

T.C

YEDİTEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

PHARMACEUTICAL TOXICOLOGY DOCTORAL PROGRAM

**TOXICOLOGICAL EXAMINATION OF
CHILDREN'S FINGER PAINTS**

DOCTORAL THESIS

NESLIHAN ŞAHİN, B.PHARM.

İSTANBUL-2023

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

09.02.2023

Neslihan ŞAHİN

BEYAN

Bu tezin kendi çalışmam olduğunu, planlanmasından yazımına kadar hiçbir aşamasında etik dışı davranışımın olmadığını, tezdeki bütün bilgileri akademik ve etik kurallar içinde elde ettiğimi, tez çalışmasıyla elde edilmeyen bütün bilgi ve yorumlara kaynak gösterdiğimi ve bu kaynakları kaynaklar listesine aldığımı, tez çalışması ve yazımı sırasında patent ve telif haklarını ihlal edici bir davranışımın olmadığını beyan ederim.

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LIST OF SYMBOLS AND ABBREVIATIONS

HET-CAM	Hen's Egg Test on Chorioallantoic Membrane
F.P	Finger Paints
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
H.M	Heavy Metal
Hg	Mercury
Pb	Lead
Ni	Nickel
As	Arsenic
Cd	Cadmium
Sb	Antimony
B.C	Before Christ
BCOP	Bovine Corneal Opacity and Permeability Assay
F-AAS	Flame-atomic absorption spectrometry
EDXRF	Energy-dispersive X-ray Fluorescence
PCA	Principal Component Analysis
PCR	Principal Component Regression Method
PLS	Partial Least Squares
TITCK	Turkish Medicines and Medical Devices Agency
EFSA	European Food Safety Authority and Health Organization
WHO	World Health Organization
FDA	U.S. Food and Drug Administration
DEP	Diethyl Phthalate
DPP	Di-N-Propyl Phthalate
DBP	Dibutyl Phthalate
BBP	Benzyl Butyl Phthalate
DEHP	Diethyl Hexyl Phthalate
DCHP	Di-Cyclohexyl Phthalate
DOP	Di-Octyl Phthalate
MPB	Methylparaben
EPB	Ethylparaben
PPB	Propylparaben
BPB	BPB
EU	European Union
BIS	Bureau of Indian Standards
EPA	United States Environmental Protection
NCCLS	The National Committee for Clinical Laboratory Standards
DNA	Deoxyribonucleic Acid
ICP-MS	Inductively coupled plasma mass spectrometry
APC	Aerobic Plate Count
SED	Systemic Exposure Dose
MoS	Margin of Safety
PoD	Point Of Departure
NO(A)EL	No Observed (Adverse) Effect Level
LO(A)EL	Lowest Observed (Adverse) Effect Level
BMD	BenchMark Dose
SCCS	Scientific Committee on Consumer Safety
DA	Dermal Absorption
SSA	Skn Surface Area
SD	Safety Directive

ppm	parts per million
kg	kilogram
L	Liter
mg	miligram



ABSTRACT

Şahin, N. (2023). Toxicological Examination of Children's Finger Paints. Yeditepe University, Institute of Health Science, Department of Toxicology PhD thesis, İstanbul.

This thesis study was carried out to toxicologically examine children's finger paints and to evaluate the risk factors for health. 12 different brands of yellow and red finger paints were purchased and analyzed by HET-CAM method to perform skin irritation test. As a result of the research, it was determined that none of the paints had the potential to cause irritation. Microbiological analysis of the samples inoculated on days 2, 7, 14 and 28 was performed. As a result of incubation of microorganisms for *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*, logarithmic reduction of $> \log 2$ on 2nd day and logarithmic reduction of $> \log 3$ on 7th day were observed. No increase was observed till 28th day. For *Candida albicans* and *Aspergillus brasiliensis*, logarithmic reduction of $> \log 2$ on 14th day was observed. In addition, finger paints are obliged not to contain inherently hazardous substances such as heavy metals, as specified in Chapter 7 of the Toy Safety Standard. Health Sciences University, Gulhane Faculty of Pharmacy analyzed the samples by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for their heavy metal content. As a result of the analysis of 12 different brands of yellow and red finger paints, it was found that almost all brands contained Hg, Pb, Ni, As, Cd, Sb heavy metals. This situation reflects the situation in Turkey and fulfills the purpose of the study. Finger paints, which are not recognized as cosmetic products in the Republic of Turkey and all over the world, are not considered to be safe, especially for children, due to their skin contact. As a result, it is thought that finger paints should be subjected to a much more comprehensive examination and the Republic of Turkey should establish a more comprehensive standard for finger paints or the scope of the existing standard should be expanded. This research is expected to raise awareness by demonstrating the importance of the issue.

Key words: Paint, Finger Paint, Heavy Metal, Health, Safety

ABSTRACT (TURKISH)

Şahin, N. (2023). Çocuk Parmak Boyalarının Toksikolojik İncelenmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Toksikoloji ABD., Doktora Tezi. İstanbul.

Bu tez çalışması, çocuk parmak boyalarının toksikolojik açıdan incelenmesi ve sağlık açısından risk faktörlerinin değerlendirilmesi amacıyla gerçekleştirilmiştir. 12 farklı marka sarı ve kırmızı parmak boyası satın alınmış ve HET-CAM yöntemi ile analiz edilerek cilt tahriş testi gerçekleştirilmiştir. Araştırma sonucunda analiz edilen boyaların tahrişe neden olma potansiyeline sahip olmadığı tespit edildi. 2, 7, 14 ve 28. günlerde inoküle edilen örneklerin mikrobiyolojik analizleri yapıldı. Mikroorganizmaların *Pseudomonas aeruginosa*, *Escherichia coli* ve *Staphylococcus aureus* için inkübasyonu sonucunda 2. günde logaritmik olarak $>\log 2$ ve 7. günde logaritmik olarak $>\log 3$ azalma gözlenmiştir. 28'inci güne kadar herhangi bir artış gözlenmemiştir. *Candida albicans* ve *Aspergillus brasiliensis* için 14. günde $>\log 2$ logaritmik azalma gözlenmiştir. Ayrıca parmak boyaları, Oyuncak Güvenliği Standardı Bölüm 7'de belirtildiği üzere ağır metaller gibi doğası gereği tehlikeli maddeler içermemekle yükümlüdür. Sağlık Bilimleri Üniversitesi Gülhane Eczacılık Fakültesi, örnekleri ağır metal içerikleri açısından İndüktif Eşleşmiş Plazma Kütle Spektrometresi (ICP-MS) ile analiz etmiştir. Sarı ve kırmızı renkli 12 farklı marka parmak boyasının analizi sonucunda neredeyse tüm markaların Hg, Pb, Ni, As, Cd, Sb ağır metalleri içerdiği tespit edildi. Bu durum Türkiye'deki durumu yansıtmakta ve çalışmanın amacını yerine getirmektedir. Türkiye Cumhuriyeti'nde ve tüm dünyada kozmetik ürün olarak kabul edilmeyen bu ürünler cilt ile teması nedeni ile özellikle çocuklar için güvenli olduğu düşünülmemektedir. Sonuç olarak, parmak boyalarının çok daha kapsamlı bir incelemeye tabi tutulması ve Türkiye Cumhuriyeti'nin parmak boyaları için daha kapsamlı bir standart oluşturulması veya mevcut standardın kapsamının genişletilmesi gerektiği düşünülmektedir. Bu araştırmanın konunun önemini ortaya koyarak farkındalık yaratması beklenmektedir.

Anahtar Kelimeler: Boya, Parmak Boyası, Ağır Metal, Sağlık, Güvenlik

1. INTRODUCTION AND PURPOSE

The subject of the thesis has been changed from ‘The toxicological examination of children's face and finger paints and temporary tattoos as well as make-up products designed for applying to children's toys’ to ‘The toxicological examination of children's finger paints. The reason for updating the subject of the thesis is because the face paints or the children’s make-up products are already accepted as a cosmetic product because of the application area and the intended usage. But when the issue is children’s finger paints, they don’t need to comply with the EU cosmetic legislation accordingly with the Turkish cosmetic legislation since the intended usage is to draw through fingers but any other surface then the human skin. These paints got accepted as children’s toys therefore they only need to comply with the EU directive for toy safety. But since both the EU-cosmetic legislation [1] and the EU directive for toy safety [2] are regulations aimed at ensuring the safety of consumer products, it may be confusing when the two of the boundary products in this scope are applicable and the definition between toys and cosmetics is not clear. This is since children are expected to use finger paints not only to draw or paint somewhere but also on their own faces and as such should also be considered as cosmetics [3].

The aim in this project is to examine the composition of finger paint products offered on shelves for children. This would be a study looking to the situation in Turkey and to evaluate the safety of them through the toxicological examination. So this study will include, a literature study on what is known and particular not known, the development of methodology to analyse the composition of these finger paint products (for example for the content of heavy metals), and then followed by analysis via *in vitro* methodology which will evaluate the safety of these products.

If the thesis ends successfully, a broader perspective and data will be collected regarding to Children’s finger paints and which will help us to have information about the safety, reliability, and compliance of these products. It is very necessary to have these data since the Turkish market and the Turkish web sites has many different products in this scope under the different brands through import or through local manufacturers. Because of this reason, it is thought that this study can contribute to the fulfilment of the legislation and requirements of the products in this scope in our country or to regulate them in case of need.

2. LITERATURE REVIEW

2.1 Color

Color emerges as a result of the material changing the light or some kind of process that the light undergoes. In terms of the psychological system, color is expressed by the effects of different light effects on the human brain. Color is part of the perceived object and is a sensation that occurs in the human brain. The perception of the object by the human brain in the act of seeing is considered as a psychological phenomenon. Therefore, it is known that different colors cause severe emotional effects on people [4].

Color is formed by light structure and propagation. Therefore, the primary factor affecting color is light. According to Smith, “value or luminance refers to how light or dark a color is. Differences in value can also be achieved by mixing the color with different proportions of white or black [5]. Other than the primary and secondary colors, other colors have received new names (very dark blue; dark blue, light purple; magenta, etc.) rather than light and dark. In addition, colors can also be named by analogy: babymouth, lemon yellow, coral, aqua green, ivory.

Of the six basic colors yellow, red and blue; primary colors, as they are colors found in nature on their own and cannot be obtained from a mixture; green, orange and purple are called intermediate colors because they consist of a mixture of primary colors. In the universal paint industry, colors are named according to their tones. The most commonly used colors; cadmium lemon yellow, medium cadmium yellow, yellow, burnt cyena, burnt ombra, light fire red, dark garans red, permanent green, emerald green, dark teal, dark cobalt blue, Prussian, titan white, and ivory black [6]. Giving color is one of the purposes of painting. The choice of color depends on the person's current feelings and the effect of the color on him [7].

Colors affect people psychologically. As a result of this effect, it is classified into two groups as cold and hot. Warm colors of red, yellow and orange; Green, blue and purple are cold colors. Despite the warming effect of warm colors, it has been determined that cold colors have a more spacious effect. For example, red is often described as a warm or exciting color, while blue is cold and distant [8]. The colors that create vitality

and warmth in human psychology are warm colors. Usually the colors in this classification are orange, red and yellow. These colors reflect the endless energy of the sun and the warmth of the lava coming out of the volcano. These colors, which instill vitality, appetite, movement and joy, evoke a feeling of intimacy in the eye as they have less refractive angles. Colors that evoke coolness and stillness in human psychology are cold colors. These colors are so named because they are dark and dull. The colors in this group are green, purple and blue. Inspiring comfort and tranquility, these colors make people rest and make the surface appear farther than they really are. [9].

Colors are also decisive in people's self-expression. Emotions determine the color preferences in life. Many choices, from the color of the clothes chosen to the color of the walls of the house, are a reflection of people's tastes. Colors can have different effects on people depending on both their lifestyle and personal tastes. It is also a key design element because of its ability to evoke an emotional response in the viewer. [8].

Color is the senses formed in the eye by the reflection of light hitting objects as rays [7]. Everything needed for decoration, furniture and architecture with elements of painting such as colour, proportion and unsymmetrical balance [10]. In terms of modern science, color is defined as the effect of light consisting of electromagnetic waves on the eye in relation to its own structure and its propagation on objects [11]. Although the definitions for the concept of color differ, color is generally handled in three ways in the literature [9]:

- Psychological Color

This is a sensation that usually occurs in the human brain. However, it is not possible to analyze and prove this situation. Accordingly, even if a similar type of light and stimulus is sent to two different people with normal vision and these people express what they see as the same color, the perception formed in the brains of these people is not exactly the same.

- Physiological Color

It is the effect of different types of light on the nerves of the eye through the retina of the eye.

- Physical Color

It is the color that is measured and determined precisely by numbers. By measuring the wavelength of the light reflected, it is easily understood which color this color is. The exact relationship between physical color and other color types is difficult to prove. Accordingly, two physically different lights can create a perception in the form of a psychologically similar color. For this reason, it is not always possible to make an inference about the actual color with the psychological color.

The colors formed in the objects are formed as a result of refracting or absorbing the rays reflected on the objects. The color of an object emerges by absorbing the wavelengths of the rays reflected on that object. According to this, the main reason why an object that is seen as yellow from the outside is perceived in this way is that the light on the object absorbs the blue wavelengths inside [12]

One of the first researches on color was made by Newton, an English physicist, in 1666. Newton let sunlight leak in a thin beam through a small hole into a dark room, passing this light through a triangular glass prism. Colors known as rainbow colors have emerged. These colors reflected on the white screen are called Solar Spectrum. the colors in the reflection are in the direction of the refraction angles; listed as orange, red, green, yellow, purple, blue [13].

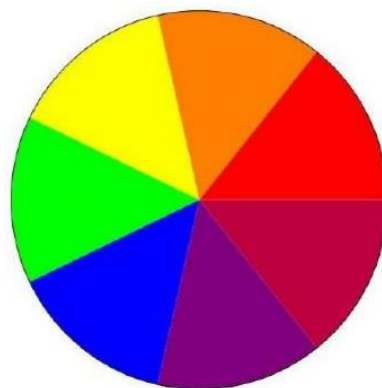


Figure 2.1. Newton's Solar Spectral System [14]

German artist and poet Goethe (1749-1832) published a work on the theory of colors in 1810 on the function, psychology and nature of colors. In this work, Goethe criticized Newton and stated that there are no seven primary colors; reported that there are two primary colors. These colors are yellow and blue. other colors are degrees of yellow and blue. [14].

Mariotte, on the other hand, argued that blue, yellow and red are the primary colors, while the other colors are shades of blue, yellow and red. These colors combine to form green, purple and orange. In the work published in 1766, he created a color wheel in which he gave color pigments [15].

- Primary Colors

These are the colors that cannot be obtained with any color mixture and that make up the light. These are red, blue and yellow.

- Mid-colors

New colors formed by mixing at least two primary colors are defined as intermediate colors. Accordingly, a mixture of blue and yellow creates green, a mixture of blue and red creates purple and a mixture of red and yellow, and orange is formed. These colors are intermediate colors [16].

It has been demonstrated by many academic studies that colors have different effects on human psychology. Therefore, different colors can have different effects on people, and colors can have similar effects on people. When evaluated in terms of human physiology, 80% of what people perceive with their senses is visual, and all of the visuals they perceive in daylight are color. A human eye perceives 7 million colors. Some of the colors in such a wide spectrum can cause discomfort for the eyes. In addition, some colors can have a calming and relaxing effect. For this reason, the correct use of colors reduces visual fatigue and increases productivity [14].

2.2 Paint

In general, paints are defined as a liquid or powder product containing pigments and forming a protective, decorative or technical covering layer when applied to suitable surfaces in thin coats. [17]. It can be characterized as a material that can cover surfaces by spreading in liquid or dry form, and can change the color and sometimes texture qualities of the surfaces by forming a thin layer. While it is used in its most natural form by applying it directly to the surface, depending on the characteristics of the paint used, some transfer materials are often needed. A wide variety of painting tools are available, such as sponges, spatulas, knives, scrapers, brushes and spray guns. Brushes are the most used materials for applying fluid paints such as watercolor, gouache, acrylic paint and oil paint [5].

Paint has a unique chemistry as content. They are pigments formed by small molecule particles that make up the paint and give color to the material [6]. The size and shape of the pigment particles; It affects the appearance of the paint in terms of light intensity, material density, opacity, fluidity, compatibility with the brush. The size of the particle also affects the balance in the picture; As the particle gets smaller, it becomes more difficult to give density and shape to the surface. Therefore, the differences in the structural properties of the material determine the types of paint [5].

People may have made the paintings seen on cave walls to communicate with each other, a religious ritual or to express their simplest thoughts and feelings. For whatever reason, they felt the need to color the pictures they drew. Those who accompanied the painting with a ceremonial activity in the caves may have carried out the same activity during paint production. The main reason for people who develop the material they use as paint is the desire to prolong the life of the created paintings. This desire has led to the discovery of a substance that can stabilize color molecules. However, it is impossible to determine the composition of this substance. However, it is known that oil, resin and herbal extracts are used. [18]. The paintings in the Chauvet cave were discovered in 1994, and the materials used as dyes were examined by radiocarbon method. It is observed that an advanced and confident graphic language is used in the illustrations [19]

The first materials used for painting in history were soil, stone, plants, and even blood. Over time, various mixtures were obtained and the paint was made more effective. Thus, they managed to reach the present day in the caves where they were painted. Red

ocher obtained from iron ore was used in cave paintings in 15000 BC. The first natural dyes were used in China to color touch in 3000 BC. After India, which used indigo (indigo) in addition to madder in 2500 BC, yellow, red and green linen mummy cover was obtained in Egypt in 2000 BC [20].

The use of paint is not limited to this area. The reason why people need paint was their visual pleasure, but this need has changed over time. Houses were whitewashed for hygiene purposes, wooden items were painted in order not to get insect and metal items were not rusted. Thus, people turned to different experiments in the method of obtaining the dye and the function of the dye obtained. This has brought diversity to paints. The methods developed in paint production and the developing industry have brought groundbreaking innovations and new products, as in every field. In the past, artists used muscle strength and plates while making their own paints, and the images made with inconsistent colors were subjected to spreading and softening processes [5]. After the developments, it has become very easy to access any paint type and desired color today. In general, regardless of the type of paint, seven different materials are used in the paint content: pigments, oils, resins, gums, starches and gums, waxes, solvents and various binders.

●Pigments

Pigments, due to their coloring properties, are the most important elements that make up the paint in the form of tiny particles. The nature or artificial nature of these particles, which are attached to each other, constitutes the quality of the paint. Pigments are substances that are insoluble in any solution and their grain sizes are generally below 1 micron. They are water-insoluble inorganic or organic substances that gain importance especially because of their colors and are therefore mostly used in surface coating processes. Although paints were obtained from natural pigments due to limited possibilities in the past, today it is common to produce them as a waste of art. For dyes produced from various animals, plants and minerals throughout history, examples of obtaining brown sepia from cuttlefish and ultramarine blue from navy blue can be given [21]. Dye technology offers a wide range of colors to users and, except in very special cases, natural dyes are not needed thanks to synthetic dyes. however, it is possible to categorize pigments as artificial and natural. In terms of the minerals it contains, the dyes

obtained from plants, animals and elements in nature are natural; those produced in the laboratory environment with various chemical mixtures are artificial dyes [22].

- Oils

Oils help bring the paint to the proper consistency for better application to the painting. Another reason why oil is used by artists in addition to its thickening properties in paint is the brightness it adds to the painting. Oil paint, which was previously obtained by mixing the existing oil with powder paint, is sold in boxes where the necessary mixture is ready. The oil in it provides fluidity with the consistency it gives to the paint, and also prevents the paint from deteriorating by being constantly wet. The paints in the tube also harden over time with the effect of oxygen in the air. Although such dried and hardened paints can be crushed again with oil and turpentine to make a paste, it would be better not to use them anymore [23].

- Connectors

Preservatives, plasticizers, chemical composition surfactants and thickeners; They are binding agents that allow the paint to adhere to the surface on which it is applied in a healthier way. Preservatives are mostly used in aqueous and acrylic paint applications, to slow the growth of bacteria in the paint and to delay the formation of mold on the surface [5]. Plasticizers are products that are also applied to the canvas surface, which means plasticizer. They help the paint to adhere better to the surface. They are used extensively in the production of aqueous, gouache and acrylic paints. Surfactants (active surface agents), which are chemical compounds, are used to reduce the tension between two surfaces (such as the tension between the pigment and the binder or the liquid paint and the paint supporting material). Surfactants can cause some reactions such as moistening, purification and foaming [24]. Thickeners are used to add consistency to the paint, especially in acrylic and oil paints. Its feature is that although it increases the consistency, it does not cause any delay in drying. The binder is the main pillar of the paint, and the paint is named according to the type of resin used. Some types of resins used in paints:

Alkyd resins: It is an ester resin composed of vegetable and animal oil, acids and alcohols with two carboxyl groups. Their composition can be very different. The most

produced alkyl resin is made from phthalic anhydride and glycerine. They are widely used in the production of organic paints, lacquers and lacquers (high-gloss furniture polish) [24].

Cellulosic resins: Carboxymethyl cellulose, cellulose acetate, ethyl cellulose, nitrocellulose are obtained by acetylation and nitration of almost all of the hydroxyl groups of insoluble cellulose. Its usage area is mainly furniture and automobile paints.

Acrylic resins: Depending on the type of acrylic monomer used, the type of compound and the conditions of the process, hard and transparent, soft and flexible solids or sticky, viscous liquid products can be obtained. It is generally used in auto topcoats, high quality durable houseware paints.

Epoxy resins: Epoxy resins are prepolymers containing two or more epoxy groups. Epoxy resins are cured and converted into thermoset products. Curing is the process of forming three-dimensional thermoset structures by cross-linking epoxy groups with various agents such as hardeners and catalysts. Epoxies can adhere strongly to many surfaces such as plastic, metal, glass, wood.

Polyurethane resins: These two-component resins, consisting of diol and diisocyanate, are very resistant to UV rays, as well as showing mechanical and chemical resistance. They are used in many areas such as elastomer, fiber, coating, soft and hard foam where these properties are desired [25].

Polyester resins: They are almost colorless liquids with a thick consistency and hardly flowing. It is a two-component resin, and organic peroxides, which act as catalysts, must be added to harden. Polyester resins are sensitive to cold and humidity.

Vinyl resins: Obtained by radical polymerization of various vinyl monomers. Vinyl resins have durability, corrosion resistance, thermal efficiency, color retention, ease of maintenance, safety and recycling properties.

Silicone resins: Its most important feature is its resistance to high temperatures. They are used in chimneys, steel mills, furnaces, as a waterproof covering, as an electrical insulator, in motors and similar equipment.

●Resins

Except for the resins listed above; drying oils, urethane oils, polyamines, hydrocarbons, phenolic binders, chlorinated rubber resins are also available. Resin is a substance that acts as a retainer between the particles that make up the paint. It is used in the production of natural adhesives and in the paint industry [11]. Resins, like pigments, are classified as natural and artificial (synthetic) depending on their raw materials. Natural resins are obtained from living things, dead trees or resin fossils. Although they are darker than oil and less clear, they are useful for polishing finished paintings. Resins are solid materials that do not have crystalline properties such as hard, glass-like structures. Artificial resins, on the other hand, are created in a laboratory environment by chemical processes. Oil paint is the type of paint that resin is used most in its composition. While insoluble in water, they can be partially and/or completely dissolved in contact with organic solvents. At this stage, they should not be mixed with water-soluble gums, which are also obtained from plants [5].

●Gums, Cokes and Gums

Gums, starches and gums also act as important mediums for dyes. These ingredients can also be obtained from both plants and animals. Unlike resin, it is water soluble. It is generally discarded as a binder in adhesives and watercolors [26]

●Waxes (Waxes)

Candles, which are produced for lighting and then an accessory function in daily life, consist of intense fatty acid and fatty alcohol esters containing free acids, alcohol and hydrocarbons. As they are heavy, permanent and durable, they survive as long as they are protected from heat and mechanical damage [5]. Although candles are also used in oil paint, watercolor and dry paint, they are mostly used as pastel paint raw materials. Candle is the basic material of encaustic painting technique [6].

●Solvents

Solvents are solvents. They are liquids that allow a solid dense substance to dissolve and take a finer form. Being able to be dissolved and thinned depends on the electrical properties and molecular density of the substance. Small-molecule structures dissolve faster than large-molecule structures [5]. All liquid paint types have solvents. Commonly used solvents are water, turpentine, thinner and naphtha. The vast majority of solvents are hazardous substances. That's why they have a warning sign on them that they are flammable [27].

2.2.1.Types of Paints Children Are Interested In

It is possible to classify different types of paints according to the place of use and the way of use. Paints vary according to their rust-proofing properties, binder type, drying condition, thinning conditions, application methods, effects on humans, areas of use and top coat color. According to their usage areas, paints are also divided into types such as construction paints, food paints, automotive paints, wood paints, textile paints, art paints.

When the paints that children are related to are evaluated over the art paints, the paint types are listed in the continuation of the content (Seymour, 2003, s. 11);

Table 2.1. Paint Types

Dry Paint [9, 28, 29]	Crayons [29, 30, 31, 6]	Watercolor [30, 28, 32, 6]
Gouache [33, 5]	Acrylic Paint [28]	Oil Painting [5, 28, 30]
Powder Coating [34]	Marker pen [19]	Glass PAint [11]
Marbling Paint [35]	Fabric Dye [36]	Finger Paint [37]

However, when it comes to children's age group, especially finger paints in artistic paint have a different production and standard. In the continuation of the content, firstly, information about finger paints is shared, and then international standards are mentioned.

2.3 Finger Paint

Finger paint is the type where the paint is applied by feeling the most. Any liquid paint can be applied with fingers without a brush and it is possible to see fingerprints in the picture. Finger paint is commonly sold in 25 ml containers in the market. There are sets of 3, 6 and 12. Top quality brands; It is known as Faber Castel, Nova Color, Fatih,

Südor and Lets. In its simplest form, finger paint can be made from a mixture of glue and powder paint at home, and there are different recipes for this type of paint.

Finger paint is applied more easily on slippery surfaces. For this, one side is slippery paper or oilcloth, etc. surfaces can be used. If you are going to work on normal paper, choosing a little thick paper ensures easy application of the paint [9].

The production purpose of the paint is to develop psychomotor skills, to provide freedom without the effort to fill the predetermined limits. It is mostly used by preschool children. Finger paint is formulated to be applied by hand. Finger paint, produced for use by artists of all ages, especially children in early childhood; It is applied on non-absorbent, smooth, glossy white paper. For this reason, it is produced odourless and harmless to health [38].

Painting can sometimes be applied as a therapy method with various techniques. Finger paint is among the paints used for this purpose. The importance of this technique in terms of healthy children takes place as a useful method in the field of education. It is known by most people as therapeutic treatment (treatment) among adults in Western countries such as England, France, and the United States [39].

Children have difficulty in holding and using stick paints with a thin diameter in painting studies, since their psychomotor skills are not yet sufficiently developed at a young age. That's why finger painting is more fun for them, or thick crayons are more suitable for younger pre-schoolers to paint because they are easier to grasp. In the school starting period, pen-like dry and felt paints are started to be used more by children with the guidance of their classroom teachers. The goal here is to engage in activities that help develop proper pencil holding skills. It is both entertaining and instructive for children to paint with pencil-like paints so that they do not get bored during this period when they learn to read and write, while improving their psychomotor skills [40].

Originally introduced as a form of play and a means of artistic expression, finger painting is now a recognized psychotherapeutic technique. It enables children to overcome their shyness and express their imagination. Painting moments of anger serves as a catharsis tool for conflicts. In addition to the therapeutic possibilities as a game

technique, finger painting has many special advantages [41]. Finger painting is a socially sanctioned form of playing with mud. Finger painting requires little technical skill and allows the use of larger muscle groups [40]. In neurotic cases, personality analysis was made based on picture movements; finger painting has been tried to be interpreted through a universal form and color symbolism. It has even been suggested that certain colors are associated with certain clinical entities. However, it was also stated that it was objectionable to make these comments arbitrarily [38].

Fine motor development is about the child's ability to observe something, to perform movements involving only certain body parts with the help of small muscles and which require careful coordination of the eyes, hands, and fingers. Fine motor skills are the fine coordination of small muscles that play an important role. Fine motor skills are affected by the maturity of motor function and good neuromuscular coordination. Thus, visual functioning and nonverbal intellectual abilities depend on their learning and the stimulation they receive. The environment (parents) has a huge impact on fine motor intelligence. These factors can increase or decrease the intelligence level of children, especially in the first days of life [42]. Children with good fine motor intelligence can perform activities such as wearing clothes, drawing, writing, and cutting more easily. Therefore, otherwise, these activities become more difficult [40]. It is possible with interesting activities to develop fine motor skills and enable children to learn with enthusiasm while overcoming boredom in children. Activities that can be done at this point are solving puzzles, cutting and pasting pictures, drawing, mixing colors and finger painting [42].

According to Pamadi (2008), finger painting is a direct painting technique without using various tools. Children change brushes directly with their fingers. In finger painting activities, children express their imagination freely. According to Montolalu (2009), finger painting can improve expression, creativity, or imagination through hand movements, can exercise hand/finger muscles, muscle and eye coordination, and increase the skills of combining colors [43].

2.3.1.Finger Paint and Safety

Despite the electronic age and the fact that auditory sensory toys are more popular, finger painting is often used when emotional games are aimed at brain and motor development. As a matter of fact, the painting made by the early age groups with their finger's dates to 13 thousand years ago. Finger paints, which enable the understanding of colors, encourage imagination and creativity, develop motor skills, and support emotional integration, are among the toys that will always be important in child development. For this reason, finger paints are protected by numerous test standards and legislation for their production, sale and use. Known and popular brands are known to benefit from EN 71 Toy Safety Standards (Part 7: Finger paints - Requirements and test methods; EN 71-7:2014+A3:2020) developed by the European Union. Within the scope of safety legislation, finger paint is categorized as aqueous, semi-solid or liquid-colored mixtures; It is specially designed for children under the age of 3 to perform activities with their fingers on suitable surfaces. Except for the EU Toy Safety Directive (2009/48/EC), which is still valid in this context [44];

- Regulation (EC) No 1907/2006 (EU REACH)
- Directive 2001/95/EC General Product Safety Directive (GPSD)
- Directive 2005/29/EC Unfair Commercial Practices or POP Recast Regulation (EU) 2019/1021 on Persistent Organic Pollutants, which relates to restricted chemicals.

Regulations on packaging and labelling.

- Directive 94/62/EC on Packaging and Packaging Waste
- CLP Regulation (EC) No 1272/2008
- Commission Decision 97/129/EC

Finger paints are subject to the Substances of Very High Concern (SVHC) obligations under Regulation (EC) No 1907/2006 (EU REACH). Some of the restricted chemicals are;

- Asbestos fibres- EU REACH Entry 6 Annex XVII
- Benzene (EU REACH Entry 5- Annex XVII)
- Cadmium and its compounds (EU REACH Entry 23 Annex XVII),
- Dimethylfumarate (EU REACH Entry 61 Annex XVII)

- Organotin compounds (EU REACH Entry 20 Annex XVII),
- Phthalates (EU REACH Entry 51 XVII and 52 Annex XVII),
- Polycyclic Aromatic Hydrocarbons (PAH) (EU REACH Entry 50 Annex XVII)

In addition, prohibited chemicals (within the scope of EU Toy Safety Directive) except heavy metals in finger paints, which are important for microbial contamination and protective effectiveness values and Toxicological Risk Assessment (TRA). [45];

- Aniline
- BIT (1,2-benzisothiazol-3(2H)-one)
- Bisphenol A
- CMIT (5-Chloro-2-methyl-isothiazolin-3(2H)-one),
- CMIT/MIT Reaction mass of: 5-chloro-2- methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1)
- Formaldehyde
- MIT (2-methylisothiazolin-3(2H)-one)
- Phenol
- TCEP (tris (2-carboxyethyl) phosphine)
- TCPP (tris (1-chloro-2-propyl) phosphate)
- TDCP (tris (1,3-dichloro-2-propyl))

In the third part of the standard (EN 71-3), heavy metals that can be taken into the body through saliva and sweat are included. These heavy metals, which are attributed as toxic in terms of child and animal health, are given in Table 1 because they are semi-replaced with vital minerals. [45].

Table 2.2. Finger Paint Heavy Metal Limit Values

No	Chemical / Heavy Metal	Limit Value (micrograms)
1	Antimony	0,2
2	Arsenic	0,1
3	Barium	25,0
4	Cadmium	0,6
5	Chromium	0,3
6	Lead	0,7

7	Mercury	0,5
8	Selenium	5,0

Source: [45]

Paints are not only for children if they are contacted for a long time, inhaled or accidentally tasted due to the chemicals they contain; It can pose a risk to everyone that may adversely affect health. Therefore, the manufacturers have to specify the age group that the paint material to be put on the market poses a risk and the possible dangers on the packaging. Applying the paint according to the instructions for use and technique helps to prevent possible risks. Everything chemical, including dyes, is harmful to human health. Since most artistic materials contain chemicals, their production, sale, use and disposal are regulated by certain laws and regulations that change from time to time as the information obtained increases [5]. Accordingly, the manufacturer is to blame if there is information required for use in the paint package, in the face of any health problems arising from the misuse of the paint. Even how it will be disposed of should be notified to the consumer. While high school and secondary school children can work alone with artistic paints, it is recommended that primary school and especially preschool children work with parents or teachers.

As a result, finger paint should be produced with cosmetic raw materials in accordance with international safety standards and designed for skin applications. For this reason, finger paints evaluated within the scope of cosmetic paint should not contain common allergens such as latex, egg, gluten, casein and milk, almonds, peanuts, and soy [46]

2.3.2.Skin irritation and Test Methods

Some of the reasons why human skin is an important organ are that it resists the chemicals contained in cosmetic products and pharmaceutical formulations and protects the organism against environmental factors. However, some general and local toxic effects may occur because of chemical exposure. The type of local toxic effects that occurs in the stratum corneum and has a high prevalence is skin irritation [47]. Considering cosmetic products and pharmaceutical formulations, this is a health and aesthetic hazard.

According to Article 1 of Council Directive 76/768/EEC of the European Union, the term "cosmetic product" means any substance or mixture prepared for application to the external surface of the human body (epidermis, hair, nails, lips and external genitalia), the mucous membranes of the oral cavity and the teeth, for the purpose of cleansing, deodorizing, fragancing or changing the appearance [48]

Natural cosmetic products are produced using various substances derived from plant, animal and mineral sources and have a history dating back thousands of years. Today, synthetic and semi-synthetic ones have been added to these products with modern technology [49]. As a matter of fact, the safe use of cosmetic products is of great importance for public health, especially for sensitive populations such as children. For many years, cosmetics have been tested on animals due to their potential to cause skin irritation and corrosion. However, this has been considered ethically painful and uncomfortable. Therefore, many alternative methods have been developed, validated and accepted by regulatory bodies. On March 11, 2013, it was announced that cosmetic products cannot be sold in the European Union if they are tested on animals (Draize Test) [50]. As a result of the prohibition of *in vivo* methods, yes *in vitro* tests have achieved high success by means of epidermis tissue models with close morphological resemblance to human skin or reconstructed epidermis tissue models. As the results obtained from some of these alternative methods were found to correlate well with *in vivo* methods, the frequency of use of these methods increased and efforts to develop new and more advanced models accelerated [51].

The validation criteria of the studies and new toxicological test methods are carried out under the supervision of 4 different organizations: ECD (Organisation for Economic Co-operation and Development- member states),

- ECVAM (The European Centre for the Validation of Alternative Methods- European countries),
- JACVAM (Japanese Center for the Validation of Alternative Methods- Japan),
- ICCVAM (The Interagency Coordinating Committee on the Validation of Alternative Methods-USA).

The following methods have been validated by EURL ECVAM [52]:

- EpiSkin Skin Irritation Test (SIT)
- EpiDerm Skin Irritation Test (SIT)
- SkinEthic Skin Irritation test (SIT)
- epiCS Skin Irritation Test (SIT)

Alternative methods [53]; Corrositex ®, BCOP (Bovine Corneal Opacity and Permeability Assay) and HET-CAM (Hen's Egg Test on Chorioallantoic Membrane).

- HET-CAM, one of the most important biological properties of consumer products but also of many raw materials, is local compatibility with mucus membranes. One of the most important *in vitro* tests developed following the decisions taken is the HET-CAM test method, the chicken egg test on the chorioallantoic membrane (CAM) of fertilized chicken eggs. HET-CAM is a very useful *in vitro* test method applied to evaluate the eye irritation properties of cosmetic formulations and ingredients [54]

This method has been pre-validated with approximately 100 test substances covering a wide range of chemical structures and physical appearance and representing the range of chemicals in the cosmetics industry. HET-CAM has proven to be a robust test with a good prediction of irritation potential. Briefly, HET-CAM test is applied to determine the irritation potential of substances. In this test method, incubating hen eggs are carefully opened on day nine and the chorioallantoic membrane (CAM) is exposed. Test samples, pure or diluted solutions, are placed directly on the exposed chorioallantoic membrane and the membrane is visually examined with a microscope. The time for the reaction to occur on the membrane is 5 minutes. The reaction results in bleeding, coagulation, and disintegration [55]. Based on the reaction time, an irritation score is calculated [53,54] :

- 0-0.9 points non-irritating
- Mild irritant between 1-4.9 points
- Moderately irritating between 5-8.9 points
- 9-21 points very irritating

Data on eye irritation are often needed for hazard identification of chemicals. HET-CAM method is used for routine testing of a wide range of chemicals and

formulations. This test method has no global regulatory acceptance. But the ICCVAM-recommended HET-CAM protocol for prospective studies is the IS(A) analysis method, which is based on development of each of the three HET-CAM endpoints at fixed time intervals of 0.5, 2, and 5 minutes [56]

2.3.3 Cosmetic Products and Toxicological Concern

The word cosmetics comes from the Greek and is defined as the beautification of the external appearance and external care in ancient Greece. [57]

According to the degree of their participation in biological processes, heavy metals are divided into vital and non-vital. The vital ones must be present in the organism in a certain proportion. Since these metals participate in biological reactions, they must also be taken regularly through food. For example, in animals and humans, copper is the most essential element of red blood cells and many oxidation and reduction processes [58].

Non-vital heavy metals can cause health problems by disrupting the psychological and physiological structure even at low concentrations. Mercury, which binds to sulphurous enzymes, is the best example of this group. The effects of heavy metals on life depend on the organism. For example, nickel, which is toxic to plants, must be present in animals as a trace element. The effects of heavy metals in some systems also vary depending on their concentration. Heavy metals can be harmful when they exceed a certain level. In addition, heavy metals do not influence organisms only depending on their amount. Their effects depend on the species and the solubility value of the metal ion, its chemical structure, its ability to form redox and complexes, the way it is taken into the body, the frequency of its presence in the environment and the local pH value. For this reason, the limit values of heavy metals in drinking water and food are restricted and subject to regular control by legal institutions [59].

Academic studies on cosmetic products have been followed with interest all over the world. In addition, developing technology and information sharing accelerated the progress in this regard and shed light on the cosmetics industry. Aslam et al. [60] studied Cu, Zn, Hg, Pb, As measurements in medical cosmetics in Asia. In 2012, Volpe et al. measured Cu, Pb, Mn, Cr, Ni and Al concentrations in eyeshadows from China, Italy and

the USA with flame-atomic absorption spectrometry (F-AAS) [61]. Reyhanlıoğlu, in his study in 2011, used the EDXRF method in some cosmetic products (blush, eye shadow, lipstick, nail polish, shampoo, hand cream, celluloid cream, diaper rash cream, baby powder, soap, toothpaste) using Cu, Pb, Mn, Cr, Ni determined Al concentrations [62].

Öztaş, in his study in 2015, determined the lead concentration with hydride forming AAS. In addition, the levels of heavy metals such as Pb, Ni, Co, Cr, As, Cd, Sb were toxicologically examined in 35 lipsticks and 52 foundation groups [63].

Alkan, in his study in 2016, successfully determined Quinoline yellow and Indigotin without using any separation process at the same time, using chemometric calibration methods, principal component analysis (PCA), principal component regression method (PCR), partial least squares (PLS) methods in some cosmetic products. evaluated chemo metrically with the data obtained [63]

Alsaffar and Hussein found Cu, Ni, Pb, Cd concentrations in eyeshadows sold in Baghdad [64], Ekere et al. [65], Pb, Cd, Fe in powder, eyeliner and lipstick samples used in Nigeria in their study in 2014. and Co concentrations were measured in their study in 2014.

Orisakwe ve Otaraku [66] measured Pb, Ni concentrations in powder, eyeliner and lipstick samples used in Nigeria in 2013. Nasirudeen and Amaechi found Pb, Cd, As and Hg concentrations [67], in lipstick, powder, hair dye and eye shadow they collected from markets in Kaduna metropolis of Nigeria in 2016. Ni and Pb concentrations [67], Sueliman and Labaran determined the Pb, Cr, Ni concentrations in lip paint in their study in 2017 [68].

El-aziz et al. measured Cu, Cr, Hg, Zn, Fe, Cd, Ni, Pb and Mn concentrations in some cosmetic products (such as eye shadow, lip paint, eyeliner) used in Alexandria, Egypt, in 2017 [69].

Ullah et al. In 2017, heavy metal concentrations in cosmetic products of different countries marketed in Kyher Pakhtunkhwa province of Pakistan were calculated and compared [70].

Zafarzadeh et al. determined the cadmium and lead concentrations in different types of cosmetic products used in Iran in their study in 2018 [71].

In this context, there are legal regulations in the world and in Turkey to control the safety of cosmetic products and to monitor the products. In this regard, the cosmetic law numbered 5324 and dated 24.03.2005, published by the Ministry of Health in 1994, draws attention. There is a definition of cosmetics in the law. "Cosmetic; It is prepared to be applied to different external parts of the human body such as the epidermis, nails, hairs, hair, lips and genital organs, mouth and teeth or mucous membranes. all preparations or substances, including hair dyes and hair lighteners, which are to straighten" [72]

Within the scope of the Cosmetic Law No. 5324 and dated 24.03.2005, the manufacturers are followed up under the responsibility of the Turkish Medicines and Devices Agency. The purpose of the law is stated in the first article of the relevant law. Accordingly, the purpose of the law; To ensure that cosmetic products reach the public in a safe, effective and high-quality manner, to determine the principles regarding notification before the products are placed on the market, market surveillance and inspection, and inspection of the production sites of these products [72].

Concerns about the possible negative or toxic effects of chemicals in the content of cosmetics, which have been referred to as "toxicological concerns" in the literature for years, continue. From the raw materials used in the production of cosmetics, to the substances used as preservatives, and from the beginning of the production until they reach the consumer, each of the harmful substances that may contaminate the products should be evaluated in terms of safety. As a matter of fact, in most countries today, the control of the safety of these substances used in the production of cosmetics is followed by the relevant official institutions, organizations or committees. They are followed by "Turkish Medicines and Medical Devices Agency (TİTCK)" in Turkey, "European Food Safety Authority and Health Organization (EFSA)" in Europe, and "World Health Organization (WHO)" in America. In our country, protective effectiveness tests, dermatological tests, stability tests, microbiological tests, and genotoxicity tests are carried out in accredited private and official laboratories in accordance with the "General Requirements for the Sufficiency of TS EN ISO/IEC 17025 Experiment and Calibration Laboratories" standard. Thanks to these control tools, the safety assessment of the

substances used in cosmetic products is carried out. This term, which has taken its place as "Toxicological Concern" in the literature, is included in many studies to prevent and minimize possible contamination in studies to determine the source and concentration of possible contamination.[73,74,75].

2.3.4 Cosmetic and Heavy Metal

In a study by Sainio et al. (2001), 75% of the eyeshadows they analyzed contained more than 5 ppm of at least one of the metals Co, Ni, Cr, Pb or As, and that at least one of these metals was 1 ppm in all the products. It was stated that it was included as a component in higher amounts than [76].

In the analyzes performed by Al-Saleh et al. (2009), it was observed that all lipsticks and eyeshadows collected from the Saudi market contained Pb. In four of them, it was determined that the color additive limit of 20 ppm allowed by the FDA was exceeded. It has been stated that this level will create a risk of poisoning[76].

Hepp et al. (2009) found the average Pb amount in lipsticks to be 1.07 µg/g in their study with lipsticks[77].

Al-Dayel et al. (2011) analyzed 28 mascaras and eyeshadows in their study in Saudi Arabia. It was determined that the Ni level reached 46.8 ppm, the Co level reached 31.3 ppm, and the Pb level reached 11.9 ppm in the samples they examined [78].

Volpe et al. (2012) examined eyeshadows produced in different countries (China, Italy and America) and measured Cd, Co, Cr, Ni and Pb values. In particular, the Pb level in Chinese-made products increased up to 81.5 ppm. However, very high levels such as Cd 0.033 ppm, Co 0.30 ppm, Cr 0.287 ppm and Ni 4.148 ppm were detected [79].

Khalid et al. (2013) examined different brands of lipsticks purchased from local markets in Pakistan in terms of Pb, Cd, Cr and Ni. Detected heavy metal levels Pb, 0.29-6.23 ppm; Cd, 0.2-0.5 ppm; Cr is 0.222-5,430 ppm and Ni is 0.6-5.95 ppm [80].

Liu et al. (2013) found high concentrations of Titanium (Ti) and Aluminium (Al) in many lip products they analyzed. They found measurable levels of manganese (Mg) in all the products they examined and Pb in 75% of the products. The highest values of Ni and Cr were found to be 9.73 and 9.72 ppm, respectively. It has been determined that approximately half of the products exceed the 0.1 ppm lead limit recommended by the FDA in sugar-like products that are frequently consumed by children [81].

On the other hand, Ullah et al. (2013) analyzed Pb in 15 cosmetic products, 141.6 ± 0.016 ppm; Cd, 0.238 ± 0.001 ppm; Cu, 26.62 ± 0.012 ppm; Co, 0.527 ± 0.002 ppm; Fe, 860.8 ± 0.061 ppm; Cr, 0.074 ± 0.002 ppm; Ni, 0.674 ± 0.002 ppm; They found Zn to be 268.6 ± 0.086 ppm [80].

In the study conducted by Campaign for Safe Cosmetics in the USA, it was stated that 61% of 33 different lipstick brands contain Pb and the Pb level is up to 0.65 ppm. In addition, the FDA stated that all lipsticks they tested contained Pb at the level of 0.09 to 3.06 ppm [82].

The Canadian Ministry of Health pointed out that the lipsticks it tested contained Pb at the level of 0.079 to 0.86 ppm and revealed that there was 6.3 ppm in one of the samples. Although the deliberate use of heavy metals as components in cosmetic products is completely prohibited, research results in many countries indicate that these metals can be included in these products as a source of pollution. This is partly a factor in the contact of human beings with the products in question, which inevitably occurs through environmental sources and is impossible to avoid. Other factors are the state of impurities in the raw materials used in cosmetic production or the negativities arising from the production processes. Since these heavy metals are known to be persistent in their environment for many years, it would not be wrong to state that these metals are found as natural components in resources such as stones, soil and water. Another consequence of this is that metal pollution takes place in many raw materials and pigments used in industrial production, including the cosmetics industry [83].

Shen et al. (2007), in 15 examples including cosmetics such as hairspray, perfume, deodorant, cream, lotion, diethyl phthalate (DEP), di-n-propyl phthalate (DPP), Di-n-butyl phthalate (DBP), butyl They examined phthalates such as benzyl phthalate (BBP),

di-cyclohexyl phthalate (DCHP) and di-octyl phthalate (DOP), parabens such as methylparaben (MPB), ethylparaben (EPB), propylparaben (PPB) and butylparaben (BPB), and only 1 sample. They stated that it does not contain phthalates and parabens [84].

2.3.5 Current Legal Regulations Regarding Cosmetic Products

- European Union

The cosmetic legislation of the European Union is based on the cosmetics directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the member states. Manufacturers are responsible for ensuring that the cosmetics they produce comply with the law before placing them on the market. The manufacturer or importer is responsible for demonstrating that the product is safe for its intended use. Regulations are implemented at the national level and each EU member state has a responsibility to maintain compliance. The EU has banned the use of approximately 1328 chemicals in cosmetics. Chemicals prohibited for use in cosmetics are listed in Appendix II of the Cosmetics Regulation. The use of arsenic, lead, human tissue and similar substances in cosmetics is prohibited. [85].

- USA

In the US, cosmetics are regulated by the Federal Food, Drug, and Cosmetic Act (FD&C Act). It is the FDA's job to oversee compliance with these regulations. But unlike drugs and color additives, cosmetics and their ingredients are not subject to pre-sale FDA approval. The substances in the 21st title (21 CFR) in the regulations published by the FDA can be applied to cosmetics. Sections 700 to 740 in Title 21 define cosmetic ingredients as “any chemical entity or mixture used in the manufacture of a cosmetic product”. FDA&C law prohibits any toxic or harmful substance that may harm the user under the conditions of use stipulated on the product label [86].

- Canada

The Canadian Food and Drug law (F&DA) states that “no one:

- a) When cosmetics are used, containing components that will harm the health of the user,
- b) Wholly or partially dirty or spoiled or containing any foreign substance,
- c) It cannot sell products that are produced, prepared, preserved, packaged or stored under unhygienic conditions.” forms a condition. In December 2008, Health Canada prepared a draft for heavy metal (Pb, As, Cd, Hg and Sb) impurities in cosmetics. [87].

●India

Cosmetic products in India are regulated by Law 1940 and 1945 and the Bureau of Indian Standards (BIS). For cosmetic products specified under Act 1945, the BIS has set the standards required. According to these regulations; 2 ppm Arsenic; 20 ppm Lead; Total heavy metals other than 100 ppm lead is the upper limit that can be found in cosmetics [88].

●Turkey

The Cosmetic Law No. 5324 enacted in 2005 was enacted in order to inspect the cosmetic products produced and sold in our country and to bring them to certain standards. The Cosmetics Regulation, which was issued based on the Cosmetics Law No. 5324 and prepared in parallel with the Council Directive 76/768/EEC of the European Union Cosmetics Legislation and the Commission Decision No. 96/335/EC, is the most up-to-date regulation regarding cosmetics. Therefore, the regulations in the European Union and the regulations in Turkey are very close to each other. In addition, the “Guideline on the Safety Evaluation of Cosmetic Products”, which was prepared for the safe use of cosmetics, is a regulation that includes the issues related to the finished product. In our country, TITCK fulfills the necessary functions in terms of establishing and supervising the relevant regulations [89].

2.3.6 Legal Limit Values

“Guide on Heavy Metal Impurities in Cosmetic Products” has been prepared to inform the manufacturer and the consumer about possible heavy metal impurities in cosmetic products and the legal limits of these impurities. TITCK monitors the up-to-datedness of this guide. In article 11 of this guideline, limit values are given for cosmetic products [85]. Relevant values are shown in Table 2.3.

Table 2.3. TITCK limit values for cosmetic products

Heavy metal	Limit Value (mg/L)
Lead	20
Arsenic	5
Cadmium	5
Mercury	1
Antimony	10

Source: [85].

United States Environmental Protection (EPA) limit value data are also used in this guide. For this reason, when evaluating the analysis data in our study, the values in the table named "Maximum metal contents that can be found in personal cosmetic products (excluding dyes containing chromium 3)" for the limit values of Co, Pb, Cd, Ni, Sb and Cr included in the study of Çağlar et al. used as reference. Relevant values are shared in Table 2.4.

Table 2.4. Limit Metal Contents

Heavy metals	The highest concentration that cosmetic products can contain (mg/L)
Antimony	10
Arsenic	5
Cadmium	5
Cobalt	70
Chromium III	100
Chromium IV	25
Mercury	1
Nickel	200

Yellow colors are essential major elements; green colors essential trace elements; blue-colored elements used for therapeutic purposes; red colors are non-essential toxic elements and extractable heavy metals; Arsenic (As), Copper (Cu), Mercury (Hg), Zinc (Zn), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Lead (Pb), Nickel (Ni). Heavy metals have a wide range of uses in many different industries. Some usage areas can be listed as follows;

- Heavy Industrial Activities
- Alloy Production
- Paint Industry
- Photography Industry
- Catalysts
- Lead Production Processes
- Metal covering
- Battery Production
- Ceramic Production

2.4.1 Effects of heavy metals on humans and the environment

Heavy metals, which are the natural components of the earth's crust, are found in surface waters as colloidal, granular, and dissolved. Colloidal and particulate metal can be found adsorbed to hydroxides, oxides, silicates, sulfides or clay, silica and organic matter, showing a high affinity for humic acids, organoclays and oxides coated with organic matter. Soluble forms are usually ions or combined organometallic chelates or complexes, although soluble concentrations are usually low [92].

Important causes of pollution of lakes, rivers, gulfs, oceans, their sediments and most importantly our drinking water; heavy metals, metal compounds and various minerals. The source of minerals can be shown as being present in these structures or being produced and somehow transported to these places because of human activities [93].

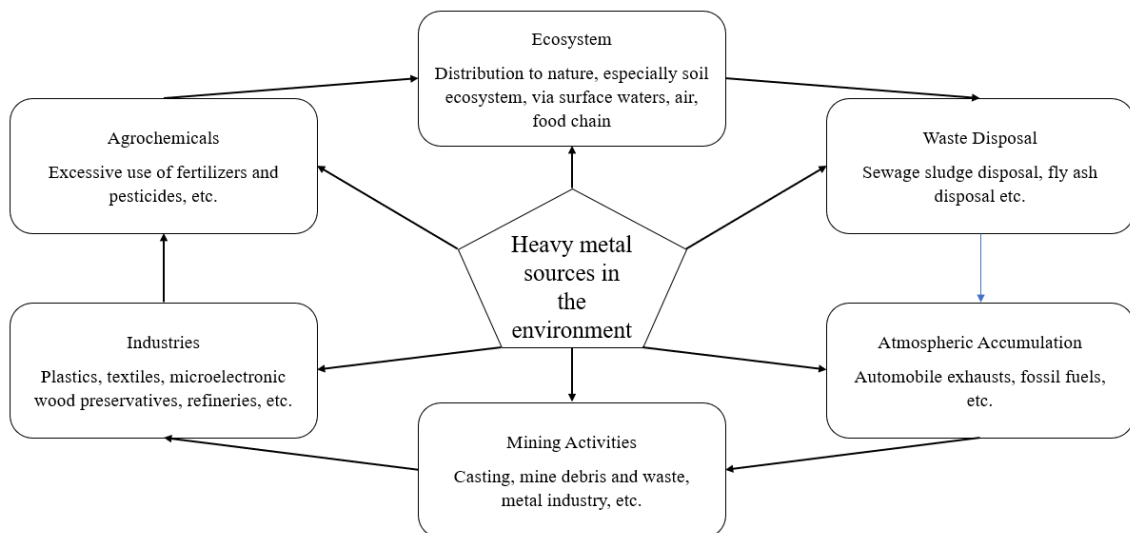
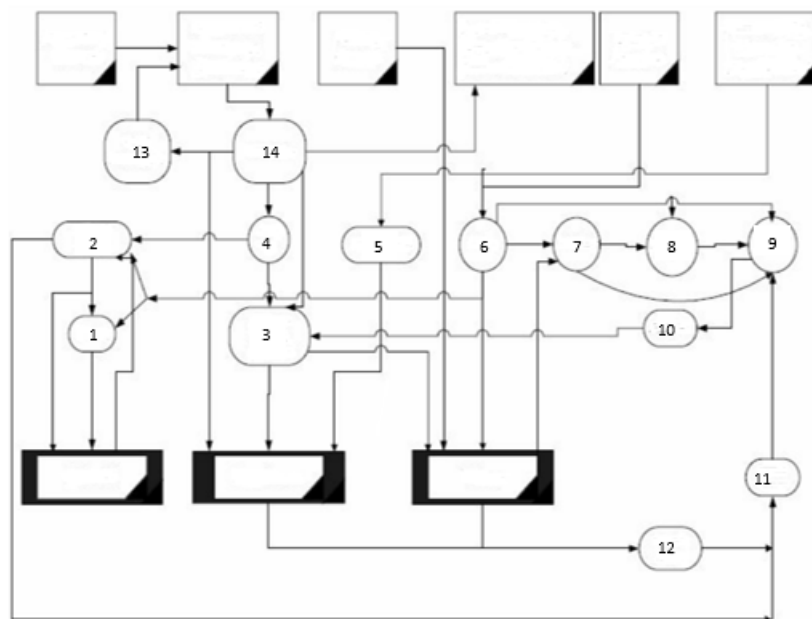


Figure 2.3. Distribution of Heavy Metals in Nature - Source: [94].

Heavy metals can reach sea creatures, soil and then people by being carried by water. Heavy metals are dangerous for life, especially when it exceeds a certain level. [95]. Heavy metals become very effective in our ecological environment by mixing industrial wastewater into our drinking water. The vital ones of heavy metals, at a certain concentration, must be taken regularly through food as they participate in biological reactions. For example, Cu is an essential part of many oxidation and reduction processes of red blood cells in animals and humans. It should also be known that non-essential heavy metals, even in very low concentrations, can cause great problems in the health of living things. [96]. The interaction of heavy metal sources with nature is shown in Figure 2.3 and Figure 2.4.



	Flow
	Intermediate Storages (sea, surface waters, treatment sludge, wastewater, filter waste, air, plants, animals, humans, wastewater, drinking water, underground waters, consumption, recycle)
	Aggregation / Settlement (sediment, storage, bottom)
	Resources (heavy metal production, enterprises using heavy metals, fertilizer industry, "thermal power plant, garbage, waste sludge incineration plants", transportation vehicles, "iron-steel, cement, glass production")

Figure 2.4. Distribution of Heavy Metals in Nature Source: [97].

Our current knowledge indicates the toxic effects of heavy metals on the ecosystem and human health, which are extremely dangerous [98, 99, 81]. Even in low concentrations, these elements can cause toxic effects on many organs and tissue systems in living things. It has been reported that there is a relationship between exposure to heavy metals and many types of cancer detected in humans, respiratory disorders, organ failure and mental retardation [100, 101]. For example, one of the possible reasons for the increase in cancer cases detected in humans is the DNA of Cd, which we are frequently exposed to from environmental sources. [102]. Cadmium also causes damage to the target organ kidney in terms of toxic effects and deterioration in bone structure due to the irregularity in calcium metabolism, while nickel, chromium and cobalt are the most common causes of allergic contact dermatitis [103]. exposure can cause eczema in humans (Table 2.4).

Table 2.5. Effects of Some Heavy Metals on Human Health and Toxicity Levels

HM	Effects	Toxicity level
Pb	Polyneuropathy, encephalitis, anemia, hypertension, cancer effect	Highly toxic
Hg	Neurological findings, goiter, tachycardia, irregular pulse, forgetfulness, impatience	Highly toxic
Cd	High blood pressure, lung cancer, osteoporosis, anemia	moderately toxic
As	Kidney, liver and vision disorders, cancer effect	Highly toxic
Li	Neurological findings, muscle weakness	mildly toxic
Cu	Abdominal pain, vomiting, anemia, respiratory distress	Highly toxic
Co	Very rare effect	mildly toxic
Cr	Skin problems, ulcers, cancer, respiratory system problems	Highly toxic

Source: [104].

2.4.1.1 Mercury

- What is Mercury?

Mercury is metal that naturally occurred and it is a odorless liquid which is insoluble in water. There is no any positif role in the human body. All forms of mercury is toxic to human. It exist in the environment different type of forms (Elemental, Inorganic (monovalent or divalent) and organic which is called methyl mercury) which effects the level of toxicty of the mercury and this toxicity beside the chemical form it also depends to the route, amount, duration and timing of exposure as well as the exposed person's vulnerability [105].

- Source of Exposure

Mostly exposure for the organic mercury for human is through the fish consumption and the mostly exposure for the metallic mercury is through the vaporization of dental amalgam. But also the exposure might be through the devices that has the mercury in it. As an example broken thermometers or equipments for science. Further the salts of mercury used as pigments in paints.

- ADME

Absorption: 70-80% of vapor of elementary mercury absorbed through the lungs. Contrast to that Gastrointestinal absorption negligible. The salts of mercury absorption through the GI is about 10-15% depends on the patient and the specific salt. In case of an oral ingestion of methyl mercury then its completely absorbed through the GI. [106].

Distribution: it distributes in the body and pass to blood brain barrier and placenta via diffusion and then to the tissues. The highest concentrations usually in the liver and kidney but also occurs in the brain. Half-life ($t_{1/2}$) for inorganic mercury is ~1-2 months. Half-life ($t_{1/2}$) for Methyl mercury is ~2 months.

Metabolism: The elementary mercury in order to form inorganic mercury it catalase in the erythrocytes. When mercury combine with the Sulfur or oxygen the inorganic mercury compounds are formed and when mercury combine with carbon then the organic mercury compounds are formed. Through the conjugation of migroorganisms to inorganic mercury will form Methyl mercury so called organic mercury. [105].Also through the demethylation of organic mercury inorganic mercury can be formed.

Elimination: Primarily is through the feces and urine but also all mercury types excreted through the sweat and the breast milk and as a result of that deposits in hair and nails.

- Toxicity and Human health Effects

The most well-known case example for mercury is the poisoning of hundreds of fishermen and their families as a result of the accumulation of methyl mercury in fish, which went down in history as the minamata disease which caused many severe effects such like paresthesia, abnormality of walking, hearing even though deads. [105]. Central Nervous System is the main target when the toxicity is through the organic mercury which is called as methyl mercury. In general mercury exposures, respiratory tract damage occurs through inhalation, fever and liver damages in oral exposure, and burns and irritations occur as a result of contact with the skin. [106].

2.4.1.2 Lead

- What is Lead?

Bluish-gray metal that is not soluble in water. It has no characteristic taste or smell. There are various colors in salts. Lead has many properties such like softness, malleability, ductility, poor conductivity etc. and that makes hard to give up for the uses of it. But since it is not biodegradable nature and there is constant use results in accumulation in the environment with the increase hazards. [107].

- Source of Exposure

Contribution of Source of exposure is 10% from water and other sources, 15% from air, 20% from foods and 55% from dust and soil. [108].

- ADME

Absorption: Absorption of the lead is through the gastrointestinal tract and children absorb the lead about 40-50% while this level is poor in adults which about 3-10%. Organic lead absorption is through the skin and Inorganic lead absorption is through the respiratory tract and while submicron particles can be

absorbed well, the larger particulates might be moved after deposition in the respiratory tract and swallowed after reaching oropharynx. [107]

Distribution: the distribution of lead 70-95% in the bone, 4% in the brain liver, kidneys and bone marrow and 1% in blood which primarily in the red blood cells. Lead is able to cross placenta. The elimination of half-time of lead 25 days in the blood which may vary with age and history of exposure, 40 days in the soft tissues and 20 years in the bone.

Metabolism: Inorganic lead is not metabolized and contrast to that organic or alkyl lead forms triethyl-lead and trimethyl lead which are the very high neurotoxins through the oxidative dealkylation by P-450 enzyme.

Elimination: The major excretion route is through the kidney, about 65% via urine and feces and about 35% via bile. [108]

- Toxicity and Human health Effects

Lead has no physiological role in the body and has toxicity in any level of it. Lead toxicity damage the functions of nervous system, digestive system, respiratory system, reproductive system and so on. More than that it even prevents the normal activities of the enzymes. Further it harms the usual DNA transcription process that results in disability of bones. Based on the International Agency for Research on Cancer (IARC), Inorganic lead compounds has been classified as category 2A as probably carcinogenic to humans while organic lead compounds categorized in group 3 because of they are not classifiable as to their carcinogenicity to human. [107]

2.4.1.3 Nickel

- What is Nickel?

Nickel is a hard and ductile transition metal with a color of silvery white. It is 24th most abundant element. It has oxidative states from -1 to +4 however most found oxidation state is +2 in environment and the biological system. Nickel is widely distributed in nature and the 6 to 8,5% of earth's core is nickel. There are soluble and insoluble forms of nickel. Water insoluble compounds of nickel are carbonate, sulfides and oxides but they can dissolve in biological fluids while water soluble salts of nickel are chloride, sulfate and nitrate. [109]

- Source of Exposure

Major source of exposure for nickel is food but also breathing air, drinking water and the cigarettes containing nickel can be the other sources of exposure. Furthermore skin contact with metals plated with nickel and soil and bath water can also result in exposure. One of the most widely use of nickel is stainless steel production.

- ADME

Nickel might enter the body via breathing of air which contain nickel or via drinking water or food that contaminated with nickel as well when the skin contacts with nickel. [110] In case of via breathing the inhaled amount of nickel will go to lungs and the blood. But that is differs based on the particule of nickels. If the particules of nickel is large then it will remain in the nose but if small particles then it reaches deep of lungs. In case of nickel particules dissolve easily in water then more nickel will be asorbed from the lungs but in case of nickel particules cant be dissolve then the nickel might remain in the lungs for a long time. Although some of the nickel might leave the lungs via swallowing or spit out of mucus. Through the skin contact some amount of nickel may enter the bloodstream and after nickel enters the body primarily goes to kidneys but it can go to all organs as well. The excrition of nickel that entered the bloodstream is through urine and feces. [109].

- Toxicity and Human health Effects

The most common harmful health effect of nickel in humans is an allergic reaction. A person can become sensitive to nickel when jewelry or other items containing nickel are in direct contact and prolonged contact with the skin. Once a person is sensitized to nickel, further contact with the metal may produce a reaction. The most common reaction is a skin rash at the site of contact. The most serious harmful health effects from exposure to nickel, such as chronic bronchitis, reduced lung function, and cancer of the lung and nasal sinus, have occurred in people who have breathed dust containing certain nickel compounds while working in nickel refineries or nickel processing plants. [110].

2.4.1.4 Arsenic

- What is Arsenic?

Known as King of Poison and it is an element that naturally occurred and much distributed in the Earth's crust. [107] Even though it is classified as a metalloid because of having the both properties of a metal and nonmetal still it is referred as metal. [111] Arsenic mostly found in the environment together with the elements such like oxygen, chlorine and sulfur. When arsenic combined with those elements is called inorganic arsenic and when arsenic combined with carbon and hydrogen then it is called as organic arsenic. Most of them are white or colorless powders with no vapor facility, with no smell and no special taste. Thus make arsenic invisible and can't be understandable if it is present in the air, food or water. [107].

- Source of Exposure

Exposure to arsenic could be through the groundwater, arsenic containing mineral ores, industrial processes, semiconductors and light-emitting diodes called LED, commercial products such as pesticides, herbicides, fungicides, wood preservatives as well as foods such like seafood and fish. [111].

- ADME

Absorption: The arsenic absorption is through the Gastrointestinal tract about 80-90% and through swallowing of water, food or soil which contains arsenic in it and the amount that enters the body will differ according to amount of swallow and the type of arsenic. Dermal contact and so skin is also a route of exposure and it is reported that exposure with an inorganic arsenic cause systemic toxicity. [107]

Distribution: No studies exist for the distribution of both arsenic forms through inhalation exposure but Rhoads and Sanders study of arsenic trioxide administration to the rats showed that the distribution of arsenic to liver, kidney skeleton, gastrointestinal tract and other tissues. And that might be the indication **Metabolism:** There are two processes are involved in the metabolism which are reduction/oxidation reactions that interconvert As(III) and As(V), and methylation reactions, which convert arsenite to MMA and

DMA respectively. [112] If necessary then the reduction of inorganic arsenate to arsenate, methylation to MMA(V), reduction to MMA(III) and methylation to DMA(V) are the reactions that end up for the resulting series. Whether the exposure by inhalation, oral or parenteral route the processes are appear to be similar to eachother. of the absorbed arsenic is distributed in the body.

Elimination: The main route of excretion for arsenic is by the kidneys and both forms (inorganic and organic) of the arsenic leaves the body through urine. Average human urine contains 10-30% inorganic arsenic, 10-20% methylarsonic acid and 60-80% dimethylarsinic acid. [106,110] Almost all of the inorganic arsenic will be gone within several days, although some could remain in the body for several months or even longer. In case of an exposed to organic arsenic, mostly of it will leave the body within several days. The half life of arsenic is 10 hours and 30 hours in the blood for inorganic and organic arsenic respectively. Several weeks after exposure to arsenic the remain arsenic in the body could be found in nails, hair and skin.

- Toxicity and Human health Effects

Based on the International Agency for Research on Cancer (IARC), arsenic and inorganic arsenic compounds has been classified as category 1 carcinogens. The major targed organ for the chronic arsenic exposure is skin. It may result in the hyperpigmentation or contrast to that hypopigmentation within the 6 months to three years. Via high level of arsenic exposure could result as skin cancer. Palmar-Plantar hyperkeratosis most known condition of arsenic induced pigmentation changes within a period of years. [111]. There is almost no information regarding to effects of organic arsenic but based on the animal studies organic arsenic compounds are less toxic comparision the inorganic forms of it. This well known human carcinogen generally associates with the tumors of the skin, lung and urinary bladder and possibly kidney, liver and prostate. [111]

2.4.1.5 Cadmium

- What is Cadmium?

A metal that found in the Earth's crust and exist in the air as small particules through mostly after high temperature processes as a by product such as production of zinc, lead and copper ores. Cadmium as a consumer product can be found in batteries, pigments, coatings and platings as well as a stabilizer for plastics. [105]

- Source of Exposure

Exposure to Cadmium is primarily through the food and smoking. Cigarette smoke significantly increases the cadmium exposure. One packet of cigarettes per day associates with the 2-4 μg daily intake of cadmium. [105] Inhalation of cadmium can be negligible except living near a cadmium emitting industries. Water can also be a source of cadmium exposure if fished and eaten from water sources near industries that emit cadmium. Highest risk of exposure is still belong to occupational exposure.

- ADME

Administration: About 5-50% of Cadmium might enter the body via breathing through the lungs and about 1-10% of Cadmium might enter the body via food and water through the gastrointestinal tract. Hardly any cadmium might enter the body via dermal contact. [112].

Distribution: Cadmium that enters the body binds to red blood cells or high molecular weight proteins in plasma, and most of it accumulates in the kidneys and liver, where it can persist for years. The concantraion of cadmium, which is low in the kidneys at birth, rises as a result of chronic exposure through diet or smoking. Parallel to that the highest cadmium content is seen in the renal cortex.

Metabolism: Although cadmium binds to many macromolecules and proteins, it still has almost no metabolism. Metallothionein mostly involved for binding of cadmium which thought that reduces the toxicity of cadmium. Beside that, the body can change most cadmium to a form that is not harmful, but too much cadmium can damage the ability of the liver and kidney to change the cadmium to a harmless form. [112].

Elimination: While the excretion of cadmium entering the body orally is via the feces, the excretion of cadmium entering the body through the respiratory tract is eliminated equally with urine and feces.

- **Toxicity and Human health Effects**

Based on the IARC, Cadmium and cadmium compounds has been classified as Category 1 carcinogens. Through the inhalation; breathing air with high cadmium can cause lung damage and resulting death, and breathing air with low levels of cadmium can result in accumulation of cadmium in the kidney, resulting in kidney disease. [105]

Regarding to ingestion of cadmium the results may vary depending on the level of exposure to cadmium through water and food. For example, exposure to low levels of cadmium can result in vomiting, insomnia and sometimes death, while exposure to high levels of cadmium causes cadmium accumulation in the kidney and causes kidney diseases. [112]. In addition, long-term exposure to low level of cadmium can cause bones to become fragile and break. As an example of a well known case itai-itai disease which is the first disease found to be due to cadmium poisoning could be given. The syndrome is characterized with the pain in the back and joints, osteomalacia (adult rickets), bone fractures and occasional renal failure. As a reason for that, accumulation of cadmium in the kidney might effect the vitamin D metabolism and thus might increase the excretion of calcium and phosphorus into the urine. This may lead to a disruption of the calcium balance which results in osteoporosis, osteomalacia, and spontaneous fractures. [105].

2.4.1.6 Antimony

- **What is Antimony?**

Antimony (Sb) is a semi-metal chemical element and exist in a two forms which are metallic form is a silver-white brittle solid and the non-metallic form is a dark-gray lustrous powder. [113]. Naturally present in the earth's crust and antimony as well as most of its compounds known as toxic.

- **Source of Exposure**

Source of exposure to antimony can be through inhalation of particulate matter which contains antimony within the ambient air also can occur via contaminated water, food, and soil contact. [113]. Occupational exposures might occur as well through the smelters, coal-fired plants and refuse incinerators that process or release antimony.

- **ADME**

Absorption: Antimony is poorly absorbed chemical element that absorption through the gastrointestinal tract is about 1% for antimony trioxide and 10% for antimony potassium tartrate.

Distribution: Antimony distributes throughout the body and the most high concentrations in the lungs, gastrointestinal tract, red blood cells, liver, kidney, bone, spleen, and thyroid. [113].

Metabolism: As far as known antimony has no metabolism but transformation of pentavalent antimony and trivalent antimony could be suggested for it.

Elimination: the excretion of antimony entering the body is through the urine and feces. Mostly of the trivalent antimony excreted in the feces while pentavalent antimony primarily excreted in the urine.

- **Toxicity and Human health Effects**

Exposure to high concentrations of antimony for a long period can result irritation of the eyes, skin and lungs. Continue of the exposure may cause more serious health effects such like lung disease, heart problems, diarrhea, severe vomiting and stomach ulcers. There is no information regarding to whether antimony may cause reproductive failure or cancer. [114].

2.4.2 Removal methods of heavy metals

Many economical and effective methods are used for heavy metal removal. Today, new treatment techniques are still being developed. The general methods used for heavy metal removal from wastewater can be diversified as chemical precipitation, ion exchange, adsorption, coagulation-flocculation, phytoremediation, flotation, and neutralization [115]. The advantages and disadvantages of some of these methods used are summarized in Table 2.6.

Table 2.6. Advantages and disadvantages of heavy metal removal methods

Method	Advantage	Disadvantage
Chemical Precipitation and Filtration	The process is simple, and the equipment is inexpensive	Difficult separation at high concentrations, sludge formation requiring extra treatment and increased cost
Electrochemical Methods	Metal recovery possible	Expensive and only effective at high concentrations
Chemical Oxidation and Reduction	is inactivation	It has ambient sensitivity
Ion Exchange	Recovery of pure waste metal and effective treatment is ensured	Sensitive to particles and expensive resins
Evaporation	Pure waste is obtained	It requires more energy, is expensive and has waste sludge formation.
Reverse Osmosis	Pure waste is obtained	Under high pressure, the membrane is large and expensive
Adsorption	Activated carbon can be used	Not applicable for all metals

Source: [95].

•Coagulation-flocculation

It is the removal of soluble heavy metals in wastewater by converting them into insoluble metal hydroxides and then precipitating them. In principle, the coagulation process destabilizes the colloidal particles by adding a coagulant, resulting in precipitation. After the acidic and basic wastewaters are adjusted to the appropriate pH value with the neutralization process, the following applications are carried out respectively for the precipitation process:

Coagulation: It is the process of getting the particles in the wastewater ready to form flocs with the addition of coagulants.

Flocculation: It is the process of forming flocs that can easily collapse by forming flocs by growing and combining with each other in the wastewater [115].

•Phytoremediation

It is an effective breeding method used to remove heavy metals from the soil with the help of genetically adjusted and specially selected plants. This breeding method can also be used as a completion phase with other methods. [116].

- Neutralization

Industrial wastewater with high acidity and alkalinity must be neutralized before being discharged into the receiving environment or biological environments. While lime is generally used in the neutralization of acidic wastewater, strong acids such as sulfuric acid are used to neutralize basic wastewater. [117].

- Chemical precipitation

It is the process of precipitation of heavy metals as metal hydroxides at a suitable pH using a suitable precipitator such as lime or calcium hydroxide. Using lime or calcium hydroxide is very cheap for metal removal costs. However, the disposal of heavy metal sludge formed after settling is an important disadvantage of the system. [118].

- Ion exchange

It is the process of replacing heavy metal ions with ions of ion exchange resins with the help of electrostatic forces [119]. In addition to the advantages of all these methods, the disadvantages are more noticeable. In the adsorption method, The most important advantages of this method are the cheap and abundant availability of the used adsorbent, fast results, repeatability, easy applicability at very low concentrations, and recovery of the used adsorbent. This process is of global importance to minimize water and air pollution [120].

3. MATERIALS AND METHODS

3.1 Chemicals

- **Chemicals For HET-CAM**

- 0.9% (w/v) sodium chloride (NaCl) in deionized/distilled water
- 1% (w/v) sodium dodecyl sulfate (SDS) in deionized/distilled water
- 0.1% sodium hydroxide (NaOH) in deionized/distilled water
- 1AS Yellow Color of Finger Paint
- 1AK Red Color of Finger Paint
- 2AS Yellow Color of Finger Paint
- 2AK Red Color of Finger Paint
- 3AS Yellow Color of Finger Paint
- 3AK Red Color of Finger Paint
- 4AS Yellow Color of Finger Paint
- 4AK Red Color of Finger Paint
- 5AS Yellow Color of Finger Paint
- 5AK Red Color of Finger Paint
- 6AS Yellow Color of Finger Paint
- 6AK Red Color of Finger Paint
- 7AS Yellow Color of Finger Paint
- 7AK Red Color of Finger Paint
- 8AS Yellow Color of Finger Paint
- 8AK Red Color of Finger Paint
- 9AS Yellow Color of Finger Paint
- 9AK Red Color of Finger Paint
- 10AS Yellow Color of Finger Paint
- 10AK Red Color of Finger Paint
- 11AS Yellow Color of Finger Paint
- 11AK Red Color of Finger Paint
- 12AS Yellow Color of Finger Paint
- 12AK Red Color of Finger Paint

- **Chemicals For Heavy Metals**

- 1AS Yellow Color of Finger Paint
- 1AK Red Color of Finger Paint
- 2AS Yellow Color of Finger Paint

- 2AK Red Color of Finger Paint
- 3AS Yellow Color of Finger Paint
- 3AK Red Color of Finger Paint
- 4AS Yellow Color of Finger Paint
- 4AK Red Color of Finger Paint
- 5AS Yellow Color of Finger Paint
- 5AK Red Color of Finger Paint
- 6AS Yellow Color of Finger Paint
- 6AK Red Color of Finger Paint
- 7AS Yellow Color of Finger Paint
- 7AK Red Color of Finger Paint
- 8AS Yellow Color of Finger Paint
- 8AK Red Color of Finger Paint
- 9AS Yellow Color of Finger Paint
- 9AK Red Color of Finger Paint
- 10AS Yellow Color of Finger Paint
- 10AK Red Color of Finger Paint
- 11AS Yellow Color of Finger Paint
- 11AK Red Color of Finger Paint
- 12AS Yellow Color of Finger Paint
- 12AK Red Color of Finger Paint
- Ultra Pure Water Aqua Nova Hepta Distillated, resistivity 0.34 MΩ-cm, Kristianstad, Sweden
- HNO₃ (65 %) and HCl (37 %) from Merck supra-pure grade (Darmstadt, Germany)
- IS Solution 0.025-10 ppm for mercury in deionized/distilled water
- IS Solution 0.1-10 ppm for nickel in deionized/distilled water
- IS Solution 0.1-10 ppm for Arsenic in deionized/distilled water
- IS Solution 0.1-10 ppm for Cadmium in deionized/distilled water
- IS Solution 0.1-10 ppm for Antimony in deionized/distilled water
- IS Solution 0.1-10 ppm for Lead in deionized/distilled water
- **Chemicals For Microbiology test**
 - Sterile Phosphate Buffer Saline
 - Soybean Casein Digest Broth

- Sabouraud Dextrose Broth
- Soybean Casein Digest Agar
- Sabouraud Dextrose Agar
- 1BM Blue Color of Finger Paint
- 2BM Blue Color of Finger Paint
- 2BM Blue Color of Finger Paint
- 3BM Blue Color of Finger Paint
- 4BM Blue Color of Finger Paint
- 5BM Blue Color of Finger Paint
- 6BM Blue Color of Finger Paint
- 7BM Blue Color of Finger Paint
- 8BM Blue Color of Finger Paint
- 9BM Blue Color of Finger Paint
- 10BM Blue Color of Finger Paint
- 11BM Blue Color of Finger Paint
- 12BM Blue Color of Finger Paint
- **Chemicals For Challenge test**
 - Sterile Phosphate Buffer Saline
 - Soybean Casein Digest Agar
 - Sabouraud Dextrose Agar
 - 8BM Blue Color Of Finger Paint

3.2 Equipments

- **Equipments for HET-CAM**
 - Fresh (not older than 7 days), clean, fertile 50-60 g
 - White Leghorn chicken eggs 96 pcs
 - Incubator
 - Disposable glass pipette
 - Electronic Chronometer
 - Candling light
 - Deionized/Distilled Water
 - Dentist's rotating saw blade
 - Incubator with an automatic rotating device
 - Micropipette(s) and disposable tips appropriate for recommended volumes
 - Mortar and pestle (or comparable grinding tools for test substances)

- Standard general biological laboratory equipment
- microcentrifuge tubes
- Tapered forceps
- Volumetric flasks
- **Equipments for Heavy Metal**
 - Plastic bottles
 - Autosampler cups
 - Teflon vessels
 - Drying Oven
 - Whatman 41 filter paper
 - High density polypropylene bottles
 - iCAP Qc Series ICP-MS system (Bremen, Germany)
 - Milestone Ethos Up, High Performance Microwave Digestion System (Serisole, Italy).
- **Equipments for Microbiology test**
 - Sterile loopful
 - Sterile Petri
 - Sterile Swab
 - Sterile 50 ml Conical Tube
 - Sterile Serological Pipette
 - Incubator (Aerobic at $36^{\circ}\text{C} \pm 1$ and $24 - 26^{\circ}\text{C}$)
 - Autoclave (121°C 15 minutes)
 - Biological Safety Cabinet
- **Equipments for Challenge test**
 - Sterile loopful
 - Sterile Petri
 - Sterile Swab
 - Sterile 50 ml Conical Tube
 - Incubator (Aerobic at $36^{\circ}\text{C} \pm 1$ and $24 - 26^{\circ}\text{C}$)
 - Autoclave (121°C 15 minutes)
 - Biological Safety Cabinet

3.3 Sample Selection

Information about the products is available on websites that sell toys. Based on this information, the toy stores selling the most toys in Turkey were visited and the finger paints to be purchased were decided. The red and yellow colors of the products purchased in packages were separated for analysis. The separated colors were listed and coded. 24 test samples were obtained as a result of choosing the most commonly used yellow and red colors among the finger paint products of 12 brands. The finger paints, which were coded to represent popular brands and divided into color-appropriate groups, were started to be analyzed.

Codes of the Selected Yellow Color F.P		Codes of the Selected Red Color F.P		The Brand of The F.P
Group A ¹	Group B ²	Group A ¹	Group B ¹	
1AS	1BS	1AK	1BK	NA ³
2AS	2BS	2AK	2BK	NA
3AS	3BS	3AK	3BK	NA
4AS	4BS	4AK	4BK	NA
5AS	5BS	5AK	5BK	NA
6AS	6BS	6AK	6BK	NA
7AS	7BS	7AK	7BK	NA
8AS	8BS	8AK	8BK	NA
9AS	9BS	9AK	9BK	NA
10 AS	10BS	10 AK	10BK	NA
11 AS	11BS	11 AK	11BK	NA
12 AS	12BS	12 AK	12BK	NA

1: The first group of selected F.P 2: The second group of selected F.P 3: Not Applicable (Secret Information) Numbers 1-12: Numbers represents the given codes for popular brands S: Refers to Yellow colors of F.P, K: Refers to red colors of F.P.

3.4 HET-CAM

First, skin irritation analysis was performed by means of the HET-CAM (Hen's Egg Test on Chorioallantoic Membrane protocol). After the preparation of the media and colors, the analysis was started. The basis of this model is that the inflammatory processes involved in irritation (e.g., erythema, edema) depend on vascular changes, which may be monitored via the chorioallantois membrane. This method is mainly used in Europe for ocular irritancy testing; however, its basic principles can be employed for skin irritancy.

- **Experimental Design**

For each finger dye, 4 eggs were used (negative and positive controls, test substance and, if included, benchmark and solvent controls). To the extent possible, eggs from the same hen were randomized between treatment groups.

Fresh (not older than 7 days), clean, fertile 50-60 g White Leghorn chicken eggs were selected for CAM preparation. Selected eggs were mummified. Unsuitable eggs, eggs with defects, thin shells, cracked or excessively misshapen eggs were not used. During preparation, care was taken to ensure that the eggs were not shaken or bumped and that mechanical dates were avoided.

Eggs were placed in an incubator with a rotating tray. They were incubated in a still air incubator. During incubation, eggs were incubated at $38.3 \pm 0.2^{\circ}\text{C}$ and $58 \pm 2\%$ relative humidity. Eggs were turned by hand five times a day until day 8.

On day 8, the eggs were re-mummified on similar principles, and the non-viable and defective eggs were excluded from the experiment. For an additional day, eggs were returned to the incubator (without hand rotation) with the large end upwards.

On day 9, the eggs were removed from the incubator to perform the test and the non-viable and defective eggs were excluded from the experiment.

The air cell of the egg was marked. The marked part was cut and removed with a dentist's rotary saw blade. It was made sure that the inner membrane was not injured.

The inner membrane was moistened with 0.9% NaCl. A disposable glass pipette was used to apply the solution.

After 30 minutes in the incubator, the egg was removed from the incubator before analysis. 0.9% NaCl solution was drained. The inner membrane was carefully removed with forceps, making sure that the inner membrane was not injured.

- **Methods of Analyse**

Depending on the physical form of the test item, the following form-specific administration protocols were applied.

For liquid or diluted test substances or formulations, 0.3 mL of liquid or diluted substances should be applied directly to the CAM surface. For solid, particulate or granular test substances or formulations, the following procedures are required. Apply 0.3 mL of solid, particulate, or granular substances (ground to a fine powder) directly onto the CAM, ensuring that at least 50% of the CAM surface area is covered. Where the total weight of the test substance in this volume is greater than 0.3 g, 0.3 g of solid, particulate, or granular test substance shall be used. In both cases, the weight of the test substance shall be recorded. As the finger paint test samples are in a directly applicable form, the test is started with the first step.

The next stage of the test is the bonding of the test substances or formulations. 0.3 mL of the paste agent or formulations were applied directly onto the CAM. It was ensured that at least 50% of the CAM surface area was covered. The total weight of the test substance in this volume was not greater than 0.3 g.

The reactions on the CAM were then observed for 300 seconds. The appearance time of each of the indicated endpoints was determined and recorded. Observed endpoints:

- Bleeding (bleeding from veins)
- Vascular lysis (blood vessel breakdown)
- Coagulation (intra and extravascular protein denaturation)
- Bleeding time = observed onset of bleeding reactions on CAM (in seconds)
- Lysis time = observed onset of vessel lysis on CAM (in seconds)
- Coagulation time = observed onset of clot formation on the CAM (in seconds)

All endpoints were photographed to contribute to future analyses and retrospective studies and to maximize the possibility of obtaining reproducible results. Photographs that can be used as a reference for endpoints are included in the conclusion.

Finally, the results were evaluated. The numerical time-dependent scores for lysis, bleeding, and coagulation (Table 3.1) were summed to give a single numerical value indicating the irritation potential of the test item on a scale with a maximum value of 21.

Table 3.1. Scoring Scheme for Irritation Testing with the HET-CAM

Effect	Score		
	0.5 min	2 min	5 min
Lysis	5	3	1
Haemorrhage	7	5	3
Coagulation	9	7	5

For retrospective analyses, data from the HET-CAM test method protocol using the IS(B) analysis method (ICCVAM 2006) could be converted to fixed time points like those used for the IS(A) analysis method.

A test is considered acceptable if the negative and positive controls each induce a response that falls within the classification of nonirritating and severely irritating, respectively. Historical control studies indicate that using 0.9% NaCl, as a negative control, the IS value was 0.0. Historical control studies indicate that using 1% SDS and 0.1 N NaOH, as positive controls, the IS values ranged between 10 and 19, respectively.

When using the IS analysis method, the severe irritancy classification for a test substance is assigned when the value is greater than nine.

3.5 Heavy Metal Analysis

Samples from the same sample container were prepared for analysis of heavy metal contents. Heavy metal levels were determined by spectrophotometric analysis. Atomic absorption spectroscopy is the method used to calculate heavy metal levels.

Heavy metal (As, Cd, Ni, Sb, Pb, Hg) content was analyzed in finger paint samples. An Inductively coupled plasma mass spectrometry (ICP-MS) technique with a Thermo Scientific iCAP Qc Series (Bremen, Germany) was used for metal investigations. Digestion was carried out using Milestone Ethos Up, High Performance Microwave Digestion System (Serisole, Italy).

All reagents were of analytical grade. All aqueous solutions were prepared with deionized water obtained by using ultra-pure water system (Aqua Nova Hepta Distillated, resistivity 0.34 MΩ-cm, Kristianstad, Sweden). HNO₃ (65 %) and HCl (37 %) from Merck supra-pure grade (Darmstadt, Germany) were used for digestion of the samples and dilution. Plastic bottles, autosampler cups, Teflon vessels, vials for collecting samples and glassware were cleaned by soaking in HNO₃ (10 % v/v) for a day, rinsing four times with ultra-pure water and drying in an oven at 40 °C. All prepared solutions were stored in high density polypropylene bottles. Stock standard solutions of analytes (1 g/L each) were obtained from Merck. Mixed standard solutions were freshly prepared by diluting the stock standard solutions to the desired calibration ranges in 0.2 % HNO₃.

The samples were analyzed using a method based on ICP-MS using acidic microwave digestion. The dyes were made ready for measurement by subjecting them to microwave acid digestion. Briefly, approximately 400 mg of each sample was taken into Teflon vessel and digested with 8 mL 65 % HNO₃ and 1 mL 37 % HCl (Table 1). Filtration was performed on Whatman 41 filter paper to remove insoluble particles and samples were transferred to polypropylene tubes. Then, 1 mL was taken from each sample and the final volume was brought to 10 mL with ultra-pure water. The blind sample is prepared in a similar way. The samples were stored at +4°C in polypropylene tubes with lids until the analysis. The operation parameters for the investigated elements were set as recommended by the manufacturer (Table 3.2).

Table 3.2. Test Standard Conditions

Parameter	Value
The microwave oven program	
Maximum power (Watts)	1600
% Power	100
Time (minutes)	15
Pressure (bar)	90
Temperature (°C)	200
Standby (minutes)	15
Instrument operating parameters	
Peristaltic pump speed	40 rpm

Pump tubing	orange/green tubing for both carrier (sample) and internal standard
Nebulizer	PFA-ST
Interface cones	Nickel
RF Power	1550 W
Cool gas flow	14 L/min
Auxiliary gas flow	0.8 L/min
Nebulizer gas flow	0.97 L/min
Number of sweeps	20
Dwell Times	0.001 - 0.02 ms
Replicates per analysis	3

The concentrations of As, Cd, Ni, Sb, Pb and Hg elements were determined with an ICP-MS (Thermo Scientific ICAP Qc Series, Bremen, Germany). A solution of mixed internal standards (IS), including arsenic (As), cadmium (Cd), nickel (Ni), antimony (Sb), lead (Pb) and mercury (Hg) was used for calibration procedures. The standards were prepared using the serial dilution technique between 0.025-10 ppm for mercury and 0.1-10 ppm for nickel, arsenic, cadmium, antimony, and lead.

3.6 Microbiological Test

Samples from the same sample container were prepared for microbiological analysis and the samples were subjected to microbiological analysis according to European Pharmacopoeia 6.0 01/2008:20601.

Microbiological analysis is the use of biological, biochemical, molecular, or chemical methods to detect, identify or enumerate microorganisms in a material. It is generally applied to disease-causing and spoilage microorganisms.

Methods used within the framework of microbiological analyzes;

- **Aerobic Plate Count (APC):** This test determines the total number of aerobic bacteria present per mL or gram of personal care or cosmetic product. The test is performed for 48-72 hours.

- **Yeast and Mold Count:** This test determines the total number of yeasts and fungi present per mL or gram of personal care or cosmetic product. It is performed over five days.
- **Enrichment Method for Specific Pathogen Screening:** This test determines whether any harmful bacteria are present (even in very low numbers or quantities). The product is enriched by growing in the enrichment water for seven days. Thus, pathogenic bacteria in the product, although present in very low numbers, multiply and become detectable.

- **Enumeration of Aerobic Mesophilic Bacteria, Yeast and Mould**

1g of product sample was transferred into 9 ml of diluent, using the direct inoculation of the culture medium method. 1 ml of the suspension was spreaded onto the surface of the agar growth medium, using the surface spread method and then incubated at 20-25°C and 30-35°C for 3 - 5 days (Table 3.3).

Table 3.3. Enumeration of Microorganisms

Test Microorganisms	Diluent	Agar Growth Medium	Incubation Conditions
Enumeration of Aerobic Mesophilic Bacteria	Soybean Casein	Soybean Casein	Aerobic at 30-35°C
	Digest Broth	Digest Agar	3 days
Enumeration of Yeast and Mould	Sabouraud Dextrose	Sabouraud Dextrose	Aerobic at 20- 25°C
	Broth	Agar	5 days

- **Detection of Specific Microorganisms**

The enrichment was prepared from a sample of at 1 g of the well-mixed product under test, which was dispersed in 9 ml of enrichment broth. The suspension was incubated at 37°C for at least 20 h. Using a sterile loop, an aliquot of the incubated enrichment broth was streaked on the surface of selective agar medium to obtain isolated colonies and then incubated at 37°C for 24 h to 48 h. Characteristic colonies were checked (Table 3.4).

Table 3.4. Detection of Specific Microorganisms

Microorganism	Enrichment Broth	Selective Agar Medium
Staphylococcus aureus ATCC	Eugon LT 100 Broth	Baird Parker Agar Medium

<i>Pseudomonas aeruginosa</i> ATCC 9027	Eugon LT 100 Broth	Cetrimide Agar Medium
<i>Escherichia coli</i> ATCC 11229	Eugon LT 100 Broth	Macconkey Agar Medium
<i>Candida albicans</i> ATCC 10231	Sabouraud Dextrose Broth	Sabouraud Dextrose Chloramphenicol Agar Medium
<i>Aspergillus brasiliensis</i> ATCC 16404	Sabouraud Dextrose Broth	Sabouraud Dextrose Agar Medium

3.7 Challenge-Sterility Test

Samples from the same sample container were prepared and the challenge test performed through the Efficacy of Antimicrobial Preservation according to European Pharmacopoeia 7.0 01/2011:50103.

Once organisms are isolated from a product and a manufacturer wants to know which organisms they are, they order the Microbial Identification Test of their products. Isolates are identified starting with observing their morphology. Isolates are Gram stained and observed under the microscope. This gives an initial clue about whether they are Gram negative or Gram positive. Isolates are then identified by Commercial identification kits, e.g., API.

This test looks for gram-negative organisms (*Pseudomonas aeruginosa*, *E. coli*, *Salmonella*, *Serratia*, *Enterobacter*, *Klebsiella pneumoniae* and others) and gram-positive organisms (*Staphylococcus* (cocci), *Streptococcus* (cocci), *Enterococcus* ((diplococci), *Bacillus* (rod-shaped), *Clostridium* (rod-shaped), *Listeria* (bacilli/rods) and others) of medical interest.

The test sample was contaminated with microorganisms shown in Table 3.5 at a concentration of 10^5 cfu/ml. The inoculated sample was shaken well to obtain a homogeneous distribution. For the 0th hour, 1 ml sample was taken from each container and incubated. Inoculated samples were stored at ambient temperature (20–25°C) avoiding sunlight. 1 ml of the inoculated sample on the 2nd, 7th, 14th and 28th days were plated on agar medium, and the logarithmic reduction of the microorganisms were calculated at the end of incubation.

Table 3.5. Incubation of Test Microorganisms

Test Microorganisms	Test Agar Medium	Incubation Conditions
<i>Staphylococcus aureus</i> ATCC 6538	Soybean Casein Digest Agar	30-35°C
<i>Pseudomonas aeruginosa</i> ATCC 9027		
<i>Escherichia coli</i> ATCC 11229		
<i>Candida albicans</i> ATCC 10231	Sabouraud Dextrose Agar	20-25°C
<i>Aspergillus brasiliensis</i> ATCC 16404		

3.8 Safety Assessment

Next step is the making a safety assessment of these amounts detected in finger paints. MoS Calculations require the calculation of the daily amount applied as well as the frequency of application based on the EU Cosmetics Regulation. SED (Systemic Exposure Dose) and MOS (Margin of Safety) calculations are performed to see an assessment of the safety of the finger paints analysed.

Safety assessment: For a cosmetic product; It refers to the evaluation report made on the finished product in accordance with Article 12 of the Cosmetics Regulation, [121] taking into account the toxicological character, chemical structure and exposure levels of the product components, the specific exposure characteristics of the target group to which the product is offered for use or the area where the product will be applied.

Exposure assessment: Human exposure is calculated with appropriate data according to the specified functions of the cosmetic product and the use of the cosmetic ingredient, the amount present in the product and the frequency of use. Determination of the level of exposure is done by calculating the amount that enters the systemic circulation and is subsequently distributed to tissues/organs, i.e. by calculating the systemic exposure dose (SED) for cosmetic products. Single product exposure refers to a single route exposure to a single cosmetic ingredient in a product category. Multiple exposure refers to the sum of single exposures to the same ingredient as specified in the guidelines. It therefore describes exposure to the same cosmetic ingredient in all product categories through all routes of exposure. The amount or percentage of absorption through the skin needs to be determined. Where necessary, a detailed assessment should be made for sensitive consumer groups (pregnant women, children).

The systemic exposure dose (SED) is calculated considering dermal exposure only. It is the amount of the cosmetic ingredient expected to enter the bloodstream. The unit is expressed in mg/kg body weight/day. The SED can be calculated in two ways, depending on the way dermal absorption is reported (knowing the amount of dermal absorption per unit surface ($\mu\text{g}/\text{cm}^2$) and knowing the amount of dermal absorption in % of the applied product). It is considered that not more than 50% of an orally administered dose is systemically available. Thus, in the absence of data, 50% of the administered dose is used as the default oral absorption value for a cosmetic ingredient and the PoDsys is derived from the PoD by dividing with a factor 2. If there is information to suggest poor oral bioavailability, a default value of 10% oral absorption could be considered.

A total value of 17.4 g/day or 269 mg/kg body weight/day is reported as the most serious rate used in MoS calculation. Acceptable levels for finger paint and the SED calculation formula are shared in the rest of the content [122]

In terms of application and exposure, the type closest to finger paint is observed in the hand cream product (Table 3.6)

Table 3.6 Sample Evaluation: Hand Cream

Tip	Hand cream
Type Estimated Amount Applied Daily q_x (g/day) (Amount of product exposure)	2,16 g
Relative Amount Applied (mg/kg bw/day) q_x	32,7
Retention Factor (Frequency of application) F_{ret}	1
Calculated Daily Exposure (g/day)	2,16
Calculated Relative Daily Exposure (mg/kg/weight/day (A)/ ($E_{product}$))	32,7

MoS (Margin of Safety): It is the limit of safety. The margin of safety (MoS) for an ingredient is calculated by dividing the value found in a toxicological effect study on a group of test animals by the systemic exposure dose of that ingredient. In order for animal test results to represent human results, these values must be converted to human values. The value of 100, which is accepted as the uncertainty factor used for this purpose, is obtained by multiplying the value of 10, which is assumed for the transition from experimental animals to humans, and the value of 10, which is assumed to express individual differences between humans ($100=10*10$). Therefore indicates that a cosmetic

ingredient is considered safe for use. Systemic effects and the limit of safety (MoS) are calculated based on the NO(A)EL, LOAEL, BMD values determined in the light of the toxicological data observed as a result of the experiments, taking into account all significant absorption pathways. If this assessment is not possible, the reason should be appropriately justified. The final step in the safety assessment of cosmetic ingredients is the MoS calculation. It is usually the ratio between NO(A)EL or BMD values from oral exposure studies (PODsys) and estimated exposure. For a study with mostly dermal exposure, only a repeated dose toxicity study at oral exposure can be used as a proxy. For comparison with PODsys, an SED for the dermal route is usually derived as an estimate of exposure.

NO(A)EL (No Observed Adverse Effect Level): The results of long-term repeated dose chronic toxicity, carcinogenicity, teratogenicity test studies such as 28 days, 90 days in rats, mice, rabbits or dogs. The highest application amount expressed in mg/kg body weight/day of the substance in which no undesirable effect is observed due to the application,

For the relationship between the exposure and the toxic response, a Point of Departure (PoD) is determined.

POD (Point of Departure): Dose-dependent response point to be used in risk assessment for threshold and non-threshold compounds.

PODsys: Dose-dependent response point for risk assessment of systemic exposure to a substance calculated from the oral POD using the proportion of a substance absorbed systemically. [122]

In most Opinions a No Observed Adverse Effect Level (NOAEL) has been used as PoD. The SCCS considers that, where usable *in vivo* data are available, the preferred method for both threshold and non-threshold cosmetic ingredients is to express the dose metric as BenchMark Dose (BMD). Both the European Food Safety Agency (EFSA) and the World Health Organization (WHO) also recommend that the BMD approach for deriving the PoD should be used as a starting point for human health risk assessment.

The BMD approach should preferentially be used as the dose descriptor for the PoD and the MoS calculations (EFSA, 2009). When no BMD can be calculated, only then the historical NOAEL values should be applied. Because the BMD approach has a

number of advantages over using NOAEL. It makes complete use of the available dose - response data. [122]

- it takes into account the shape of the dose - response curve
- it is less dependent on dose spacing
- it enables quantification of the uncertainties in the dose - response data using statistical methodology [122].

If a BMD or a NOAEL cannot be identified from the available data, other dose descriptors such as the Lowest Observed (Adverse) Effect Level (LOAEL) may be used in the MoS calculation.

For compounds with a threshold, the PoD can be a NOAEL, a Lowest Observed Adverse Effect Level (LOAEL), or a BMD Lower limit (BMDL).

For non-threshold effects (e.g. a non-threshold carcinogenic effect), the lifetime risk is often based on the BMD10 (benchmark dose response for a 10% response).

When the PoD is based on a LOAEL, often an additional assessment factor of 3 is added in the calculation of the MoS.

In case a 90-day repeated dose toxicity study is not available, a NOAEL or BMDL from a 28-day repeated dose toxicity study can be used in the MoS calculation for a cosmetic ingredient. In this case, a default assessment factor of 3 for exposure duration may be used in the calculation of the MoS [122].

It may not be possible to find NOAEL values for all product components. In this case, NOAEL values should be determined using alternative methods. The more MoS values that can be calculated for the product components in a formulation, the better the safety assessment. It is particularly important that MoS values of active substances can be calculated. However, if the MoS values of the product components cannot be calculated to a large extent because NOAEL values cannot be reached, this assessment should be supported by skin irritation, eye irritation (mucous membrane irritation), skin sensitivity tests according to the application site and the intended use of the product.

- **Systemic exposure dose (SED) Calculation**

$$SED = (DA_a (\mu\text{g}/\text{cm}^2) * 10^{-3} \text{mg}/\mu\text{g} * SSA (\text{cm}^2) * f (\text{day}^{-1})) / \text{Body weight}$$

SED (mg/kg body weight/day): Systemic exposure dose.

DA_a (μg/cm²): Amount of absorption through the skin per unit surface area (obtained from an experiment simulating conditions of use).

SSA (cm²): Skin surface area contacted by the finished product (860cm²).

The ratio between the SSA/BW (The surface area/body weight) ratios of children and adults changes from 0 to 10 years and is as follows; 2.3-fold higher in newborns than in adults, changing to 1.8-fold, 1.6-fold, 1.5-fold and 1.3 fold at 6 and 12 months and at 5 and 10 years respectively. This is in general covered by the intra-species factor of 10 (3.2 x 3.2) used in the calculation of MoS [122].

f (day⁻¹): Frequency of application of the finished product (2/day)

Body weight (kg): Adult human weight considered to be standard (60 kg). Since the average weight of 3-year-old children is between 13-14 kg, 13.5 kg was assumed.

- **Calculation of the Limit of Safety (MoS)**

$$MoS = \frac{(POD_{sys} (NO(A)EL / BMDL))}{SED}$$

Products with a toxicological limit of safety (MoS) of substances above 100 are considered safe for adults and for children, from birth; newborn 230, 6 months 180, 12 months 160, 5 years 150, up to 10 years 130 considered as safe. Since this safety assesment performed for the children's finger paints and the targeted population by considering that it is suitable for use in children over 3 years old, the margin of safety is accepted as 160 which refers to 12 months babies in order to make worst case scenario.

- **Exposure to the Finger Paints**

1) The site(s) of application: area hands

2) The surface area(s) of application (cm²): 860 cm²

3) The amount of product applied (g): 2,16 g

4) The duration and frequency of use: 2/day.

5) The normal and reasonably foreseeable exposure route(s): Exposure with direct skin and Rinse-off.

6) The targeted (or exposed) population(s). Potential exposure of a specific population shall also be taken into account: The targeted population is Children.

7) Possibility of secondary exposure: There is no possibility of secondary exposure way.

- **Exposure to the Heavy Metal content of Finger paints**

$$SED = A \text{ (mg/kg body weight/day)} \times C(\%) / 100 \times DAp (\%)/100$$

SED (mg/kg body weight/day): The Systemic Exposure Dosage of a cosmetic ingredient is the amount expected to enter the blood stream (and therefore be systemically available) per kg body weight and per day. It is expressed in mg/kg body weight/day.

A (mg/kg body weight/day): Estimated daily exposure to a cosmetic product per kg body weight, based upon the amount applied and the frequency of application.

C(%): Concentration of the ingredient under study in the finished cosmetic product on the application site

DAp (%): Dermal Absorption expressed as a percentage of the test dose assumed to be applied in real-life conditions.

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4. RESULTS

The results of the research are shared in the following section of the content in the order in which they were presented in the materials and methods section.

4.1 HET-CAM results

Tabulation of data from individual test samples (e.g., irritancy scores for the test substance and the various controls, including data from replicate repeat experiments as appropriate, and means and $\pm$ the standard deviation for each test).

1AS and 1AK. Which mean; 1AS is to represent the first study finger paint brand with the yellow color and the 1AK is represent the first study finger paint brand with the red color.

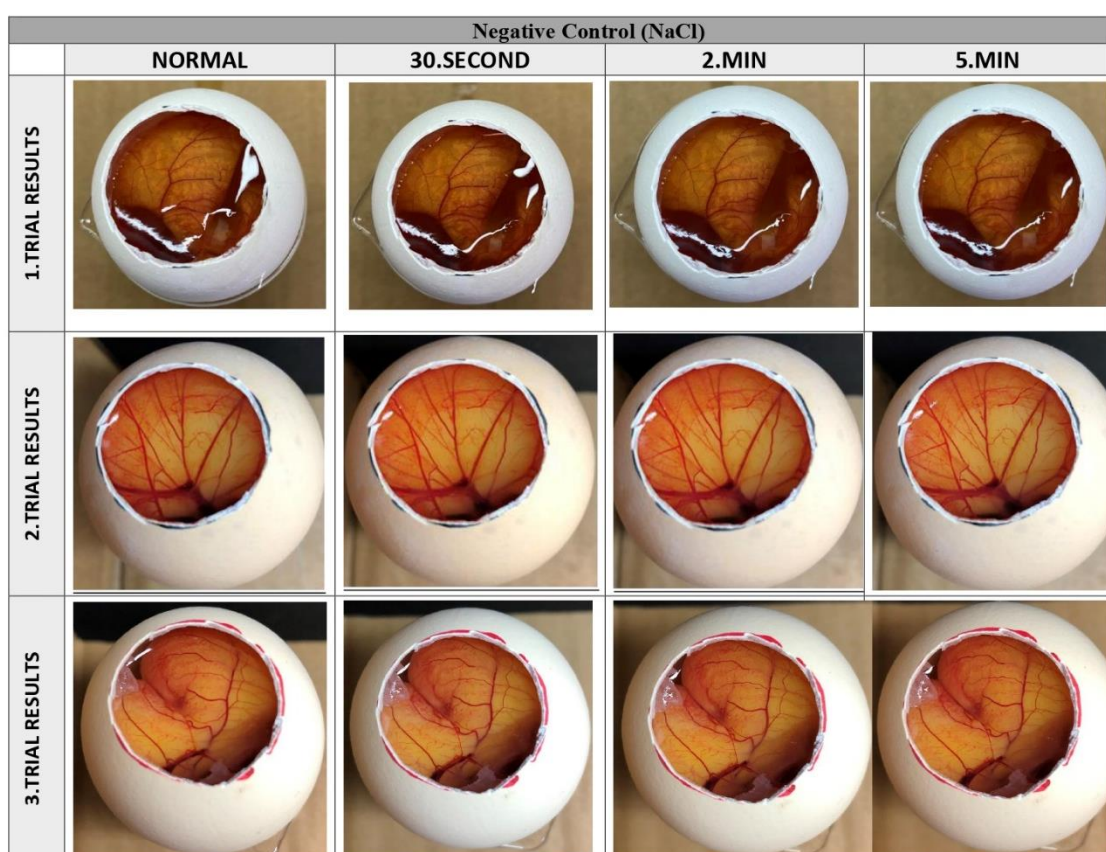


Figure 4.1. Negative Control 1-3 Trial

Table 4.1. HET-CAM Result for NaCl (Negative Control) of 1-3 Trial

	Effect	Score 0		
		0,5 min.	2 min.	5 min.
NC-NaCl	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

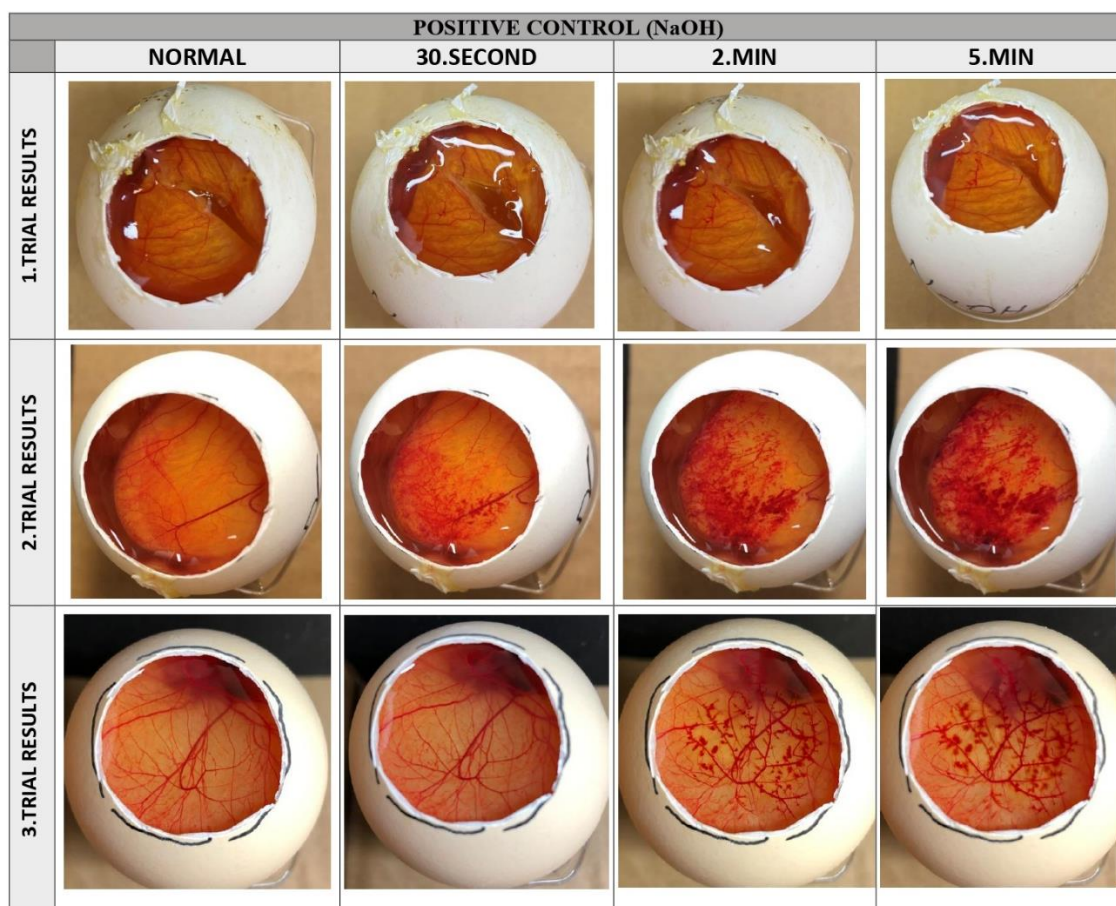


Figure 4.2. Positive Control 1-3 Trial

Table 4.2. HET-CAM Results for NaOH (Positive Control) of 1-3 Trial

1st	Effect	Score 19		
		0,5 min.	2 min.	5 min.
NaOH (PC)	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

2nd	Effect	Score 19		
		0,5 min.	2 min.	5 min.
NaOH (PC)	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

3rd	Effect	Score 19		
		0,5 min.	2 min.	5 min.
NaOH (PC)	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

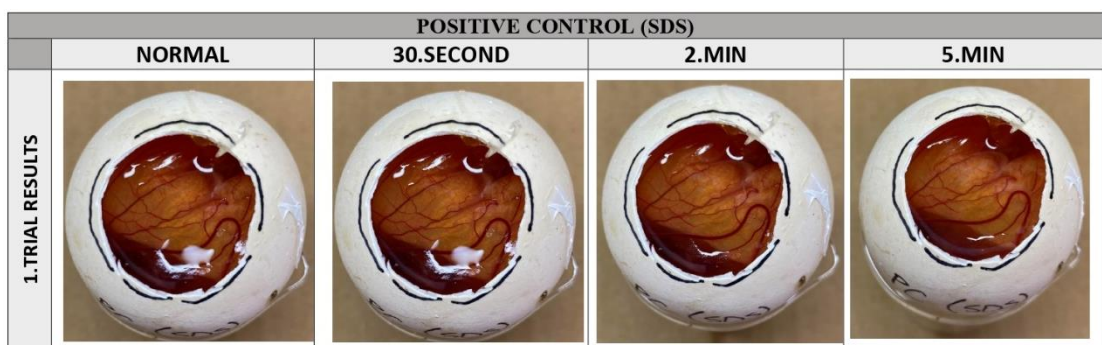


Figure 4.3. Positive Control 1st Trial

Table 4.3. HET-CAM Result for SDS (Positive Control) of 1st Trial

	Effect	Score 0		
		0,5 min.	2 min.	5 min.
SDS (PC)	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

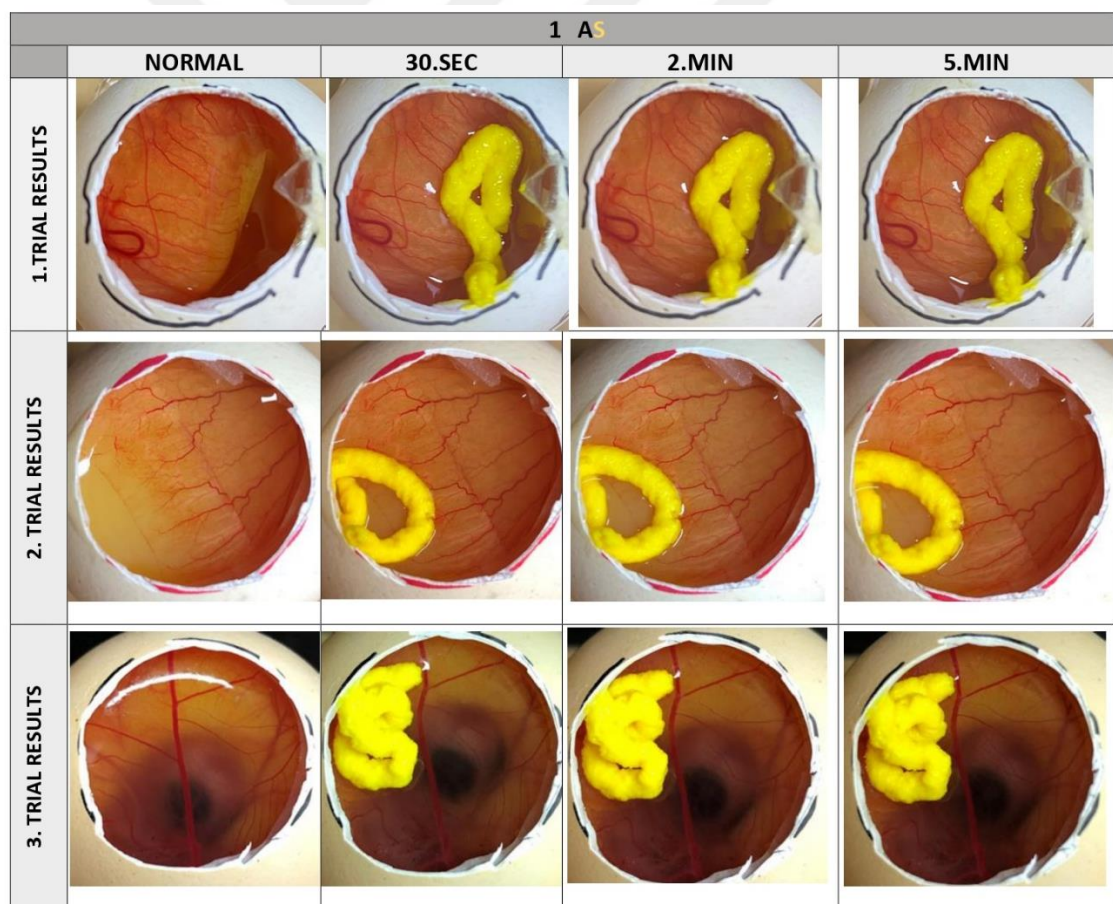


Figure 4.4. 1 AS Yellow 1-3 Trial

Table 4.4. HET-CAM Result for 1 AS Yellow of 1-3 Trial

1 AS 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

1 AS 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

1 AS 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

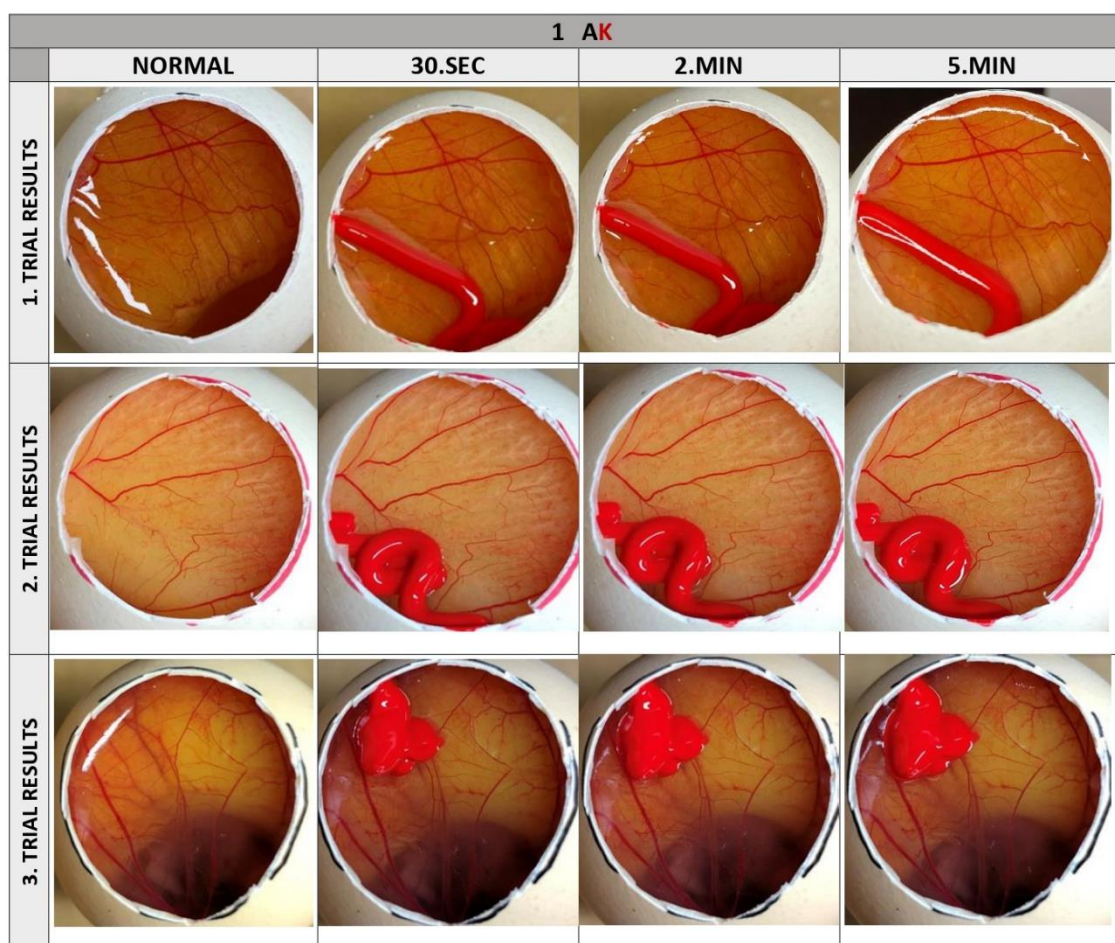


Figure 4.5. 1 AK Red 1-3 Trial

Table 4.5. HET-CAM Result for 1 AK Red of 1-3 Trial

1AK 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

1AK 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

1AK 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

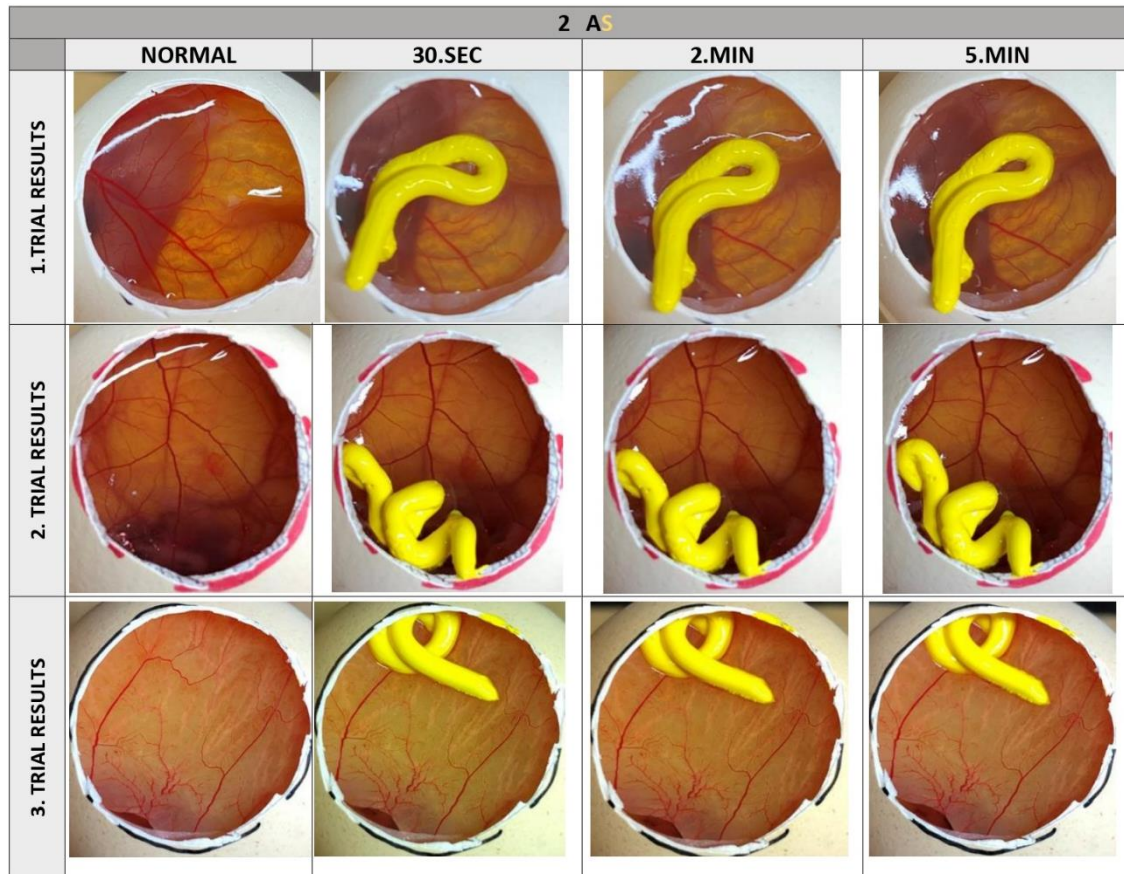


Figure 4.6. 2 AS Yellow 1-3 Trial

Table 4.6. HET-CAM Result for 2 AS Yellow of 1-3 Trial

2AS 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

2AS 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

2AS 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

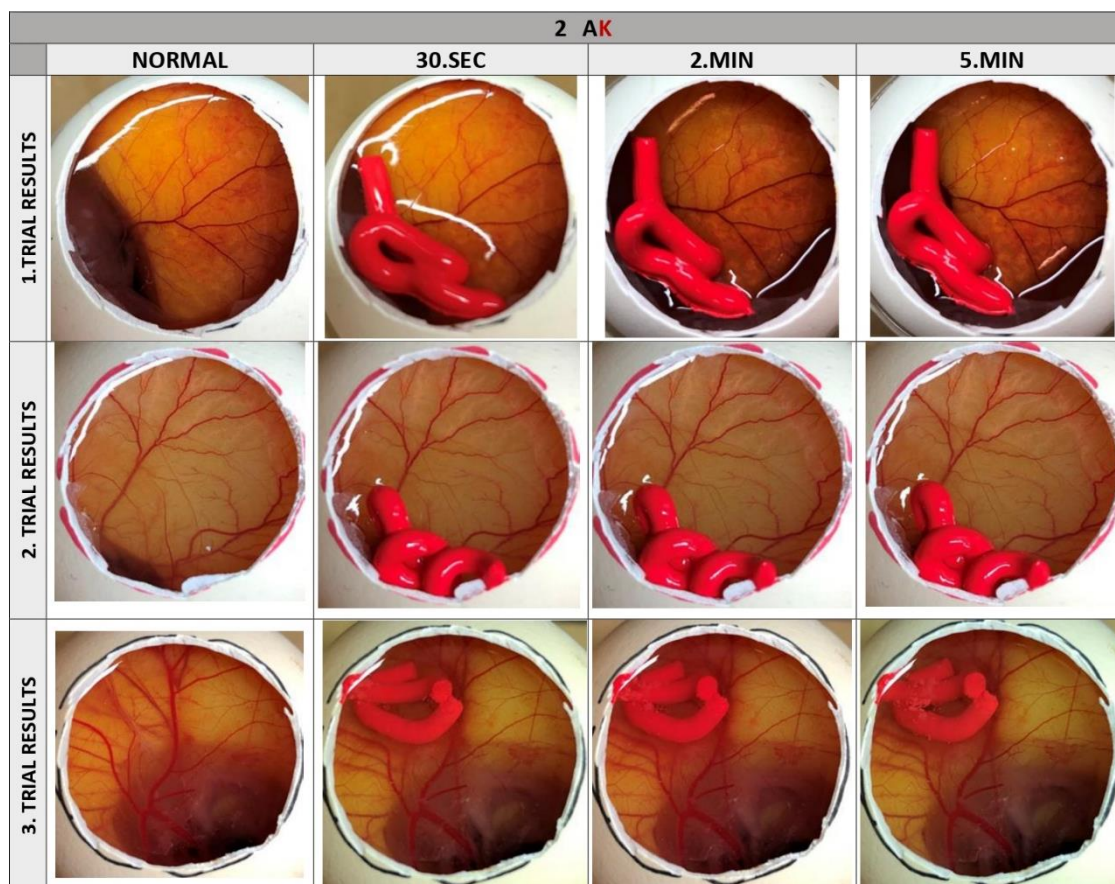


Figure 4.7. 2 AK Red 1-3 Trial

Table 4.7. HET-CAM Result for 2 AK Red of 1-3 Trial

2AK 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

2AK 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

2AK 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

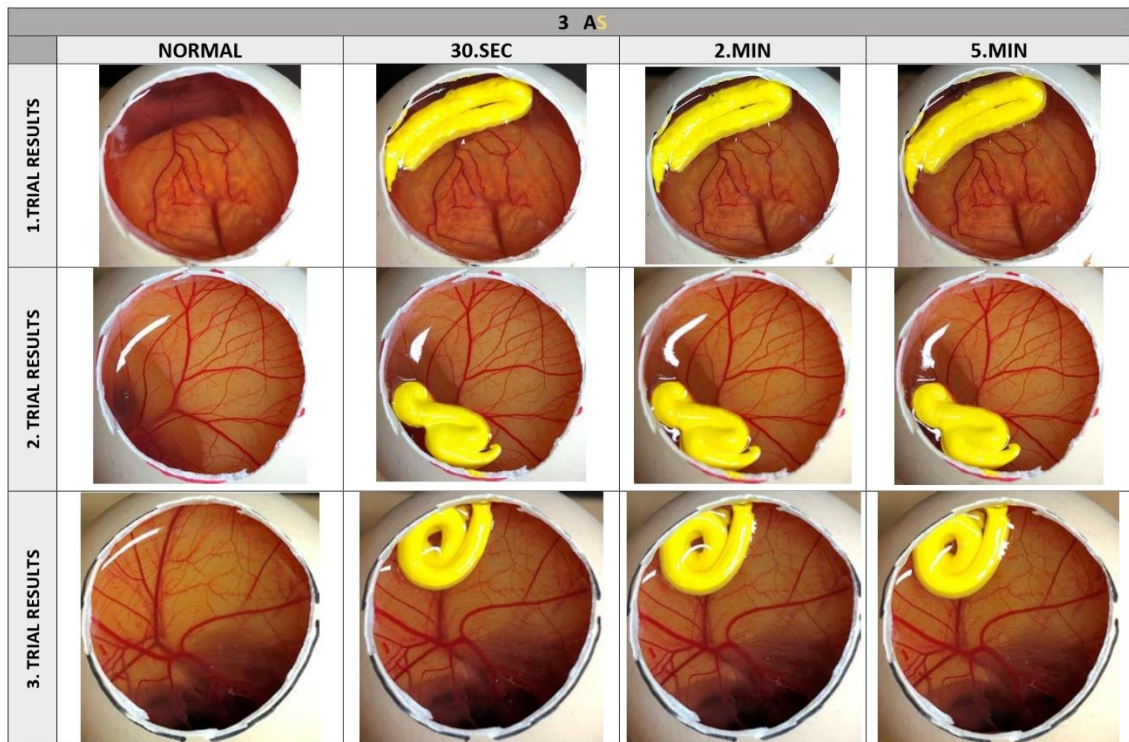


Figure 4.8. 3 AS Yellow 1-3 Trial

Table 4.8. HET-CAM Result for 3 AS Yellow of 1-3 Trial

3AS 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

3AS 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

3AS 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

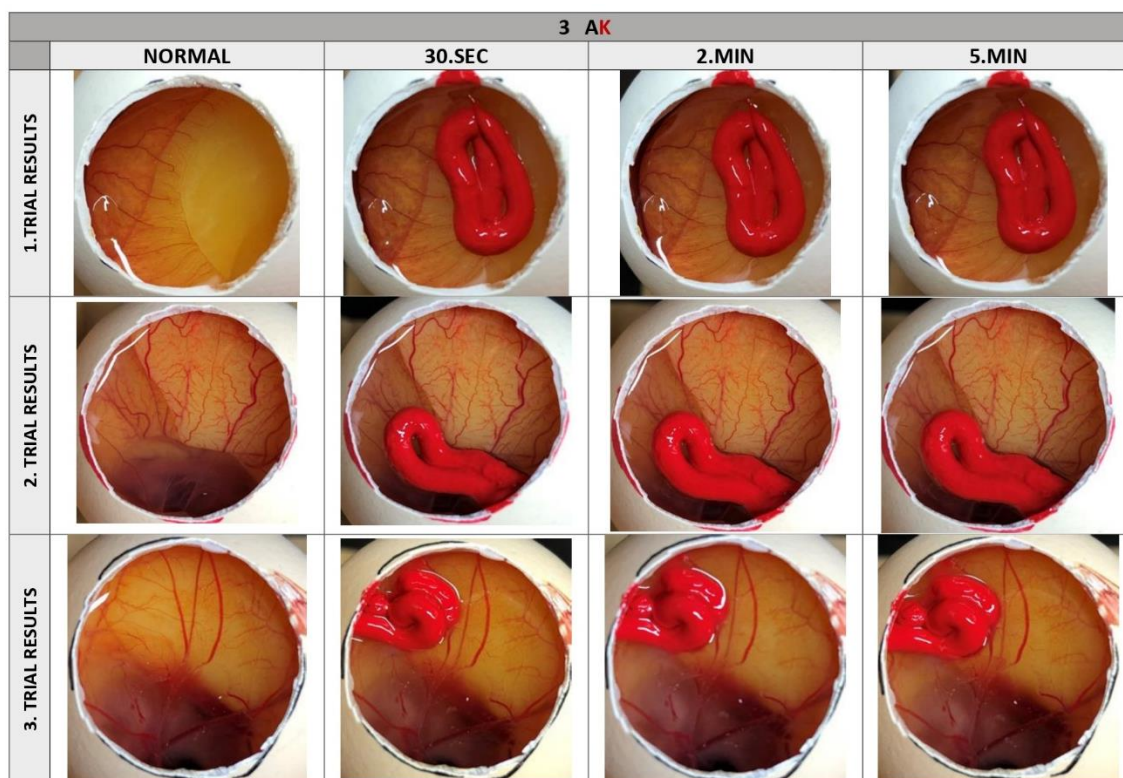


Figure 4.9. 3 AK Red 1-3 Trial

Table 4.9. HET-CAM Result for 3 AK Red of 1-3 Trial

3AK 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

3AK 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

3AK 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

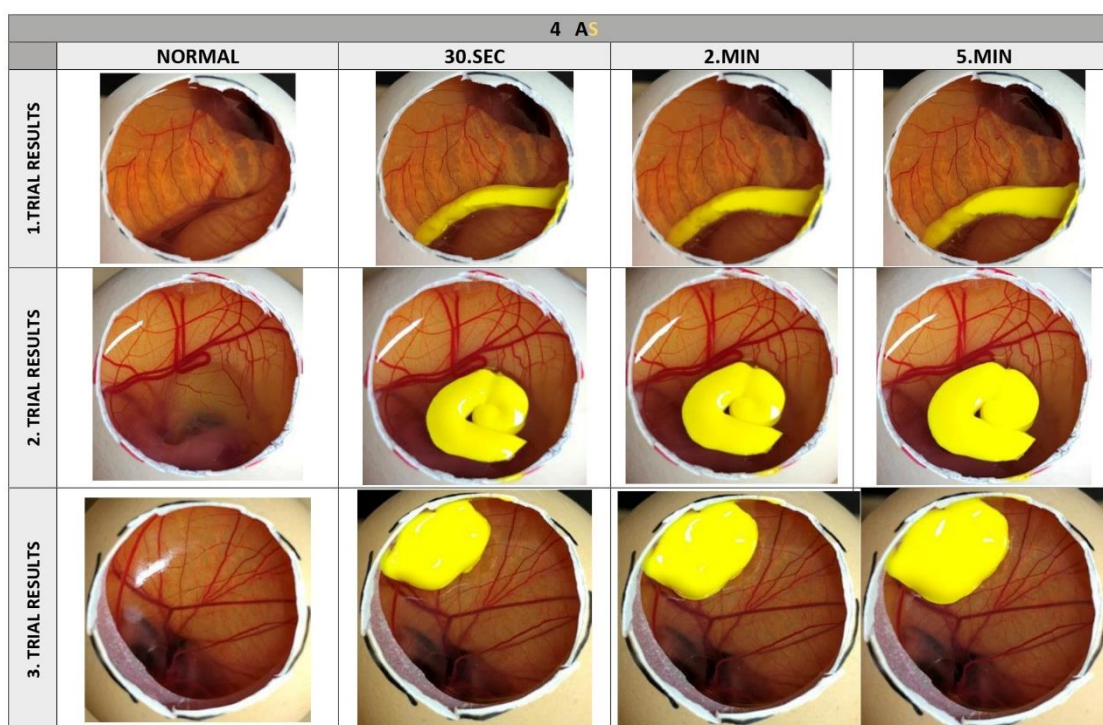


Figure 4.10. 4 AS Yellow 1-3 Trial

Table 4.10. HET-CAM Result for 4 AS Yellow of 1-3 Trial

4AS 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

4AS 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

4AS 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

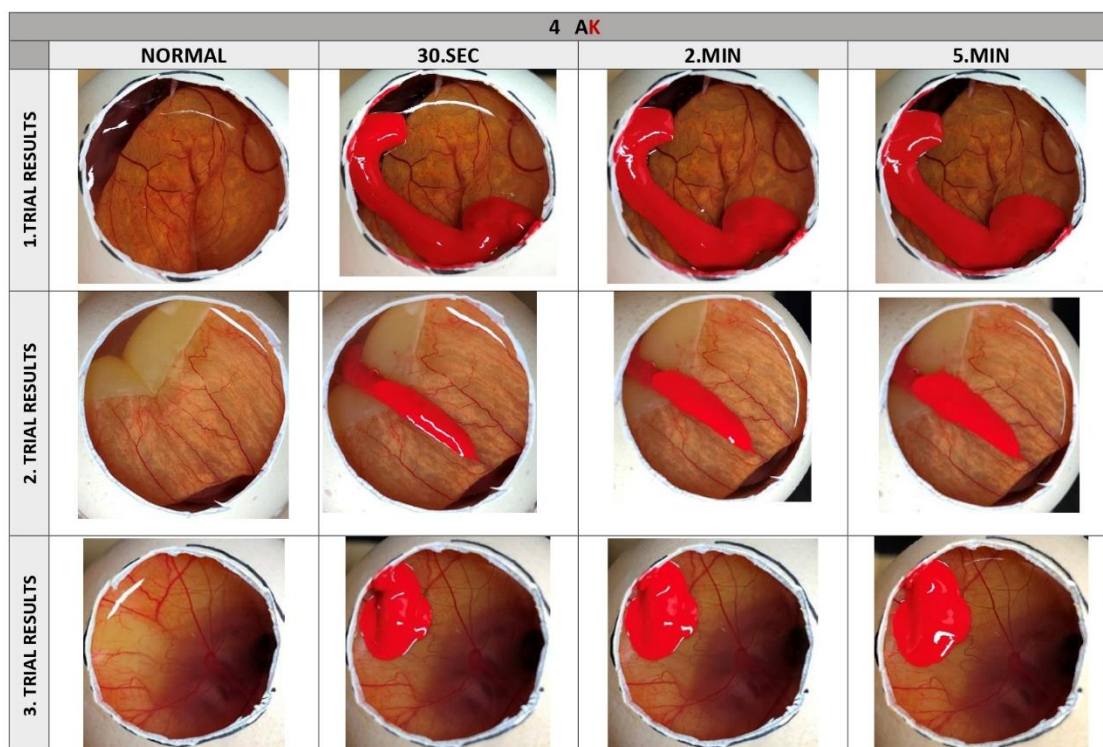


Figure 4.11.– 4 AK Red 1-3 Trial

Table 4.11. HET-CAM Result for 4 AK Red of 1-3 Trial

4AK 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

4AK 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

4AK 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

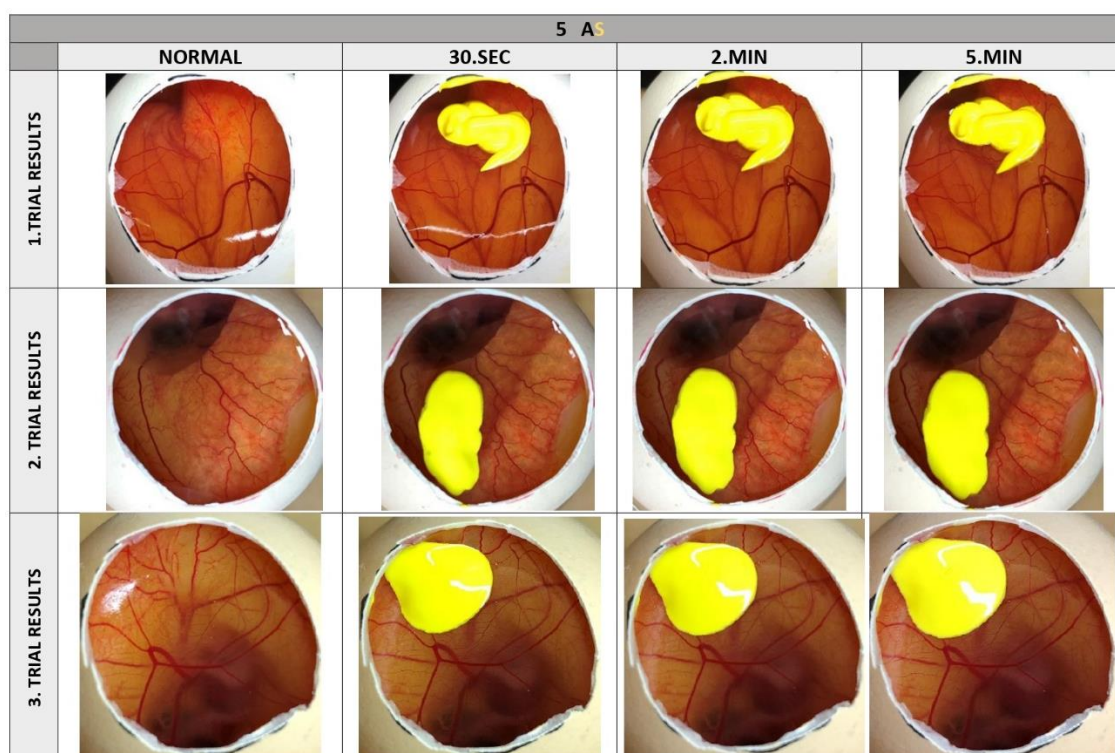


Figure 4.12.– 5 AS Yellow 1-3 Trial

Table 4.12. HET-CAM Result for 5 AS Yellow of 1-3 Trial

5AS 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
5AS 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
5AS 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
5AS 4 th Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3

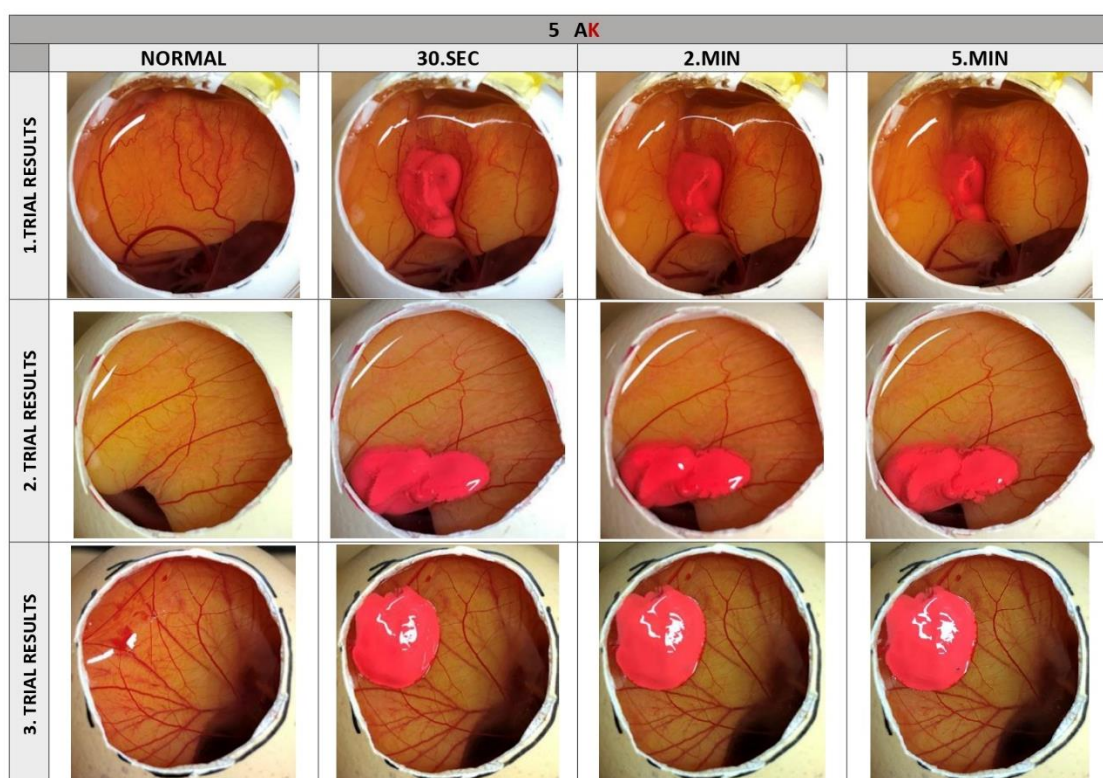


Figure 4.13. 5 AK Red 1-3 Trial

Table 4.13. HET-CAM Result for 5 AK Red of 1-3 Trial

5AK 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

5AK 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

5AK 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5



Figure 4.14. 6 AS Yellow 1-3 Trial

Table 4.14. HET-CAM Result for 6 AS Yellow of 1-3 Trial

6AS 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

6AS 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

6AS 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

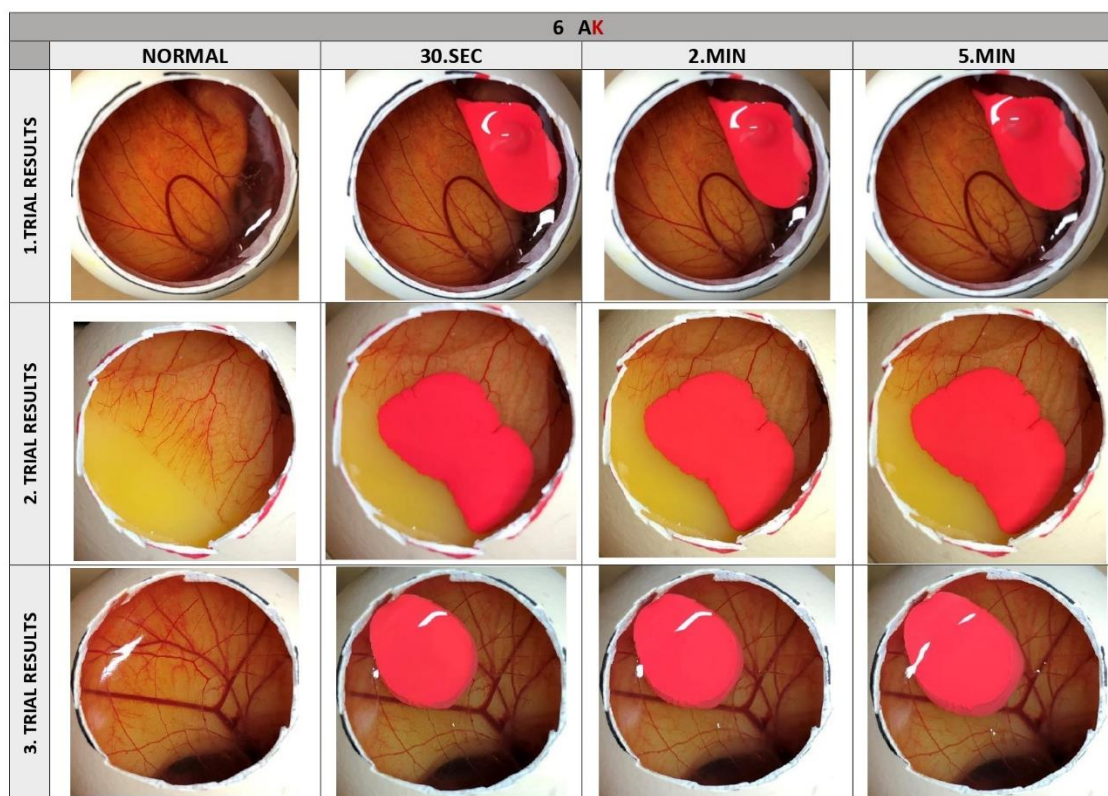


Figure 4.15.– 6 AK Red 1-3 Trial

Table 4.15. HET-CAM Result for 6 AK Red of 1-3 Trial

6AK 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

6AK 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

6AK 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

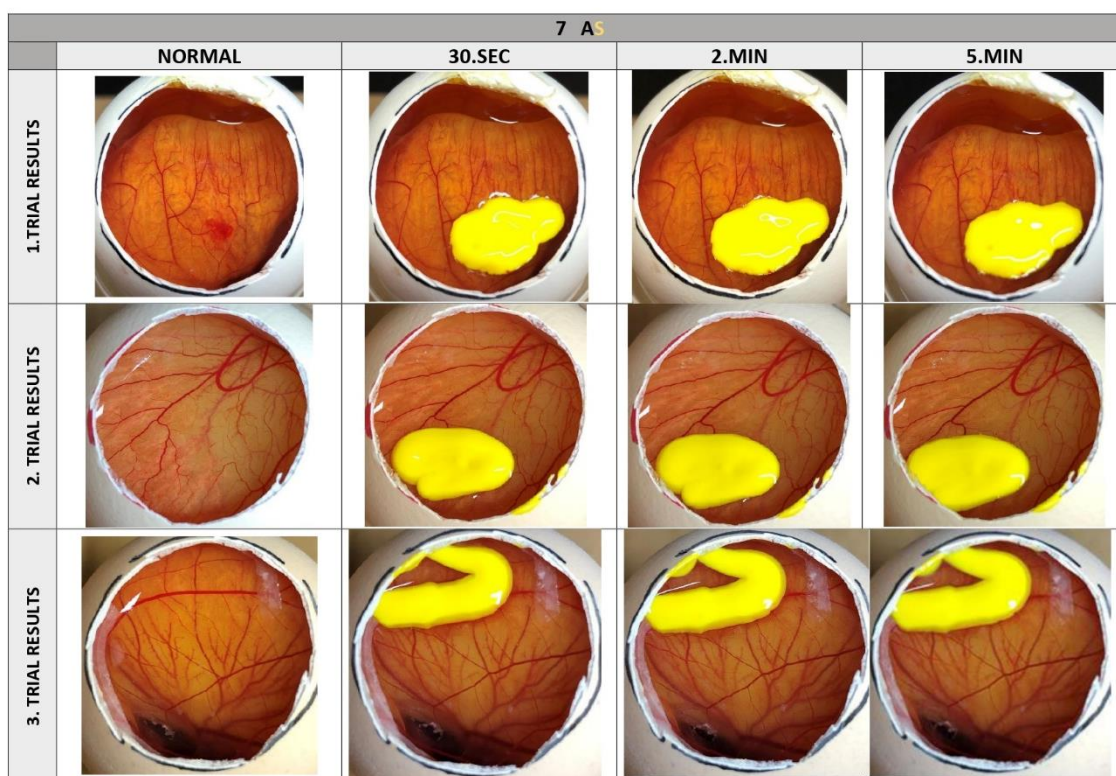


Figure 4.16. 7 AS Yellow 1-3 Trial

Table 4.16. HET-CAM Result for 7 AS Yellow of 1-3 Trial

7AS 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

7AS 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

7AS 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

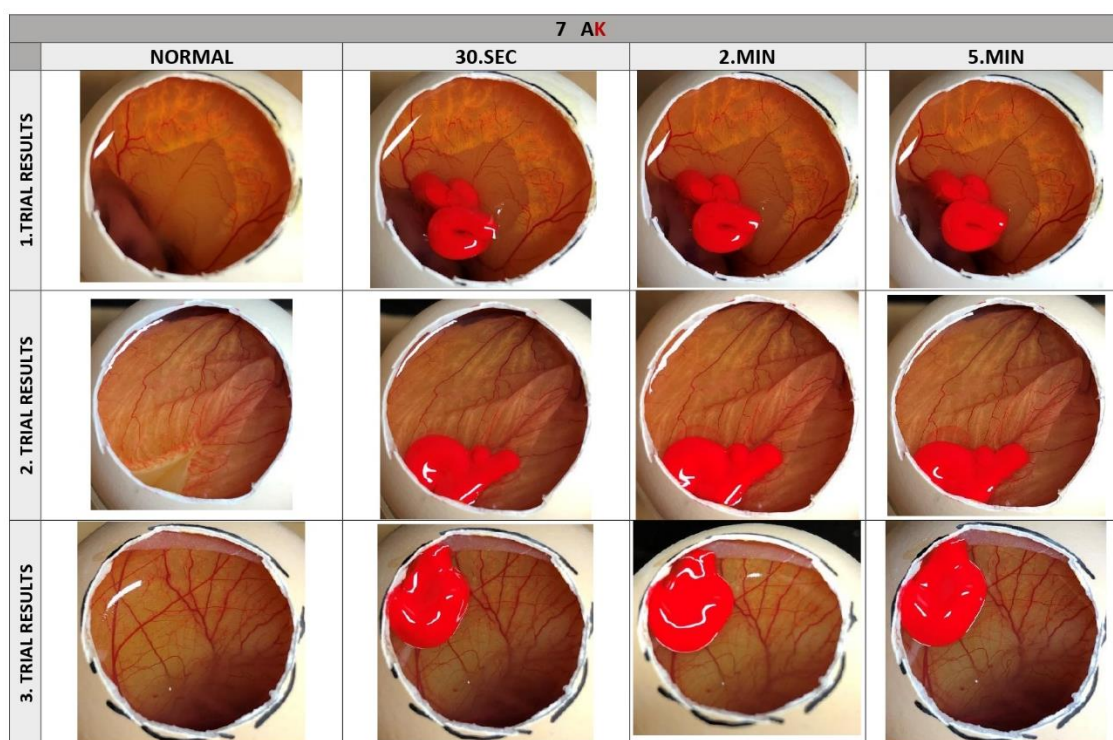


Figure 4.17.– 7 AK Red 1-3 Trial

Table 4.17. HET-CAM Result for 7 AK Red of 1-3 Trial

7AK 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

7AK 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

7AK 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

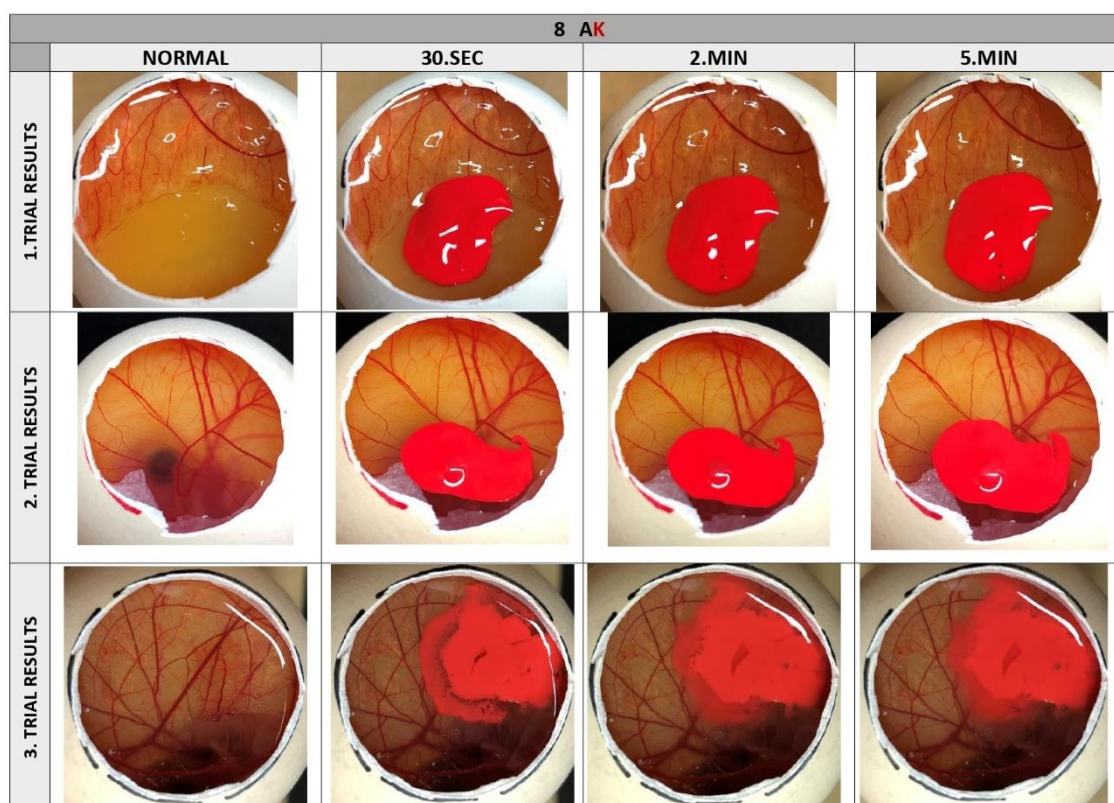


Figure 4.19. 8 AK Red 1-3 Trial

Table 4.19. HET-CAM Result for 8 AK Red of 1-3 Trial

8AK 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

8AK 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

8AK 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

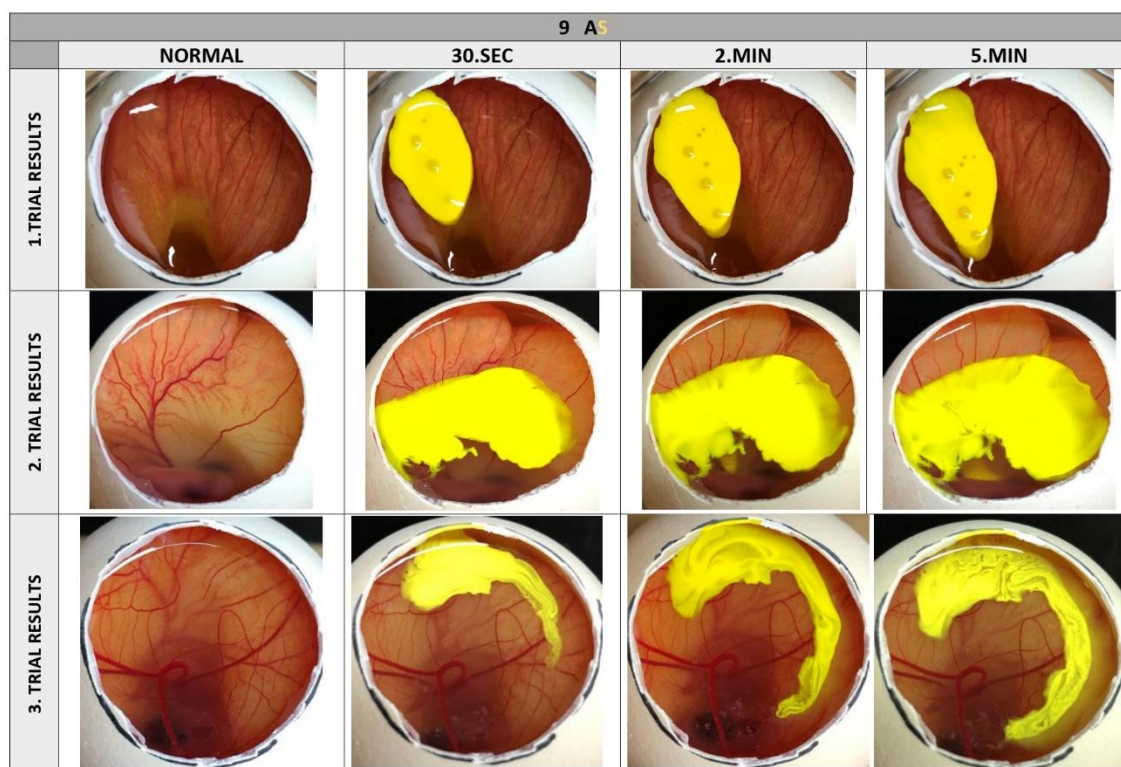


Figure 4.20.– 9 AS Yellow 1-3 Trial

Table 4.20. HET-CAM Result for 9 AS Yellow of 1-3 Trial

9AS 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

9AS 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

9AS 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

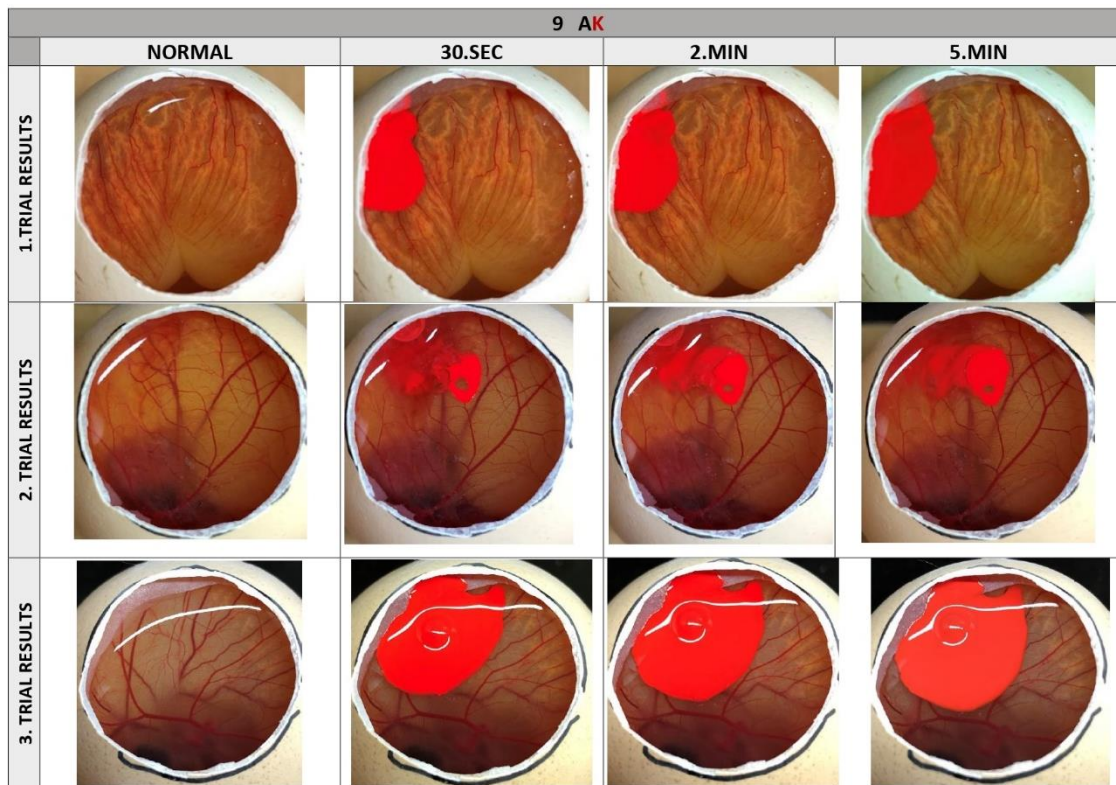


Figure 4.21. 9 AK Red 1-3 Trial

Table 4.21. HET-CAM Result for 9 AK Red of 1-3 Trial

9AK 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

9AK 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

9AK 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5



Figure 4.22. 10 AS Yellow 1-3 Trial

Table 4.22. HET-CAM Result for 10 AS Yellow of 1-3 Trial

10AS 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

10AS 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

10AS 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

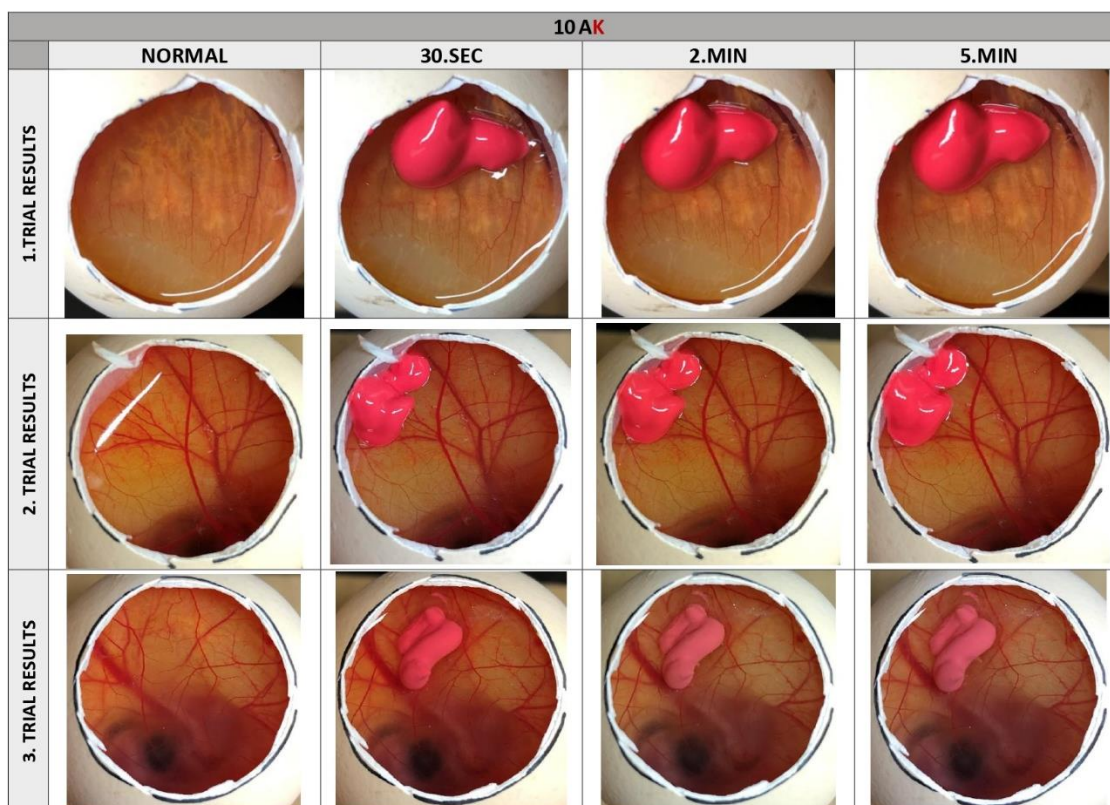


Figure 4.23. 10 AK Red 1-3 Trial

Table 4.23. HET-CAM Result for 10 AK Red of 1-3 Trial

10AK 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

10AK 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

10AK 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

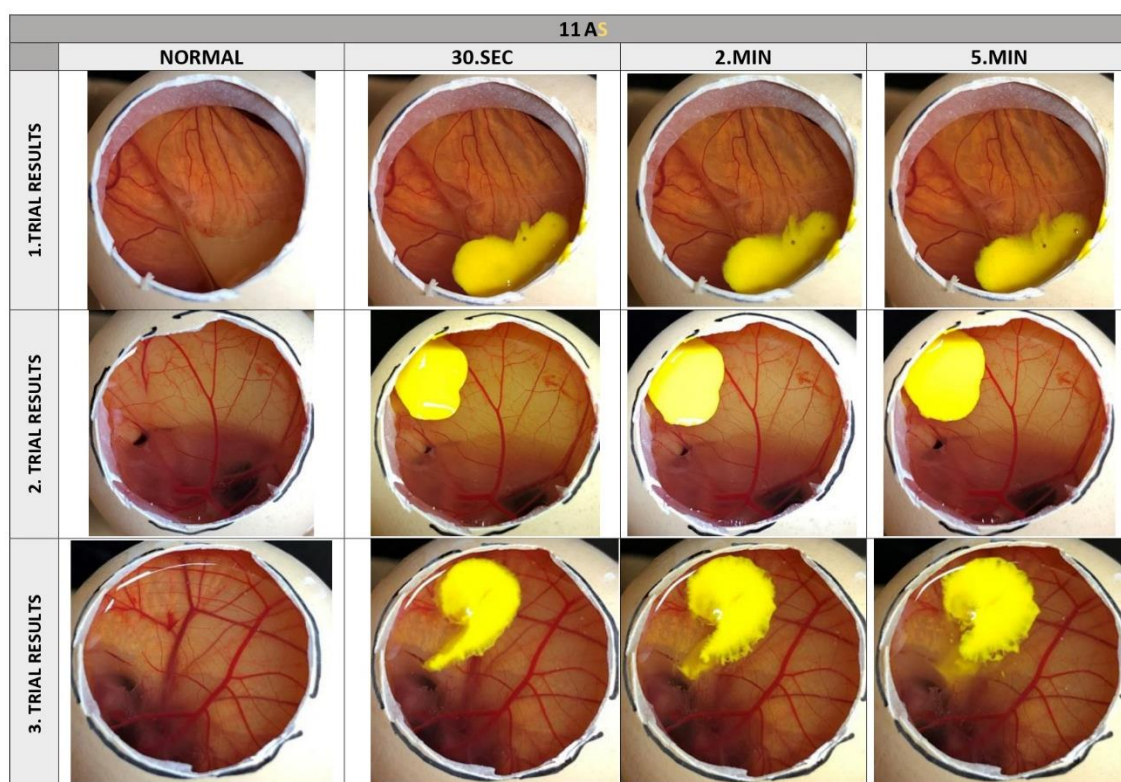


Figure 4.24.– 11 AS Yellow 1-3 Trial

Table 4.24. HET-CAM Result for 11 AS Yellow of 1-3 Trial

11AS 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

11AS 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

11AS 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

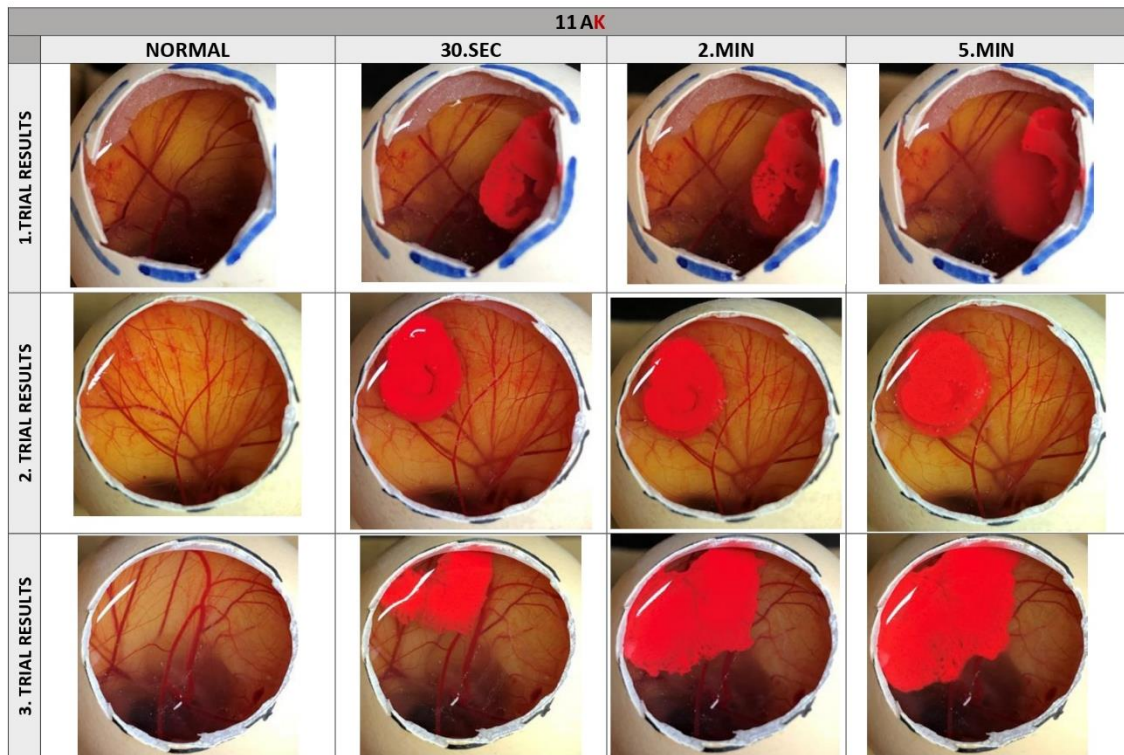


Figure 4.25.– 11 AK Red 1-3 Trial

Table 4.25. HET-CAM Result for 11 AK Red of 1-3 Trial

11AK 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

11AK 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

11AK 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

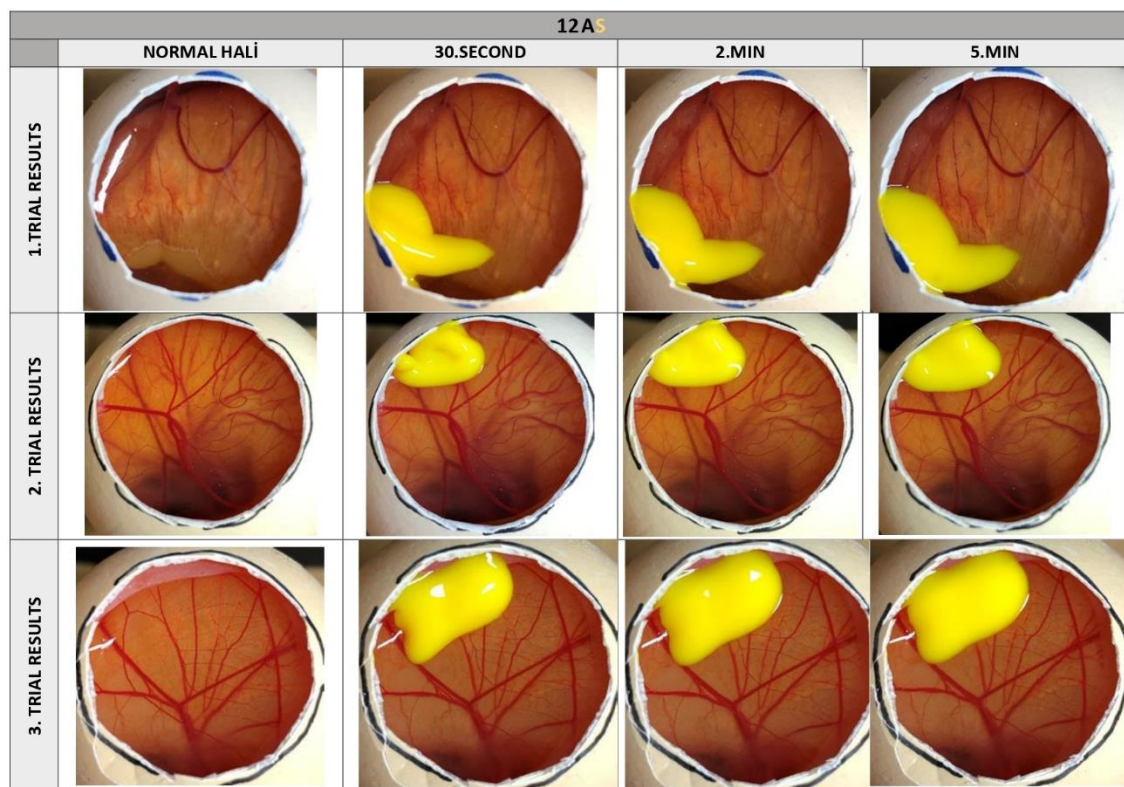


Figure 4.26. 12 AS Yellow 1-3 Trial

Table 4.26. HET-CAM Result for 12 AS Yellow of 1-3 Trial

12AS 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

12AS 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

12AS 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

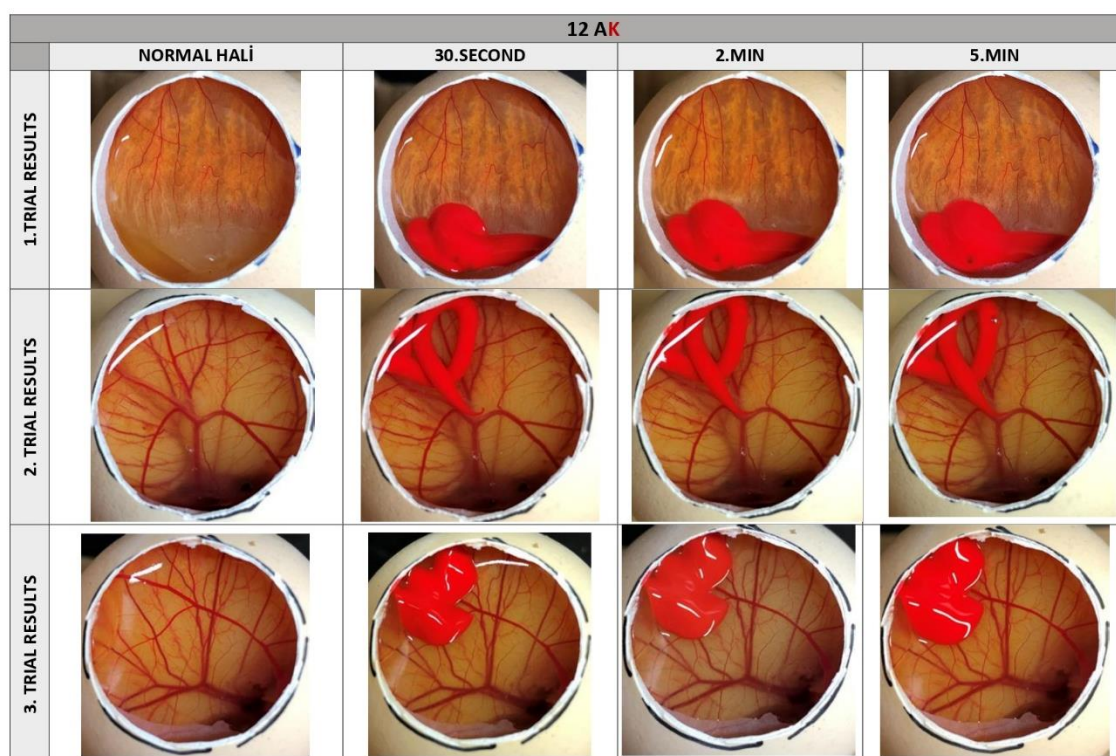


Figure 4.27. 12 AK Red 1-3 Trial

Table 4.27. HET-CAM Result for 12 AK Red of 1-3 Trial

12AK 1 st Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

12AK 2 nd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

12AK 3 rd Trial	Effect	Score 0		
		0,5 min.	2 min.	5 min.
	Lysis	5	3	1
	Bleeding	7	5	3
	Coagulation	9	7	5

4.2 Heavy Metal Analysis Results

Table 4.28. Standard Concentrations for calibration plot

	Hg	Pb	Ni	As	Cd	Sb
STD1	10	10	10	10	10	10
STD2	5	5	5	5	5	5
STD3	2,50	2,50	2,50	2,50	2,50	2,50
STD4	1	1	1	1	1	1
STD5	0,5	0,5	0,5	0,5	0,5	0,5
STD6	0,1	0,1	0,1	0,1	0,1	0,1
STD7	0,05					
STD8	0,025					

Table 4.29. Finger Paints Heavy Metal Content Analysis Results

No	Code	Brand	Group	Color	Heavy Metal Content (mg/kg, ppm)					
					Hg	Pb	Ni	As	Cd	Sb
1	1BK	1	B	Red	0,588	0,09	ND	6,355	ND	0,73
2	1BS	1	B	Yellow	0,264	2,63	4,733	5,119	0,225	1,718
3	2BK	2	B	Red	0,396	0,166	ND	7,931	ND	0,536
4	2BS	2	B	Yellow	1,533	0,47	1,767	8,561	0,11	4,351
5	3BK	3	B	Red	0,219	1,436	1,576	6,187	0,004	1,058
6	3BS	3	B	Yellow	0,338	0,319	ND	4,697	ND	0,518
7	4BK	4	B	Red	0,181	2,834	2,283	10,364	0,062	1,052
8	4BS	4	B	Yellow	0,166	1,669	2,057	5,99	0,081	1,153
9	5BK	5	B	Red	0,393	ND	2,433	11,978	0,033	1,956
10	5BS	5	B	Yellow	0,293	0,162	0,448	7,143	ND	1,578
11	6BK	6	B	Red	1,094	0,689	0,77	9,203	0,164	2,506
12	6BS	6	B	Yellow	0,869	0,104	1,23	5,941	ND	1,86
13	7BK	7	B	Red	0,338	ND	0,399	6,032	ND	1,279
14	7BS	7	B	Yellow	1,032	0,311	0,182	12,75	ND	1,486
15	8BK	8	B	Red	0,708	ND	ND	6,038	ND	1,174
16	8BS	8	B	Yellow	0,465	0,647	ND	4,854	ND	0,568
17	9BK	9	B	Red	0,846	ND	0,809	5,052	0,024	1,407
18	9BS	9	B	Yellow	0,122	1,322	2,169	4,299	0,037	1,03
19	10BK	10	B	Red	2,645	ND	ND	0,374	0,014	3,841
20	10BS	10	B	Yellow	0,252	0,777	1,124	4,914	ND	1,125
21	11BK	11	B	Red	0,525	ND	ND	5,518	ND	0,654
22	11BS	11	B	Yellow	0,252	3,698	1,826	8,72	0,004	1,111
23	12BK	12	B	Red	0,224	3,826	2,529	6,444	0,029	1,172
24	12BS	12	B	Yellow	0,226	1,971	1,599	7,199	0,074	1,102

Table 4.30. Metal content measurements (ppm)*

	Red	Yellow
Hg	0,68 ± 0,648	0,484 ± 0,414
Pb	1,507 ± 1,391	1,173 ± 1,079
Ni	1,543 ± 0,824	1,713 ± 1,183
As	6,79 ± 2,805	6,682 ± 2,319
Cd	0,047 ± 0,05	0,088 ± 0,07
Sb	1,447 ± 0,893	1,467 ± 0,954

* The averages ± standard deviation of red and yellow dyes of different brands

For the comparison of yellow and red colors in accordance with brand codes, it was first tested whether the data showed normal distribution. IBM SPSS Statistic Viewer 23.0 program was used. The results were considered significant at $p < 0.05$ level.

Table 4.31. Tests of Normality

	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
HgRed	,236	12	,063	,691	12	,001
PbRed	,342	12	,000	,665	12	,000
NiRed	,225	12	,094	,803	12	,010
AsRed	,214	12	,137	,921	12	,298
CdRed	,287	12	,007	,644	12	,000
SbRed	,267	12	,018	,813	12	,013

a. Lilliefors Significance Correction

As can be seen, PbRed and PbYellow and CdRed and CdYellow are not normally distributed. Therefore, nonparametric analysis was performed.

Table 4.32. Paired Samples Test

		Mean	Std. Deviation	t	Sig. (2-tailed)
Pair 1	HgRed - HgYellow	,19542	,84640	,800	,441
Pair 2	PbRed - PbYellow	-,41992	1,57781	-,922	,376
Pair 3	NiRed - NiYellow	-,52800	1,81469	-1,008	,335
Pair 4	AsRed - AsYellow	,10742	3,51220	,106	,918
Pair 5	CdRed - CdYellow	-,01675	,09052	-,641	,535
Pair 6	SbRed - SbYellow	-,01958	1,49333	-,045	,965

As a result of the parametric analysis in Table 4.32 no statistically significant difference was found between different colors of the same brands in terms of Hg, Pb, Ni, As, Cd, Sb content.

4.3 Microbiology Test Results

No growth of microorganism has been observed within the sample's product tested. Results are shown in Table 4.31.

Table 4.33. Counting of Microorganisms

Test Product	<i>Aerobic Mesophilic Bacteria</i>	<i>Yeast and Mould</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>	<i>Aspergillus brasiliensis</i>
1 BM	0	0	0	0	0	0	0
2 BM	0	0	0	0	0	0	0
3 BM	0	0	0	0	0	0	0
4 BM	0	0	0	0	0	0	0
5 BM	0	0	0	0	0	0	0
6 BM	0	0	0	0	0	0	0
7 BM	0	0	0	0	0	0	0
8 BM	0	0	0	0	0	0	0
9 BM	0	0	0	0	0	0	0
10 BM	0	0	0	0	0	0	0
11 BM	0	0	0	0	0	0	0
12 BM	0	0	0	0	0	0	0

B: refers to Blue color of F.P

4.4 Sterility Test Results

For *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*, logarithmic reduction of $> \log 2$ on 2nd day and logarithmic reduction of $> \log 3$ on 7th day were observed. No increase was observed till 28th day. For *Candida albicans* and *Aspergillus brasiliensis*, logarithmic reduction of $> \log 2$ on 14th day was observed. No increase was observed till 28th day. Therefore, the test sample is proved to be effective for the test microorganisms listed Table 4.34.

Table 4.34. Results of Log Reduction

Test Microorganisms	Inoculum (cfu/ml) (N)	Viable Count In Sample cfu/g-ml (N = IC / (V x d))					Log Reduction (Rx= lgN0 – lgNx)			
		N0	N2	N7	N14	N28	R2	R7	R14	R28
		1st d	2d	7d	14d	28d	2d	7 d	14d	28d
<i>Staphylococcus aureus</i> ATCC 6538	1x10 ⁸	1.6x10 ⁵	1.2x10 ³	1.5x10 ²	-	NI	>2	>3	-	NI
<i>Pseudomonas aeruginosa</i> ATCC 9027	1x10 ⁸	1.7x10 ⁵	1.3x10 ³	1.6x10 ²	-	NI	>2	>3	-	NI
<i>Escherichia coli</i> ATCC 11229	1x10 ⁸	1.5x10 ⁵	1.1x10 ³	1.2x10 ²	-	NI	>2	>3	-	NI
<i>Candida albicans</i> ATCC 10231	1x10 ⁷	1.4x10 ⁴	-	-	1.2x10 ²	NI	-	-	>2	NI
<i>Aspergillus brasiliensis</i> ATCC 16404	1x10 ⁷	1.3x10 ⁴	-	-	1,1x10 ²	NI	-	-	>2	NI

Criterias	Bacteria (<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i>)				Fungi (<i>Candida albican</i> , <i>Aspergillus brasiliensis</i>)	
	2d	7d	14d	28d	14d	28d
Criteria A	≥2	≥3	-	NI	≥2	NI
Criteria B	-	-	≥3	NI	≥1	NI

(-): No Test, (NI): No increase

4.5 Safety Assessment Results

4.5.1 SED calculations of Heavy Metals for Finger Paints

4.5.1.1 Mercury

-SED calculation of Mercury of Finger Paint is below;

A (mg/kg body weight/day):2,16 (In terms of application and exposure, the type closest to finger paint is observed in the hand cream product as recommended by the SCCS's Notes of Guidance)

C (%): 0,000068 for red color of finger paint and 0,0000484 for yellow color of finger paint (The average of metal content measurement of red and yellow colors of different brands are 0,68 ppm and 0,484 ppm respectively) (* %1 vol = 10.000 ppm)

D_{Ap} (%): 50 (The dermal absorption rate was searched for mercury and it has been found that Absorption rates through the skin vary according to the form of mercury. For

Methylmercury (CH_3Hg^+) : Dermal: in guinea pigs, 3 – 5% of applied dose absorbed in 5 h. For Elemental mercury (Hg^0), Dermal: average rate of absorption of Hg^0 through human skin, 0.024 ng/cm^2 for every 1 mg/m^3 in air and For Mercuric mercury (Hg^{2+}): Dermal: in guinea pigs, 2 –3% of applied dose of HgCl_2 absorbed.[123] Since no precise absorption data is available, the conservative default value of 50% is used as recommended by the SCCS's Notes of Guidance [122].

- SED calculation of Mercury for red color of Finger Paint is below;

A: 2,16 mg/kg bw/day

C: % 0,000068

$\text{SED} = A \text{ (mg/kg bw/day)} \times C(\%) / 100 \times \text{DAp} (\%)/100$

$\text{SED} = 2,16 \times 0,000068/100 \times 50/100$

$\text{SED} = 0,0000007344 \text{ mg/kg bw/day}$

- SED calculation of Mercury for yellow color of Finger Paint is below;

A: 2,16 mg/kg bw/day

C: % 0,0000484

$\text{SED} = A \text{ (mg/kg bw/day)} \times C(\%) / 100 \times \text{DAp} (\%)/100$

$\text{SED} = 2,16 \times 0,0000484/100 \times 50/100$

$\text{SED} = 0,00000052272 \text{ mg/kg bw/day}$

Table 4.35. SED Table for mercury of finger paints for red and yellow colors.

Name of the heavy metal of finger paint	Name of the color for heavy metal of finger paint	Amount of Use (% Concentration)	Retention Factor (R)	Dermal Absorption DAa ($\mu\text{g/cm}^2$)	Dermal Absorption DAp (%)	SED (mg/kg bw/day)	A (mg/kg bw/day)
Mercury	Red	0,000068	1	-	50	0,0000007344	2,16
Mercury	Yellow	0,0000484	1	-	50	0,00000052272	2,16

4.5.1.2 Lead

- SED calculation of Lead of Finger Paints

A (mg/kg body weight/day):2,16 (In terms of application and exposure, the type closest to finger paint is observed in the hand cream product as recommended by the SCCS's Notes of Guidance)

C (%): 0,0001507 for red color of finger paint and 0,0001173 for yellow color of finger paint (The average of metal content measurement of red and yellow colors of different brands are 1,507 ppm and 1,173 ppm respectively) (* %1 vol = 10.000 ppm)

DAp (%):50 (The percutaneous absorption of lead acetate following the use of hair coloring preparations was almost nil with a range between 0 and 0.3% of the dose applied onto the skin. [124]. Therefore it seems reasonable to use the DA of 0,3 %. But still its better to accept the conservative default value of 50% as recommended by the SCCS in order to evaluate the worst case scenario.

SED calculation of Lead for red color of Finger Paint is below;

A: 2,16 mg/kg bw/day

C: % 0,0001507

$SED = A \text{ (mg/kg bw/day)} \times C(\%) / 100 \times DAp (\%)/100$

$SED = 2,16 \times 0,0001507 / 100 \times 50/100$

SED= 0,00000162756 mg/kg bw/day

- SED calculation of Lead for yellow color of Finger Paint is below;

A: 2,16 mg/kg bw/day

C: % 0,0001173

$SED = A \text{ (mg/kg bw/day)} \times C(\%) / 100 \times DAp (\%)/100$

$SED = 2,16 \times 0,0001173 / 100 \times 50/100$

SED= 0,00000126684 mg/kg bw/day

Table 4.36. SED Table for Lead of finger paints for red and yellow colors.

Name of the heavy metal of finger paint	Name of the color for heavy metal of finger paint	Amount of Use (% Concentration)	Retention Factor (R)	Dermal Absorption DAa (µg/cm²)	Dermal Absorption DAp (%)	SED (mg/kg bw/day)	A (mg/kg bw/day)
Lead	Red	0,0001507	1	-	50	0,00000162756	2,16
Lead	Yellow	0,0001173	1	-	50	0,00000126684	2,16

4.5.1.3 Nickel

- SED calculation of Nickel of Finger Paints

A (mg/kg body weight/day):2,16 (In terms of application and exposure, the type closest to finger paint is observed in the hand cream product as recommended by the SCCS's Notes of Guidance)

C (%):0,0001543 for red color of finger paint and 0,0001713 for yellow color of finger paint (The average of metal content measurement of red and yellow colors of different brands are 1,543 ppm and 1,713 ppm respectively) (* %1 vol = 10.000 ppm)

DAP (%):50 (For oral absorption, it is known that soluble Ni-compounds are more absorbable than insoluble ones. In the fasted state up to 50% can be absorbed from the gastrointestinal tract, but the extent of absorption can be reduced dramatically in the presence of several food constituents. Thus most Ni in food remains unabsorbed; about 10% of Ni in food is absorbed [125]. From a study of Tanojo et al.,the absorption rate of the four “soluble” Nickel compounds (sulfate, chloride, nitrate and carbonate) in the EU Risk Assessment Report (RAR) was derived from a study on soluble Nickel compounds using human skin in vitro, which showed that about 98% of the dose remained in the donor solution, whereas 61% was found in the receiving fluid, and <1% was retained in the stratum corneum. However, the RAR reached the conclusion that 0.2% should be used for risk characterization [124]. Therefore it seems its appropriate to use the DA of 0,2 % but still its better to accept the conservative default value of 50% as recommended by the SCCS in order to evaluate the worst case scenario.

- SED calculation of Nickel for red color of Finger Paint is below;

A: 2,16 mg/kg bw/day

C: % 0,0001543

$SED = A \text{ (mg/kg bw/day)} \times C(\%) / 100 \times DAP (\%)/100$

$SED = 2,16 \times 0,0001543 / 100 \times 50/100$

SED= 0,00000166644 mg/kg bw/day

- SED calculation of Nickel for yellow color of Finger Paint is below;

A: 2,16 mg/kg bw/day

C: % 0,0001713

$SED = A \text{ (mg/kg bw/day)} \times C(\%) / 100 \times DAP (\%)/100$

$SED = 2,16 \times 0,0001713 / 100 \times 50/100$

SED= 0,00000185004 mg/kg bw/day

Table 4.37. SED Table for Nickel of finger paints for red and yellow colors.

Name of the heavy metal of finger paint	Name of the color for heavy metal of finger paint	Amount of Use (% Concentration)	Retention Factor (R)	Dermal Absorption DAa ($\mu\text{g}/\text{cm}^2$)	Dermal Absorption DAp (%)	SED (mg/kg bw/day)	A (mg/kg bw/day)
Nickel	Red	0,0001543	1	-	50	0,00000166644	2,16
Nickel	Yellow	0,0001713	1	-	50	0,00000185004	2,16

4.5.1.4 Arsenic

- SED calculation of Arsenic of Finger Paints

A (mg/kg body weight/day):2,16 (In terms of application and exposure, the type closest to finger paint is observed in the hand cream product as recommended by the SCCS's Notes of Guidance)

C (%):0,000679 for red color of finger paint and 0,0006682 for yellow color of finger paint (The average of metal content measurement of red and yellow colors of different brands are 6,79 ppm and 6,682 ppm respectively) (* %1 vol = 10.000 ppm)

DAp (%):50 (Based on the study of Wester et al., “The extent of percutaneous absorption of arsenic was found to be 6.4% for trace doses and 2.0% for high doses in the monkey, and 1.9% when applied in trace doses onto human skin samples”[124]. Therefore it seems reasonable to use the DA of 1,9 % but still its better to accept the conservative default value of 50% as recommended by the SCCS in order to evaluate the worst case scenario).

- SED calculation of Arsenic for red color of Finger Paint is below;

A: 2,16 mg/kg bw/day

C: % 0,000679

$SED = A \text{ (mg/kg bw/day)} \times C(\%) / 100 \times DAp (\%)/100$

$SED = 2,16 \times 0,000679 / 100 \times 50/100$

$SED = 0,0000073332 \text{ mg/kg bw/day}$

- SED calculation of Arsenic for yellow color of Finger Paint is below;

A: 2,16 mg/kg bw/day

C: % 0,0006682

$SED = A \text{ (mg/kg bw/day)} \times C(\%) / 100 \times DAp (\%)/100$

$SED = 2,16 \times 0,0006682 / 100 \times 50/100$

$SED = 0,00000721656 \text{ mg/kg bw/day}$

Table 4.38. SED Table for Arsenic of finger paints for red and yellow colors.

Name of the heavy metal of finger paint	Name of the color for heavy metal of finger paint	Amount of Use (% Concentration)	Retention Factor (R)	Dermal Absorption DAa ($\mu\text{g}/\text{cm}^2$)	Dermal Absorption DAp (%)	SED (mg/kg bw/day)	A (mg/kg bw/day)
Arsenic	Red	0,000679	1	-	50	0,0000073332	2,16
Arsenic	Yellow	0,0006682	1	-	50	0,00000721656	2,16

4.5.1.5 Cadmium

- SED calculation of Cadmium of Finger Paints

A (mg/kg body weight/day):2,16 (In terms of application and exposure, the type closest to finger paint is observed in the hand cream product as recommended by the SCCS's Notes of Guidance)

C (%):0,0000047 for red color of finger paint and 0,0000088 for yellow color of finger paint (The average of metal content measurement of red and yellow colors of different brands are 0,047 ppm and 0,088 ppm respectively) (* %1 vol = 10.000 ppm)

Dap (%): 50 (About 0.3–0.8% of cadmium is absorbed through the skin [124]. Therefore it seems reasonable to use the DA of 0,8 % but still its better to accept the conservative default value of 50% as recommended by the SCCS in order to evaluate the worst case scenario.)

- SED calculation of Cadmium for red color of Finger Paint is below;

A: 2,16 mg/kg bw/day

C: % 0,0000047

SED = A (mg/kg bw/day) $\times$ C(%) / 100 $\times$ DAp (%) / 100

SED = 2,16 $\times$ 0,0000047 / 100 $\times$ 50 / 100

SED= 0,00000005076 mg/kg bw/day

- SED calculation of Cadmium for yellow color of Finger Paint is below;

A: 2,16 mg/kg bw/day

C: % 0,0000088

SED = A (mg/kg bw/day) $\times$ C(%) / 100 $\times$ DAp (%) / 100

SED = 2,16 $\times$ 0,0000088 / 100 $\times$ 50 / 100

SED= 0,000000009504 mg/kg bw/day

Table 4.39. SED Table for Cadmium of finger paints for red and yellow colors.

Name of the heavy metal of finger paint	Name of the color for heavy metal of finger paint	Amount of Use (% Concentration)	Retention Factor (R)	Dermal Absorption DAa ($\mu\text{g}/\text{cm}^2$)	Dermal Absorption DAp (%)	SED (mg/kg bw/day)	A (mg/kg bw/day)
Cadmium	Red	0,0000047	1	-	50	0,0000005076	2,16
Cadmium	Yellow	0,0000088	1	-	50	0,0000009504	2,16

4.5.1.6 Antimony

- SED calculation of Antimony of Finger Paints

A (mg/kg body weight/day):2,16 (In terms of application and exposure, the type closest to finger paint is observed in the hand cream product as recommended by the SCCS's Notes of Guidance)

C (%):0,0001474 for red color of finger paint and 0,0001467 for yellow color of finger paint (The average of metal content measurement of red and yellow colors of different brands are 1,447 ppm and 1,467 ppm respectively) (* %1 vol = 10.000 ppm)

DAP (%): 50 ((Based on the studies of Iffland and Bösche, 1987; Lauwers et al., 1990; WHO, 2003; Analyses performed in four individuals following antimony intoxication have proved that gastric absorption amounts to 5% and based on the study of Roper and Stupart, an absorption rate of 0.26% of Sb trioxide through human skin has been suggested by an *in vitro* percutaneous study [124]. Therefore it seems reasonable to use the DA of 0,26 % but still its better to accept the conservative default value of 50% as recommended by the SCCS in order to evaluate the worst case scenario.)

- SED calculation of Antimony for red color of Finger Paint is below;

A: 2,16 mg/kg bw/day

C: % 0,0001474

SED = A (mg/kg bw/day) $\times$ C(%) / 100 $\times$ DAp (%) / 100

SED = 2,16 $\times$ 0,0001474 / 100 $\times$ 50 / 100

SED= 0,00000159192 mg/kg bw/day

- SED calculation of Antimony for yellow color of Finger Paint is below;

A: 2,16 mg/kg bw/day

C: % 0,0001467

SED = A (mg/kg bw/day) $\times$ C(%) / 100 $\times$ DAp (%) / 100

$$SED = 2,16 \times 0,0001467 / 100 \times 50/100$$

$$SED = 0,00000158436 \text{ mg/kg bw/day}$$

Table 4.40. SED Table for Antimony of finger paints for red and yellow colors.

Name of the heavy metal of finger paint	Name of the color for heavy metal of finger paint	Amount of Use (% Concentration)	Retention Factor (R)	Dermal Absorption DAa ($\mu\text{g}/\text{cm}^2$)	Dermal Absorption DAp (%)	SED (mg/kg bw/day)	A (mg/kg bw/day)
Antimony	Red	0,0001474	1	-	5	0,00000159192	2,16
Antimony	Yellow	0,0001467	1	-	5	0,00000158436	2,16

4.5.2 MoS calculations of Heavy Metals for Finger Paints

4.5.2.1 Mercury

- MoS calculation of Mercury for red color of Finger Paint is below

NOAEL: 0,23 mg/kg bw/day (According to EPA and WHO, NOAEL value of mercury is 0.23 mg/kg. [126].)

SED: 0,0000007344 mg/kg bw/day

Mos= NO(A)EL / SED

Mos= 0,23 / 0,0000007344 mg/kg bw/day

Mos= 313.180,82 mg/kg bw/day

Mos>160 mg/kg bw/day

- MoS calculation of Mercury for Yellow color of Finger Paint is below

NOAEL: 0,23 mg/kg bw/day

SED: 0,00000052272 mg/kg bw/day

Mos= NO(A)EL / SED

Mos= 0,23 / 0,00000052272 mg/kg bw/day

Mos= 440.006,12 mg/kg bw/day

Mos>160 mg/kg bw/day

Table 4.41. MoS Table for Mercury of finger paints for red and yellow colors.

Name of the heavy metal of finger paint	Name of the color for heavy metal of finger paint	SED (mg/kg bw/day)	NOAEL (mg/kg bw/day)	MoS (mg/kg bw/day)
Mercury	Red	0,00000007344	0,23	313.180,82

Mercury	Yellow	0,000000052272	0,23	440.006,12
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4.5.2.2 Lead

- MoS calculation of Lead for red color of Finger Paint is below

BMDL10: 0,00063 mg/kg bw./day (In adults lead affects mainly blood pressure and the kidney, resulting in chronic renal disease. Indeed in adults, the effects on the CNS (the major target during developmental stages and childhood) occur at much higher doses. From data available in humans, EFSA derived a BMDL10 of 36 µg /L for the 1% (1.2 mmHg) increase in mean systolic blood pressure, which is not considered an adverse effect per se, but is a marker for cardiovascular effects. For renal effects, defined as 50% reduction in Glomerular Filtration Rate, the BMDL10 of 15 µg /L, corresponds to a 10% prevalence. The sample size was large enough to cover any inter-individual differences, so no assessment factor was applied. The daily dietary intake of lead in adults corresponding to BMDL10 for blood pressure is 1.50 µg /kg b.w./day, whereas the corresponding BMDL10 for renal effects is 0.63 µg /kg b.w./day. [124].)

SED: 0,00000162756 mg/kg bw/day

Mos= BMDL10 / SED

Mos= 0,00063 / 0,00000162756 mg/kg bw/day

Mos= 387,082 mg/kg bw/day

Mos>160 mg/kg bw/day

- MoS calculation of Lead for Yellow color of Finger Paint is below

NOAEL: 0,00063 mg/kg bw/day

SED: 0,00000126684 mg/kg bw/day

Mos= BMDL10 / SED

Mos= 0,00063 / 0,00000126684 mg/kg bw/day

Mos= 497,300 mg/kg bw/day

Mos>160 mg/kg bw/day

Table 4.42. MoS Table for Lead of finger paints for red and yellow colors.

Name of the heavy metal of finger paint	Name of the color for heavy metal of finger paint	SED (mg/kg bw/day)	BMDL10 (mg/kg bw/day)	MoS (mg/kg bw/day)
Lead	Red	0,00000162756	0,00063	387,082
Lead	Yellow	0,00000126684	0,00063	497,300

4.5.2.3 Nickel

- MoS calculation of Nickel for red color of Finger Paint is below

NOAEL: 5 mg/kg bw/day (The mostly used NOAEL for Ni is 5 mg/kg body weight/day, measured for Ni SO₄ in a 2-year oral rat study [124].)

-MoS calculation of Nickel for Red color of Finger Paint is below

NOAEL: 5 mg/kg bw/day

SED: 0,00000166644 mg/kg bw/day

Mos= NO(A)EL / SED

Mos= 5 / 0,00000166644 mg/kg bw/day

Mos= 3.000.408,05 mg/kg bw/day

Mos>160 mg/kg bw/day

- MoS calculation of Nickel for Yellow color of Finger Paint is below

NOAEL: 5 mg/kg bw/day

SED: 0,00000185004 mg/kg bw/day

Mos= NO(A)EL / SED

Mos= 5 / 0,00000185004 mg/kg bw/day

Mos= 2.702.644,26 mg/kg bw/day

Mos>160 mg/kg bw/day

Table 4.43. MoS Table for Nickel of finger paints for red and yellow colors.

Name of the heavy metal of finger paint	Name of the color for heavy metal of finger paint	SED (mg/kg bw/day)	NOAEL (mg/kg bw/day)	MoS (mg/kg bw/day)
Nickel	Red	0,00000166644	5	3.000.408,05
Nickel	Yellow	0,00000185004	5	2.702.644,26

4.5.2.4 Arsenic

- MoS calculation of Arsenic for red color of Finger Paint is below

BMDL₁₀: 0.0003 mg/kg b.w./day (Based on the EFSA's CONTAM Panel, they concluded that "a range of benchmark dose lower confidence limit (BMDL₁₀) values between 0.3 and 8 µg/kg b.w./day was identified for cancers of the lung, skin and bladder, as well as skin lesions". The overall range of BMDL₁₀ should be used instead of a single reference point in the risk characterization for inorganic arsenic. Arsenic exposure may include contributions from As(III), As(V), and organic species. Inorganic As(III) form of arsenic is more toxic than As(V) and organic arsenic, but so far most data on cosmetics

are still reported as total arsenic. The need for specification data is evident: as organic forms are less toxic, a risk assessment considering total arsenic as inorganic arsenic would lead to a considerable overestimation of the health risk related to arsenic exposure [127].)

SED: 0,0000073332 mg/kg bw/day

Mos= BMDL10 / SED

Mos= 0,0003 / 0,0000073332 mg/kg bw/day

Mos= 40,909 mg/kg bw/day

Mos<160 mg/kg bw/day

- MoS calculation of Arsenic for Yellow color of Finger Paint is below

NOAEL: 0,0003 mg/kg bw/day

SED: 0,00000721656 mg/kg bw/day

Mos= BMDL10 / SED

Mos= 0,0003 / 0,00000721656 mg/kg bw/day

Mos= 41,57 mg/kg bw/day

Mos<160 mg/kg bw/day

Calculated MoS value Of Arsenic of Finger paint is lower than 160 mg/kg bw/day which is the threshold value for children. When calculated SED value, dermal absorption rate is accepted 50% of conservative default value as recommended by the SCCS. Actually this condition is considered in order to evaluate the worst case scenario. Because of this reason, the dermal absorption rate was searched for Arsenic and it has been found 1.9 %. The extent of percutaneous absorption of arsenic was found to be 6.4% for trace doses and 2.0% for high doses in the monkey, and 1.9% when applied in trace doses onto human skin samples [205]. Therefore when we accept the Dermal absorption 1.9% the SED value for red color of Arsenic is 0,0000002786616 mg/kg bw/day and the SED value for yellow color of Arsenic is 0,00000027422928 mg/kg bw/day. According to the revised SED values for the red and yellow colors of Arsenic; the BMDL10 value for Arsenic remaining as 0,003 mg/kg bw/day and the Calculated MoS values are 10.765,74 mg/kg bw/day and 10.939,75 mg/kg bw/day for the red and yellow colors of arsenic of finger paints respectively. Calculated MoS values are greater than 160 mg/kg bw/day.

Table 4.44. MoS Table for Arsenic of finger paints for red and yellow colors.

Name of the heavy metal of finger paint	Name of the color for heavy metal of finger paint	SED (mg/kg bw/day)	BMDL10 (mg/kg bw/day)	MoS* (mg/kg bw/day)	MoS (mg/kg bw/day)
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Arsenic	Red	0,0000073332	0,003	40,909	10.765,74
Arsenic	Yellow	0,00000721656	0,003	41,57	10.939,75

*When the conservative default value of 50% is used as recommended by the SCCS.

4.5.2.5 Cadmium

- MoS calculation of Cadmium for red color of Finger Paint is below

BMDL5: 0.004 mg/kg b.w. (EFSA Contaminant Panel calculated the value of BMDL5 that produces a specific change in B2M (beta-2-microglobulin) urinary levels; EFSA Contaminant Panel applied a 3.9 adjustment factor to a BMDL5 of 4 micrograms of cadmium/g creatinine in urine to account for data variability, deriving a urinary concentration of 1 lg cadmium/g creatinine as the new reference point. To remain below 1 µg cadmium/g creatinine in urine, Contaminant EFSA Panel calculated that the average daily dietary intake of cadmium should not exceed 0.36 µg/kg b.w.)

SED: 0,00000005076 mg/kg bw/day

Mos= NO(A)EL / SED

Mos= 0,004 / 0,00000005076 mg/kg bw/day

Mos= 78.802,20 mg/kg bw/day

Mos>160 mg/kg bw/day

- MoS calculation of Cadmium for Yellow color of Finger Paint is below

BMDL5: 0.004 mg/kg b.w

SED: 0,00000009504 mg/kg bw/day

Mos= NO(A)EL / SED

Mos= 0,004 / 0,00000009504 mg/kg bw/day

Mos= 42.087,54 mg/kg bw/day

Mos>160 mg/kg bw/day

Table 4.45. MoS Table for Cadmium of finger paints for red and yellow colors.

Name of the heavy metal of finger paint	Name of the color for heavy metal of finger paint	SED (mg/kg bw/day)	BMDL5 (mg/kg bw/day)	MoS (mg/kg bw/day)
Cadmium	Red	0,00000005076	0,004	78.802,20
Cadmium	Yellow	0,00000009504	0,004	42.087,54

4.5.2.6 Antimony

- MoS calculation of Antimony for red color of Finger Paint is below

NOAEL: 6 mg/kg bw/day (Based on the study of Poon et al., the NOAEL of 0.5 ppm antimony administered in drinking water for 13 weeks to rats (average intake of 0.06 mg/kg b.w./day) was proposed, based on decreased body weight gain and minimal biochemical and histological organ changes observed at 5.0 ppm. However, in the study of Lynch et al. proposed a subchronic NOAEL of 6 mg/kg b.w. based on the decreased body weight gain and reduced food and water intake observed at the LOAEL of 60 mg antimony/kg b.w./day. As a conclusion based on the National toxicology program of National Institute of health , since none of the subtle histological reversible changes recorded by Poon et al. were detected in a NTP chronic study, it seems reasonable to use the NOAEL of 6 mg/kg b.w. [127].)

SED: 0,00000159192 mg/kg bw/day

Mos= NO(A)EL / SED

Mos= 6 / 0,00000159192 mg/kg bw/day

Mos= 3.769.033,61 mg/kg bw/day

Mos>160

- MoS calculation of Antimony for Yellow color of Finger Paint is below

NOAEL: 6 mg/kg bw/day

SED: 0,00000158436 mg/kg bw/day

Mos= NO(A)EL / SED

Mos= 6 / 0,00000158436 mg/kg bw/day

Mos= 3.787.018,10 mg/kg bw/day

Mos>160

Table 4.46. MoS Table for Antimony of finger paints for red and yellow colors.

Name of the heavy metal of finger paint	Name of the color for heavy metal of finger paint	SED (mg/kg bw/day)	NOAEL (mg/kg bw/day)	MoS (mg/kg bw/day)
Antimony	Red	0,00000159192	6	3.769.033,61
Antimony	Yellow	0,0000000158436	6	3.787.018,10

5. DISCUSSION AND CONCLUSION

In this study, which was carried out to examine the composition of finger paint products that marketed in Turkey for children and to evaluate their safety, firstly, through the HET-CAM method was used to investigate whether children's finger paints cause irritation. Total 12 brand's yellow and red finger paints were included in this study. As a result of this test, there were no findings such as lysis, hemorrhage or coagulation which showed that whether yellow dyes nor red dyes could be irritant. Considering that this study was repeated three times in order to obtain more accurate results and the results did not change, it was concluded that a total of 24 children's finger paints, yellow and red, belonging to 12 different brands, were non-irritant. Studies have shown that there is no other similar study and therefore no compatibility comparison can be made. It made us think that this study could serve as an example for future studies.

In the other part of our study, metal levels were searched and measured in a total of 24 products belonging to 12 different brands, consisting of yellow and red colors. The metal levels of red paints of different brands are 0.680 ± 0.648 ppm for mercury, 1.507 ± 1.391 ppm for lead, 1.543 ± 0.824 ppm for nickel, 6.790 ± 2.805 ppm for arsenic, 0.047 ± 0.050 ppm for cadmium, and 1.447 ± 0.893 ppm for antimony. The metal levels of yellow paints of different brands are 0.484 ± 0.414 ppm for mercury, 1.173 ± 1.079 ppm for lead, 1.713 ± 1.183 ppm for nickel, 6.682 ± 2.319 ppm for arsenic, 0.088 ± 0.070 ppm for cadmium and 1.467 ± 0.954 for antimony.

Comparison of the metal content values of the finger paints determined in this study with the heavy metal requirement of the European toy SD (2009/48/EC) and the harmonized standard EN-71-3, which adopts the same migration limits, it was determined that the Lead (Pb) and Arsenic (As) values were higher than the British Standard and exceeded the 0.5 ppm limit and 0.9 ppm limit, respectively.

Comparison of the metal content values of finger paints determined in this study with the TITCK heavy metal impurity limit values for cosmetic products, it was determined that the Arsenic (As) values were higher than the standard and exceeded the 5 ppm limit.

Comparison of the metal content values of finger paints determined in this study with the WHO Heavy Metal Limits For Color Additives Used In Cosmetics, it was

determined that the Arsenic (As) values were higher than the standard and exceeded the 3 ppm limit.

Comparison of the metal content values of finger paints determined in this study with the US FDA Heavy Metal Limits For Color Additives, it was determined that the Arsenic (As) values were higher and exceeded the 3 ppm limit.

Comparison of the metal content values of finger paints determined in this study with the Health Canada Heavy Metal Limits For Cosmetic Products, it was determined that the Arsenic (As) values were higher and exceeded the 3 ppm limit.

With these results, it was determined that the Arsenic levels in each of the selected children's finger paints were above the specified standards while the other determined metals are at low concentrations. Only based on the British standard the Lead levels exceeding the limit while these values were compatible with the other determined standards. But since Lead or cadmium accepted as dangerous for children in the blood at any level this study shows that the heavy metals content of the children finger paints still carries a risk for the health of children. Although arsenic is seen as a less important pollutant in cosmetics than other heavy metals, it can cause hyperpigmentation and keratosis as a result of long-term dermal exposure but systemically it may lead to carcinogenesis and vascular diseases. When considering the target population was children this study suggests that having a broader perspective on the issue, collecting more data, and knowing more about the safety and suitability of these products can play an important role in preventing potential health risks.

In the study titled "Heavy metal contents of play dough, face and finger paint samples sold in Turkish markets" conducted by Erbaş, Karatepe and Soylak, the metal ions contained in the finger paints were determined by flame atomic absorption spectrometry (FAAS). The samples analyzed were 2 face paints, 7 finger paints, and 8 playdoughs purchased from the markets in Kayseri province and when the researcher compared the results to European Toy Safety Directive (2009/48/EC), they have been found that three of the play dough samples have high cadmium concentrations while the other determined metals are at low concentrations. Four finger paint samples have high cadmium, copper, and lead content. Both of the face paint samples contain lead, cadmium, and copper at high concentrations.

The author of this article determined that such toys samples contain heavy metals in varying concentrations and thus exhibits the threat to children exposed to such kind of toys and these kinds of toys carry a risk for the health of children. [128].

With this study, the determined Lead values were compatible and similar results were obtained in our results but since the author did not analyze the Arsenic levels of finger paints it's not possible to talk about the compatibility of the studies regarding to Arsenic content of finger paints. It was the only study similar to ours due to heavy metal analyzing of finger paints.

Since there are not enough studies regarding to determination of metal content values of finger paints, this study might be a sample and a spot of light to the potential future studies regarding to children finger paints.

In the other part of our study, while SED and MOS calculations were made for the safety assessment of the heavy metals of analyzed finger paints, hand cream was accepted as the closest type to finger paint in terms of application and exposure. A value of 2.16 (g/d) is accepted and included in the calculations which is the given value for a hand cream in SCCS's table of daily exposure levels for different cosmetic product categories in Europe.

Taking into consideration that a cosmetic product type if its rinse-off or leave-on therefore the chosen retention factor value is one of the parameter which describe an exposure scenario; The definition of terms 'Rinse-off product' means a cosmetic product which is intended to be removed after application on the skin, the hair or the mucous membranes and the 'Leave-on product' means a cosmetic product which is intended to stay in prolonged contact with the skin, the hair or the mucous membranes as explained in Cosmetics Regulation (EC) No 1223/2009. For leave-on cosmetic products mostly a fraction of 1 (100%) is used while for rinse-off cosmetics the retention factor value vary from 0,001-0,1 %. Even though the finger paints intended to be removed after application on the skin still the retention factor of 1 (such like in hand creams) were accepted and used in the calculations in order to evaluate the worst case scenario for considering the targeted population is children and so called as sensitive consumer.

Regarding the choice of dermal absorption rates of heavy metals of finger paints, the scientific committee on consumer's safety recognizes that in many conventional calculations of MoS, oral bioavailability of an element is assumed to be 100%, if oral absorption data are not available. However, it is considered as appropriate to assume not

more than 50% of an orally administered dose is systemically accessible. Although the rate of Dermal Absorption has been investigated and found for the each heavy metal, still the dermal absorption ratios were accepted as 50% the conservative default value as recommended by the SCCS.

And finally the MoS value were determined as 160 (mg/kg bw/day) instead of 100 (mg/kg bw/day) which is the acceptable safety threshold value for adults by considering the exposed population is children. The accepted MoS value of 160 (mg/kg bw/day) is relevant with the 12 months babies threshold value and the reason of choosing that age is again related with the interest of worst case scenario. Therefore we can assume that MoS value above 160 is considered safe for use only in a conservative way.

According this evaluation, Mercury, Lead, Nickel, Cadmium and Antimony were above the determined safety threshold while Arsenic was under and considered not safe. As a result, the actual DA values found for Arsenic (1.9%) and SED and MOS calculations were made again, and this time it was found that the threshold values for Arsenic was also appropriate.

Still knowing that arsenic is associated with impacts on child health and exposure in early childhood has been linked to increases in mortality in young adults due to multiple cancers, lung disease, heart attacks and kidney failure, it is hard to talk about the safety of these toys even though the MoS results seem compatible.

In the other part of our study, in order to evaluate the microbiological contamination of the children finger paints, the microbiological test has been performed according to the European pharmacopeia for the blue colors of 12 selected children finger paints. The presence of Total Microorganism Count, Total Yeast and Molds Count, *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli* are searched in the finished products. According to microbiological test result, no growth of microorganism has been observed within the samples product tested and pathogen microorganisms such as *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus brasiliensis* are not found in the finished product. The microbiological test results were appropriate to Cosmetic Regulation.

As last, challenge test has been performed according to the European pharmacopeia for one of the selected blue color of finger paint in order to evaluate the efficacy of antimicrobial preservation. In none of the microorganisms (*Pseudomonas*

aeruginosa, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus brasiliensis*) increase was observed till 28th day. Therefore, the test sample is proved to be effective for the test microorganisms and the test results were appropriate to Cosmetic Regulation.

As a conclusion,

- In this study, HET-CAM method skin irritation test was performed to see whether children's finger paints cause skin irritation and it was seen that the total 24 pieces of paints used in the analysis did not cause skin irritation.
- Heavy metal measurements were made in a total of 24 children's finger paints, yellow and red, belonging to 12 different brands, and it was determined that almost all brands contained Hg, Pb, Ni, As, Cd, Sb heavy metals.
- It was determined that the Arsenic levels in each of the selected children's finger paints were above the specified standards while the other determined metals are at low concentrations.
- Only based on the British standard the Lead levels of analysed finger paints were exceeding the limit while the same values were compatible with the other determined standards.
- SED and MOS calculations were performed of a total of 24 children's finger paints, yellow and red, belonging to 12 different brands for the heavy metals in children's finger paints. It has been observed that the calculated MOS values are greater than 160 (mg/kg bw/day), which is the safe limit, in case the real dermal absorption values considered the calculation.
- Microbiology test was performed on children's blue colors of finger paints belonging to 12 different brands, and as a result, no microbiological growth was observed.
- Challenge test was performed for one of the selected blue color of finger paint, and test result was appropriate and proved to be effective for the test microorganisms.
- Although HET-CAM, MoS, Microbiology test and challenge test analysis results seem to be positive, shouldn't be forgotten that, heavy metals were detected in almost all brands of children finger paints. Thus reflects the situation in Turkey which fulfills the purpose of the study. This research is expected to raise awareness by demonstrating the importance of the issue so that those finger paints should be subjected to a much more comprehensive examination and might be accepted as

cosmetics only then we can talk about the safety of this children toys certainly.
Until then, we cannot consider children's finger paints safe.



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7. APPENDICES

CV

Personal Informations

Name	Neslihan	Surname	Şahin
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Education

Degree	Department	The name of the Institution Graduated From	Graduation year
Doctorate	Pharmaceutical Toxicology	Yeditepe University Institute of Health Science	2023
Master	Cosmetology	Yeditepe University Institute of Health Science	2016
University	Pharmacy	Semmelweis University	2012

Languages	Grades (#)
1 English	Advanced
2 Italian	Beginner
3 Hungarian	Beginner

All the grades must be listed if there is more than one (KPDS, ÜDS, TOEFL; EELTS vs),

Work Experience (Sort from present to past)

Position	Institute	Duration (Year - Year)
CEO	KOZAŞ KOZMETİK AŞ	Since 2013
Founder / Owner	NŞ GRUP DANIŞMANLIK	Since 2014
Founder / Owner	ELNES Cosmetic Consulting KFT.,	Since 2016
Founder / Owner	ELNES Cosmetic Consulting Limited	Since 2022

Computer Skills

Program	Level
Microsoft Office Programs	Advanced
CS5 Photoshop and Adope Illustrator and Corel	Intermediate

***Excellent , good, average or basic**