Application of Dose-Response Model of Infection in The Risk Assessment for Contamination Health-Hazard from Pharmaceutical Dosage Forms

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ABSTRACT

The applications of the dose-response models of infection for various pathogens and objectionable microbes have been developed to assess the probability of the infection for specific microbes. The use of such models has been implemented in water safety for human consumption and food industry. But till now, none of these models have been used in assessing the risk of infections from contaminated pharmaceutical products. Multi-dose medicines with significant water activity are especially products that need careful formulation as they are liable to microbial spoilage and proliferation. This deterioration of the multiple-use dosage forms may impacts their quality, efficacy, stability and safety. While manufacturing conditions of pharmaceutical products are controlled and microbiological cleanliness is monitored through quality control tests, the in-use behavior and attitude influenced by the consumers on the dosage forms are beyond the control of pharmaceutical manufacturing firms. The mishandling of drugs by the healthcare professional or the patient himself may affect the health of the both hospitalized and outdoor users. The new methodology provides quantitative evaluation. This review article investigates and highlights the limitations of the reliance on the preservative efficacy test (PET) alone and extends its usefulness by combined application with in-use contamination simulation study of infection model from pharmaceutical products. This imitation study will provide new prospective for the design and evaluation of the new pharmaceutical dosage forms. This novel simulation approach may assume either single or multiple spots contamination models with different intervals using suitable indicator microbe for the route of administration of the drug.

Keywords: *Dose-Response Model; Multi-Use; Water Activity; PET; Simulation Stu*

INTRODUCTION TO THE RISK ASSESSEMENT

Risk analysis is a process or methodology that is used in the risk management and decision making through risk assessment. The risks associated with the exposures to the health-affecting environmental hazards are usually cannot be directly separated and measured, environmental protection agency (EPA) researchers and other scientists have worked hard for more than two decades in creating a numerous arrays of risk assessment roadmaps, tools, and data to determine environmental health risks. Although there is appreciable degree of uncertainties remaining, this risk assessment methodology has been extensively revised, is vastly applied and well-known by the scientific society and continues to grow and develop as scientific awareness progresses [1].

The framework for evaluating and managing risks presents the risk assessment and risk management models set forth by the National Research Council (NRC) in 1983, demonstrated in Figure 1. The NRC concluded that risk assessment and risk management are two separate entities between which agencies should preserve an obvious conceptual distinction. In 1983, NRC reported that any risk assessment can be segregated into four basic complementary steps viz, hazard identification, dose-response assessment, exposure assessment and risk characterization [2, 3].

The infection hazard associated with water and food pathogens has stimulated the development of special type of the risk analysis. Simultaneously

with the expressive investigation of clinical or epidemiological data or information, designed scientific models has been pushed to give help with building up a dose-response relationship, specifically when extrapolation to low dosages is important. A quantitative measurement (such as dose of chemical substance that kills 50% of the test group i.e. LD50) of undesirable or adverse effects of chemicals at certain defined endpoints [4]. While *in vivo* (experimental animals) models have been used for toxicology, *in vitro* testing has been applied favorably due to the ability to achieve greater output results [5]. On the other hand, toxicity assessment can be performed using *in silico* studies by applying computational tools [6, 7]. Numerical (quantitative) models have been utilized for quite a few years as a part of the field of toxicology. In the field of aquatic and nourishment microbiology, it is presently perceived that scientific models may encourage the dosage reaction appraisal work out, and give valuable data. Meanwhile, the accountability of variability and uncertainty are preserved [8].

In the quantitative microbiological risk assessment (QMRA) system, the dose-response estimation stage is the quantitative criterion for the assessment of hazard, as this period estimates a risk of specific reaction, either infection, morbidity or mortality with respect to a predetermined number of a specific microbe. The core of the dose-response phase is the dose-response modeling, which are mathematical equations that describe the relation between both the response and the dose for specific pathogens. Therefore, for a certain endpoint (response), a particular objectionable micro-organism and route of administration there is a singular dose-response relation and accordingly a dose-response pattern. Dose-response models are necessary as it is not conceivable to implement a direct research (even with animals) to determine the dose level corresponding to an acceptable threshold of minimal hazard [9].

ESSENTIALS OF THE DOSE-RESPONSE MODELS

Dose-response relationships should be fitted to the results obtained from experimental studies where the required risks and the microbial levels that that should be used in protecting public health are much lower than can be assessed directly from the subjects in the study. Accordingly, the extrapolation of the parametrically-fitted curve is essential to determine the dose level in the low-dose area. Several experiments have been made to determine the microbial dose-response profile for micro-organisms in QMRA. It was reported previously that this relationship between both dose and response could be described by one of either type of two “semi-mechanistic” models that fits the process of infection. The first model is exponential, which was applied successfully for infections associated with *Bacillus anthracis*, *Francisella tularensis*, *Legionella pneumophila*, *Listeria monocytogenes*, *Yersinia pestis* and other types of bacteria and other microorganisms [10-14]. This model is based on assuming a constant probability of starting infection by single microbial particle coupled with random occurrence of micro-organisms, the possibility of infection (P) is given as a function of the ingested dose (D) by [15-18]:

$$P = 1 - \exp(-\mathrm{k}\,.\,D) \ldots\ldots\ldots\ldots \mathrm{eq.}\,1$$

When certain level of non-homogeneity in the interaction between micro-organism and the host is expected, then the curve slope will be shallower than that expressed by equation 1. In such instance, if the probability of infection can be described by a beta distribution, then the previous equation can be used to develop the appropriately fitted beta-Poisson model for this situation [19-21]. Examples of such situations of application of this type of

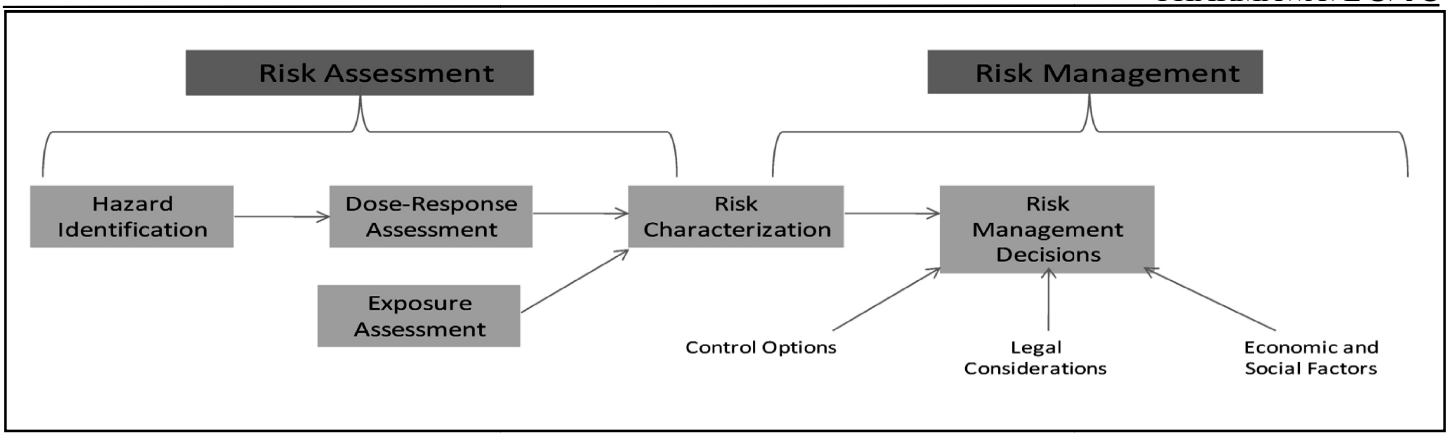

Figure 1. National Academy of Sciences risk assessment/risk management paradigm [1].

model for infections include *Burkholderia pseudomallei*, *Rickettsia rickettsi*, *Salmonella anatum*, *Vibrio cholerae* and as with exponential model its use can be extended to other types of microorganisms such as viruses, perions, protozoans [22-25,15]. This is the second model which is described by two parameters, a median infective dose (N_{50}) and a slope parameter (α):

$$P = 1 - \left[1 + D.\frac{\left(2^{\frac{1}{\alpha}} - 1\right)}{\mathrm{N50}}\right]^{-\alpha} \quad \ldots\ldots\ldots\ldots \mathrm{eq.2}$$

If $\alpha \rightarrow \infty$, then equation 2 approaches equation 1. The two previously demonstrated equations provided general framework for feasible models. Different tentative models are also applicable, three of them have been used (mainly in chemical risk assessment), are the log-logistic, the Weibull, and the log-probit [15]. However, the dilemma is that several models may fit available data in a statistically acceptable sense, and yet provide very different estimates for the risk at an extrapolated low dose. This is a very common situation that has been found in chemical risk assessment [26]. To solve this problem for QMRA, The most appropriate dose-response function can be obtained by validation with outbreak data. Standard maximum likelihood techniques are used to determine the optimum parameters that fit a dose-response functions by using series of observations about the doses of micro-organisms versus responses e.g. infections for the exposed populations. This technique has been demonstrated for human rotavirus [2728] and protozoa [29]. Dose-response models of infections have been deduced from studies on healthy individuals and hence they may not represent the actual responses that could be obtained from the normal populations [15].

PHARMACEUTICAL PRODUCTS CONTAMINATION AND HUMAN HEALTH HAZARD

Microbial contamination is a risk to product quality and safety [30]. Pharmaceutical products contamination can be divided into types based on the origin of the microbial intrusion into the product as follows.

MANUFACTURING SITE CONTAMINATION SOURCE

Microbiological contamination may be one of the sources of the "cost of poor quality" of product if inefficient control was encountered. The effect may be tremendous which may stop the production pull chain with possible shut down for significant time in order to resolve the root cause, conduct corrective action and preventive action (CAPA) to avoid the relapse in the future. Awareness about the possible route of contamination intrusion, the associated hazards and the required control

measures to protect the process and the product is crucial. Figure 2 shows possible routes of microbial contamination. Appropriate control on microbiological contamination begins with the design of the facility, machine, sterilization and cleaning validation programs, thorough preventive maintenance (PM) plans, regular and robust monitoring for bioburden in the process with clearly defined alert and action limits and strict control over the process to minimize the bioburden. For example, regulatory references mandate the monitoring of whole production locations to maintain control of both viable and non-viable particles. Moreover, if aseptic procedures are executed, monitoring should be at high frequency, using a different tools and techniques such as passive air sampling, active air sampling, and surface sampling (for working area and operators monitoring), especially before and after critical activities [31].

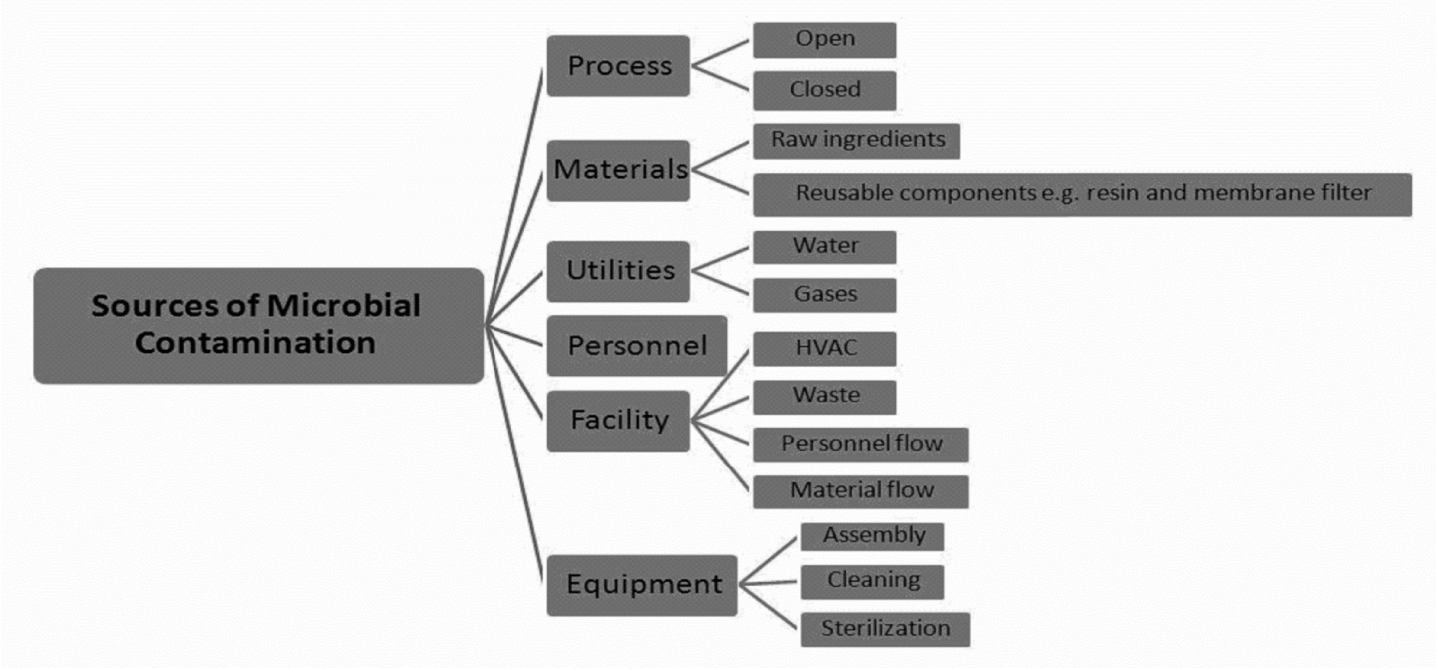

Figure 2. Sources of contamination for medicinal products in pharmaceutical manufacturing facility [32].

In addition, environmental monitoring (EM) program should reflect the risk of bioburden to product with both warning and action levels are well established [32]. Another important consideration to control bioburden level is related to process equipment. This includes efficient and validated cleaning program, validated sanitization (for non-pressure vessels) or validated steam-in-place (SIP) (for pressure vessels) [33, 34]. All of these procedures must be ensured to minimize potential microbial load. This should be complemented by careful and appropriate equipment design [35]. Water quality monitoring is another concern of the regulatory authorities as it harbor different types of microorganisms (especially Gram-negative bacteria) and well-established limits are required accordingly [36-38]. A well designed plan for handling contamination problem will be very important for the current good manufacturing practice (cGMP) and helpful in decreasing the stoppage duration period of the production train. In case of any excursions,

thorough and inclusive investigations should be conducted including careful estimation of all possible roots of microbial entry. If the investigation did not lead to any specific assignable cause, then all possible sources of out-of-control must by identified and addressed appropriately incorporated into corrective actions [30].

IN-USE CONTAMINATION SOURCE

Contamination of multi-dose pharmaceutical products can occur either for hospitalized or outdoor patients. Sources of contamination vary from environmental to human-born microbes. In addition to contamination arising from raw materials and during manufacture, products may become contaminated during storage and use. Types and levels of in-use contamination are almost impossible to predict and only limited published data are available. For instance, *Erwinia, Enterobacter* and *Pseudomonas* genera were found contaminating intravenous liquid products when spray-cooled using tap water after sterilization and in another similar case infusion reservoir was contaminated with *P. thomasii* [39, 40]. In the same line, different studies showed that minute amount of water may get trapped above the rubber and in the screw threads of the neck of the bottles [41]. Robertson (1970) notarized intrusion of glucose saline infusion with *Trichoderma* and *Penicillium* spp. due to cracks in infusion bottles [42]. Comparable situations were documented by Sack (1970) and Daisy et al. (1979) [43, 44]. Contamination of ophthalmic products by in-use application with bacteria and fungi in addition to non-sterile products such as topical and oral products were found to be contaminated with Gram-positive and Gram-negative bacteria [45-49]. However, extensive surveys have been made about sterile and non-sterile products contamination. In assessment of data from studies of in-use microbiological contamination, two important factors must be considered. The degree of contamination in the consumed medicines reflects not only the microbial load introduced by the consumer but also the permanence properties of the contaminant in the dosage forms [31].

The potential risk of medicinal product spoilage may be intensified when both sources of microbial contamination are involved simultaneously and the bioburden load may overcome, exhaust and/or deplete the preservation system of multi-dose drugs.

PRESERVATION OF MULTI-DOSE MEDICINAL PRODUCTS

Additives that are added as components of formulae for pharmaceutical products serve varieties of functions for the medicines. These functions include but are not limited to: 1- Stability/absorption enhancers. 2- Manufacturing, consumption and administration facilitators. 3- Product differentiation facilitators. 4- Compliance improvement components. 5- Aesthetic appearance enhancers. All of these pharmaceutical ingredients share a common characteristic of biological inertness [50]. Compounds that are used as preservatives can be considered as deviation from that rule as they are included in the pharmaceutical formulae to enhance the antimicrobial properties of the product. The use of preservatives is a must for multi-use products either liquid or semisolid (medicinal products with relatively high water activity) and pharmacopeias have specified in their monographs specific and defined tests for performance and efficiency assessments [51, 52]. Meeting these compendial requirements is a hard duty that cannot be accomplished easily.

Pharmacopoeial antimicrobial effectiveness tests (AET) or preservative efficacy tests (PET) involve challenging a product with a defined number of colony forming units (CFU) of a variety of test microorganisms (bacteria, yeasts and fungi),

enumeration at time zero and then monitoring kill / survival rate at defined time intervals up to 28-days [53-55]. Test organisms that are recommended by all pharmacopoeias include: Gram-positive coccus (*Staphylococcus aureus*), Gram-negative rod (*Pseudomonas aeruginosa*), fungi/mold (*Aspergillus niger*), yeast (*Candida albicans*) [56].

In addition, USP [53] and Ph. Eur. [54] recommend the use of *Escherichia coli.* The list may be supplemented by additional organisms that may be associated with a particular process, facility or material, e.g. *Burkholderia cepaceia* an opportunistic pathogen often isolated in manufacturing environments [57], *Bacillis subtilis* a spore-forming bacteria, etc [56].

Acceptance criteria for USP [53] and JP [55] are broadly similar with some differences between product type and presentation. All require satisfactory reduction for each challenge organism with no subsequent increase from the initial count after 14- and 28-days. However, it is widely recognized that the criteria of the Ph. Eur. [54] are the more stringent and challenging to meet. The Ph. Eur. requires a specified reduction in bacterial count within the first 14-days with no subsequent increase from the initial count after 14- and 28-days [56].

SIMULATION OF DOSAGE FORM CONTAMINATION

The novel approach in the assessment of the microbiological safety of the multi-dose medicinal product is principally based on the assumption that contamination was introduced within the pharmaceutical primary packaging to the internal contents either accidentally once or several times successively due to mishandling of the drug during use and application. The study combines the simulated contamination model, either single spot or multiple spots with data of PET and the probability of infection for the customer from the consumed product [57-61]. Hence, the contamination imitation analysis will take into account other factors of the entire product and not only the preservation ability of the formula. Thus, the reliance on only one measure such as AET may be not quite sensitive for the preferential selection of specific formulae to be transferred from the test phase to the production phase [62].

The new method provides a mean for the risk assessment and the determination of the microbiological safety of the pharmaceutical products especially when new formulae being designed or comparative studies being conducted between equivalent product forms. While the range of the approved preservatives by regulatory agencies is limited for multi-dose products either topical or oral and becomes more restricted for parenteral drugs, yet the criteria for the assessment of the true preservation power need review to include other influential factors. Performance criteria and assessment techniques, based on type of pharmaceutical product, dosage regime, the history of environmental exposure in the manufacturing site and experience during in-use activity of the consumer might be more suitable than applying a solitary compendial test literally as defined in pharmacopoeias that represent over-killing capabilities in a microbiological and common sense context for many products. At the same time, attempts of designing products that are free from preservatives are still in their initial phases and they are lagging behind those medicinal products that are preserved chemically. However, promising results were obtained with several preservative-free intranasal and ophthalmic devices [56].

CONCLUSION

Dose-response models of infections may found a useful application in the evaluation of the microbiological safety of multi-dose products such as ophthalmic products, parentral medicines, oral

liquid dosage forms and topical pharmaceuticals and they provide a scientific means for selection of most appropriate formulae during the design and test phases. They provides new horizon for decision making based on health risks assessments obtained from quantitative data. They take into consideration the physical integrity of the product, dosage form size, dose size, dosage frequency, route of administration, contamination density and PET.

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