AN OVERVIEW ON CURRENT REGULATION AND EVALUATION OF BIOCIDAL PRODUCTS

ABSTRACT

A biocidal product is a substance or mixture prepared to limit, destroy, neutralize or control the effects of a harmful microorganism, plants and animals. Biocides consist of four main groups: disinfectants, preservatives (wood, paint, etc.), pest control and other type of biocidal products.In this study biocidal products have been overviewed in the scope of current European Unionregulations, product types and conformity tests.

Keywords: Biocidal products,Disinfectants,Regulation (EU) 528/2012, Conformity tests for biocidal products.

INTRODUCTIONAccording to the Biocidal Products Regulation (BPR, Reg. (EU) 528/2012)of European Commission (EC), a biocidal product is defined as a product or substance intended to eliminate, control or prevent the effects of harmful organisms for human and animal health and to control organisms harmful to natural or manufactured materials [1].The use of chemical biocides is a fundamental protection in the prevention and control of microbial growth in medical, veterinary, domestic and industrial environments [2]. Biocides are used a great extent in the healthcare environment for the disinfection of equipments, surfaces, water, and for antisepsis of skin and wound. Besides biocides are used for the preservation of pharmaceuticals and sterilization of medical devices [3]. In the 20th century, the development of cationic biocides such as quaternary ammonium compounds, biguanides, aldehydes, peroxigens and phenolics hadbeen an enormous increase in the number of active compounds used for disinfection, sterilization and preservation[4].

Biocides are the main armoury in the disinfection programme of food industry to control pathogenic and spoilage micro-organisms [5].Biocidal products also constitute the antimicrobial component of nanomaterials used in food packaging in recent years [6].Preservatives are an important part of biocides and used to protect industrial products, cosmetics, metal and wood materials, textile products from microbial spoilage. Isothiazolinone biocides are broadly used in various industrial applications for the control of microbial growth. Between the biocides,5-chloro-2-methyl-4-isothiazolin-3-one (CMIT), 2-methyl-4-isothiazolin-3-one (MIT),1,2-Benzisothiazolin-3-one (BIT),4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT)are the most commonly used as preservative active substance [7].

Effective and proper use of insecticide and rodenticide group biocides is also important for the protection of human and environmental health. Permethrin,tetramethrin, cypermethrin are commonly used insecticide active substance for product type [8].

Misuse of biocidal products brings about some problems such as resistance development and toxicity. The spreading usage of products containing low concentrations of commonly used biocides have raised some concerns about the possible development of microbial resistance. Laboratory studies have demonstrated that bacteria can become resistant to a biocide, and that resistant bacteria can develop cross-resistance to other biocides and antibiotics [9].High concentrations of biocides generally have toxic effects not only for humans but also for the

environment.Therefore, a legal guideline for environmental risk evaluationfor biocidal products is being prepared [10]. The toxicity of some biocides associated with dermatitis, occupational asthma and irritation has been reported [11-13].

The use of commercially available biocidal products is regulated according to the EU Biocidal Products Regulation. In this study biocidal products have been overviewed in the scope of current EU regulations, product types and conformity tests.

RULES FOR BIOCIDAL PRODUCTS

The aim of the Biocidal Products Regulation (Reg. (EU) 528/2012)is to determine the rules for the production and the use of biocidal products, as well as to provide a protection fromhealth and environmental risks. The main rule is that biocidal products must be authorized before they are placed on the market. This regulation applies to biocidal products containing or forming one or more active substances.

A biocidal product is a substance or mixture designed to limit, destroy, neutralize or control the effects of a harmful organism. All biocidal products contain one or more active substances. The active ingredients give the product the desired biocidal properties. Active substances used in biocidal products must be authorised within the EU before they can be put on the market. Before the approval of an active substance, its effect on the environment and human health is assessed. To ensure legal certainty, Active substances from the Union list approved for use in biocidal products has been established. In order to market an active substance that has not been previously authorized or under examination,It must be applied for authorization to The European Chemical Agency (ECHA) [14].

The following conditions must be met in order to license a biocidal product:

- The active substances must be approved for the type of product concerned and the conditions specified for these active substances must be met.
- It must be sufficiently effective.
- The biocidal product must not produce unacceptable resistance or cross-resistance on target organisms. There should be no unacceptable effects such as unnecessary pain and suffering for vertebrates.
- The biocidal product or its residues must not have an unacceptable effect on food and feed, directly or indirectly.
- The biocidal product or its residues must not have an unacceptable effect on the environment, groundwater, surface water, drinking water, soil and air.
- It should not have undesirable effects on non-target organisms.
- It should not have undesirable effects on the ecosystem andbiodiversity.
- The chemical identity, quantities and technical equivalence of the active and inactive substances in the biocidal product should be determined. Toxicological and ecotoxicological information should be provided where appropriate.

PRODUCT TYPES

In order to make a suitable assessment of exposure and risk to health and the environment as a result of a biocidal product’s use, the EU has determined the different product types of biocidal products. According to the Biocidal Products Regulation (EU) 528/2012, biocides are classified in four main categories and twenty-two specific product types [1].

1) Disinfectants

PT 1 Human hygiene: Products are usedfor human hygiene. The primary purpose of these products is to disinfect human skin and scalp.

PT 2 Disinfectants and algaecides: The aim of these products isto disinfect surfaces and equipment that are not contact with food or feeding stuff. Areas of use include air conditioning systems, aquariums,swimming pools, and private, public and industrial areas. It is also used for disinfection of air, waterother than human or animal consumption, waste water, hospital waste and soil. They are incorporated into textiles, textures, paints and other articles to produce treated products.

PT 3 Veterinary hygiene: Antiseptics and disinfectants that are used for veterinary hygiene. Used for disinfecting animal shelters and transport equipment and surfaces.

PT 4 Food and feed area: It is used disinfecting equipment, surfaces or piping related to the production, transportation,storage of food or feed.

PT 5 Drinking water: Products in this group are used for disinfection of drinking water for human and animal consumption.

2) Preservatives

PT 6 In-can preservatives: It is used for the protection of cosmetic products, medical products or medical devices and manufactured products with microbial deterioration and control to ensure shelf life.

PT 7 Film preservatives: These products are used for the protection of films and coatings by controlling microbial deterioration or algae growth for the purpose of protecting the objects such as paint, plastic, wall adhesives, paper.

PT 8 Wood preservatives: These products are used for the protection of wood and wood products by controlling organisms that damage wood, including insects.

PT 9 Fibre, leather, rubber and polymerised materials preservatives: These preservatives are used for the preservation of polymers and fibres, such as textile, rubber,leather or paper by the control of microbiological degradation.

PT 10 Construction material preservatives: The purpose of these products are to preserve masonry, construction materials andcomposite materials except for wood by the control of bacterial, fungal and algal attack.

PT 11 Preservatives for liquid-cooling and processing systems: Products in this group are used to protect liquids used in cooling water and other water systems from microorganisms.

PT 12 Slimicides: This group is used to control or prevent the formation of slime in equipment and materials used in industrial areas.

PT 13 Working or cutting fluid preservatives: Theseproducts aim to control microbial deterioration in fluids used for metal, glass or other materials.

3) Pest control

PT 14 Rodenticides: These products aim to control of rodents, except forattraction or repulsion.

PT 15 Avicides: Purpose of use is to control birds, except for attraction or repulsion.

PT 16 Molluscicides, vermicides and products to control other invertebrates: Used for the control of invertebrates such as molluscs, worms and other, by means other than attraction or repulsion.

PT 17 Piscicides: These products purpose is to control of fish.

PT 18 Insecticides, acaricides and products to control other arthropods: These products are used for the control of arthropods such as insects, crustaceans and spiders.

PT 19 Repellents and attractants: Theseare used to control harmful organisms by attracting or repelling, are used for directly on the skin or indirectly in environments of human or animal.

PT 20 Control of other vertebrates: These products are used for controlling vertebrates other than those included by the other product types.

4) Other biocidal products

PT 21 Antifouling products: These products aim to control the growth of fouling microorganisms, plant or animal species on vessels, aquaculture equipment or other construction used in water.

PT 22 Embalming and taxidermist fluids: These products are used for the disinfection and preservation of human or animal corpses and parts.

CONFORMITY TESTS OF BIOCIDAL PRODUCTS

Physical and Chemical Properties of the Active Substance

When evaluating the physicochemical properties of the active substances in the biocidal product, the appropriate method should be selected and experimental results given priority, provided that it operates within the validity range. Data on physicochemical properties should be reliable[15].

Physical Parameters

Appearance: The physical parameters of the product should be measured and reported at 20 °C ambient temprature, and 101.3 kPa atmospheric pressure. The physical stateof the product may be solid, liquid, or gaseous. The colour and odour must be reported at parameters mentioned above,Odour associated with the active substance describedin laboratories or production plants, must be reported. This can be e.g. odourless, characteristic of aromatic compounds, ammonia-like, biting, faint, pungent, slight, sweetish or other. Substances that are hazardous by inhalationshould not be investigated for their odour properties[15].

Melting and freezing points: The melting point must be measured up to 360 °C. Generally, it should be determined if the freezing point of liquid substances is above –20 °C. EC method A.1 (Melting / Freezing Temperature) should be used as the test method. The use of Differential Scanning Calorimetry (DSC) or Differential Thermo Analysis (DTA) is recommended [16].

Acidity and alkalinity: For water-containing active substances, the pH of the active substance itself must be tested according to the CIPAC method MT 75.3 [17]. If solid and

nonaqueous liquid active substances are to be used in biocidal products, the pH of an aqueous dilution of 1% of the active substance should be determined. Acidity(pH <4) or alkalinity(pH >10) of the products should be determined according to the CIPAC method MT 191 [18].

Boiling point: The boiling point must be measured up to 360 °C according to EC method A.2 (Boiling temperature) [19].The boiling point should be measured at of 101.3 kPa (Standart atmospheric pressure) unless decomposition occurs.

Density: Density for liquids and solids is tested according to OECD Test Guideline 109 (Density of Liquids and Solids) [20]. The relative density of gases can be calculated from their molecular weight and the Ideal Gas Law. Polymer density should be determined by buoyancy methods.

Additional physical tests may be performed depending on the product type.

Chemical Properties

Approved analytical methods should be used for the determination of active substances and, where necessary, residues. Methods should be able to fully characterize analytes and quantitatively identify them. Therefore, the methods should be validated by laboratories. The method validation parameters should include recovery, repeatability, selectivity/specificity, limit of detection and limit of quantification. Methods should avoid hazardous substances and where possible, use commonly available techniques/equipment.

The active substance analysis is carried out using titrimetric, spectroscopic or chromatographic methods according to the chemical properties of the substance. HPLC, GC, GC-MS, AAS, ICP systems are the most commonly used analytical methods. In the analyzes, it is evaluated whether the content of the active substance is in accordance with the manufacturer's declaration. It is accepted that the content of the active substance will vary with each batch and as a result of sampling and analytical errors. These changes must be within a certain limit. The tolerance limits applied to the active substances are given in the table below [15].

Table 1. Tolerance limits of the active substance content.

Declared nominal content of active [g/kg or g/L]	**Tolerance limit**
≤25	±15%(homogenous formulations) ±25% (non-homogenous preparations)
25 - 100	±10%
100 - 250	±6%
250 - 500	±5% o
> 500	±2.5%

Storage Stability Tests

Tests to prove that the biocidal product is stable during storage and shelf life.

Accelerated storage test

Stability tests are performed with CIPAC method MT 46.3 accelerated stability results are used to indicate that the biocidal product will remain stable over a two-year shelf life. However, the biocidal product must also be tested under ambient temperature conditions.

Accelerated stability data are also indicative of the stability of the biocidal product when exposed to a higher temperature than optimum temperatures. If the biocidal product should not be stored at high temperatures, the label should contain a warning statement.

If the active substance is sensitive to high temperature, the accelerated stability test can be carried out at lower temperatures for longer periods[21].

Long term storage test

Long term stability test is carried out at 25 °C for 2 years to determine whether the product will remain stable in the commercial packaging during the shelf life. During this time the condition of the product is supported by chemical analysis data.

Low temperature stability test (liquids)

The relevant test method for low temperature stability is the CIPAC method MT 39.3. The product is stored for seven days at 0 °C, and its stability is examined.Some types of formulations may need to be investigated for stability to freeze/thaw [22].

Efficacy Tests

To determine the claimed efficacy of the biocidal product or active substance, its biological/microbiological activity needs to be evaluated using appropriate methods.

Efficacy is described as the ability of a biocidal product to perform the claimed activity when used according to the instructions recommended on the product label. Studies should be conducted using appropriate methods to demonstrate that the product is sufficiently effective against organisms under conditions of use (concentration, duration, application method, etc.).

There are different types of studies to determine the efficacy of the product [23]:

Screening tests: Screening tests are generally not related to practical/field conditions and are only performed with the active substance. Therefore, such tests are used during product development.

Laboratory studies: These are the studies performed according to the standard criteria for determining the efficiency in the laboratory.

Simulation tests in the laboratory: Simulation tests are more appropriate to demonstrate effectiveness. It simulates the field conditions where the product will be used under laboratory conditions. For example, for a product intended for disinfection of hard surfaces, a suspension test and surface test with the relevant EN standards is sufficient.

Field tests: Field tests are a good indicator to see how the efficacy of the biocidal product is affected by field conditions. The results of biocidal treated samples or areas are compared with those of untreated control samples or areas.

Efficacy tests should be carried out in according to CEN, ISO, OECD, ASTM standard protocols. If standard method are not available, validated in-house methods can be used.For the disinfectant group, the product must have at least “cidal” effect on the target organisms of the relevant standard when tested according to the phase 1 and phase 2 step 1 suspension methods of the EN standards. An appropriate method should be selected considering the area where the disinfectant is used [24-27].

For preservatives group, biocidal activity is mostly a static activity showed on challenge tests on some target organisms, in the relatedproduct matrix. When a curative effect is claimed, it is sufficient to show that the microbial reduction in the treated samples is significantly greater than that of the untreated control samples.

For pest control products, only biological activity can be demonstrated for a target organism (eg, control of mice or mosquito control).

Testing for Skin Irritation

Disinfectants used for human hygiene and insecticides that contact with the skin should be tested for irritation. *In vitro* and *in vivo* assays are used to study the skin irritation potential of a biocidal product [28].

The EC method B.46 and OECD Test Guideline 439, known as Reconstructed Human Epidermis Model Test, are the methods used for *in vitro* skin irritation [29-30].

Animal testing should be used as a last resort to determine the irritant potential of the products. *In vivo* tests can be used if there are certain limitations to conducting the in vitro test to study the irritation potential of the biocidal product. EC method B.4 Acute Toxicity: Dermal Irritation/Corrosion, OECD Test Guideline 404: Acute Dermal Irritation/Corrosion test methods may be used[31-32].

CONLUSION

As a conclusion, the use of chemical biocides is a fundamental protection in the prevention and control of microbial growth in medical, veterinary, domestic and industrial environments [2]. Biocides are used a great extent in the healthcare environment for the disinfection of equipments, surfaces, water, and for antisepsis of skin and wound. Misuse of biocidal products brings about some problems such as resistance development and toxicity. The spreading usage of products containing low concentrations of commonly used biocides has raised some concerns about the possible development of microbial resistance. Laboratory studies have demonstrated that bacteria can become resistant to a biocide, and that resistant bacteria can develop cross-resistance to other biocides and antibiotics [9]. High concentrations of biocides generally have toxic effects not only for humans but also for the environment. In this context biocidal products need to be enlarged in regulations and control in terms of traceability due to their extensive and increase in use.

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