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Potential Health Risks of Worker Exposure to Synthetic and Natural Dyes in the Textile Industry

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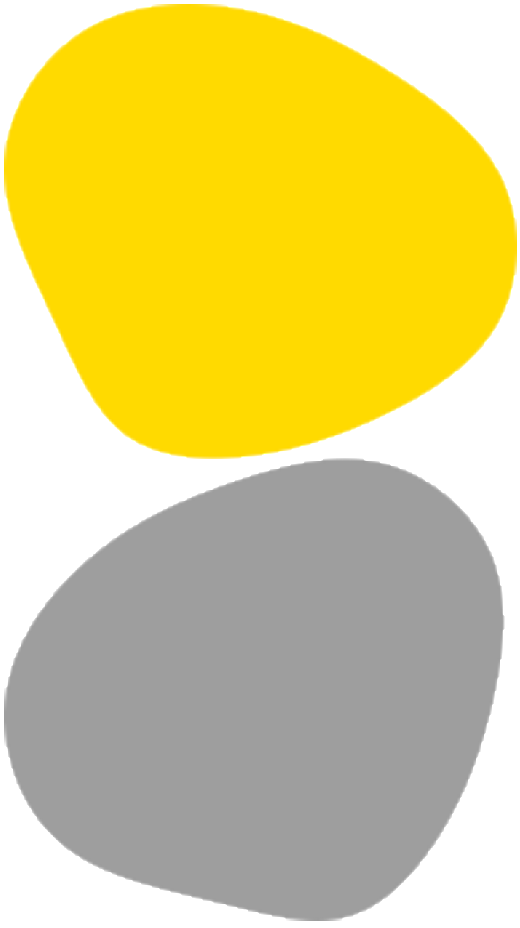

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Resumo

A Indústria Têxtil (IT) está associada a um elevado impacto ambiental, sendo a etapa de tingimento considerada um ponto crítico, devido às preocupações ambientais pela poluição gerada e aos potenciais efeitos adversos dos corantes sintéticos na saúde nos trabalhadores. A reintrodução de corantes naturais pode constituir uma alternativa face às restrições cada vez mais rigorosas impostas ao uso de corantes sintéticos. No entanto, o conhecimento dos seus potenciais efeitos para a saúde dos trabalhadores é ainda limitado, devido a escassez de investigação sobre o tema. O presente estudo visa aprofundar o conhecimento sobre os potenciais riscos associados à exposição dos trabalhadores a corantes sintéticos e naturais na IT. O estudo foi desenvolvido em 3 empresas (A, B e C), que figuram a realidade das indústrias de tingimento em Portugal. De forma a caracterizar os potenciais efeitos na saúde reportados pelos trabalhadores, foi aplicado um questionário a uma amostra de 33 profissionais do setor. Embora este estudo não inclua a avaliação da exposição ocupacional dos trabalhadores envolvidos, nas áreas de pesagem de corantes, foi realizada a monitorização ambiental de parâmetros físico químicos, com destaque para a quantificação de PM_{10} e $PM_{2,5}$. Paralelamente procedeu-se à avaliação in vitro da viabilidade de linhagens celulares representativas da pele (HaCaT, 3T3-L1 e B16F10), expostas a classes de corantes sintéticos e naturais de maior relevância para as indústrias em estudo. Os resultados da caracterização dos potenciais efeitos na saúde reportados pelos trabalhadores demonstraram que o sintoma respiratório mais declarado foi a tosse matinal (30,3%), onde 20% dos trabalhadores referiram agravamento no local de trabalho. A nível dérmico, o eczema foi o sintoma mais referido (42.4%), onde 57.1% dos trabalhadores indicaram agravamento no local de trabalho. Nas empresas B e C, observaram-se concentrações elevadas de PM_{10} e $PM_{2.5}$, com picos durante a pesagem dos corantes. Os ensaios de viabilidade celular revelaram que a maioria dos corantes sintéticos induziu citotoxicidade para as linhagens HaCaT e B16F10 (a concentrações de 500µg/mL e 250µg/mL) enquanto para linhagem 3T3-L1, os corantes sintéticos demonstraram uma viabilidade a rondar os 70% para todas as concentrações depois de 48h de exposição. Os corantes naturais apresentaram uma taxa de viabilidade celular maior (entre os 80%-100%), para tempos de exposição de 24h nas linhas celulares HaCaT e 3T3-L1. No caso da linhagem B16F10, a citotoxidade dos corantes sintéticos e naturais poderá traduzir-se um efeito positivo, pela potencial redução da proliferação de células cancerígenas. Este estudo preliminar sugere que os trabalhadores experienciam sintomas de natureza respiratória e cutânea, bem como o seu agravamento durante a manipulação de corantes, não se verificando uma distinção clara entre os efeitos associados a corantes sintéticos e naturais. A monitorização ambiental evidenciou que a tarefa de manipulação de corantes constitui uma fonte significativa de emissão de partículas, o que representa um fator agravante perante a potencial toxicidade destes corantes por via inalatória e dérmica. A mitigação destes possíveis riscos deve considerar uma abordagem integrada para garantir a segurança e saúde dos mesmos, independentemente da origem do corante utilizado.

Palavras-chave: Indústria Têxtil, Corantes Sintéticos, Corantes Naturais, Exposição Ocupacional, Efeitos na Saúde

Abstract

The Textile Industry (TI) is associated with a high environmental impact, with the dyeing stage considered a critical point due to environmental concerns related to pollution and the potential adverse health effects of synthetic dyes on workers. The reintroduction of natural dyes may represent an alternative in light of increasingly stringent restrictions on the use of synthetic dyes. However, knowledge of their potential health effects on workers remains limited, due to the scarcity of research on the subject. In this context, the present study aims to expand understanding of the potential risks associated with worker exposure to synthetic and natural dyes in the TI. The study was conducted in three companies (A, B and C), representative of the dyeing industry in Portugal. To characterize the potential health effects reported by workers, a questionnaire was administered to a sample of 33 professionals in the sector. Although this study does not include an assessment of the workers' occupational exposure, environmental monitoring of physicochemical parameters was carried out in dye weighing areas, with particular focus on the quantification of PM_{10} and $PM_{2.5}$. In parallel, in vitro evaluation of the viability of cell lines representative of the skin (HaCaT, 3T3-L1 and B16F10) was performed, following exposure to classes of synthetic and natural dyes most relevant to the industries under study. The results of the characterization of potential health effects reported by workers showed that the most frequently declared respiratory symptom was morning cough (30.3%), with 20% of workers reporting worsening at the workplace. At the dermal level, eczema was the most reported symptom (42.4%), with 57.1% of workers indicating worsening in the workplace. In companies B and C, high concentrations of PM_{10} and $PM_{2.5}$ were observed, with peaks during dye weighing. The cell viability assays revealed that most synthetic dyes induced cytotoxicity in HaCaT and B16F10 cell lines (at concentrations of 500 µg/mL and 250 µg/mL), whereas in the 3T3-L1 line, synthetic dyes showed viability around 70% at all concentrations after 48 hours of exposure. Natural dyes exhibited higher cell viability rates (between 80%–100%) after 24 hours of exposure in HaCaT and 3T3-L1 cell lines. In the case of the B16F10 line, the cytotoxicity of both synthetic and natural dyes may represent a positive effect, due to the potential reduction in the proliferation of cancerous cells. In summary, this preliminary study suggests that workers experience respiratory and skin-related outcomes, as well as worsening of these conditions during dye handling, with no clear distinction observed between the effects associated with synthetic and natural dyes. Environmental monitoring demonstrated that dye handling constitutes a significant source of particle emissions, representing an aggravating factor in view of the potential inhalation and dermal toxicity of these dyes. Mitigating these potential risks should involve an integrated to ensure their safety and health, regardless of the origin of the dye used.

Keywords: Textile Industry, Synthetic Dyes, Natural Dyes, Occupational exposure, Health effects

Index

List of Acronyms

ACD – Allergic Contact Dermatitis

ACGIH – American *Conference of Governmental Industrial Hygienists*

AD – Atopic Dermatitis

ATCC– American Type Culture Collection

CO_2 – Carbon Dioxide

CO – Carbon Monoxide

CPE – Collection Protective Equipment

DMEM – Dulbecco's Modified Eagle Medium

EAC – Economic Activity Code

EC – European Commission

ECHRS – European Community Respiratory Health Survey

ELV – MC –Exposure Limit Value – Maximum Concentration

ELV – STE – Exposure Limit Value – Short-Term Exposure

ELV – TWA– Exposure Limit Value – Time-Weighted Average

ELV – Exposure Limit Values

GA²LEN – Global Allergy and Asthma European Network

ICD – Irritant Contact Dermatitis

MTT– 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide

PBS – Phosphate Buffer Solution

PM_{10} – Particulate Matter which is <10µm in diameter

$PM_{2.5}$ - Particulate Matter which is <2.5µm in diameter

PPE – Personal Protective Equipment

REACH – Registration, Evaluation, Authorization and Restriction of Chemicals

SDS – Safety Data Sheets

TCI – Textile and Clothing Industry

List of Figures

List of tables

1. Introduction

Globalization and population growth have created the need for large-scale production across various industries to meet consumer demands, markedly the Textile Industry (Carvalho & Santos, 2016). Currently, the textile and clothing industry (TCI) is one of the most globalised industries in the world, contributing approximately 7% of total global exports and employing around 35 million workers globally (Desore & Narula, 2018). In Portugal, TCI holds significant economic importance, contributing positively to job creation (Costa Maia et al., 2014).

TCI sector of activity encompasses the production of yarns and fabrics, finishing processes and fabric manufacturing. In the case of finishing processes, four sub-steps stand out: (1) pre-treatment, (2) dyeing, (3) printing and (4) final finishing. The dyeing and printing processes are used in the conversion of pre-treated raw textile fibres into finished products that aim to enhance the appearance of fabrics for consumers (Abrahart & Whewell, 2024). These processes, though somewhat distinct, involve the fusion of dye molecules into textile materials in various forms, such as fibres, yarns, and fabrics, in an aqueous solution with the aid of appropriately equipped machines (Ammayappan et al., 2016). However, the global textile industry is responsible for having a serious environmental impact across the entire supply chain, with remarkable greenhouse gas emissions (over 3.3 billion metric tons per year), significant land and water consumption, pollution of the soil, air and water, and increasing waste production (Pizzicato et al., 2023). The dyeing and finishing stages represent a significant hotspot in the textile supply chain due to their high water and energy demands, as wet processing requires large volumes of heated water for operations such as preparing dye baths and washing fabrics after dyeing. Moreover, there are growing ecological concerns about the release of these effluents into the environment, given the potential mutagenic, toxic and carcinogenic effects of certain dyes (Pizzicato et al., 2023). Therefore environmental, public health and sustainability issues are constantly under debate, with legislation becoming increasingly restrictive, as revelled by the recent banish of some synthetic dyes by the European Commission (EC). In fact, beyond environmental concerns, several studies have highlighted potential health effects of synthetic dyes on both industry workers and end consumers (Al-Tohamy et al., 2022). Indeed, exposure to certain types of dyes may pose health risks, with some identified as respiratory or skin sensitizers (HSE, 2024). Among these, azo dyes, once commonly used to colour cotton and now banned by various EC regulations, are regarded as carcinogenic, allergenic and detrimental to reproductive health (Md. T. Islam et al., 2022). Likewise, disperse dyes, typically applied to synthetic fabrics, are known to cause allergic reactions (KEMI, 2014). The EC has investigated the potential link

between chemicals used in textile finishing and allergic responses, finding that textile finishing resins and other auxiliaries can trigger allergic contact dermatitis (Van der Putte et al., 2013).

Given the importance of the textile industry and its products to society, it is essential to find alternatives with lower environmental impact, sustainable and equally effective dyeing processes. The reintroduction of natural dyes into the textile industry, specifically in the dyeing process, is an alternative aimed at addressing the restrictions increasingly imposed on the use of synthetic dyes (Rungruangkitkrai & Mongkholrattanasit, 2012). Although innovative methods like low-water or waterless dyeing and chemical-free technologies are being developed to lessen the environmental impact of dyeing, there is a growing renewed interest in natural dyes as a more sustainable alternative to synthetic options, offering the potential to decrease chemical use and reduce environmental harm. The use of natural dyes in the dyeing process is often considered an environmentally friendly alternative. However, the potential for adverse health effects, such as allergies, remain largely unknown due to the scarce research available on this topic. More in-depth research on the safety of these materials for both humans and the environment is needed before promoting their widespread use, as not everything of natural origin is guaranteed to be safe (Pizzicato et al., 2023). Care, therefore, is required when introducing new dye sources and detailed toxicological studies must be carried out to determine their safety for both humans and the environment (Muthu, 2014).

In this regard, it becomes crucial to address the risks associated with both synthetic and natural dyes used in the textile industry, as they have a direct impact on workers' health. Thus, this study aims to deepen the understanding of the potential human health risks associated with worker exposure to synthetic and natural dyes in the textile industry, through the integration of worker-reported health effects, environmental monitoring and *in vitro* toxicological evaluation.

The specific objectives of this work were:

- To characterize the potential effects on health reported by workers, based on their perspective of occupational exposure to synthetic and natural dyes in the textile industry.
- To monitor environmental physicochemical parameters associated with the handling of synthetic and natural dyes in the most representative workstations, namely weighing areas.
- To evaluate the *in vitro* effects on cell viability of different classes of synthetic and natural dyes, specifically on skin representative cells namely keratinocytes, fibroblasts and melanoma cells.

2. Literature Review

2.1. Textile Industry

In Portugal, the textile industry is one of the most significant sectors, having long played an important role in both the national and international economy (Bullon Pérez et al., 2017). The TCI is represented by the following Economic Activity Codes (EAC) (INE, 2007):(1) EAC 13, which represents the manufacturing of textiles, including the preparation of textile fibres, washing, combing, spinning, twisting and weaving of wool, as well as cotton, linen, hair, artificial and synthetic fibres. This EAC also includes textile finishing, production of home textiles and other textile articles; (2) EAC 14, which encompasses the manufacturing of clothing items, such as fabric, knitwear, leather and furs, excluding fur garments. This sector includes the transformation of textile materials into clothing, covering activities such as cutting, sewing and finishing. According to Bullon Pérez et al. (2017), despite the diversity of working methods in the TCI, they can be summarized into four main interrelated stages, as shown in **Table 1**.

Table 1. Production processes of the textile industry sector.

Designation	Process Description
Raw Material Production Processes	
1. Spinning	The production of yarns or filaments that will be prepared for the weaving stage
2. Weaving	The manufacturing of woven fabrics, knitted fabrics (knitwear), and non-woven fabric technology
Finishing Processes	
3. Finishing	A stage that gives fabrics durability and comfort through their visual, physical, and aesthetic properties, achieved through subprocesses such as bleaching, dyeing, printing, coating, among others.
Garment Manufacturing Process	
4. Garment Manufacturing	The stage involves design, pattern making, grading, layout, cutting, and sewing for various purposes.

Given the TCI production cycle, where the final product of each phase becomes the raw material for the next phase, the textile industry sector is attributed to a highly diverse character with different dynamics and differentiating segments. In this sense, textile fibres and their respective categorization are central to the production cycle.

2.2. Legal framework and standards applied to textile industry

Understanding the regulatory context surrounding the textile industry, particularly regarding dyeing using synthetic and natural dyes, involves defining a set of laws, regulations and standards aimed at ensuring the protection of workers' health, the safety of production processes and the preservation of the environment. Within the scope of this study, which addresses the worker exposure to dyes and their potential health effects, it is essential to identify and analyse the main legal provisions governing the use of chemical substances, occupational health and safety as well as the specific technical standards of the textile sector. **Table 2** provides a summary of the key legal and regulatory instruments, organised by areas of application.

Table 2. Legal framework and certifications in the field of health and safety, with a focus on chemical agents used in the textile industry.

Domain	Legal/Normative Reference	Description	Application
Occupational Health and Safety	Law No. 102/2009, of 10 de September and its subsequent amendments	Legal framework for the promotion of safety and health at work	Rights and duties of textile sector workers
Chemical agents	Decree-Law No. 24/2012 of 6 February	Requirements for the protection of workers against risks to safety and health arising from exposure to chemical agents at work	Define exposure limits and employer obligations
	Regulation (EC) No 1907/2006, of 18 de December 2006 (amended by Regulation (EC) No 2020/878, of 18 June 2020)	Registration, evaluation, authorization, and restriction of chemical substances	Restriction of hazardous dyes and provision of Safety Data Sheets (SDS)
	Regulation (EC) No 1272/2008, of 16 December 2008	Classification, Labelling and Packaging of substances and mixtures	Classification of the hazardous properties of substances
Certifications	GOTS – Global Organic Textile Standard	Certification for organic fibres and approved natural dyes	Guarantee of safe and sustainable practices
	OEKO-TEX® Standard 100	Certification limiting harmful chemical substances	Impact of dyes on health

2.3. Textile Dyes

Since prehistoric times, natural dyes have been used to colour natural fibres, cosmetics and to produce some paints and watercolours. With Perkin's discovery in 1856, certain industries, such as textiles, printing, rubber and plastics, began widely using synthetic dyes to colour their products (Kant, 2012).

A dye consists of a synthetic and/or organic compound that, due to its chemical structure, is capable of colouring various products. Dyes are composed of chromophores, auxochromes and a solubilizing group. According to Ardila-Leal et al. (2021), the chromophore group is responsible for the dye's colour due to its saturation. Chromophores contain heteroatoms such as N, O and S, and include bonds like –N=N– (azo), =C=O (carbonyl), NO or N–OH (nitroso), $-NO_2$ or NO–OH (nitro), and C=S (sulphur). Chromophore groups are unsaturated and consist of atoms or groups of atoms in which the arrangement of successive single and double bonds resonates, allowing the absorption of light rays. On the other hand, the auxochrome group controls the affinity of the product that the dye will colour. Some of these include $-NH_3$ (amine), –COOH (carboxyl), HSO_3 (sulfonate) and –OH (hydroxyl). As the name suggests, the solubilizing group allows the dye molecule to dissolve in water. Unlike dyes, pigments are highly water insoluble and contain particles ranging from 1–2 μm. In contrast, dyes, in addition to their water solubility, have particle sizes ranging from 0.025–1.0 μm and absorb light in the visible spectrum (400–700 nm) (Ardila-Leal et al., 2021).

Based on how they are obtained, dyes are classified into two major groups: synthetic dyes and natural dyes. Natural dyes may be derived from three main sources: (1) plants, (2) animals or insects and (3) minerals (Affat, 2021; Wardman, 2018). In case of synthetic, their subclassification can be based on other various factors, such as method of application and chemical structure, as shown in **Table 3 and 4** (Affat, 2021).

Table 3. Classification of dyes according to their chemical structure.

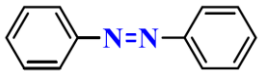
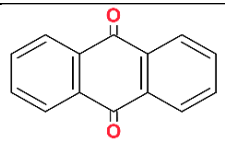
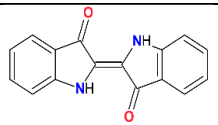
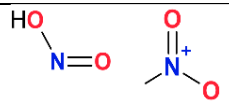
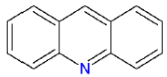
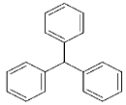
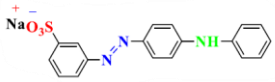
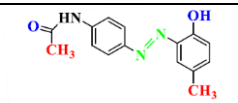
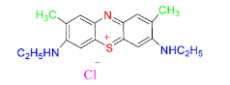
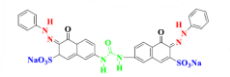
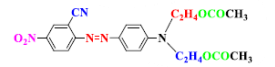
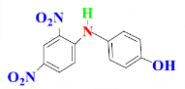
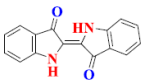
Classification of Dyes		Chromophore Structure	Example of Dyes	Characteristics	Reference
Chemical Structure	Azo dyes		Methyl Orange, Congo Red.	Most common chemical class (60%). The chromophore is the azo group (–N=N–).	(Ardila-Leal et al., 2021; Benkhaya et al., 2020; Wardman, 2018)
	Anthraquinone dyes		Reactive Bright Blue X-BR, Reactive Blue 4	Low price, high accessibility and good performance. Used as base for acid, vat and disperse dyes.	
	Indigoid dyes		Indigo Carmine, Ciba Blue 2B	Highly resistant to light and high temperatures.	
	Nitro dyes Nitroso dyes		Disperse Yellow 26, Disperse Yellow 14	Nitro ($-NO_2$) group incorporated in dye molecules as an auxochrome, acts as the chromophore.	
	Acridine		Acridine orange, Basic Yellow 9	Heat-resistant, although they have low lightfastness.	
	Triphenylmethane dyes		Crystal Violet, Light Green SF	Central sp3 hybridised carbon atom, bonded to three aryl groups. Most used synthetic dyes in the textile industry.	

Table 4. Classification of dyes according to their application class.

Classification of Dyes		Chromophore Structure	Example of Dyes	Characteristics	Reference
Application class	Acid dyes (Anionic)		Acid Yellow 36	Need for an acid dyebath for its application rather. Anionic dyes with substantivity for protein fibres.	(Benkhaya et al., 2020; Wardman, 2018)
	Azoic dyes		Disperse Yellow 3	Creating an insoluble azo dye in situ within the fibre (usually cotton).	
	Basic dyes (Cationic)		Basic Blue 24	Applied to acrylic fibres where they provide good lightfastness.	
	Direct dyes (Anionic)		Direct Orange 26	Substantivity for cellulosic fibres, their attachment being through both hydrogen bonds and van der Waals forces.	
	Disperse dyes		Disperse Red 60	Non-ionic in character and applied to hydrophobic fibres from an aqueous dispersion. Simplicity of application.	
	Mordant dyes (Anionic)		Disperse Red 82	Contain suitably disposed chemical groups with which the chromium can combine.	
	Reactive dyes (Anionic)		Reactive Red 6	High wet fastness, brilliance and range of hues. Second largest class of dyes.	
	Sulphur dyes		Sulfur Blue Dye, CI 53235	Cover a limited shade range. Used on cellulosic fibres and their blends.	
	Vat dyes		Vat Blue 1 (synthetic indigo)	Intended for application to cellulosic fibres.	

Due to population growth, increased industrial production and the high costs associated with extracting natural dyes, led to the search for synthetic alternatives, a pursuit that culminated in 1856 with the discovery of the first Synthetic Dye (Kant, 2012).The colour obtained from these types of dyes began to be used in materials such as plastics, textiles, food and printing materials. The properties of these dyes vary depending on the specific dye; however, in summary, synthetic dyes exhibit the following properties (Ardila-Leal et al., 2021): (1) brightness and consistency, these dyes have bright and vibrant colours, consistent across different batches; (2) stability, as they offer greater stability in use, being more resistant to exposure to certain environmental factors; (3) accessibility, generally, synthetic dyes are more accessible compared to natural dyes, making them a more economical option; (4) chemical properties, that vary from dye to dye.

Regarding Natural Dyes, these are defined as organic compounds derived from natural sources such as plants (e.g., saffron), insects (e.g., beetles), animals (notably some species of molluscs) and minerals (e.g., clay), without the use of any chemical treatment (Affat, 2021). These organic molecules exhibit various colours and mixtures derived from light absorption in the visible region of 400–800 nm. Thus, achieving the desired colour with these dyes depends on the characteristics of the source, as well as the extraction method and application technique. Natural dyes have been used for various purposes, notably in colouring natural fibres (wool, cotton, and silk), leather, in cosmetics, and in the production of paints and watercolours (Wardman, 2018).

The use of synthetic and natural dyes considers the balance between their advantages and disadvantages in terms of price, production capacity, and effects on the environment and human health (**Table 5**).

Table 5. Advantages and disadvantages/challenges of synthetic and natural dyes in terms of price, production capacity, and effects on the environment and human health

Classification of Dye	Advantages	Disadvantages/Challenges	Reference
Synthetic dyes	– Offer a wider range of colours and shades. – More consistent in colour, ensuring that the same shade of colour can be achieved repeatedly. – Generally, less expensive, making them a more cost-effective option for large-scale production. – More durable and can withstand harsh environmental conditions and washing. – Can be produced in large quantities, making them more widely available and accessible. – Different chemical properties that make them suitable for different types of materials and applications, allowing for greater versatility and flexibility.	– Can cause pollution of water and soil due to their synthetic origin and complex molecular structures. – Can be harmful to humans, especially those who work in their production. – Most dyes are not biodegradable, meaning they can persist in the environment for a long time.	(Affat, 2021; Nambela et al., 2020. Pizzicato et al., 2023. Samanta, 2018)
Natural dyes	–Fairly non-polluting and have lower toxicity. – Natural dyestuffs produce rare colour ideas and are automatically harmonizing. –The vegetable based natural dyes are replaceable and at the same time biodegradable. –Fabrics dyed with natural dyes can provide good protection against ultraviolet rays without altering wear properties. –Naturally dyed materials have good resistance to moths;	– The extraction, preparation of textile materials requires and mordanting and natural dyeing need expertise and is thus expensive. – The natural dyed textile fabric may change colour when exposed to the sun, sweat and air and moisture. –Lack of availability of precise scientific/technical knowledge on extraction. – Poor solubility in water may require a more complex extraction procedure.	

2.4. Dyeing process

The term textile finishing encompasses the set of operations, sequential or not, to which a fabric is subjected after its manufacturing until it is ready for garment production. These operations, which can also be referred to as textile ennoblement, can be subdivided as shown in **Figure 1** (Broadbent, 2001; Wardman, 2018):

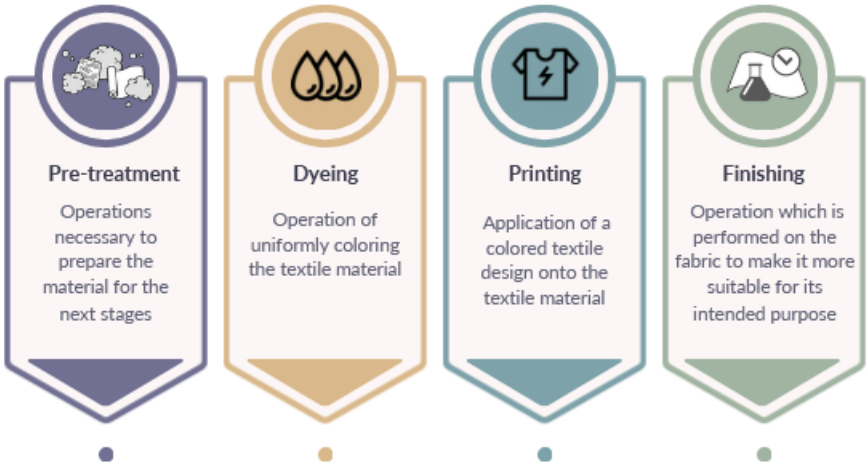


Figure 1. Textile finishing stages.

In the textile finishing stages, raw materials involved include not only the textile material but also various products such as water, resin, dyes, detergents, among others (Broadbent, 2001; Wardman, 2018). This work will focus only on the dyeing process using dye products. Dyeing is a process aimed at uniformly colouring textile materials by converting raw textile fibres into finished products. During this process, the fabric is immersed in an aqueous solution, called a bath, containing chemical products (dyes) and auxiliaries (dispersing agents, mordants, among others). Dyeing is based on physicochemical equilibrium mechanisms, through the absorption of the dye molecule by the fibre via ionic forces, hydrogen bonds and covalent chemical bonds (Abrahart & Whewell, 2024; Schönberger & Schäfer, 2002).

The dyeing process can be carried out by different methods based on the application technique, specifically: (1) continuous, (2) discontinuous, and (3) semi-continuous processes (Broadbent, 2001).
In discontinuous process, the movement of the dye into the fibres is driven by the dye's substantivity. In this sense, the textile material is in constant contact with the bath, and the fibres gradually absorb the

dyes. In this process, the following stages can be distinguished: (1) disaggregation of dye aggregates in the bath, (2) diffusion in the bath, (3) surface absorption onto the fibre, (4) diffusion into the fibre and (5) fixation of the dyes to the fibre (Wardman, 2018). In this sequence, the dyeing equilibrium depends on temperature, time, pH and the appropriate auxiliary chemicals for the dye/fibre system to achieve level and well-penetrated dyeing. After the dyeing process is completed, finishing processes are carried out to ensure the quality of the substrate (Broadbent, 2001; Wardman, 2018). In semi-continuous process, dyes are applied from the same bath, and the fabric is rolled onto a perforated or non-perforated beam, covered with a plastic sheet to prevent drying, and then stored to allow dye diffusion and fixation. The coloration process is completed by washing the dyed fabric (Wardman, 2018). In the continuous process, the dyes are applied through a filling nozzle, followed by direct fixation of the dye onto the fabric. In this case, a uniform distribution of the dye occurs across the width and length of the fabric through processes involving steaming and temperature action. The stages of the continuous dyeing process are as follows: (1) impregnation of the fibres with the dye bath, (2) uniform distribution of the bath within the fibres, followed by appropriate (3) post-treatment processes, which involve the diffusion and fixation of the dye (Broadbent, 2001; Wardman, 2018). After the dyeing process is completed (in any of the processes), the textile material undergoes the following stages: (1) washing with a detergent, usually in boiling water, to remove any unfixed dye, and (2) the addition of chemicals to improve the dye's fastness and finishing properties, such as softeners (Wardman, 2018). In these processes, factors such as the material to be dyed, dye classes, water, auxiliary products, dyeing machines, and human resources are crucial in determining the quality of the dyeing process, which initially limits the type of process that textile industry units intend to apply (Broadbent, 2001; Wardman, 2018).

Dyeing units have a laboratory focused on conducting dyeing tests to develop formulas capable of reproducing the color desired by the customer on the intended textile material (Broadbent, 2001). The personnel responsible for dye preparation begin the process according to the previously lab defined formulas, detailing the quantities of each dye. Before the dye (powder) is transferred to the bath, it is necessary to ensure that all dye particles are fully dissolved in water to prevent the possibility of staining certain parts of the textile material. With the aid of some wetting agents, the dye powder is dissolved by agitation, and the solution is then added to the dye bath. In some cases, the aqueous solution is added through a fine sieve to ensure the removal of any particles that may not have dissolved.

2.5. Potential worker exposure associated with dyeing process

The definition of a chemical agent consists of any chemical element or compound, whether alone or in a mixture, that occurs naturally or is produced, used, or released because of work activity, including in the form of waste, whether it is intentionally produced or marketed. Chemical agents can exist in different forms, for example as solids (dust, fumes, fibers, powders), as liquids (vapor, mists) or gases (ACT et al., 2016). In the dyeing process, hazardous chemical substances are present, whether of synthetic or natural origin, and may be used for different purposes to confer specific effects to a product. In this context, they can be categorized as functional chemical agents, auxiliaries and non-intentionally added substances. Functional chemical substances are intended to contribute to the design and/or provide certain properties to the final article, such as dyes. In the case of auxiliaries, these agents are only necessary to ensure the production processes function properly, such as salts, acids and bases. Non-intentionally added substances are degradation products or contaminants, such as certain toxic metals released as impurities from the raw materials (ACT et al., 2016; CE, 2005).

Chemical contaminants, during their handling, transport, or storage, potentially cause harm to human health (occupational diseases) for exposed individuals or leading to personal and material accidents (Eurisko, 2008). The effects resulting from this exposure are related to numerous factors, including the following (Eurisko, 2008; ILO, 2023): (1) chemical composition , (2) ability to penetrate the body and its solubility in the blood, (3) quantity of chemical substance exposed, (4) duration and frequency of exposure, (5) characteristics of the functions/tasks performed by the worker, (6) routes of exposure and (7) individual characteristics of the worker. The routes of exposure are considered a crucial factor in the development of potential health risks associated with exposure to chemical substances. The typical routes of occupational exposure are categorized into the classes outlined in **Table 6** (ILO, 2023).

Table 6. Routes of exposure to textile dyes.

Absorption	Inhalation	Ingestion
The main source of exposure to various chemical substances through the skin and eyes. In many cases, absorption occurs without the knowledge of the worker. Pregnant women and young people are considered vulnerable groups.	The preferred route for materials that generate dust. Additionally, gases and vapours are also inhaled and absorbed in the respiratory tract.	Occurs typically when particles reach the back of the throat and are swallowed. This happens in situations such as handling food or cigarettes without hands.

In the dyeing process, occupational exposure is mainly by dermal contact, but substances might also reach the respiratory system and eyes, especially during the manual preparation of dye baths near work machines (Eurisko, 2008). Dust consists of small, spheroidal airborne particles formed through the handling of certain materials and mechanical disintegration processes. According to the type of harm they cause, different categories can distinguish: (1) Inert particles, that do not cause significant physiological changes, although they may be retained in the lungs. They generally pose problems only at very high concentrations; (2) Fibrogenic particles, capable of triggering chemical reactions at the level of the pulmonary alveoli ; (3) Sensitizing particles, may act on the skin (cutaneous sensitizers) or on the respiratory system (inhalation sensitizers) ; (4) Toxic particles , can cause damage to one or more internal organs, either rapidly and at high concentrations (acute intoxication) or slowly and at relatively low concentrations (chronic intoxication) (Miguel, 2014).As previously mentioned, during the dyeing process, dyes in their primary physical state, i.e., powder, are dissolved in a bath to form a saturated solution. To ensure rapid dissolution, the dyes are composed of very small particles. In the case of disperse dyes, the particles range in size from 0.5 μm to 1.0 μm (Wardman, 2018).

2.5.1. Effects on human health due to exposure to textile dyes

Hazardous textile dyes can cause both acute and chronic health effects in workers. In the case of acute effects, signs and symptoms occur immediately after exposure and can be either local or systemic. Local effects occur at the point of contact (for example, skin and eye irritation, which will be detailed later), while systemic effects require absorption and distribution from the entry route to other parts of the human body (ILO, 2023). Regarding chronic and/or long-term effects, health consequences may develop after several years or decades, caused by constant exposure to a harmful substance over a prolonged period. In such cases, the effects are usually permanent and may include organ damage, weakening of the immune system, allergies and occupational asthma, reproductive problems and cancer (ILO, 2023). Health effects tend to be specific to certain agents, so the information provided in the Safety Data Sheets (SDS) (as required by Regulation (EC) No. 1272/2008, of 16 December 2008) for these substances is essential to understand their hazardous properties with respect to human health. According to this regulation, hazards to human health are organised into hazard categories based on the adverse effects they may cause to the body **(Table 7).** These categories are crucial for identifying and communicating risks associated with exposure to chemical substances, including those used in the textile industry at dyeing facilities.

Table 7. Health hazard categories based on the adverse effects specified by Regulation (EC) No1272/2008, of 16 December 2008.

Health Hazard Categories	Description
Acute toxicity	Immediate toxicity after a single exposure via oral, dermal, or inhalation routes
Skin corrosion/irritation	Substances that cause irreversible damage to the skin (corrosion) or reversible inflammation (irritation
Serious eye damage/eye irritation	Covers products causing permanent eye damage or temporary eye discomfort
Respiratory or skin sensitisation	Substances that may induce allergic reactions after inhalation or skin contact
Germ cell mutagenicity	Potential to cause inheritable genetic changes
Carcinogenicity	Substances with the potential to induce cancer
Reproductive toxicity	Covers adverse effects on fertility, fetal development, and reproductive functions
Specific target organ toxicity – single or repeated exposure	Adverse effects on organs following single or prolonged exposure

Workers in the textile industry are exposed to a variety of chemicals including dyes, solvents, optical brighteners, finishing agents and numerous types of synthetic and natural fibre dusts, all of which can affect their health. Additionally, textile workers are exposed to airborne dust containing infectious, allergic and toxic substances. Dyes used in dyehouses are very harmful to human health. The most common symptoms resulting from this exposure focus on problems such as skin, eye and respiratory tract irritations (Elhadidy et al., 2022). In general, the diseases provided by textile dyes comprise from dermatitis to disorders of the central nervous system (Khan & Malik, 2018) or may be related to the substitution of enzymatic cofactors that result in the inactivation of the enzymatic activities themselves (Copaciu et al., 2013).The acute toxicity to textile dyes is caused by oral ingestion and inhalation, especially by exposure to dust (Clark, 2011), triggering irritations to the skin and eyes (Gregory, 2007). The workers who produce or handle reactive dyes may have contact dermatitis, allergic conjunctivitis, rhinitis, occupational asthma or other allergic reactions (Hunger, 2003). **Table 8** summarizes reported health effects observed in workers exposed to dyes.

Table 8. Overview of the general health effects associated with exposure to different classes of dyes among workers.

Health effects	Class of Dyes	References
Allergic dermatitis	Reactive dyes, Basic dyes, Disperse dyes, Acid dyes, Direct dyes, Mordant dyes, Vat dyes, Sulphur dyes	(Chavan, 2023; KEMI, 2014; Van der Putte et al., 2013)
Allergic conjunctivitis	Reactive dyes	(Chavan, 2023)
Rhinitis	Reactive dyes	
Asthma	Reactive dyes, Disperse dyes	
Respiratory sensitizers	Reactive dyes, Sulphur dyes, Direct dyes	
Skin irritation	Reactive dyes, Disperse dyes, Basic dyes	(Chavan, 2023; Van der Putte et al., 2013)
Skin sensitization	Reactive dyes, Disperse dyes, Acid dyes, Vat dyes, Sulphur dyes, Direct dyes	
Mutagenic effects	Reactive dyes, Disperse dyes	(T. Islam et al., 2022)
Carcinogenic effects	Acid dyes, Direct dyes, Azo dyes	

According to the study of Elhadidy et al. (2022), there is a reported prevalence of respiratory complaints among the workers studied, with cough and shortness of breath being the most common symptoms identified among exposed workers to synthetic dyes. The respiratory problems identified are caused by the inhalation of certain particles from synthetic dyes handled by workers, as well as other chemicals like formaldehyde, leading to respiratory sensitization. Inhalation of respiratory sensitizers can cause allergic symptoms such as nasal discharge, nasal congestion, and/or watery and irritated eyes, as well as obstruction of the airways, even from exposure to small quantities of the dye (Van der Putte et al., 2013). Available literature describes dye powder as a potent respiratory sensitizer; however, it is challenging to differentiate whether health problems are caused by the particles of this powder or by chemically induced toxicity (KEMI, 2014). Furthermore, textile dyes have been implicated in respiratory effects, such as asthma, particularly in workers exposed to occupational settings (NM et al., 2022). According to Tang et al. (2018), occupational asthma results from specific etiological agents present in the work environment. Several studies have evaluated the pulmonary function of workers employed in the textile dyeing industry. Reactive dyes have been identified as one of the contributing factors to occupational asthma, as they comprise a chromogen linked to a reactive group that

facilitates covalent bonding with hydroxyl groups on cellulose fibres. These dye–fibre conjugates function as haptens, potentially triggering an immune response. According to the study carried out by Elhadidy et al. (2022) another common health issue among the studied workers was various skin manifestations, such as rashes, burns, colour changes and dryness of the hands. Skin sensitization and irritation are very common symptoms resulting from exposure to dyes. Dermatitis related to textiles can be classified into four groups consisting of: (1) atopic dermatitis (AD), (2) allergic contact dermatitis (ACD), (3) irritant contact dermatitis (ICD) and (4) mimics of textile pattern dermatitis. The primary sensitisation due to textile-related ACD is rarely due to textiles, but more often due to occupational exposure to chemicals that cross-react with allergens found in textiles; dye is one of the main culprits, especially disperse dye. Reactive dyes, vat dyes and disperse dyes are considered skin sensitizers, causing redness and rashes usually between the fingers and wrists (HSE, 2024). Some textile dyes are cytotoxic, genotoxic, carcinogenic and mutagenic; for instance, dyes containing an azo group (–N=N–) can split apart genotoxic and carcinogenic amines (e.g., CI Acid Red 85, which releases benzidine). In the case of azo dyes, the Registration, Evaluation, Authorization and Restriction of Chemicals, Regulation (EC) No. 1907/2006, of 18 de December 2006 (REACH) establishes strict restrictions on their manufacture, placing on the market and use. Several aromatic amines associated with this type of dye have been classified as carcinogenic, mutagenic and/or toxic to reproduction. Accordingly, the regulation includes a list of 22 aromatic amines that can be released from azo dyes, whose presence in textile articles is prohibited at detectable concentrations, i.e., above 0.003% by weight (KEMI, 2014).

According to HSE (2024), it is important to highlight that symptoms associated with dye exposure may appear immediately after contact with a specific dye, in which case the correlation is relatively easy to identify. However, a common pattern is that symptoms are delayed for several hours, often worsening at the end of the workday or even during the night. In such cases, the affected worker may not realize that their health condition is work-related. The first indication may come when the individual is away from the workplace for an extended period (e.g., during vacation) and notices that the symptoms have improved or even disappeared. Sensitization to dyes presents several key points relevant to occupational health surveillance for exposed workers. Workers operating under the same conditions and with similar job profiles (such as dyeing operators, weighing technicians,

among others) may become sensitized to dyes; in most cases, sensitization occurs within the first two years of exposure, although in some instances it may develop years or even decades after beginning work with dyes. Same author mentioned, dye sensitization is irreversible, and a worker sensitized to one class of dyes is more likely to experience adverse reactions to other dye classes as well. Although natural dyes are often perceived as safer and more environmentally friendly, this assumption is not always supported by scientific evidence. In fact, natural dyes do not guarantee safety and comprehensive toxicological data for many of these substances are lacking. Conversely, synthetic dyes that pass eco-standards such as Bluesign, GOTS standard and OekoTex 100 have been assessed thoroughly for toxicity and should be chosen over dyes that have not. Moreover, the synthetic dyes are supported more detailed SDS and scientific testing, compared to many natural dyes (Affat, 2021).

2.6. Risk assessment associated with the dyeing process.

To ensure control of the effects resulting from exposure to chemical agents on workers' health, Directive 98/24/EC of 7 April 1998 establishes the employer's obligation to identify the presence of hazardous chemicals in the workplace, eliminate them, and, where elimination is not possible, assess the risk they pose and minimize their impact. Chemical risk assessment allows for setting priorities, implementing necessary prevention measures, and evaluating the effectiveness of existing measures. This assessment is an informative process that involves studying the properties of chemical agents, as well as the working conditions and the people exposed to these agents. The risk assessment process can be carried out with varying degrees of detail, from more detailed and complex evaluations to simplified assessment methodologies (CE, 2005). The following section will describe assessment methodologies and tools.

The chemical risk assessment process should include the following steps presented in **Figure 2**:

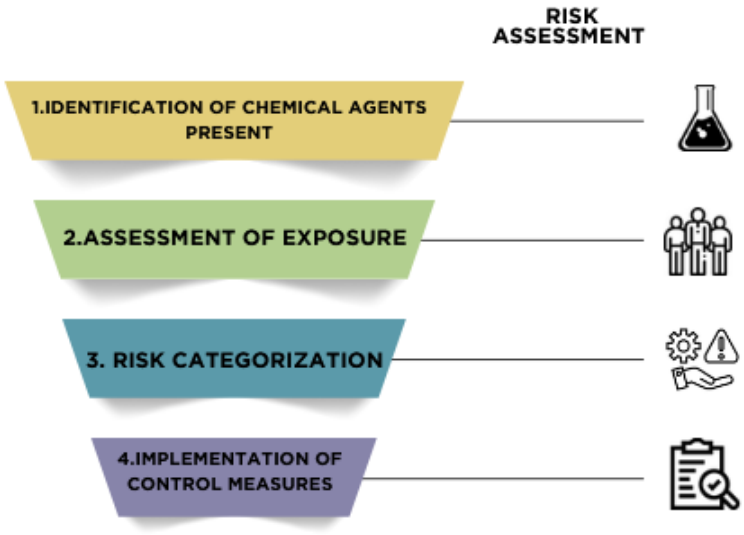


Figure 2.Steps of Chemical risk assessment.

The identification of chemical elements present in the workplace can be achieved through the analysis of manufacturing processes (such as dyeing), raw materials used and products produced, identifying locations where chemical contaminants are released as well as consulting SDS (Eurisko, 2008). After identifying the chemical elements present, the next step is to assess workers' exposure, considering aspects: routes and duration of exposure, risk of penetration trough damaged skin or infiltration through Personal Protective Equipment (PPE), levels of concentrations of hazardous substances and influence of other environmental factors (ILO, 2023). Risk categorization through quantitative assessments should include results from environmental measurements concerning Exposure Limit Values (ELV). These values represent the concentrations of various substances in the air and indicate conditions under which nearly all workers can be exposed, day after day, without adverse health effects. Therefore, to ensure that a chemical contaminant does not cause irreversible long-term effects, its concentration in the air must be below the established value. It is important to note that these values should never be used as a dividing line between hazardous and non-hazardous situations. The Portuguese standard NP 1796:2007, establishes exposure limit values for chemical agents in the air at workplaces, based on the guidelines from the American *Conference of Governmental Industrial Hygienists* (ACGIH). There are three classes of limit values:

- Exposure Limit Value – Time-Weighted Average (ELV– TWA): The average concentration over an 8-hour workday and a 40-hour workweek to which it is considered that nearly all workers can be exposed, day after day, without adverse health effects.
- Exposure Limit Value – Short-Term Exposure (ELV – STE): The concentration to which it is considered that nearly all workers can be repeatedly exposed for short periods, provided the ELV – TWA is not exceeded, and without adverse effects such as irritation, chronic or irreversible tissue damage, dose-dependent toxic effects, and absorption rate.
- Exposure Limit Value – Maximum Concentration (ELV – MC): The concentration that should never be exceeded during any period of exposure.

Thus, risk categorization involves comparing the existing environmental concentration with the exposure limit values stipulated for various chemical elements. However, organizations also often resort to simplified qualitative assessments, which provide a semi-quantitative approach to risk dimensioning in the absence of exposure limit values. The risk categorization should include the results of environmental measurements concerning the established occupational exposure limit values. Whenever an occupational exposure limit value is exceeded, the employer must act immediately, considering the nature of the limit, to eliminate or reduce the exposure through the implementation of appropriate prevention and protection measures. However, as provided in Directive 98/24/EC of 7 April 1998, it is possible to forgo environmental measurements for risk categorization, provided that the employer can demonstrate the use of alternative assessment methods and that appropriate prevention and protection measures have been implemented. The adoption of simplified methodologies may be a viable option, as they allow for a semi-quantitative estimation of risk magnitude in the absence of an established exposure limit. These methodologies combine values for the probability and severity of existing risks, aiming to provide an approximate understanding of risk magnitude, which is often sufficient for prioritizing risks and, consequently, establishing preventive action priorities.

On the other hand, *in vitro* methods can be used in risk assessment, as they can help to understand the mechanisms by which a substance may cause adverse biological effects. *In vitro* methods allow for expert technical evaluation of whether an existing exposure represents a risk to human health. In the risk assessment paradigm, the identification of toxicological properties is referred to as hazard identification, and the process of linking the effect to the dose is called hazard characterization. Based on the above information, a set of prevention and/or correction measures at the source, the environment, and the individuals exposed should be defined and implemented to eliminate or minimize risks from exposure to

chemical contaminants. The organization should include a monitoring and follow-up program to verify the effectiveness of the implemented measures (ILO, 2023).

As previously mentioned, preventing risks from exposure to chemical agents essentially involves actions at three levels, respecting the General Principles of Prevention. The **Table 9** includes the main measures that should be adopted according to the level of intervention.

Table 9. Key measures for preventing and controlling chemical hazards, based on the nature of the source, environmental conditions, and affected receptors.

Level of action	Measure of prevention/control of chemical risk	References
Source	– Use of localized exhaust systems on machines that involve the release of contaminants.	(CE, 2005; Eurisko, 2008; ILO, 2023)
	– Preventive maintenance of work installations and equipment. Lack of maintenance tend to increase the risk of leaks and deficiencies in materials that can favor the presence of chemical agents in the workplace.	
	– Replacement of products that have toxicological characteristics such as carcinogens and/or sensitizers, justifying the search for alternatives to the chemicals used.	
Environment*	– Periodic cleaning of work areas stations, as the presence of spills and the accumulation of dust can generate new sources of contamination.	
	– Use of suction systems for cleaning machines and work areas instead of compressed air.	
Receptors (exposed workers)	– Training and information on safe work practices, occupational hazards, among other topics related to workplace safety and health.	
	– Reduction of exposure time.	
	– Provision of Collective Protective Equipment (CPE) (such as eyewash stations, showers, and uniforms.)	
	– Use of PPE, such as masks and protective gloves.	

*Action in the environment requires a joint of support measures, as they do not resolve problems in isolation, however, together with measures from other levels of action, they can reduce risk.

Regarding the conditions at the workplace (source and environment level), it is important to highlight the Storage Areas, since these areas should be safe, ventilated and accessible only to authorized personnel. The facilities should have structural characteristics resistant to fire and be designed to prevent leaks in the event of spills with a containment system. The storage method for each chemical product must follow the manufacturer's instructions, and all containers holding chemicals must be properly identified and labeled with the essential information from the SDS (Eurisko, 2008; ILO, 2023). Changing rooms and showers facilities should allow workers to maintain personal hygiene to prevent the spread of chemicals and other substances affecting their health (Eurisko, 2008; ILO, 2023). Regarding measures for the receptor level (workers exposed to chemical agents) highlighted: Eyewash Stations and Emergency Showers, once these facilities should provide clean running water and be available for workers contaminated by splashes or chemical spills. They should also be easy to operate in emergencies, such as using levers, handles, or pedals (ILO, 2023). Training an information, all workers should be trained and informed about the occupational risks arising from their exposure to chemical agents in their work practices. Workers should be informed about their duties, work procedures and health and safety measures, including knowledge of the chemicals being handled, proper handling procedures, SDS information, and the use of personal and CPE. In addition to initial training when a worker starts, periodic refresher training should be conducted whenever necessary (CE, 2005; Eurisko, 2008; ILO, 2023). Use of PPE (5) that protects workers from hazardous chemicals primarily includes respiratory protection, eye protection and skin protection. For respiratory protection, properly certified filter masks with filters suitable for the chemical contaminant should be provided. The employer should provide various models and sizes according to the worker's needs and tasks. Respiratory protection in certain situations should be accompanied by eye protection to prevent contact between the eyes and chemical agents in various states. Eye protection should also be certified with CE marking and indicate the level of protection against chemical risks. Lastly, skin protection includes using protective gloves and clothing (such as aprons and suits) to prevent skin contact with chemicals. This type of protection should have characteristics that prevent chemical penetration and may vary depending on the chemical being handled. All equipment should have a protection index, so it is important to read the information leaflet provided by the manufacturer which details these aspects (types, protection indices, and applicable substances). As mentioned earlier, equipment should have CE certification and the appropriate pictogram indicating protection against chemical risks (CE, 2005; Eurisko, 2008; ILO, 2023).

3. Materials and Methods

3.1. Type of study

The present study adopts a cross-sectional design with both descriptive and analytical components. The descriptive component involves the application of a questionnaire, aimed at characterizing the potential health effects reported by workers in dyeing units who handle synthetic and natural dyes. The analytical component includes the measurement of chemical parameters in textile industry (dyeing units) as well as *in vitro* assays using cell lines.

3.2. Assessment of potential effects on health caused by textiles dyes

3.2.1. Sample characterization

To characterize the potential health effects resulting from worker exposure to synthetic and natural dyes, the sample was drawn from three dyeing units belonging to the textile sector in the Northern Region of Portugal. Although these units cover a range of activities, this study focused specifically on production tasks directly associated with the textile dyeing process. These three large, well-established companies (**Table 10**) hold a strong position within the textile industry and belong to business groups of considerable commercial significance. Their inclusion adds robustness to the data collected and enables a meaningful characterization of the sector's practices and conditions.

The data collection instruments (described in the following sections) were applied to a sample of 33 workers involved in these specific activities. Regarding the monitoring of exposure to the chemical agents, namely Particulate Matter (PM), the tests were carried out in the dye weighing areas, located within the production zone, as these represent a critical point for emission and potential exposure during the handling of dyes.

Table 10. Identification of company, number of workers and work shifts.

Company Identification	Number of Workers	Work Shifts
A	5	08h00-18h00
B	18	06h00–14h00, 14h00–22h00, 22h00–06h00
C	10	06h00–14h00, 14h00–22h00

3.2.2. Characterization of health effects reported by workers in dying units

To characterize the potential health effects reported by workers, data were collected using the Portuguese version of the 21-item questionnaire used in the Global Allergy and Asthma European

Network (GA²LEN) survey. Its original version includes the European Community Respiratory Health Survey (ECHRS) questions related to asthma outcomes. The questionnaire was adapted to suit the objectives of the present study. Accordingly, additional questions were included, mainly related to socio-educational variables, use of healthcare resources, respiratory, skin and eye symptoms, as well as the worsening of these symptoms in the workplace among workers handling synthetic and natural dyes (ANNEX II). The questionnaire consisted of a total of 59 questions divided into two sections covering the following topics: Section 1: Sociodemographic Data and Section 2: Health Effects.

The study was approved by the Ethics Committee of the School of Health of the Polytechnic Institute of Porto (CE0038E). All participants gave informed consent and were informed that they could abandon the study whenever they pleased, without any implication for their work. Data confidentiality was guaranteed by storing personal information separately from the study data.

3.2.3. Monitoring of physicochemical agents in dyeing areas

Sampling was conducted at the workstations designated for dye weighing, i.e., areas where workers manually weigh powdered dyes according to the specific formulations outlined in the dyeing recipes. For this task, workers use a precision scale to accurately record the required quantities of each dye. Operational procedures vary slightly between the companies assessed. In companies A and C, there is a dedicated dye weigher per shift, whose sole responsibility is to carry out the weighing of all dyes required during that period. Otherwise at company B, each worker responsible to a specific set of dyeing is responsible for weighing the dyes needed for their own processes. Once the weighing is completed, the dyes are transferred into buckets and manually transported by the workers to the corresponding dyeing jets, where they are subsequently introduced into the dyeing machines. This area, weighing workstation, was identified as critical, as the handling and transfer of substances may lead to the release of particles into the air, posing a potential health risk to workers. Furthermore, this sampling location was considered representative for the purposes of this study, which aimed not to assess the occupational exposure of workers involved in dye weighing, but rather to carry out environmental monitoring of physicochemical parameters using direct-reading instruments and thereby quantify the concentration of particles released into the air during the dye weighing process. In all companies, this single sampling point was defined and the sampling equipment was positioned next to the scale used for weighing dyes, i.e., the location with the highest likelihood of particle emissions due to the handling of the powders. The selection of this point considered the nature of the task (manual weighing of powdered substances), the absence of physical barriers between the emission source (powdered dyes) and the worker, and the existing

ventilation in the area (natural or mechanical), which could influence the dispersion of particles. Measurements in all companies were carried out across the different work shifts: morning shift (06:00–14:00), afternoon shift (14:00–22:00) and night shift (22:00–06:00). The following physicochemical parameters were measured: Suspended Particulate Matter (PM_{10} and $PM_{2.5}$), which include mineral and/or organic substances that may be present in the atmosphere in solid form. The size of suspended particles can vary significantly and the smaller their diameter, the greater the likelihood of penetration into the respiratory system and the more severe the potential health effects; Carbon Monoxide (CO), it has an anthropogenic origin in the incomplete combustion of fossil fuels or other carbon-containing organic materials, and may be present in emissions from industrial combustion; Carbon Dioxide (CO_2) that are colourless and odourless gases naturally present in the atmosphere, with an average outdoor concentration typically above 300 ppm (300 µmol/mol), equivalent to approximately 600 mg/m^3. Its concentration in indoor air in workplaces serves as an indicator of the ventilation rate and Temperature and Relative Humidity, these parameters are considered valuable for the interpretation of results and the assessment of thermal comfort, potentially contributing to the detection of anomalous non-compliance situations, such as blockages, stagnation, or the presence of emission sources within the ventilation system. In the present study, direct reading equipment were used for the measurement of the above mentioned parameters, involving real-time air sampling and immediate determination using appropriate sensors. The following instruments were used for direct-reading measurements: (1) DustTrak™ II Aerosol Monitor 8533, this instrument continuously draws ambient air into a detection chamber using an internal pump, where it is illuminated by a laser light beam. Within the detection chamber, a gold-coated spherical mirror collects the light scattered by the airborne particles and focuses it onto a photodetector. The resulting voltage signal is proportional to the mass concentration of atmospheric particles and is used to determine particulate levels. The equipment was programmed to record 1-minute average concentrations of $PM_{2.5}$ (respirable fraction) and PM_{10}. Measurements were conducted over a five-day period during the respective work shifts. Subsequently, the data recorded by the equipment were downloaded using TrakPro™ software, and corrections were applied based on the analytical control described in the following section. (2) VelociCalc® Model 9565 Series, this multifunctional instrument allows for precise measurement of CO_2 and CO when equipped with the appropriate indoor air quality probe. The probe integrates gas-specific sensors, enabling continuous real-time monitoring of concentrations. Additionally, the equipment also measures Temperature and Relative Humidity. The devices were set to log 1-minute average concentrations of the aforementioned parameters. They were operated over a five-day period during the respective work shifts. Data were then downloaded using

TrakPro™ software and subsequently corrected based on the analytical quality control procedures detailed in the next section.

3.3. Cytotoxicity analysis

3.3.1. Collection of dye samples

For the cytotoxicity assays, samples of synthetic and natural dyes were collected from all participating companies. The sampling criteria were based on the most frequently used dyes in each facility and the inclusion of different dye classes. The quantities collected were determined by each company according to their availability and operational practices. The companies supplied the available SDS for the collected dyes. Based on the information contained in these documents, the dyes fall into the health hazard categories presented in **Table 11 and 12**, reflecting their potential adverse effects on human health.

Table 11. Health hazard classification outlined in SDS of the collected synthetic dyes.

Dye category	Dye classification	Dye name	Hazard warning	Health hazard classification
Synthetic	Acid dye	Bezaktiv Cosmos Carmine s-c	H412 – Harmful to aquatic life with long-lasting effects.	Chronic (long-term) aquatic hazard, Category 3.
		Bemaplex Black D-R, Neutrilan Black M-RX	H317- May cause an allergic skin reaction.	Skin sensitisation, Category 1.
			H411- Toxic to aquatic life with long-lasting effects.	Chronic (long-term) aquatic hazard, Category 2.
			H319: Causes serious eye irritation.	Eye irritation, Category 2.
		Colocid Yellow 3RL	Acid dye.	H373 – May cause damage to organs through prolonged or repeated exposure.
	Reactive dye	Bezaktiv Navy S-W, Bezaktiv Blue S-Matrix 150 01, Sunfix Yellow SSR, Sunfix Dark-Blue S-R, Jakazol Navy	H317- May cause an allergic skin reaction.	Skin sensitisation, Category 1.
			H334- May cause allergies, asthma symptoms, or breathing difficulties if inhaled.	Respiratory sensitisation, Category 1.
		Bezaktiv Black S-NN 02, Remazol Black ® Ultra NN	H318: Causes serious eye damage.	Serious eye damage, Category 1.
			H334- May cause allergies, asthma symptoms, or breathing difficulties if inhaled.	Respiratory sensitisation, Category 1.
			H317- May cause an allergic skin reaction.	Skin sensitisation, Category 1.
		Sunfix Blue SPR, Sunfix Intense Blue SS	H317: May cause an allergic skin reaction.	Skin sensitisation, Category 1.
		Remazol Yellow RR	H319: Causes serious eye irritation.	Eye irritation, Category 2.
		Remazol Red RR 01	H315: Causes skin irritation.	Skin irritation, Category 2.
			H319: Causes serious eye irritation.	Eye irritation, Category 2.
			H350: May cause cancer.	Carcinogenicity, Category 1B.
		Levafix Ambar CAN, Levafix Blue CA, Levafix Rubine CA, Jakofix Orange ME2RLC, Jakozol Yellow, Sunfix Yellow G4GL 200%, Sunfix Yellow S3R 150%, Sunfix Red SPR-F	Not a hazardous substance or mixture. No hazard pictogram, no signal word, no hazard warnings.	
	Direct dyes	Imacel Bright Pink FR	Not a hazardous substance or mixture. No hazard pictogram, no signal word, no hazard warnings.	

Table 12. Health hazard classification outlined in SDS of the collected natural dyes.

Dye category	Dye classification	Dye name	Hazard warning	Health hazard classification
Natural	Vegetal/Plants	AquiNat Mallow	H319- Attention.	Eye irritation, Category 2.
	No defined	Rialterra Orange, Rialterra Soya, Rialterra Sun, Amarelo Neart, Rialterra Caribe, Rialterra Peach	Not a hazardous substance or mixture. No hazard pictogram, no signal word, no hazard warnings.	

3.3.2. Preparations of dyes for testing

The concentrations tested in cellular assays were based in data provided by the companies. The concentrations provided were expressed as a percentage (%) of dye relative to the fibre weight (g/g). To determine the concentration in grams, this value was divided by the bath ratio (**Table 13**).

Table 13. Name, concentration and dyebath of the selected dyes in study.

Class of dye	Dye	Maximum concentration applied % (µg/mL)	Dyebath	Tested Concentration (µg/mL)		
Synthetic dye	Bezaktiv Blue S-MATRIX 150 01, Bezaktiv Navy S-W, Bezaktiv Black SNN 02, Sunfix Yellow G4GL 200%, Sunfix Yellow SSR, Intense Blue Sunfix SS, Jakofix Orange ME2RLC, Jakozol Navy CE, Jakozol Yellow PP	2(2000)	1/10	500	250	125
	SunfixYellow S3R 150%	1(1000)	1/10			
	SunfixRed SPR-F					
	Sunfix Ble SPR					
	Levafix Ambar CAN	2(2000)	1/6			
	Levafix Blue CA	1(1700)	1/6			
	Levafix Rubine CA					
Natural dye	Rialterra Orange, Rialterra Sun, Rialterra Soya	1(1000)	1/10			
	Rialterra Peach, Neart Yellow, Rialterra Caribe	6(6000)	1/6			

The dyes were diluted in 1 mL of phosphate buffer solution (PBS) (Gibco, Germany) and subsequently filtered using 0.2 µm filters into autoclaved vials.

3.3.3. Cell culture

For cytotoxic assays, keratinocytes, fibroblasts and melanoma cells were selected. Keratinocytes are the fundamental cells of the epidermis and fibroblasts are the primordial cells of the connective tissue of the dermis. The cell lines include in the study were the HaCat keratinocytes cell line, the 3T3-L1 fibroblasts cell line and the B16-F10 melanoma cell line, all obtained from the American Type Culture Collection (ATCC). Cells were cultured in DMEM Glutamax medium (Dulbecco's Modified Eagle Medium GlutaMAXTM–Gibco, Germany), supplemented with 10% (*v/v*) fetal bovine serum (Gibco,USA), 0,1% Amphotericin B (Gibco, Germany) and 1% of penicillin-streptomycin (Pen-Strep 100 IU/ml and 10 mg/ml, respectively) (Gibco, Germany) in a humidified atmosphere containing 5% CO_2, 95% humidity and at 37ºC. Culture medium was renewed every two days. At 80-90% cell confluence, adherent cells were washed with PBS and enzymatically released with TrypLE express enzyme(1x) (Gibco, Denmark). Supplemented medium was added to neutralize the enzyme and the cell suspension was centrifuged at 1250 rpm for 5 minutes at 20 ºC. The supernatant was discarded, eliminating possible enzyme residues and cell debris. Two mL of fresh medium were added and the cell suspension gently homogenized. A 20 µL aliquot was collected and mixed with 20 µL of trypan blue to confirm cell viability. Cell quantification was performed using a Neubauer chamber, under an inverted phase contrast microscope.

3.3.4. Cytotoxicity assay: MTT assay

To study the effect of dyes extracts in cell viability the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) tetrazole salt assay was performed. Keratinocytes, fibroblasts and melanoma cells were seeded in 96 well plates at a density of $2{,}5x10^4$ cells mL^{-1}; $3{,}3x10^4$ cells mL^{-1} and $5{,}0 \times 10^4$ cells mL^{-1} respectively and incubated for 24 hours for cell adhesion. After cell adhesion, the medium was replaced with a new medium containing the dye extracts. In preliminary assays, concentrations of 2000 µg/mL, 1000 µg/mL, and 500 µg/mL were tested. Considering the inconsistent results obtained (negative values) (ANNEX IV), the final concentrations tested were 500 µg/mL, 250 µg/mL, and 125 µg/mL (**Table 13**). The positive control consisted in 20% DMSO and negative control consisted in 1% PBS. After each incubation time, an aliquot of 20µL of a 1 mg mL^{-1} MTT solution (Sigma-Aldrich) was added to each well and incubated at 37ºC for 3h. After the incubation time, the medium was carefully aspirated, and the purple-colored formazan salts dissolved in dimethyl sulfoxide (DMSO). Absorbance was read at 550 nm using a Multiskan SkyHigh Microplate Spectrophotometer (Thermofisher Scientific Inc, Singapore) running Thermo Scientific SkanItTM (version 7.0.2) software.

The assay was run in quadruplicate and averaged. Cytotoxicity was expressed as a percentage of cell viability considering 100% viability in the negative control. For reproducibility of the results each assay was independently repeated two times.

3.4. Statistical analysis

Statistical analysis was performed using the R environment (v4.4.1) in RStudio IDE. The distribution of continuous variables was assessed using histograms and Q-Q plots. For descriptive statistics, continuous variables were described as mean, respective standard deviation, and maximum values. Since Gaussian distributions were observed, parametric tests were applied (student's t-tests) for inferential statistics and categorical variables were expressed as proportions. Student's t-tests were used to assess differences in pollutant distributions across questionnaire items with binomial responses. For the analysis of pollutant concentrations at different dye weighing timepoints, graphical analyses, spearman correlation and linear regression were conducted to visualize patterns. In cell viability assays, the viability rate (%) was calculated by dividing the absorbance of the sample by the absorbance of the negative control and multiplying by 100.

4. Results and Discussion

4.1. Characterization of health effects reported by workers exposed to synthetic and natural dyes

This study comprised 33 workers from the textile dyeing sector, distributed across three companies (A, B, and C). The sociodemographic profile of the workers surveyed as part of this study is presented in (Table 14). The majority of participants were male (90.9%), with Company C consisting exclusively of male workers. The most represented age group was 31–50 years (51.5%), followed by 18–30 age group (33.3%). In terms of educational attainment, most workers had completed secondary education (42.4%) or upper secondary education (30.3%), while only a small proportion (9.1%) reported holding higher education qualifications. A notable variation was observed in the average duration of professional experience across companies: Company C presented the highest mean length of service (13 years), followed by Company B (5 years) and Company A (2 years). These differences may reflect distinct organizational profiles or workforce turnover rates and potentially contribute to differential patterns of occupational exposure. Overall, the sociodemographic and occupational characteristics of the sample are consistent with the known profile of workers in the textile dyeing industry and provide relevant context for interpreting the reported health effects.

Table 14. Socio-demographic characterization of the sample of workers surveyed through the questionnaire regarding health effects associated with potential occupational exposure to synthetic and natural dyes.

Variable	Company A	Company B	Company C	Total
Gender % (N)				
Male	60.0% (3)	94.4% (17)	100.0% (10)	90.9% (30)
Female	40.0% (2)	5.6% (1)	0.0% (0)	9.1% (3)
Age groups				
18-30 years old	40% (2)	27.8% (5)	40% (4)	33.3% (11)
31-50 years old	40% (2)	55.6% (10)	50% (5)	51.5% (17)
51-70 years old	20% (1)	16.7% (3)	10% (1)	15.2% (5)
Educational Qualifications, % (N)				
Primary Education	0.0% (0)	5.6% (1)	0.0% (0)	3.0% (1)
Lower Secondary Education	0.0% (0)	16.7% (3)	20.0% (2)	15.2% (5)
Upper Secondary Education	20% (1)	33.3% (6)	30.0% (3)	30.3% (10)
Secondary Education	40.0% (2)	38.9% (7)	50.0% (5)	42.4% (14)
Bachelor's Degree	20% (1)	0.0% (0)	0.0% (0)	3.0% (1)
Master's Degree	20% (1)	5.6% (1)	0.0% (0)	6.1% (2)
Years of Work (Mean)	2	5	13	7

The analysis of respiratory symptoms reported by workers from the three companies in the textile dyeing sector is presented in **Table 15.** The most frequently reported symptom was morning cough, with 30.3% of workers indicating its presence, of whom 20% reported aggravation at the workplace. Wheezing was reported by 9.1% of participants, with 33.3 of these experiencing worsening symptoms in the work environment. Symptoms such as waking with chest tightness and breathlessness were less common, occurring in 12.1% and 6.1% of workers, respectively, with variable proportions reporting symptom aggravation at work. Asthma attacks were less frequent, affecting 18.2% of the sample. Allergic rhinitis and sinusitis had prevalences of 6.1% and 12.5%, respectively, with lower percentages reporting aggravation associated with the occupational environment.

Table 15. Characterisation of respiratory symptoms and their aggravation at the workplace reported by workers potentially exposed to synthetic and natural dyes.

Respiratory symptoms	Company A	Company B	Company C	Total
	Reporting Symptoms (n=5)	Reporting Symptoms (n=18)	Reporting Symptoms (n=10)	Reporting Symptoms (n=33)
Wheeze	Y-0% N-100%	**Y-5.6% (100%*)** N-94.4%	**Y-20%** N-80%	Y-9.1% N-90.9%
Waking with tightness in the chest	**Y-20% (100% *)** N-80%	**Y-11.1%** N-88.9%	**Y-10%** N-90%	Y-12.1% N-87.9%
Waking with breathlessness	**Y-20%** N-80%	**Y-5.6%** N-94.4%	Y-0% N-100%	Y-6.1% N-93.9%
Waking with cough	**Y-60% (33.3%*)** N-40%	**Y-27.8% (20%*)** N-72.2%	**S-20%** N-80%	Y-30.3% N-69.7%)
Attack of asthma	Y-0% N-100%	**Y-33.3%** N-66.7%	Y-0% N-100%	Y-18.2% N-81.8%
Allergic Rhinitis	**Y-40% (50%*)** N-60%	Y-0% N-100%	Y-0% N-100%	Y-6.1% N-93.9%
Sinusitis	Y-40% N-60%	**Y-11.8% (50%*)** N-88.2%	Y-0% N-100%	Y-12.5% N-87.5%

Legend: Y *Indicate that the workers reported (%) a worsening of these symptoms in the workplace.

These results indicate that, although some respiratory symptoms are infrequent, a portion of workers experience worsening of these symptoms in the occupational context. These findings are consistent with the results reported by NM et al., (2022) ,who described a high frequency of respiratory complaints, such as cough, wheezing, and dyspnoea, among workers exposed to dyes. The inhalation of reactive dye particles has been identified as a potential mechanism of respiratory sensitization, which may explain the patterns observed in the present study. Similarly, Mohammed et al. (2019) demonstrated that workers exposed to dyes in the textile industry exhibited a significantly higher prevalence of respiratory symptoms and pulmonary dysfunction compared to non-exposed individuals.

The analysis of ocular and dermal symptoms reported by workers from the three companies in the textile dyeing sector, as well as their aggravation in the workplace environment, is presented in **Table 16**. Eczema/atopic dermatitis was reported by 42.4% of the total workers surveyed, with 57.6% of these workers indicating symptom worsening at the workplace. The highest predominance was observed in Company B (55.6%), followed by Company A (40.0%) and Company C (20.0%). Symptoms related to eye irritation, including tearing and redness, were reported by 30.3% of workers, of whom 60% reported symptom exacerbation during work. Company B showed the highest prevalence of these symptoms (27.8%). Pain or a sensation of pressure in the frontal, nasal, or orbital region was reported by 33.3% of workers, with half of the cases indicating worsening at the workplace. Company B had the highest frequency (38.9%), followed by Companies C (30.0%) and A (20.0%). These findings indicate that ocular and dermal symptoms are common among workers in this sector.

Table 16. Characterisation of ocular and dermal symptoms and their aggravation at the workplace reported by workers potentially exposed to synthetic and natural dyes.

Ocular and dermal symptoms	Company A	Company B	Company C	Total
	Reporting Symptoms (n=5)	Reporting Symptoms (n=18)	Reporting Symptoms (n=10)	Reporting Symptoms (n=33)
Eczema/atopic dermatitis	**Y-40.0% (50%*)** N- 60.0%	**Y-55.6% (50%) *** N- 44.4%	**Y-20.0% (100%) *** N- 80.0%	**Y-42.4%** N- 57.6%
Eye irritation, watery eyes, and redness	**Y-40.0% (50%*)** N- 60.0%	**Y-27.8% (40%) *** N- 72.2%	**Y-30.0% (100%) *** N- 70.0%	**Y-30.3%** N- 69.7%
Pain or a sensation of pressure in the forehead, nasal, or orbital region	**Y-20.0%** N- 80.0%	**Y-38.9% (42.9%) *** N- 61.1%	**Y-30.0% (100%) *** N- 70.0%	**Y-33.3%** N-66.7%

Legend: *Indicate that the workers reported (%) a worsening of these symptoms in the workplace.

These results are aligned with the findings of Tounsadi et al. (2020), who identified a statistically significant association between the duration of exposure to chemical agents and the occurrence of dermatitis symptoms ($p = 0.011$), particularly among workers with more than 10 years of occupational experience. The same study also demonstrated that the presence of otorhinolaryngological symptoms was significantly associated with the use (or improper use) of personal protective equipment (PPE), suggesting that inadequate or insufficient PPE usage may compromise its preventive effectiveness. According to Jabeen & Jabeen (2017) and NM et al. (2022), chronic and repeated exposure to dyes, especially those based on anthraquinone or benzidine, may lead to both respiratory and dermal sensitization. The variations observed among companies regarding the prevalence and severity of

symptoms may reflect differences in task organization, ventilation conditions, PPE usage practices and worker awareness. It was not possible to distinguish the effects reported by workers between synthetic and natural dyes, as the workers themselves were unable to make this differentiation. In some cases, workers reported that it is not always possible to identify the type of dye even in the workplace, as they merely follow the work procedure ('recipe') based on the dye code and the specified quantities to be weighed.

4.2. Environmental monitoring of physicochemical parameters in textile dyeing workstations: PM_{10}, $PM_{2.5}$, CO_2, CO, T. and HR

According to CDC & NIOSH (2018) the available data on potential exposure levels of workers associated with the weighing or mixing or powder dyes are limited, and they are not always representative of textile dyeing operations. The purpose of this study was not to assess the occupational exposure of workers involved in dye weighing, but rather to carry out environmental monitoring of physicochemical parameters using direct-reading instruments. The physicochemical parameters presented in **Table 17** were monitored through direct-reading measures conducted exclusively in the dye weighing areas of the three companies studied. In all three companies, the dye weighing area was isolated from the other production zones and was occupied exclusively by workers assigned to this specific task. However, in Companies A and B, it was observed that synthetic and natural dyes were also stored in these areas. Regarding the size of the space, the dimensions varied between companies: Company A (80m²), Company B (50m²), and Company C (120m²).

Table 17. Environmental physicochemical parameters measured in textile dyeing workstations in Company A, B and C.

Parameter	Company A		Company B		Company C	
	Mean (SD)	Max.	Mean (SD)	Max.	Mean (SD)	Max
CO_2 (ppm)	373.81(±39.62)	475.0	370.63(±33.42)	582.0	301.19(±20.18)	428.0
CO (ppm)	0.01(±0.03)	0.1	0.02(±0.07)	1.0	0(0)	0.1
Temp.(ºC)	15.82(±1.05)	17.3	24.17(±0.95)	26.4	14.93(±2.49)	18.5
HR (%)	60.31(±0.90)	65.2	51.01(±5.68)	65.0	67.15(±6.37)	80.6
PM_{10} (µg/m³)	18.91(±7.58)	52.0	116.63(±53.19)	471.0	33.00(±20.00)	206.0
$PM_{2.5}$(µg/m³)	26.92(±8.18)	46.0	55.95(±19.98)	168.0	33.48(±19.38)	249.0

Legend: Max. = Maximum

Indoors, CO_2 concentration is generated through human metabolism and depends on the level of physical activity, making it an indicator of the ventilation rate of a given space. Its average concentration in the atmosphere is typically above 300 ppm; therefore, the results obtained in the three companies (values

<400 ppm) may reflect poor baselining (Jardim et al., 2015). However, considering the low occupancy of the dye weighing areas and the absence of significant internal sources of CO_2 emissions, low CO_2 levels were expected.

In case of PM_{10} and $PM_{2.5}$, the methodology adopted in this study, as well as its primary objective, does not allow for direct comparison with the reference values established for inhalable particles (PM_{10}) and respirable particles ($PM_{2.5}$), according to NIOSH guidelines and the Portuguese Standard NP 1796:2007. Nevertheless, the elevated concentrations of PM_{10} and $PM_{2.5}$ observed in Companies B and C suggest a high level of environmental exposure to these contaminants. Although existing studies focus on the monitoring of total dust in similar sampling locations, the results obtained in this study are consistent with the findings reported in Burroughs (2002); Edmonds & Heitbrink (1993) and by the NIOSH and Centres for Disease Control and Prevention (CDC). Exposure to such elevated levels of particulate matter in dye weighing areas may result in adverse health effects for workers. Furthermore, the toxicity of many powdered dyes is not yet well defined, which underscores the importance of implementing measures to control occupational exposure. This approach allows a preliminary analysis of the environmental conditions associated with these workstations, which may be relevant for understanding potential risks and for developing prevention strategies.

According to several authors, the manual weighing of dyes represents the task with the highest potential for worker exposure (Burroughs, 2002; Edmonds & Heitbrink, 1993) .Through the analysis of the environmental sampling period, where PM_{10} and $PM_{2.5}$ concentrations were represented over time and dye weighing moments were marked, it was possible to visually detect perceptible variations in airborne particulate concentrations associated with these events.

In Companies B and C (**Figures 3 and 4**), a pattern was observed, with higher mean concentrations of PM_{10} and especially $PM_{2.5}$ during dye weighing, reinforcing the potential role of this task as a direct source of particle emissions. The spearman correlation analysis revealed a strong positive correlation between PM_{10} and $PM_{2.5}$ levels, confirming the common coexistence of these pollutants in industrial environments. Although, the correlation between dye weighing activity and particle levels was weak, through linear regression analysis, it was observed that dye weighing activity was associated with a significant increase in airborne particle concentrations. On average, during weighing periods, PM_{10} and $PM_{2.5}$ levels were, respectively, 28.47 $\mu g/m^3$ and 11.21 $\mu g/m^3$ higher, compared to non-weighing periods ($p < 0.001$ for both models). Despite the low coefficient of determination ($R^2 < 0.01$), which indicates that other factors also

contribute to concentration variability, the findings strengthen the evidence of an association between weighing activity and increased exposure to inhalable particles.

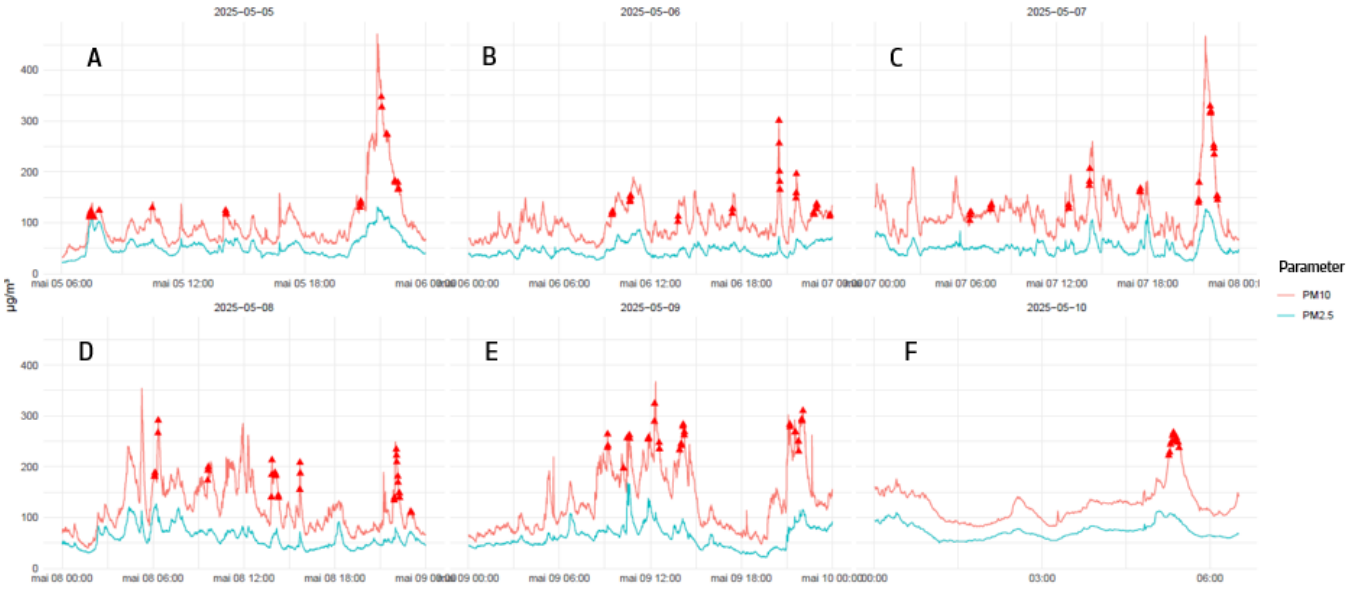


Figure 3. Concentration of PM10 and PM2.5 parameters over the sampling period, with marked timepoints corresponding to dye weighing activities in Company B: (A) 1st sampling day (05 may 2025), (B) 2nd sampling day (06 may 2025), (C) 3rd sampling day (07 may 2025)), (D) 4th sampling day (08 may 2025) , (E) and (F) 5th sampling day (09-10 may 2025).

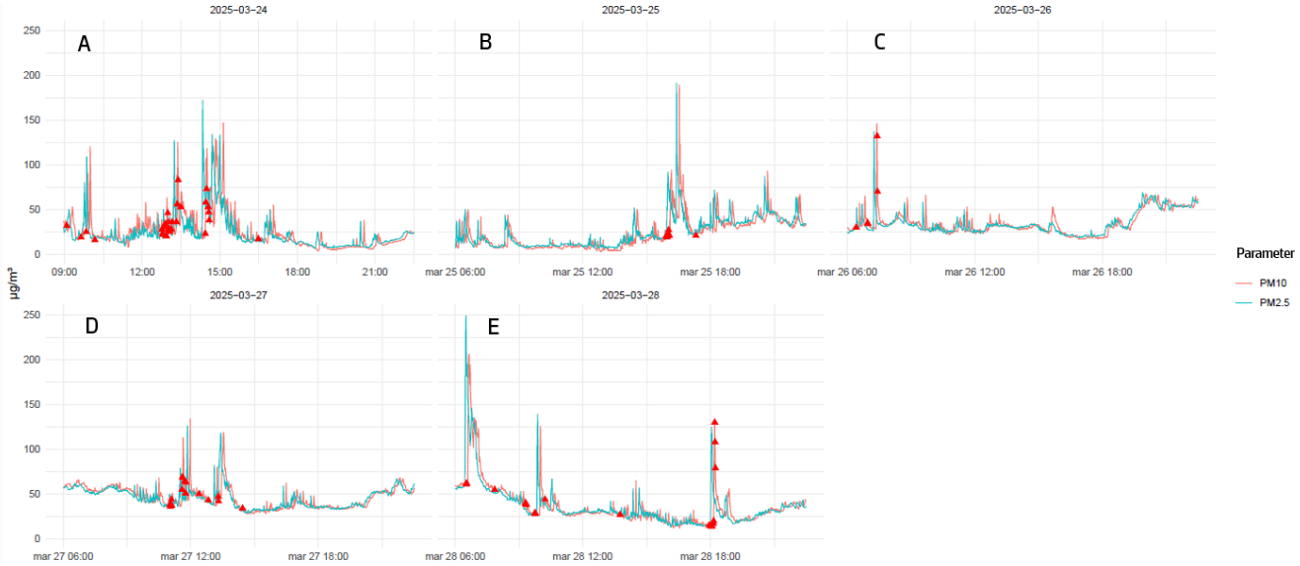


Figure 4. Concentration of PM10 and PM2.5 parameters over the sampling period, with marked timepoints corresponding to dye weighing activities in Company C: (A) 1st sampling day (24 march 2025), (B) 2nd sampling day (25 march 2025), (C) 3rd sampling day (26 march 2025), (D) 4th sampling day (27 march 2025) , (E) 5th sampling day (28 march 2025).

Additionally, the analysis using the dye weighing maps revealed that the majority of these concentration peaks correspond to the weighing of reactive dyes, with only one instance observed in Company B, during the night shift, involving the weighing of a direct dye. The weighing of natural dyes was not observed during the sampling period in any of the three companies. It is important to highlight that in Company A, it was reported that the weighing timestamps recorded in the software may not accurately reflect the actual moment of the operation, with possible temporal discrepancies and therefore these data were not considered. This inaccuracy can compromise the precise identification of potential causal relationships between the weighing activity and the observed increases in particulate concentrations, particularly in time-interval analyses. These findings reinforce the importance of an integrated exposure assessment, which considers not only the weighing moment itself, but also ventilation conditions, task sequencing, and operator behaviours. The Engineering Control Technology Branch (ECTB) conducted several field studies that evaluated the effectiveness of three techniques for controlling exposure to powdered dyes. Based on the reviewed studies, the following recommendations were provided to minimize dust exposure during powder handling operations: (1) tasks should be performed within a semi-downdraft ventilated hood; (2) workers should use slow, controlled movements when handling dyes to limit airborne dust concentrations; and (3) skin contact with dyes should be avoided by wearing appropriate protective clothing, such as gloves, long-sleeved shirts and aprons (CDC & NIOSH, 2018). They further emphasise the need for individual monitoring and more accurate operational records. Such measures are essential to identify critical sources of exposure and to implement these effective strategies to protect workers' health.

To assess a potential association between environmental parameters and the outcomes reported by workers from Companies A, B, and C, a comparative statistical analysis was conducted for each of the 11 outcomes identified in the administered questionnaire. The sample was divided into two groups: Group "Reported Outcomes" and Group "No Reported Outcomes." Mean, minimum, maximum, and interquartile values (Q1, median, Q3) of environmental variables were compared between the two groups. Although all outcomes variables were analysed, the majority did not exhibit statistically significant differences between groups (**Table 18**). On the other hand, it was observed that the concentrations of environmental parameters were higher in the group without outcomes compared to the group with outcomes, which may suggest that these parameters do not have a direct impact on the outcomes reported by the workers. However, in the case of asthma or asthmatic bronchitis, despite the small total sample size (n =

33) and the fact that only six workers reported a history of this condition, statistically significant differences were observed between individuals with and without this outcomes ($p<0.05$).

Table 18. Comparative analysis of environmental parameters according to the outcomes reported by workers from Companies A, B, and C.

Outcomes	Parameter	Reported Outcomes Mean (SD)	No reported Outcomes Mean (SD)
Asthma	$PM_{2.5}$ (mg/m^3)	55.2(±0.0) *	41.2(±12.8)
	PM_{10} (mg/m^3)	103.0(±0.0) *	59.3(±39.9)
Wheeze	$PM_{2.5}$ (mg/m^3)	38.3 (±14.7)	44.3 (±12.7)
	PM_{10} (mg/m^3)	58.1 (±49.2)	76.5 (±44.7)
Allergic Rhinitis	$PM_{2.5}$ (mg/m^3)	30.3 (0)	44.6 (±12.7)
	PM_{10} (mg/m^3)	21.2 (0)	78.3 (±43.8)
Eczema/atopic dermatitis	$PM_{2.5}$ (mg/m^3)	48.0 (±11.8)	40.6 (±12.8)
	PM_{10} (mg/m^3)	89.3 (±42.0)	64.2 (±44.5)
Eye irritation, watery eyes, and redness	$PM_{2.5}$ (mg/m^3)	42.6 (±13.3)	44.2 (±12.8)
	PM_{10} (mg/m^3)	70.6(±46.8)	76.7 (±44.7)

Legend: * Significant differences between mean ± SD of reported and no reported outcomes at a $p<0.05$ significance level.

Workers with asthma were exposed to higher levels of PM_{10} and $PM_{2.5}$, as well as higher mean and maximum temperatures. According to Malo & Vandenplas (2011), Occupational Asthma (OA) is a work-related condition characterized by variable airflow obstruction and/or by bronchial hyperresponsiveness to the conditions of a specific working environment. Positive skin prick tests have suggested that airborne dye molecules may act as haptens and aggravate the production of reactive dye specific immunoglobulin E (IgE) antibodies (Ozkurt et al., 2012). Ozkurt et al (2012) mentioned that reactive dyes are common causative agents for respiratory symptoms among dyehouse workers. The weighers showed the highest prevalence of respiratory and nasal symptoms and IgE mediated allergy to reactive dyes. However, Clofent et al. (2020) mentioned that there are few reports of their relationship to OA and it is likely that dyestuff-related OA is underdiagnosed.

Additionally, lower average relative humidity was recorded in these environments, which may contribute to discomfort and worsening of respiratory symptoms. Although these findings are limited by the small

number of individuals with asthma, they suggest a relationship between unfavourable environmental conditions and the presence of chronic respiratory diseases among workers. Nevertheless, these results should be interpreted with caution, and further research with larger sample sizes is warranted to validate these associations.

4.3. Cytotoxicity assessment of synthetic and natural dyes: *In vitro* cell viability assay using skin cell lines

Another common health problem among the studied textile dyeing workers is different skin manifestations including rash, burns, itching, colour change, dryness and thickening (NM et al., 2022). Recognizing the potential hazards of skin exposure, the NIOSH characterized skin disease as one of the most pervasive occupational health problems in the United States (Cohen & Rice, 2001). Chemical agents, such as textile dyes, cause cellular toxicity through different mechanisms, such as the destruction of cell membranes, inhibition of protein synthesis, and/or interference with enzymatic reactions (Wattenberg, 2005). However, just simply being exposed to these hazardous chemicals, such as textile dyes, does not necessarily translate into a toxicological response. The mammalian body has several inherent defence mechanisms and membrane barriers that tend to prevent the entry or absorption and distribution of these toxicants once an exposure event has occurred (Baynes & Hodgson, 2004; Cohen & Rice, 2001). However, if the toxicant is readily absorbed into the body, there are still other anatomical and physiological barriers that may prevent distribution to the target tissue to elicit a toxic response (Baynes & Hodgson, 2004).

The skin represents the largest organ in the human body, and one of its primary functions can be seen as a physical barrier to absorption of toxicants. The skin consists of two major components: the outer epidermis and the underlying dermis, which are separated by a basement membrane (**Figure 5**).

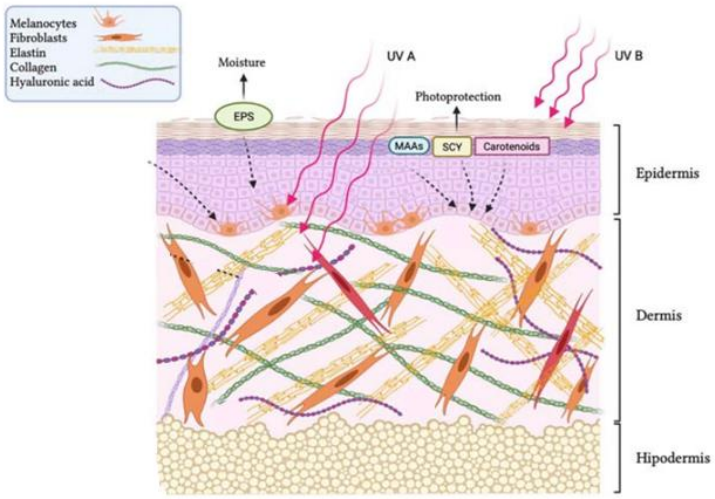


Figure 5. Schemactic representation of skin structure. Adapted by Morone et al. (2022)

According to Baynes & Hodgson (2004) ; Cohen & Rice (2001) , the epidermis is a stratified squamous epithelium primarily composed of keratinocytes, for which the HaCat cell line serves as a representative model and is highly used in cytotoxicity cell assays. The outer layer of epidermis, the stratum corneum, is constituted by corneocytes, enriched with proteins and lipids and in a constant process of cell renewal. The dermis is mostly composed by connective tissue. In thickness, the dermis makes up approximately 90% of the skin and has a supportive function. Its extracellular matrix has a high content of collagen and elastin, secreted by scattered fibroblasts thus providing the skin with elastic properties. Fibroblasts are the main dermis cells since there are responsible for the production and manutention of the matrix. In this work murine fibroblasts cell line 3T3-L1 was used instead of human ones just due to logistic issues. However, this cell line is largely used in cell assays involving fibroblasts. To extend the study to other skin cells, melanocytes were initially considered. However, it was not possible to teste the dyes on normal melanocytes; therefore, the B16-F10 melanoma cell line was used as an alternative. This cell line, derived from the skin tissue of a mouse with melanoma, exhibits a morphology characterized by spindle-shaped and epithelial-like cells and is widely used in skin cancer research (ATCC, 2024).

In vitro cell viability assays are widely used for cytotoxicity testing of chemical substances. Although the main objective was to evaluate the effects of dyes on human skin, due to logistical issues, fibroblasts, keratinocytes and melanoma cells were used as abovementioned.

The results regarding cell viability (%) in the HaCaT, 3T3-L1 and B16-F10 cell lines after exposure to synthetic dyes Sunfix Blue SPR, Levafix Ambar and Bezaktiv Navy are presented in **Figures 6,7 and 8**. In

this section, only the cell viability figures of 2 out of the 19 synthetic dyes tested are shown, for illustrative purposes. The remaining figures are presented in Annex V. For natural dyes the results regarding cell viability (%) in the HaCaT, 3T3-L1 and B16-F10 cell lines after exposure to Rialterra Orange are presented in **Figure 9**. In this section, only the cell viability figures of 1 out of the 6 natural dyes tested are shown, for illustrative purposes. The remaining figures are presented in Annex V.

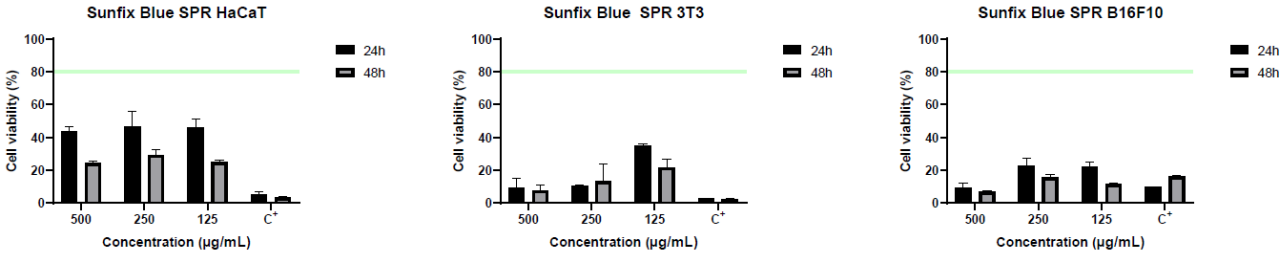


Figure 6. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Sunfix Blue SPR.

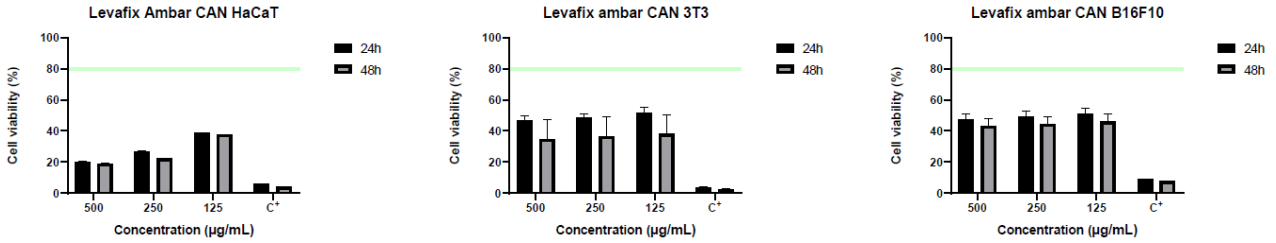


Figure 7.Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Levafix Ambar CAN.

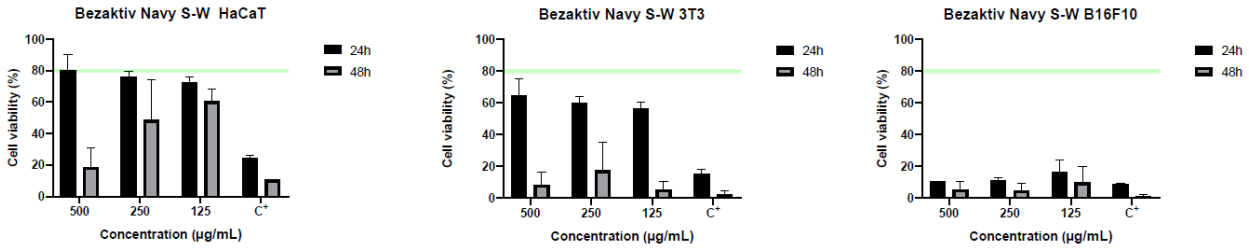


Figure 8. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Bezaktiv Navy S-W.

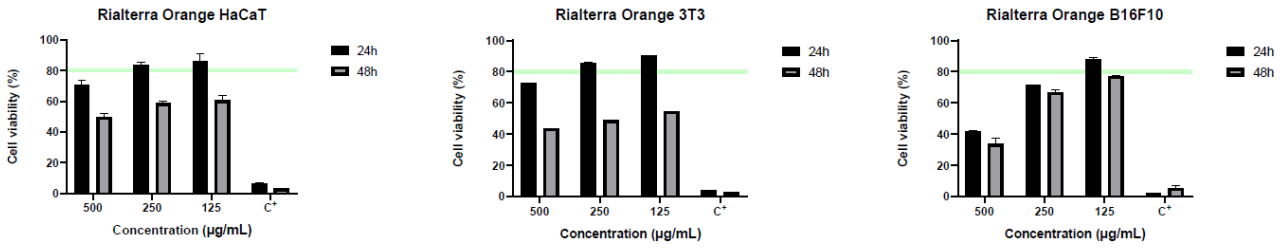


Figure 9. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Rialterra Orange.

A summary of the effects of each synthetic and natural dye on the three cell lines is presented in **Tables 19 to 21**. In 3T3-L1 cells, Yellow Sunfix G4GL 200%, Yellow Sunfix S3R 150% and Yellow Sunfix SSR dyes exhibited moderate cytotoxicity, with viability around 70% at all concentrations after 48h exposure, while Jakozol Yellow PP and Levafix Rubine showed mild cytotoxic effects. Most of other tested dyes induced high cytotoxicity in this cell line, with viability below 70% across all concentrations and time points (**Table 19**).

Table 19. Summary the effects of each synthetic and natural dyes on the 3T3-L1 cell line.

Class of dye	Dyes	3T3-L1					
		500 µg/mL		250 µg/mL		125 µg/mL	
		24h	48h	24h	48h	24h	48h
Synthetic dye	Yellow Sunfix G4GL 200%, Yellow Sunfix S3R 150%, Yellow Sunfix SSR	++	+	++	+	++	+
	Intense Blue Sunfix SS, Blue Sunfix SPR, Bezaktiv Black SNN 02, Bezaktiv Blue S-Matrix 150-01, Bezaktiv Navy S-W, Jakofix Orange ME2RLC, Jakozol Navy CE, Levafix Ambar CAN, Levafix Blue CA, Red Sunfix SPR-F	-	-	-	-	-	-
	Jakozol Yellow PP	+	-	+	+	+	+
	Levafix Rubine	-	+	+	+	+	+
Natural dye	Yellow Neart, Rialterra Caribe, Rialterra Peach	-	-	-	-	-	-
	Rialterra Sun, Rialterra Soya	++	+	++	+	++	+
	Rialterra Orange	-	-	++	-	++	-

Legend: + indicate percentage of viability, where: +++ higher than 100 %; ++ viability 80-100%; + viability 70-80%; - viability lower than 70%.

The natural dyes, Yellow Neart, Rialterra Caribe, and Rialterra Peach consistently exhibited low cell viability (<70%) across all tested concentrations (125, 250 and 500 µg/mL) and exposure times (24h and 48h) in the 3T3-L1 cell line, indicating significant cytotoxicity. In contrast, Rialterra Sun and Rialterra Soya showed no cytotoxicity since cell viability was higher than 80% at most concentrations and time points,

with a slight decrease at 48h exposure (70–80% viability). Rialterra Orange demonstrated a variable response in 3T3-L1 cells, with viability above 80% at 125 and 250 µg/mL after 24h exposure but falling below 70% under other conditions.

In HaCaT cells, most dyes caused significant cytotoxicity, except for Bezaktiv Black SNN 02, which displayed variable effects ranging from mild to high viability depending on dose and exposure time, as well as Bezaktiv Blue S-Matrix 150-01 and Bezaktiv Navy S-W, which demonstrated moderate cytotoxicity under specific conditions. The dye Sunfix Intense Blue SS exhibited mild toxicity only at the lowest concentration and time point but was highly cytotoxic in other scenarios (**Table 20**).

Table 20. Summary the effects of each synthetic and natural dyes on the HaCaT cell line.

Class of dye	Dyes	HaCaT					
		500 µg/mL		250 µg/mL		125 µg/mL	
		24h	48h	24h	48h	24h	48h
Synthetic dye	Sunfix Yellow G4GL 200%, Sunfix Yellow S3R 150%, Sunfix Yellow SSR, Sunfix Blue SPR, Jakofix Orange ME2RLC, Jakozol Navy CE, Jakozol Yellow PP, Levafix Ambar CAN, Levafix Blue CA, Levafix Rubine, Red Sunfix SPR-F	-	-	-	-	-	-
	Sunfix Intense Blue SS	-	-	-	-	+	-
	Bezaktiv Black SNN 02	+	-	++	-	+++	-
	Bezaktiv Blue S-Matrix 150-01	-	-	-	-	++	-
	Bezaktiv Navy S-W	++	-	+	-	+	-
Natural dye	Yellow Neart, Rialterra Caribe, Rialterra Peach	++	+	++	++	++	++
	Rialterra Orange, Rialterra Soya	+	-	++	-	++	-
	Rialterra Sun	-	-	++	-	++	-

Legend: + indicate percentage of viability, where: +++ higher than 100 %; ++ viability 80–100%; + viability 70–80%; - viability lower than 70%.

Yellow Neart, Rialterra Caribe, and Rialterra Peach maintained viability between 80–100% (++) or slightly lower (70–80%) depending on the concentration and exposure time. Rialterra Orange and Rialterra Soya presented intermediate cytotoxic effects, with viability ranging from 70% to 100% in most conditions but dropping below 70% at some concentrations and times. Rialterra Sun showed lower viability (<70%) at 500 µg/mL but improved viability (80–100%) at lower concentrations after 24h exposure.

In B16-F10 cells, all synthetic dyes showed strong cytotoxicity, except for Bezaktiv Black SNN 02, which revealed a slight increase in viability at low concentration after 24 hours yet maintained toxicity in other conditions (**Table 21**).

Table 21. Summarizing the effects of each synthetic and natural dye on the B16F10 cell line.

Class of dye	Dyes	B16-F10					
		500 µg/mL		250 µg/mL		125 µg/mL	
		24h	48h	24h	48h	24h	48h
Synthetic dye	Sunfix Yellow G4GL 200%, Sunfix Yellow S3R 150%, Sunfix Yellow SSR, Sunfix Intense Blue SS, Sunfix Blue SPR, Bezaktiv Blue S-Matrix 150-01, Bezaktiv Navy S-W, Jakofix Orange ME2RLC, Jakozol Navy CE, Jakozol Yellow PP, Levafix Ambar CAN, Levafix Blue CA, Levafix Rubine, Red Sunfix SPR-F	-	-	-	-	-	-
	Bezaktiv Black SNN 02	-	-	-	-	+	-
Natural dye	Yellow Neart, Rialterra Caribe, Rialterra Peach, Rialterra Soya	-	-	++	+	++	++
	Rialterra Orange	-	-	+	-	++	+
	Rialterra Sun	-	-	-	-	+	-

Legend: + indicate percentage of viability, where: +++ higher than 100 %; ++ viability 80-100%; + viability 70-80%; - viability lower than 70%.

Regarding the B16-F10 cell line, Yellow Neart, Rialterra Caribe, Rialterra Peach, and Rialterra Soya exhibited low cell viability (<70%) at higher concentrations and longer exposure times, but an increase in viability (>80%) at lower concentrations and shorter exposure periods. The Rialterra Orange dye showed a similarly variable profile, with viability values ranging from <70% to 70–80%, whereas Rialterra Sun induced cytotoxic effects, with cell viability below 70%, except at the lowest concentration after 24 hours. In this context, the observed decrease in cell viability may suggest a potential anticancer activity against skin cancer, considering the characteristics of this cell line. Several studies have demonstrated that natural dyes (such as bacterial, fungal, and microalgal dyes) can confer various functional properties, such as antibacterial, antifungal, and UV-protective activity, as well as the ability to inhibit the proliferation of cancer cells (Li et al., 2022; Pizzicato et al., 2023).

The cytotoxic effects observed in the HaCaT cell line are consistent with the findings of Silva et al. (2022), who demonstrated organ-specific toxic responses induced by reactive dyes, highlighting a greater susceptibility of epidermal cells compared to hepatic cells. This suggests that dermal exposure to reactive dyes may pose a higher cytotoxic risk, in agreement with the significant cytotoxicity observed in HaCaT cells in the present study. However, Tsuboy et al (2007), reported that cell viability in response to varying dye concentrations remained above 80%, indicating that cytotoxic effects vary according to commercial concentration and dye category, which may explain the viability patterns observed at higher concentrations used in this study. Therefore, in this study, although some dyes exhibited cytotoxicity in the tested cell lines, it is important to note that this does not necessarily translate directly into skin toxic effects. In skin keratinocytes and fibroblasts are protected by the corneal layer of the epidermis and the

extracellular matrix respectively. The skin possesses membrane barriers that tend to prevent the entry, absorption and distribution of such toxicants once exposure has occurred. For future studies, it is recommended that these assays be conducted using full-thickness skin models, which more accurately reflect the conditions of exposure during dye handling activities.

According to Chavan (2023), natural dyes are not always inherently safe. In fact, the toxicological properties of synthetic dyes are very well tested scientifically, and safety data sheets are available for each dye. No comparable studies have been conducted specifically on natural dyes. Many of the natural dyes are quite safe but few can be toxic. The major hurdles for using natural dyes are serious gap and lack/non-availability of scientific data base and knowledge base required for their successful application on textiles. The majority of studies in this field offer only broad insights, lacking comprehensive information on the chemical composition and technical specifications (SDS) of the materials. The SDS of the tested natural dyes (**Table 12**) indicate that these dyes are not considered hazardous substances or mixtures and therefore do not warrant a health hazard classification. However, in the present study, it was demonstrated that at certain concentrations, these dyes can induce toxicity in cell lines. Despite the skin's inherent protective mechanisms and the potential discrepancy between the tested concentrations and real-world exposure levels, the findings highlight the need for further investigation into the occupational toxicology of these natural compounds. Furthermore, workers' perception during handling of these natural compounds often leads to a lack of PPE usage, and thus these results may support the implementation of preventive measures during handling processes (Jabeen & Jabeen, 2017).

The integration of the different results obtained in this study allows for a comprehensive understanding of the potential impact of occupational exposure to synthetic and natural dyes in the textile dyeing sector. In this context, the recommendations developed by institutions such as NIOSH and ETAD are particularly relevant. These emphasize the importance of using local exhaust ventilation systems, performing slow and smooth movements when handling dyes to maintain low dust concentrations, minimizing the transport distance of dyes between storage and process containers, and using PPE, such as gloves, long-sleeved shirts and aprons (CDC & NIOSH, 2018) .

5. Conclusion

The reintroduction of natural dyes in the textile industry, even on a limited scale, has been recognized as a promising alternative to address the increasing restrictions on synthetic dyes and to minimise their potential impacts on both human health and the environment. The present study sought to advance knowledge in this area through an integrated approach that combined the analysis of self-reported symptoms by workers in the dyeing sector with environmental monitoring in the most critical work areas (dye weighing stations), alongside toxicity assessments of the dyes used.

Based on the symptoms reported by surveyed workers, the most frequent respiratory complaint was morning cough (30.3%), with 20% of affected individuals indicating symptom worsening at the workplace. Eczema or atopic dermatitis was reported by 42.4% of workers, of whom 57.6% of these noted that symptoms worsened while at the work. Symptoms of ocular irritation, including tearing and redness, were reported by 30.3% of workers, 60% of whom noted that these symptoms worsened during work. These findings indicate that, although some symptoms are relatively infrequent, a portion of workers experience worsening of these conditions in the workplace. Environmental monitoring carried out in this study revealed that Companies B and C exhibited elevated concentrations of PM_{10} and $PM_{2.5}$ in the dye weighing areas. Analysis of weighing maps further indicated that most concentration peaks were associated with the handling and weighing of reactive dyes. Regarding the cell viability assays, results differed across the cell lines and dye classes. In 3T3-L1 cells, most dyes (both synthetic and natural) induced high cytotoxicity, with cell viability below 70% at all concentrations and exposure times. In the HaCaT cell line, most synthetic dyes caused significant cytotoxicity. However, the natural dyes Yellow Neart, Rialterra Caribe, and Rialterra Peach maintained cell viability between 70% and 100%. In the B16-F10 cell line, all synthetic dyes demonstrated high cytotoxicity, except for Bezaktiv Black SNN 02, which showed a slight increase in viability at low concentrations after 24 hours. In contrast, some natural dyes displayed increased viability (>80%) at lower concentrations and shorter exposure times. Although the results appear promising for natural dyes regarding effects on skin cell lines, they should be be interpreted with caution, as the cell lines are derived from dermal and epidermal layers, whereas intact human skin is protected by additional barrier mechanisms.

This study provided a preliminary overview of the potential health risks faced by workers exposed to both synthetic and natural dyes. Nevertheless, clear differences between synthetic and natural dyes were observed only in the cell viability assays. In occupational settings, natural dyes are used only sporadically compared with synthetic dyes, and the lack of differentiation in handling limits workers from linking adverse health effects to a specific dye. Still, environmental monitoring in dye weighing areas, combined

with the systematic collection of worker-reported outcomes, can be highly valuable for enhancing risk understanding and supporting the development of more effective prevention strategies. Mitigating these risks require an integrated approach that includes improved work practices, adequate ventilation, the use of personal protective equipment, and worker training and awareness, to safeguard health and safety regardless of the dye origin. Considering the limitations of this study, future research should adopt a broader approach, including a larger and more representative sample of workers, comprehensive occupational exposure monitoring, and physicochemical characterisation of natural dyes. Furthermore, since inhalation is a primary route of exposure, it is recommended that *in vitro* toxicity assays be extended to respiratory tract cell lines to complement the existing skin models.

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ANNEX

ANNEX I – Supplementary information –Materials and equipment associated with dyeing process.

The automation of machines and equipment has been expanding in recent times to optimize the dyeing process and minimize the possibilities of errors. The machines involved in this process fundamentally consist of a container that holds the dye bath, equipped with temperature regulators, agitators for the bath, and the material to be dyed (Abrahart & Whewell, 2024; Araújo & Melo e Castro, 1986). The machines and equipment in each industrial unit vary according to the manufacturing stage of the textile material, such as loose fibers, yarn, fabrics, and finished articles:

Classification of dyeing machines	Description	Example	References
Machines for dyeing width	– Discontinuous process – The bath circuit is carried out through the action of a centrifugal pump and a valve, which allows the bath circuit to be reversed. – In case of a continuous process, there are machines that allow dyeing by passing a conveyor belt.	– Autoclave	(Araújo & Melo e Castro, 1986)
Dyeing machines for silver	– The silvers are placed in perforated pots and compressed, then inserted into autoclaves;	– Conical-pan machine	(Araújo & Melo e Castro, 1986; Broadbent, 2001; Wardman, 2018)
Machines for dyeing yarn	– Used for multicolor effects in weaving or knitting;	– Package dyeing machines – Dyeing beams	(Araújo & Melo e Castro, 1986; Broadbent, 2001; Wardman, 2018)
Machines for dyeing fabric	– Discontinuous process;	– Winch dyeing – Jig dyeing machines – Beam dyeing machines – Jet dyeing machines	(Araújo & Melo e Castro, 1986; Broadbent, 2001; Wardman, 2018)
Dyeing machines for specific articles	–Performed in specific cases. – The machines have a device for agitating the material;	– Side-paddle machines – Rotating drum machines	(Araújo & Melo e Castro, 1986; Broadbent, 2001; Wardman, 2018)
Continuous dyeing equipment	– Dyeing is performed in a continuous sequence, until the entire length of textile passes through.	–*Foulard Machine* with a steaming or high-temperature process	(Araújo & Melo e Castro, 1986; Broadbent, 2001; Wardman, 2018)

ANNEX II – Questionnaire used for Characterization of the health effects reported by the workers.

Proteção de dados: Após a recolha dos dados, os mesmos ficarão armazenados num armário fechado à chave. Após o tratamento dos dados e no final do estudo, estes serão encriptados e destruídos. Durante todo o processo, nunca será possível identificar qualquer um dos participantes garantindo assim o anonimato e a proteção de cada um dos participantes.

1. **DADOS SOCIODEMOGRÁFICOS**

1.1. Género

Feminino ☐ Masculino ☐ Indefinido ☐

1.2. Idade

(responder um nº)

1.3. Habilitações Literárias

1º Ciclo ☐ Licenciatura ☐

2º Ciclo ☐ Mestrado ☐

3º Ciclo ☐ Doutoramento ☐

Ensino Secundário ☐

1.4. Estatuto profissional

Trabalhador Permanente ☐ Trabalhador Temporário ☐

1.5. Anos de Trabalho

(responder em nº)

1.6. Local de trabalho

Laboratório ☐ Cozinhas Tingimento – Produção ☐ Local de Pesagem ☐

2. EFEITOS NA SAÚDE

2.1. Alguma vez teve chiadeira ou pieira ou "gatinhos" no peito nos últimos 12 meses?

Sim	1
Não	2

2.1.1. Teve falta de ar quando a chiadeira estava presente?

Sim	1
Não	2

2.1.2. Teve a chiadeira ou a pieira sem estar constipado?

Sim	1
Não	2

2.1.3. A chiadeira ou pieira piora quando se encontra no seu local de trabalho?

Sim	1
Não	2

2.2. Acordou com a sensação de aperto no peito nos últimos 12 meses?

Sim	1
Não	2

2.2.1. Se sim, essa sensação piora quando se encontra no seu local de trabalho?

Sim	1
Não	2

2.3. Alguma vez foi acordado devido an um ataque de falta de ar nos últimos 12 meses?

Sim	1
Não	2

2.3.1. Essa falta de ar piora quando se encontra no seu local de trabalho?

Sim	1
Não	2

2.4. Alguma vez foi acordado devido a um ataque de tosse nos últimos 12 meses?

Sim	1
Não	2

2.4.1. Esse sintoma- ataque de tosse- piora quando se encontra no seu local de trabalho?

Sim	1
Não	2

2.5. Num ano produziu pelo menos durante 3 meses muco do seu peito na maioria dos dias?

Sim	1
Não	2

2.6. Já alguma vez teve asma? (ou "Bronquite asmática")

Sim	1
Não	2

2.6.1. Que idade tinha quando teve o seu primeiro ataque de asma? (Se estiver indeciso, assinale a sua melhor estimativa!)

	anos

2.6.2. Alguma vez esteve hospitalizado (internado) por asma?

Sim	1
Não	2

2.6.3. Teve um ataque de asma nos últimos 12 meses?

Sim	1
Não	2

2.6.4. Presentemente, está a tomar remédios (inaladores/" bomba," aerossóis/nebulizador ou comprimidos/xarope) para a asma ou falta de ar?

Sim	1
Não	2

2.6.5. E nos últimos 12 meses, usou algum inalador ("bomba") para a asma ou falta de ar?

Sim	1
Não	2

2.6.6. De que cor?

Azul (p.ex. Bricanyl, Ventilan, Foradil, Oxis, Formoterol)	1
Cinza (p.ex. Spiriva)	2
Verde (p.ex. Serevent, Dilamax, UltraBeta)	3
Lilás/roxo (p.ex. Seretaide, Brisomax, Maizair)	4
Vermelho (p.ex. Symbicort, Assieme)	5
Castanho (p.ex. Pulmicort)	6
Laranja (p.ex. Flixotaide, Brisovent, Veraspir)	7
Não sei	8

2.6.7. Nos últimos 12 meses, fez uma ou mais vezes aerossóis/nebulizações (fuminhos/respirar um vapor/fumo feito por uma máquina ou num serviço de saúde) para a asma ou falta de ar?

Sim	1
Não	2

2.6.8. Este sintoma- asma- piora quando se encontra no seu local de trabalho?

Sim	1
Não	2

2.7. Alguma vez fez um exame para avaliar a sua função respiratória (a capacidade de respirar, p.ex. espirometria, soprar com muita força para uma máquina ou respirar dentro de uma caixa de vidro. Não "conta" ter só soprado rapidamente para um tubo de plástico)

Sim	1
Não	2

2.8. Teve rinite incluindo febre dos fenos ou alergias do nariz?

Sim	1
Não	2

2.8.1. Foi perturbado pela rinite nos últimos 12 meses?

Sim	1
Não	2

2.8.2. Alguma vez teve problemas de rinite que durassem mais do que 4 dias numa semana?

Sim	1
Não	2

2.8.3. Se sim, isso aconteceu mais do que 4 semanas (1 mês) continuamente?

Sim	1
Não	2

2.8.4. Este sintoma -renite – piora quando se encontra no seu local de trabalho?

Sim	1
Não	2

2.9. O seu nariz esteve entupido mais de 12 semanas (3 meses) nos últimos 12 meses?

Sim	1
Não	2

2.9.1. Este sintoma – nariz entupido – piora quando se encontra no seu local de trabalho?

Sim	1
Não	2

2.10. Teve dor ou pressão na zona da testa, nariz ou olhos durante mais de 12 semanas (3 meses) nos últimos 12 meses?

Sim	1
Não	2

2.10.1. Este sintoma – dor ou pressão na zona da testa, nariz ou olhos – piora quando se encontra no seu local de trabalho?

Sim	1
Não	2

2.11. Os seus olhos estiverem irritados (olhos vermelhos ou secos, olhos com comichão) últimos 12 meses?

2.11.1. Estes sintomas – olhos vermelhos ou secos, olhos com comichão – pioram quando se encontra no seu local de trabalho?

2.12. O seu médico alguma vez lhe disse que tem rinite alérgica?

Sim	1
Não	2

2.12.1. A renite alérgica piora quando se encontra no seu local de trabalho?

Sim	1
Não	2

2.13. O seu médico alguma vez lhe disse que tem sinusite crónica?

Sim	1
Não	2

2.13.1. Este sintoma – sinusite crónica– piora quando se encontra no seu local de trabalho?

Sim	1
Não	2

2.14. Nos últimos 12 meses usou alguma vez sprays (gotas) nasais durante mais de 2 semanas seguidas? (p.ex. Pulmicort nasal, Flutaide, Nasomet, Eustidil, Rontilona, Aeromax, Avamys)

Sim	1
Não	2

2.15. Nos últimos 12 meses usou alguma vez comprimidos/xarope para as alergias (anti-histaminicos – p.ex. Zyrtec, Aerius, Rinialer, Xyzal, Claritine, Telfast, Atarax, Levrix, Ceterizina, Loratadina, ...)

Sim	1
Não	2

2.16. Alguma vez teve eczema ou qualquer tipo de alergia de pele?

Sim	1
Não	2

2.16.1. Este sintoma – eczema ou alergia na pele – piora quando se encontra no seu local de trabalho?

Sim	1
Não	2

2.17. Alguma vez algum médico lhe disse que tinha alergias a medicamentos?

Sim	1
Não	2

2.17.1. Já fez algum teste ou prova para diagnóstico da alergia a medicamentos?

Sim	1
Não	2

2.18. Alguma vez algum médico lhe disse que tinha alergias alimentares?

Sim	1
Não	2

2.18.1. Já fez algum teste ou exame para alergias alimentares?

Sim	1
Não	2

2.19. Alguma vez fez testes cutâneos (na pele do braço) para despiste de alergia?

Sim	1
Não	2

2.20. Alguma vez fez análises ao sangue para despiste de alergia?

Sim	1
Não	2

2.21. Sente que desenvolveu esse tipo de alergias no seu local de trabalho?

Sim	1
Não	2

2.22. Já alguma vez fumou, pelo menos 1 cigarro por dia (ou um charuto por semana) durante 1 ano? [SIM significa pelo menos 1 cigarro por dia ou um charuto por semana durante 1 ano]

Sim	1
Não	2

2.22.1. Que idade tinha quando começou a fumar?

	anos

2.22.2. Em média, por dia quantos cigarros fumou?

	Cigarros por dia

2.22.3. Em média, por dia quantos cigarros fuma?

	Cigarros por dia

2.23. Na sua residência alguém fuma?

Sim	1
Não	2

2.21A. Sofre de alguma doença do coração?

Sim	1
Não	2

ANNEX III – Work instructions for measuring equipment DustTrak™ DRX Aerosol Monitor 8533 and VelociCalc® Model 9565 Series

1. Âmbito e objetivo

Nesta instrução de trabalho está descrito o modo como se opera com o *DustTrak* II *Aerosol Monitor*, para proceder a medições de qualidade do ar interior.

2. Responsabilidades

Técnico encarregue das medições.

3. Equipamento

O *DustTrak* II *Aerosol Monitor* realiza leituras de massa dos aerossóis através dos fotómetros de dispersão de luz a laser e o registo dos dados obtidos em tempo real. Este equipamento utiliza um sistema de bainha de ar que isola o aerossol na câmara ótica para maior confiabilidade e baixa manutenção.

A utilização deste instrumento é adequada para ambientes de escritório limpos, bem como locais de trabalho em ambientes industriais agressivos, locais de construção, entre outras aplicações externas. Os monitores *DustTrak* II medem aerossóis contaminantes como poeiras, fumos e névoas e medem as concentrações de aerossóis correspondentes a PM1, PM2,5, PM10 e fração respirável.

3.1. Componentes

1) Mala de transporte

2) Monitor Desktop II

3) Filtro ZERO CAL

4) Impactor Kit

5) Impactor Oil

6) Tampa da entrada de ar

7) Carregador

8) Ferramenta tampa filtro interno

9) Caneta ecrã touch

10) Software CD-ROM

11) Cabo USB

12) Ferramenta Cassete 37 mm

4. Preparação do equipamento para medições

Ao preparar o equipamento para a realização de medições deve:

1º Coloque o equipamento sobre uma superfície estável e segura, de preferência já no local em que será realizada a medição;

2º Ligue o equipamento no botão On/Off;

On/Off

3º O ecrã *START UP* é exibido inicialmente quando o equipamento é ligado, verifique o estado da bateria para garantir que é suficiente para as medições a realizar;

Nota: o equipamento **não pode** realizar medições enquanto carrega!

4º Selecione o menu **SETUP** para aceder à opção **ZERO CAL** (deve ser realizado antes de cada medição) e carregue em **START** para iniciar;

5º Retire a tampa de proteção da entrada de ar e substitua pelo filtro *ZERO CAL*;

Nota: Nunca realizar o zero sem o filtro indicado!

6º Após terminar no ecrã irá aparecer a mensagem "*Zero Cal complete*", deve desconectar o filtro e colocar novamente a tampa de proteção ou substitur pelo *Size-Selective Impactor* que irá utilizar nas medições.

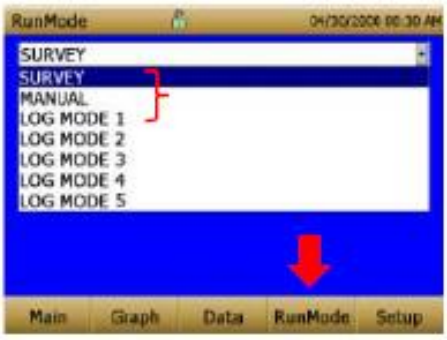
5. Medições

1º No menu RUN MODE, deve selecionar o modo/teste para a medição:

SURVEY, permite realizar medições em tempo real, mas não regista os dados.

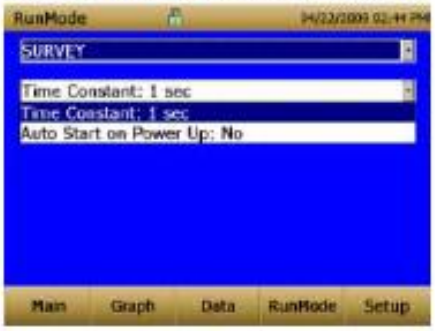
MANUAL, permite configurar o equipamento para registar os dados por um tempo especificado.

LOG MODE, o modo de registo inicia e interrompe o equipamento em horários específicos.



2º Defina as variáveis necessárias para o modo selecionado:

SURVEY – Time Constant, Auto Start on Power Up



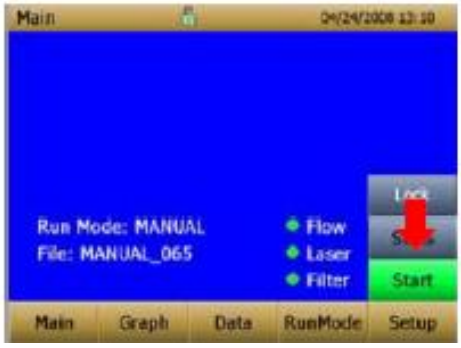
MANUAL – Log interval, Test length, Time constant

LOG MODE – Log name, Start date, Start time, Log interval, Auto zero interval, Test length, Number of tests, Time between tests, Time constant, Use start date, Use start time

3º Selecione o *Size-Selective Impactor* adequado para o parâmetro a medir (PM_1, $PM_{2.5}$, PM_4 – fração respirável, PM_{10}) e prepare-o: retire a tampa, coloque 2 gotas de *Impactor oil* no filtro de impactação e coloque novamente a tampa;

5º Retire a tampa de proteção da entrada de ar e substitua pelo *Size-Selective Impactor* a utilizar;

6º Após acoplar o filtro ao equipamento, para iniciar a medição deve carregar em START. A medição terminará automaticamente quando for atingido o tempo de medição definido anteriormente;



Nota: Durante a medição preste atenção aos indicadores de erro para assegurar que estão reunidas todas as condições necessárias à medição!

7º Terminadas todas as medições retire o *Size-Selective Impactor* e coloque a tampa de proteção na entrada de ar;

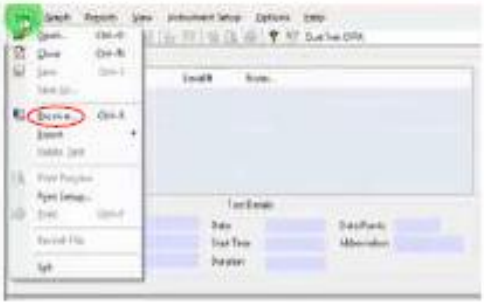
8º Desligue o equipamento e coloque-o na mala de transporte.

6. Transferência de dados para o PC

O software para a transferência de dados deve ser instalado no PC utilizando o CD-ROM incluído no Kit.

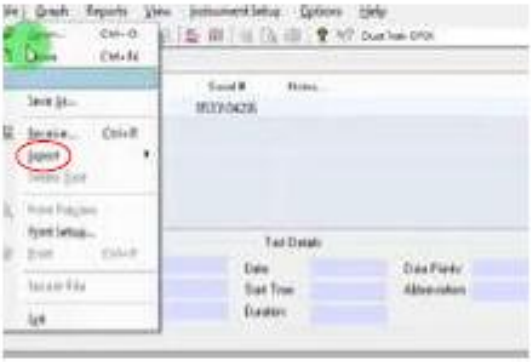
1º Para realizar a transferência dos dados basta conectar o equipamento ao PC utilizando o respetivo cabo USB e abrir o programa;

2º No menu *File* selecione a opção *Receive* para transferir os dados;



3º Selecione os dados que quer transferir na janela *Receive Tests From Instruments*;

4º Para guardar os dados no menu *File* selecione *Export* e guarde os dados no PC;



7. Limpeza e manutenção

Componente	Frequência
Zero Cal	Antes de cada utilização
Entrada de ar	A cada 350h de amostragem a 1 mg/m^3
Size-Selective Impactors	Depois de cada utilização
Filtros internos	A cada 350h de amostragem a 1 mg/m^3 ou quando surja no ecrã mensagem de erro referente ao filtro
Limpeza e calibração de fábrica	Anualmente

7.1. Entrada de ar

1º Desaperte o tubo da entrada de ar do equipamento;

2º Utilize um cotonete de algodão para limpar a entrada. Pode utilizar água ou um solvente leve (p.e. isopropanol);

3º Para limpar o interior do tubo utilize uma pequena escova com um solvente leve;

4º Seque o tubo com ar comprimido ou deixe secar completamente ao ar antes de voltar a colocar no equipamento;

Nota: Não sopre ar para dentro do equipamento!

5º Coloque o tubo da entrada de ar novamente no equipamento.

7.2. *Size-Selective Impactors*

1º Desaperte o *Impactor* e retire o filtro para limpeza;

2º Limpe o interior e o exterior do Impactor e o filtro utilizando uma escova e um solvente leve;

3º Seque as partes do Impactor com ar comprimido ou deixando secar totalmente ao ar;

4º Monte novamente o *Impactor*.

7.3. Filtros internos

1º Abrir o compartimento na parte de trás do equipamento;

2º Utilize a ferramenta adequada presente no Kit do equipamento para retirar a tampa do filtro;

3º Retire o filtro cilíndrico e, se o poço do filtro se encontrar sujo limpe com ar comprimido;

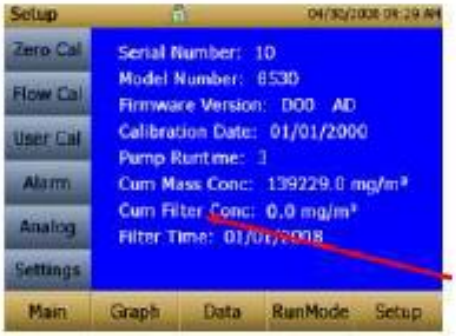
4º Coloque um novo filtro e volte a aparafusar a tampa;

5º Abra o clipe de retenção azul e remova a cassete de filtro 37 mm;

6º Abra a cassete utilizando a ferramenta adequada presente no Kit e troque o filtro;

7º Feche novamente a cassete e coloque-a no equipamento;

8º Faça *Reset* no menu *SETUP* para eliminar o erro do filtro e reiniciar a contagem até à próxima manutenção.



1. Âmbito e objetivo

Nesta instrução de trabalho está descrito o modo como se opera com o *Velocicalc – Multi-Function Ventilation Meter Model 9565*, para proceder a medições de qualidade do ar interior.

2. Responsabilidades

Técnico encarregue das medições.

3. Equipamento

O *VelociCalc® Multi-Function Ventilation Meter* 9565 apresenta uma interface de utilizador orientada por menus para uma fácil operação no seu idioma local. As instruções na tela e as instruções passo a passo guiam o usuário através da configuração do instrumento, operação e calibração em campo. O 9565 também apresenta um design ergonómico de caixa moldada com suporte de sonda e um bloqueio de teclado para evitar a manipulação durante a utilização sem vigilância.

Estes instrumentos estão disponíveis com ou sem sensor de pressão diferencial e são projetados para funcionar com uma ampla gama de sondas plug-in. Desta forma permite a medição da temperatura, humidade relativa, velocidade do ar, ponto de orvalho, COV's totais, CO e CO_2.

Atendendo à sua multifuncionalidade pode ser utilizado para avaliações de Qualidade do Ar Interior (QAI), teste de exaustão de laboratórios, teste de sistemas AVAC, estudos de conforto térmico, avaliações de ventilação e testes de fluxo de ar de processo.

3.1. Componentes

1) *VelociCalc®*

2) Mala de transporte

3) Carregador

4) Software CD-ROM

5) Cabo USB

6) Sonda Termohigrómetro

8) Sonda COV

7) Sonda CO_2

4. Preparação do equipamento para medições

1º Deve ligar o equipamento no botão On/Off e verifique o estado da bateria:

O *VelociCalc® Multi-Function Ventilation Meter* 9565 funciona a pilhas ou com o carregador.

Caso opte pelas pilhas deve inserir 4 pilhas AA no compartimento na parte traseira do equipamento.

No caso de utilizar o carregador deve conectá-lo na entrada correta (indicada na figura abaixo).

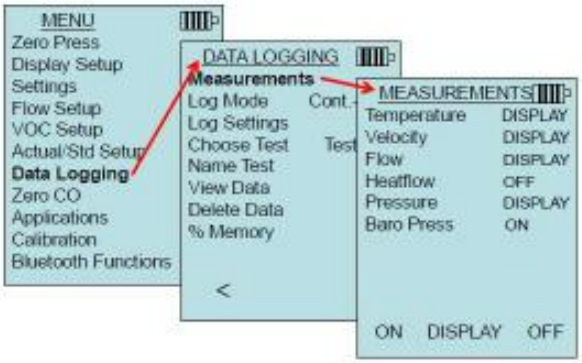
2º Conecte ao equipamento a sonda que irá utilizar para o parâmetro a analisar (Termohigrómetro, CO_2 ou COV's):

As sondas devem ser conectadas na entrada central (ilustrado na figura à direita).

5. Medições

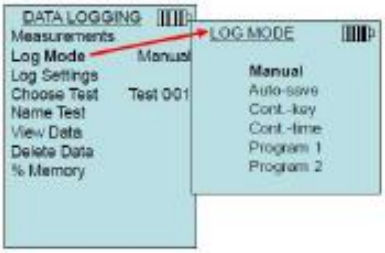
1º Ligue o equipamento no botão On/Off  ;

2º Selecione a tecla central *MENU* e selecione a opção *DATA LOGGING* para confirmar quais os parâmetros serão medidos, em *MEASUREMENTS*;

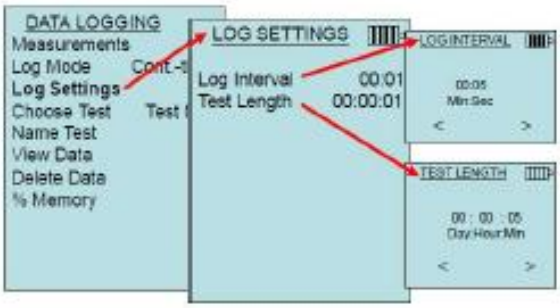


3º Ainda no menu *DATA LOGGING* deve ainda selecionar as variáveis pretendidas para a medição:

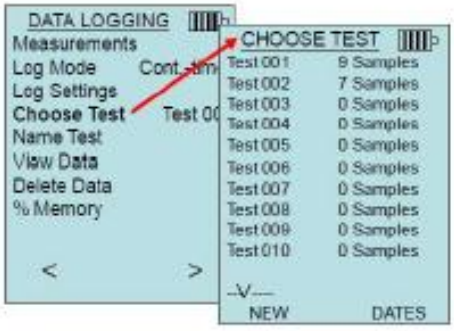
Log Mode



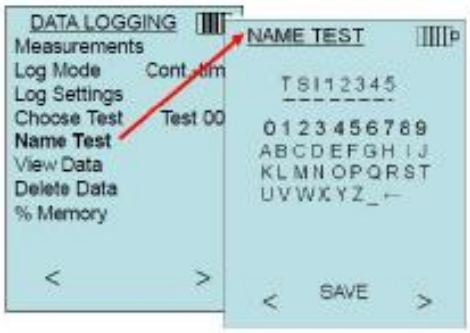
Log Interval e *Test Lenght*



Choose Test

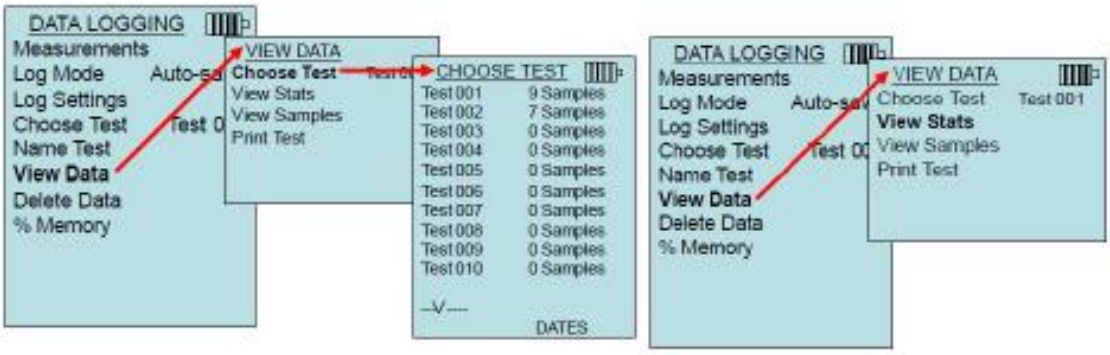


Nota: Pode alterar o nome do teste para ser mais fácil identificar.



4º Para iniciar a medição, é necessário regressar ao ecrã inicial, através da tecla ESC e pressionar a tecla ENTER

5º Para ver os resultados obtidos na medição, no menu DATA LOGGING, selecionar a opção VIEW DATA e escolher o teste que se pretende verificar (CHOOSE TEST). Depois selecionar a opção VIEW STATS.

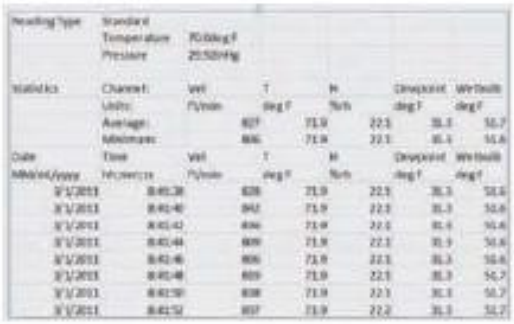


6. Transferência de dados para o PC

O software para a transferência de dados deve ser instalado no PC utilizando o CD-ROM incluído no Kit.

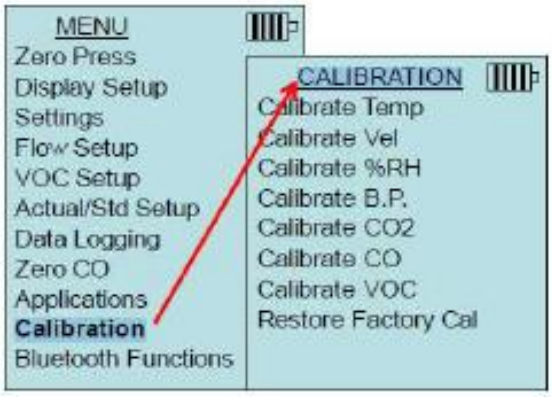
1º Para realizar a transferência dos dados basta conectar o equipamento ao PC utilizando o respetivo cabo USB;

2º Ao abrir o programa instalado os dados serão transferidos para o PC numa folha de cálculo excel.



7. Limpeza e manutenção

O equipamento e as respetivas sondas devem ser calibração de fábrica anualmente. No entanto, estes podem também ser calibrados em campo recorrendo ao menu *CALIBRATION*, não substituindo a calibração mencionada anteriormente.



Nota: Para calibração de campo as respetivas sondas devem estar conectadas ao equipamento quando selecionado o parâmetro a calibrar.

ANNEX IV – Results of preliminary MTT assays with the concentrations of 2000 µg mL^{-1}; 1000 µg mL^{-1}; 500 µg mL^{-1}

Class of dye	Dyes	3T3-L1					
		2000 µg/mL		1000 µg/mL		500 µg/mL	
		24h	48h	24h	48h	24h	48h
Synthetic dye	Yellow Sunfix G4GL 200%, Yellow Sunfix S3R 150%, Yellow Sunfix SSR Intense Blue Sunfix SS, Blue Sunfix SPR, Bezaktiv Black SNN 02, Bezaktiv Blue S-Matrix 150-01, Bezaktiv Navy S-W, Jakofix Orange ME2RLC, Jakozol Navy CE, Levafix Ambar CAN, Levafix Blue CA, Red Sunfix SPR-F	0					
	Jakozol Yellow PP	+	-	+	+	+	+
	Levafix Rubine	-	+	+	+	+	+
Natural dye	Yellow Neart, Rialterra Caribe, Rialterra Peach	+++	+++	+++	++	++	++
	Rialterra Sun, Rialterra Soya	0					
	Rialterra Orange	+++	+++	+++	+++	+++	++

Legend: + indicate percentage of viability, where: +++ higher than 100 %; ++ viability 80-100%; + viability 70-80%; - viability lower than 70%; 0 indicate negative values.

Class of dye	Dyes	HaCaT					
		2000 µg/mL		1000 µg/mL		500 µg/mL	
		24h	48h	24h	48h	24h	48h
Synthetic dye	Yellow Sunfix G4GL 200%, Yellow Sunfix S3R 150%, Yellow Sunfix SSR Intense Blue Sunfix SS, Blue Sunfix SPR, Bezaktiv Black SNN 02, Bezaktiv Blue S-Matrix 150-01, Bezaktiv Navy S-W, Jakofix Orange ME2RLC, Jakozol Navy CE, Levafix Ambar CAN, Levafix Blue CA, Red Sunfix SPR-F	0					
	Jakozol Yellow PP	+	-	+	+	+	+
	Levafix Rubine	-	+	+	+	+	+
Natural dye	Yellow Neart, Rialterra Caribe, Rialterra Peach	+++	+++	+++	++	++	++
	Rialterra Sun, Rialterra Soya	++	+++	++	++	++	+++
	Rialterra Orange	+++	+++	+++	+++	+++	++

Legend: + indicate percentage of viability, where: +++ higher than 100 %; ++ viability 80-100%; + viability 70-80%; - viability lower than 70%; 0 indicate negative values.

Class of dye	Dyes	B16F10					
		2000 µg/mL		1000 µg/mL		500 µg/mL	
		24h	48h	24h	48h	24h	48h
Synthetic dye	Yellow Sunfix G4GL 200%, Yellow Sunfix S3R 150%, Yellow Sunfix SSR Intense Blue Sunfix SS, Blue Sunfix SPR, Bezaktiv Black SNN 02, Bezaktiv Blue S-Matrix 150-01, Bezaktiv Navy S-W, Jakofix Orange ME2RLC, Jakozol Navy CE, Levafix Ambar CAN, Levafix Blue CA, Red Sunfix SPR-F	0					
	Jakozol Yellow PP	+	-	+	+	+	+
	Levafix Rubine	-	+	+	+	+	+
Natural dye	Yellow Neart, Rialterra Caribe, Rialterra Peach	+++	++	+++	+++	+++	+++
	Rialterra Sun, Rialterra Soya	0	+++	+++	++++	+++	+++
	Rialterra Orange	+++	+++	+	0	+	+

Legend: + indicate percentage of viability, where: +++ higher than 100 %; ++ viability 80-100%; + viability 70-80%; - viability lower than 70%; 0 indicate negative values.

ANNEX V – Results of MTT assays with the concentrations of 500 µg mL^{-1}; 250 µg mL^{-1}; 125 µg mL^{-1}

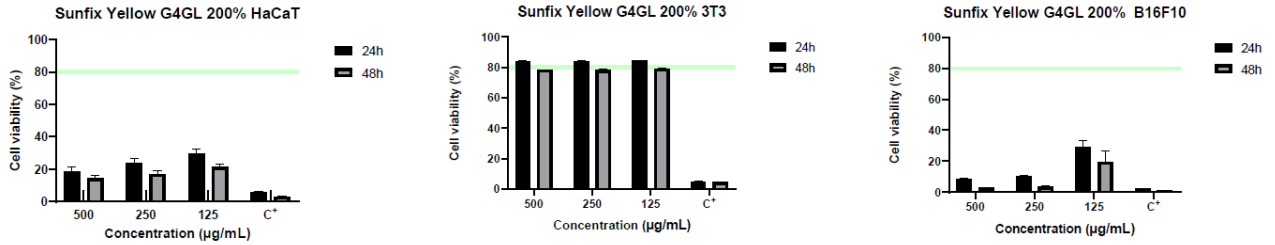


Figure 10. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Sunfix Yellow G4GL 200%.

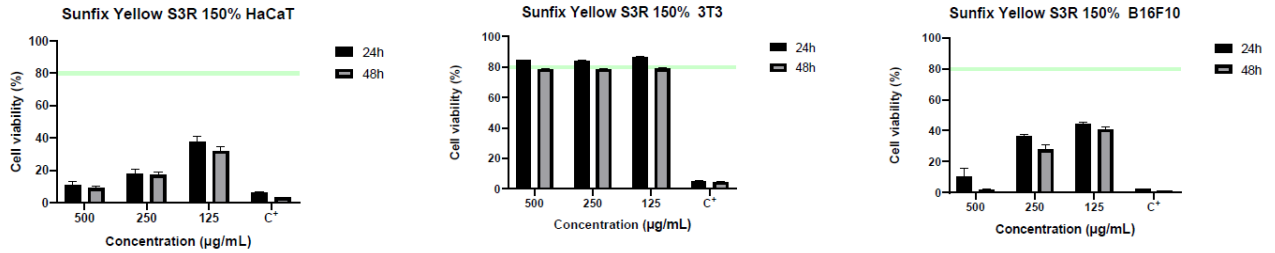


Figure 11. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Sunfix Yellow S3R 150%.

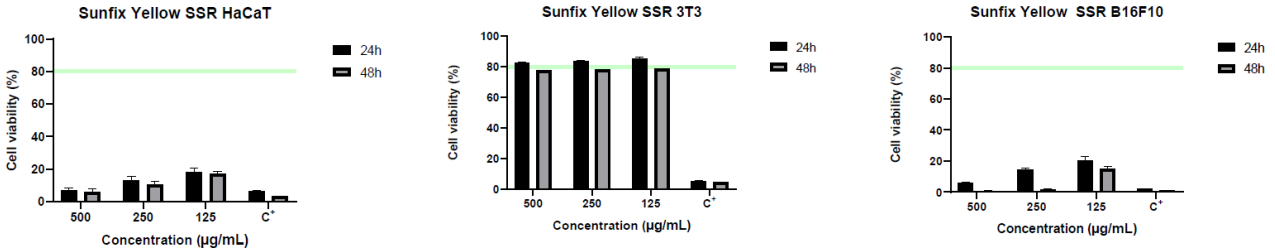


Figure 12. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Sunfix Yellow SSR.

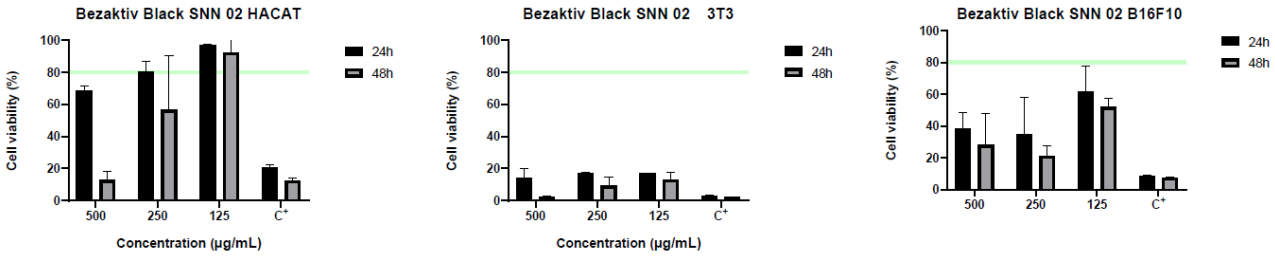


Figure 13. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Bezaktiv Black SNN 02.

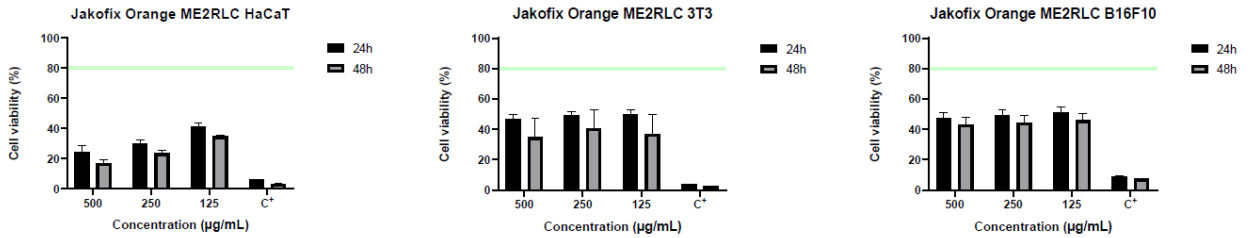


Figure 14. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Jakofix Orange ME2RLC.

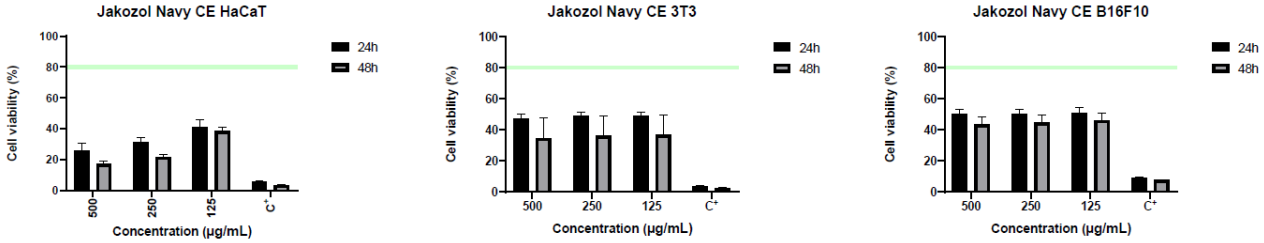


Figure 15. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Jakozol Navy CE.

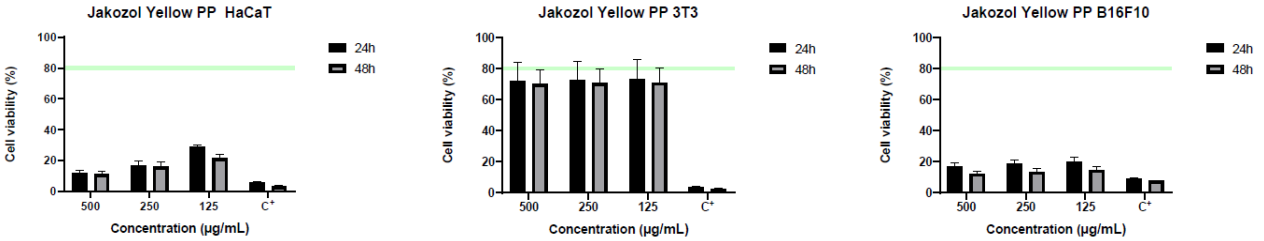


Figure 16. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Jakozol Yellow PP.

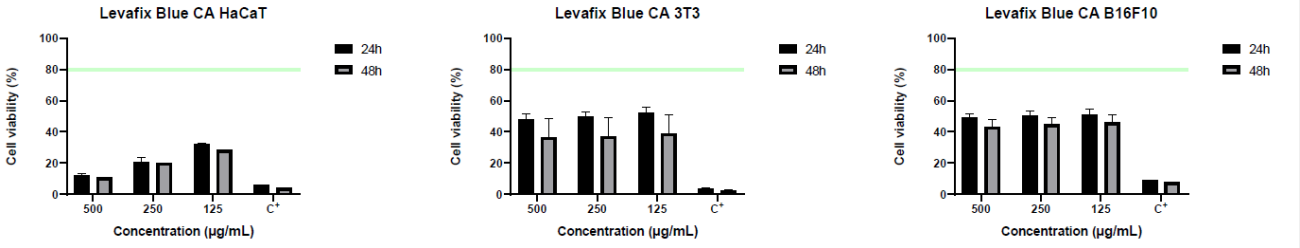


Figure 17. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Levafiz Blue CA.

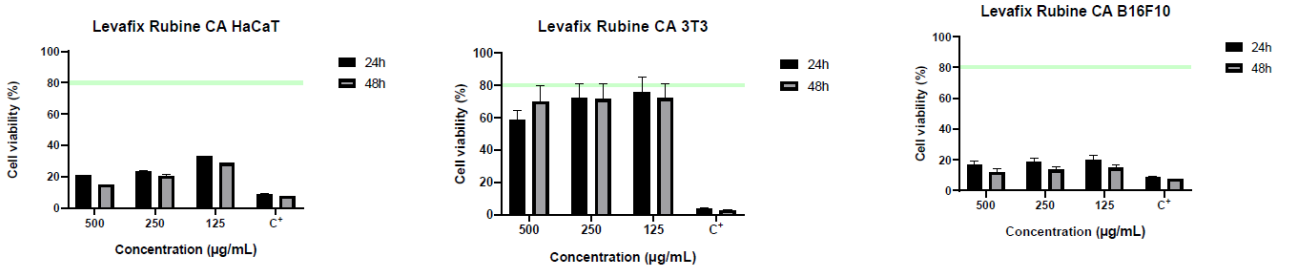


Figure 18. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Levafix Rubine.

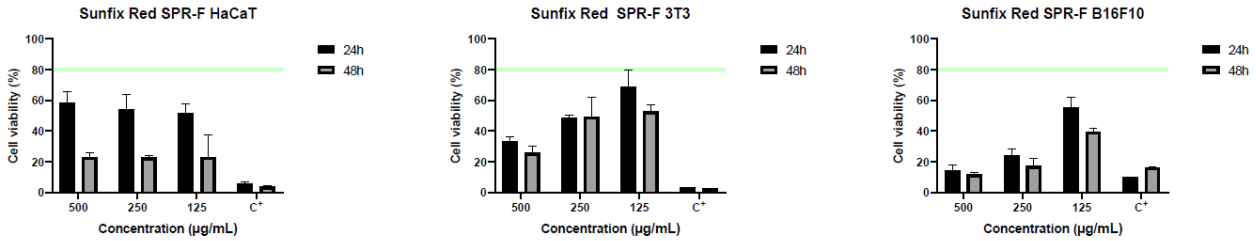


Figure 19. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Sunfix Red SPR-F.

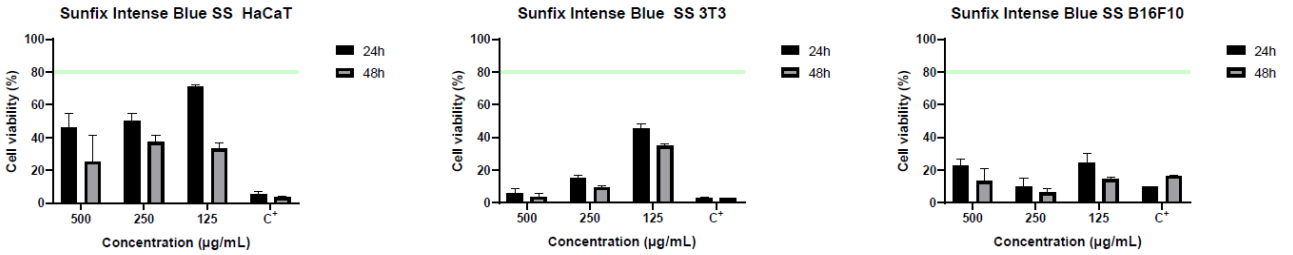


Figure 20. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Sunfix Intense Blue SS.

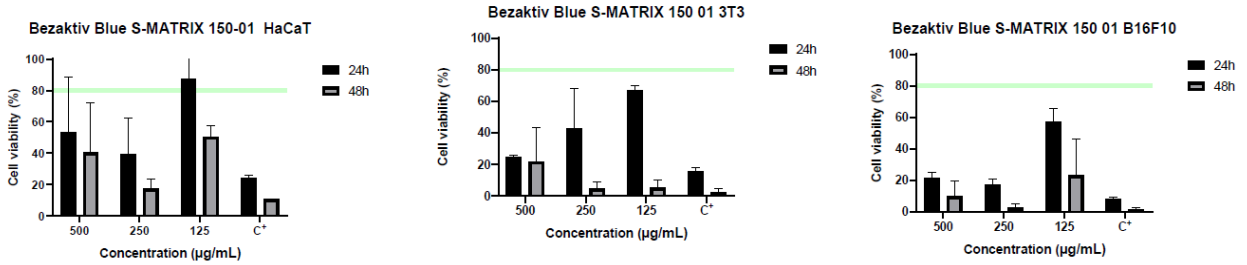


Figure 21. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Bezaktiv Blue S-MATRIX 150-01.

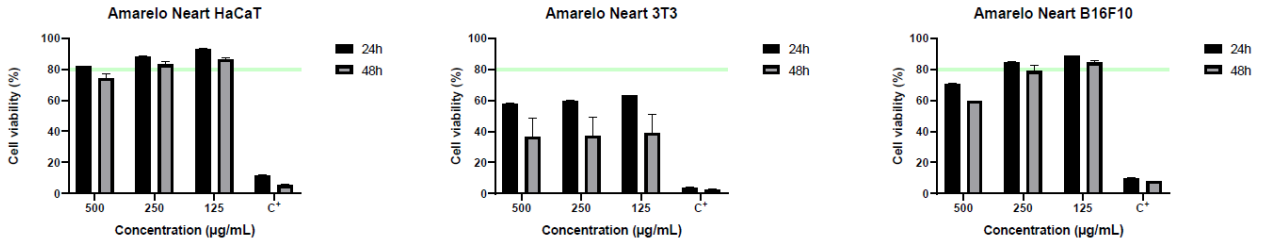


Figure 22. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Amarelo Neart.

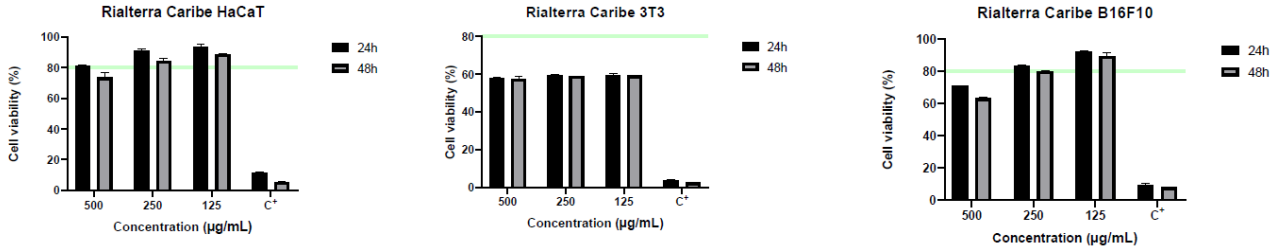


Figure 23. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Rialterra Caribe.

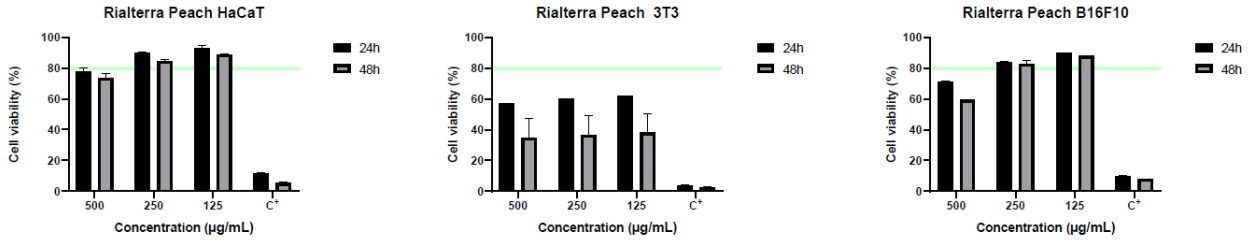


Figure 24. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Rialterra Peach.

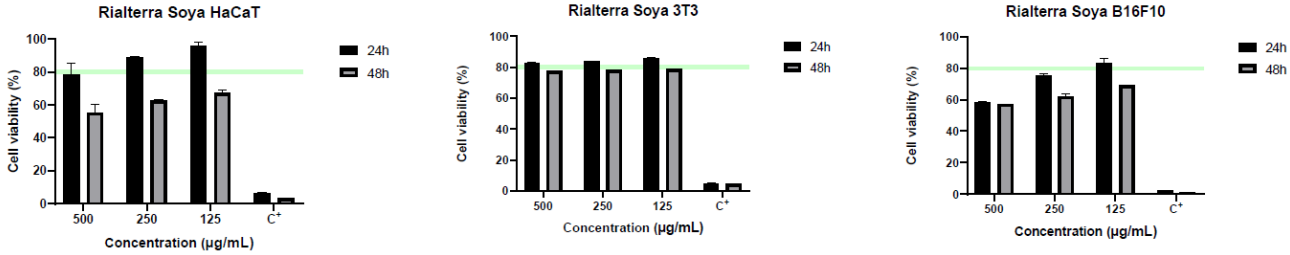


Figure 25. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Rialterra Soya.

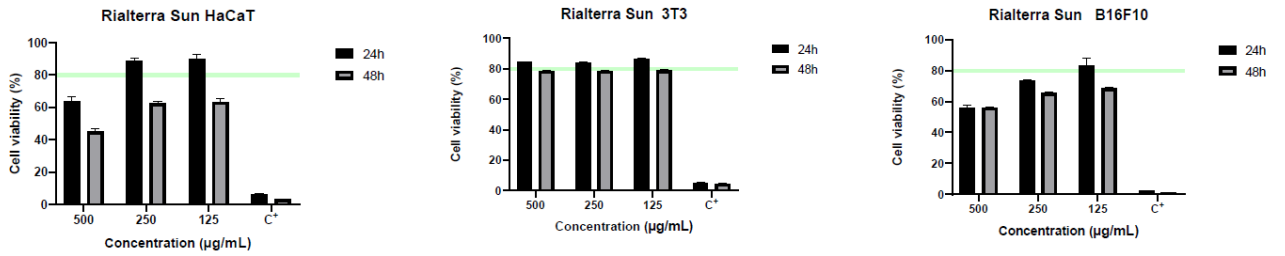


Figure 26. Viability of HaCaT, 3T3-L1 and B16F10 cells by MTT assay on exposure to Rialterra Sun.

P.PORTO

ESCOLA SUPERIOR DE SAÚDE

M

MESTRADO

HIGIENE E SEGURANÇA NAS ORGANIZAÇÕES