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微宇宙理论及其在生态毒理学研究中的应用

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摘要:简述微宇宙理论、发展史和主要类型,并着重综述了微宇宙在生态毒理学研究中的主要应用情况,以及与实验室单物种试验和野外试验比较微宇宙的优缺点,并对微宇宙的未来发展趋势也作了简要讨论。

关键词:微宇宙理论;微宇宙;模型生态系统;生态毒理学

The theory of microcosm and its application in ecotoxicology

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Abstract: Microcosms, generally including mesocosms, are small experimental ecosystems or model ecosystems. In recent years, microcosms have been widely used in ecological field. "Biosphere 2", a huge microcosm even including human within, was constructed in 1986 in USA for study of the Earth ecosystem. Microcosms have played an important role in protecting the Earth eco-environment. Microcosms are also useful methods in ecotoxicological research because they provide a possibility to study the effects of pollutants on the ecosystems under artificially controlled conditions. This paper emphasized the application of microcosms in ecotoxicology.

The concept of microcosm originated from philosophic field in ancient Greeks. It means that portions varying in size of the world exhibit similarities in structures and functions, and one portion imitates another or others at different levels. Such analogy extrapolation was refereed in philosophy as microcosm theory. Although in the middle of nineteenth century Warrington published the first scientific paper on microcosms, until the first of century 20 microcosmic thought did not shifted really from philosophy to natural sciences, especially ecological science. In 1970's microcosms drew attention in ecotoxicology as its development. Metcalf was the first to use microcosms in the studies of the fate of insecticides and some toxic substances in ecosystems. In 1980, Giesy and Odum introduced the microcosmology. Recently, the Chinese scientists have widely applied microcosms into ecotoxicological research.

Microcosms are generally divided into three kinds, namely terrestrial, wetland and aquatic microcosms. Relatively satisfactory terrestrial microcosms were 'farm pond' used by Metcalf to test the toxicity of pesticides, food additives and industrial chemicals. However, since terrestrial microcosms have limited test animals and longer turnover period, it is difficult to get statistic data of toxicity, such as LD_{50} value. Wetland microcosms are suitable for studies of pesticide residues in wetland systems and treatment of pollutants by wetland systems. Aquatic microcosms have been studied popularly much and developed more perfectly. Various types of microcosms simulating river, lake, estuary and marine ecosystems have been developed. Aquaria are the first of microcosms, and they can be simply constructed and controlled. Probably millions of these microcosms have been set up around the world, however few were seriously studied. Stream microcosms include channel and circulating microcosms. In particular, circulating

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microcosms have compact structures and they occupied small area, they are useful for the environmental hazard assessment of chemicals though they have poor reality. Pond and pool microcosms have been widely used to investigate the processes of silf-organization including eutrophication and ecotoxicological effects of pollutants. Thy may be useful facilities for the tests of the environmental risk assessment at middle or higher levels. Enclosed column microcosms include enclosures in lakes and oceans. They are realistic, but cost expensive and they are difficult to operate for a long time. Land-based marine microcosms simulate marine or estuary ecosystems. They can operate for a longer time than enclosures in oceans (e.g. pelagic marine microcosms), but they need a longer turnover time to reach a stable state. Reef and benthic microcosms are significant for protecting biodiversity.

Ecological microcosms provide an important tool to research on ecological effects of toxic pollutants and the response of ecosystems to pollutants at ecosystem levels. They have been used to trace the distribution in space and time, the transfers and the transformations of pollutants labeled with radioactive tracers and their metabolites. Institute of Zoology, Chinese Academy of Sciences (CAS) set up pond microcosms in their laboratory and pond mesocosms for research of the effects of pesticides on the structures and functions of ecosystems. They drew the conclusions as following: All these microcosms showed ecological effects of chemicals at ecosystem levels. Pond microcosms had simple structure and small volume, and their conditions were easily controlled, they were suitable for careful studies on mechanism in laboratory. Pond mesocosms were larger in scales and they had complex structures and stable functions. They were similar to the natural practice and suitable to test environmental behavior of chemicals and to analyze the trends of ecological effects. Institute of Botany, CAS constructed wetland mesocosms to study the effects of insecticides on the waters through surface runoff. They used terrestrial mesocosms to study the extent of effects of the pesticides on the structures and functions of the terrestrial ecosystems after the pesticides entered into the ecosystems. They have learnt the residual characteristics of the pesticides in soil-plant systems, and estimated their harmfulness, also posed the measurements for using pesticides properly. Microcosms have been used to study the variations of primary productivity of plant, behavior and interaction between species, the effects of pollutants on biodiversity, the relationship between doses and effects, response and recovery, as well as pre-assessment of ecological impacts of chemicals on the ecosystems etc. Since microcosms provide the information of exposure as well as fate of toxic pollutants, they have been used in ecological risk assessment. Microcosms can be used also in study of the remediation engineering of ecosystems and design of parameters.

Microcosms have many advantages, such as reality, replicability, flexibility and safety. However, the disadvantages, for example, not operating for a longer time and no clear response end, should be studied carefully.

Microcosms have brilliant perspectives. They are developing forward to standardization, automatization of control and measurement. Microcosms may become screening tools for ecotoxicological effects of chemicals, and they could compose a barrier for testing toxicity at different end levels together with mono-species assays and field tests.

Key words: microcosm theory; microcosm (mesocosms); model ecosystems; ecotoxicology

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微宇宙(Microcosm),**一般也包括中宇宙**(Mesocosm),**是指应用小生态系统或实验室模拟生态系统进行试验的技术。近年来,该技术在生态学研究领域得到越来越广泛的应用。应用微宇宙技术不仅可以研究一般生态系统,而且便于对那些人们难于到达的沙漠、远洋和火山口等特殊生态系统进行研究。人们甚至**

设计了包括人类在内的微宇宙系统,例如,1992 年美国亚利桑那州的"第二生物圈(Biosphere 2)",用来模拟研究地球生态系统,对于保护全球生态环境做出了重大贡献。由此可见,微宇宙的设计、研究和应用具有重要意义。本文仅重点综述微宇宙在生态毒理学研究中的主要应用情况。

生态毒理学是一门新兴的学科,其研究和实验方法除沿用药物学和毒理学的方法外,还发展了独特的单一物种毒性试验、多物种模拟试验和野外试验等方法。其中微宇宙技术则被认为是生态毒理学研究中很有价值的试验方法,因为它提供了在人工控制条件下模拟研究生态系统对污染物反应的可能性[1]。

1　关于微宇宙理论

微宇宙概念起源于古希腊哲学界[2],其中心思想是:自然界一切单元,不论其大小,在结构和功能上均具有相似性,一种水平上的结构和功能与另一水平上的相似。这种相似推理的法则在哲学上叫做"微宇宙理论"。

19 世纪中叶,Warrington[3]发表了第一篇有关微宇宙的自然科学论文。他在 12 加仑的水族箱中放入两尾金鱼和一些苦草,又引入 5～6 只螺蛳,建立了与天然大鱼塘相似的简单的生态平衡系统,即微宇宙。他指出,根据微宇宙理论,即相似推理的原理,微宇宙试验结果可以推广到野外真实世界。

20 世纪初,微宇宙的思想逐渐从哲学界过渡到自然科学领域,特别是生态学领域。例如,Woodruff[4]应用微宇宙成功地进行了经典的生物演替试验。到 20 世纪 40～50 年代,许多生态学家对于应用微宇宙技术研究生态系统中群落代谢及有关现象的独到之处得到共识。例如,Odum 等[5]建立的一套实验程序,证明微宇宙的确是一个多生物的微小世界,它在许多方面与其代表的真实世界非常相似。

20 世纪 70 年代,随着生态毒理学的发展,微宇宙在生态毒理学领域受到重视。Metcalf[6]率先用微宇宙研究了农药在生态系统中的归宿。此后,有关微宇宙应用的学术讨论多有报道。于是,微宇宙也在数量、类型和研究项目等方面进入迅速发展阶段,并于 1980 年,由 Giesy 等[7]将微宇宙理论命名为微宇宙学(Microcosmology)。近年来,我国学者[8～10]也对微宇宙理论进行研究和探讨,并应用微宇宙技术研究生态毒理学问题.

2　微宇宙的主要类型

微宇宙总体可分为三大类,即陆生、水生和水陆生(湿地)微宇宙。

2.1　陆生微宇宙(Terrestrial microcosm)

陆生微宇宙通常是在容器内装入土壤,移植(殖)陆生植物和动物。系统中含有空气,能与外界交换,O_2 和 CO_2 水平保持与外界相通。比较完善的陆生微宇宙是 Metcalf[11]的"farm pond",用于农药、食品添加剂和工业化学品的毒性试验。DeCatanzaro[12]建立了 30 个林地微宇宙,模拟镍冶炼厂污水排放对林地生态系统的影响。近几年中国科学院植物所设置了包含 6 个 $2\times2\times1.1m^3$ 培植池陆生中宇宙,研究农药对陆生生态系统各组分的影响[10]。陆生微宇宙不乏是研究有毒污染物环境归宿和效应的好方法。但是,总的来说,很严密的典型的陆生微宇宙研究较少。由于陆生微宇宙的试验生物数量有限和生物对污染物暴露历史的差异性,较难获得有效的具有统计意义的毒性数据,如,半致死计量 LC_{50} 值。陆生微宇宙平衡稳定期较长,在几年的研究期间,很难得出具有代表性的生态系统变化过程。应用陆生微宇宙还需仔细了解边界条件对系统的影响。

2.2　湿地微宇宙(Wetland microcosm)

湿地微宇宙即人工沼泽系统。早先主要用于研究农药在农业湿地环境中的持久性[13～15],近年来利用湿地微宇宙研究污染物的迁移和转化[16,17],并研究湿地系统用于处理污染物的可能性与条件。同时,对于了解地表径流对水域生态系统的影响也有重要意义。

2.3　水生微宇宙(Aquatic microcosm)

水生微宇宙包括模拟河流、湖泊、河口及海洋生态系统的各种类型[18]。

2.3.1　水族箱系统(Aquaria)　水族箱系统是模拟水生生态系统的最早应用的微宇宙。Warrington(1857)[3]最早建立的水族箱系统,曾用于放射性物质、农药等毒物的降解性研究[14]和大气中重金属粒子进入水体表面微层的规律性研究[19]。水族箱系统的设计和操作简单,在全世界的数量多得惊人。然而,也许正

因为它过于简单,真实性差,严格进行研究的并不多。

2.3.2 溪流微宇宙(Stream microcosm) 溪流微宇宙是用来模拟河流生态系统的微宇宙。主要有水渠系统[20,21]和循环水系统[10]。循环水系统,结构紧凑,占地面积小,可作为化学品危险性评价的有用试验设施,能满足建立有毒物质控制法规的要求[22]。现在,美国、德国、加拿大、日本等国家广泛采用这类微宇宙研究化学污染物慢性暴露的行为及其生态学效应。循环式溪流微宇宙比较经济,但真实性较差。

2.3.3 池塘与水池式微宇宙(Pond and pool microcosms) 池塘与水池式微宇宙的特点是其直径远远大于深度,多用于模拟湖泊、水库和河口等生态系统,以了解它们的自组织过程、系统特征、富营养化过程以及污染物的生态效应等等。这类微宇宙结构简单,规模小,条件易于控制,因此而得到较多的研究和较广泛的应用。中国科学院动物所曾用含多种水生生物种子、孢子、卵子的干河泥和自来水组成池塘微宇宙来研究单甲脒农药的生态效应[10]。普遍认为这类微宇宙可用作化学品危险性评价中的中间或较高层次的试验与研究的模拟系统[23]。

2.3.4 围隔水柱微宇宙(Enclosed column microcosm) 在海洋中的围隔水柱即所谓“大袋子”,也称远洋微宇宙(Pelagic marine microcosm),一般不含沉积物,水柱体积与上表面积的比例不少于 $2\sim4m^3/m^2$。最有名的大袋子是加拿大的CEPEX[24]和苏格兰的Loch Ewe[25]。二者的结构很相似,但规模大小不同,CEPEX的容积为68 000L,而Loch Ewe达100 000～300 000L,均获得满意的试验结果。这类微宇宙真实性强,但耗费大,难于维持很长时间。

在湖泊中的围隔水柱微宇宙,最简单的例子是测定浮游植物光合生产力的“黑白瓶”,也有较大的围隔水柱,用于研究湖泊“水华”控制等问题。这种围隔系统也同样具有真实性强而耗费大的利弊。

2.3.5 陆基海洋微宇宙(Land-Based marine microcosm) 陆基海洋微宇宙是指在陆地上构建的模拟海洋生态系统的微宇宙,它较“大袋子”系统费用低,条件相对容易控制,运行时间长,当然真实性差一些。最有名的是美国罗德岛海洋实验室的MERL微宇宙[26],由14个圆柱体水槽组成,水槽中水深5m,底部沉积物厚0.37m,总体积 $13.1m^3$。该实验室对MERL微宇宙进行过多年的研究,也用这类微宇宙作过多种模拟试验。他们的结论是微宇宙的体积越大,达到稳态所需要的时间越长,正如上述MERL微宇宙运行3a才达稳态。但是,一旦大系统形成稳态,它可以向别的微宇宙转接而使其在较短的时间内达到相同的稳态条件。

2.3.6 珊瑚礁和底栖生物微宇宙(Reef and benthic microcosms)[27～29] 海洋珊瑚礁和湖泊、河口沉积物是多种水生生物栖息的地方,构建珊瑚礁和底栖生物微宇宙对于研究生物多样性、保护生物多样性具有重要意义。

3 微宇宙在生态毒理学研究中的应用

微宇宙提供了在生态系统水平上研究毒物对生态系统影响和生态系统对毒物适应能力的有用工具。

3.1 用于化学物质在环境中的迁移与归宿的研究

微宇宙中化学物质的迁移、转化是一重要特征,因此,利用放射性标记物可跟踪污染物的时空分布、迁移转化过程及其转化产物。这方面较典型的例子是Metcalf[6]的微宇宙研究。该系统经反复构建和修正,试验了约200种化学物质的归宿及生态效应。Metcalf及其同事还把辛醇-水分配系数 Kow 与全生物体的生物积累联系起来,可以根据 Kow 的对数值 $\mathrm{Log}P$ 安全地预测生物浓缩效应 EM 值。生物降解是化学物质归宿的一个重要方面,而该系统对于测量生物降解指数 BI 和生态放大指数 EI 非常有用。Fuhr[16]总结了有机污染物在湿地微宇宙中变为生物无效性的归宿,他指出,生态系统以紧密结合的机制部分地中和毒性,这种机制是生态系统对人类经济活动产生的毒物的适应过程中形成的,从而起到保护生物圈的作用。近年来,关于化学物质在环境中的迁移与归宿的研究,微宇宙得到普遍应用[30～32]。

3.2 用于毒物的生态毒理学效应研究

中国科学院动物所和植物所曾成功地运用实验室循环水生微宇宙、池塘微(中)宇宙、水陆生中宇宙和陆生中宇宙研究了农药及其它污染物对生态系统结构与功能的影响[10]。中宇宙(Mesocosm)是一种规模较大的室外模型生态系统,其性质与微宇宙相同,人们习惯上把它们统称为微宇宙。他们的结论是:含有多种水生生物种子、孢子和卵子的干河泥和自来水组成的池塘微(中)宇宙和水陆生中宇宙,对外来化学污染物

质均具有生态系统水平上的效应,且具有各自的代表性。池塘微宇宙体积小,结构简单,条件易于控制,适用于室内作较细致的机理研究;池塘中宇宙规模较大,结构复杂,功能稳定,较接近自然实际,适用于化学物质的环境行为和生态效应趋势分析;水陆生中宇宙适用于研究农药等化学污染物质通过地表径流对水域生态系统的影响;陆生中宇宙可用于研究农药对陆生生态系统结构与功能的影响,且研究结果与现场农地生态系统的研究结果一致,可较全面地评价农药进入陆生生态系统后对其结构和功能的影响程度,摸清在作物-土壤系统的残留特点,进行农药危害性的预测,提出合理使用农药的措施。总之,微宇宙系统的组建与管理技术简便,费用较低,是生态系统水平上研究化学污染物质生态效应评价及预测的良好工具,可根据不同目的和条件选择使用。

微(中)宇宙还用于研究生物遗传型的改变[33],植物生长、初级生产力和产量的变化[34],生物行为和种间相互作用的变化[35,36],对生物多样性的影响[37,38],剂量-效应关系[39],反应与恢复[20],化学品的预评价[36]等等诸多生态毒理学问题。

3.3 生物对毒物的反应

微宇宙不仅可用于试验一般性生物对于毒物的反应,而且有助于发现生物对毒物的特异性反应。Sander and Cibik[40]研究低水平镉对河口优势硅藻种群的影响,发现 5μg/L 的镉对种群组成、生长速率、种群密度及 C/N 比率几乎没有影响,但优势硅藻的孢子数量明显减少,使孢子减少的镉浓度大约比硅藻生长受抑制的量低一个数量级。由于孢子形成和萌发是硅藻维持优势的机制所在,孢子持续减少必然会导致群落组成和优势种的改变。Phelps 等[41]研究了微宇宙中海洋沉积物间隙水中铜对蛤子行为的影响。他们发现,蛤子掘洞速度与蛤所在沉积物间隙水中铜的浓度直接相关。掘洞是蛤逃避被捕食的主要功能,如果掘洞速度减慢,其整个种群的生存就会受到威胁。

3.4 生态风险评价

由于微宇宙系统比单一生物实验提供更完整的信息,可同时提供暴露和归宿的信息;它不仅能提供母化合物的、而且能提供降解产物的信息。所以,近年来在农药及其它毒物的生态风险评价中,微宇宙系统的应用越来越普遍[42~45]。确定一种试验方法是否适用,其敏感性是一重要标准,敏感的试验方法得出的 NOEC 值更有助于保护物种和群落。为发展可用于 2 级风险评价的水生毒性试验方法,欧洲共同实验室研究计划对单一生物种实验室方法和微(中)宇宙试验作了比较,发现用单一生物种实验室方法测得的二氯苯胺的慢性 NOEC 值比微(中)宇宙试验高约 200 倍[46]。这说明微宇宙试验方法对特定化合物可能更敏感。

3.5 用于修复生态工程的研究与参数设计

近年来,微宇宙技术还广泛用于原位修复生态工程或治理工程的研究与参数设计[47,48]。Huddleston 等[47]精心构建了 2 个湿地微宇宙,对炼油厂废水进行净化处理试验。结果表明,48 h 内,炼油厂废水平均 BOD5 去除 80%以上,NH_3-N 去除 90%,同时,对湿地特征参数设计提供了有用的参考。

4 微宇宙与其它试验方法的比较

4.1 微宇宙的优点

微宇宙与实验室单一物种试验、野外试验及数学模型相比较,具有许多独特的优点。

真实性。微宇宙具有与自然生态系统非常相似的生物学组成、营养循环及多种物理参数,可在控制条件下对功能完全的生态系统进行研究,可观测到多种过程和组分的相互作用,它比单一物种的实验室研究提供更完整的信息[42]。微宇宙不仅可以了解污染物对生态系统影响的全面结果,而且可以合理地将结果外推到自然生态系统,而单一生物的毒性试验结果很难直接外推到复杂的自然生态系统。例如,2-甲基萘的降解速率,在试验瓶中试验的结果比微宇宙测得的结果低的多。因为单一生物的作用过程与生态系统完成的过程不同。污染物质在全功能生态系统中是由不同营养级多种生物经过一系列选择、适应等正常自组织过程之后的共同作用的结果[49]。野外试验直接研究毒物对自然生态系统的效应,往往很难得到明确的结果,而且,从某一地区、某一时期得出的结果很难用于其它地区和其它时间。数学模拟是设法把实验室数据同野外试验数据综合起来进行预测的工具,但是常因数据量太少而受局限,而且很难找到决定一个生态系

统所必须的所有变量,因此很难准确地预测毒物的生态效应。

重复性和再现性。重复性和再现性问题是所有生态毒理试验中的重要问题,无论单一物种试验还是微宇宙试验、还是野外试验。试验表明,微宇宙与天然系统之间以及微宇宙与微宇宙之间存在的差异,均在自然环境偏差限度范围内,一般小于30%[50]。

灵活性。微宇宙的应用相对灵活,可对不同环境条件下的多种类型的生态系统进行探究。例如,对沙漠、海洋等难于研究的自然环境,利用微宇宙进行研究,其结果可以定量地直接外推到自然环境中去。微宇宙还可以灵活地选择一两个营养级、甚至可以仅用一种指示生物进行仔细研究。

成本·效益比高。花费比野外试验低的多的代价获得与野外试验相当的效果[51]。

安全性。使用放射性标记化合物、毒物或未知致病性的工程生物进行试验,微宇宙技术可以避免环境遭受危害。

微宇宙可用来验证和校准实验室试验结果与野外观测结果之间的差异。通过标准化的单物种试验,已经积累了大量有用的数据,这些数据如何与野外观测结果联系起来,它们与野外观测结果究竟有多大差距,通过微宇宙试验可以来验证。因为微宇宙的生态结构和功能介于二者之间,它在二者之间架起了一座桥梁。

总之,微宇宙技术的应用,使我们能够在控制条件下研究全功能的生态系统,能在生态水平上研究污染物的效应,推动了生态毒理学的发展。但是,它仍有许多问题值得大家认真思考和研究。

4.2 微宇宙的缺点

微宇宙虽然可以延续数月或几年,但自身不可能维持太长时间,因此,无法显示重大的生态过程,如生物演替过程,多代生物种的传代过程等等[11]。这只是针对某些微宇宙而言,如某些陆生微宇宙。大多数发展较完善的水生微宇宙可以显示生物演替过程和传代过程。

微宇宙虽不是完全的天然系统,但也不是完全可控制的,因此只能在自然差异限度内重复,却不能像实验室试验那样严格重复。随着微宇宙向标准化方向的发展,微宇宙的重复性会逐步提高。

人为控制可能影响生物间的相互作用,反过来也影响物理条件,因此,微宇宙中观测的结果往往不易解释。这就要求试验人员有较高的素质,能随时识别人为控制对生物间相互作用的影响。

微宇宙中群落结构比较复杂,对毒物反应的终点不够明确。这正是向我们提出了要求,要求人们对结构复杂的微宇宙进行更加深入的研究,试验并识别系统中针对不同毒物的最敏感的反应终点,包括生物学的、生物化学的和分子生物学的。

目前微宇宙虽然有许多不足之处,但是,随着微宇宙的不断发展和应用,定会变得更加完善。

5 微宇宙的未来发展趋势

随着生态毒理学的发展,微宇宙理论及微宇宙技术在生态毒理学中也将得到更加充分的应用和发展。

向标准化发展。微宇宙的标准化是指应用相同的生物组合、生物培养介质和试验条件。世界上许多污水处理厂都具有相同的生物组成,它们都是世界范围的标准微宇宙。因此,通过择生生物(Gnotobiotic)的培养,或者按一套标准化的条件引入多种生物,通过自组织而产生标准化的混合种,从而实现实验室内可重复的、实验室间可再现的标准微宇宙试验结果。Taub等[52]对水生微宇宙组分和方法作了规定,包括培养基质,生物组成,数据处理与统计方法等,经标准化的水生微宇宙试验结果较为一致,重现性也较好。

向高度自动化控制发展。随着计算机技术和仪器技术的发展,以及PCR和生物技术的广泛应用,微宇宙技术也会向自动化控制和终点的自动化测量发展。在这方面,自动控制的植物吸收室就是很好的一例[53].

在应用上微宇宙系统将作为化学品生态毒理效应的筛选工具[23],可与单物种毒性试验、野外试验相结合,组成不同终点水平上的毒性试验栅。而且,试验表明微宇宙试验用于测定某些化合物的生态毒性比单物种毒性试验更敏感[46]。

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