups are usually made by the thermoforming process, because only thin walls are required as they do not have to withstand high-speed filling operations. Several containers have multilayer structures. These consist of a layer of HIPS fit in between layers of GPPS. The GPPS layer offer “barrier” properties between the HIPS and the food or beverage, and an attractive “glossy” outer appearance. Other multilayer composites contain layers with barrier resins such as ethylene vinyl alcohol and polyesters. EPS products are produced from GPPS that has been treated with a blowing agent such as pentane or butane to produce EPS beads. These beads can be expanded or extruded to form a variety of food packaging products. A few EPS trays, cups and containers have surface layers of GPPS which provides a “barrier” layer between the plastic and the foodstuff. These modifications of polystyrene, and the addition of modifiers and other additives, may influence the diffusion coefficients of migrants from the food packaging [9].

GPPS has the properties of hardness, stiffness, brilliant transparency, high clarity, colorlessness, but is rather brittle with low impact strength. Polystyrene plastics are some of the most versatile, cost-effective and easily fabricated plastics. They combine many excellent properties, such as superior foaming ability, good chemical resistance, shape reproducibility, excellent dielectric property, high processability and good dimensional stability. Also, they have been generally used in the electrical application, automotive, kitchen appliances, thermal insulating materials, bottled water and food packaging industries [10].

1.3 Polystyrene Food Container

Polystyrene is suitable for a variety of food-contact applications as a result of its light weight. It is generally used in the food-service industry as rigid trays and containers, egg cartons, take-out containers, disposable eating utensils as well as foamed trays, cups, bowls and plates. EPS are widely employed as general protective packaging, called cushioning packaging, but they also find wide use as packaging for food formed into disposable beverage cups and trays for packaging meat, fish, produce, poultry, cheese, biscuit, fruits and vegetables. GPPS are mainly used as packaging material where its “crystal clear” properties can be applied to advantage. They are containers for a range of foods and disposable “plastic glasses” for hot drinks or alcoholic beverages. HIPS are used in the form of containers for cream, cottage cheese, ice cream, fruit juice, coffee, tea, chocolate, soup, sandwich clamshells packaging, with the biggest single application being yogurt containers.

Bi-axially oriented polystyrene films in thin gauges are employed for food packaging carton windows. They are also used as “breathable” films for over-wrapping fresh produce, for example, lettuce. The thicker gauges are utilized to manufacture clear vending cups, and tubs for desserts and preserves, by the thermoforming process. For most of these applications, the food is in contact with polystyrene for a relatively short period of time at moderate temperatures up to 130°F. For example, vending cups and instant noodle bowls, or for longer periods of time at refrigerated temperatures of 40°F such as packaged dairy and meat products. Polystyrene plastics have been used for food packaging longer than poly (vinyl chloride). As at 2012, 50% of the domestic consumption of polystyrene was related to food packaging and food service articles. In the United Kingdom, disposable drinking containers and cups used for vending machines are projected to account for about 45% of the total production of food-grade rigid polystyrene [11]. The amount of polystyrene used per year in food contact packaging and consumables increased from 2000 million to 2500 million pounds (lbs) in 1999, and the domestic consumption was 2600 million pounds in 2012 [12].

In modern living, food packaging plays a crucial role in improving safe transportation, delivery, distribution, storage of food and providing safety assurance from microorganisms, biological and chemical alterations which gives the packed food a longer shelf life. It is believed to be an imperative element in the food manufacturing process. Plastic packaging is applied on a large scale in addition to traditional packaging materials including ceramic, metal, paper, wood, and cardboard. Amongst the most important polymers used in food

packaging, polystyrene has made up a large amount of consumption of plastic containers. Nowadays, plastic packaging is mostly utilized among other packaging materials, due to its relatively cheap price, convenience and outstanding service properties. To meet up with the swift and convenient living demands of recent times, fast food has become increasingly popular. Hence, a lot of studies have been conducted out on the suitability of a variety of polymer materials for use in food packaging and stringent legislation has been adopted. Polymer materials commonly used in the food packaging industry are poly(ethylene terephthalate) for soft-drink or water bottles; polypropylene for glass caps, etc.; polyethylene (low or high density) for films, bags, etc.; and polystyrene, both rigid or foamed, for frozen materials and disposable cups. Disposable polystyrene containers are mainly used for packing of take-away foods in some fast-food joints, hawkers, and food court outlets in order to save time.

Recently, polypropylene plastics are now used in place of HIPS plastics in some of the above-mentioned uses, but for some types of food packaging, the opposite has occurred due to benefits of ease of processing and low shrinkage properties of polystyrene plastics. There may be limits on the use of polystyrene and HIPS plastics for food packaging because of its physical properties and performance. For instance, polystyrene plastics cannot physically survive “high” temperatures, thus, cannot be used for oven cooking of foods. Other drawbacks of polystyrene plastics include brittle, low impact strength, prone to UV degradation, poor chemical resistance, flammability and weak barrier properties to water vapor and gases, including oxygen and carbon dioxide. GPPS is susceptible to stress cracking caused by organic liquids and oils, which excludes it from being used with foodstuffs having high concentrations of fats and vegetable oils. It has high permeability to oxygen which causes spoilage of the oil, and therefore cannot be used as a film wrap or as containers for vegetable oils [13].

EPS is extremely flammable and can get ignited easily. Also, the use of flame retardant in all polystyrene building insulation has been banned by the European Union (EU). It is known that polymers with aliphatic backbones tend toward the low smoke generation, whereas polystyrene polymers and those with pendant aromatic groups generate more smoke, which indicates that polystyrene also produces smoke in high quantity. Nevertheless, the fire hazard of polystyrene restricts its usage in some areas, due to the release of a significant amount of heat and toxic smoke during combustion, which will cause heavy casualties and property losses in case of fire accidents. Hence, reducing the fire hazard of polystyrene is an urgent

need to stir up wide concerns. Heat is released in fire accidents, which increases temperature and helps the spreading of fire. To be able to decrease the peak heat release rate and the total heat release, flame retardants are mostly utilized in the polymer composites production [14].

In recent times, despite the convenience polystyrene packaging presents to the consumer, there have been concerns about the wholesomeness and safety of food, and this has been the subject of many debates in the environmental and health sector. With the growing awareness of consumers regarding health issues, the implication of the migration of styrene from polystyrene containers used for food packaging attracted the attention of the scientific and legislative communities. It has been found from numerous studies that polystyrene food packaging is a source of contamination as the polystyrene material releases styrene via diffusion process into the food. In food packaging, the term "migration" describes interaction and exchange of mass between the packaging material and food component through a diffusion process.

Migration is a process where polymerization residues or stabilizers can disperse through the polymer matrix to the surface. Diffusion is one of the major mechanisms for the movement and migration of chemical compounds from packaging materials into food. The migration of components from packaging material into food is not desired. Yet, the transfer is inevitable, since most foodstuffs are packed ahead of the consumer purchase. The contamination is as a result of the dissolution in the food that meet the migrated stabilizers on the surface of the polymer. During the production of polymers, it is probable that not all the monomer will be transformed into long chain/high molecular weight polymer. Having only a small amount of the monomer, reaction may stop after only some molecules have combined, producing very low molecular weight polymer units, called oligomers. When only two monomer elements are connected, the oligomer is known as a dimer. A trimer is formed by linking three-monomer oligomer together. Oligomers are formed as by-products of incomplete polymerization during production of polystyrene and by degradation after irradiation or thermal treatment of polystyrene during downstream applications.

1.4 Styrene Toxicity

Toxicity is the degree to which a compound or substance can be harmful to humans and animals. According to [13], the United States Food and Drug Administration (US-FDA) accepted limit of residual styrene in polystyrene food container is 10,000 ppm for water-based foods and 5,000 ppm for fatty foods. Exposure of human to vapors of styrene may cause irritations of eye, nose, throat, and skin. Styrene has shown a toxic effect on the liver, acts as a depressant on the central nervous system and cause neurological impairment. The chronic effects of styrene monomer and metabolite of styrene are chromosomal aberrations in lymphocytes of humans and damage to the liver and nervous system [15].

Styrene was re-classified from a Groups 3 (not classifiable as to its carcinogenicity) to a Group 2B carcinogen that is possibly carcinogenic to humans by the World Health Organization's International Agency for Research on Cancer (IARC) [16,17]. Styrene is biotransformed into styrene-7,8-oxide (Figure 1.4) by the mixed function oxidase system, it binds with DNA and induces tumors and probably causes carcinogenicity. Therefore, styrene-7,8-oxide, a reactive metabolite of styrene, was classified by IARC as a Group 2A carcinogen, probably carcinogenic to humans [18].

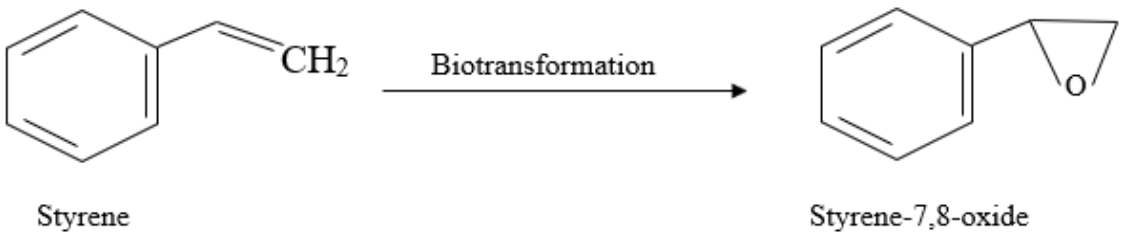

Figure 1.4: Biotransformation of styrene into styrene-7,8-oxide

Oligomers of styrene are also present in polystyrene packaging samples intended for food packaging. These oligomers contribute to non-intentionally added substances that can migrate into food or food simulants and thus must be considered for the potential risk to health [19]. There are no oligomers larger than dimers and trimers of styrene found in polystyrene. Oligomers of styrene are quantitatively an important part of non-intentionally added

substances that may potentially migrate into food [20] . According to the European Union (2011) (Article 19), the potential risk to health of non-intentionally added substances must be measured in addition to the basic monomers.

A universal limit was established by the European Union [21]. This stated that migration of substances should not be detectable at a limit of detection (LOD) of 10 mg/kg food or food simulant. They introduced this as a result of the current state of analytic technology instead of toxicological considerations. Though the concentrations of dimers, trimers and the sum of both may occasionally exceed this limit, particularly when certain hostile food simulants are employed, a toxicological assessment was then undertaken. It was reported from a toxicological assessment that a single intraperitoneal injection of 1000 mg/kg of a waste stream from polystyrene production comprising a mixture of styrene oligomers (13.3% dimers, 69.5% trimers; other components: styrene 4.5%, ethylbenzene 3.2%, cumene 1.6%, n-propylbenzene 1.5%, mineral oil 6%) produced a substantial decrease in the number of days required for vaginal opening in rats indicating estrogenic activity of this mixture [22].

1.5 Meta-Analysis

Meta-analysis is a quantitative review where clinical efficiency is determined by calculating the weighted average of results in at least two separate studies. It is a two-stage process that involves (a) estimating the appropriate summary statistic for each study, (b) calculating the weighted average of the statistics across the studies [23]. It is a statistical analysis that combines or integrates the results of several independent clinical trials to create one single report that has a high statistical power. It emerged in literature in the 1970s and the term was first used by Dr. Gene Glass in 1976 to describe the methods of combining findings from individual studies [24]. In the period between the mid-1980s and 1990s, researchers in health, social sciences and statistics endorsed meta-analysis as a research approach. In more recent times, there has been an exponential growth in the application of meta-analysis, and it is most frequently cited in clinical reports.

It is also an important aspect of evidence-based healthcare, as it is mostly used to measure the clinical effectiveness of healthcare interventions. In toxicology, meta-analysis is used in the systematic determination of the mechanisms by which chemicals exert toxic effects on living organisms, descriptive toxicity testing and regulatory determination of the risk a drug or

chemical poses. It is essential in assessing therapies and integrating the results of individual studies in order to inform clinical study. When the result of a study is consistent in different studies, meta-analysis is used to calculate the absolute effect. Also, when the result of a study varies in different studies, meta-analysis is used to calculate the reason for the variation. It is relevant in validating hypothesis. Results obtained from a single study cannot be considered valid, because there are variations from one study to another. A mechanism is needed to synthesize data across studies. Individual studies can be combined to increase data and improve the precision and accuracy of the estimates. Combining underpowered individual studies can increase the overall statistical power to detect an effect.

Before starting a meta-analysis, the main elements are agreed upon, including the scope of the research question, inclusion and exclusion criteria, and means of resolving disagreements regarding risk of bias and research quality assessment. These are important in order to produce detailed and transparent meta-analysis report. The steps in conducting meta-analysis are:

Step 1: Develop Research Questions

Starting a meta-analysis begins with asking questions using the PICOS (Participant, interventions, comparisons, outcomes and study design) framework. Research questions help to guide the meta-analysis in the right direction.

Step 2: Define Inclusion and Exclusion Criteria and Potential for Bias

The inclusion and exclusion criteria are formed as a result of assessed outcomes, length of follow up, types of publication, study designs and methods, date of publication and language of publication. When a study is removed from the meta-analysis, the reason for such exclusion should be provided. The assessors independently decide the studies to include or exclude using a well-defined checklist and there should be a procedure to follow when the assessors disagree. This step is followed by a quality assessment by two people familiar with the research topic. Afterwards, a general meeting to discuss the studies excluded or included is conducted.

In order to reduce the potential for bias, a quality assessment protocol should be created. Some quality assessment protocols allow numerous irrelevant research papers to be added into a meta-analysis and it can be sometimes misleading. It is therefore preferable for researchers to use individual components of quality assessment to identify the trials and experiments that do not meet quality standards and exclude them from the study [27]. In order to minimize the potential bias, identifying information such as authors' names, title of journal, institutional affiliations, acknowledgements, as well as funding sources, should be removed when assessing the research article for inclusion [24].

Step 3: Search for Literature

The next step in meta-analysis is to search for literature. Having a clearly stated research question is essential in this process. There is no limit to the amount of studies to be reviewed and used for meta-analysis. This step begins with identifying databases with peer-reviewed articles and search for relevant studies. Collecting all relevant study is important because any loss of studies can cause a bias in the meta-analysis. Published papers and previous abstracts can be found on databases such as MEDLINE, EMBASE, PubMed, SCOPUS, Web of Science, Ovid, Google Scholar, ScienceDirect, Springer Protocols, Springer Link, NCBI Database, Federal Science Library (Canada), ECOTOX Database and Cochrane library. The use of key words is essential while searching for literature. This helps to refine the search and save time.

Furthermore, a research question that addresses the participants, interventions, comparisons, outcomes and study design (PICOS) is essential in the search strategy. In addition, the library also has resources such as relevant papers, books, abstracts, and conference proceedings. It is important that each reference should be checked properly along with citations in review papers, and communication with scientists who have been working in the relevant field should be checked as well.

Step 4: Screen Literature

Two reviewers at minimum perform the first screening. This involved screening of titles and abstracts based on the research question and study design, population, intervention and outcome desired to be studied. A second screening is then performed on the selected studies

by at least two reviewers. The selected studies are ready for data extraction. Screened literature is recorded using the PRISMA standard.

Step 5: Extract Data

Data can be extracted using various statistical tools. The statistical programs that can be used in meta-analysis are Excel spreadsheet, STATA, SAS, R, Review manager or comprehensive meta-analysis. For proper data extraction, all reviewers must be trained under a consensus standard.

Step 6: Statistical Analysis

1. *Effect Measure*

Studies included in a meta-analysis must have common statistics that allow their results to be combined. When all the studies to be included in a meta-analysis have the same outcome measurement, an effect size in the original units may be calculated [27]. For example, if all studies measure the amount of styrene monomer leachate into hot drinks in $\mu g/cm^3$, the mean difference can be used as the effect size. Standardization of the effect size is necessary when the results of studies analyzed are not calculated with the same units. When the effect sizes are in the original units, the interpretation is clearer. When the effect sizes are in standardized units, the interpretation is more difficult and published guidelines for interpreting effect sizes may be used. Whether standardized or not, the overall effect size derived from the meta-analysis is calculated by combining the effect sizes of all the included studies [23].

Use of the confidence interval can give insight into the precision of the treatment estimates of the included studies. A wider confidence interval may be a function of a small sample size, as well as inaccuracy in the measurement. Larger sample sizes provide more accuracy of the effect size, whereas smaller studies are less accurate, unless these smaller studies have little variance. Confidence intervals, which are reported as a probability (for example, 95% confidence interval), provide a range (upper and lower bounds) that indicate the accuracy of the estimate of the effect size [28]. When the confidence interval of effect size is within an area considered as clinically meaningful, the applications of the results in clinical care may be justified. Equally, large confidence intervals suggest less precise estimates and combined

with a small sample size, can lead to questions about the stability of the effect size estimates. By combining the results of small studies, a meta-analysis may provide a more accurate estimate of the treatment effect [29].

2. Data Model

The fixed-effect model is used where results of studies are consistent, and the associated effects are also consistent. It compares exact or nearly exact replications of the same experiment. The estimation methods used to calculate fixed-effect are 1) inverse variance-weighted estimation 2) Mantel Haenszel estimation 3) Peto estimation. The random-effect model assumes heterogeneity between studies. This means that the size of the effect of treatment differs among studies. The estimation methods used to calculate random-effect are 1) the Dersimonian and Laird method for dichotomous variables 2) inverse variance-weighted estimation for continuous variables [25].

The model used is dependent on the presence or absence of heterogeneity. When there is no heterogeneity (heterogeneity $P \geq 0.10$), a fixed-effect model is used. However, when the Q value is significant ($p < 0.10$) showing heterogeneity, a random-effect model is used [26]. When study groups are homogenous, both models offer similar results. Nonetheless, in heterogeneity, the random-effect model typically provides wider confidence intervals than the fixed-effect model [29].

3. Heterogeneity

Heterogeneity is the inconsistency among different studies. Meta-analysis determines the presence of heterogeneity amongst primary studies and calculates the variance in the results of different studies. Meta-analysis heterogeneity is the degree of variation in the individual study results. Meta-analysis makes use of Cochran's Q test and I^2 value statistical tests to detect and quantify heterogeneity. The Cochran's Q test is used to determine whether there are differences between primary studies or if the variation seen is due to chance [27].

Cochran's Q-value is calculated by adding up squared deviations of the estimate of each study from the overall estimate and then comparing it with the chi-square distribution with K–1 df (degrees of freedom), K represents the number of studies [29]. However, using the

Cochran's Q test may be unreliable when the meta-analysis contains a small number of studies. Heterogeneity $p < 0.10$ indicates the existence of heterogeneity, given that Cochran's Q test has low statistical strength and is insensitive [24]. Another commonly used method for testing heterogeneity is the I^2 value, which quantifies the effect of heterogeneity, and does not depend on the number of studies or the type of outcome data. I^2 values range between 0% and 100% and represent the proportion of inter-study variability that can be attributed to heterogeneity rather than chance [$I^2 = 100\% \times (Q - df)/Q$]. I^2 values of 25%, 50%, and 75% are considered low, moderate, and high estimates, respectively [23].

The different types of heterogeneity are clinical heterogeneity, methodological heterogeneity and statistical heterogeneity. In statistical heterogeneity, there are variability in treatment effects, resulting from clinical and methodological diversity. Statistical heterogeneity is present if the observed treatment effect is more different from each other than would be expected due to chance alone [27].

Step 7: Report Results

The information obtained is represented graphically by converting effect sizes into real numbers. These numbers are analyzed with multilevel model and plotted on a graph. This graph is called a 'Forest plot'. Forest plot use point estimate of the individual studies with confidence interval [28]. A meta-analysis will generally include a Forest plot, in which the results from each study are displayed as a square and a horizontal line, representing the intervention effect estimated together with its confidence interval. The weight that each study contributes to the meta-analysis is reflected by the area of the square. The combined effects estimate, and its confidence interval are represented by a diamond.

Reporting results from meta-analysis study in a clear manner is necessary. There are well-established instruments, such as the PRISMA and Meta-analysis of Observational Studies in Epidemiology (MOOSE), aimed at assisting researchers to improve the reporting of meta-analysis to help readers of study review and appraise meta-analysis studies with greater transparency [30]. PRISMA offers a checklist of items that should be included when conducting meta-analyses of randomized controlled trials. On the other hand, MOOSE criteria are widely used to assess meta-analysis of observational studies. High-quality meta-analysis adheres to and report items outlined in the instruments.

Step 8: Publication Bias

Publication bias is publishing only positive results and leaving out some results. Numerous methods have been established to provide an assessment of publication bias; however, the most used is the funnel plot [23]. The funnel plot provides a graphical evaluation of the potential for bias and it also shows a scatterplot of treatment effect against a measure of study size. The purpose of meta-analysis is to include all studies which meet inclusion criteria; however, it is not always possible to obtain these [27].

One crucial concern is that papers that might have been missed. There is good reason to be concerned about this possible loss because studies with significant positive results are more likely to be published than studies with negative results. Studies that yield a positive result, most especially large studies, are more likely to have been published and, equally, there is a reluctance to publish small studies that have non-significant results [25]. Publication bias is not only the concern of editorial policy as there is unwillingness among researchers to publish results that were either uninteresting or randomized [28]. There are also problems with including all studies that have failed to meet peer-review standards. It is important to examine the results of each meta-analysis for indication of publication bias. Estimating the likely size of the publication bias in the review and an approach to dealing with the bias is essential in conducting meta-analysis.

Studies which have no publication bias, larger studies having lower standard error, tend to cluster closely to the point estimate [23]. When studies become less accurate, for example, smaller trials having higher standard error, the result is expected to be more varied and scattered across both sides of the more precise larger studies. The asymmetry of funnel plots is not exclusively attributable to publication bias; it may also come from clinical heterogeneity among studies [26]. Still, the funnel plot is not without problems. When high precision studies are more varied than low precision studies with respect to effect size as a result of different populations examined, a funnel plot may give a wrong impression of publication bias [30].

1.6 Motivation for Current Research

Research has established the migration of styrene from polystyrene packaging and the toxic effects of styrene. Literature describing the migration of styrene from polystyrene food container is diverse. Crucial experimental factors like group sizes, study instrumentation, experimental approaches, and methodologic details are different across studies. Consequently, a meta-analysis of the literature using a quantitative analysis of the combined data from several individual study reports can help unite the literature by improving a) accuracy, by reducing the effect of single, perhaps inconsistent reports; b) precision, by comprising a large amount of subjects; and c) consistency, by collecting several studies with different group sizes and experimental factors. Therefore, this meta-analysis research will help polystyrene manufacturers keep up-to-date with the level of toxicity of styrene monomer; provide evidence for policy makers to judge risks, benefits, and harms of health care behaviors and interventions; provide summaries of previous study for funders wishing to support new studies; and help editors judge the merits of publishing reports of new studies.

CHAPTER 2: OBJECTIVES

This research is aimed at consolidating the varied research data and determining styrene migration into food simulants. It is established that several factors like fat content in food, temperature of food, how long the food is in contact with polystyrene and diffusion coefficient of the polystyrene container enhance the migration of styrene from polystyrene container into food. High temperature allows the weakened styrene monomer bonds to be broken and they migrate into the food they are in contact with.

2.1 Thesis Objective

The goal of this research is to synthesize data from several studies and use meta-analysis to obtain a cohesive amount of styrene migrating from polystyrene food containers into food simulants. This is important to improve accuracy, precision and uniformity of the combined studies. The migration of styrene from polystyrene containers into food established this objective:

Objective

To investigate the migration of styrene from polystyrene food containers into food simulants and observe the rate of styrene migration.

The objective of this research was to study the migration of styrene from polystyrene containers into food simulants. This was carried out by collecting the styrene migration data from each study analyzed. Other variables that were collected from the studies are fat content of food sample, time of exposure, temperature of food sample, type and size of the polystyrene material used for the analysis. The rate of styrene migration from polystyrene containers was also observed. The total time taken for styrene migration from polystyrene containers and the amount of migrated styrene was obtained. Migration of styrene was plotted against the square root of time.

CHAPTER 3: LITERATURE REVIEW

This chapter gives an overview of the current research on the migration of styrene from polystyrene containers and the health impact of styrene consumed from food. The mechanism of styrene migration from food containers will be reviewed, such as diffusion of materials from the polymer, fat content of the food, temperature of the food in contact with the polystyrene container and time of contact between the food and the polystyrene container. The toxic effects of styrene consumption will also be discussed.

3.1 Diffusion of styrene from polystyrene

According to Crank, mass transfer from polystyrene materials into food obeys Fick's second law of diffusion [32]. This is explained in the equation below:

$$\frac{\delta Cp}{\delta t} = Dp \cdot \frac{\delta^2 C_P}{\delta x^2} \qquad (1)$$

Where D_p is the diffusion coefficient of migrant in the polymer, C_p is the migrant concentration in the polymer, χ is the space coordinate measured normal to the polymer–food interface, and t is the elapsed time, and these are the parameters that affects the rate of migration. This equation was further simplified by [32] to predict the extent of migration from polymer into food simulant.

$$M_t = 2C_{pO}\sqrt{\frac{D_P t}{\pi}} \qquad (2)$$

Where M_t is the total migrant from the polymer in time t, and C_{P0} is the initial migrant concentration in the polymer. It is assumed in equation 2 that the solvent used to dissolve the polystyrene material is well mixed and the polymer is suitably thick for the migrant concentration at the middle of the polymer to remain at its original value C_{P0} [32].

3.2 Styrene Migration Studied with Temperature

Miltz & Rosen-doody, determined the migration of styrene from polystyrene cups to cheese and yogurt. The styrene migration values indicated that high temperatures lead to high rate of migration and throughput because of weakened monomer/polymer interaction and increased solubility of styrene [39]. Furthermore, Ahmad & Bajahlan discovered that hot water stored in Styrofoam and polystyrene cups were contaminated with styrene and other aromatic compounds. Thus, they concluded that paper cups were found to be safe for hot drinks and temperature played a major role in migration of styrene monomer from Styrofoam cups [40].

Sanagi et al., determined the migration of volatile organic compounds (VOCs) from food packaging materials into food simulant. They established that at higher temperature, the analyte being extremely volatile can be easily released from the matrix [41]. Furthermore, in 2009, a study by Khaksar & Ghazi-Khansari clarified that the migration of styrene into hot foods and drinks is dependent on the fat content, storage temperature and time. Hence, they concluded that migration of styrene monomer at 20°C is minimal, but when temperature increased, the migration in the first 10 minutes is considerable, and increases as the time of exposure increases [42]. Also, the concentration of styrene observed was above the EPA recommended level. In addition, Choi et al., examined the migration of styrene monomer and oligomers from polystyrene to food stimulants. They found that all the styrene monomer and oligomer migrated completely into n-heptane in 72 hours and high temperature yielded faster migration [43].

Gennari et al., discovered from their experiment that styrene migration level varied from 0.61-8.15 μg/L for hot tea, 0.65-8.30 μg/L for hot milk, and 0.71-8.65 μg/L for hot cocoa milk in clear polystyrene cups and from 0.48-6.85 μg/L for hot tea, 0.61-7.65 μg/L for hot milk, and 0.72-7.78 μg/L for hot cocoa milk EPS cups at different temperatures and times. They also found that there was no migration from the cups into water, except for drinking water at 80 °C, with level ranging from 2.07-9.03 μg/L and dependent on the storage period. Furthermore, their results also showed no styrene migration from polystyrene glasses into water, even at elevated temperatures of 100 °C [44].

3.3 Styrene Migration Studied with Fat Content

Tawfik & Huyghebaert, studied the level of styrene migration into whole milk, half-fat milk and skimmed milk at different temperatures and storage times. They found that the quantity of styrene migrating from EPS cups in milk of 3.6% fat was higher than milk of 1.5 and 2.5% fat content. Also, the maximum rate of styrene migration occurred in the first ten minutes of the experiment [19]. Similarly, Miltz & Rosen-doody stated that when residual styrene in polystyrene package is as low as 0.1%, some styrene can still migrate into fatty foods [39].

Varner & Breder, discovered styrene levels of 60-2250 ppm were present in polystyrene food packaging [45]. Also, Withey, described styrene detected in dairy products at levels up to 245 ppb in sour cream [13]. In hot chocolate and chocolate spread, Gilbert & Startin found 13 ppb and 2 ppb, respectively. They also found separate levels of styrene in food products, for example 180 ppb in chopped candied peel [46]. Varner et al., analyzed the styrene migration into margarine and they found that there was no detectable migration [47]. Snyder & Breder, studied the migration of styrene from polystyrene into various solvents. They established that the rate of styrene migration was similar into 20% ethanol, corn oil, and HB-307 (a synthetic triglyceride) [48].

3.4 Styrene Migration Studied with Exposure Time

Ahmad & Bajahlan, analyzed polystyrene bottles for residual styrene. They discovered that the concentration of styrene in polystyrene bottles increased after one year of storage [40]. Carillo-Carrió et al., determined styrene and other volatile compounds in olive oil. They discovered that olive oil stored in plastic bottles showed higher styrene levels, ranging from 2.29-102.8 ng/mL, which increased with storage time [49]. Furthermore, Verzera et al., studied the migration of styrene from polystyrene packaging materials into commercial yoghurt from an Italian dairy industry immediately after manufacturing and during the storage at 4°C. They identified styrene in the volatile fraction of yogurt and quantified it using the headspace solid phase microextraction coupled with fast high-resolution capillary gas chromatography (HS SPME/fast GC). Therefore, they concluded that styrene was present in trace amounts at production time and its level increased up to 23 days of refrigeration reaching 15.9 ng g^{-1} [50].

3.5 Quantity of Styrene Consumed by Humans

The FDA established a “threshold of regulation” process in the Federal Register of July 17, 1995 [6]. The probability of a substance causing a health hazard depends on its dietary concentration and toxic potency. Therefore, the FDA considered both factors in establishing a threshold of regulation level. Non-carcinogenic compounds would be unlikely to cause harm at levels lower than 1 mg/kg. However, in order to provide adequate safety margin, the dietary concentration should be well below 1 mg/kg. It should however be noted that a 0.5 ppb threshold is 2000 times lower than the dietary concentration at which the majority of examined compounds are likely to cause noncarcinogenic toxic effects and 200 times lower than the chronic exposure level at which potent pesticides display toxic effects. Thus, the FDA has determined that most known carcinogens pose less than a one in a million-lifetime risk if present in the diet at 0.5 μg/kg [6].

The Brazilian legislation maximum permitted quantity of styrene in the finished material or article is 250 mg/100 g plastic polymer [51]. The FDA requires that styrene monomer level remaining in basic styrene polymer products planned for fatty food must not be more than 0.5 % (5000 mg/kg) [52]. Lickly et al., stated that rate of styrene intake of the daily diets in the USA is 9 μg/day, and in UK from 1-4 μg/day, and this rate is four times less than the daily allowed intake as computed by Styrene Information Research Center (SIRC) [38]. Food and Agriculture Organization (FAO) and World Health Organization (WHO) stated that the maximum permissible intake of styrene in different diets is 40 μg/day/person. This limit is computed based on 60 kg body weight [53].

Because styrene can adversely affect humans in numerous ways raises serious public health and safety questions. According to Verzera et al., examining styrene exposure through human daily intake via food, estimated about 3-7 ng/kg body weight [50]. Tawfik & Huyghebaert, estimated the dietary intake of styrene to range from 0.012-0.123 μg/kg of the body wt/day, which is equivalent to 0.76 -7.4 μg/day [19]. However, the styrene intake of each tested food according to the present results is less than the international allowed level of 40 μg/day/person [53]. Compiling those foods in a single diet the consumption styrene figure will be amounted to 11.31 μg/day/person. Addicted consumption of certain types of food regularly packaged in polystyrene cups could lead to public health hazard. In a study by Vitrac & Leblanc, evaluating the consumers exposure to styrene from yogurt for more than 5400 houses in France with a purchasing power of 2 million, it was revealed that the rate of

dietary intake per person is approximately 12 µg/day and average of house or family exposure to styrene is in the range of 1-35 µg/person/day [54].

Cao et al., found that dietary exposures to styrene is about 12.9–46.3% in dairy, 11.8–39.4% in grain-based foods and 2.9–36.2% in nuts. Generally, dietary exposures to styrene are lower for children, ranging from 1.4 µg/day for infants of 6–11 months, 5.7 µg/day for toddlers of 2–3 years. There is an increase for older age groups as exposure estimates range from 8.1-11 µg/day for adults [55]. They also estimated dietary exposure to styrene by comparing with the intake estimates from ambient and indoor air determined in the early 1990s for non-smokers (0.096–0.24 µg/kg body weight/day for 7 months to 4 years; 0.107–0.27 µg/kg body weight/day for 5 to 11 years; 0.096–0.23 µg/kg body weight/day for 12 to 19 years; and 0.085–0.21 µg/kg body weight/day for 20 to 70 years) [56].

German Society for Nutrition in 1996, used the average per capita consumption figures of the general population in Germany to estimate the amount of human styrene intake via food [57]. It was found that consumption of milk and milk products together comprised a total amount of 338 g/person/day, fat and oil contribute another 72 g/person/day. Hence, if these foods were packed in polystyrene materials, allowing styrene contents resulting from migration of 5–30 ppb, the daily styrene intake via such foods would reach 2–12 µg, which corresponds to an annual intake of about 0.7–4.4 mg/person. Additionally, styrene intake might come from wine consumption. According to the German Society for Nutrition, the annual consumption of wine is 24.5 l/person. Therefore, if all wines had an average styrene content of 1–3 ppb, the total intake from wine reaches about 25–75 mg/year [57]. Tang et al., applied the US-FDA consumption factor (CF) which assumes that only 10% of the food is packed in polystyrene CF polystyrene-0.1 (10%) results in an average annual intake of styrene of 0.08–0.45 mg/person or 1.1–6.5 mg/kg body weight for adults (70 kg body weight). This corresponds to an average daily intake of 0.2–1.2 mg/person or 3–17 ng/kg body weight [20].

3.6 Health Effects of Styrene

Several adverse health effects are attributed to styrene. Humans experience eye, nose, throat, and skin irritation when exposed to the vapor. Styrene has a toxic effect on the liver, acts as a depressant on the central nervous system, and causes neurological impairment. An increase in

the frequency of chromosomal aberrations has been observed in the lymphocytes of human subjects occupationally exposed to styrene [47].

According to IARC, styrene itself exhibits low genotoxic effects unless it is metabolically activated to styrene 7,8-oxide, which has been classified as a probable human carcinogen group 2A. Styrene 7,8-oxide is exclusively the metabolite responsible for genotoxicity and its major site of metabolic formation is the liver. Since the human exposure to styrene is predominantly via inhalation, the lung represents one major organ of entry and first contact [18]. Styrene is a relatively weak genotoxicant and mutagen, however, styrene-specific DNA adducts have been found in humans. This represents an important initial step in the carcinogenic process and should not be underestimated [58].

Kawamura et al., stated that three Styrene dimers including 2,4-diphenyl-1-butene (NSD-01), cis-1,2-diphenylcyclobutane (NSD-08), and trans-1,2-diphenylcyclobutane (NSD-09), two styrene trimers including 2,4,6-triphenyl-1-hexene (NST-01) and 1-phenyl-4-(1-phenylethyl)tetralin (NST-03), and two unknown compounds were detected from an extract of a polystyrene food container [59]. Styrene dimers and styrene trimers have been listed as endocrine disrupters by [60] in the Wingspread Statement, Yamada et al., from several studies on the safety of polystyrene food containers, have shown that one unknown compound was 1-phenyl-4-(2-phenylethyl)tetralin (NST-12) [61], and explained that styrene dimers and styrene trimers showed no endocrine disrupting action by in vivo and in vitro assays, which were compared in detail by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) [62]. Yet, the other unknown compound has not been identified or tested for bioassays. Although, it was presumed to be 1,3,5-triphenylcyclohexane based on GC-MS data by [59].

Prinsen & Gouko, determined from their uterotrophic assays in rats with extracts of polystyrene samples that under the exaggerated exposure conditions (which is a maximum dose level of styrene trimers that was a factor of 1000 higher than the estimated maximum human daily intake), there was no evidence of migration of styrene oligomers from food containers that may have an adverse effect on human health with respect to oestrogenicity [63]. The experiments of [64] show that styrene oligomers extracted from GPPS did not induce gene mutation nor chromosomal aberration, indicating that the risk of the genotoxicity of styrene oligomers migrating from polystyrene food container into food is possibly very low.

Date et al., investigated the endocrine-disrupting effects of styrene dimer and trimer that eluted from polystyrene containers and migrated into instant noodles [65]. It was found in the estrogen and androgen receptor binding assays, styrene monomer, dimer and trimer did not show any binding affinity for the estrogen and androgen receptor binding assay. Therefore, they concluded that styrene does not have influence on the sex hormone feedback system via these receptors. A uterotrophic assay using prepubertal and ovariectomized adult rats was conducted to evaluate estrogenic activity in vivo. As a result, styrene monomer, dimer and trimer did not induce an increase in uterine weight and had no estrogenic activity in vivo. However, Ohyama et al., described that certain styrene dimer and trimer in high concentrations showed estrogenic effects in the estrogen binding assay and E-SCREEN pointing out the fear that they may disrupt the endocrine system [66].

CHAPTER 4: COMPARATIVE OVERVIEW OF STYRENE TOXICITY STUDIES

This chapter outlines a comparative overview of each study included in this meta-analysis. The sampling methods utilized in analyzing styrene migration in each study were reviewed and compared, each studies result was analyzed, and the findings stated. The different sampling methods for styrene analysis are described and their impact on the amount of styrene migration was evaluated. The variables considered in each study were identified, studies with similar variables were categorized and their results were compared. The overall impact of these variables on styrene migration and styrene toxicity was investigated and discussed.

4.1 Different Sampling Methods for Styrene Migration Analysis

There are three sampling methods used in analyzing residual styrene and styrene migration from polystyrene containers into food, in the studies included in this meta-analysis. They are Immersion sampling method, Vapor-phase sampling method and Cell sampling method. These sampling methods can be utilized to measure both the amount of residual styrene in the polystyrene containers and the amount of styrene migrating from polystyrene containers into food. The sampling methods were compared in order to identify their similarities and differences. Also, this is important to discover the effect of these sampling methods on the overall result obtained from each study.

4.1.1 Immersion Sampling Method

For this sampling method, polystyrene materials were cut into smaller sizes and immersed in a vial filled with the food simulant or solvent for a period and at desired temperatures. Some portion of the supernatant solution is then analyzed either as a liquid in the High-Performance Liquid Chromatography (HPLC) or as gas in the Gas Chromatograph Mass Spectrometry (GCMS). The immersion sampling method was most used amongst the studies, because all surfaces of the polystyrene material is submerged and exposed to the food simulant. This method was used in 24 studies included in this meta-analysis.

4.1.2 Vapor Phase Sampling Method

This sampling method requires polystyrene materials to be cut into smaller sizes and placed in a vial without contact with the food simulant for a period and at a desired temperature. Some portion of the vapor is collected with a gas syringe and then analyzed in the GCMS. This method was utilized in 4 studies included in this meta-analysis.

4.1.3 Cell Sampling Method

In this method, polystyrene materials are cut into round discs of about 14 pieces and connected at the centre with a wire. This cell of polystyrene material is placed in a vial filled with the food simulant or solvent and tight sealed. The cell is then placed on a shaker bath for a period and at a desired temperature. Some portion of the solution is then analyzed either as a liquid in the HPLC or as vapor is then analyzed in the GCMS. This method was utilized in 8 studies included in this meta-analysis.

4.2 Effect of These Sampling Methods on the Styrene Migration Analysis

Each of these sampling methods influence the styrene migration levels observed in the studies analyzed. Immersion sampling was found to give the most precise analysis of styrene migration into food simulant, closely followed by vapor-phase sampling method and then cell sampling method. This was supported by the study of Lessen et al., 1991, which compared the 3 sampling methods and concluded that immersion sampling resulted in the highest level of migrated styrene monomer. Vapor-phase sampling was only slightly lower than immersion sampling and cell sampling gave the lowest levels of styrene monomer in oil [71].

The study of Linssen et al., 1992, demonstrated that styrene migration analysis in the cell sampling method results in much lower levels of migrated styrene compared to immersion sampling [72]. Lickly et al., compared the immersion sampling and the vapor-phase sampling methods. They concluded that amount of styrene migrating from vapor-phase sampling is slightly lower than with immersion sampling [38].

4.3 Comparison of Each Study and Their Impact

While comparing the studies included in this meta-analysis, there were some agreements in the results of each study, despite the different variables applied. Studies with the same variables are categorized below.

4.3.1 Effect of Time on Styrene Migration

The studies of Withey & Collins, Eiceman & Carpen, Snyder & Breder, Durst & Laperle, Linssen et al., 1991, Lickly et al., Tawfik & Huyghebaert, Brunelli et al., Choi et al., Khaksar & Ghazi-Khansari, Condurso et al., and Amirshaghaghi et al., describes that the migration of styrene is dependent on time. The amount of styrene migration increased as time increased [19], [34], [38], [42], [43], [48], [67], [69], [70], [71], [70], [80], [81].

Snyder & Breder stated that after 2 weeks at 40^{o}C all the standard cells, irrespective of solvent, contained at least 90% of the residual styrene. For styrene migration into water, which was used as the worst case, contained 80% of the residual styrene after 2 months at 40^{o}C. A significant amount of styrene was lost when the cells were held at 70^{o}C than at 40^{o}C [48]. Durst & Laperle noticed that the amount of migrated styrene increased with time, and the rate was quickened by increased storage temperature. Highest styrene levels were at high temperatures and long period of time [70].

Lickly et al., observed that the rise in styrene migration levels observed from 1 day to 4 or 10 days is proportional to the square root of the increase in time at a particular temperature, which was expected if migration was diffusion controlled (Fickian) and not altered by equilibrium partitioning [38]. Choi et al., found that an elevated temperature produced a faster migration rate and the larger molecules of styrene were slower to completely migrate. The amount of migrated styrene is seen to increase as both time and temperature increases [43]. Till et al., noticed the rise of styrene concentration into milk at 4^{o}C as time increased, as shown in Figure 4.1 below, the values in the plot were obtained from their study [34].

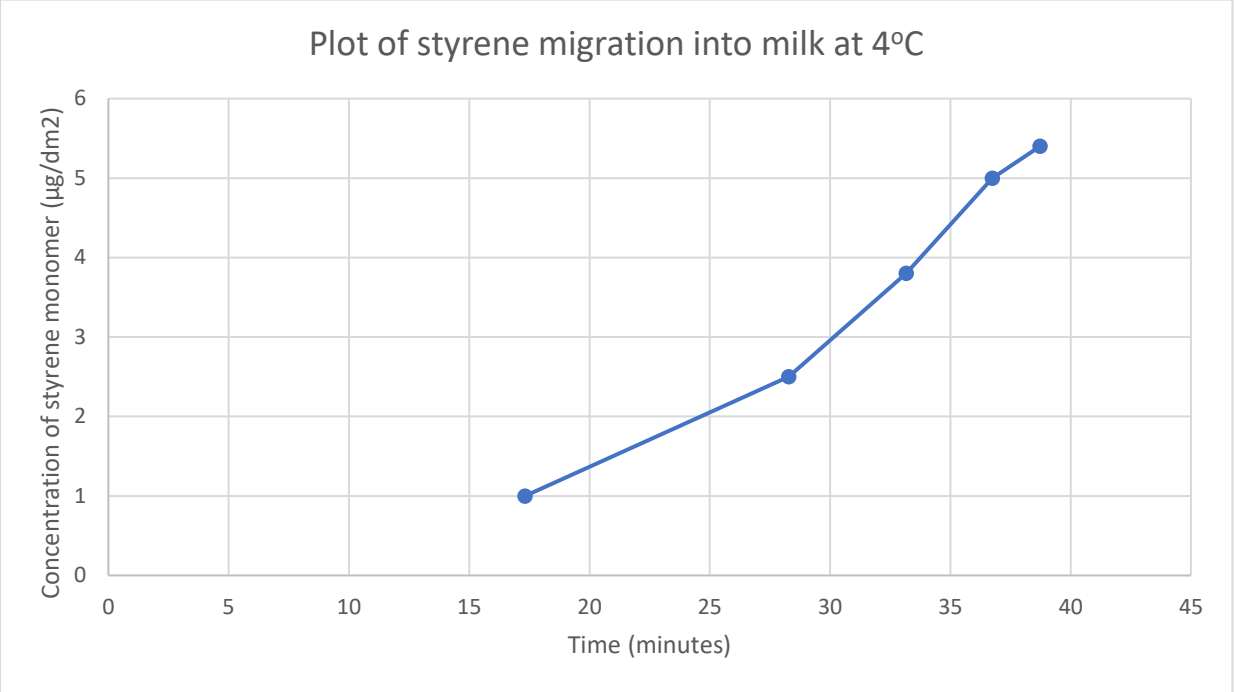

Figure 4.1: Plot of styrene migration into milk at 4°C [34]

4.3.2 Effect of Temperature on Styrene Migration

The studies of Withey, Miltz et al., Miltz & Rosen-Doody, Snyder & Breder, Durst & Laperle, Lau et al., Tawfik & Huyghebaert, Brunelli et al., Choi et al., Ahmad & Bajahlan, Sanagi et al., Khaksar & Ghazi-Khansari, Amirshaghaghi et al., and Parasekevopulou et al., illustrates that increase in temperature leads to an increase in styrene migration [13], [19], [36], [39], [40], [41], [42], [43], [48], [68], [70], [72], [77], [79], [81].

Miltz et al., stated that polystyrene cups have high residual styrene when hot water is used as the migrating medium [68]. Miltz & Rosen-Doody observed that the higher the temperature, the higher the rate of amount of styrene migration. This is because of weakened monomer/polymer interaction and to increased solubility of the styrene in the oil [39]. Snyder & Breder stated that after 2 weeks at 40°C all the standard cells, irrespective of solvent, contained at least 90% of the residual styrene. For styrene migration into water, which was used as the worst case, contained 80% of the residual styrene after 2 months at 40°C. A significant amount of styrene was lost when the cells were held at 70°C than at 40°C [48].

Durst & Laperle noticed that the amount of migrated styrene increased with time, and the rate was quickened by increased storage temperature. Highest styrene levels were at high temperatures and long period of time [70]. Choi et al., found that a elevated temperature produced a faster migration rate and the larger molecules of styrene were slower to completely migrate. The amount of migrated styrene is seen to increase as both time and temperature increases [43]. Ahmad & Bajahlan observed the effect of temperature at 100°C and found that the highest amount of styrene was detected in Styrofoam cups allowed to stand for 60mins. The quality of Styrofoam cups is also essential in migration of styrene monomer [40]. Linssen et al., 1992, observed the rise in the amount of migrated styrene into corn oil after 14 days, as the temperature increased, as shown in Figure 4.2 below, the values in the plot were obtained from their study [72].

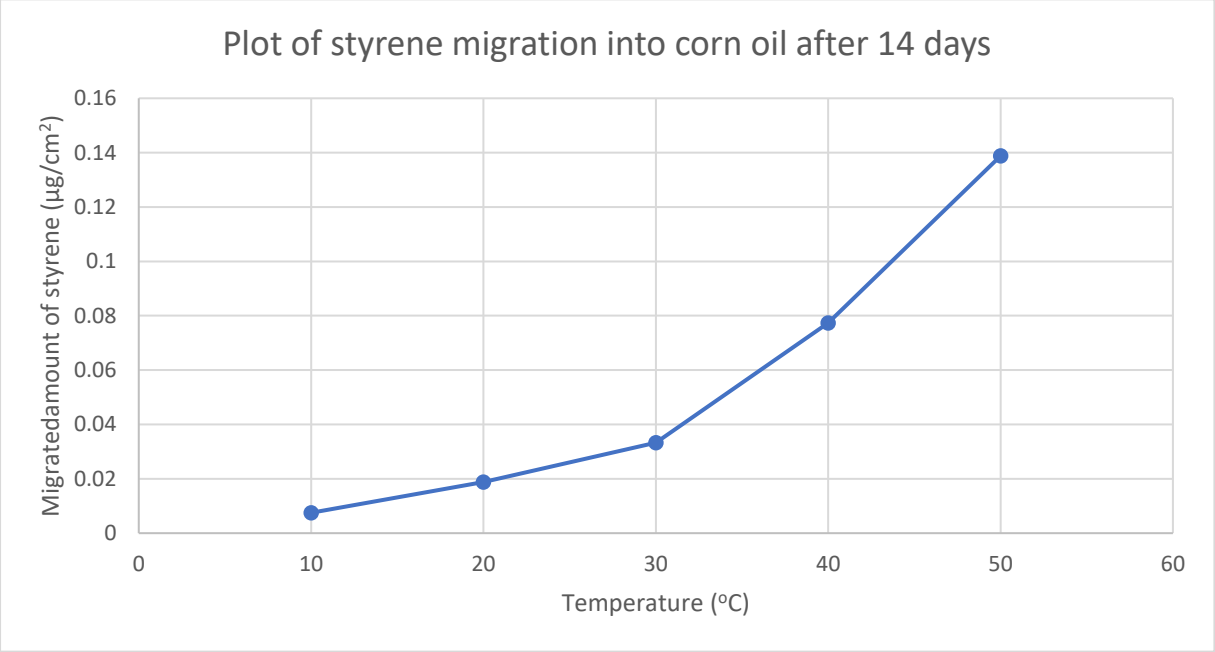

Figure 4.2: Plot of styrene migration into corn oil after 14 days [72]

4.3.3 Effect of Fat Content on Styrene Migration

The studies of Miltz et al., Varner & Breder, Miltz & Rosen-Doody, Linssen et al., 1992, Murphy et al., Lehr et al., O'Neil & Tuohy, Linssen & Reitsma, Lau et al., Tawfik & Huyghebaert, Khaksar & Ghazi-Khansari, Abolghasemi-Fakhri et al., and Song et al.,

describes that migration of styrene is dependent on fat content. More styrene is seen to migrate into oil faster than water as a result of its high fat content [19], [39], [42], [45], [68], [72], [73], [74], [75], [76], [77], [84], [85].

Miltz et al., stated that the migration of styrene into oil was higher when observed for cheese than from yoghurt package. Also, the amount of styrene in oil was much higher than that in the water. Polystyrene cups have high residual styrene when hot water is used as the migrating medium [68]. O'Neill & Tuohy noticed that the migration of styrene from polystyrene containers was strongly dependent on the fat content of the milk and on the ethanol concentration in the simulant. They observed that 50% ethanol correlates approximately with 3.5% fat milk [75]. Song et al., observed from analyzing migration results that the most migrating compound in the experiment was styrene. Comparing its amount to the amount of other migrants it can be concluded that styrene migrated from 4 to 132 times more in 10% ethanol, and from 2 to 257 times more in 3% acetic acid, than any other migrant [85].

Tawfik & Huyghebaert indicated that the migration of styrene from polystyrene cups into different beverages does not increase more than 0.08% in whole milk kept at 100°C for 2 hours of the quantity of residual styrene in the cup. The degree of styrene migration largely depends upon the fat content, storage temperature, and time. Also, the migration of styrene into hot beverages was higher than into cold beverages, this was influenced by the time of contact. There is a correlation between the amount of migration and food composition when comparing the fat content. Styrene migration from the cups into whole milk (3.6% fat) was more than into half-fat milk (1.55% fat) and both were more than into skimmed milk (0.5% fat) at different temperatures and storage times. The differences between the three fat percentages were distinct at the highest temperatures of 60 and 100°C, after 2 hours of storage, but there was no major difference between them at 20 and 40°C [19].

Khaksar & Ghazi-Khansari observed that styrene migration from polystyrene containers into hot and fatty drinks was highly dependent on fat content and temperature of drinks. Also, the migration of styrene from polystyrene cups into different beverages does not increase by more than 0.05% of the total amount of styrene in the cup. The migration of styrene into hot beverages basically depended upon the fat content, storage temperature, and time [42]. Linssen & Reitsma discovered that oil in water emulsions and fatty food products show an increasing amount of styrene migration with increasing fat content as shown in Figure 4.3

below, the values in the plot were obtained from their study. The migration of styrene increases as the fat content of the food simulant increases. The lowest concentration for migrated styrene was observed in water and the highest concentration was found in corn oil. They concluded that the general recommended migration circumstances for fatty foods of vegetable oil for 10 days at 40°C, results in an overestimation of styrene migration in fatty foods [76].

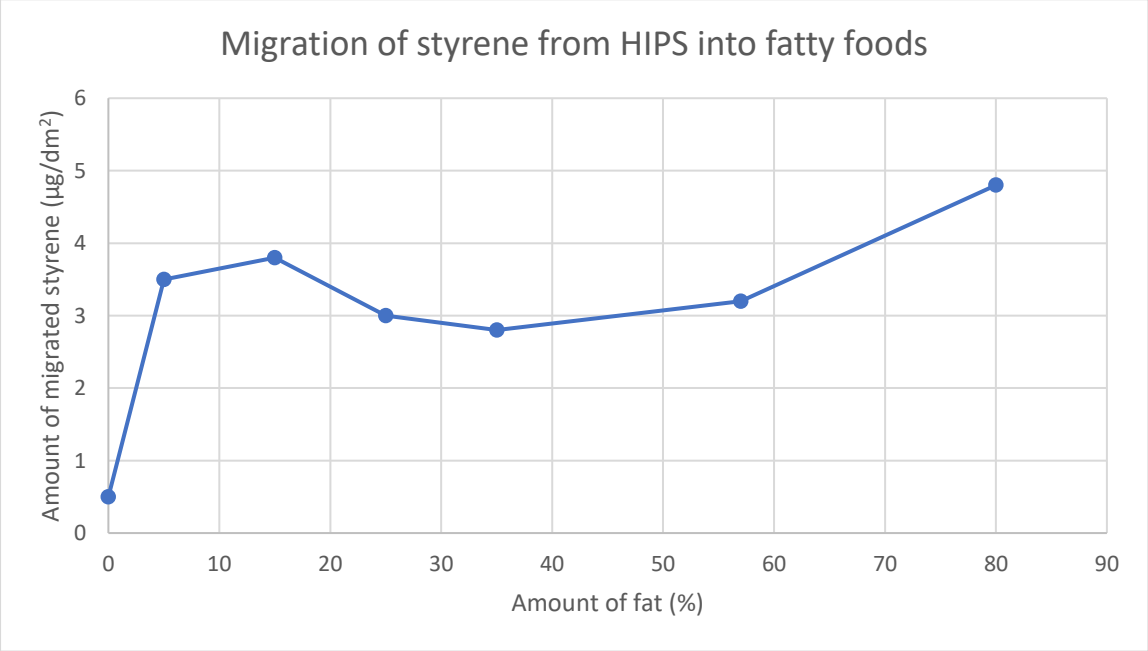

Figure 4.3: Migration of styrene from HIPS into fatty foods after incubation for 3 days at 40°C in migration cell [76].

4.3.4 Effect of Residual Styrene in Polystyrene Container on Styrene Migration

The studies of Withey, and Withey & Collins showed that polystyrene containers with the lowest amount of residual styrene leads to the most styrene migration into food simulants. Smaller containers for example yoghurt cups, migrate more styrene than other polystyrene materials analyzed. The authors explained that the extent to which styrene can migrate is proportional to the original styrene content in the polystyrene material [13], [67].

Withey, found that yoghurt cup with internal surface area of 107 cm^2 and 0.0518 ppm residual styrene, there was a total of 6.01 µg of styrene that migrated into cold water in 24 hours while for beer glass with internal surface area of 270 cm^2 and 0.0112 ppm residual styrene, there was a total of 4.46 µg styrene that migrated into hot water. Also, for yoghurt cup with internal surface area of 107 cm^2 and 0.3264 ppm residual styrene, there was a total of 33.29 µg of styrene that migrated into cold water in 24 hours while for beer glass with internal surface area of 270 cm^2 and 0.0859 ppm residual styrene, there was a total of 32.63 µg styrene that migrated into hot water [13].

Withey & Collins discovered that the amount of residual styrene in sour cream, raspberry yoghurt and butter fat cream containers were 745 ppm, 2589 ppm and 1219 ppm, respectively. While the amount of styrene migrating into sour cream was 210.46 ppb, raspberry yoghurt was 46.66 ppb and butter fat cream was 40.75 ppb. The highest extent of migration was observed for the sour cream which, surprisingly, had the lowest styrene content in the package [67].

4.3.5 Effect of Sampling Method on Styrene Migration

The studies of Linssen et al., 1991, Linssen et al., 1992 and Lickly et al., indicated that immersion sampling method results in the highest styrene migration analysis from polystyrene containers into food simulants, closely followed by the vapor-phase sampling method. The lowest amount of migrated styrene in oil was obtained in cell sampling [38], [71], [72].

Linssen et al., 1991, found that the concentration of styrene migration into oil at 40ºC from HIPS was 2.84 ppm for immersion sampling method, 2.02 ppm for vapor-phase sampling method and 0.8 ppm for cell sampling method. For HIPS-GPPS (1:1) the concentration of styrene migration into oil at 40ºC was 2.31 ppm for immersion sampling method, 1.57 ppm for vapor-phase sampling method and 0.74 ppm for cell sampling method. For GPPS the concentration of styrene migration into oil at 40ºC was 0.81 ppm for immersion sampling method, 0.78 ppm for vapor-phase sampling method and 0.58 ppm for cell sampling method [71].

Linssen et al., 1992, discovered that quantity of styrene migration into oil from GPPS:HIPS 1:1 after 21 days and 41ºC was 1.43 ppm for immersion sampling method and 0.19 ppm for

cell sampling method [72]. Lickly et al., found that the amount of styrene migrating into oil from meat tray (polystyrene foam article) after 10 days and 21°C was 0.14 µg/cm^2 for immersion sampling and 0.06 µg/cm^2 for vapor-phase sampling after 10 days and 4.4°C [38].

4.3.6 Effect of Diffusion Coefficient on Styrene Migration

The studies of Till et al., Linssen et al., 1992, Murphy et al. and Lehr et al., 1993 explains that diffusion coefficient is a function of temperature. The diffusion coefficient for styrene in polystyrene in contact with the food simulant increases with increase in temperature [34], [72], [73], [74].

In the experiment of Linssen et al., 1992, they analyzed the amount of styrene migrating from GPPS:HIPS 1:1 into corn oil after 14 days. It was observed that the diffusion coefficient obtained at 10°C was 4.7 x 10^{-16} cm^2/s and at 50°C was 1.5 x 10^{-13} cm^2/s. This supported that diffusion coefficient increases with temperature [72]. Lehr et al., compared the diffusion coefficients for styrene in HIPS and GPPS. They observed that the diffusion coefficients of HIPS appeared to be greater than the equivalent GPPS values. This is because HIPS comprise of polybutadiene rubber segments, the diffusion of styrene in rubber segment occurs at a greater rate than through the crystalline polystyrene segment. Also, they speculated that there might be presence of significant fraction of low molecular weight plasticizer; mineral oil in the HIPS and the diffusion of styrene through this material is higher than GPPS [74].

4.3.7 Effect of Polystyrene Container and Food Simulant Contact

The studies of Withey & Collins, Eiceman & Carpen, and Condurso et al., describes that the migration of styrene from the polystyrene container occur immediately it encounters the food simulant [67], [69], [80] .

Withey & Collins discovered that reasonably high concentration of styrene migrates during a short time after the food simulants meets the polystyrene container. They found that the plot of mean migrating styrene against time for the food sample honey gave a value of 11 ppb when the extrapolation of the approximate linear relationship to the coordinate axis was calculated. Also, from their previous experiment, they observed that water poured into GPPS drinking cup and immediately analyzed had a relatively high styrene concentration [67].

Eiceman & Carpen noticed that styrene migration was found in hot water in detectable levels as early as 2 minutes after initial contact of water and polystyrene cup. There was a rapid increase in the amount of migrated styrene and then a slow decrease with increased time [69]. Condurso et al., found that styrene was present in trace amount at the time of production, afterwards, its level increased up to 23 days of refrigeration reaching 15.91 ppb and finally the amount decreased to 7.03 ppb at the end of the shelf-life [80].

4.3.8 Effect of Polystyrene Container Type on Styrene Migration

The studies of Varner & Breder, Brunelli et al., Amirshaghaghi et al., Genualdi et al., and Lin et al., revealed that the most styrene migration occurs in polystyrene foam materials. Therefore, the highest amount of migrated styrene was obtained from EPS, then GPPS, and then HIPS [9], [45], [79], [81], [83].

Varner & Breder discovered that the highest migration of styrene was observed for 8% ethanol in GPPS container. There was about 3 times as much styrene migrated from GPPS containers with residual levels of 2261 ppm than from the HIPS cups with residual levels of 771 ppm. In comparison to the residual concentration, the amount of migrated styrene from the EPS cups with residual levels of 70.8 ppm styrene was about 6 times that from either of the other polystyrene types [45]. Brunelli et al., observed that mainly GPPS released more residual styrene, more quickly than the HIPS. This can be as a result of the higher molecular weight of GPPS than HIPS. A higher molecular weight implies a higher average chain length, thus breaking into fragments is statistically more likely for a longer chain than for a shorter one [79].

Amirshaghaghi et al., observed that styrene migration is supported by increased temperature and exposure time. When comparing the type of polystyrene material, the quantity of styrene migrated from EPS cups is greater than that migrated from GPPS and HIPS cups, for the same experimental conditions. This results directly from the fact that the initial measured quantity of styrene is greater in the EPS cups than in GPPS and HIPS cups [81]. Genualdi et al., observed that the most residual styrene was found in water pitcher (3042 mg/kg) made from Acrylonitrile-butadiene-styrene (ABS), followed by GPPS cups (770.8 mg/kg) [9].

4.3.9 Effect of Nanoparticles on the Polystyrene Matrix on Styrene Migration

The study of Abolghasemi-Fakhri et al., analyzed the addition of nanoparticles to polystyrene matrix leads to a reduction in the transfer of styrene monomer from polystyrene into the food simulant. They discovered that the presence of nanoparticles could substantially ($p \leq 0.05$) hinder the release of styrene monomer, causing low amount of styrene migration compared to the polystyrene without nanoparticles. Also, migration was discovered to follow Fickian diffusion principles. The diffusion coefficient of styrene was assessed and was found to be decreased with nanoparticles content [84].

4.3.10 Effect of Styrene Consumed in the Human Body

The studies of Withey, Ahmad & Bajahlan, and Verzera et al., reveals that constant intake of migrated styrene from polystyrene container into food leads to the bioaccumulation of styrene in the body [13], [40], [50].

Withey observed that the body of the animal analyzed for styrene uptake acted as a sink for styrene monomer until the section containing lipids in the animal body becomes either saturated and led to death or the tissues are at equilibrium with the exposure environment [13]. Ahmad & Bajahlan found that there was evidence of styrene monomer and some related aromatic compounds migrating into water. Though, there may not be immediate toxic effect from drinking the contaminated water, but chronic effects may be observed as a result of repeated ingestion of a number of small doses, each in itself is inadequate to cause an instantaneous acute reaction but in the long term having a cumulative toxic effect [40].

4.3.11 Effect of Modified Instruments for Analysis on Styrene Migration

The studies of Lau et al., Condurso et al., Nerin et al., and Verzera et al., reveals that the use of modified instruments for styrene analysis along with the GCMS leads to a faster and effective way of determining migration of styrene from polystyrene containers into food simulants [50], [77], [78], [80].

Nerin et al., examined analytical conditions for the determination of styrene in yoghurt. They found that the automatic, commercially available purge-and-trap system can be used for the

analysis of volatile organic compounds in thick liquids such as yoghurt for which it was not initially designed. They achieved excellent results and reproducibility with the system at low cost. The detection limit of styrene reached by the dynamic purge-and-trap coupled to GC-MS-SIM procedure is very low with high accuracy and sensitivity [78]. Condurso et al., found that the Headspace Solid Phase Microextraction/fast Gas Chromatograph procedure was suitable for determining styrene migrating into food simulants. The technique was rapid, sensitive enough for migration control and showed a good repeatability in terms of peak areas and retention times [80].

Verzera et al., compared the conventional SPME/GC with the SPME/fast GC method. The experiment was carried out by reducing both SPME equilibration and analyte separation time. This led to the determination of styrene and ethylbenzene in about 15 min (including sampling, extraction and analysis). The quantity of styrene and ethylbenzene, observed in the analyzed yoghurts, even if in small quantities, increases the daily total intake of substances classified as carcinogenic to humans. They concluded that the proposed HS-SPME/fast GC procedure is suitable for the determination of styrene and ethylbenzene migrating into yoghurt from polystyrene containers. The proposed method is fast, does not need sample manipulation, shows good repeatability in terms of peak areas and retention times, and has a higher sensitivity [50].

CHAPTER 5: METHODOLOGY

The method used in carrying out this meta-analysis is in line with the PRISMA standard [30]. The statistical analysis was carried out following the guide from Harrer et al., [86]. R is the statistical technique used in this meta-analysis research. It was chosen because it is an open-source software and it is widely used for applied research across many fields. The R statistical tool is vastly used in the field of toxicology and its recommended by the Cochrane group for meta-analysis in toxicology. Also, it is more accurate to use for meta-analysis because of its meta-analytic package functionality.

5.1 Develop Research Questions

The research questions evaluated while carrying out this meta-analysis are:

- What is the evidence that styrene migrates from polystyrene food containers into food?
- How much of styrene do humans consume from food in polystyrene food containers?
- How much of styrene consumed is harmful to human health?

5.2 Define Inclusion and Exclusion Criteria

The inclusion and exclusion criteria considered for this meta-analysis are:

- All studies should be considered, excluding case reports, letters, tutorials, editorials, and review articles.
- Only English studies should be considered.
- Each study should provide enough data to be able to obtain a single estimate. It should also provide information on the control experiment.
- Papers published multiple times with either the same or overlapping data set, the paper with the larger data set will be considered.

5.3 Search for Literature

The studies collected for this research were obtained by searching electronic databases and scanning reference lists of articles. The electronic databases searched were PubMed-Medline, SCOPUS, Springer link, Google scholar, EMBASE, Medline at OVID, Federal Science Library, ScienceDirect, PubMed Central (PMC) and Web of Science. The last search was run on 13 January 2020. The key words used for the search are polystyrene food container, styrene leachate, styrene migration and styrene toxicity.

5.4 Screen Literature

Three sets of screening were done to obtain studies that will be included in the meta-analysis. This was done according to the method of [30]. The studies were first identified, then they were screened to remove duplicate studies, followed by a check for eligibility; using the inclusion and exclusion criteria as a standard, and the selected studies were included in the meta-analysis.

5.5 Extract Data

Extraction of data from the selected articles was conducted using Excel spreadsheet. The data from the studies were compiled according to the identified variables and arranged in separate columns. The data obtained for the control experiments were also compiled under the set variables. The variables identified amongst the study compiled are percentage of fat content, time of contact between the polystyrene container and food, type of polystyrene container, size of polystyrene container and the temperature of food in the polystyrene container. The raw extracted data are included in Appendix A.

The data from each study was standardized into a uniform unit as each individual study reported their results differently. The fat content of the food simulant was obtained from each study. The time measured in each study was converted to minutes for uniformity, the temperature was also reported in degrees Celsius. The type of polystyrene material analyzed in each study was determined and the size was recorded in cm^2. The amount of styrene migrating from polystyrene container into food from all 33 studies considered were converted

into µg/100cm^2, for uniformity. Results of experimental analysis obtained from the determination of styrene concentration in polystyrene container was used as the control experiment and measured in µg/100 cm^2.

R statistical program was used to perform the meta-analysis. It was chosen because it is an open-source software and it is widely used for applied research across many fields. In addition, R is more accurate to use for meta-analysis because of its meta analytic packages functionality. Therefore, the dataset compiled in Excel spreadsheet were restructured using the method of [86] in order to be imported into RStudio (Figure 5.1). The data was arranged for a "Standardized Mean Meta-Analysis". This requires the mean, standard deviation, and sample size from both groups in a study and for all studies considered.

Here is how you should name the data columns in your EXCEL spreadheet containing your Meta-Analysis data

Column	Description
Author	This signifies the column for the study label (i.e., the first author)
Me	The mean of the experimental/intervention group
Se	The standard deviation of the experimental/intervention group
Mc	The Mean of the control group
Sc	The Standard Deviation of the control group
Ne	The number of participants in the experimental/intervention group
Nc	The number of participants in the control group
Subgroup	This is the label for one of your subgroup codes. It is not that important how you name this column, so you can give it a more informative name (e.g. population). In this column, each study should then be given an subgroup code, which should be exactly the same for each subgroup, including upper/lowercase letters. Of course, you can also include more than one subgroup column with different subgroup codings, but the column name has to be unique

Figure 5.1: Naming data column in Excel spreadsheet [86]

The dataset in Excel spreadsheet was saved using the comma-separated-values (.csv) file format. This is a working directory which is a folder from which RStudio can use data and save results. Importing of dataset from the computer into RStudio was done following the method of [86].

Figure 5.2 below shows the extracted dataset in the Excel spreadsheet ready to be imported into R. The names of each author are listed in the first column titled **AUTHOR**. The second column **Ne** contains the total number of experiment each study performed on the migration of styrene from polystyrene containers into food simulant. The third column **Me** shows the mean amount of migrated styrene from polystyrene containers into food simulant for each study. The fourth column **Se** describes the standard deviation of migrated styrene from polystyrene containers into food simulant for each study.

The fifth column **Nc** represents the total number of experiments to determine the amount of residual styrene present in polystyrene food containers for each study. The sixth column **Mc** refers to the mean amount of residual styrene present in polystyrene food containers for each study. The seventh column **Sc** denotes the standard deviation of residual styrene present in polystyrene food containers for each study. Method describes the specific sampling method utilized to determine the amount of migrated styrene from each study. Subgroup identifies the categories of food sample applied by each study.

For this meta-analysis, the **Experimental Group** represents the quantity of styrene migrating from polystyrene containers into food simulant while the **Control Group** signifies the amount of residual styrene present in polystyrene food containers. Residual styrene can be obtained from polystyrene containers by dissolving them in aromatic hydrocarbon solvents, such as diethylbenzene, and butylbenzene.

Author	Ne	Me	Se	Nc	Mc	Sc	Method	Subgroup
Withey	15	0.0891	0.09795	11	22.45	20.11	Vapour phase	Food & Simulant
Withey & Collins	9	166.43	253.23	14	12.45	6.93	Vapour phase	Food
Miltz et al	3	2296	1946.44	2	166	39.598	Immersion sampling	Food
Varner & Breder	6	0.03044	0.03997	3	16.34	11.19	Vapour phase	Food & Simulant
Eiceman & Carpen	11	0.9598	0.41556	2	0.07857	0.004964	Vapour phase	Food
Till et al	11	0.35477	0.451717	3	142.41	170.14	Cell sampling	Food
Miltz & Rosen-Doody	6	3925	2552.78	5	8.02	2.928	Immersion sampling	Food
Snyder & Breder	16	0.0000953	0.00328	5	116.937	160	Cell sampling	Food & Simulant
Durst & Laperle	4	5.1797	3.434	4	49812	27864.69	Vapour phase	Food
Linssen et al 1991	9	0.125746	0.07546	3	32.512	4.02055	Vapour phase, Cell sampling, Immersion	Food
Linssen et al 1992	15	0.0677	0.07665	5	13.981	8.3012	Immersion & Cell sampling	Food & Simulant
Murphy et al	14	92.1037	168.72	6	0.81696	0.56514	Cell sampling	Food
Lehr et al	8	31.7534	30.85	4	0.90896	0.56196	Vapour phase	Food
O'Neill & Tuohy	7	19.392	21.1597	2	0.2727	0.3851	Vapour phase	Food & Simulant
Linssen & Reitsma	28	0.0455	0.025196	2	26.0883	36.786	Cell sampling	Food & Simulant
Lau et al	9	1.3792	2.9746	8	0.4901	0.6304	Vapour phase	Food
Lickly et al	39	49.277	78.933	6	0.51169	0.3229	Vapour phase	Food & Simulant
Tawfik & Huyghebaert	75	0.0279	0.055617	3	10.75	18.4032	Vapour phase	Food & Simulant
Nerin et al	5	76.16	41.07	2	0.065	0.0495	Vapour phase	Food
Brunelli et al	110	223.49	222.73	2	0.02523	0.035	Immersion sampling	Food & Simulant
Choi et al	3	0.2554	0.01365	2	0.254	0.3479	Cell sampling	Simulant
Ahmad & Bajahlan	18	0.5684	0.6567	5	0.01115	0.01533	Vapour phase	Food
Sanagi et al	15	4.4333	2.246	3	13.77	6.0274	Immersion sampling	Food
Khaksar & Ghazi-Khansari	30	0.003295	0.00412	2	0.0347	0.0485	Vapour phase	Food & Simulant
Condurso et al	8	17.456	8.81411	2	0.0006	0.000566	Vapour phase	Food
Verzera et al	7	21.2551	11.546	2	0.0006	0.00057	Vapour phase	Food
Amirshaghaghi et al	3	2.51	0.3161	2	2.24	3.055	Immersion sampling	Simulant
Paraskevopoulou et al	12	136261.99	158768.5	2	0.8496	0.63583	Cell sampling	Simulant
Saim et al	20	0.13019	0.042735	4	0.004738	0.00501	Vapour phase	Food
Genualdi et al	10	0.025496	0.02889	24	2.795	3.822	Vapour phase	Food
Lin et al	3	158.23	52.716	2	0.458	0.0202	Vapour phase	Simulant
Abolghasemi-Fakhri et al	16	0.08093	0.03979	2	0.0665	0.082737	Vapour phase	Simulant
Song et al	16	0.43774	0.21303	2	0.1833	0.00806	Immersion sampling	Simulant

Figure 5.2: Extracted data to be run in R

5.6 Assessment of Risk of Bias

This research was conducted by an individual. There was no assessment to evaluate the risk of bias. The individual studies excluded and included in the meta-analysis were not verified. The overall risk of bias was assessed by using the PRISMA standard checklist.

5.7 Statistical Analysis

The meta-analysis was performed by computing the Standardized Mean Difference (SMD) using the random-effects model, and followed the method proposed by Harrer et al., see Appendix B for more details.

The outcome measures used in the meta-analysis were for continuous outcomes, as observed from the extracted data. The amount of migrated styrene detected from each of the study was measured in different units and the migration experiments were carried out using different instruments and variables. Hence, the summary effect measure used was the standardized mean difference, the statistical method used was the inverse variance and the random-effects model was used. The method used for testing heterogeneity; the variance between each study, is the I^2 value. This was chosen because it signifies the percentage of inter-study variability that can be ascribed to heterogeneity rather than chance. The Hartung-Knapp-Sidik-Jonkman method was used to estimate I^2 in the random-effects model. This method was chosen because it proposed a way to produce more robust estimates of var(Θ_F). It can also be very easily applied in R and typically leads to more conservative results, indicated by wider confidence intervals.

5.8 Test for Heterogeneity

The between-study heterogeneity test was carried out to know the level of heterogeneity between the studies. This indicates the presence of very extreme effect sizes called outliers. In order to assess the heterogeneity of the pooled effect size, three heterogeneity measures were carried out. They are detecting outliers & influential cases, influence analysis and Graphic Display of Heterogeneity (GOSH) plot analysis.

5.9 Publication Bias

The possibility of publication bias was assessed by using the method of [86]. For each study, the effect was plotted by its standard error. The symmetry of the funnel plots obtained were assessed both visually, and formally with Egger's test, to see if the effect decreased with increasing sample size, see Appendix B for more details.

5.10 Flux of Styrene Migration

The quantity of migrated styrene from polystyrene container into food simulant was plotted against the square root of time to obtain the rate of styrene migration from polystyrene containers into food simulants. The amount of migrated styrene obtained from studies is dependent on the diffusion coefficient of styrene from the polystyrene material and the initial concentration of styrene in the polystyrene material.

CHAPTER 6: RESULTS AND DISCUSSION

6.1 Meta-analysis Study Selection

Articles on the migration of styrene from polystyrene containers into food were obtained from 10 databases. After the search, a total of 21,990 papers were found. The first screening was done to remove the papers titles not related to the research questions from the total number of papers. After the first screening, a total of 21,608 were excluded and a total of 382 papers were left for review. The second screening was to remove duplicate papers from within and between the database. A total of 278 papers were removed because they were in duplicates after the second screening and a total of 104 papers were left for review. The third screening was to review the abstract. After the abstract review, at total of 44 papers were excluded because they did not meet inclusion criteria and a total of 60 papers were left for full review. After the full review was completed, 27 papers were excluded. 18 studies out of the 27 studies were discarded because the studies were not written in English and could not be possibly translated into English. No unpublished relevant studies were obtained. A total of 33 papers were included in the meta-analysis study (Figure 6.1). Table 1 below display details of studies included in the meta-analysis.

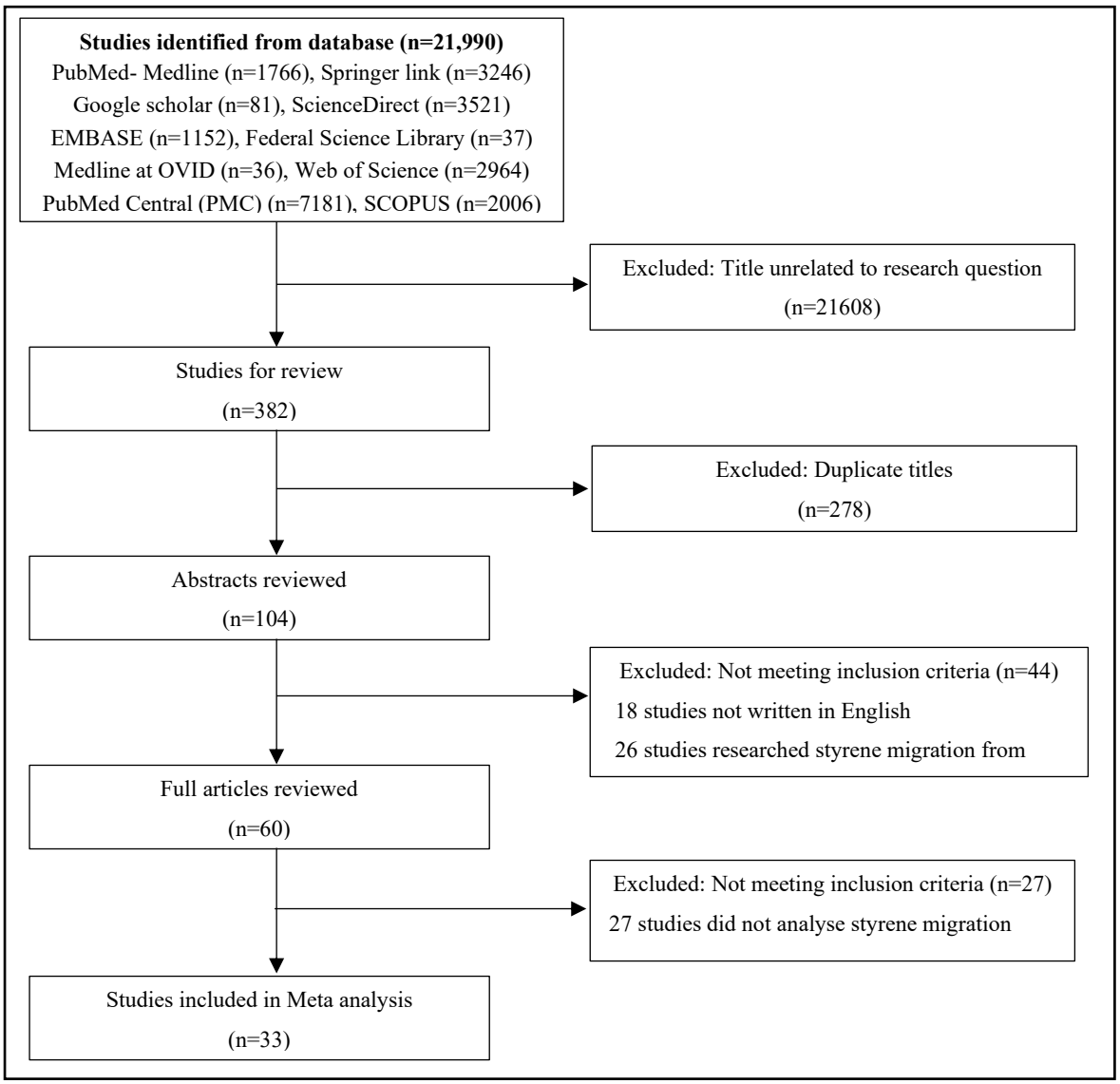

Figure 6.1: Inclusion flow diagram which illustrates the study selection process

Table 1: Studies investigating the migration of styrene from polystyrene containers into food.

Reference	Study code	Control group	Sampling method	Unit	Food sample
Withey, 1976 [13]	1	Yes	Vapor phase	ppm	Food & Simulant
Withey & Collins, 1978 [67]	2	Yes	Vapor phase	ppb	Food
Miltz et al., 1980 [68]	3	Yes	Immersion sampling	µg	Food
Varner & Breder, 1981 [45]	4	Yes	Vapor phase	ppb	Food & Simulant
Eiceman & Carpen, 1982 [69]	5	Yes	Vapor phase	µg/L	Food
Till et al., 1982 [34]	6	No	Cell sampling	$\mu g/dm^2$	Food
Miltz & Rosen-Doody, 1984 [39]	7	Yes	Immersion sampling	g x 10^6	Food
Snyder & Breder, 1985 [48]	8	Yes	Cell sampling	cm x 10^{-6}	Food & Simulant
Durst & Laperle, 1990 [70]	9	Yes	Vapor phase	µg/L	Food
Linssen et al., 1991 [71]	10	Yes	Vapor phase, Cell sampling, Immersion	ppm	Food
Linssen et al., 1992 [72]	11	Yes	Immersion & Cell sampling	$\mu g/cm^2$	Food & Simulant
Murphy et al., 1992 [73]	12	Yes	Cell sampling	ppb	Food
Lehr et al., 1993 [74]	13	No	Vapor phase	ppm	Food & Simulant
O'Neill & Tuohy,1994 [75]	14	Yes	Vapor phase	$\mu g/cm^2$	Food
Linssen & Reitsma, 1995 [76]	15	Yes	Cell sampling	$\mu g/dm^2$	Food & Simulant
Lau et al., 1995 [77]	16	Yes	Vapor phase	µg/g	Food
Lickly et al., 1995 [38]	17	Yes	Vapor phase	$\mu g/cm^2$	Food & Simulant
Tawfik & Huyghebaert, 1998 [19]	18	No	Vapor phase	mg/kg	Food & Simulant
Nerin et al., 1998 [78]	19	No	Vapor phase	µg/kg	Food
Brunelli et al, 2002 [79]	20	No	Immersion sampling	mg/dm^2	Food & Simulant
Choi et al., 2005 [43]	21	Yes	Cell sampling	µg/g	Simulant
Ahmad & Bajahlan, 2007 [40]	22	No	Vapor phase	µg/L	Food
Sanagi et al., 2008 [41]	23	Yes	Immersion sampling	µg/L	Food
Khaksar & Ghazi-Khansari, 2009 [42]	24	No	Vapor phase	µg/L	Food & Simulant
Condurso et al., 2009 [80]	25	No	Vapor phase	ppb	Food
Verzera et al., 2010 [50]	26	No	Vapor phase	ng/g	Food
Amirshaghaghi et al., 2011 [81]	27	No	Immersion sampling	mg/g	Simulant
Paraskevopoulou et al., 2012 [36]	28	Yes	Cell sampling	$\mu g/cm^2$	Simulant
Saim et al., 2012 [82]	29	No	Vapor phase	µg/L	Food
Genualdi et al., 2014 [9]	30	Yes	Vapor phase	ng/g	Food
Lin et al, 2017 [83]	31	Yes	Vapor phase	µg/L	Simulant
Abolghasemi-Fakhri et al., 2019 [84]	32	No	Vapor phase	$\mu g/cm^2$	Simulant
Song et al., 2019 [85]	33	No	Immersion sampling	µg/kg	Simulant

Table 1 above lists the selected studies included in the meta-analysis alongside their year of publication. The study code represents the numbering of the studies according to their year of publication. The control group denotes the studies which indicated the amount of residual styrene present in polystyrene containers. Sampling method describes the specific method utilized to determine the amount of migrated styrene from each study. The unit and food sample applied by each study were stated.

6.2 Statistical Analysis

The meta-analysis output indicates the Standardized Mean Difference (SMD), 95% Confidence Interval (CI) and the percentage weight (%W) of each study. The total number of studies combined (k) were 33 and the test for heterogeneity was reported by the p-value, Q-test and I^2 value. This meta-analysis output was then converted to a Forest Plot, Figure 6.2 below.

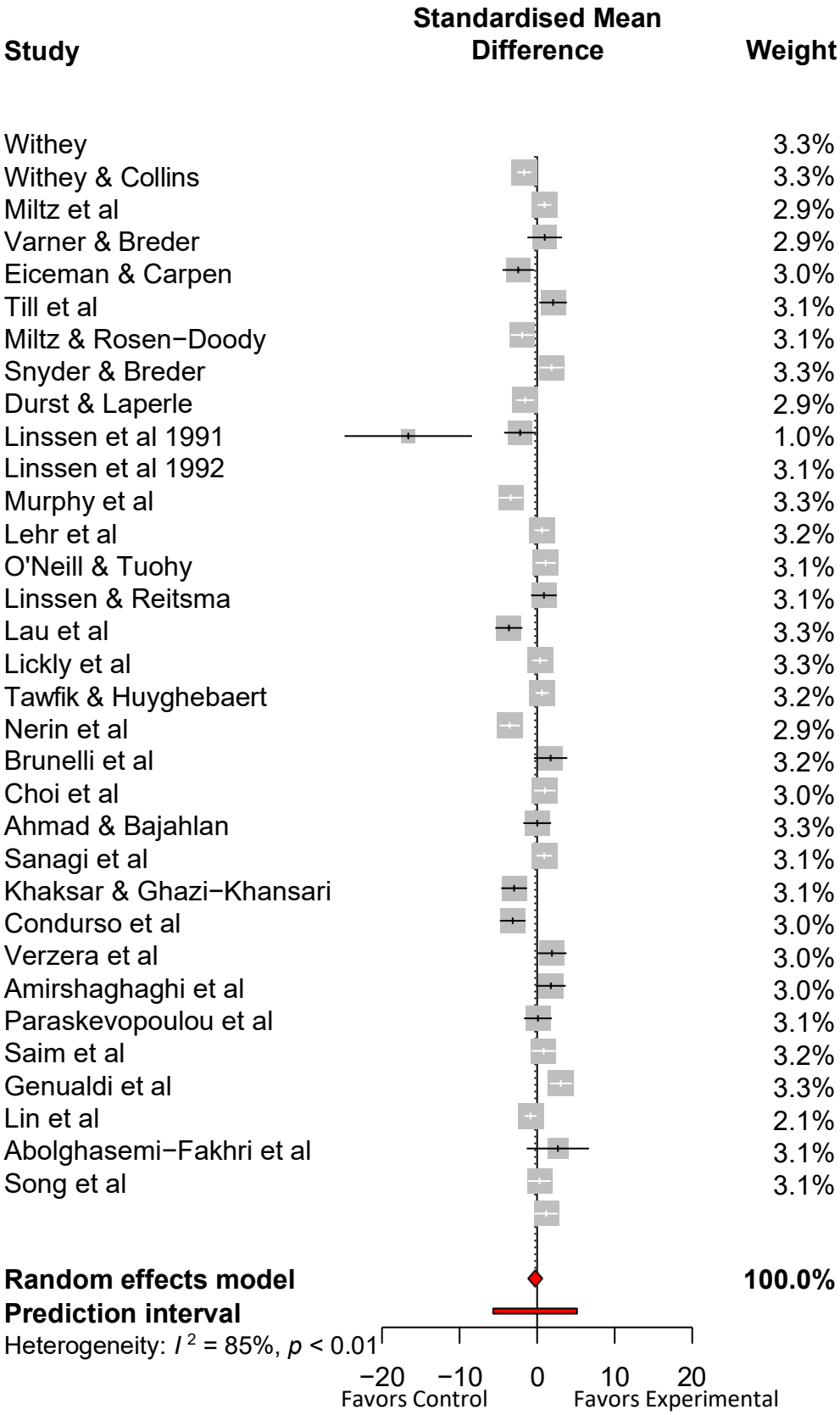

Figure 6.2: Meta-analysis Forest Plot

6.3 Forest Plot

This Forest Plot in Figures 6.2 above illustrates the meta-analysis output. The author of each study considered is on the left side of the plot with the heading **STUDY**. Each box on the plot represents the point estimate for each study and the size signifies the weight of the study. The horizontal lines through the box demonstrates the length of the 95% CI. The vertical line is the line of no effect, the position where there is no clear difference between the experimental and control. The diamond symbolizes the meta-analytic summary of all the studies. The peak of the diamond and dotted lines represents the overall estimate while the width of the diamond shows the 95% CI. The left side of the plot favors the experimental group while the right side of the plot favors the control group. The information on the right side of the Forest Plot is similar to the plot in the middle.

The overall effect size obtained was -0.26, with the 95% CI from -1.15 to 0.64 and p-value 0.56. The overall effect sizes are absolute values [87], hence this can be interpreted that the average residual styrene that migrated in the control group is 26% exceeding the average styrene migrating from polystyrene containers into food simulants in the experimental group. The negative value of the overall effect indicates that the amount of migrated styrene in the control group surpasses that of the experimental group [88]. I^2 measuring statistical heterogeneity indicates the presence of substantial heterogeneity (I^2 = 84.5%, p-value <0.01), supporting the use of random-effects model.

6.4 Test for Heterogeneity

The R function was rerun, identifying and excluding the outliers. The function has detected 7 outliers and removed them by giving them 0.0% weight. These outliers are: ‘Linssen et al 1991’, ‘Linssen et al 1992’, ‘Linssen and Reitsma’, ‘Tawfik & Huyghebaert’, ‘Sanagi et al’, ‘Khaksar & Ghazi-Khansari’ and ‘Saim et al’.

After these outliers were removed, the overall effect was 0.35, the 95% CI was from -0.18 to 0.89 and p-value 0.19. It is observed that the overall effect changed from negative to positive, thus signifying that the amount of migrated styrene in the experimental group exceeds that of the control group by 35%. This meta-analysis output was then reported as a Forest Plot in Figures 6.3 below.

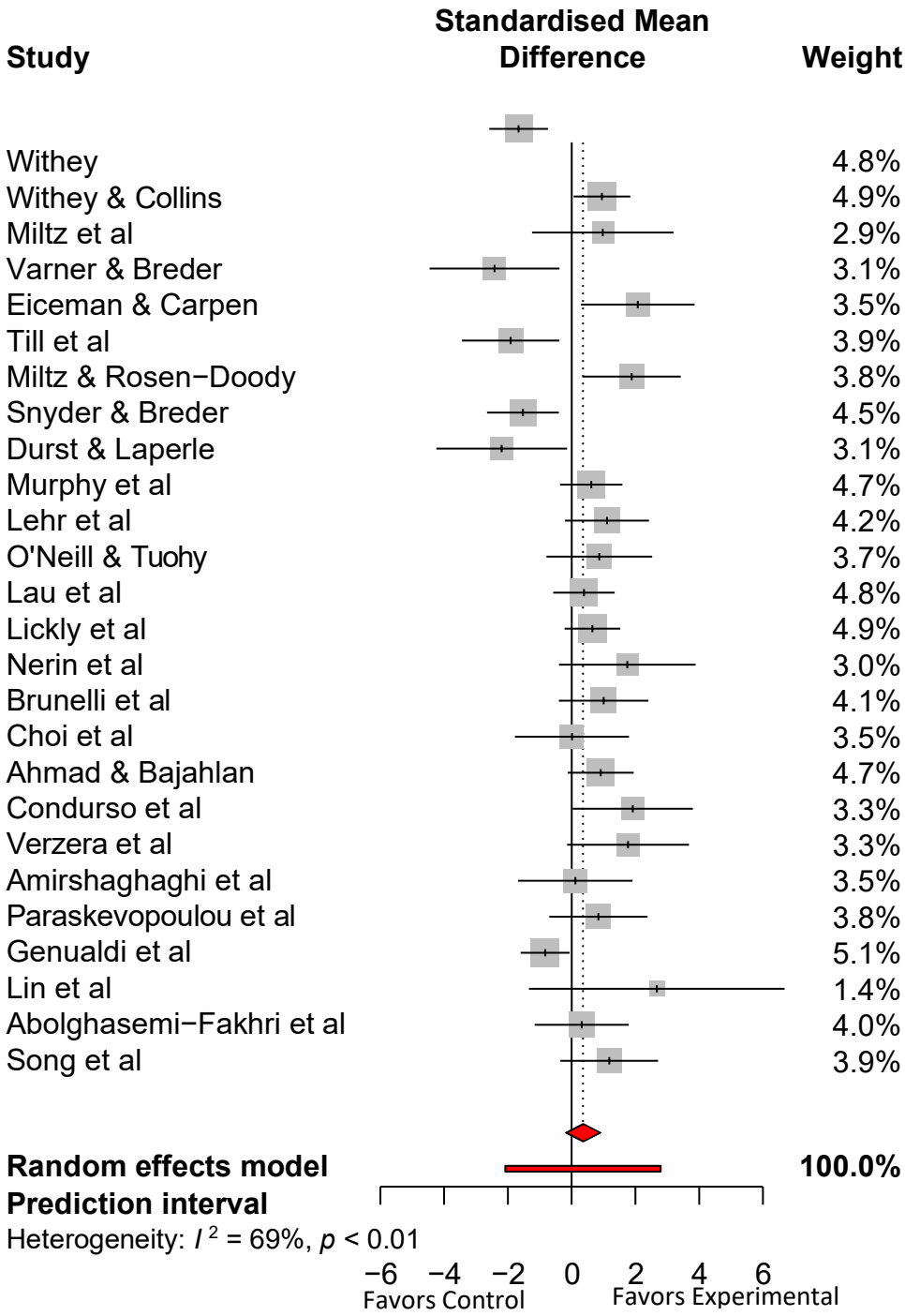

Figure 6.3: Meta-analysis Forest Plot without outliers

This Forest Plot in Figures 6.3 above illustrates the meta-analysis output without outliers. It was observed that overall effect changed from -0.26 to 0.35 and the I^2 value reduced from 84.5% to 69.4%, which is a moderate level of heterogeneity. This change in the result is because of the removal of outliers from the meta-analysis. Also, the overall effect (diamond) overlaps the line of no effect, this indicates that there is no significant difference between the amount of migrated styrene from polystyrene containers into food simulant and the amount of residual styrene present in polystyrene containers.

6.5 Publication Bias

The funnel plot of the meta-analysis of published studies was obtained. Each plotted represents the standard error and standardized mean difference between each study. The black dash lines forming a triangle represent the region of 95% confidence interval, meaning 95% of the study would lie in this region in the absence of a publication bias. The vertical line represents the average standardized mean difference of -0.26 found in the meta-analysis. The asymmetry of the funnel plot in Figure 6.4 suggests the presence of publication bias or small study effects. The Egger's test is then carried out to confirm this finding.

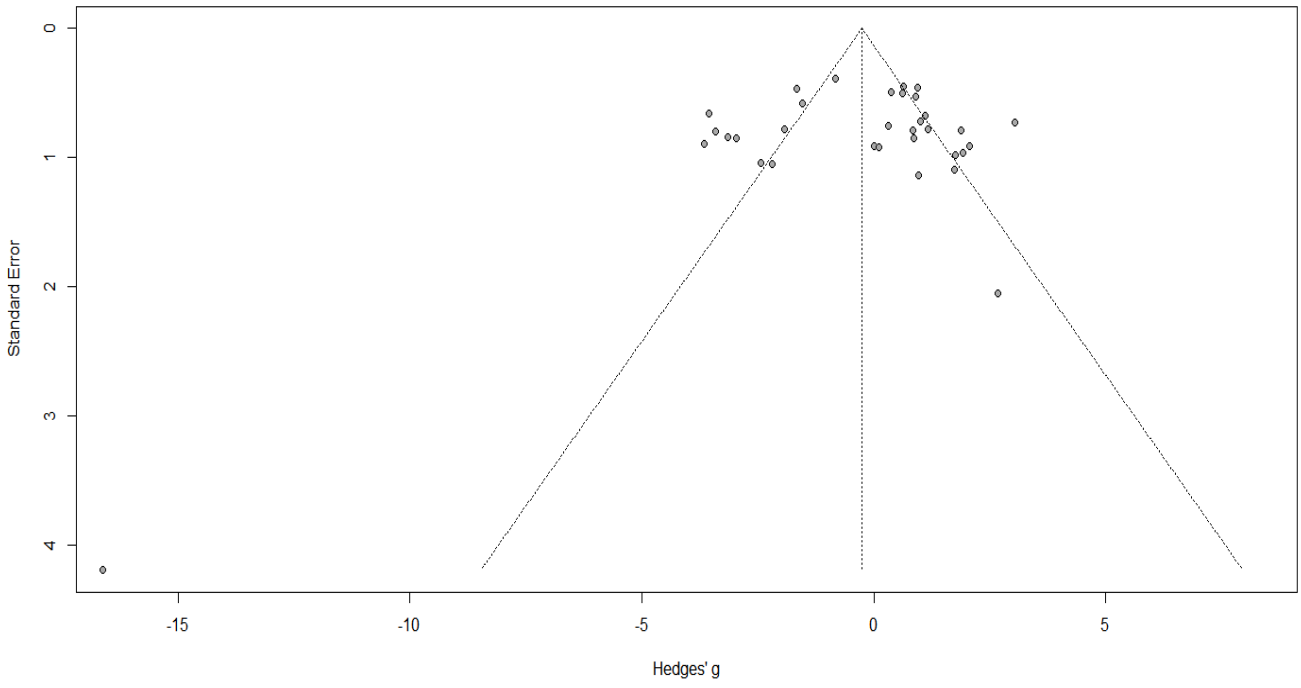

Figure 6.4: Funnel Plot

The Egger's test for publication bias p = 0.57 is not statistically significant as the p-value is (p>0.05). This is plotted using the contour-enhancing funnel plot, in order to differentiate publication bias from other cause of asymmetry (Figure 6.5).

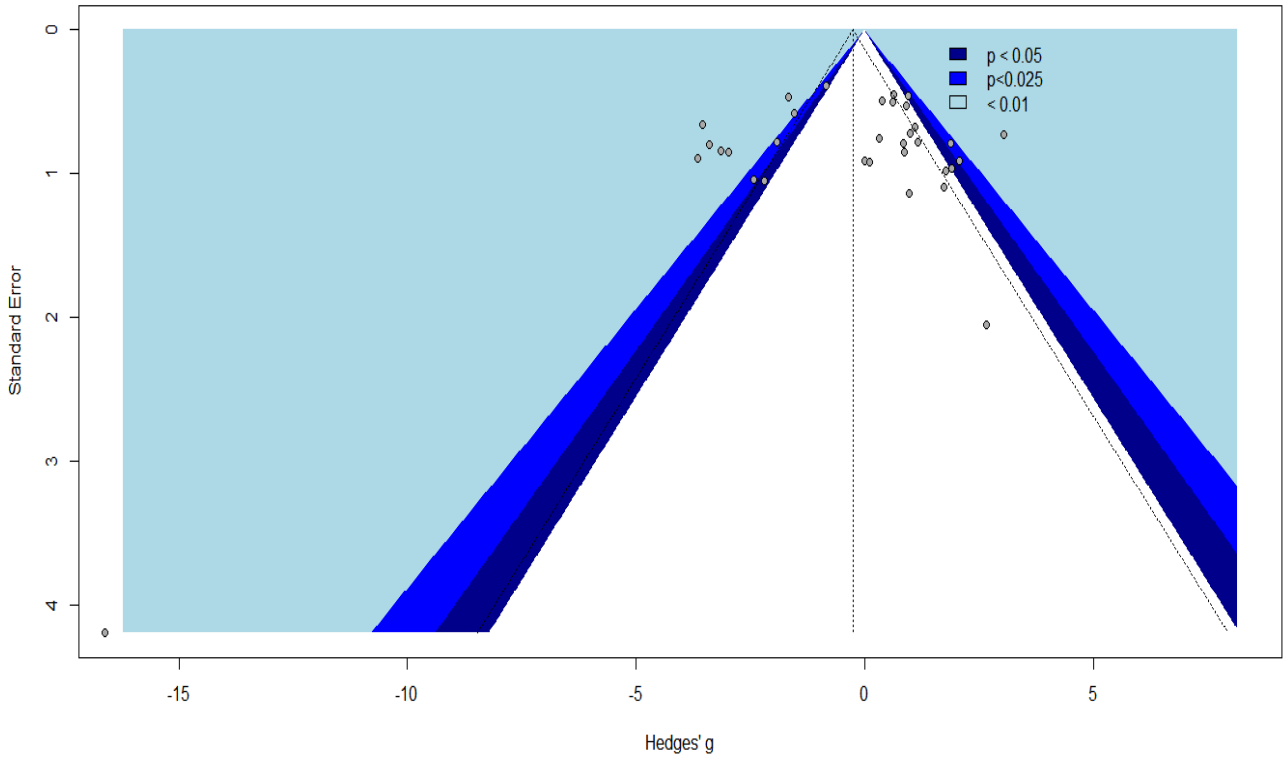

Figure 6.5: Funnel Plot displaying p-value

6.6 Flux of Styrene Migration

The plot of migrated styrene against the square root of time was done in R (Figure 6.6). It was observed from the Flux of styrene plot that the amount of migrated styrene from polystyrene container into food simulant M_t, increased as the square root of time increases. The migration of styrene obeys the Fick's second law of diffusion. The Fick's second law of diffusion states that the rate of change of concentration at a point in space is proportional to the second derivative of concentration with space [89].

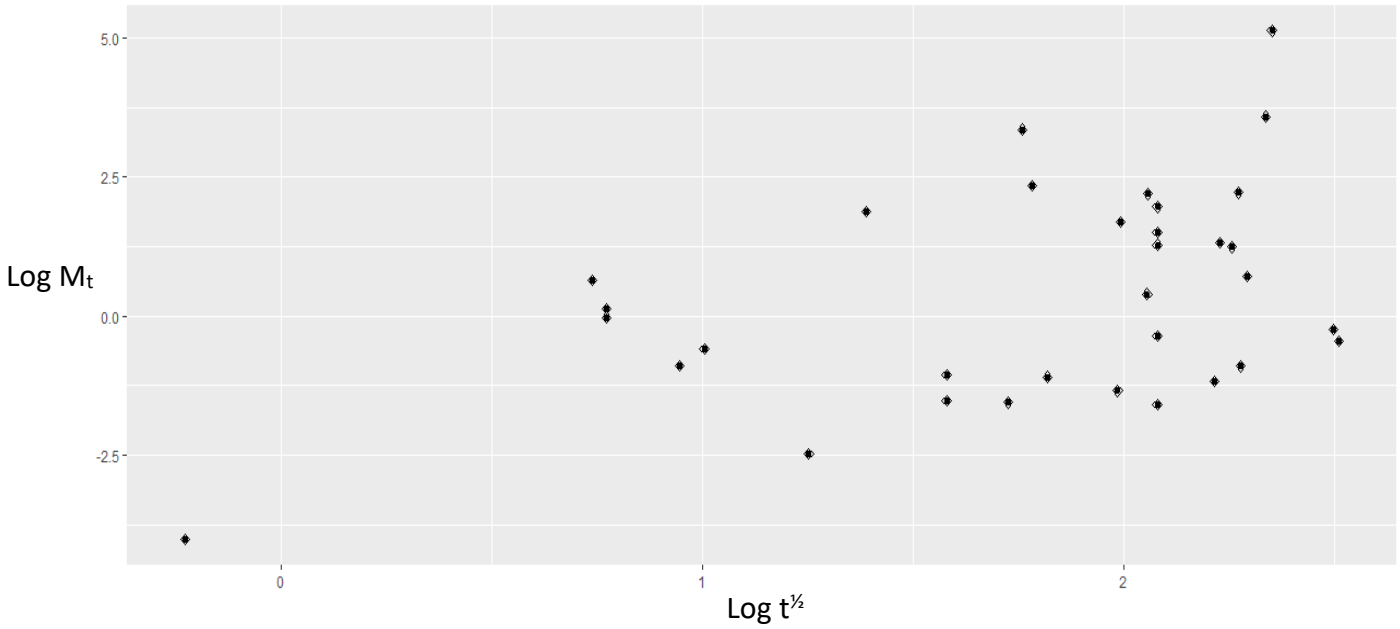

Figure 6.6: Flux of styrene migration per square root of time

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

A meta-analysis has been performed in order to consolidate several study data on the migration of styrene from polystyrene container into food simulants. This chapter specifies the various conclusions that can be drawn from this research, as well as suggesting directions for future work in this area.

7.1 Conclusions

Each of the studies included in this meta-analysis established the migration of styrene from polystyrene food containers into food simulants. However, the report from these studies had styrene migration levels less than the US-FDA standard. The studies combined in this meta-analysis established that the immersion sampling method yielded the highest styrene migration from polystyrene containers into food simulants. The migration of styrene from the polystyrene container occur immediately it meets food and increases with time, temperature and fat content. Addition of nanoparticles to polystyrene matrix causes to a reduction in the transfer of styrene monomer from polystyrene into the food simulant. Constant intake of food contained in polystyrene containers leads to the bioaccumulation of styrene in the body.

The result of the meta-analysis in this study indicates that higher amount of residual styrene is present in polystyrene containers than the amount of styrene migrating from polystyrene containers into food simulants. The 35% more residual styrene in the polystyrene container is not statistically significant as shown in Figure 6.3. The rate of styrene migration into the food simulant was observed to obey Fick's second law of diffusion. There was an increasing amount of styrene from polystyrene containers into food simulants as the square root of time increases.

7.2 Recommendations

This thesis analyses the study of migration of styrene from polystyrene containers into food simulants by a meta-analysis. It is evident that the study on styrene migration is very broad

and cannot be finalized in a single study. Therefore, it is recommended that there should be a continuous analysis of styrene migration from polystyrene containers as new production materials and techniques are designed, so as to keep up with the harm that might be associated with the indirect consumption of styrene migrating into food from polystyrene containers.

Additionally, a study on possible compounds and additives that can lead to complete polymerization of styrene monomers in order to eliminate the presence of residual styrene from polystyrene containers should be carried out. The absence of residual styrene in polystyrene container will completely eradicate the migration of styrene into food.

Lastly, though the aim of this research was to evaluate styrene migration, an exhaustive study can be carried out on the determination of other volatile products not examined here for example, ethylbenzene and benzene.

REFERENCES

[1] Isowall group. (2018, April 26). A Brief History of Polystyrene. Retrieved January 24, 2020, from https://www.isowall.co.za/a-brief-history-of-polystyrene/

[2] Bellis, M. (2020, January 06). A History of Polystyrene and Styrofoam. Retrieved January 24, 2020, from https://www.thoughtco.com/invention-of-polystyrene-and-styrofoam-1992332

[3] Polymerization of Styrene (2015). Polymer Properties Database. Retrieved January 24, 2020, from http://polymerdatabase.com/polymer%20chemistry/Polystyrene.html

[4] Food Surveillance Information Sheet No. 83. (March 1996). Food Safety Directorate, Ministry of Agriculture Fisheries and Food, London.

[5] Castle, L., Sharman, M., & Gilbert, J. (1988). EEC Relating to Plastics Materials and Articles In-274. J. Chromatogr. 437(766).

[6] Ioannis, S. A., & Loulouda, A. B. (2004). Migration of Substances from Food Packaging Materials to Foods. Critical Reviews in Food Science and Nutrition, 44(2), 63-76. https://doi.org/10.1080/10408690490424621

[7] Lau, O., & Wong, S. (2000). Contamination in food from packaging material. Journal of Chromatography A, 882(1–2), 255-270. https://doi.org/10.1016/S0021-9673(00)00356-3

[8] International Life Sciences Institute. (2002). Polystyrene for Food Packaging Applications. ILSI Europe Report Series. Retrieved January 24, 2020, from https://ilsi.eu/publication/packaging-materials-2-polystyrene-for-food-packaging-applications-updated-version/

[9] Genualdi, S., Nyman, P., & Begley, T. (2014). Updated evaluation of the migration of styrene monomer and oligomers from polystyrene food contact materials to foods and food simulants. Food Additives & Contaminants: Part A, 31(4), 723-733. https://doi.org/10.1080/19440049.2013.878040

[10] Gu, J. W., Zhang, Q. Y., Zhang, J. P., & Wang, W. W. (2010). Studies on the preparation of polystyrene thermal conductivity composites. Polym Plast Technol Eng, 49(13), 1385-1389.

[11] Castle, L., Kelly, M., & Gilbert, J. (1991). Migration of mineral hydrocarbons into foods. 1. Polystyrene containers for hot and cold beverages. Food Additives and Contaminants, 8, 693-700. DOI: 10.1080/02652039109374026

[12] American Chemistry Council. (2013). The Resin Review: The Annual Statistical Report of the North American Plastic Council. Retrieved January 24, 2020, from https://plastics.americanchemistry.com/Jobs/EconomicStatistics/Plastics-Statistics/

[13] Withey, J. R. (1976). Quantitative Analysis of Styrene Monomer in Polystyrene and Foods Including Some Preliminary Studies of the Uptake and Pharmacodynamics of the Monomer in Rats. Environmental Health Perspectives, 17, 125-133.

[14] Sabet, M., Soleimani, H., Mohammadian, E., & Hosseini, S. (2019). Impact of inclusion graphene oxide nanosheets on polystyrene properties. International Journal of Plastics Technology, 1.

[15] Ohyama, K. I., Nagai, F., & Tsuchiya, Y. (2001). Certain styrene oligomers have proliferative activity on human breast tumor cells. Environmental Health Perspective, 109(7), 699–703.

[16] Agency for Toxic Substances and Disease Registry (ATSDR). (2007). Toxicological profile for styrene (draft for public comment). US Department of Public Health and Human Services, Public Health Service. Atlanta, GA, 140. Retrieved January 24, 2020, from http://www.atsdr.cdc.gov/ToxProfiles/tp53.pdf.

[17] International Agency for Research on Cancer (IARC). (1994). Styrene-7,8-oxide. World Health Organization, Lyon. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, 60, 321-346.

[18] International Agency for Research on Cancer. (IARC). (2002). Styrene. World Health Organization, Lyon. IARC monographs on the evaluation of the carcinogenic risks to humans 82, 437-550.

[19] Tawfik, M. S., & Huyghebaert, A. (1998). Polystyrene cups and containers: Styrene migration. Food Additives & Contaminants, 15(5), 592-599. DOI: 10.1080/02652039809374686.

[20] Tang, W., Hemm, I., & Eisenbrand, G. (2000). Estimation of human exposure to styrene and ethylbenzene. Toxicology, 144(1-3), 39–50. DOI: 10.1016/S0300-483X(99)00188-2.

[21] Commission regulation (EU) No. 10/2011. (2011). Plastic materials and articles intended to come into contact with food. Retrieved January 24, 2020, from https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32011R0010

[22] Blair, E. H. (1978). Estrogenic potential of a polystyrene waste stream; evaluation of the estrogenic potential of condensate from polystyrene manufacturing plant. EPA TSCA 8(e) reported by Dow Chemical Co., 88-7800245; letter dated 1978 Oct 2.

[23] Deeks, J. J., Altman, D. G., & Bradburn, M. J. (2008). Statistical methods for examining heterogeneity and combining results from several studies in meta-analysis. In Systematic Reviews in Health Care: Meta-analysis in Context (2nd edition). Egger M, Smith GD, Altman DG (eds) BMJ Publishing Group, London, UK. doi: 10.1002/9780470693926. ch15.

[24] Chertok, I. R. A. & Haile, Z. T. (2018). Meta-Analysis. Journal of Human Lactation. 34(3), 420–423. DOI: 10.1177/0890334418776619.

[25] Lee, Y. (2018). An overview of meta-analysis for clinicians. Korean J Intern Med, 33, 277-283. https://doi.org/10.3904/kjim.2016.195

[26] Stewart, S. (2017). Meta-Analysis. Retrieved on 26, April 2019, from https://health.ucdavis.edu/ctsc/area/Resource_Library/documents/Stewart%20-%20Meta-analysis%20-2017.pdf

[27] Haidich, A. B. (2010). Meta-analysis in medical research. Department of Hygiene and Epidemiology, Aristotle University of Thessaloniki School of Medicine, Thessaloniki, Greece. 14 (Suppl 1): 29-37

[28] Liu, Z., Yao, Z., Li, C., Liu, X., Chen, H. & Gao, C. (2013). A step-by-step guide to the systematic review and meta-analysis of diagnostic and prognostic test accuracy evaluations. British Journal of Cancer, 108, 2299–2303. doi: 10.1038/bjc.2013.185

[29] Israel, H. & Richter, R. R. (2011). A Guide to Understanding Meta-Analysis. Journal of Orthopaedic & Sports Physical Therapy, 41(7), 496-504. doi:10.2519/jospt.2011.3333

[30] Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gotzsche, P., Ioannidis, J. P., . . . Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. PLoS Med, 6(7). doi: 10.1371/journal.pmed.1000100

[31] Bollinger, T. (2020, January 14). Does Plastic Cause Cancer? How to Avoid Cancer Causing Plastics. The Truth About Cancer. Retrieved January 24, 2020, from https://thetruthaboutcancer.com/cancer-causing-plastics

[32] Crank, J. (1975). Mathematics of Diffusion (2nd edition). Oxford Science Press, Oxford.

[33] Baner, A., Brandsch, J., Franz, R., & Piringer, O. (1996). The application of a predictive migration model for evaluating the compliance of plastic materials with European food regulations. Food Addit. Contam, 13(5), 587-601. DOI: 10.1080/02652039609374443

[34] Till, D. E., Reid, R. C., Schwartz, P. S., Sidman, K. R., Valentine, J. R., & Whelan, R. H. (1982). Plasticizer migration from polyvinyl chloride film to solvents and foods. Food Chem. Toxic, 20, 95-104.

[35] Franz, R., Huber, M., Piringer, O. (1997). Presentation and experimental verification of a physico-mathematical model describing the migration across functional barrier layers into foodstuffs. Food Addit. Contam. 14(6-7), 627-40.

[36] Paraskevopoulou, D., Achilias, D. S., & Paraskevopoulou, A. (2012). Migration of styrene from plastic packaging based on polystyrene into food simulants. Polym Int, 61, 141–148. doi:10.1002/pi.3161

[37] Thaysen, C., Stevack, K., Ruffolo, R., Poirier, D., De Frond, H., DeVera, J., Sheng, G., Rochman C. (2018). Leachate from Expanded Polystyrene Cups is Toxic to Aquatic Invertebrates (Ceriodaphnia dubia). Front. Mar. Sci., 5(71). doi.org/10.3389/fmars.2018.00071

[38] Lickly, T. D., Lehr, K. M., & Welsh, G. C. (1995). Migration of Styrene from Polystyrene Foam Food-contact Articles. Fd Chem. Toxic, 33(6), 475-481.

[39] Miltz, J., & Rosen-doody, D. (1984). Migration of styrene monomer from polystyrene packaging materials into food simulants. Journal of Food Processing and Preservation, 8, 151 – 161.

[40] Ahmad, M., Bajahlan, A. S. (2007). Leaching of styrene and other aromatic compounds in drinking water from PS bottles. Journal of Environmental Sciences. 19, 421–426.

[41] Sanagi, M. M., Ling, S. L., Nasir, Z., Ibrahim, W. A., Naim, A. A. (2008). Determination of residual volatile organic compounds migrated from polystyrene food packaging into food simulant by headspace solid phase microextraction–gas chromatography. The Malaysian Journal of Analytical Sciences, 12(3), 542–551.

[42] Khaksar, M., & Ghazi-Khansari, M. (2009). Determination of Migration monomer Styrene from GPPS (general purpose polystyrene) and HIPS (high impact polystyrene) cups to hot drinks. Toxicology Mechanisms and Methods, 19(3), 257-261. https://doi.org/10.1080/15376510802510299

[43] Choi, J. O., Jitsunari, F., Asakawa, F., & Lee, D. (2005). Migration of styrene monomer, dimers and trimers from polystyrene to food simulants. Food Additives and Contaminants, 22(7), 693-699. doi: 10.1080/02652030500160050

[44] Gennari, O., Albrizio, S., & Monteiro, M. (2012). A GC–FID Method to Determine Styrene in Polystyrene Glasses. Food Anal. Methods, 5, 1411–1418. DOI 10.1007/s12161-012-9395-5

[45] Varner, S. L. & Breder, C. V. (1981). Headspace sampling and gas chromatographic determination of styrene migration from food-contact polystyrene cups into beverages and food stimulants. J. Assoc. Off. Anal. Chem., 64, 1122-1130.

[46] Gilbert J., & Startin, J. R. (1983). A survey of styrene monomer levels in foods and plastic packaging by coupled mass spectrometry automatic headspace gas chromatography. J. Sci. Food Agric, 34, 647-652.

[47] Varner S. L., Breder, C. V., & Fazio, T. (1983). Determination of styrene migration from food-contact polymers into margarine, using Azeotropic distillation and head space gas chromatography. J. Ass. Offic. Anal. Chem. Int., 66, 1067–1073.

[48] Snyder R. C. & Breder, V. (1985). New FDA migration cell used to study migration of styrene from polystyrene into various solvents. J. Asso. Offic. Anal. Chem. Int., 68, 770-775.

[49] Carillo-Carrión, C., Lucena, R., Cárdenas, S., & Valcárcel, M. (2007). Liquid–liquid extraction/headspace/gas chromatographic/mass spectrometric determination of

benzene, toluene, ethylbenzene, (o-, m- and p-) xylene and styrene in olive oil using surfactant-coated carbon nanotubes as extractant. J Chromatogr A, 1171(1–2), 1–7.

[50] Verzera, A., Condurso, C., Romeo, V., Tripodi, G., & Ziino, M. (2010). Solid-Phase microextraction coupled to fast gas chromatography for the determination of migrants from polystyrene-packaging materials into yoghurt. Food Anal. Methods, 3, 80–84. doi: 10.1007/s12161-009-9088-x

[51] National Health Surveillance Agency (ANVISA). (1999, May 19). Technical regulations: general provisions for packaging and plastic equipment in contact with food and its attachments. Annex XII. Determination of residual styrene monomer. Resolution no. 105. Retrieved January 24, 2020, from http://www.anvisa.gov.br/legis/resol/105_99.htm.

[52] Food and Drug Administration (FDA) (2004). Code of federal regulations (CFR): GPO. Retrieved January 24, 2020, from https://www.fda.gov/medical-devices/medical-device-databases/code-federal-regulations-title-21-food-and-drugs

[53] BASF (2002). Styrene (C8H8) Chemical Emergency Medical Guidelines. Code: EO 27-001: 1-4. Retrieved January 24, 2020, from https://collaboration.basf.com/portal/basf/en/dt.jsp?setCursor=1_1032616

[54] Vitrac, O., & Leblanc, J. C. (2007). Consumer exposure to substances in plastic packaging 1- Assessment of the contribution of styrene from yogurt pots. Food Addit. Contam. 24(2), 194-215.

[55] Cao, X. L., Sparling, M., Pelletier, L., & Dabeka, R. (2018). Styrene in foods and dietary exposure estimates. Food Additives & Contaminants: Part A, 35(10), 2045-2051. https://doi.org/10.1080/19440049.2018.1512760

[56] Government of Canada. (1993). Canadian Environmental Protection Act (CEPA) Priority Substances List Assessment Report – Styrene. Retrieved January 24, 2020, from https://www.canada.ca/content/dam/hc-sc/migration/hcsc/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/contaminants/psl1-lsp1/stryene/styrene-eng.pdf.

[57] German Society for Nutrition. (1996). Nutritional Report. Retrieved January 24, 2020, from https://www.dge.de/en/nutrition-reports/

[58] Vodicka, P., Koskinen, M., Naccarati, A., Oesch-Bartlomowicz, B., Vodickova, L., Hemminki, K., & Oesch, F. (2006). Styrene Metabolism, Genotoxicity, and Potential Carcinogenicity. Drug Metabolism Reviews, 38(4), 805-853. DOI:10.1080/03602530600952222

[59] Kawamura, Y., Sugimoto, N., Takeda, Y., & Yamada, T. (1998). Identification of unknown substance in food contact polystyrene. J. Food Hyg. Soc. Jpn., 39, 110-119.

[60] Colborn, T., Dumanoski, D., Myers, J. P. (1996). The Wingspread Consensus Statement. Our Stolen Future, Penguin Group. New York. p 253.

[61] Yamada, T., Hirano, S., Kobayashi, K., Sakurai, T., Takaki, I., Tanaka, M., Nagao, Y., Azuma, Y., Date, K., Ohno, K., & Nobuhara, Y. (2000). Identification, determination and biological evaluation of novel styrene trimer in polystyrene container. Bunseki Kagaku, 49, 493-501.

[62] U.S. Environmental Protection Agency (U.S. EPA). (1998). Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), Final draft report; U.S. GPO: Washington, DC. Retrieved January 24, 2020 from https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-and-testing-advisory-committee-edstac-final

[63] Prinsen, M. K., & Gouko, N. (2001). Determination of the Osteogenic (Uterotrophic) Activity of Extracts of 'General Purpose Polystyrene (GPPS)' using Immature Female Rats. J. Appl. Toxicol, 21, 235–239.

[64] Nakaia, M., Tsubokuraa, M., Suzukia, M., Fujishimaa, S., Watanabeb, Y., Hasegawab, Y., Oyamab, K., & Oguraa, S. (2014). Genotoxicity of styrene oligomers extracted from polystyrene intended for use in contact with food. Toxicology Reports, 1, 1175–1180.

[65] Date, K., Ohno, K., Azuma, Y., Hirano, S., Kobayashi, K., Sakurai, T., Nobuhara, Y., & Yamada T. (2002). Endocrine-disrupting effects of styrene oligomers that migrated from polystyrene containers into food. Food and Chemical Toxicology, 40, 65–75.

[66] Ohyama, K., Nagai, F., & Tsuchiya, Y. (2001). Certain styrene oligomers have proliferative activity on MCF-7 human breast cancer cells and binding affinity for human estrogen receptor. Environmental Health Perspectives, 109, 699–703.

[67] Withey, J. R., & Collins, P. G. (1978). Styrene monomer in foods a limited Pharmacodynamics

survey. Environmental Contamination & Toxicology, 78, 0019-0086.

[68] Miltz, J., Elisha, C., & Mannhem, C. H. (1980). Sensory threshold of styrene and the monomer migration from polystyrene food packages. Journal of Food Processing and Preservation, 4, 281-289.

[69] Eiceman, G. A., & Carpen, M. (1982). Determination of volatile organic compounds as impurities in polystyrene food containers and polystyrene cups. Analytical Letters, 15, 1169-1177.

[70] Durst, G. L., & Laperle, E. A. (1990). Styrene monomer migration as monitored by purge and trap gas chromatography and sensory analysis for polystyrene containers. Journal of Food Science, 55(2).

[71] Linssen, J. P. H., Reitsma, J. C. E., & Roozen, J. P. (1991). Effect of sampling method on the level of styrene monomer migrated from polystyrene packaging material. Packaging Technology and Science, 4, 171-175.

[72] Linssen, J. P. H., Reitsma, J. C. E., & Roozen, J. P. (1992). Polystyrene sheet Composition and Temperature as Parameters for Migration of Styrene Monomer into Corn Oil. Packaging technology & science, 5(4), 217-222. https://doi.org/10.1002/pts.2770050408

[73] Murphy, P. G., MacDonald, D. A., & Lickly, T. D. (1992). Styrene Migration from General-Purpose and High Impact Polystyrene into Food-Simulating Solvents. Fd Chem. Toxic, 30(3), 225-232.

[74] Lehr, K. M., Welsh, G. C., Bell, C. D., & Lickly, T. D. (1993). The Vapour-Phase Migration of Styrene from General Purpose and High Impact Polystyrene into Cooking Oil. Fd. Chem. Toxic, 31(11), 793-708.

[75] O'Neill, E. T., & Tuohy, J. J. (1994). Comparison of Milk and Ethanol/Water Mixtures with respect to Monostyrene Migration from a Polystyrene Packaging Material. Int. Dairy Journal, 4, 271-283.

[76] Linssen, J. P. H., & Reitsma, J. C. E. (1995). Comparison of Migration of Styrene Monomer from High Impact Polystyrene in Oil in Water Emulsions and Fatty Foods. Journal of Polymer Engineering, 15, 133-138.

[77] Lau, O., Lung, M., & Mok, C. S. (1995). Distillation-extraction of styrene migrating from polystyrene containers to foods by gas chromatography. International Journal of Food Science and Technology, 30, 397-404.

[78] Nerin, C., Rubio, C., Cacho, J., & Salafranca, J. (1998). Parts-per-trillion determination of styrene in yoghurt by purge-and-trap gas chromatography with mass spectrometry detection. Food Additives & Contaminants, 15(3), 346-354. DOI:10.1080/02652039809374650

[79] Brunelli, N., Nocci, R., & Magno, F. (2002). Global Migration from Styrenics to Liquid Food Simulants. Annals of Chemistry, 92, 637-648

[80] Condurso, A., Verzera, A., Romeo, V., Tripodi, G., & Ziino, M. (2009). Rapid Determination of Styrene and Ethylbenzene in Packaged Yoghurt. Italian Journal of Food Science. 187-190.

[81] Amirshaghaghi, Z., Djomeh, E.Z., & Oromiehie, A. (2011). Studies of Migration of Styrene Monomer from Polystyrene Packaging into the food Simulant. Iranian Journal of Chemical Engineering, 8(4), 43-49.

[82] Saim, N., Osman, R., Sabian, H. A., Zubir, M. R., & Ibrahim, N. (2012). A Study on the Migration of Styrene from Polystyrene Cups to Drinks using Online Solid-Phase Extraction Liquid Chromatography (SPE-LC). The Malaysian Journal of Analytical Sciences, 16(1), 49-55.

[83] Lin, Q., Song, X., Fang, H., Wu, Y., & Wang, Z. (2017). Migration of Styrene and Ethylenebenzene from Virgin and Recycled Expanded Polystyrene Containers and Discrimination of these two kinds of Polystyrene by Principal Component Analysis. Food Additives & Contaminants: Part A, 34(1), 126-132.

[84] Abolghasemi-Fakhri, L., Ghanbarzadeh, B., Dehghannya, J., & Farhang, A. (2019). Styrene monomer migration from polystyrene based food packaging nanocomposite: Effect of clay and ZnO nanoparticles. Food and Chemical Toxicology. 129, 77-86.

[85] Song, X., Wrona, M., Nerin, C., Lin, Q., & Zhong, H. (2019). Volatile non-intentionally added substances (NIAS) identified in recycled expanded polystyrene containers and their migration into food simulants. Food Packaging and Shelf Life, 20, 2214-2894.

[86] Harrer, M., Cuijpers, P., Furukawa, T.A, & Ebert, D. D. (2019). Doing Meta-Analysis in R: A Hands-on Guide. Retrieved October 6, 2019, from https://bookdown.org/MathiasHarrer/Doing_Meta_Analysis_in_R/

[87] Durlak, J.A. (2009). How to Select, Calculate, and Interpret Effect Sizes, Journal of Pediatric Psychology, 34(9), 917–928. https://doi.org/10.1093/jpepsy/jsp004

[88] Coe, R. (2002). It's the Effect Size, Stupid: What effect size is and why it is important. Retrieved on April 10, 2020, from https://www.leeds.ac.uk/educol/documents/00002182.htm

[89] Multiphysics Cyclopedia. (January 14, 2015) Diffusion Equation. Retrieved on April 10, 2020, from https://www.comsol.com/multiphysics/diffusion-equation

APPENDIX A

Paper	Food simulant	%fat	time	temp (°C)	PS type	contact size (cm^2)	styrene per unit area, ug/cm^2	styrene per 100 cm^2, ug/cm^2
1	cold water	0	24 hrs	25	yoghurt cups	107 cm^2	0.0563	0.0526
	cold water	0	24 hrs	25	styrofoam cup	199 cm^2	N.D	N.D
	cold water	0	24 hrs	25	styrofoam cup	109 cm^2	N.D	N.D
	cold water	0	24 hrs	25	ABS container	257 cm^2	N.D	N.D
	cold water	0	24 hrs	25	beer glass	270 cm^2	0.0165	0.0061
	hot water	0	24 hrs	100	yoghurt cups	107 cm^2	0.3119	0.2915
	hot water	0	24 hrs	100	styrofoam cup	199 cm^2	0.0387	0.01945
	hot water	0	24 hrs	100	styrofoam cup	109 cm^2	0.0204	0.0187
	hot water	0	24 hrs	100	ABS container	257 cm^2	0.0323	0.0126
	hot water	0	24 hrs	100	beer glass	270 cm^2	0.1209	0.0447
	50% ethanol-water	0	24 hrs	25	yoghurt cups	97 cm^2	0.266	0.2742
	50% ethanol-water	0	24 hrs	25	styrofoam cup	93 cm^2	0.111	0.1194
	50% ethanol-water	0	24 hrs	25	styrofoam cup	90 cm^2	0.11	0.12
	50% ethanol-water	0	24 hrs	25	ABS container	114 cm^2	0.077	0.0675
	50% ethanol-water	0	24 hrs	25	beer glass	93 cm^2	0.04	0.043
2	yoghurt (plain)	3.5	21.8	60	PS container	0.16	0.0661	41.31
	yoghurt (raspberry)	3.5	31	60	PS container	0.16	0.2916	182.25
	butter - fat cream	40	14.25	60	PS container	0.16	0.25469	159.18
	Homogenised milk	3.4	10.75	60	PS container	0.16	0.00252	1.575
	Honey	0	60.58	60	PS container	0.16	0.106188	66.37
	cottage cheese	9	22.3	60	PS container	0.16	0.04875	30.47

	yopi	4	18.25	60	PS container	0.16	0.205	128.13
	sour cream 1	15	17.25	60	PS container	0.16	0.10638	66.49
	sour cream 2	15	23	60	PS container	0.16	1.3154	822.11
3	water	0	54.8 hours	110	Cheese cup	0.5 cm^2	3.2	640
	soybean oil	100	55.4 hours	125	Yogurt cup	0.5 cm^2	9.04	1808
	soybean oil	100	55 hours	125	Cheese cup	0.5 cm^2	22.2	4440
4	8% ethanol	0	24 hrs	25	Foam cup	168 cm^2	0.036	0.02143
	8% ethanol	0	24 hrs	25	Impact cup	157 cm^2	0.064	0.04076
	8% ethanol	0	24 hrs	25	crystal cup	196 cm^2	0.209	0.1066
	water	0	24 hrs	65.6	Foam cup	168 cm^2	0.0077	0.004583
	tea	0	24 hrs	65.6	Foam cup	168 cm^2	0.0078	0.004643
	coffee	0	24 hrs	65.6	Foam cup	168 cm^2	0.0078	0.00464
5	Hot water	0	35	90	PS cup	20.08	0.19273	0.9598
6	Lean beef	30	7 days	4	PS coupon	20 cm^2	0.027	0.135
	beef fat	15	7 days	4	PS coupon	20 cm^2	0.005	0.025
	beef fat	15	14 days	4	PS coupon	20 cm^2	0.032	0.16
	margarine	82	90 days	4	PS coupon	20 cm^2	0.045	0.225
	margarine	82	90 days	4	PS coupon	20 cm^2	0.049	0.245
	mayonnaise	81	91 days	4	PS coupon	20 cm^2	0.042	0.21
	mayonnaise	81	91 days	21	PS coupon	20 cm^2	0.25	1.25
	mayonnaise	81	104 days	21	PS coupon	20 cm^2	0.25	1.25

	gelatin	0	38 days	4	PS coupon	20 cm^2	0.002	0.01
	vanilla frosting	16	194 days	21	PS coupon	20 cm^2	0.061	0.305
	enrobing chocolate	20	78.5 days	21	PS coupon	20 cm^2	0.0175	0.0875
7	soybean oil	100	5.05 weeks	15	yoghurt cups	1 cm^2	18	1800
	soybean oil	100	5.05 weeks	15	cheese cups	1 cm^2	13.5	1350
	soybean oil	100	4.5 weeks	25	yoghurt cups	1 cm^2	37	3700
	soybean oil	100	4.5 weeks	25	cheese cups	1 cm^2	29.1	2910
	soybean oil	100	4.5 weeks	35	yoghurt cups	1 cm^2	80.6	8060
	soybean oil	100	4.5 weeks	35	cheese cups	1 cm^2	57.3	5730
8	50% ethanol	0	500 $s^{½}$	40	PS discs	30.2 cm^2	0.00000529	0.00001752
	hexadecane	0	450 $s^{½}$	40	PS discs	30.2 cm^2	0.00000252	0.000008344
	decanol	0	500 $s^{½}$	40	PS discs	30.2 cm^2	0.00000286	0.00000947
	20% ethanol	0	558.33 $s^{½}$	40	PS discs	30.2 cm^2	0.00000275	0.00000911
	corn oil	100	558.33 $s^{½}$	40	PS discs	30.2 cm^2	0.00000218	0.000007219
	Synthetic triglyceride (HB-307)	0	558.33 $s^{½}$	40	PS discs	30.2 cm^2	0.00000275	0.000009106
	8% ethanol	0	678.57 $s^{½}$	40	PS discs	30.2 cm^2	0.00000156	0.000005166
	3% acetic acid	0	558.33 $s^{½}$	40	PS discs	30.2 cm^2	0.00000145	0.000004801
	water	0	558.33 $s^{½}$	40	PS discs	30.2 cm^2	0.00000132	0.00000437
	decanol	0	350 $s^{½}$	70	PS discs	30.2 cm^2	0.00004	0.001325
	50% ethanol	0	350 $s^{½}$	70	PS discs	30.2 cm^2	0.00000176	0.00000583
	20% ethanol	0	237.5 $s^{½}$	70	PS discs	30.2 cm^2	0.000008	0.0000265

	Synthetic triglyceride (HB-307)	0	237.5 $s^{½}$	70	PS discs	30.2 cm^2	0.00000105	0.00000348
	8% ethanol	0	425 $s^{½}$	70	PS discs	30.2 cm^2	0.00000125	0.0000414
	corn oil	100	425 $s^{½}$	70	PS discs	30.2 cm^2	0.000008	0.0000265
	water	0	350 $s^{½}$	70	PS discs	30.2 cm^2	0.0000064	0.00002119
	water	0	26.7 days	24	PS container	15.4 cm^2	0.342078	2.22
9	water	0	26.7 days	38	PS container	15.4 cm^2	0.522078	3.39012
	water	0	26.7 days	52	PS container	15.4 cm^2	0.783896	5.0902
	water	0	26.7 days	66	PS container	15.4 cm^2	1.54286	10.01855
	Corn oil	100	25.8 days	40	HIPS PS sheet	9.09 g	0.25816	0.25816
	Corn oil	100	25.7 days	40	HIPS PS sheet	9.09 g	0.18362	0.18362
	Corn oil	100	25.6 days	40	HIPS PS sheet	9.09 g	0.07272	0.07272
10	Corn oil	100	21.5 days	40	HIPS : GPPS 1:1	9.09 g	0.20998	0.20998
	Corn oil	100	22.1 days	40	HIPS : GPPS 1:1	9.09 g	0.14271	0.14271
	Corn oil	100	27.1 days	40	HIPS : GPPS 1:1	9.09 g	0.06727	0.06727
	Corn oil	100	22.4 days	40	GPPS PS sheet	9.09 g	0.07363	0.07363
	Corn oil	100	27.9 days	40	GPPS PS sheet	9.09 g	0.070902	0.070902
	Corn oil	100	25.1 days	40	GPPS PS sheet	9.09 g	0.052722	0.052722
	cooking oil	100	10 days	21.1111	GPPS	1.632 cm^2	0.424	25.98
	cooking oil	100	10 days	48.8889	GPPS	1.632 cm^2	0.382	23.407
11	cooking oil	100	10 days	65.5556	GPPS	1.632 cm^2	0.872	53.43
	cooking oil	100	10 days	82.2222	GPPS	1.632 cm^2	8.416	515.69
	cooking oil	100	10 days	21.1111	HIPS	1.632 cm^2	0.637	39.031
	cooking oil	100	10 days	48.8889	HIPS	1.632 cm^2	0.319	19.546

	cooking oil	100	10 days	65.5556	HIPS	1.632 cm^2	1.625	99.57
	cooking oil	100	10 days	82.2222	HIPS	1.632 cm^2	7.410	454.044
	8% ethanol	0	10 days	40	GPPS	1.632 cm^2	0.093	5.698
	8% ethanol	0	10 days	48.8889	GPPS	1.632 cm^2	0.088	5.392
	8% ethanol	0	10 days	65.5556	GPPS	1.632 cm^2	0.336	20.588
	8% ethanol	0	10 days	40	HIPS	1.632 cm^2	0.084	5.147
	8% ethanol	0	10 days	48.8889	HIPS	1.632 cm^2	0.068	4.16
	8% ethanol	0	10 days	65.5556	HIPS	1.632 cm^2	0.29	17.769
12	corn oil	100	14 days	10	GPPS:HIPS 1:1		0.0075	0.0075
	corn oil	100	14 days	20	GPPS:HIPS 1:1		0.0188	0.0188
	corn oil	100	14 days	30	GPPS:HIPS 1:1		0.0333	0.0333
	corn oil	100	14 days	40	GPPS:HIPS 1:1		0.0773	0.0773
	corn oil	100	14 days	50	GPPS:HIPS 1:1		0.1388	0.1388
	corn oil	100	21 days	41	GPPS:HIPS 1:1	9.09 g	0.253611	0.253611
	corn oil	100	21 days	41	GPPS:HIPS 1:1	9.09 g	0.182709	0.182709
	corn oil	100	21 days	41	GPPS:HIPS 1:1	9.09 g	0.14453	0.14453
	corn oil	100	21 days	41	GPPS:HIPS 1:1	9.09 g	0.05999	0.05999
	corn oil	100	21 days	41	GPPS:HIPS 1:1	9.09 g	0.01091	0.01091
	corn oil	100	21 days	41	GPPS:HIPS 1:1	9.09 g	0.031815	0.031815
	corn oil	100	21 days	41	GPPS:HIPS 1:1	9.09 g	0.02182	0.02182
	corn oil	100	21 days	41	GPPS:HIPS 1:1	9.09 g	0.019089	0.019089
	corn oil	100	21 days	41	GPPS:HIPS 1:1	9.09 g	0.010908	0.010908
	corn oil	100	21 days	41	GPPS:HIPS 1:1	9.09 g	0.004909	0.004909
13	milk	0.5	10 days	30	PS cups	9.09 g	0.1818	3.636
	milk	3.5	10 days	30	PS cups	9.09 g	0.3636	7.272

	milk	10	10 days	30	PS cups	9.09 g	1.818	36.36
	0% ethanol	0	10 days	30	PS cups	9.09 g	N.D	N.D
	15% ethanol	0	10 days	30	PS cups	9.09 g	0.1818	3.636
	50% ethanol	0	10 days	30	PS cups	9.09 g	0.5454	10.908
	100% ethanol	0	10 days	30	PS cups	9.09 g	2.727	54.54
14	cooking oil	100	10 days	21	GPPS	1.664 cm^2	0.043	2.5841
	cooking oil	100	10 days	49	GPPS	1.664 cm^2	0.195	11.7187
	cooking oil	100	10 days	66	GPPS	1.664 cm^2	0.411	24.6995
	cooking oil	100	10 days	82	GPPS	1.664 cm^2	1.11	66.707
	cooking oil	100	10 days	21	HIPS	1.664 cm^2	0.063	3.7861
	cooking oil	100	10 days	49	HIPS	1.664 cm^2	0.284	17.067
	cooking oil	100	10 days	66	HIPS	1.664 cm^2	0.671	40.325
	cooking oil	100	10 days	82	HIPS	1.664 cm^2	1.45	87.1394
15	O/W-emulsions	0	3 days	40	HIPS	100 cm^2	0.0071	0.0071
	O/W-emulsions	5	3 days	40	HIPS	100 cm^2	0.0346	0.0346
	O/W-emulsions	10	3 days	40	HIPS	100 cm^2	0.0367	0.0367
	O/W-emulsions	25	3 days	40	HIPS	100 cm^2	0.0305	0.0305
	O/W-emulsions	30	3 days	40	HIPS	100 cm^2	0.0298	0.0298
	O/W-emulsions	50	3 days	40	HIPS	100 cm^2	0.0308	0.0308
	O/W-emulsions	100	3 days	40	HIPS	100 cm^2	0.0493	0.0493
	O/W-emulsions	0	10 days	40	HIPS	100 cm^2	0.0085	0.0085
	O/W-emulsions	4	10 days	40	HIPS	100 cm^2	0.0402	0.0402
	O/W-emulsions	10	10 days	40	HIPS	100 cm^2	0.0449	0.0449
	O/W-emulsions	25	10 days	40	HIPS	100 cm^2	0.0536	0.0536

	O/W-emulsions	30	10 days	40		HIPS	100 cm^2	0.0674	0.0674
	O/W-emulsions	50	10 days	40		HIPS	100 cm^2	0.0706	0.0706
	O/W-emulsions	100	10 days	40		HIPS	100 cm^2	0.0984	0.0984
	fatty foods	0	3 days	40		HIPS	100 cm^2	0.0059	0.0059
	fatty foods	5	3 days	40		HIPS	100 cm^2	0.0325	0.0325
	fatty foods	15	3 days	40		HIPS	100 cm^2	0.0385	0.0385
	fatty foods	25	3 days	40		HIPS	100 cm^2	0.0309	0.0309
	fatty foods	35	3 days	40		HIPS	100 cm^2	0.0292	0.0292
	fatty foods	57	3 days	40		HIPS	100 cm^2	0.0318	0.0318
	fatty foods	80	3 days	40		HIPS	100 cm^2	0.0503	0.0503
	fatty foods	0	10 days	40		HIPS	100 cm^2	0.0097	0.0097
	fatty foods	8	10 days	40		HIPS	100 cm^2	0.0716	0.0716
	fatty foods	12	10 days	40		HIPS	100 cm^2	0.0695	0.0695
	fatty foods	25	10 days	40		HIPS	100 cm^2	0.0621	0.0621
	fatty foods	38	10 days	40		HIPS	100 cm^2	0.0651	0.0651
	fatty foods	57	10 days	40		HIPS	100 cm^2	0.0759	0.0759
	fatty foods	80	10 days	40		HIPS	100 cm^2	0.0984	0.0984
16	Elegante' ice-cream (small size)	11	35	100	PS	food container	11 cm^2	N.D	N.D
	Elegante' ice-cream (large size)	11	35	100	PS	food container	11 cm^2	0.0026	0.0236
	Tappuri soft ice-cream	11	35	100	PS	food container	11 cm^2	0.00348	0.0317
	Yakult	0	35	100	PS	food container	11 cm^2	N.D	N.D
	Yogo	0	35	100	PS	food container	11 cm^2	N.D	N.D
	Doll instant noodles	22	35	100	PS	food container	11 cm^2	0.0109	0.0992

	Nissin Japanese instant fried noodles	13	35	100	PS	food container	11 cm^2	0.0045	0.0413
	Chinese sweet bean curd dessert*	0	35	100	PS	food container	11 cm^2	0.737	6.7
	Chinese sliced pork soup*	12	35	100	PS	food container	11 cm^2	N.D	N.D
17	oil	100	10 days	21		cup		0.42	93.3
	oil	100	1 day	49		cup		0.51	113.3
	oil	100	4	49		cup		0.8	117.7
	oil	100	10	49		cup		0.99	220
	oil	100	1	66		cup		1.01	224.4
	oil	100	4	66		cup		1.21	268.8
	oil	100	10	66		cup		1.39	308.8
	oil	100	10	21		plate		0.03	2.3023
	oil	100	1	49		plate		0.05	3.8373
	oil	100	4	49		plate		0.1	7.675
	oil	100	10	49		plate		0.15	11.512
	oil	100	1	66		plate		0.12	9.2095
	oil	100	4	66		plate		0.3	23.023
	oil	100	10	66		plate		0.54	41.44
	oil	100	10	21		hinged container		0.08	4.624
	oil	100	1	49		hinged container		0.12	6.936
	oil	100	4	49		hinged container		0.22	12.717
	oil	100	10	49		hinged container		0.36	20.809
	oil	100	1	66		hinged container		0.23	13.295
	oil	100	4	66		hinged container		0.46	26.59
	oil	100	10	66		hinged container		0.79	45.665
	oil	100	10	21		meat tray (1)		0.14	5.34
	oil	100	1	49		meat tray (1)		0.21	8.015

	oil	100	4	49	meat tray (1)		0.35	13.358
	oil	100	10	49	meat tray (1)		0.55	20.99
	oil	100	1	66	meat tray (1)		0.5	19.083
	oil	100	4	66	meat tray (1)		0.8	30.534
	oil	100	10	66	meat tray (1)		1.13	43.129
	oil	100	10	21	meat tray (2)		0.09	3.169
	oil	100	1	49	meat tray (2)		0.14	4.9296
	oil	100	4	49	meat tray (2)		0.26	9.155
	oil	100	10	49	meat tray (2)		0.41	14.437
	oil	100	1	66	meat tray (2)		0.36	12.676
	oil	100	4	66	meat tray (2)		0.65	22.887
	oil	100	10	66	meat tray (2)		1.03	36.268
	oil	100	10	4	meat tray (1)		0.06	2.2901
	oil	100	10	4	meat tray (2)		0.03	1.0563
	8% ethanol	0	10	4	Egg carton		N.D	N.D
	8% ethanol	0	31	4	Egg carton		N.D	N.D
	distilled water	0	1h	100	PS cup	131	0.01	0.007634
	distilled water	0	2h	100	PS cup	131	0.02	0.015267
	distilled water	0	3 days	60	PS cup	131	0.026	0.019847
	distilled water	0	3 days	40	PS cup	131	0.02	0.015267
	distilled water	0	3 days	20	PS cup	131	0.002	0.0015267
18	distilled water	0	3 days	4	PS cup	131	0.002	0.0015267
	Wholemilk (3.6% fat)	3.6	2h	100	PS cup	131	0.11	0.08397
	Wholemilk (3.6% fat)	3.6	2h	60	PS cup	131	0.022	0.01679
	Wholemilk (3.6% fat)	3.6	2h	40	PS cup	131	0.001	0.0007634
	Wholemilk (3.6% fat)	3.6	24h	40	PS cup	131	0.036	0.02748
	Wholemilk (3.6% fat)	3.6	2h	20	PS cup	131	0.001	0.0007634
	Wholemilk (3.6% fat)	3.6	24h	20	PS cup	131	0.019	0.0145

Wholemilk (3.6% fat)	3.6	24h	4	PS cup	131	0.001	0.0007634
Wholemilk (3.6% fat)	3.6	3 days	4	PS cup	131	0.004	0.003053
half-fat milk (1.55% fat)	1.55	2h	100	PS cup	131	0.102	0.07786
half-fat milk (1.55% fat)	1.55	2h	60	PS cup	131	0.006	0.0045802
half-fat milk (1.55% fat)	1.55	2h	40	PS cup	131	0.001	0.0007634
half-fat milk (1.55% fat)	1.55	24h	40	PS cup	131	0.028	0.02137
half-fat milk (1.55% fat)	1.55	2h	20	PS cup	131	0.001	0.0007634
half-fat milk (1.55% fat)	1.55	24h	20	PS cup	131	0.01	0.007634
half-fat milk (1.55% fat)	1.55	24h	4	PS cup	131	0.001	0.0007634
half-fat milk (1.55% fat)	1.55	3 days	4	PS cup	131	0.001	0.0007634
skimmed milk (0.5% fat)	0.5	2h	100	PS cup	131	0.05	0.03816
skimmed milk (0.5% fat)	0.5	2h	60	PS cup	131	0.001	0.0007634
skimmed milk (0.5% fat)	0.5	2h	40	PS cup	131	0.001	0.0007634
skimmed milk (0.5% fat)	0.5	24h	40	PS cup	131	0.022	0.01679
skimmed milk (0.5% fat)	0.5	2h	20	PS cup	131	0.001	0.0007634
skimmed milk (0.5% fat)	0.5	24h	20	PS cup	131	0.007	0.005344
skimmed milk (0.5% fat)	0.5	24h	4	PS cup	131	0.001	0.0007634
skimmed milk (0.5% fat)	0.5	3 days	4	PS cup	131	0.001	0.0007634
Apple juice	1	16h	20	PS cup	131	0.007	0.005344
orange juice	2	16h	20	PS cup	131	0.005	0.0038168
carbonated water	0	16h	20	PS cup	131	0.009	0.00687023
cola	0	16h	20	PS cup	131	0.007	0.005344
beer	0	16h	20	PS cup	131	0.009	0.00687023
drinking chocolate	2.3	16h	20	PS cup	131	0.007	0.005344
drinking yoghurt (3% fat)	3	3 days	4	PS cup	131	0.001	0.0007634
drinking yoghurt (3% fat)	3	7 days	4	PS cup	131	0.01	0.007634
drinking yoghurt (3% fat)	3	14 days	4	PS cup	131	0.01	0.007634
jelly	1	1 day	4	PS cup	131	0.002	0.0015267
jelly	1	3 days	4	PS cup	131	0.008	0.00610687

jelly	1	7 days	4	PS cup	131	0.01	0.007634
pudding with whole milk	3.6	1 day	4	PS cup	131	0.001	0.0007634
pudding with whole milk	3.6	3 days	4	PS cup	131	0.012	0.0091603
pudding with whole milk	3.6	7 days	4	PS cup	131	0.014	0.01069
ice-cream	11	30 days	-10	PS cup	131	0.02	0.015267
ice-cream	11	30 days	-10	PS cup	131	0.03	0.0229
tea	0	1h	100	PS cup	131	0.01	0.007634
coffe	0	1h	100	PS cup	131	0.01	0.007634
chocolate	0	1h	100	PS cup	131	0.01	0.007634
soup (3.6% fat)	3.6	1h	100	PS cup	131	0.02	0.015267
(2% fat)	2	1h	100	PS cup	131	0.019	0.014504
(1% fat)	1	1h	100	PS cup	131	0.017	0.012977
(0.5% fat)	0.5	1h	100	PS cup	131	0.01	0.007634
(0.0% fat)	0	1h	100	PS cup	131	0.004	0.0030534
3% acetic acid	0	1h	100	PS cup	131	0.04	0.030534
3% acetic acid	0	24h	40	PS cup	131	0.014	0.010687
15% ethanol	0	1h	100	PS cup	131	0.02	0.015267
15% ethanol	0	24h	40	PS cup	131	0.04	0.030534
15% ethanol	0	1h	100	PS cup	131	0.32	0.24427
15% ethanol	0	24h	40	PS cup	131	0.05	0.038168
15% ethanol	0	1h	100	PS cup	131	0.51	0.38931
15% ethanol	0	24h	40	PS cup	131	0.09	0.068702
olive oil	100	1h	100	PS cup	131	0.15	0.114504
olive oil	100	24h	40	PS cup	131	0.11	0.08397
Yogurt	3.5	1 h	100	PS container	131	0.06	0.0458015
Yogurt	2.6	1 h	100	PS container	131	0.04	0.030534
Mixed Yogurt	2.9	1 h	100	PS container	131	0.04	0.030534
Biograde	3.4	1 h	100	PS container	131	0.09	0.068702
rice with milk	1.5	1 h	100	PS container	131	0.03	0.0229

	fromage	1.5	1 h	100	PS container	131	0.03	0.0229
	fromage	2.6	1 h	100	PS container	131	0.05	0.038168
	mozzarella	14	1 h	100	PS container	131	0.11	0.08397
	cheese	13.5	1 h	100	PS container	131	0.1	0.076336
	cheese	8.9	1 h	100	PS container	131	0.08	0.061069
19	yoghurt No 1	3.5	10 h	90	PS cup	10 g	0.068	27.2
	yoghurt No 2	3.5	10 h	90	PS cup	10 g	0.196	78.4
	yoghurt No 3	3.5	10 h	90	PS cup	10 g	0.128	51.2
	yoghurt No 4	3.5	10 h	90	PS cup	10 g	0.34	136
	yoghurt No 5	3.5	10 h	90	PS cup	10 g	0.22	88
20	ethanol 10% v/v	0	1 h	40	GPPS1	4.36 cm^2	11	252.29
	ethanol 10% v/v	0	2 h	40	GPPS1	4.36 cm^2	7	160.55
	ethanol 10% v/v	0	24 h	40	GPPS1	4.36 cm^2	11	252.29
	ethanol 10% v/v	0	240 h	40	GPPS1	4.36 cm^2	9	206.42
	ethanol 10% v/v	0	1 h	40	GPPS2	4.36 cm^2	14	321.101
	ethanol 10% v/v	0	2 h	40	GPPS2	4.36 cm^2	16	366.97
	ethanol 10% v/v	0	24 h	40	GPPS2	4.36 cm^2	30	688.07
	ethanol 10% v/v	0	240 h	40	GPPS2	4.36 cm^2	28	642.202
	ethanol 10% v/v	0	1 h	40	HIPS1	4.36 cm^2	7	160.55
	ethanol 10% v/v	0	2 h	40	HIPS1	4.36 cm^2	2	45.87
	ethanol 10% v/v	0	24 h	40	HIPS1	4.36 cm^2	11	252.29
	ethanol 10% v/v	0	240 h	40	HIPS1	4.36 cm^2	11	252.29
	ethanol 10% v/v	0	1 h	40	HIPS2	4.36 cm^2	7	160.55
	ethanol 10% v/v	0	2 h	40	HIPS2	4.36 cm^2	5	114.67

ethanol 10% v/v	0	24 h	40	HIPS2	4.36 cm^2	5	114.67
ethanol 10% v/v	0	240 h	40	HIPS2	4.36 cm^2	11	252.29
ethanol 10% v/v	0	24	40	GPPS2 rec	4.36 cm^2	2	45.87
ethanol 10% v/v	0	24	40	HIPS1 rec	4.36 cm^2	N.D	N.D
ethanol 10% v/v	0	24	40	GPPS2 n.a	4.36 cm^2	11	252.29
ethanol 10% v/v	0	24	40	HIPS1 n.a	4.36 cm^2	16	366.97
acetic acid 3% m/v	0	1 h	40	GPPS1	4.36 cm^2	7	160.55
acetic acid 3% m/v	0	2 h	40	GPPS1	4.36 cm^2	11	252.29
acetic acid 3% m/v	0	24 h	40	GPPS1	4.36 cm^2	11	252.29
acetic acid 3% m/v	0	240 h	40	GPPS1	4.36 cm^2	7	160.55
acetic acid 3% m/v	0	1 h	40	GPPS2	4.36 cm^2	9	206.42
acetic acid 3% m/v	0	2 h	40	GPPS2	4.36 cm^2	16	366.97
acetic acid 3% m/v	0	24 h	40	GPPS2	4.36 cm^2	16	366.97
acetic acid 3% m/v	0	240 h	40	GPPS2	4.36 cm^2	16	366.97
acetic acid 3% m/v	0	1 h	40	HIPS1	4.36 cm^2	5	114.67
acetic acid 3% m/v	0	2 h	40	HIPS1	4.36 cm^2	2	45.87
acetic acid 3% m/v	0	24 h	40	HIPS1	4.36 cm^2	5	114.67
acetic acid 3% m/v	0	240 h	40	HIPS1	4.36 cm^2	N.D	N.D
acetic acid 3% m/v	0	1 h	40	HIPS2	4.36 cm^2	7	160.55
acetic acid 3% m/v	0	2 h	40	HIPS2	4.36 cm^2	9	206.42
acetic acid 3% m/v	0	24 h	40	HIPS2	4.36 cm^2	5	114.67
acetic acid 3% m/v	0	240 h	40	HIPS2	4.36 cm^2	5	114.67
distilled water	0	1 h	40	GPPS1	4.36 cm^2	N.D	N.D
distilled water	0	2 h	40	GPPS1	4.36 cm^2	N.D	N.D
distilled water	0	24 h	40	GPPS1	4.36 cm^2	N.D	N.D

distilled water	0	240 h	40	GPPS1	4.36 cm^2	7	160.55
distilled water	0	1 h	40	GPPS2	4.36 cm^2	N.D	N.D
distilled water	0	2 h	40	GPPS2	4.36 cm^2	N.D	N.D
distilled water	0	24 h	40	GPPS2	4.36 cm^2	5	114.67
distilled water	0	240 h	40	GPPS2	4.36 cm^2	11	252.29
distilled water	0	1 h	40	HIPS1	4.36 cm^2	5	114.67
distilled water	0	2 h	40	HIPS1	4.36 cm^2	N.D	N.D
distilled water	0	24 h	40	HIPS1	4.36 cm^2	N.D	N.D
distilled water	0	240 h	40	HIPS1	4.36 cm^2	14	321.101
distilled water	0	1 h	40	HIPS2	4.36 cm^2	N.D	N.D
distilled water	0	2 h	40	HIPS2	4.36 cm^2	N.D	N.D
distilled water	0	24 h	40	HIPS2	4.36 cm^2	N.D	N.D
distilled water	0	240 h	40	HIPS2	4.36 cm^2	2	45.87
olive oil	100	24 h	40	GPPS1	4.36 cm^2	9	206.42
olive oil	100	24 h	40	GPPS2	4.36 cm^2	50	1146.79
olive oil	100	24 h	40	HIPS1	4.36 cm^2	11	252.29
olive oil	100	24 h	40	HIPS2	4.36 cm^2	N.D	N.D
ethanol 10% v/v	0	1 h	70	GPPS1	4.36 cm^2	11	252.29
ethanol 10% v/v	0	2 h	70	GPPS1	4.36 cm^2	7	160.55
ethanol 10% v/v	0	24 h	70	GPPS1	4.36 cm^2	2	45.87
ethanol 10% v/v	0	240 h	70	GPPS1	4.36 cm^2	11	252.29
ethanol 10% v/v	0	1 h	70	GPPS2	4.36 cm^2	14	321.101
ethanol 10% v/v	0	2 h	70	GPPS2	4.36 cm^2	16	366.97
ethanol 10% v/v	0	24 h	70	GPPS2	4.36 cm^2	7	160.55
ethanol 10% v/v	0	240 h	70	GPPS2	4.36 cm^2	9	206.42

ethanol 10% v/v	0	1 h	70	HIPS1	4.36 cm^2	7	160.55
ethanol 10% v/v	0	2 h	70	HIPS1	4.36 cm^2	2	45.87
ethanol 10% v/v	0	24 h	70	HIPS1	4.36 cm^2	2	45.87
ethanol 10% v/v	0	240 h	70	HIPS1	4.36 cm^2	9	206.42
ethanol 10% v/v	0	1 h	70	HIPS2	4.36 cm^2	7	160.55
ethanol 10% v/v	0	2 h	70	HIPS2	4.36 cm^2	5	114.67
ethanol 10% v/v	0	24 h	70	HIPS2	4.36 cm^2	2	45.87
ethanol 10% v/v	0	240 h	70	HIPS2	4.36 cm^2	N.D	N.D
ethanol 10% v/v	0	24 h	70	GPPS2 rec	4.36 cm^2	5	114.67
ethanol 10% v/v	0	24 h	70	HIPS1 rec	4.36 cm^2	5	114.67
acetic acid 3% m/v	0	1 h	70	GPPS1	4.36 cm^2	7	160.55
acetic acid 3% m/v	0	2 h	70	GPPS1	4.36 cm^2	7	160.55
acetic acid 3% m/v	0	24 h	70	GPPS1	4.36 cm^2	9	206.42
acetic acid 3% m/v	0	240 h	70	GPPS1	4.36 cm^2	N.D	N.D
acetic acid 3% m/v	0	1 h	70	GPPS2	4.36 cm^2	5	114.67
acetic acid 3% m/v	0	2 h	70	GPPS2	4.36 cm^2	9	206.42
acetic acid 3% m/v	0	24 h	70	GPPS2	4.36 cm^2	11	252.29
acetic acid 3% m/v	0	240 h	70	GPPS2	4.36 cm^2	2	45.87
acetic acid 3% m/v	0	1 h	70	HIPS1	4.36 cm^2	5	114.67
acetic acid 3% m/v	0	2 h	70	HIPS1	4.36 cm^2	7	160.55
acetic acid 3% m/v	0	24 h	70	HIPS1	4.36 cm^2	9	206.42
acetic acid 3% m/v	0	240 h	70	HIPS1	4.36 cm^2	N.D	N.D
acetic acid 3% m/v	0	1 h	70	HIPS2	4.36 cm^2	2	45.87
acetic acid 3% m/v	0	2 h	70	HIPS2	4.36 cm^2	2	45.87
acetic acid 3% m/v	0	24 h	70	HIPS2	4.36 cm^2	9	206.42

	acetic acid 3% m/v	0	240 h	70	HIPS2	4.36 cm^2	9	206.42
	distilled water	0	1 h	70	GPPS1	4.36 cm^2	1	22.94
	distilled water	0	2 h	70	GPPS1	4.36 cm^2	N.D	N.D
	distilled water	0	24 h	70	GPPS1	4.36 cm^2	N.D	N.D
	distilled water	0	240 h	70	GPPS1	4.36 cm^2	11	252.29
	distilled water	0	1 h	70	GPPS2	4.36 cm^2	2	45.87
	distilled water	0	2 h	70	GPPS2	4.36 cm^2	2	45.87
	distilled water	0	24 h	70	GPPS2	4.36 cm^2	N.D	N.D
	distilled water	0	240 h	70	GPPS2	4.36 cm^2	7	160.55
	distilled water	0	1 h	70	HIPS1	4.36 cm^2	2	45.87
	distilled water	0	2 h	70	HIPS1	4.36 cm^2	N.D	N.D
	distilled water	0	24 h	70	HIPS1	4.36 cm^2	2	45.87
	distilled water	0	240 h	70	HIPS1	4.36 cm^2	16	366.97
	distilled water	0	1 h	70	HIPS2	4.36 cm^2	5	114.67
	distilled water	0	2 h	70	HIPS2	4.36 cm^2	7	160.55
	distilled water	0	24 h	70	HIPS2	4.36 cm^2	5	114.67
	distilled water	0	240 h	70	HIPS2	4.36 cm^2	11	252.29
	olive oil	100	24 h	70	GPPS1	4.36 cm^2	60	1376.15
	olive oil	100	24 h	70	GPPS2	4.36 cm^2	5	114.67
	olive oil	100	24 h	70	HIPS1	4.36 cm^2	18	412.84
	olive oil	100	24 h	70	HIPS2	4.36 cm^2	50	1146.79
21	n-heptane	0	8.8 $h^{0.5}$	10	PS sheet	0.1 g	0.00995	0.2475
	n-heptane	0	1.4 $h^{0.5}$	24	PS sheet	0.1 g	0.00995	0.2475
	n-heptane	0	0.9 $h^{0.5}$	40	PS sheet	0.1 g	0.0109	0.27114

	water	0	4 months	25	PS bottles	32.05 cm^2	0.15874	0.4953
	water	0	4 months	25	PS bottles	32.05 cm^2	0.15639	0.48795
	water	0	3 months	25	PS bottles	32.05 cm^2	0.124805	0.38941
	water	0	10 months	25	PS bottles	32.05 cm^2	0.12699	0.39622
	water	0	3 months	25	PS bottles	32.05 cm^2	0.15639	0.48796
	water	0	4 months	25	PS bottles	32.05 cm^2	0.12356	0.385523
	water	0	8 months	25	PS bottles	32.05 cm^2	0.230109	0.71796
	water	0	4 months	25	PS bottles	32.05 cm^2	0.20507	0.63984
22	water	0	39 days	25	PS bottles	32.05 cm^2	0.09587	2.83802
	water	0	60 mins	25	styrofoam cups	25.64 cm^2	N.D	N.D
	water	0	60 mins	50	styrofoam cups	25.64 cm^2	N.D	N.D
	water	0	60 mins	70	styrofoam cups	25.64 cm^2	0.009048	0.035289
	water	0	60 mins	90	styrofoam cups	25.64 cm^2	0.07293	0.28444
	water	0	60 mins	100	styrofoam cups	25.64 cm^2	0.1355	0.52847
	water	0	60 mins	100	styrofoam A	25.64 cm^2	0.13798	0.53814
	water	0	60 mins	100	styrofoam B	25.64 cm^2	0.06903	0.2692278
	water	0	60 mins	100	PS cup	50 cm^2	0.00812	0.031669
	water	0	60 mins	100	paper cups	25.64 cm^2	N.D	N.D
	water	0	30 mins	80	PS bowl	30.3 cm^2	1.4191	4.6836
23	water	0	30 mins	24	PS bowl	30.3 cm^2	0.825	2.723
	water	0	30 mins	60	PS bowl	30.3 cm^2	N.D	N.D
	water	0	30 mins	24	PS bowl	30.3 cm^2	N.D	N.D

	water	0	30 mins	24	PS bowl	30.3 cm^2	N.D	N.D
	water	0	30 mins	80	PS bowl	22.5 cm^2	1.724	7.6642
	water	0	30 mins	24	PS bowl	22.5 cm^2	0.8	3.5
	water	0	30 mins	60	PS bowl	22.5 cm^2	N.D	N.D
	water	0	30 mins	24	PS bowl	22.5 cm^2	N.D	N.D
	water	0	30 mins	24	PS bowl	22.5 cm^2	N.D	N.D
	water	0	30 mins	80	PS cup	28.6 cm^2	2.0489	7.16398
	water	0	30 mins	24	PS cup	28.6 cm^2	1.5664	5.47704
	water	0	30 mins	60	PS cup	28.6 cm^2	0.88112	3.08084
	water	0	30 mins	24	PS cup	28.6 cm^2	0.335664	1.17365
	water	0	30 mins	24	PS cup	28.6 cm^2	N.D	N.D
24	hot tea	0	33.3	20	GPPS cups	120 cm^2	0.00103	0.000861
	hot tea	0	33.3	60	GPPS cups	120 cm^2	0.006583	0.005486
	hot tea	0	33.3	100	GPPS cups	120 cm^2	0.01305	0.010875
	hot milk	3.4	33.3	20	GPPS cups	120 cm^2	0.00093	0.0007
	hot milk	3.4	33.3	60	GPPS cups	120 cm^2	0.00173	0.0014
	hot milk	3.4	33.3	100	GPPS cups	120 cm^2	0.013416	0.011181
	hot cocoa milk	3.4	33.3	20	GPPS cups	120 cm^2	0.001625	0.001354
	hot cocoa milk	3.4	33.3	60	GPPS cups	120 cm^2	0.006816	0.0056805
	hot cocoa milk	3.4	33.3	100	GPPS cups	120 cm^2	0.01386	0.0115
	hot tea	0	33.3	20	HIPS cups	120 cm^2	0.00125	0.001042
	hot tea	0	33.3	60	HIPS cups	120 cm^2	0.00575	0.00479
	hot tea	0	33.3	100	HIPS cups	120 cm^2	0.01063	0.008861
	hot milk	3.4	33.3	20	HIPS cups	120 cm^2	0.00156	0.001305

	hot milk	3.4	33.3	60	HIPS cups	120 cm^2	0.00663	0.005527
	hot milk	3.4	33.3	100	HIPS cups	120 cm^2	0.01206	0.01005
	hot cocoa milk	3.4	33.3	20	HIPS cups	120 cm^2	0.001716	0.001431
	hot cocoa milk	3.4	33.3	60	HIPS cups	120 cm^2	0.006916	0.005764
	hot cocoa milk	3.4	33.3	100	HIPS cups	120 cm^2	0.01226	0.0102
	acetic acid	0	1 h	100	GPPS cups	120 cm^2	0.000123	0.0001027
	acetic acid	0	24 h	40	GPPS cups	120 cm^2	0.000146	0.00012
	acetic acid	0	1 h	100	HIPS cups	120 cm^2	0.00009	0.000075
	acetic acid	0	24 h	40	HIPS cups	120 cm^2	0.000113	0.000094
	15% ethanol	0	1 h	100	GPPS cups	120 cm^2	0.000086	0.000072
	15% ethanol	0	24 h	40	GPPS cups	120 cm^2	0.0001116	0.00009305
	15% ethanol	0	1 h	100	HIPS cups	120 cm^2	0.0000683	0.0000569
	15% ethanol	0	24 h	40	HIPS cups	120 cm^2	0.000085	0.00007083
	olive oil	100	1 h	100	GPPS cups	120 cm^2	0.000046	0.000038
	olive oil	100	24 h	40	GPPS cups	120 cm^2	0.000056	0.0000472
	olive oil	100	1 h	100	HIPS cups	120 cm^2	0.0000416	0.00003472
	olive oil	100	24 h	40	HIPS cups	120 cm^2	0.0000483	0.0000403
	yoghurt	3.5	1 day	4	yoghurt container	0.314	N.D	N.D
	yoghurt	3.5	10 days	4	yoghurt container	0.314	0.020191	6.43028
	yoghurt	3.5	14 days	4	yoghurt container	0.314	0.026306	8.37762
25	yoghurt	3.5	18 days	4	yoghurt container	0.314	0.06344	20.2
	yoghurt	3.5	23 days	4	yoghurt container	0.314	0.10134	32.273
	yoghurt	3.5	35 days	4	yoghurt container	0.314	0.05739	18.2766
	yoghurt	3.5	38 days	4	yoghurt container	0.314	0.0702547	22.374
	yoghurt	3.5	42 days	4	yoghurt container	0.314	0.044777	14.26

26	yoghurt	3.5	1 day	4	yoghurt container	0.314	N.D	N.D
	yoghurt	3.5	10 days	4	yoghurt container	0.314	0.02019	6.43028
	yoghurt	3.5	14 days	4	yoghurt container	0.314	0.026114	8.31677
	yoghurt	3.5	18 days	4	yoghurt container	0.314	0.063694	20.2847
	yoghurt	3.5	23 days	4	yoghurt container	0.314	0.10127	32.2528
	yoghurt	3.5	35 days	4	yoghurt container	0.314	0.09363	29.8187
	yoghurt	3.5	38 days	4	yoghurt container	0.314	0.09554	30.4272
27	ethanol	0	192 hrs	25	PS glass	0.16 g	151.703	187287.65
	ethanol	0	232 hrs	40	PS glass	0.16 g	270.62	334095.41
	ethanol	0	180 hrs	60	PS glass	0.16 g	381.23	470659.96
	isooctane	0	225.7 hrs	4	PS glass	0.16 g	91.65	113153
	isooctane	0	225.7 hrs	25	PS glass	0.16 g	137.09	169242.49
	isooctane	0	224.1 hrs	40	PS glass	0.16 g	242.17	298978.81
	ethanol	0	232.1 hrs	25	EPS glass	0.064 g	84.83	14233.59
	ethanol	0	216.2 hrs	40	EPS glass	0.064 g	115.44	1936.497
	ethanol	0	180.2 hrs	60	EPS glass	0.064 g	150.66	25278.14
	isooctane	0	225.7 hrs	4	EPS glass	0.064 g	17.3423	2909.78
	isooctane	0	225.7 hrs	25	EPS glass	0.064 g	27.597	4630.422
	isooctane	0	224.1 hrs	40	EPS glass	0.064 g	75.9195	12738.16
28	10% ethanol	0	35 days	5	PS dishes	11 cm^2	0.24	2.18
	10% ethanol	0	35 days	20	PS dishes	11 cm^2	0.28	2.54
	10% ethanol	0	35 days	40	PS dishes	11 cm^2	0.31	2.81
29	water	0	35	30	PS cups	14.28 cm^2	0.01541	0.10789
	water	0	35	80	PS cups	14.28 cm^2	0.02381	0.16673

	water	0	120	80	PS cups	14.28 cm^2	0.027311	0.19125
	water	0	78	55	PS cups	14.28 cm^2	0.18201	0.127501
	water	0	120	30	PS cups	14.28 cm^2	0.01891	0.1324
	water	0	78	55	PS cups	14.28 cm^2	0.01891	0.1324
	water	0	120	30	PS cups	14.28 cm^2	0.019607	0.13731
	water	0	78	55	PS cups	14.28 cm^2	0.019608	0.13731
	water	0	35	80	PS cups	14.28 cm^2	0.029412	0.20596
	water	0	78	55	PS cups	14.28 cm^2	0.021708	0.152021
	water	0	120	80	PS cups	14.28 cm^2	0.021709	0.15202
	water	0	35	30	PS cups	14.28 cm^2	0.008403	0.05885
	water	0	78	97	PS cups	14.28 cm^2	0.02591	0.18144
	water	0	78	55	PS cups	14.28 cm^2	0.002101	0.014712
	water	0	78	55	PS cups	14.28 cm^2	0.017507	0.12259
	water	0	78	55	PS cups	14.28 cm^2	0.016807	0.11769
	water	0	78	13	PS cups	14.28 cm^2	0.018201	0.127501
	water	0	6	55	PS cups	14.28 cm^2	0.016801	0.11769
	water	0	78	55	PS cups	14.28 cm^2	0.01681	0.11769
	water	0	149	55	PS cups	14.28 cm^2	0.014705	0.10298
	yoghurt	3.5	10 days	280	PS packaging	17.7 cm^2	0.0003375	0.00191
	raw chicken	15	10 days	280	PS packaging	17.7 cm^2	0.0002347	0.001326
30	bakery croissants	7	10 days	280	PS packaging	17.7 cm^2	0.0040205	0.02271
	sandwich cookies	20	10 days	280	PS packaging	17.7 cm^2	0.0148	0.08377
	chocolate candies	30	10 days	280	PS packaging	17.7 cm^2	0.003529	0.01994
	noodles soup	1.2	10 days	280	PS packaging	17.7 cm^2	0.0003939	0.0022252

	raw ground beef	30	10 days	280	PS packaging	17.7 cm^2	0.0005094	0.002878
	chocolate chip cookies	28	10 days	280	PS packaging	17.7 cm^2	0.0097323	0.05499
	chewing gum #1	3	10 days	280	PS packaging	17.7 cm^2	0.00199203	0.01125
	chewing gum #2	3	10 days	280	PS packaging	17.7 cm^2	0.0095508	0.05396
31	isooctane	0	9 days	40	Virgin EPS	0.05 g	6.573	219.1
	isooctane	0	12 days	25	Recycled EPS	0.02 g	3.842	128.06
	isooctane	0	6 days	40	Recycled EPS	0.02 g	3.826	127.53
32	10% ethanol/water	0	10 days	5	PS	113 cm^2	0.139	0.12301
	10% ethanol/water	0	10 days	5	PS/ZnO	113 cm^2	0.107	0.09469
	10% ethanol/water	0	10 days	5	PS/C15A	113 cm^2	0.074	0.06549
	10% ethanol/water	0	10 days	5	PS/ZnO/C15A	113 cm^2	0.055	0.04867
	10% ethanol/water	0	24 h	40	PS	113 cm^2	0.053	0.046902
	10% ethanol/water	0	24 h	40	PS/ZnO	113 cm^2	0.047	0.04159
	10% ethanol/water	0	24 h	40	PS/C15A	113 cm^2	N.D	N.D
	10% ethanol/water	0	24 h	40	PS/ZnO/C15A	113 cm^2	0.033	0.0292
	10% ethanol/water	0	0.5 h	100	PS	113 cm^2	N.D	N.D
	10% ethanol/water	0	0.5 h	100	PS/ZnO	113 cm^2	N.D	N.D
	10% ethanol/water	0	0.5 h	100	PS/C15A	113 cm^2	N.D	N.D
	10% ethanol/water	0	0.5 h	100	PS/ZnO/C15A	113 cm^2	N.D	N.D
	50% ethanol/water	0	24 h	40	PS	113 cm^2	0.175	0.1548
	50% ethanol/water	0	24 h	40	PS/ZnO	113 cm^2	0.084	0.07434
	50% ethanol/water	0	24 h	40	PS/C15A	113 cm^2	0.137	0.12123
	50% ethanol/water	0	24 h	40	PS/ZnO/C15A	113 cm^2	0.102	0.090265
33								

10% ethanol	0	10 days	60	VC1	3 cm^2	0.016335	0.5445
10% ethanol	0	10 days	60	VC2	3 cm^2	0.012339	0.4113
10% ethanol	0	10 days	60	VC3	3 cm^2	0.023895	0.7965
10% ethanol	0	10 days	60	RC1	3 cm^2	0.009459	0.3153
10% ethanol	0	10 days	60	RC2	3 cm^2	0.010305	0.3435
10% ethanol	0	10 days	60	RC3	3 cm^2	0.008253	0.2751
10% ethanol	0	10 days	60	RC4	3 cm^2	0.003096	0.1032
10% ethanol	0	10 days	60	RC5	3 cm^2	0.006282	0.2094
3% acetic acid	0	10 days	60	VC1	3 cm^2	0.022365	0.7455
3% acetic acid	0	10 days	60	VC2	3 cm^2	0.017928	0.5976
3% acetic acid	0	10 days	60	VC3	3 cm^2	0.023166	0.7722
3% acetic acid	0	10 days	60	RC1	3 cm^2	0.015912	0.5304
3% acetic acid	0	10 days	60	RC2	3 cm^2	0.012834	0.4278
3% acetic acid	0	10 days	60	RC3	3 cm^2	0.010152	0.3384
3% acetic acid	0	10 days	60	RC4	3 cm^2	0.008001	0.2667
3% acetic acid	0	10 days	60	RC5	3 cm^2	0.009792	0.3264

APPENDIX B

Statistical Analysis in R

The analysis began in R by loading meta and metaphor packages that were previously installed. The command below was used to install and load the packages:

```
install.packages("tidyverse")
install.packages("meta")
install.packages("metafor")

library(meta)
library(metafor)
```

The structure of the data was pulled and viewed in the console with the command below:

```
str(madata)
```

The random-effects model meta-analysis was then coded using the command below:

```
m.hksj.raw <- metacont(Ne,
                       Me,
                       Se,
                       Nc,
                       Mc,
                       Sc,
                       data = madata,
                       studlab = paste(Author),
                       comb.fixed = FALSE,
                       comb.random = TRUE,
                       method.tau = "SJ",
                       hakn = TRUE,
                       prediction = TRUE,
                       sm = "SMD")
m.hksj.raw
```

The output of this command (Figure A1) gave the individual effect sizes for each study, and their percentage weight, the total number of included studies, the overall effect size, and the

95% confidence interval value. Measures of between-study heterogeneity values, such as τ^2, I^2 and a Q-test of heterogeneity were reported.

```
                                  SMD             95%-CI %W(random)
Withey                        -1.6682 [ -2.5888; -0.7475]        3.3
Withey & Collins               0.9490 [  0.0591;  1.8389]        3.3
Miltz et al                    0.9746 [ -1.2440;  3.1933]        2.9
Varner & Breder               -2.4237 [ -4.4611; -0.3864]        2.9
Eiceman & Carpen               2.0689 [  0.2864;  3.8514]        3.0
Till et al                    -1.9146 [ -3.4409; -0.3883]        3.1
Miltz & Rosen-Doody            1.8822 [  0.3419;  3.4224]        3.1
Snyder & Breder               -1.5291 [ -2.6568; -0.4015]        3.3
Durst & Laperle               -2.1981 [ -4.2491; -0.1471]        2.9
Linssen et al 1991           -16.6147 [-24.8300; -8.3994]        1.0
Linssen et al 1992            -3.4047 [ -4.9574; -1.8521]        3.1
Murphy et al                   0.6098 [ -0.3696;  1.5891]        3.3
Lehr et al                     1.1030 [ -0.2125;  2.4185]        3.2
O'Neill & Tuohy                0.8675 [ -0.7924;  2.5274]        3.1
Linssen & Reitsma             -3.6449 [ -5.3876; -1.9021]        3.1
Lau et al                      0.3811 [ -0.5825;  1.3446]        3.3
Lickly et al                   0.6457 [ -0.2251;  1.5164]        3.3
Tawfik & Huyghebaert          -3.5554 [ -4.8436; -2.2671]        3.2
Nerin et al                    1.7444 [ -0.4001;  3.8890]        2.9
Brunelli et al                 1.0010 [ -0.4038;  2.4058]        3.2
Choi et al                     0.0051 [ -1.7841;  1.7943]        3.0
Ahmad & Bajahlan               0.9090 [ -0.1230;  1.9409]        3.3
Sanagi et al                  -2.9714 [ -4.6276; -1.3153]        3.1
Khaksar & Ghazi-Khansari      -3.1439 [ -4.7947; -1.4930]        3.1
Condurso et al                 1.9122 [  0.0255;  3.7990]        3.0
Verzera et al                  1.7674 [ -0.1445;  3.6793]        3.0
Amirshaghaghi et al            0.1102 [ -1.6852;  1.9055]        3.0
Paraskevopoulou et al          0.8392 [ -0.7020;  2.3804]        3.1
Saim et al                     3.0466 [  1.6179;  4.4753]        3.2
Genualdi et al                -0.8345 [ -1.6018; -0.0672]        3.3
Lin et al                      2.6658 [ -1.3440;  6.6756]        2.1
Abolghasemi-Fakhri et al       0.3143 [ -1.1603;  1.7888]        3.1
Song et al                     1.1748 [ -0.3580;  2.7075]        3.1

Number of studies combined: k = 33

                         SMD             95%-CI     t p-value
Random effects model -0.2587 [-1.1544; 0.6371] -0.59  0.5605
Prediction interval          [-5.6604; 5.1431]

Quantifying heterogeneity:
 tau^2 = 6.8214 [2.5230; 12.0741]; tau = 2.6118 [1.5884; 3.4748];
 I^2 = 84.5% [79.2%; 88.5%]; H = 2.54 [2.19; 2.94]

Test of heterogeneity:
      Q d.f.  p-value
 206.63   32 < 0.0001

Details on meta-analytical method:
- Inverse variance method
- Sidik-Jonkman estimator for tau^2
- Q-profile method for confidence interval of tau^2 and tau
- Hartung-Knapp adjustment for random effects model
- Hedges' g (bias corrected standardised mean difference)
```

Figure A1: Output of meta-analysis

This output was converted into a Forest plot (Figure A2) using the command below:

forest(m.hksj.raw)

Study	Experimental Total	Experimental Mean	Experimental SD	Control Total	Control Mean	Control SD	SMD	95%-CI	Weight
Withey	15	0.09	0.0980	11	22.45	20.1100	-1.67	[-2.59; -0.75]	3.3%
Withey & Collins	9	166.43	253.2300	14	12.45	6.9300	0.95	[0.06; 1.84]	3.3%
Miltz et al	3	2296.00	1946.4400	2	166.00	39.5980	0.97	[-1.24; 3.19]	2.9%
Varner & Breder	6	0.03	0.0400	3	16.34	11.1900	-2.42	[-4.46; -0.39]	2.9%
Eiceman & Carpen	11	0.96	0.4156	2	0.08	0.0050	2.07	[0.29; 3.85]	3.0%
Till et al	11	0.35	0.4517	3	142.41	170.1400	-1.91	[-3.44; -0.39]	3.1%
Miltz & Rosen-Doody	6	3925.00	2552.7800	5	8.02	2.9280	1.88	[0.34; 3.42]	3.1%
Snyder & Breder	16	0.00	0.0033	5	116.94	160.0000	-1.53	[-2.66; -0.40]	3.3%
Durst & Laperle	4	5.18	3.4340	4	49812.00	27864.6900	-2.20	[-4.25; -0.15]	2.9%
Linssen et al 1991	9	0.13	0.0755	3	32.51	4.0206	-16.61	[-24.83; -8.40]	1.0%
Linssen et al 1992	15	0.07	0.0766	5	13.98	8.3012	-3.40	[-4.96; -1.85]	3.1%
Murphy et al	14	92.10	168.7200	6	0.82	0.5651	0.61	[-0.37; 1.59]	3.3%
Lehr et al	8	31.75	30.8500	4	0.91	0.5620	1.10	[-0.21; 2.42]	3.2%
O'Neill & Tuohy	7	19.39	21.1597	2	0.27	0.3851	0.87	[-0.79; 2.53]	3.1%
Linssen & Reitsma	28	0.05	0.0252	2	26.09	36.7860	-3.64	[-5.39; -1.90]	3.1%
Lau et al	9	1.38	2.9746	8	0.49	0.6304	0.38	[-0.58; 1.34]	3.3%
Lickly et al	39	49.28	78.9330	6	0.51	0.3229	0.65	[-0.23; 1.52]	3.3%
Tawfik & Huyghebaert	75	0.03	0.0556	3	10.75	18.4032	-3.56	[-4.84; -2.27]	3.2%
Nerin et al	5	76.16	41.0700	2	0.06	0.0495	1.74	[-0.40; 3.89]	2.9%
Brunelli et al	110	223.49	222.7300	2	0.03	0.0350	1.00	[-0.40; 2.41]	3.2%
Choi et al	3	0.26	0.0136	2	0.25	0.3479	0.01	[-1.78; 1.79]	3.0%
Ahmad & Bajahlan	18	0.57	0.6567	5	0.01	0.0153	0.91	[-0.12; 1.94]	3.3%
Sanagi et al	15	4.43	2.2460	3	13.77	6.0274	-2.97	[-4.63; -1.32]	3.1%
Khaksar & Ghazi-Khansari	30	0.00	0.0041	2	0.03	0.0485	-3.14	[-4.79; -1.49]	3.1%
Condurso et al	8	17.46	8.8141	2	0.00	0.0006	1.91	[0.03; 3.80]	3.0%
Verzera et al	7	21.26	11.5460	2	0.00	0.0006	1.77	[-0.14; 3.68]	3.0%
Amirshaghaghi et al	3	2.51	0.3161	2	2.24	3.0550	0.11	[-1.69; 1.91]	3.0%
Paraskevopoulou et al	12	136261.99	158768.5000	2	0.85	0.6358	0.84	[-0.70; 2.38]	3.1%
Saim et al	20	0.13	0.0427	4	0.00	0.0050	3.05	[1.62; 4.48]	3.2%
Genualdi et al	10	0.03	0.0289	24	2.80	3.8220	-0.83	[-1.60; -0.07]	3.3%
Lin et al	3	158.23	52.7160	2	0.46	0.0202	2.67	[-1.34; 6.68]	2.1%
Abolghasemi-Fakhri et al	16	0.08	0.0398	2	0.07	0.0827	0.31	[-1.16; 1.79]	3.1%
Song et al	16	0.44	0.2130	2	0.18	0.0081	1.17	[-0.36; 2.71]	3.1%
Random effects model	**561**			**146**			**-0.26**	**[-1.15; 0.64]**	**100.0%**
Prediction interval								**[-5.66; 5.14]**	

Heterogeneity: $I^2 = 85\%$, $\tau^2 = 6.8214$, $p < 0.01$

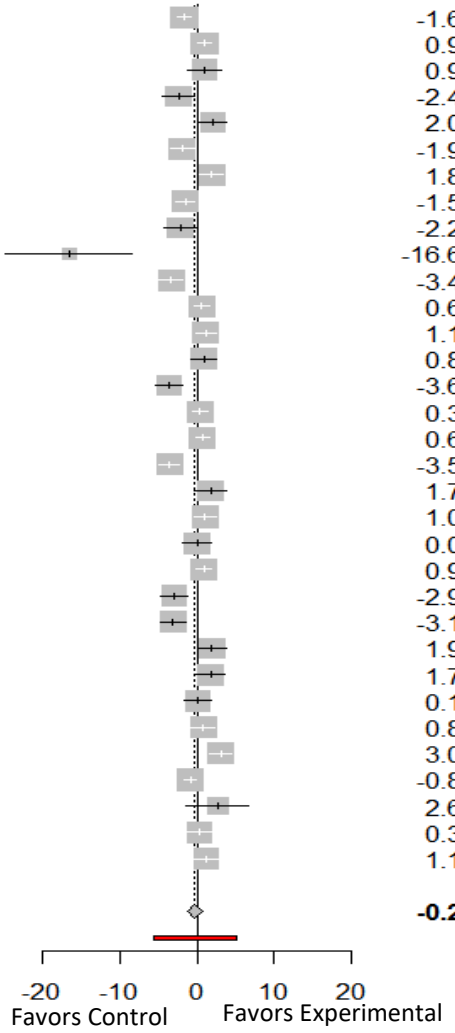

Figure A2: Meta-Analysis Forest Plot

The **EXPERIMENTAL** represents the quantity of styrene migrating from polystyrene containers into food simulant while **CONTROL** signifies the amount of residual styrene present in polystyrene food containers. The **TOTAL** column indicates the total number of experiment each study performed for the experimental and control. The **MEAN** column shows the mean amount of migrated styrene for the experimental and control. The **SD** column represents the standard deviation of migrated styrene for the experimental and control.

Test for Heterogeneity

Detecting outliers & influential cases allows outliers and influential studies to be identified and removed (Figure A3). These are studies whose confidence interval does not overlap with is pooled effect confidence interval. The command run in R console is below:

```
library(dmetar)
find.outliers(m.hksj.raw)
```

```
Results with outliers removed
-----------------------------
                                SMD             95%-CI %w(random) exclude
Withey                      -1.6682 [ -2.5888; -0.7475]        4.8
Withey & Collins             0.9490 [  0.0591;  1.8389]        4.9
Miltz et al                  0.9746 [ -1.2440;  3.1933]        2.9
Varner & Breder             -2.4237 [ -4.4611; -0.3864]        3.1
Eiceman & Carpen             2.0689 [  0.2864;  3.8514]        3.5
Till et al                  -1.9146 [ -3.4409; -0.3883]        3.9
Miltz & Rosen-Doody          1.8822 [  0.3419;  3.4224]        3.8
Snyder & Breder             -1.5291 [ -2.6568; -0.4015]        4.5
Durst & Laperle             -2.1981 [ -4.2491; -0.1471]        3.1
Linssen et al 1991         -16.6147 [-24.8300; -8.3994]        0.0        *
Linssen et al 1992          -3.4047 [ -4.9574; -1.8521]        0.0        *
Murphy et al                 0.6098 [ -0.3696;  1.5891]        4.7
Lehr et al                   1.1030 [ -0.2125;  2.4185]        4.2
O'Neill & Tuohy              0.8675 [ -0.7924;  2.5274]        3.7
Linssen & Reitsma           -3.6449 [ -5.3876; -1.9021]        0.0        *
Lau et al                    0.3811 [ -0.5825;  1.3446]        4.8
Lickly et al                 0.6457 [ -0.2251;  1.5164]        4.9
Tawfik & Huyghebaert        -3.5554 [ -4.8436; -2.2671]        0.0        *
Nerin et al                  1.7444 [ -0.4001;  3.8890]        3.0
Brunelli et al               1.0010 [ -0.4038;  2.4058]        4.1
Choi et al                   0.0051 [ -1.7841;  1.7943]        3.5
Ahmad & Bajahlan             0.9090 [ -0.1230;  1.9409]        4.7
Sanagi et al                -2.9714 [ -4.6276; -1.3153]        0.0        *
Khaksar & Ghazi-Khansari    -3.1439 [ -4.7947; -1.4930]        0.0        *
Condurso et al               1.9122 [  0.0255;  3.7990]        3.3
Verzera et al                1.7674 [ -0.1445;  3.6793]        3.3
Amirshaghaghi et al          0.1102 [ -1.6852;  1.9055]        3.5
Paraskevopoulou et al        0.8392 [ -0.7020;  2.3804]        3.8
Saim et al                   3.0466 [  1.6179;  4.4753]        0.0        *
Genualdi et al              -0.8345 [ -1.6018; -0.0672]        5.1
Lin et al                    2.6658 [ -1.3440;  6.6756]        1.4
Abolghasemi-Fakhri et al     0.3143 [ -1.1603;  1.7888]        4.0
Song et al                   1.1748 [ -0.3580;  2.7075]        3.9

Number of studies combined: k = 26

                         SMD            95%-CI    t p-value
Random effects model 0.3527 [-0.1809; 0.8863] 1.36  0.1856
Prediction interval         [-2.0847; 2.7901]

Quantifying heterogeneity:
 tau^2 = 1.3276 [0.4526; 2.7977]; tau = 1.1522 [0.6727; 1.6726];
 I^2 = 69.4% [54.2%; 79.5%]; H = 1.81 [1.48; 2.21]

Test of heterogeneity:
     Q d.f.  p-value
 81.61   25 < 0.0001

Details on meta-analytical method:
- Inverse variance method
- Sidik-Jonkman estimator for tau^2
- Q-profile method for confidence interval of tau^2 and tau
- Hartung-Knapp adjustment for random effects model
- Hedges' g (bias corrected standardised mean difference)
```

Figure A3: Result with outliers removed

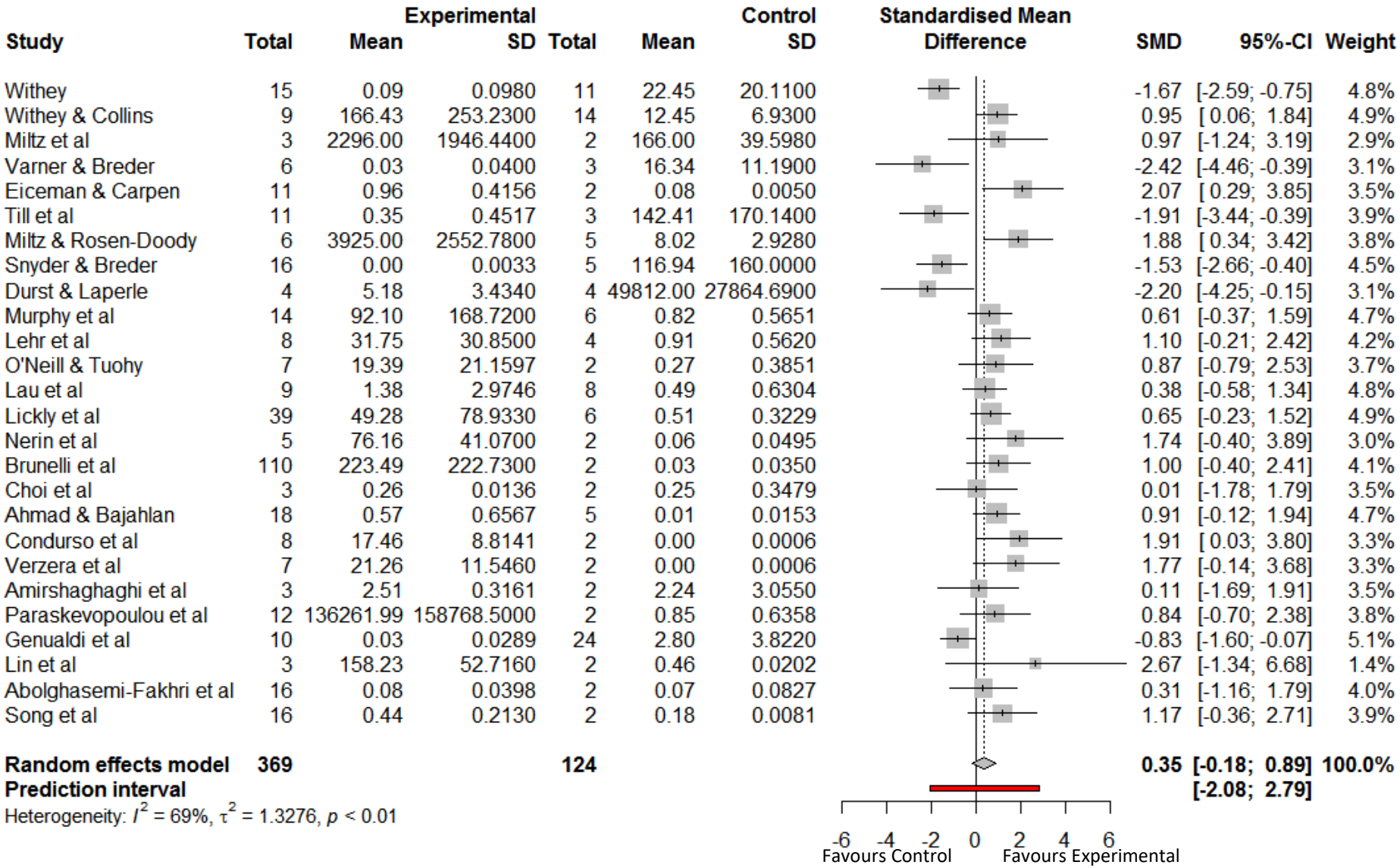

Figure A4: Meta-analysis Forest Plot without outliers

The result obtained were converted into a Forest plot (Figure A4) above, using the command below:

```
m.hksj <- find.outliers(m.hksj.raw)
forest(m.hksj)
```

There was an error from this code which prevented the Forest plot. The error is below.

```
Error in forest.default(m.hksj) :
  Must specify either 'vi', 'sei', or ('ci.lb', 'ci.ub') pairs.
```

However, the Forest plot was obtained by excluding the outliers from the Excel spread sheet and pooling effect size without the outliers. The command used is below:

```
m.hksj_outlier <- metacont(Ne,
                   Me,
                   Se,
                   Nc,
                   Mc,
                   Sc,
                   data = madata_outlier,
                   studlab = paste(Author),
                   comb.fixed = FALSE,
                   comb.random = TRUE,
                   method.tau = "SJ",
                   hakn = TRUE,
                   prediction = TRUE,
                   sm = "SMD")
m.hksj_outlier

forest(m.hksj_outlier)
```