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The Safety Assessment of Food Additives by Reproductive and Developmental Toxicity Studies

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1. Introduction

The overall consumption of food additives is 139 lbs/year/person. If the common additives like spices, sugars, salt, honey, pepper, mustard, dextrose etc. are excluded, the consumption decreases to 5 lbs/year. Due to widespread consumption, it is necessary to evaluate the implications for the health of consumers because of the presence of newly synthesized food additives before commence production according to accepted guidelines such as Food and Drug Administration (FDA), U.S. Environmental Protection Agency (EPA) and European Food Safety Authority (EFSA). "Redbook 2000" is one of the revised form of Redbook II guideline published in 1993 by FDA; also defined as "Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food". This document is a guidance for determining toxicity studies, for designing and reporting the results of toxicity studies, conducting statistical analyses of data, the review of histological data and the submission of this information to FDA. The toxicological testing should provide not only information relevant to the average consumer, but also relevant to those population groups whose pattern of food consumption, physiological or health status may make them vulnerable such as young age, pregnancy and other metabolic disorders. Possible toxicological effects due to additive consumption should be tested especially in reproduction and developmental studies which are designed to evaluate effects on sexuality and fertility of males and females, developing organisms (mortality, structural abnormality and functional deficiencies). Besides, multigenerational reproductive toxicity studies provide information about the effects of a test substance on gonadal function, estrous cycle, mating behavior, lactation and development of the offspring.

For new food additives, a safety evaluation is obtained generally from experimental data derived from investigations in laboratory animals. Although it may be possible to use human data derived from medical use, occupational epidemiology or from volunteers, the obtained data would be limited. Therefore, the likely effects on man can be estimated by intentive extrapolation from laboratory animals. The end points and the indices obtained must provide sufficient information and statistical power to permit FDA to determine whether the additive is associated with changes in reproduction and fertility.

* Both authors have equal contribution in the chapter.

This chapter will highlight the possible health effects of newly synthesized food additives on market and focus on their reproductive and developmental toxicity perspectives. Considering that food technology is a complex area in which even the simplest additive interacts with all the others to produce qualified food; we are expecting that this chapter will help to examine food additives that can probably affect the human life.

2. Overview of food additives

Advances in food technology have resulted in an increased number of modified foods and additives in 20th century. An additive is a substance which may intentionally become a component of food or affect its characteristics. There are about 3000 different food additives defined up to date. Food additives may be divided in several groups; although there is some overlap between them. Main six categories of food additives are classified as preservatives, nutritional supplements, flavoring agents, colorings, texturing agents and miscellaneous. According to the functional classes, definitions and technological functions, food additives are summarized in Figure 1 (COABISCO, 2011).

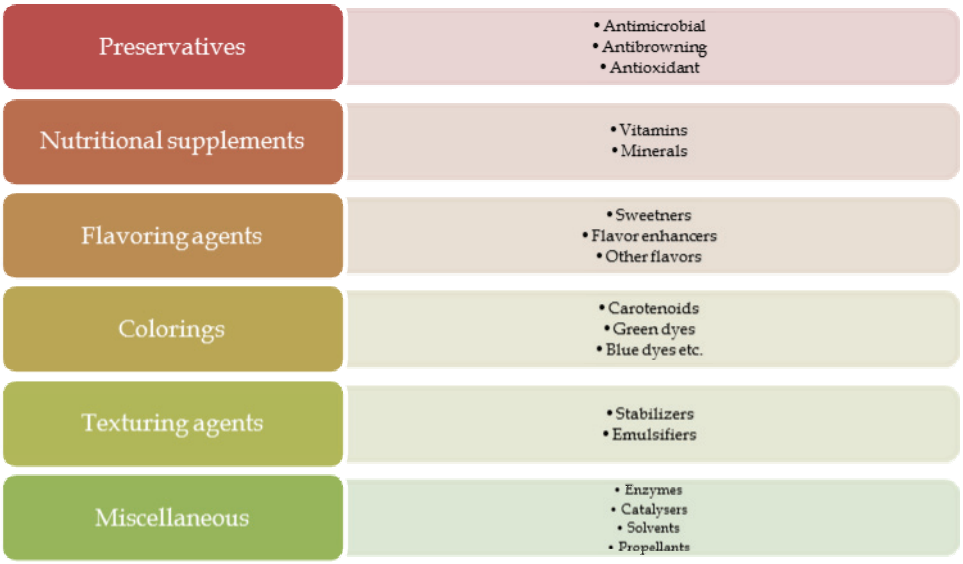

Fig. 1. Six main categories of food additives

Each food additive is assigned a unique E number, which have been assessed for use within the European Union (EU) to inform consumers (Figure 2). E numbers for European countries are all prefixed by "E"; on the other hand non-European countries do not use this prefix. E letter stands for the approval of the food additive in Europe. The numbering scheme fallows that of the International Numbering System (INS) as determined by Codex Alimentarius Committee (Codex Alimentarius, 2009). Though, only a subset of INS additives are approved for use in the EU. The United States Food and Drug Administration listed these items as "generally recognized as safe" or GRAS. As an example, additive E 341 (Tricalcium phosphate) is approved by US so has an "E" prefix and 341 numbering which stands for 340-349 subset known as "phosphates" under Antioxidants and Acidity Regulators group.

Fig. 2. Classification of additives by numeric range

In the EU, to authorize a substance as a food additive, a reasonable case of technological need, no hazard to consumers at level of proposed use and no misguidance to consumers should be demonstrated. To evaluate whether the newly released food additive has an effect on health; The European Commission is required a consultancy from Scientific Committee on Food (SCF). In this context, SCF deals with questions relating to the toxicology and hygiene in the entire food production chain for consumer health and food safety issues. For submission of a new food additive, the evaluation process by the SCF requires administrative, technical, toxicological data and references (European Commission Health & Consumer Protection Directorate, 2011). Among these, toxicological data obtained from experimental studies have a crucial role for consumers' health due to the presence of any additive in food.

During the general toxicological evaluation of food additives, the SCF first issued Guidelines for the Safety Assessment of Food Additives in 1980 (Scientific Committee for Food, 1980). However, new guidance documents have been published as Joint FAO/WHO Expert Committee on Food Additives (JECFA) (IPCS/JECFA, 1987), SCF guideline is still applicable. The aim of toxicological testing should provide sufficient information relevant to average consumer and vulnerable populations such as young age, pregnancy, diabetes, etc. The testing conditions depend on the chemical structure, proposed levels of use in food. The human data is derived from occupational epidemiology, medical use and volunteers but; for newly submitted food additives experimental data is commonly derived from laboratory animals. For evaluation of the safety of food additives, core studies are required such as; metabolism/toxicokinetics, subchronic toxicity, genotoxicity, chronic toxicity, carcinogenicity, reproduction and developmental toxicity. In this chapter, we are going to discuss the significant role of reproductive and developmental toxicity studies for evaluating new food additives.

3. Traditional and newly released food additives

"Toxicological Principles for the Safety Assessment of Food Ingredients" (Redbook 2000) is the new name of Redbook I which was previously published in draft form in 1983. Redbook 2000 provides information about toxicological data of food ingredients which are submitted to Center for Food Safety and Applied Nutrition and Office of Food Additive Safety for industry and other stakeholders. Food and color additives, food contact substances (which are also defined as indirect food additives) and substances classified as generally recognized as safe (GRAS) are the components of food ingredients (U.S. Food and Drug Administration, 2007).

At the end of the toxicological studies data derived from animal studies can be used to extrapolate to give information on human exposure. Therefore, defining the Acceptable Daily Intake (ADI), described as the dose level at which the additive causes effects on the health of the animals, is important. The highest level at which no adverse effect on the health of the animals is observed is called the NOAEL (No-Observed-Adverse-Effect-Level). An ADI is derived by dividing the NOAEL obtained from these studies, by an appropriate 'uncertainty' factor, which is intended to take account of differences between the animals on which the additive was tested and humans, in order to reduce further possibility of risk to humans. This uncertainty factor is commonly 100 (assuming that human beings are 10 times more sensitive than test animals and that the different levels of sensitivity within the human population is in a 10 fold range), but may be as much as 1,000 (if, for example, the toxic effect in animals is found to be particularly severe) or as low as 10 (where it has been found that humans are less likely than animals to be affected, based on actual data on the additive in humans) (Food Safety Authority of Ireland, 2011).

4. Assessment of potential reproductive and developmental toxicity of recently used food additives

Before a new food additive is introduced to the market, it should be tested if it causes any reproductive and developmental toxicity (EFSA, 2010a). To observe the potential effects, multigeneration reproduction studies have to be conducted. Laboratory species such as mouse, rabbit and especially rat are used at least for two generations and one litter per generation (Scientific Committee for Food, 1980). The test substance should be administrated in normal diet.

On the other hand, two laboratory species, usually a rodent and a non-rodent should be used in developmental toxicity studies. The test substance should either be in normal diet or administered by oral gavage during whole gestation period in order to detect the potential toxicological effects. In addition to a multigenerational and/or developmental toxicity study; in order to provide the possible effects after postnatal development and function (such as neurological function and behavior), examinations should be continued from the beginning of embryogenesis through to weaning.

4.1 Reproductive toxicity studies

The aim of a reproductive toxicity study is to ensure data about effects on the sexuality and fertility of males and females. These include reproductive behavior, pregnancy carriage

ability, pre-postnatal survival rate, reproductive ability-capacity of the offspring and to examine major target organs for toxicity including reproductive organs in both parents and offspring histopathologically (Scientific Committee for Food, 1980). Besides, multigeneration reproductive toxicity studies ensure information about the effects on gonadal function, estrous cycles, conception, parturition, lactation (Joint FAO/WHO Expert Committee, 2000).

Reproductive studies constitutively target multigenerational studies as a result of human exposure to most food additives and preservatives during the whole lifetime. Studies performed with multigeneration enable researchers to detect any potential effect of a specific additive on each litter per generation. The administration of the test substance to parental and offspring generations should be continuous via the diet.

The end point evaluated in the indices calculated must provide sufficient information and statistical power to permit FDA whether the additive has effects on reproduction and fertility (U.S. Food and Drug Administration, 2007). The minimal reproduction study should consist at least two generations with one litter per generation. The reproduction study generation number should be expanded if the developmental toxicity effect of food additive is observed. A brief summary of the recommended reproduction study design according to FDA was given in Figure 3.

In a basic two generation reproduction and teratology study, the first step is to find the appropriate dose range of the compound in order to conduct the main study. The second step, includes the selection of experimental animal species due to its' life span, body size, breeding conditions, gestation length, high fertility rate etc. 5-9 weeks of aged animals, preferably rats are typically chosen. Each test and control group should include approximately 20 males and 20 pregnant females with uniform weight and age. For the detection of dose-related responses, minimum three doses of the test substance should be used: high, intermediate and low doses. The administration of the test substance may be via diet, drinking water or by gavage. In the first parental group (F_0), males should be administered for the duration of spermatogenesis and epididymal transit before and throughout the mating period. First parental females should be administered for the same length of time as males and through pregnancy to the weaning of the F_{1a} litter. Litters should be exposed throughout their entire lives. A female should be mated with a single randomly selected male from the same dose group until the pregnancy occurs or three weeks have elapsed. Each morning all females should be examined for the presence of vaginal plug or sperm in the vaginal lavage which is considered as "day zero" of gestation. Each animal should be observed twice each day at predefined time intervals. All animals should be weighted before administration, once weekly thereafter and at necropsy. During necropsy, the organs of reproductive system belonging to weanlings and parental animals (males-females) should be examined histopathologically. Uterus and ovaries for females; testis, seminal vesicle, prostate, epididymis for males should be weighted and evaluated separately. Brain, thymus and spleen tissues should also be examined for weanlings. Organ weights should be recorded both as absolute and relative weights. Indices which are the animal number responding to the test substance during conception until weaning period should be calculated for each reproduction study. To evaluate the endpoints of male reproductive toxicity, apart from counting testicular spermatid numbers, minimum 200 sperm per sample from cauda epididymis or proximal vas deferens should be examined. Acquired data from control and test groups of animals should be compared statistically using suitable statistics program.

Fig. 3. A reproduction study design by FDA

According to the recommendations of FDA (U.S. Food and Drug Administration, 2007), a toxicity study should be conducted according to Good Laboratory Practice Regulations. The animals used in the study should be well cared and housed according to the recommendations of Guide for the Care and the Use of Laboratory Animals. All test animals should be categorized according to their species, strain, sex and weight or age.

4.2 Pre- and post-natal developmental toxicity studies

Developmental toxicity studies assess the effects of the test substance on the developing organism including the death, structural abnormalities, altered growth and functional deficiencies (Joint FAO/WHO Expert Committee, 2000).

Lethal, teratogenic and other toxic effects on the embryo and fetus are examined under the consideration of prenatal developmental toxicity studies (Scientific Committee for Food,

1980). Besides, postnatal developmental toxicity studies deal with the observed side effects caused by maternal or maternal milk way exposure.

Embryonic and fetal resorptions, death, fetal weight, sex ratio, external and visceral skeletal morphology are in the context of prenatal developmental toxicity. The major objectives of postnatal developmental studies are physical, functional and behavioral development in animals exposed from embryogenesis through to weaning. These tests include neurological function, behavior during both early postnatal and adulthood phases, the measurement of vaginal opening in female pups, etc.

4.3 Current studies about recently used food additives

All food additives prior to their authorization should be evaluated for their safety by SCF or EFSA. According to the international legislations, all food additives must be kept under continuous observations and must be re-evaluated in the light of new scientific techniques. This part will be an overview of commonly used food additives listed below:

4.3.1 Lutein (E 161b)

Food colors are the first evaluated additives whose data are old. As soon as new available studies are conducted, the results should be renewed with the old ones. This is the reason of the evaluation priority of food color additives.

Lutein (E 161b) is a natural carotenoid dye which is approved as a food additive by the EU. In a 90 day rat study, according to NOAEL (No Observed Adverse Effect Level) of 200 mg/kg bw/day, which was defined by EFSA Panel, there were no developmental toxicity effects observed at dose levels up to 1000 mg/kg bw/day, the highest dose tested. Additionally, available reported data showed no effects on reproductive organs in oral 90-day studies. Due to the fact that lutein is a normal constituent of diet, ADI can be altered. Lutein is found as a non-genotoxic additive; but the absence of multigeneration reproductive toxicity and chronic toxicity/carcinogenicity studies caused EFSA to get the decision for ADI of lutein as 1 mg/kg bw/day with the uncertainty factor of 200 (EFSA, 2010a).

4.3.2 Caramel colours (E 150a,b,c,d)

Caramel colours are colouring substances approved as food additives according to the reactants used in their manufacture. E 150a is Class I Plain Caramel or Caustic Caramel, E 150b is Class II Caustic Sulphite Caramel, E 150c is Class III Ammonia Caramel and E 150d is Class IV Sulphite Ammonnia Caramel. EFSA determined the NOAEL as 30 g/kg bw/day with the uncertainty factor of 100 and ADI as 300 mg/kg bw/day for caramel colours. Up to date, there are no reproductive and/or developmental study of Class I and II caramels. JECFA states that there are only three developmental studies on Class III caramels. For Class III and IV caramels, studies with pregnant CD1 mice showed that, after treatment on days 6-15 of gestation, there were no effects on the number of implantation sites and resorption numbers, maternal-fetal survival and fetal skeletal defects (Morgareidge, 1974a; 1974b). In other studies conducted with pregnant Wistar rats and Dutch-belted rabbits, no treatment-related effects of Class III caramels were seen in the dams and fetal parameters (Morgareidge, 1974a). In the studies which were conducted on Wistar rats for Class IV caramels, no adverse effect was seen on female fertility, litter size, number of implantation

sites or sex ratio of the pups (Til and Spanjers, 1973). Available reproduction and developmental studies, although limited, do not reveal any effects of concern. The studies also did not reveal any effects on reproductive organs. Lymphocytopenia is the only concern reported in short term studies with caramel colours (EFSA, 2010b).

4.3.3 Erythrosine (E 127)

Erythrosine is a xanthene-dye which was evaluated by JECFA with the ADI of 0-0.1 mg/kg bw/day. It is used especially for cocktail and candied cherries. In rats (Collins et al., 1993a; 1993b) and rabbits (Burnett et al., 1974) exposed to erythrosine by gavage or in drinking water, on day 0-19 of gestation, it was found neither fetotoxic nor teratogenic. Vivekanandhi et al. conducted a study with Swiss male Albino mice in which 64, 128 and 256 mg/kg bw/day erythrosine were administered daily by gavage resulted in decreased sperm motility and increased sperm abnormalities in dose dependent manner (Vivekanandhi et al., 2006).

4.3.4 Green S (E 142)

Green S is a triarylmethane dye authorized as a food additive in the EU. JECFA has previously established an ADI of 25 mg/kg bw/day in 1975; however, this was re-evaluated and later considered to be 5 mg/kg bw/day by SCF. In a previous study established by rats, the NOAEL of Green S was derived as 500 mg/kg bw/day, based on the results as increased spleen and kidney weight. At the high dose treatment (1000 mg/kg bw/day), there was evidence that the amniotic membranes had a green colouring. However, intra-uterine and post-natal development was not affected. Within the light of these results, the NOAEL is concluded as 1000 mg/kg bw/day for fetal development. In an additional study, 15 male and 15 female Wistar rats were administrated Green S in the diet at dose levels of 250, 500 and 1500 mg/kg bw/day for 13 weeks. When compared with controls, increases in mean body weight were observed in all treatment groups (BIBRA, 1978; Clode et al., 1987). Also, adequate reproduction and embryotoxicity studies including teratology are still requested (EFSA, 2010c).

4.3.5 Amaranth (E 123)

Amaranth (E 123) is an azo dye approved as a food additive in the EU. The ADI of Amaranth was established as 0-0.5 and 0.08 mg/kg bw/day by JECFA and SCF respectively. There are several studies which examined the reproductive and developmental toxicity of Amaranth. When all of these studies are taken into account, NOAELs for Amaranth is as follows: mouse 100 mg/kg bw/day, rat 15 mg/kg bw/day and rabbit 15 mg/kg bw/day (EFSA, 2010d). According to the results of Shtenberg and Gavrilenko, oral Amaranth exposure at doses of 1.5 and 15 mg/kg bw/day for 12-14 months in parental generation causes a significantly higher percentage of unsuccessful pregnancies with no live born born pups and increases percentages of stillborns in rats (Shtenberg and Gavrilenko, 1970). In the study, 1.5 and 15 mg/kg bw/day administration of Amaranth in drinking water was reported to cause increased rate of post-implantation death with increased mortality at post-partum in both treatment groups and a higher incidence of stillbirth at 15 mg/kg bw/day group rats. Khera et al. administrated Amaranth to Wistar rats at dose levels of 15, 30, 100, 200 mg/kg bw/day on Days 0-18 of

gestation, either by gavage or in diet (Khera et al., 1974). According to the results, all dams were killed on day 19. The Panel considered that the NOAEL of this study is 200 mg/kg bw/day Amaranth, which is the highest dose tested.

4.3.6 Brilliant Blue FCF (E 133)

Brilliant Blue FCF (E 133) is another commonly used triarylmethane dye which is authorized as a food additive by the EU. Both JECFA and SCF established an ADI of 12.5 mg/kg bw/day. Depending on the newly conducted long-term studies, the ADI was revised to 10 mg/kg bw/day by SCI in 1984. The NOAEL was assigned as 2500 mg/kg bw/day by JECFA with uncertainty factor of 200. Afterwards, SCF established Brilliant Blue NOAEL as 1073 mg/kg bw/day in male and female rats with uncertainty factor of 100 (EFSA, 2010e). Among few chronic toxicity studies, the lowest NOAEL came from the most recent toxicity study (IRCD, 1981; Borzelleca, 1990). The Panel agreed with the authors and considered that the new NOAEL as 631 mg/kg bw/day. With uncertainty factor 100, the new established ADI for Brilliant Blue is 6 mg/kg bw/day. Data available up to date shows Brilliant Blue is poorly absorbed by the body and it is mainly excreted as unchanged in faeces. In addition, there have been several rat studies up to date; however in none of the studies treatment-related abnormalities were observed.

4.3.7 Curcumin (E 100)

Curcumin (E 100) is a dicinnamoylmethane dye consisting of three principal colouring components. It is also approved by the EU for the use as a food additive. JECFA allocated an ADI dose of 0-3 mg/kg bw/day and a NOAEL dose of 250-320 mg/kg bw/day with uncertainty factor of 100 depending on the results of a multigeneration study which is conducted by Ganiger et al. In the study, rats were fed with Curcumin for 24 weeks at doses of 250-320 mg/kg bw/day and 960-100 mg/kg bw/day (Ganiger et al., 2007). In the high dose group, there was a decrease in body weight gain. Garg conducted a multigeneration study in Wistar rats according to OECD Testing guideline administering curcumin (Garg, 1974). Rats were fed with diets containing 0, 1500, 3000, and 10000 mg/kg bw/day curcumin. At the end of the study, it was reported that there was a dose-related decrease on body weight gain in the dams of the parental generation during days 10-15 of gestation. However, no other effects were observed. According to its' chemical composition, Curcumin is a rapidly metabolized dye which is later excreted with faeces (EFSA, 2010f).

4.3.8 Canthaxanthin (E 161 g)

Canthaxanthin is a carotenoid pigment which is authorized by the EU as a food additive. It is mainly composed of all-*trans* β-carotene-4,4'-dione with other minor carotenoids. The ADI dose of canthaxanthin is established as 0.03 mg/kg bw/day. There is no data reporting adverse effects of xanthaxanthin on reproductive system or on the developing fetus in high doses up to 1000mg/kg bw/day in rats and in high doses up to 400 mg/kg bw/day in rabbits. Hoffmann-La Roche reported that there were no adverse effects on fertility, litter size, the number of young weaned and their weights after 0 (placebo) and 0.1% canthaxanthin exposure in rats (Hoffmann – La, 1990). In a three-generation reproduction study by Buser, male and female rats were fed with diet including 0, 250, 500 and 1000 mg/kg bw/day canthaxanthin (Buser, 1987). The results indicated that there were no

treatment-related effects in reproductive system. The EFSA Panel evaluated the highest dose tested of 1000 mg/kg bw/day as NOAEL for reproductive system, embryotoxicity and teratogenicity (EFSA, 2010g).

4.3.9 Aspartame

Nowadays, aspartame (APM) is the most commonly used artificial sweetener in the world (Hazardous Substances Data Bank, 2005). APM is approved by both FDA and the EU for the use in all foods (FDA, 1996; EFSA, 2006h). According to long term studies, ADI of APM is 2.5-5 mg/kg bw/day (Butchko et al., 2002). Long term carcinogenicity bioassays performed on rat and mice indicated that APM is a high effective carcinogenic additive causing lymphomas, leukemias and neoplastic lesions in females and Schwannomas in males (Soffritti et al., 2006). However, recently conducted lifespan studies with Swiss mice at dose levels of 0, 2000, 8000, 16000, 32000 ppm resulted that it does not affect the daily feed consumptions, mean body weights and the survival of males-females (Soffritti et al., 2010).

4.3.10 Paraben

Parabens have wide range of use in different industrial areas. One of them is its' use in food ingredients as an anti-microbial agent (Hossani et al., 2000). Parabens are lately reported to act as xenoestrogens which are a class of endocrine disruptors due to the lengths of their alkyl side chains (Okubo et al., 2001). It is known that parabens have an effect on reproductive tissues, induce aberrant estrogenic signaling in cells, cause changes in the expression patterns of multiple genes in rat fetal reproductive system (Naciff et al., 2003). Despite the potential health effects, parabens are approved as food additives by the EU with ADI dose of 0-10 mg/kg bw/day (Ishiwatari et al., 2007) and with NOAEL as 1000 mg/kg bw/day (Boberg et al., 2010). In a developmental study conducted by Thuy et. al, during juvenile-peripubertal period, female rats were administered with methyl-, ethyl-, propyl-, isopropyl-, butyl- and isobutylparabens at doses of 62.5, 250, 1000 mg/kg bw/day (Thuy et al., 2010). Their results showed that in the highest dose group there was a significant delay in the date of vaginal opening, a decrease in length of estrous cycle, morphological changes in the uterus and increased number of cystic follicles in ovaries. Additionally, after 10, 100, 1000 mg/kg bw/day polyparaben treatment, decreases in daily testis sperm production and in serum testosterone levels in a dose-dependent manner were observed in all doses (Oishi, 2002).

4.3.11 Coriander essential oil

Coriander essential oil is obtained by steam distillation of the dried fruits (seeds) of Coriandrum sativum L. In the food industry, coriander oil is used as a flavoring agent and adjuvant. Coriander oil is both approved for the use as food additive by FDA and The EU (Vollmuth et al., 1990). While maternal NOAEL of coriander oil was determined as 250 mg/kg bw/day, developmental NOAEL was established as 500 mg/kg bw/day (FFHPVC, 2002). Vollmuth et al. administered 250, 500, 100 mg/kg bw/day coriander oil to pregnant pregnant Crl CD rats (7 day before cohabitation, during gestation, 4 day post-parturition). At the highest dose significant decreases in gestation index, length of gestation, viability of pups and litter size were noted.

4.3.12 Allyl isothiocyanate (AITC)

Allyl isothiocyanate (AITC) is used both as a food additive and a flavouring agent. It can occur naturally in certain vegetables such as cabbage, mustard and horseradish (EFSA 2010i). EFSA Panel (2010) established the ADI dose as 0.02 mg/kw bw/day with an uncertainty factor of 500 (EFSA, 2010i). The Panel regarded a Low Observed Adverse Effect Level (LOAEL) as 12 mg/kw bw/day rather than the NOAEL in order to cover uncertainties resulting from extrapolation. Oral doses of AITC up to 18.5, 23.8 and 12.3 mg/kg bw/day did not cause any developmental toxicity in pregnant rats, hamsters and rabbits and may be fetotoxic to mouse at doses higher than 6 mg/kg bw/day without any teratogenic effects (EFSA, 2010i).

4.3.13 Tricalcium phosphate (E 341)

Tricalcium phosphate (E 341) is a commonly used flavor preservative, anti-caking, stabilizing and anti-souring food additive. JECFA reported the ADI dose of E 341 as 70 mg/kg bw/day (JECFA, 2001). In a recently published study, Wistar rats were treated with 175 and 350 mg/kg bw/day E 341 during gestation days 0-20. Decrease in placental weights and skeletal morphometry in fetus were observed in both doses (Güngörmüş et al., 2010). There was also a decrease in trans-umblical cord lengths in treatment group. According to these results, it was concluded that rat prenatal development during gestation is sensitive to E 341 exposure.

5. Alternative methods

To support and explore in more depth of results obtained from fundamental studies; immunotoxicity, allergenicity, neurotoxicity, genotoxicity, human volunteer studies, predictive mechanistic and special studies may also be helpful. Another recent method for developmental toxicity testing is *in vitro* studies, which are not based on the use of animals. *In vitro* studies provide sufficient data by using cellular and subcellular systems to predict the mechanisms involved in early stage of development. As a result of ethical issues about animal use, *in vitro* testing mechanisms will come into prominence in upcoming years. Over the past few decades, one of the most notable revolutionized technologies is the application of nanotechnology in food sector. Therefore, in this part, concerns and health implications on the application of nanotechnology in food will be discussed. The possible effects on reproductive system due to consumption of foods involving nanoparticles are evaluated.

5.1 Approaches of *in vitro* studies for assessing food additives

Commonly, the toxicological risk to humans from exposure to an individual chemical is evaluated using animal data from long-term or acute *in vivo* toxicity studies. Over the last 20 years, there has been a clear tendency for increased use of *in vitro* methods in toxicology as supplements to animal tests. Although such studies have previously been considered during the hazard characterization of many compounds they generally have had no direct influence on the calculation of ADI values. *In vitro* studies may be a useful perspective in bridging the gap between a test species and the human situation, thereby providing a more scientific

basis for the use of a specific data. *In vitro* testing systems are increasingly becoming an essential tools as part of integrated toxicology testing strategy and scientific progress in the fields of cellular and molecular biology. These studies are used in a wide range of processes including the determination of ADI, for reporting suggestions in safety approaches, metabolism pathways of specific compounds. According to the limited number of toxicological studies, *in vitro* applications are useful for the prospective toxicological classification and characterization of food additives. *In vitro* studies have the potential for calculation and detection of both inter-species and inter-individual variability in toxicokinetics and toxicodynamics of food additives (Walton, 1999).

5.2 Nanoparticle based applications of food additives in food industry

Public interest in the subject of nanotechnology in the food industry is growing. It opens up many new possibilities which are of interest to the food industry. Nanofood market potential was predicted as 20.4 billion US dollars for the year 2010 (IFST, 2006). More than 200 companies worldwide are already believed to be involved in this sector, especially in the USA, Japan and China. Several companies are investigating encapsulation technology for the delivery of active ingredients in food products (e.g. flavouring agents, vitamins, fatty acids). Nanotechnology in the food industry is a sensitive subject. Manufacturers fear a blanket rejection of products containing nanomaterials, similar to what has happened with genetic engineering. Food products naturally contain nano-sized ingredients. These are different from synthetically manufactured nanomaterials. Food proteins can be mentioned as examples of natural nanostructures whose size can vary between several hundred nanometers such as milk proteins and casein.

With encapsulation (Figure 4), in which active agents and substances can be encapsulated in nanostructured materials, the purpose is to enhance solubility (e.g. of colouring agents), facilitate controlled release (e.g. only in certain parts of the alimentary tract, for instance in order to prevent the bad taste of an ingredient which in itself is beneficial such as omega-3 fatty acids in fish oils), improve bioavailability, i.e. the amount of a nutritional ingredient which is actually absorbed by the body (e.g. vitamins, minerals), protect micronutrients and bioactive compounds during manufacture, storage and retail. The most important nanostructured materials are currently nano-capsules (micelles, liposomes) and nanoemulsions (Greßler et al., 2010). Nanocarrier systems can be used to mask the unpleasant tastes and flavours of ingredients and additives such as fish oils, to protect the encapsulated ingredients from degradation during processing and storage, as well as to improve dispersion of water-insoluble food ingredients. However, current studies on the application of nanoencapsulation mainly address its potential for target delivery of active ingredients of functional food and nutraceuticals (Hsieh and Ofori, 2007).

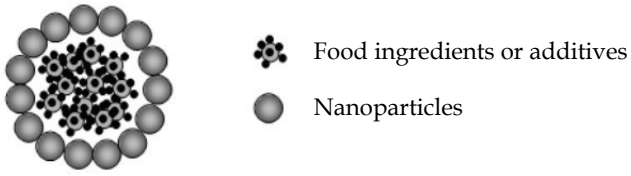

Fig. 4. Schematic diagram of nanoencapsulation (Centre for Food Safety, Hong Kong, 2010)

Experimental data demonstrated that the distribution of nanoparticles after oral administration is dependent upon particle size. Smaller-sized nanoparticles have a more widespread tissue distribution in organs like kidney, liver, lungs and brain while the bigger particles (28 nm and 58 nm) remain almost solely inside the gastrointestinal tract (The Government of the Hong Kong Centre for Food Safety Food and Environmental Hygiene Department, 2010). Studies have been performed on the ability of nanoparticles to penetrate the placental barrier. There is also information that certain nanomaterial (C60 fullerene) can pass across the placenta. However, due to the inconsistent results of some *in vitro* and animal studies, no general conclusion on the penetration power of nanoparticles across the placental barrier can be made. There is no information on whether nanomaterials are transferred into milk (Tsuchiya et al., 1996; EFSA, 2010j). Because nanoparticle food industry is a recently developing field, the reproductive and developmental toxicity studies are rare. In one of the few studies conducted by Durnev et al., silicon crystal 2-5 nm nanoparticles in the form of 1-5 μ granules in water suspension were injected intraperitoneally in a single dose to male F_1(CBA×C57Bl/6) mice or to outbred albino rats on days 1, 7, and 14 of gestation (Durnev et al., 2010). It was reported that injection of 50 mg/kg dose of silicon crystal nanoparticles reduced body weight gain in pregnant rats and newborn rats at different stages of the experiment, but had no effect on other parameters of physical development of rat progeny and caused no teratogenic effects. In a recent study it was also reported that nanosized silicon materials are generally nontoxic and biodegradable (Fucikova et al., 2011). Nowadays, in comparison to other materials currently used in medicine applications, nanosized Si based materials are the only one showing complete biodegrability and nontoxicity without any significant inflammatory reactions.

6. Discussion

Food additives are natural or manufactured substances, which are added to foods for restoring colors lost during processing, providing sweetness, preventing deterioration during storage and guarding with preservatives against food poisoning. A food additive is defined as a substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods (Food Safety Authority of Ireland, 2011).

All food additives undergo a safety assessment that may be used in the manufacture or preparation of foodstuffs in the European Union. Up to 2002, this safety assessment was carried out by the EU SCF but since 2003, the responsibilities of the SCF have been taken by EFSA.

The safety evaluation of a food additive involves examination of the chemical structure and characteristics, including its specifications, its impurities and potential breakdown products. Toxicological data is essential to identify and characterize the possible health hazards of an additive and to allow extrapolation of the findings in animals and other test systems to humans. In these studies, the additive is administered to laboratory animals.

Such tests are designed to give information on any possible effects from short-term or long-term exposure to the additive, including whether it may have any potential to cause cancer (carcinogenicity), or to affect male or female reproduction or the development of the embryo or the fetus if consumed by a pregnant woman (reproductive or developmental toxicity). Other effects include the genotoxicity potential of the compound; which is the ability to cause the development of cancer or adverse effects in future generations.

This chapter is an overview of the developmental and reproductive studies conducted by the administration of commonly used food additives. Recent studies show that there is a lot of concern about the safety of food additives in toxicological manner. With the increasing amount of progression in food additive industry, people who need more information about popular additives admit to being confused with the possible health effects. There are both safety evaluations and regulations of newly released food additives. As a matter of fact, it is inevitable to use food additives with the increasing demand of high-quality food. Excessive parental exposure of food additive throughout lifespans makes reproductive and developmental endpoints remarkable for investigating. This chapter strived to focus on the fine prints of toxicological assessments, especially the possible reproductive and developmental effects of food additives and to give perspectives for new approaches in the evaluations of food additives in concordance with the improvement of food industry.

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