miss biological eccentricities. Egg-shell thinning by DDE in birds, deformities in molluscs exposed to tributyltin, and inhibition of the mating pheromones of newts by endocrine disruptors are some of the sorts of responses that would be missed by all but the most sophisticated imaginable toxicodynamic models. This danger could become an impediment to the development of integrated risk assessment, if it is perceived that integrated assessment misses important effects on non-mammalian species. However, the current regulatory test sets also miss many effects. The development of integrated mechanistic risk assessment will make it even more apparent that eco-epidemiological monitoring is needed, along with epidemiological monitoring of public health, to reveal unanticipated effects. Integration of available knowledge in various assessments may give deeper insight and help understanding real situations as shown in examples in the section on "Benefits of Integration" for complex exposure situations.

Another danger is that integrated risk assessment will become too focused on risks to vertebrate organisms and neglect other ecological endpoints. This is already occurring in the sense that toxicity testing is disproportionately performed with vertebrates. This is problematical from the ecological point of view given the much great importance of invertebrates, plants, and microbes to the functioning of the biosphere. If risk assessment becomes fully integrated, there could be an even greater temptation to neglect mechanisms that are not shared with humans and the species possessing those mechanisms.

RECOMMENDATIONS FOR CONTINUED DEVELOPMENT AND ACCEPTANCE OF IRA

To summarize, the strengths of IRA are:

- Risk-based decision-making will be informed of all risks that are potentially significant,
- IRA may predict and diagnose previously unexpected risks,
- Assessment efficiency will increase with regard to data collection, methodology and decision-making,
- Cost effectiveness will increase in view of shared resources,
- Assessment results will be more coherent in view of shared methodology and characterization of exposure, hazards and risks, and
- Assessment uncertainty will decrease by confirmation of mechanisms of action and increased knowledge on toxicokinetics and toxicodynamics.

However, a number of serious weaknesses can also be identified, not so much in the approach *per se*, but rather in the demonstration of its benefits and in organizational backing:

- Although several cases have been studied to demonstrate the benefits of IRA, none of them have demonstrated convincingly that this approach will be efficient and cost effective.
- These case studies also revealed that an increased quality of the assessment seems likely, but hard to prove.

- Although many regulations call for protection of both human health and the environment, scientifically and institutionally these areas often have developed independently.
- The emphasis on direct effects on human health reduces the opportunities for integration.
- The knowledge of shared mechanisms, testing methods and integrated testing strategies still has to evolve to really appreciate the benefits of IRA.

It is clear that further demonstrations of the scientific, economic and regulatory benefits of the IRA approach are needed. Our analysis of opportunities for the promotion of IRA shows that, apart from scientific reasons, societal and political pressures require increased efficiency in risk assessment as well as moving away from vertebrate testing. This necessitates integration of *in silico, in vitro,* and *in vivo* methods across species. As risk assessment is becoming more mechanistic and molecular there may be new opportunities to create an integrated approach based on common mechanisms and a common systems-biological approach. This development will automatically provide the examples asked for, which subsequently have to be analyzed for economic and regulatory benefits.

ACKNOWLEDGMENTS

The contribution of all members of the IPCS Planning Group on Approaches to Integrated Risk Assessment to the project is gratefully acknowledged. The authors also thank the reviewers for their comments. This work was partly carried out with budget provided by The Netherlands' Directorate-General of Environmental Protection (project no. M/601200).

LIST OF ABBREVIATIONS

EC (European Commission), EPA (U.S. Environmental Protection Agency), ERF (Emergency Response Function), EU (European Union), FAO (Food and Agricultural Organization), IPCS (International Programme on Chemical Safety), OECD (Organization for Economic Cooperation and Development), POP (Persistent Organic Pollutant), REACH (Registration, Evaluation, Authorisation and restriction of Chemicals), SIDS (OECD Screening Information Data Set), WHO (World Health Organisation).

REFERENCES

Bontje D, Hermens J, Vermeire T, *et al.* 2005. Integrated risk assessment: nonylphenol case study. WHO/IPCS/IRA/12/04.World Health Organization/International Programme on Chemical Safety, Geneva, Switzerland

Bradbury SP, Feijtel TCJ, and van Leeuwen CJ. 2004. Meeting the scientific needs of ecological risk assessment in a regulatory context. Environ Sci Technol, December 1, 463A–70A

Briggs J. 2003. Human health and environmental risk assessment: the need for a more harmonised approach. Chemosphere 52:1347–51

EC (European Commission). 2003a. Technical Guidance Document on Risk Assessment (TGD) in support of Commission Directive 93/67/EEC, Commission Regulation (EC) No 1488/94 and Directive 98/8/EC. European Chemicals Bureau, Ispra, Italy

EC. 2003b. Communication from the Commission to the Council, the European Parliament and the European Economic and Social Committee—A European Environment and Health Strategy. COM/2003/0338. Commission of the European Communities, Brussels, Belgium

EC. 2004. Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee—"The European Environment and Health Action Plan 2004". COM(2004)416. Commission of the European Communities. Available at http://europa.eu.int/comm/environment/health/index_en.htm, Brussels, Belgium

EC. 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency and amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC and 2000/21/EC. Commission of the European Communities, Brussels, Belgium. Off. J. Eur. Union L 396/1-856.

Euling SY and Sonawane BR. 2005. A Cross-Species Mode of Action Information Assessment: A Case Study of Bisphenol A. EPA/600/R-05/044F. US Environmental Protection Agency, Washington, DC, USA

Hansen L, Hedtke SF, and Munns WR Jr. 2003. Integrated human and ecological risk assessment: a case study of ultraviolet radiation effects on amphibians, coral, humans, and oceanic primary productivity. Human Ecol Risk Assess 9:359–77

Höfer T, Gerner I, Gundert-Remy U, *et al.* 2004. Animal testing and alternative approaches for the human health risk assessment under the proposed new European chemicals regulation. Arch Toxicol 78:549–64

IPCS (International Program on Chemical Safety). 1979. DDT and its Derivatives. Environmental Health Criteria 9. International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

IPCS. 1989. DDT and its Derivatives—Environmental Aspects. Environmental Health Criteria 83. International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

MOE (Ministry of Environment). 2003. Chemicals in the Environment, Report of the Environmental Survey and Wildlife Monitoring of Chemicals in FY2001. Environmental Health Department, Ministry of the Environment, Japan, March 2003

Munns WR Jr, Suter GW II, Damstra T, *et al.* 2003. Integrated risk assessment—Results of an international workshop. Hum Ecol Risk Assess 9:379–86

Orme-Zavaleta J and Munns WR Jr. Integrating human and ecological risk assessment for the cyanobacterial harmful algal bloom problem. Adv Exper Med Biol (in press)

Ross P and Birnbaum L. 2003. Integrated human and ecological risk assessment: a case study of persistent organic pollutants (POPs) risks to humans and wildlife. Hum Ecol Risk Assess 9:303–24

Sekizawa J and Tanabe S. 2005. A Comparison between integrated risk assessment and classical health/environmental assessment: Emerging beneficial properties. Proceedings of the 10th International Congress of Toxicology. Toxicol Appl Pharmacol 207, Issue 2, Suppl. 1:617–22

Sekizawa J, Suter GW II, and Birnbaum L. 2003. Integrated human and ecological risk assessment: a case study of tributyltin and triphenyltin compounds. Hum Ecol Risk Assess 9:325–42

Suter GW II, ed. 2003. Special Issue: Framework for integration of human and ecological risk assessment. Hum Ecol Risk Assess 9(1):267–386

Suter GW II, Vermeire T, Munns WR Jr, *et al.* 2003a. A framework for the integration of health and ecological risk assessment. Hum Ecol Risk Assess 9:281–301

Suter GW II, Munns WR Jr, and Sekizawa J. 2003b. Types of integration in risk assessment and management, and why they are needed. Human Ecol Risk Assess 9:273–79

Suter GW II, Vermeire T, Munns WR Jr, *et al.* 2005. An integrated framework for health and ecological risk assessment. Proceedings of the 10th International Congress of Toxicology. Toxicol Appl Pharmacol, 207, Issue 2, Suppl. 1:611–6

USEPA (US Environmental Protection Agency). 1998. Guidelines for Ecological Risk Assessment EPA/630/R-95/002F. Washington, D.C., USA

USEPA. 2003. Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health. 2000. Technical support document volume 2: Development of National Bioaccumulation Factors EPA-822-R-03-030. Office of Water, Washington, DC, USA

USEPA. 2004. Risk Assessment Principles and Practices. Staff paper. Office of the Science Advisor, Washington, DC, USA

Van der Jagt K, Munn S, Torslov J, *et al.* 2004. Alternative Approaches can Reduce the Use of Test Animals under REACH. Joint Research Centre, European Commission, Ispra, Italy

Vermeire T, MacPhail R, and Waters M. 2003. Integrated human and ecological risk assessment: a case study of organophosphorous pesticides in the environment. Hum Ecol Risk Assess 9:343–357

Waters MD, Selkirk JK, and Olden K. 2003. The impact of new technologies on human population studies. Mutation Res. 544:349–60

WHO (World Health Organization). 2001. Pesticide Residues in Food -2000 Evaluations. Part II—Toxicological Evaluation. WHO/PCS/01.3. Geneva, Switzerland

WHO/IPCS. 2001. Report on Integrated Risk Assessment.World Health Organization/ International Programme on Chemical Safety. WHO/IPCS/IRA/01/12. Geneva, Switzerland

日本リスク研究学会誌 17(1)：79－84(2007)

【シンポジウム】

シンポジウム講演 5

内分泌かく乱化学物質による低用量影響の蓋然性

Biological Plausibility of Low-Dose Effects of Endocrine Disruptors

関澤　純*

Jun SEKIZAWA

先ほど学会総会でごあいさつをさせていただきました徳島大学総合科学部の関澤でございます。私は日本リスク研究学会のほかに日本トキシコロジー学会にも入っており，安全性評価あるいはリスク評価に関心を持って研究していますので，そういう観点からお話しを進めることにします。

このシンポジウムの企画について今日のオーガナイザーの間正さんからご相談を受けたことがありました。本日，私の前に遠山先生がおっしゃったように，ダイオキシンや環境ホルモン問題についてある程度沈静化し，あるいは巻き返しとして騒ぎすぎだったのではないかというご意見もあります。しかし最近になっていろいろ新しいことが見つかりつつあって，私はより深く検討する必要があるだろうということをお話しさせていただきたいと思います。

さてIPCSについてはご存じでない方もおられると思いますが，International Programme on Chemical Safetyの略で，日本語では国際化学物質安全性計画と呼ばれる国連の組織の一つです。私は徳島大学に移る前は国立医薬品食品衛生研究所で，化学物質のリスク評価を担当してこのIPCS関連の仕事を主にやってきました。IPCSは化学物質のリスク評価では国際的に信頼性の高い組織ですが，2002年にGlobal Assessment of the State-of-the-Science of Endocrine Disruptorsという内分泌かく乱化学物質についての国際的な専門家グループによる報告書をまとめました（IPCS, 2002)。そこに書かれている幾つかの大事なことをご紹介します。まず一つは内分泌かく乱化学物質と内分泌系をかく乱する可能性のある物質，英語ではEndocrine DisruptorsとEndocrine Disrupting potentialを持つ Hormonally Active Substancesとを概念的に区別すべきであるということです。すなわち内分泌かく乱とは生体レベルの事象であって，試験管内の現象とは概念的に区別すべきであるということです。先ほどの井口先生のお話しでもありましたが世界的にホルモン活性を持つ物質はさまざま見つかっていて，2000くらいあるというお話しもありました。しかしその中で本当に内分泌をかく乱する可能性のある物質というものは，おそらく非常に限られるだろうと思われます。次に内分泌かく乱化学物質については，当初考えられたようなエストロゲンすなわち女性ホルモン活性を持った物質か，アンチアンドロジェン，すなわち抗男性ホルモン活性を持った物質，また甲状腺ホルモンレセプターに反応する物質に限らず，さまざまな物質があり得るということは井口先生のお話しの中でも紹介されました。

さて今日私のメインテーマは，低用量影響リスクの評価ということです。これについて私は厚生労働科学研究の研究班に入れていただき，現在もその研究を続けております。今日はその研究成果の一端を紹介したいと思います。

本学会の皆さんはよくご存じかと思いますが，化学物質のリスク評価では，有害性の確認，用量―反応評価，暴露評価，リスク判定というステップをとるということが国際的に合意されています。そのうちの用量―反応評価についてですが，図1に示ししましたように，ある毒性については用量を増やしていくと毒性が増強されるというのが一般的で，ある濃度以下では毒性が見られなくなるという種類の物質と，いくら濃度を下げても非常に微少だけれども毒性が見つけられる，いわゆる

* 徳島大学総合科学部（Faculty of Integrated Arts and Sciences, University of Tokushima）

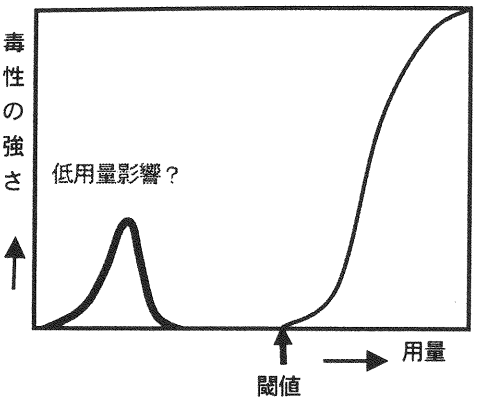

図１　通常の毒性試験における閾値以下の濃度（低用量）での反応の可能性を示す模式図

遺伝子に傷を付けるような発がん性物質の2種類に大別されています。

前者の場合に，動物実験などで毒性が見られなくなる濃度を閾値と呼んでおり，この閾値を安全係数あるいは不確実係数により割って，人における許容基準あるいは一日許容摂取量を求めています。この手法が種々の化学物質の安全性評価の基本として，食品添加物，食品汚染物，残留農薬などについて守るべき基準とされるわけです。ところが内分泌かく乱化学物質で指摘された大きな問題の一つは，図1のように閾値と言われる濃度，つまり濃度を下げていくと何の影響も見られなくなる濃度よりもさらに低い濃度で，何かの反応が見られることがあるということです。ホルモン活性を持つ物質についていえば，実はこのことは不思議でも何でもないことですが，必ずしもそのことが十分理解されていなかったことがあったほかに，この低用量での影響がもし有害ないし不可逆的なものである時にどう考えるかということが問題となりました。この問題については2000年に米国環境保護庁と，米国毒性プログラム(NTP)が共同して低用量影響評価ワークショップを開き検討しました（NTP，2001)。

ここで低用量作用とはヒトの通常の暴露の範囲あるいはアメリカ環境保護庁が，生殖・発生毒性評価のために決めた標準試験法で，一般に使用される用量よりも低い用量で起きる生物学的変化と規定しました。このワークショップの結論としては，幾つかの試験データはこの低用量影響を示唆しているが，必ずしも再現性が見られていないので，さらに深い検討が必要であるというところにとどまりました。しかしご存じのように，体内に既に天然のホルモン活性を持ったエストロゲンなどがあって，それらが時期や生体の必要に応じて増減することで体の機能を調節しています。その調節が適切に保たれ，ある範囲でバランスを取るということが非常に大事になっています。わかりやすい例が女性の生理を支えている女性ホルモン物質です。ところがこのホルモン活性物質については，生体内でシグナルクロストークという物質間の相互作用があります。先ほど紹介がありましたように，アリルハイドロカーボン受容体（ダイオキシン受容体とも通称される）と，エストロゲン受容体が相互作用することが知られています。そういった受容体間の相互作用には本来的な意味があるだろうということ，それから生体内のホルモン物質濃度の多少によるフィールドバックによる生体の恒常性（ホメオスタシスと言う）があるため，これまで考えられていた毒性評価とは違うような問題が，検討されなければいけないということです。私はこういう背景から，内分泌かく乱化学物質の問題というのは，毒性学的に新しい種類の問題であり，毒性評価の上からも生物学の基本に関係した幾つかの問題を深く検討しなければいけないと考えます。

また環境中の生物で見られた現象については，先ほどのご紹介ではメダカで陽性反応が見られたが，ヒトでは陰性の反応結果だったというお話しもありました。野生生物への影響と，ヒトの健康への影響の関係を生物学的メカニズムや，両者における暴露経路とか曝露量，そういったことを考えながら検討する必要があると考えます。次世代への影響の可能性や，リスクにおける不確実性の検討など深く考えておくべき課題が提供されたと思います。

少し長くなるので時間を節約しますが，具体的には特定の暴露時期，クリティカルウインドーあるいは臨界期と呼びますが，この時期の曝露による特別の影響，特に発生の非常に限られた時期におけるある種の薬物への暴露により，特定の臓器が影響を受ける可能性，また暴露を受けた結果が後の特定の時期に初めて検出される可能性があります。

食品中の残留農薬についての安全性試験とか，食品添加物などに課せられている試験には，多世代の繁殖試験がありますが，その場合は交配，妊娠，出産，授乳，その後の時期にわたり，継続的

に暴露するような試験になっています。あとで紹介しますが，たとえば妊娠後期だけなど，ある特定の時期に限って暴露すると，その影響が思春期など成育してから後の時期に現れる可能性があります。逆にいうと，暴露をし続けると検出できなくなる可能性があるということも，生物学的に言えば考えられます。

もう一つ，毒性学的に大事なのは影響の非可逆性，その影響が可逆的なのか非可逆的なのか，生体の調節機能を考慮した結果の解釈ですね。それから影響のエンドポイントについては前立腺の重量が増えるなどが指摘されていますが，これまでは指摘されてなかったエンドポイントについても注目する必要があるかもしれないということです。エンドポイントの選び方と暴露時期の選び方，メカニズムでは細胞のシグナル伝達による調節機構のかく乱，こういったことについて注意して標準的な試験法を見直す必要性があるのはないかが考えられました。

また野生生物との関係では，野生生物とヒトでは共通する部分と違う部分があります。このへんについても十分検討して，野生生物で見られた結果をヒトに適用していくことができるのか，できないのかを考えることが非常に大事です。

さらに天然物による寄与の考察で，当然ながら天然にヒトや畜産動物が持っているホルモン物質との関係です。すでに存在するこれら物質による影響を超えて，どんな影響があるのかということがありますし，また天然にはヒトや動物が体の中に持っているホルモン以外に，植物中にホルモン様活性を示す物質があり，これがどれだけの問題を提供するだろうかということの検討が必要です。

野生生物への影響という点では，ヒトを含む動物が排泄する天然のホルモン物質や畜産用に用いられたホルモン物質の尿中排泄物が，意味のある濃度で環境に検出されている事実があります。また従来注意されなかった医薬品や健康関連製品中の生理活性物質が一部環境中に放出され，特に病院の廃水や，下水処理場放流水，一般の河川にも検出されるという報告もあります。

最後に本学会に関連して重要なもう一つは，リスクの問題として不確実性の検討ということです。影響の違いといった場合に，どの段階に不確実性が最も大きいか，例えばヒトの個体間の影響の違いでは，遺伝的な背景によるものなのか，あるいは地域的な違い，また環境影響への感受性を左右する年齢，生活，摂食パターンの違いがどうなのか，これらがどれだけそのリスクに影響を与えているのか与えてないのか，その幅はどうなのか，こういったことの検討が要求されます。

私どもの研究のご紹介になりますが，まず低用量影響の再現性があまり見られないという問題について検討いたしました。たとえば，個々の論文では，必ずしも試験条件について詳しく記述してない場合が多く見られます。学術論文としてはそのままで掲載される場合も多くあります。しかし，たとえば飼育条件の微妙な違いや，餌中の植物エストロゲン物質の混入の可能性などにより，また投与群を何群選んだかというようなことで，安全性評価という観点から見ると不十分な論文が数多くあります。特にエストロゲン物質で，植物中のホルモン活性物質については注意されるようになりましたが，ある試験研究機関で注意深く計画された低用量のビスフェノールＡの試験が行われました。その際にきわめて低用量の投与群を設定したところ，試験物質を投与しない餌や飲料水中に設定されたと同じくらいの濃度のビスフェノールＡが検出され，試験が予定された低濃度での影響について適切な解析ができなくなったということを正直に報告された例もございました。結果の解析手法についても，動物試験では同じ母親から生まれたこどもを利用しますが，微妙な違いについては，別な母親から生まれたこどもを同じ母親から生まれたこどもと分けて，母親の違いによる影響の寄与を分析する統計解析の方法を検討することが必要になります。

前立腺重量など臓器重量の増減が，報告されていますが，体重比による補正を行うとほとんど違いが見られなくなる，いう場合もありました。私

表1　データ集のフォーマット

文献番号：
著者名：
論文題名：
出典：
チェック項目
　1．対象生物、2．影響の標的臓器、
　3．影響の種類、4．曝露方法、
　5．曝露時期、6．曝露濃度（用量段階）、
　7．観察された影響の種類と濃度
　8．観察時期、9．論文中に低用量影響への関心、
　10．試験の信頼性（ＧＬＰ基準準拠など）
論文の概要：（200～400字）
添付資料：　文献の内容を理解する上で重要な図表
評価者のコメント：　統計的な信頼性など報告の信頼性についての記述

表2　ヒトとマウス・ラットにおける母親と胎児血中のエストラジオール濃度 (Witorschら,2002)

母親
妊娠後期の血中濃度はヒト(15-20 ng/mL)
ラット・マウス妊娠後期 (30-60 pg/mL)の数百倍
胎児
胎児血中濃度はヒト（5-10 ng/mL)、
マウス（100-150 pg/mL）の50-100倍

たちはビスフェノールＡについて，内分泌かく乱の可能性が指摘された論文が多くあることから，この物質について集中的に論文を深く検討しました。NTPが行った毒性試験で，ラットに体重１キログラムあたり50ミリグラムの投与で体重減少が見られました。この値を不確実性係数1000で割って，体重１キログラムあたり50マイクログラムの参照用量が設定され，これが安全基準の目安と看做されてきました。

さてこのビスフェノールＡについて，低用量を報告した文献がどのくらいあるかを調べました。先ほどのアメリカＮＴＰの低用量影響ワークショップでは，未公表のデータを含めて2000年までの低用量報告がレビューされました。したがって，私たち厚生労働科学研究班（井上達代表）では，2000年以降に発表された文献を収集して一つ一つについて内容を検討しました。すなわちビスフェノールＡについて文献検索で得られた文献の中からヒトの健康への影響ということで，水生生物への影響や分析の文献を省いて168件を得ました。さらに昨年，2005年に追加的に調査いたしました。レビューの結果を表１のフォーマットにまとめたデータ集を作りました（関澤; 2005a; 2006a)。

その中からいくつかを紹介します。内分泌かく乱のうちエストロゲン活性による影響については，マウスやラットの胎児では母親由来のエストラジオールへの曝露濃度がヒトに比べ，ずっと低いレベルにあります。ヒトの場合の数百分の１というレベルであり，このような場合にいわゆる環境ホルモン，すなわち外から比較的微量のエストロゲン物質を与えると敏感に反応する可能性があると考えられます（表２)。マウスで微量の環境ホルモン物質を与えたときに影響が見られたとしても，ヒトの場合はもともと胎児がずっと高い濃度のエストラジオールにさらされているため，少々程度の外界からのエストロゲン物質の混入による影響は見られなくなる可能性が高いといえ，種差があると考えられます。いわゆる女性ホルモン作用については，実験結果の再現性があまり見られなかったのですが，ラットやマウスでようやく検出されるような影響は，必ずしもヒトでは影響が起こらない可能性が大きいのではないかと推測されました。

他方，よく引用されるアメリカと日本で行った広範囲の投与レベルでの３世代試験，２世代の長期繁殖試験でも，統計的に有意な影響が見られなかったということについては，先ほど申しましたようにある特定の時期に暴露するということと，ずっと暴露し続けるということで結果が異なる場合がありえます。専門家にはよく知られたことですが，受容体タンパクの濃度そのものが体の中で変動していて，活性物質に長期に暴露させると反応を示す受容体タンパクの濃度が，低くなるダウンレギュレーションという現象が見られます。この場合には，外部からいくらエストロゲン物質が来ても反応が見られなくなるという可能性があります。ずっと暴露し続けるのではなく，妊娠の後期など，ある特定の時期のみに暴露することにより，多世代の曝露試験で見られなかったような影響が見られる可能性があります。

私どもが注目したのは，これまでは女性ホルモン作用や男性ホルモン作用，あるいはせいぜい甲状腺ホルモンへの作用に着目した試験が行われてきましたが，最近になってこれまで検討されなかったエンドポイントである神経行動毒性の影響が比較的低用量の体重１キログラムあたり10マイクログラムという曝露により見られた報告が出つつある点です。このような濃度で脳内ドーパミン系の異常とか，行動における雌雄の性差の解消や変動が見られるという報告が増えています。

ここであえて指摘しておいたほうが良いと思いますことに，ビスフェノールＡについては昨年国内で詳細なリスク評価書というものが独立行政法人の産業技術総合研究所の化学物質リスク管理研究センターから出されました(NEDO/産総研, 2005)。私たち自身のデータベース作成にはこの詳細リスク評価書は利用していませんが，この図書は出版物として販売され日本におけるビスフェノールＡについての詳細なリスク評価を行った資料として重視される位置にあると思います。ほとんど同じ時期に私たちは厚生労働研究班で詳細な文献調査を行った訳ですが，どういうわけか化学物質リスク管理研究センターの詳細リスク評価書ではこのような多くの神経行動毒性の報告について指摘が

一例もなかったということです。ですからこの詳細リスク評価においてはそもそも文献調査が本当に詳細になされた上でリスク評価が，十分なされたのかどうかということの疑問が生ずる次第です。

神経行動毒性の一例ですが，九州工業大学の粟生先生たちが発表された報告では，飲料水中からビスフェノールＡを体重１キログラムあたり15マイクログラムの濃度で妊娠の13日目から出産までの間摂取させます。マウスやラットでは21日目ぐらいで出産しますが，その期間に限って投与します。出生後6ないし9週にかけて神経行動毒性試験として幾つかの試験を組み合わせて実施しますと，探索行動やストレス対処行動の性分化に障害や，うつ・不安の増強が見られました。

神経行動毒性試験は標準化されにくかったので，現在安全性評価における標準的な試験の中にはまだ組み込まれていません。形態的な催奇形性はどちらかというと客観的に評価しやすかったと思いますが，神経行動毒性については必ずしもそうではないので，あまり観察されてこなかったですが注目する必要があると私は思います。

結論として，ビスフェノールＡに関して胎児期と周産期に低用量暴露した場合に，個体レベルで神経系への影響が検出されているということです。そのためにＮＴＰの長期毒性試験を基にした体重１キログラムあたり50マイクログラムという参照用量（一日許容摂取量にあたる）を見直す必要があるかもしれません（文末注）。今後このようなデータの再現性と毒性学的な意義，ヒトへの適用可能性を十分検討する必要があります。もうひとつの課題としては生体のそういう生理作用の制御，それからホメオスタシスにかかわる内分泌系，免疫系，神経系の共同作用，こういったものに着目した毒性試験の確立とリスク評価方法を今後検討していくということが要求されます。私は日本リスク研究学会の一員として，このような新しい毒性学的問題について対応できるリスク評価の手法について考え，またこのような場合の不確実性について十分検討していく必要があると思います。

特に毒性試験における曝露期間の設定については今まで見られていなかった，暴露時期とエンドポイントの関係との組み合わせ，最終的には動物実験からヒトへの適用の可能性について，またヒトでは個体間の差というのも大きかったり，それぞれ環境が違いますので環境がどのように影響の発現を左右し，それが結果の変動要因となるかを検討する必要があると思います。

私どもは別途，実際的な応用例の研究の一つとして直接ビスフェノールＡが，比較的高濃度で直接体に取り込まれる可能性の検討を，2000年に日本リスク研究学会で発表しています。ビスフェノールＡ重合樹脂で成型された血液透析装置から重合反応で残存し溶け出すビスフェノールＡモノマーに着目し，当時在職していた国立医薬品食品衛生研究所の方たちと調査し，平均的にどの位が血液透析器から体の血液に直接入ってくる可能性があるかを調べました(関澤等，2001)。これと当時設定されていた許容量と比べてみたところ，十分余裕があるだろうと考えわけですが最近の文献調査によると必ずしもそうではなさそうだというようなところになってきます。それから実際にエストロゲン活性を持った物質のヒトへの可能性ということでは，ご存じの植物ホルモンについても私は日本リスク研究学会誌に論文発表しています（関澤，大屋，1999)。最近内閣府の食品安全委員会は，植物ホルモン物質のイソフラボノイドは体に良いが，これをサプリメントとして過剰に摂ると問題が起こりうる，つまり私は先ほどの表でもお見せしましたが，大豆製品を平均65グラム（当時）を一日取っているということが国民栄養調査から分かりました。その大豆製品の中に含まれる植物ホルモン物質であるダイゼインとか，ゲニステインの量というのは分析で分かります。その結果，平均20数ミリグラムぐらいのこういった植物ホルモン物質をとっているということが分かり，それら物質の活性との計算からおよその影響の可能性を推計できます。併せて当時私たちは女子大生の方を対象にして，小規模でしたが340人規模のアンケート調査をしました。その結果，大豆製品の摂取と不正出血との関係では一見関連があるようなデータが得られましたが，サンプルサイズが小さかったので統計的な有意差は見られませんでした。食生活のほかに睡眠時間や運動量についても聞いたところ，貧血については，睡眠時間が５時間以下の方と５時間以上の方では，明らかに有意差が見られました。そのことは，ヒトにおける影響を考察するときに，私たちの生活と環境，遺伝的な背景，性別，年齢といったさまざま要因が，私たちのリスクやベネフィットに関係してきているということです。こういったことをトータルとして考えていくということが，問題の解明にどうしても必要だと思います。日本リスク研究学会は，社

会科学や医学の専門家といわゆる自然科学の専門家などが会員となっており，ともにリスクの不確実性要因や，リスクコミュニケーションについても考えながら，これから研究を進めていく必要がますます大きくなると考えています。今回の講演は口頭なので，厳密な議論と本主題に関するより詳しい内容については，別に記しましたので，そちらを参照してください（関澤, 2005b；2006b)。

引用文献

IPCS(2002) Global Assessment of the State-of-the-Science of Endocrine Disruptors, WHO/IPCS/ EDC/02.2, World Health Organization, Geneva

NTP(2001) National Toxicology Program's Report of the Endocrine Disruptors Low Dose Peer Review, National Toxicology Program/National Institute of Environmental Health Sciences, http://ntp-server.niehs. nih.gov/htdocs/liason/LowDosePeerFinalRpt.pdf

関澤　純，大屋幸江(1999) 植物エストロゲン物質の日本人の健康への定量的リスク・ベネフィット解析，日本リスク研究学会誌11(1) 75-82

関澤　純，配島由二，土屋利江(2001)ビスフェノールＡ重合樹脂成型血液透析器使用のリスク・ベネフィト分析，日本リスク研究学会第14回研究発表会講演論文集，73-76

関澤　純監修(2005a; 2006a)ビスフェノールＡの低用量影響評価データ集，平成１５年，16年厚生労働科学研究（井上達代表）の分担研究成果

関澤　純(2005b)低用量問題―低用量影響の生物学的蓋然性，井上達・井口泰泉編「生体統御システムと内分泌撹乱」297-314，シュプリンガーフェアラーク東京，東京

関澤　純(2006b)「環境ホルモン物質」の低用量影響を考える，四国医学雑誌，62, 3/4, 113-119

NEDO/産総研(2005)　詳細リスク評価書シリーズ６，ビスフェノールＡ，丸善株式会社，東京

（文末注）

欧州連合食品科学諮問委員会(2002)は，ラットの３世代試験における無毒性量5 mg/kg体重/日に種差，個体差それぞれ10づつと，低用量影響の不確実性について５の合計500の不確実性係数をあてはめて0.01 mg/kg体重/日を暫定耐容摂取量とした。

Available online at www.sciencedirect.com
ScienceDirect
Chemosphere 70 (2008) 865–873
CHEMOSPHERE
www.elsevier.com/locate/chemosphere

The effects of pH on fluoxetine in Japanese medaka (*Oryzias latipes*): Acute toxicity in fish larvae and bioaccumulation in juvenile fish

Yuki Nakamura [a], Hiroshi Yamamoto [a,*], Jun Sekizawa [a], Takuya Kondo [b], Narisato Hirai [b], Norihisa Tatarazako [b]

[a] *University of Tokushima, Department of Mathematical and Natural Sciences, 1-1, Minamijosanjima-cho, Tokushima 770-8502, Japan*
[b] *National Institute for Environmental Studies, 16-2, Onogawa, Tsukuba, Ibaraki 305-8506, Japan*

Received 22 December 2006; received in revised form 27 June 2007; accepted 30 June 2007
Available online 4 September 2007

Abstract

Recent detection of fluoxetine in the aquatic environment and fish suggests a possibly high accumulation of fluoxetine; however, no report is available on the bioaccumulation of fluoxetine in aquatic organisms. Since bioaccumulation of fluoxetine was probably dependent on pH near the pK_a value of 10.1, experiments were conducted approximately at pH 7, 8, and 9. Distribution coefficients between 1-octanol and water (D_{ow}), and those between synthetic membrane vesicles (liposomes) and water ($D_{lip-wat}$) were determined at pH 7, 8, and 9. The D_{ow} and $D_{lip-wat}$ values increased significantly with increasing pH. Acute toxicity tests were performed using Japanese medaka (*Oryzias latipes*) prior to the bioaccumulation test, and 96-h LC_{50} values were 5.5, 1.3, and 0.20 mg l^{-1} at pH 7, 8, and 9, respectively. In the bioaccumulation test, concentrations of fluoxetine and its major metabolite, norfluoxetine, in the fish body and liver were measured. The bioconcentration factors (BCF) of fluoxetine for Japanese medaka were 8.8, 3.0×10, and 2.6×10^2 in the body and 3.3×10^2, 5.8×10^2, and 3.1×10^3 in the liver at pH 7, 8, and 9, respectively. The BCF values were lower at pH 7 and higher at pH 9 mainly because of the increase in nonionized species with significantly higher hydrophobicity than the ionized species at pH values closer to pK_a. A similar trend was obtained for the concentration of norfluoxetine in the fish but the pseudo-BCF values (the ratio of the norfluoxetine concentration in the fish and the fluoxetine concentration in test water) were higher than the BCF value of fluoxetine at all pH conditions.

Keywords: Fluoxetine; Bioconcentration factor; Ecotoxicity; Ionization

1. Introduction

Fluoxetine and its major metabolite, norfluoxetine, have recently been detected in fish tissues (Brooks et al., 2005) and have become a topic of growing public concern. Fluoxetine was detected in the fish caught from Pecan Creek (Denton, TX, USA) at 0.11 ± 0.03 ng g^{-1} in the muscle and 1.34 ± 0.65 ng g^{-1} in the liver (Brooks et al., 2005). The maximum concentration of fluoxetine found in surface water was 0.012 μg l^{-1} in the US streams (Kolpin et al., 2002), and that detected in the effluents of sewage treatment plants (STPs) was 0.099 μg l^{-1} (Metcalfe et al., 2003). The predicted environmental concentrations calculated by the US and EU guidelines with the estimated removal rate in STPs using STPWIN (USEPA v3.11) of 22.6% are 0.030 μg l^{-1} and 0.841 μg l^{-1}, respectively (Johnson et al., 2005). However, since the experimentally measured removal rate was 93.1% mainly by sorption onto sludge (Yamamoto et al., 2005), the aquatic environmental concentration is possibly lower than the prediction. Although the predicted bioconcentration factor (BCF) of fluoxetine is only 2.0 at pH 7 (Brooks et al., 2003a), the actual BCF is considered to be much higher because of the detection in fish.

* Corresponding author. Tel./fax: +81 88 656 7618.
E-mail address: hiroshi@ias.tokushima-u.ac.jp (H. Yamamoto).

0045-6535/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2007.06.089

Several investigators have initiated research into the fate and transport of fluoxetine in the natural aquatic environment. The reported pseudo first-order photodegradation constant by sunlight was 0.0126 h^{-1} and the half-life was 55.2 h (Lam et al., 2005). The sorption coefficients ($\log K_{oc}$) of fluoxetine to dissolved organic matter and a model sediment, Elliott Silt Loam Soil, were 4.72 and 4.87, respectively, and were comparable to a four-ringed hydrophobic polycyclic hydrocarbon, pyrene (Yamamoto et al., 2005). Thus, fluoxetine was predicted to be highly accumulative in soil/sediment but the transport could be accelerated by dissolved organic matter.

Several toxicity tests have been extensively performed using various aquatic organisms. While the predicted LC_{50} for fish was 2 mg l^{-1} (Sanderson et al., 2003), the measured 48-h LC_{50} for *Pimephales promelas* was 2.28 μM (705 μg l^{-1}) (Brooks et al., 2003b). The aggression of *Thalassoma bifasciatum* was decreased after 14 d of oral exposure to fluoxetine at 6 μg g^{-1} d^{-1} (Perreault et al., 2003). As far as cladocera are concerned, the predicted EC_{50} was 150 μg l^{-1} (Sanderson et al., 2003) and the measured 48 h EC_{50} for *Daphnia magna* was 2.65 μM (820 μg l^{-1}) (Brooks et al., 2003b). Brooks et al. (2003b) found 48 h EC_{50} for immobilization in *Ceriodaphnia dubia*, and no observed effect concentration (NOEC) for fecundity decrease to be 0.756 μM (234 μg l^{-1}) and 0.180 μM (56 μg l^{-1}), respectively, whereas Henry et al. (2004) found acute 48-h EC_{50} and chronic NOEC (number of broods per female) to be 510 and 447 μg l^{-1}, respectively. For algae, the predicted EC_{50} was 900 μg l^{-1} (Sanderson et al., 2003), and the measured 50% growth inhibition at 120 h was 0.077 μM (24 μg l^{-1}) and NOEC was <0.0436 μM (<13 μg l^{-1}) (Brooks et al., 2003b). These aquatic toxicities of fluoxetine were reviewed and compared with those of other human pharmaceuticals by Fent et al. (2006). They found that fluoxetine is one of the most toxic human pharmaceuticals. However, these toxicity tests were performed at a single pH or with no details about pH, and the effects of pH have never been examined. For pentachlorophenol, bioconcentration factors in goldfish (*Carassius auratus*) were strongly affected by pH near the pK_a value (Stehly and Hayton, 1990). Fluoxetine is a secondary amine, so that it may also be strongly affected by changing pH near the pK_a value of 10.1 (Fig. 1). An ionized species (AH^+) is presumably more easily dissolved in water (i.e., the octanol/water partition coefficient is lower) than a nonionized species (A), which increases as decreasing pH becomes lower than pK_a. Thus, the octanol/water distribution coefficient (D_{ow}) decreases with the reduction in pH, because of the decrease in hydrophobic nonionized species (A). Hence the accumulation and toxicity of amines, such as that of fluoxetine, are considered to vary significantly at pH values slightly lower than pK_a.

Consequently, the objectives of this study were (1) to measure the octanol/water and liposome/water distribution coefficients at different pH values, and (2) to determine the toxicity and BCF of fluoxetine using Japanese medaka (*Oryzias latipes*) at different pH values. The pH values of the test water were set at approximately 7, 8, and 9 mainly because the effects of pH near the pK_a value of 10.1 are apparently more significant than those of the acidic side. Additionally, Brooks and co-workers sampled fish at a northern Texas stream (Brooks et al., 2005) where limestone is prevalent and the pH value could be as high as 8. In fact, the pH value of the upper Trinity River basin was reported by the USGS to range from 7.3 to 8.3 (USGS, 2007).

2. Materials and methods

2.1. Materials

Japanese medaka (*Oryzias latipes*) acquired from the National Institute for Environmental Studies (Ibaraki, Japan) were used in this study. The fish were acclimated in the laboratory at the University of Tokushima for at least two months. Approximately 15-d-old larvae were used for the acute test after five days of acclimation period, and approximately 2-month-old fish were used for the BCF test.

Fluoxetine (hydrochloride), its major metabolite norfluoxetine (hydrochloride), an internal standard fluvoxamine, and 1-octanol were purchased from Sigma Chemical Co. (St Louis, MO, USA). Biochemical grade bis–tris (bis(2-hydroxyethyl) iminotris(hydroxymethyl)methane), tris (tris(hydroxymethyl)aminomethane), HPLC grade acetonitrile, hexane 5000, dietylether 5000, dichloromethane 300, and iso-amyl alcohol (3-methyl-1-butanol) for special grades were obtained from Wako Pure Chemicals Co. (Osaka, Japan). Palmitoyl-oleoyl phosphatidylcholine

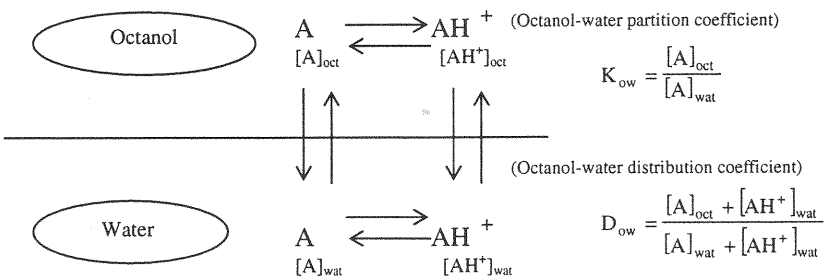

Fig. 1. Schematic diagram of the partition/distribution of the nonionized (AH^+) and ionized (A) species of amines between octanol and water.

(POPC) was obtained from Nippon Fine Chemical Co. (Osaka, Japan) to synthesize liposomes. *S*-(−)-*N*-(trifluoroacetyl)-prolyl chloride in dichloromethane (0.1 M), which was used to analyze the derivative reagent with gas chromatograph mass spectrometry (GC–MS), was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA).

2.2. Analysis

Concentrations of fluoxetine in the aqueous phase were measured using high-performance liquid chromatography (HPLC) equipped with UV/vis absorbance and fluorescence detectors. The system consisted of an SLC-10AD controller (Shimadzu, Kyoto, Japan), two LC-10AD pumps (Shimadzu, Kyoto, Japan), a CTO-10AS oven (Shimadzu, Kyoto, Japan), an SPD-10A UV/vis absorbance detector (Shimadzu, Kyoto, Japan), and an RF-10A fluorescence detector (Shimadzu, Kyoto, Japan). A 150 mm × 4.6 mm i.d. packed with 5 μm of end-capped ODS main column (VP-ODS, Shimadzu, Kyoto, Japan) was used with a 10 mm × 4.6 mm i.d. of similar ODS pre-column (GV-ODS, Shimadzu, Kyoto, Japan). The mobile phase was isocratic and consisted of acetonitrile and 10 mM potassium phosphate buffer (pH 3.0, 62:38 v/v%) and the flow rate was set at 1.0 ml min^{-1}. The UV/vis absorbance detector was used for quantification at a wavelength of 228 nm, and the fluorescence detector was used for rough identification at excitation and emission wavelengths of 230 and 293 nm, respectively. The retention time was approximately 9.0 min and the lower detection limit was 0.75 μg l^{-1} for 500 μl of injection volume.

The concentration of fluoxetine in the fish was measured using a GC–MS after the extraction (Eap et al., 1996) described in detail below. The QP-2010 (Shimadzu, Kyoto, Japan) was used with splitless injection and electron impact (EI) modes. A column of 30 m × 0.25 mm i.d. and 0.25 μm film thickness (DB-5ms, Agilent Technology, CA, USA) was used, and high-quality helium was used as the carrier gas at 1.11 ml min^{-1}. The GC oven, injection temperature, and interface temperature were set at 145 (initial temperature), 250, and 280 °C, respectively. The temperature graduation was held for 0.5 min at initial temperature (145 °C), heated at 10 °C min^{-1} to 290 °C, and held for 10 min at 290 °C. For the MS, the ion source temperature and ionization potential were set at 200 °C and 70 eV, respectively. Five microliters of solution was injected, and analysis was performed in SIM mode with collected peaks of $m/z = 117$, 166, 237, 253, 341, and 327. Quantification was conducted at $m/z = 117$ for fluoxetine and norfluoxetine, and $m/z =$ 166 for fluvoxamine. Chromatograms of a spiked blank fish and an example of exposed fish are shown in Fig. 2. Two separate peaks were found for fluoxetine and norfluoxetine, respectively, and these could be enantiomers as reported by Eap et al. (1996) because their analytical conditions/columns are similar to ours. However, no separated enantiomer standard was available and we could not identify them. Fluoxetine and norfluoxetine were quantified by adding the two peaks. The detection limits for fluoxetine and norfluoxetine were 63 and 11 μg l^{-1}, respectively.

2.3. Distribution coefficients in 1-octanol/water and liposome/water systems

The D_{ow} values were measured at pH 7.0, 8.0, and 9.0 with 10 mM buffers according to the OECD test guideline No. 107 (OECD, 1995). Bis–tris and HCl with 75:25 (v/v%) were used to prepare the pH 7.0 buffer while a mixture of tris and HCl was used for the pH 8.0 and 9.0 buffers with 67:33 and 90:10 (v/v%), respectively. The total amount of 10 ml of 10 mM buffer-saturated 1-octanol with fluoxetine and 1-octanol-saturated 10 mM buffer solution were added into 10 ml dry glass tubes and mixed in the dark using a tumbler for 24 h. The final concentration of fluoxetine in the aqueous phase was determined using a fluorescence spectrophotometer (F-2500, Hitachi, Tokyo, Japan).

Distribution coefficients between synthetic membrane vesicles (liposomes) and water ($D_{lip-wat}$) were determined using the equilibrium dialysis method developed by Escher and Schwarzenbach, (1996) and later modified by Yamamoto and Liljestrand, (2004). Liposomes were prepared from POPC dissolved in chloroform using the thin film hydration technique (Mueller et al., 1983). Ten milliliter of 20 g l^{-1} POPC in dichloromethane was evaporated to dryness and re-suspended in 100 ml Milli Q water in a sonicator. The POPC suspension was extruded with 1.2 μm Millipore polycarbonate membrane to obtain vesicles with 0.6–0.8 μm diameters (Yamamoto and Liljestrand, 2004). The total organic carbon (TOC) concentration of the liposomes was determined using a TOC analyzer (TOC-5000, Shimadzu, Kyoto, Japan). The buffer solutions described in the previous section were added to the liposome to set the concentration at 122 mg C l^{-1} and aqueous solution of fluoxetine to set the pH at 6.9, 7.9, and 8.9. Sodium azide (10 mM) was also added to minimize microbial activity. The apparatus of the combination of two glass vials, a sheet of dialysis membrane (Spectram/Por), and a PTFE connector had been developed by Escher and Schwarzenbach, (1996) and was later modified by Yamamoto and Liljestrand, (2004). The initial concentration of fluoxetine in the aqueous phase was set at 100 μg l^{-1} and was connected with the liposome phase by the PTFE connector and a dialysis membrane. After a week of rotary shaking using a tumbler in the dark, the concentrations of fluoxetine in both the liposome and the aqueous phase were measured using HPLC. One week is considered enough to attain equilibrium for this system (Yamamoto and Liljestrand, 2004). Blank samples were prepared with 10 mM buffer solution instead of liposome suspension. All tests and blanks were performed with four replicates, as were the D_{ow} tests. The values of $D_{lip-wat}$ (l kg^{-1}) were calculated as follows:

$$D_{lip\text{-}wat} = (C_{blank} - C_w)/(C_{w\times}[lip]) \quad (1)$$

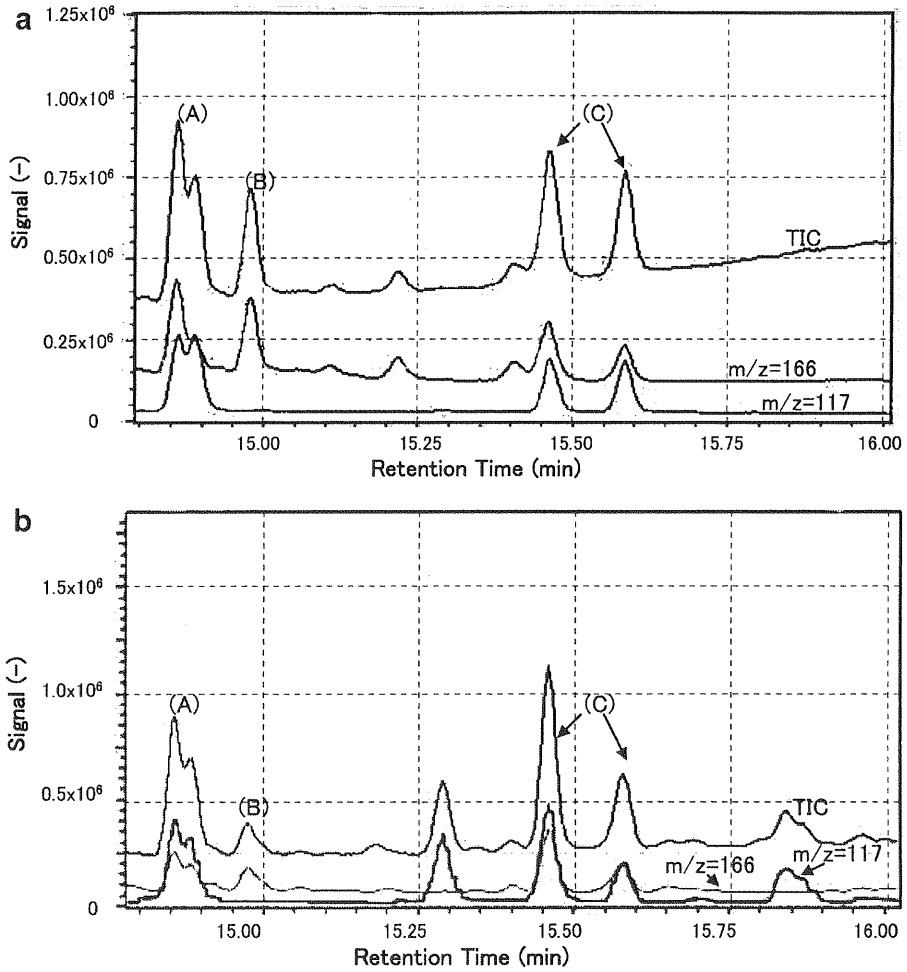

Fig. 2. Examples of selected ion chromatograms of (A) norfluoxetine, (B) fluvoxamine (internal standard), and (C) fluoxetine, of the (a) spiked blank and (b) BCF test.

where C_{blank} (mg l^{-1}) is the concentration of fluoxetine in the blank solution, C_w (mg l^{-1}) is that in the aqueous phase, and [lip] (kg l^{-1}) is the TOC of the liposome suspension.

2.4. Acute tests

Acute tests were performed in accordance with the OECD test guideline No. 203 (OECD, 1992) at pH 7.1, 7.9, and 8.8 with 10 mM buffers as presented above. Ten 15-d-old fish were exposed to 100 ml of test water in a 100 ml glass beaker. No significant toxic effects were found for the concentration of buffers (10 mM) for Japanese medaka in blank solutions during the 96-h test period and this concentration was enough to control pH. Ten fish were exposed to fluoxetine solution of each concentration for 96 h. The test water was replaced once in 48 h from the beginning of exposure, and the concentration in the tanks was monitored using HPLC at the beginning and end of the exposure period. The mean measured concentration was used for the data analysis. The maximum relative deviation of the aqueous concentration was 19%. The pH values of the test solutions were also monitored using a pH meter (D-52, HORIBA, Kyoto, Japan). LC_{50} values for 96 h were determined with probit conversion from the number of deaths in 96 h for each pH.

2.5. Bioconcentration factors

2.5.1. Exposure

A preliminary test with a single pH condition was performed before the test with three different pH conditions. Fluoxetine concentrations in the aqueous phase were set at approximately 30 and 300 μg l^{-1}, and Japanese medaka were exposed for 30 d in the preliminary test. For the test with three different pH values, test waters were adjusted at pH 7.2, 8.1, and 8.9 with 10 mM buffers as described above, and eight fish were exposed to 10 μg l^{-1} of fluoxetine, which was determined from 96-h LC_{50} and the detection limits for GC-MS (i.e., 63 μg l^{-1} or 12.6 ng per sample for fluoxetine and 11 μg l^{-1} or 2.2 ng per sample for norfluoxetine). For each pH test, blank samples with 10 mM buffer solutions with no fluoxetine addition were prepared to confirm absence of any significant effect by the buffer agent or pH on fish. The exposure period was 30 d as with the preliminary test.

The custom-made exposure system consists of glass tanks, Vivaria R 1815, purchased from Torio Co. (Osaka, Japan) filled with 2,300 ml of test solution. The fluoxetine solution was continuously fed into the tank at a flow rate of 1.7 ml min^{-1}. Aqueous concentrations of fluoxetine and pH in these tanks were measured every 10 d using HPLC. After the 30-d exposure period, the body and liver samples were collected from the fish and stored at −20 °C until the extraction process.

2.5.2. Extraction

The extraction of fluoxetine and norfluoxetine from the fish was performed using the method developed by Eap et al. (1996) and Lefebvre et al. (1999) with slight modifications. Two fish bodies (approximately 50 mg) were placed in a 2 ml plastic tube, and 30 μl of 2 mg l^{-1} fluvoxamine, an internal standard, 250 μl of Na_2CO_3, 200 μl of Milli Q water, and 500 μl of *n*-hexane-diethylether (50:50 v/v%) were added. Fluvoxamine was selected as the internal standard because of its similarity in chemical structure to clovoxamine, which is used as an internal standard for the quantification of fluoxetine and fluvoxamine by Eap et al. (1996). For the liver, the pairs identical to the body samples (approximately 1.5 mg) were similarly paired and extracted. After the homogenization, the solution was centrifuged at 4 °C and 10 000 rpm for 30 min. The organic layer was transferred into a 10 ml glass tube and 500 μl of 0.1 M HCl was added. The aqueous layer was collected followed by 1 min of shaking and centrifugation at 2500 rpm for 15 min, and then 1 ml of 1 M Na_2CO_3 and 500 μl of dichloromethane-iso-amyl alcohol (85:15 v/v%) were added. After shaking and centrifugation, the aqueous layer was removed and anhydrous sodium sulfate was added to remove the water completely. After shaking and centrifugation at 2500 rpm for 10 min again, the supernatant was transferred to another 10 ml dry glass tube to evaporate until dryness under a stream of nitrogen at 40 °C. A derivatization reagent of 500 μl was added to the glass tube and extracted in a sonicator. The derivatization reagent was prepared every day by adding 360 μl of *S*-(−)-*N*-(trifluoroacetyl)-prolyl chloride solution into 4.5 ml of dichloromethane. The glass tube was topped and sealed with PTFE tape, and the derivatization was performed in a water bath at 60 °C for 1 h. The reagent was evaporated to dryness under a stream of nitrogen at 40 °C, 500 μl of dichloromethane was added, and the derivatized fluoxetine was extracted in a sonicator. Analysis was performed using GC–MS as presented above after the solution was concentrated to 200 μl under a stream of nitrogen. The BCF value for each pH was calculated as follows:

$$BCF = C_{fish}/C_{water} \quad (2)$$

where C_{fish} (μg kg^{-1}) is the concentration of fluoxetine in fish and C_{water} (μg kg^{-1}) is the mean of the aqueous concentrations. The standard solution was prepared by following the procedure shown above after transferring from the aqueous layer to the dichloromethane–iso-amyl alcohol layer.

3. Results

3.1. Distribution coefficients between 1-octanol/water and liposome/water

The D_{ow} and $D_{lip-wat}$ values for fluoxetine are summarized in Table 1. While both coefficients increased with increasing pH, the difference in the D_{ow} value was significantly larger than that of the $D_{lip-wat}$ value.

3.2. Acute test

Table 2 shows 96-h LC_{50} values of fluoxetine for approximately 15-d-old *Oryzias latipes*. The number of dead fish in the blank solution of 10 mM buffer at any pH was at most one (i.e., 10%), which was less than the maximum lethal percentage indicated in the OECD test guideline No. 203. The higher pH obviously caused higher toxicity. The difference between LC_{50} at pH 9 and 7 was statistically significant ($p < 0.01$) as a result of *t*-tests. NOEC at pH 7.1 was 3.8 mg l^{-1} in this study. At pH 8.8, three-tenth of the fish died at 100 μg l^{-1}, the lowest concentration in the study. Therefore, the exposure concentration of the BCF test was set at 10 μg l^{-1}, one order of magnitude lower than the concentration.

3.3. Bioconcetration factors

The recovery of fluoxetine from the fish body and liver was 81.3 ± 10.8% and 92.6 ± 5.3%, respectively, and those of norfluoxetine were 71.1 ± 11.0% and 95.7 ± 2.6%, respectively. The measured pH values in the test solution during the exposure period were 7.2 ± 0.1, 8.1 ± 0.1, and 8.9 ± 0.1, and concentrations were 13.8 ± 3.8, 15.0 ± 3.2,

Table 1
D_{ow} and $D_{lip-wat}$ of fluoxetine at each pH (mean ± S.D.)

	Ratio of nonionized species [A] (%)	D_{ow}	$D_{lip-wat}$
pH7	0.079	$3.6(\pm 1.6)\times 10$	$1.7(\pm 0.6)\times 10^4$
pH8	0.79	$4.6(\pm 0.6)\times 10^2$	$2.4(\pm 0.8)\times 10^4$
pH9	7.4	$4.6(\pm 0.5)\times 10^3$	$4.0(\pm 1.6)\times 10^4$

Table 2
96-h LC50 Values of fluoxetine for *Oryzias latipes* at pH 7, 8, and 9 (mean ± 95% confidence interval)

	96 h-LC50 (mg l^{-1})
pH 7 (7.1 ± 0.1)[a]	5.5 ± 1.3
pH 8 (7.9 ± 0.1)[a]	1.3 ± 0.2
pH 9 (8.8 ± 0.2)[a]	0.20 ± 0.02*

[a] Measured pH values (pH monitoring was performed at the first and last days of the test).
* $p < 0.01$ compared with LC_{50} value of pH 7 as a result of *t*-test.

Table 3
Bioconcentration factor of fluoxetine and its major metabolite norfluoxetine in the body and liver ($n = 4$; mean ± S.D.)

		Body	Liver	Body + liver
Fluoxetine	pH 7	8.8 ± 5.2	$3.3(\pm 0.9)\times 10^2$	$1.3(\pm 0.6)\times 10$
	pH 8	$3.0(\pm 1.3)\times 10$	$5.8(\pm 1.1)\times 10^2$	$3.7(\pm 1.3)\times 10$
	pH 9	$2.6(\pm 1.5)\times 10^2$	$3.1(\pm 0.4)\times 10^3$	$3.3(\pm 1.5)\times 10^2$
Norfluoxetine	pH 7	$8.4(\pm 0.8)\times 10$	$1.5(\pm 0.2)\times 10^3$	$1.0(\pm 0.1)\times 10^2$
	pH 8	$1.3(\pm 0.7)\times 10^2$	$3.3(\pm 0.6)\times 10^3$	$1.7(\pm 0.6)\times 10^2$
	pH 9	$6.5(\pm 1.8)\times 10^2$	$3.7(\pm 2.3)\times 10^3$	$7.2(\pm 1.7)\times 10^2$
Fluoxetine + norfluoxetine	pH 7	$9.3(\pm 1.3)\times 10$	$1.8(\pm 0.3)\times 10^3$	$1.1(\pm 0.2)\times 10^2$
	pH 8	$1.6(\pm 0.6)\times 10^2$	$3.9(\pm 0.6)\times 10^3$	$2.1(\pm 0.6)\times 10^2$
	pH 9	$9.1(\pm 2.1)\times 10^2$	$6.8(\pm 0.2)\times 10^3$	$1.0(\pm 0.2)\times 10^3$

and 14.5 ± 3.3 μg/l, respectively. Table 3 shows the BCF values of fluoxetine in the body and liver of approximately 2-month-old *Oryzias latipes* at each pH. No fish died or were apparently immobilized in either test or blank solutions. Neither fluoxetine nor norfluoxetine was detected in blank fish. The measured BCF value of fluoxetine for the summation of the body and liver was 11 at pH 7.2 in this study, which was slightly higher than the predicted value of 2.0 at pH 7 (Brooks et al., 2003a). Since the major metabolite norfluoxetine was also detected from both samples, the pseudo-BCF value for norfluoxetine (i.e., the denominator is the concentration of fluoxetine in the aqueous phase and not the concentration of norfluoxetine) is also added in Table 3.

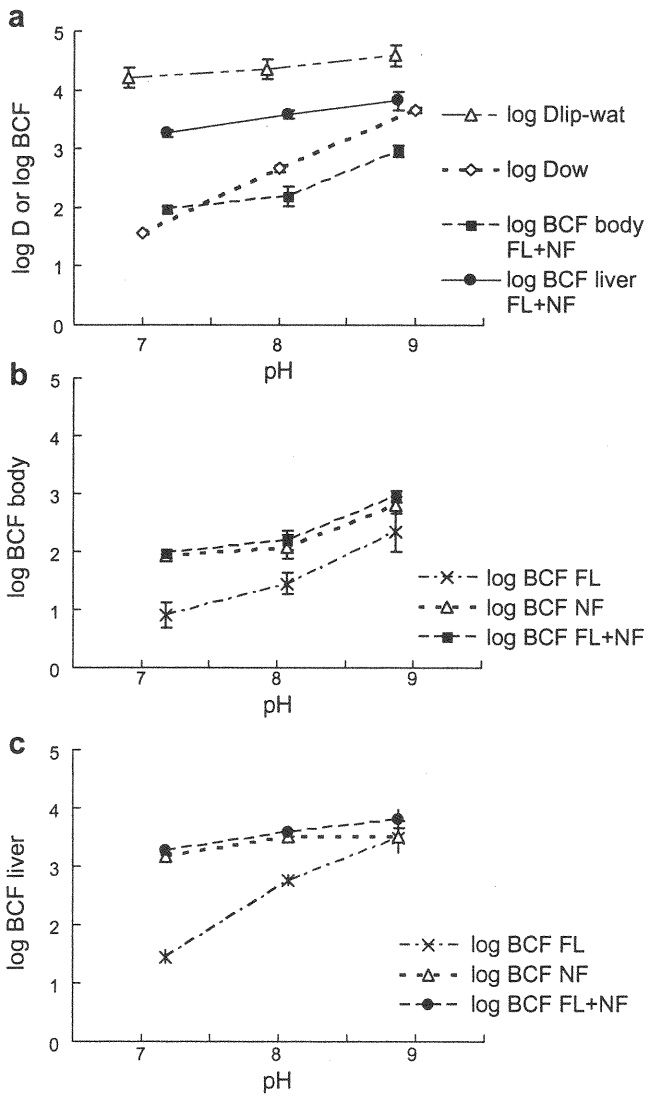

Fig. 3. Relationship between pH and D_{ow}, $D_{lip-wat}$, and BCF. (a) shows log D_{ow}, $D_{lip-wat}$, and BCF of sum of fluoxetine and norfluoxetine in body and liver, (b) shows log BCF in the body, and (c) shows these in the liver. (FL: fluoxetine, NF: norfluoxetine. Error bars show standard deviations.)

In our preliminary tests with two different aqueous concentrations, the BCF value for fluoxetine in the whole body was $4.5(\pm 1.2)\times 10$ and the pseudo-BCF value for norfluoxetine was $2.4(\pm 0.3)\times 10^2$ in exposing Japanese medaka to 30 μg l^{-1} of fluoxetine for 30 days, and $1.0(\pm 0.3)\times 10^2$ for fluoxetine and $1.1(\pm 0.2)\times 10^2$ for norfluoxetine at 300 μg l^{-1}. The ratios of norfluoxetine and fluoxetine were 5.3 and 1.1 at 30 μg l^{-1} and 300 μg l^{-1}, respectively.

Fig. 3 shows the relationships between pH and distribution coefficient in the octanol/water, liposome/water, and fish/water systems (i.e., the BCF value) in the body and liver. The BCF values of fluoxetine in the body and liver were both lower at pH 7.2 and higher at pH 8.9. The trend of the increase in the BCF values was apparently similar to that of the log D_{ow} value. Contrarily, the pseudo-BCF values for norfluoxetine were neither so increased as the increase in pH nor similar to the trend of log D_{ow} value, but were similar to that of log $D_{lip-wat}$. The sum of the BCF and pseudo-BCF values for fluoxetine and norfluoxetine increased similar to the log $D_{lip-wat}$ value. The ratios of pseudo-BCF and BCF (norfluoxetine per fluoxetine) were 8.5, 3.9, and 2.2 in the body, and 4.5, 5.9, and 1.2 in the liver at pH 7.2, 8.1, and 8.9, respectively. The ratios were relatively high at pH 7.2 and low at pH 8.9 except for the liver at pH 8.

4. Discussion

The large difference of the D_{ow} value at different pH was mainly attributed to the difference in the ratio of the concentration of ionized species ($[AH^+]$) and nonionized

species ([A]) as can be seen in the increasing ratio of [A] in Table 1. The estimated coefficient of octanol and water (K_{ow}) values of ionization (AH^+) and nonionization (A) are less than 1 and 5.2×10^3, respectively. The $D_{lip-wat}$ value, however, did not vary significantly as the D_{ow} value; the estimated partition coefficient of liposome and water ($K_{lip-wat}$) of these two are 1.7×10^4 and 3.3×10^4, respectively.

The acute toxicity of fluoxetine found in this study for Japanese medaka at pH 7.1 (i.e., $LC_{50} = 5.5 \pm 1.2$ mg l^{-1}) was greater than that in other reports, such as Brooks et al. (2003 b), where no effect was found at 28.9 μM (8.9 mg l^{-1}) in 48 h. Moreover, the toxicity was found to be as high as 1.7 mg l^{-1}, which is the 96-h LC_{50} value for fish predicted by ECOSAR (US EPA v3.12). Approximately 15-d-old fish larvae, which are apparently more sensitive than juvenile or sexually matured fish, were used in this study. Toxicity was reported slightly stronger for other species including algae, daphnids, and other species of fish as described above. The results of the toxicity tests were obviously different for various ages and species.

The strong dependence of toxicity on pH is partly because of the higher BCF at increased pH due to the higher fraction of the lipophilic nonionized species (Table 1). The toxicity shows positive correlation with the lipophilicity, so that the increasing fraction of lipophilic nonionized species caused the greater toxicity. Despite no apparent effect on blank fish at higher pH, the toxic effects of the buffer reagent and the higher pH on Japanese medaka are unclear and further investigation is necessary to reveal the complete mechanism. The slight difference in ionic strength in the test water (i.e., the estimated ionic strength at pH 7.9 was twice as high as the other two) also possibly causes the difference in the toxicity.

D_{ow} has long been used to estimate the hydrophobicity or bioaccumulation of chemical compounds. Lopes et al. (2006) calculated the BCF value of an acidic pesticide, trichlofon, referring to Kleier, (1994) and Isnard and Lambert, (1988) at different pH values, and the estimated values agreed to the measured ones. In these reports, the BCF values of the ionized (AH^+) and nonionized species (A) were calculated as per the following equation:

$$BCF = P_{ion}BCF_{ion} + (1 - P_{ion})BCF_{nonion} \quad (3)$$

where P_{ion} is the ratio of ionization calculated by following the equation below.

$$P_{ion} = \frac{1}{1 + 10^{pH} - pK_a} \quad (4)$$

BCF_{ion} and BCF_{nonion} are the bioconcentration factors of the ionized and nonionized species, respectively. These BCF values were estimated using the equation for nonionized compounds suggested by Isnard and Lambert (1988).

$$\log BCF = 0.76 = \log K_{ow} - 0.52 \quad (5)$$

where $\log K_{ow}$ was used for both the ionized and nonionized species for estimating BCF_{ion} and BCF_{nonion}. The $\log K_{ow}$ value for the ionized species was estimated using the following equation:

$$\log K_{ow\ ion} = \log K_{ow\ nonion} - 3.4 \quad (6)$$

This equation moderately agrees to our results; $\log K_{ow}$ for the ionized species based on Eq. (3) is 0.4 and that for the nonionized species is 3.0. Those of our results were 0.5 and 3.4, respectively. The formula derived from our results is

$$BCF = 0.50\ P_{ion} + 3.4(1 - P_{ion}) \quad (7)$$

The estimated log BCF values of fluoxetine based on Eq. (3) and those estimated by Eq. (7) are shown in Fig. 4. The estimated log BCF using Eq. (3) at pH 7.2, 8.1, and 8.9 were 0.60, 1.1, and 1.8, respectively, and the measured log BCF values of fluoxetine in the body were 0.94, 1.5, and 2.4, respectively, and slightly higher than the estimated log BCF values at each pH.

The smaller difference in $D_{lip-wat}$ value than D_{ow} value at pH range 7–9 agrees with those obtained by Escher and Schwarzenbach, (1996) for substituted phenols with ionizable groups. Liposome has also been used as a model of biomembrane and found to be better than 1-octanol especially for ionizable compounds, because liposomes are made of phospholipid, a main constituent of biomembrane, and closer to the real biomembrane (Escher and Schwarzenbach, 1996). The result of $D_{lip-wat}$ suggests the smaller influence of the ratio of $[AH^+]$ and [A] on the accumulation of the sum of AH^+ and A into biomembranes. In Fig. 3, however, the trend of the increase in the log BCF values was apparently similar to that of the $\log D_{ow}$ value. Contrarily, the trend of the sum of log BCF for fluoxetine and norfluoxetine is similar to that of log $D_{lip-wat}$. Since the pseudo-BCF/BCF ratio (i.e., the norfluoxetine/fluoxetine ratio) was much higher than 1, the compounds originating from fluoxetine in the aqueous phase were as highly accumulated as the metabolite norfluoxetine in the fish body and need to be carefully examined. Moreover, the prediction of BCF values from $\log D_{ow}$ values is inadequate especially for ionizable species (i.e., weak acids and

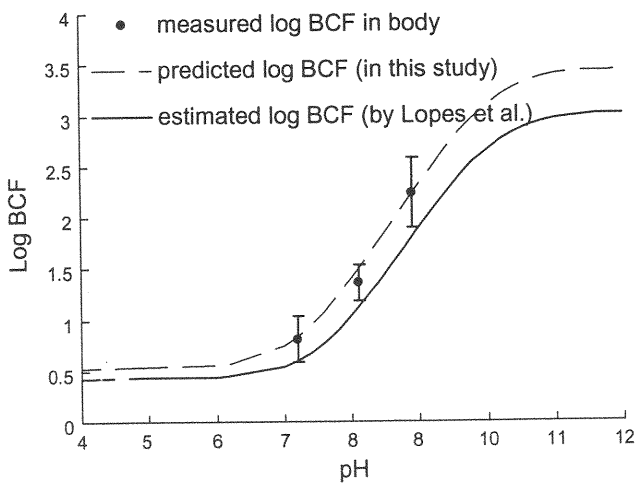

Fig. 4. Measured and predicted log BCF of fluoxetine at different pH.

bases) such as pharmaceuticals. Further investigation is necessary for other polar materials to confirm the relationships between $\log D_{ow}$, $\log D_{lip-wat}$ and log BCF.

The half lives of fluoxetine and norfluoxetine for mammals are 1–4 d and 7–15 d, respectively (Hiemke et al., 2000), and the half-life of norfluoxetine in the fish may also be much longer than that of fluoxetine. In addition, the relatively lower clearance rate of norfluoxetine resulted in a moderate change in the pseudo-BCF of norfluoxetine with increasing pH. Not only the half lives of both compounds but also the higher uptake rate at higher pH caused the higher BCF. As presented above, the fraction of the hydrophobic nonionized species (A) significantly increases with the increase in pH. In this case, the metabolism, the conversion from fluoxetine to norfluoxetine, proceeded relatively faster than the uptake. Conversely, the uptake was relatively faster at high pH because of the higher hydrophobicity of the nonionized species (A), and a part of the fluoxetine absorbed by the fish was possibly accumulated without metabolism. In our preliminary test with two different aqueous concentrations described above, the ratio of norfluoxetine and fluoxetine was higher at lower concentration and lower at higher concentration. Exposure to low concentration might result in the metabolism from fluoxetine to norfluoxetine being relatively faster than the uptake.

Both fluoxetine and norfluoxetine were detected in wild bluegill (*Lepomis macrochirus*), channel catfish (*Ictalurus punctatus*), and black crappie (*Pomoxis migromaculatus*) (Brooks et al., 2005). The concentrations of fluoxetine in the muscle and liver were approximately 0.11 and 1.3 ng g^{-1}, respectively, and those of norfluoxetine were approximately 1.1 and 10 ng g^{-1}, respectively. The ratios of norfluoxetine and fluoxetine in our results, including the preliminary tests, were 1.1 (whole body), 5.3 (whole body), and 9.5 (body) or 4.5 (liver), at aqueous concentrations of 300, 30, and 10 μg l^{-1}, respectively, while those found by Brooks and co-workers, (2005) were approximately 10 (muscle) and 8 (liver). This comparison suggests that the exposure of fish to a low concentration causes a larger ratio, and the aqueous concentration in the river, where fluoxetine was detected in the fish by Brooks and co-workers, (2005), was much less than 10 μg l^{-1}. Using the BCF value obtained in this study (i.e., 8.8 at pH 7.2), the aqueous concentration of fluoxetine exposed to the fish can be roughly predicted as 0.011 μg l^{-1}, which is similar to the maximum concentration reported by Kolpin et al. (2002). Otherwise, the concentration might be slightly lower and the pH greater than 7, e.g., 0.0033 and 0.00038 μg l^{-1} at pH 8 and 9, respectively. As presented above, the pH of the Upper Trinity River Basin in North Texas (e.g., Grand Prairie and Dallas) monitored by USGS ranged between 7.3 and 8.3 (USGS, 2007). If the pH at Brooks and co-workers' sampling point is similar to these values, the fluoxetine concentration was predicted to be a few nanograms per liter. However, further investigation is necessary to clarify the contribution of the other routes of bioaccumulation, such as food.

In this study, the accumulation of fluoxetine was dependent on pH, which agrees to the reports by Fent et al. (1995) and Looser et al. (1998), who had extensively investigated the effects of pH on the bioaccumulation of organotins. However, not much has been revealed about the effects of pH on bioaccumulation, metabolism, or toxicity of other organic micropollutants including fluoxetine, and further investigation is necessary.

References

Brooks, B.W., Foran, C.M., Richards, S.M., Weston, J., Turner, P.K., Stanley, J.K., Solomon, K.R., Slattery, M., La Point, T.W., 2003a. Aquatic ecotoxicology of fluoxetine. Toxicol. Lett. 142, 169–183.

Brooks, B.W., Turner, P.K., Stanley, J.K., Weston, J.J., Glidewell, E.A., Foran, C.M., Slattery, M., La Point, T.W., Huggett, D.B., 2003b. Waterborne and sediment toxicity of fluoxetine to select organisms. Chemosphere 52, 135–142.

Brooks, B.W., Chambliss, C.K., Stanley, J.K., Ramirez, A., Banks, K., Johnson, R.D., Lewis, R.J., 2005. Determination of select antidepressants in organisms from a municipal effluent-dominated stream. Environ. Toxicol. Chem. 24, 464–469.

Eap, C.B., Gaillard, N., Powell, K., Baumann, P., 1996. Simultaneous determination of plasma levels of fluvoxamine and of the enantiomers of fluoxetine and norfluoxetine by gas chromatography–mass spectrometry. J. Chromatogr. B 682, 265–272.

Escher, B.I., Schwarzenbach, R.P., 1996. Partitioning of substituted phenols in liposome–water, biomembrane–water, and octanol–water systems. Environ. Sci. Technol. 30, 260–270.

Fent, K., Looser, P.W., 1995. Bioaccumulation and bioavailability of tributyltin chloride: influence of pH and humic acids. Water Res. 29, 1631–1637.

Fent, K., Weston, A.A., Caminada, D., 2006. Ecotoxicology of human pharmaceuticals. Aquat. Toxicol. 76, 122–159.

Johnson, D.J., Sanderson, H., Brain, R.A., Wilson, C.J., Bestari, K.T., Solomon, K.R., 2005. Exposure assessment and microcosm fate of selected selective serotonin reuptake inhibitors. Regul. Toxicol. Pharmacol. 42, 313–323.

Henry, T.B., Kwon, J.W., Armbrust, K.L., Black, M.C., 2004. Acute and chronic toxicity of five selective serotonin reuptake inhibitors in *Ceriodaphnia dubia*. Environ. Toxicol. Chem. 23, 2229–2233.

Hiemke, C., Härtter, S., 2000. Pharmacokinetice of selective serotonin reuptake inhibitors. Pharmacol. Therapeut. 85, 11–28.

Isnard, P., Lambert, S., 1988. Estimating bioconcentration factors from octanol-water partition coefficient and aqueous solubility. Chemosphere 17, 21–34.

Kleier, D.A., 1994. Phloem mobility of xenobiotics. V.* Structural requirements for phloem-systemic pesticides. Pestic. Sci. 42, 1–11.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. Environ. Sci. Technol. 36, 1202–1211.

Lam, M.W., Young, C.J., Mabury, S.A., 2005. Aqueous photochemical reaction kinetics and transformations of fluoxetine. Environ. Sci. Technol. 39, 513–522.

Lefebvre, M., Marchand, M., Horowitz, J.M., Torres, G., 1999. Detection of fluoxetine in brain, blood, liver and hair of rats using gas chromatography–mass spectrometry. Life Sci. 64, 805–811.

Looser, P.W., Bertschi, S., Fent, K., 1998. Bioconcentration and bioavailability of organotin compounds: influence of pH and humic substances. Appl. Organomet. Chem. 12, 601–611.

Lopes, R.B., Paraiba, L.C., Ceccarelli, P.S., Tornisielo, V.L., 2006. Bioconcentration of trichlorfon insecticide in pacu (*Piaractus mesopotamicus*). Chemosphere 64, 56–62.

Metcalfe, C.D., Miao, X-S., Koenig, B.G., Struger, J., 2003. Distribution of acidic and neutral drugs in surface waters near sewage treatment

plants in the lower Great Lakes, Canada. Environ. Toxicol. Chem. 22, 2881–2889.
Mueller, P., Chien, T.F., Rudy, B., 1983. Formation and properties of cell-size lipid bilayer vesicles. Biophys. J. 44, 375–381.
OECD, 1992. OECD Test Guidelines for Chemicals No. 203.
OECD, 1995. OECD Test Guidelines for Chemicals No. 107.
Perreault, H.A.N., Semsar, K., Godwin, J., 2003. Fluoxetine treatment decreases territorial aggression in a coral reef fish. Physiol. Behav. 79, 719–724.
Sanderson, H., Johnson, D.J., Wilson, C.J., Brain, R.A., Solomon, K.R., 2003. Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECOSAR screening. Toxicol. Lett. 144, 383–395.
Stehly, G.R., Hayton, W.L., 1990. Effect of pH on the accumulation kinetics of pentachlorophenol in goldfish. Arch. Environ. Contam. Toxicol. 19, 464–470.
USEPA, 2005. EPI Suite v.3.12. <http://www.epa.gov./opptintr/exposure/docs/episuite.htm>.
USGS, 2007. Real-time data for Trinity River Basin, Texas: water quality. <http://waterdata.usgs.gov/tx/nwis/current> (checked on May 5, 2007).
Yamamoto, H., Liljestrand, H.M., 2004. Partitioning of selected estrogenic compounds between synthetic membrane vesicles and water: effects of lipid components. Environ. Sci. Technol. 38, 1139–1147.
Yamamoto, H., Hayashi, A., Nakamura, Y., Sekizawa, J., 2005. Fate and partitioning of selected pharmaceuticals in the aquatic environment. Environ. Sci. 12, 347–358.

(16) 下水道未普及地域における河川生物膜による直鎖アルキルベンゼンスルホン酸浄化作用の評価

田村　生弥[1]・太田　美菜子[2]・関澤　純[2]・山本　裕史[2*]

[1]徳島大学大学院　人間・自然環境研究科（〒770-8502 徳島県徳島市南常三島町一丁目一番地）

[2]徳島大学　総合科学部　自然システム学科（〒770-8502 徳島県徳島市南常三島町一丁目一番地）

＊ E-mail: hiroshi@ias.tokushima-u.ac.jp

わが国の下水道人口普及率は現在全国平均で約70%となっているが、地域格差が大きく下水道未普及地域の中小河川では未だに水質汚濁が深刻である。河川の自浄作用についてはT-N・T-Pといった栄養塩全体の濃度やBODのような間接的な有機汚濁指標を用いて検討した例はあるが、具体的な化学物質を用いた研究例は少ない。そこで本研究では陰イオン界面活性剤の直鎖アルキルベンゼンスルホン酸(LAS)に着目し、下水道普及率の低い徳島市近郊6河川を対象に3つの季節で生物膜を回収し、自浄作用を検討した。その結果、LAS汚染度が高い河川ほど浄化作用が大きく、LASの浄化作用とNH_4^+-Nとの間には弱い相関が見られた。また生物膜の浄化作用に対する寄与率を計算したところ、下水道未普及地域における中小河川のLAS浄化に対して大きな役割を果たすことが示唆された。

Key Words : *anionic surfactant, biodegradation, biofilm, household effluent, urban streams*

1. はじめに

河川等の水環境の自浄作用を調べる際はこれまで、間接的な有機汚濁指標であるBODやT-N、T-Pといった栄養塩全体の濃度を用いることがほとんどであった[1]。しかしながら、家庭用合成洗剤等に含まれる陰イオン界面活性剤の一種である直鎖アルキルベンゼンスルホン酸(Linear Alkylbenzene Sulfonate: LAS)（図-1）は、PRTR(環境汚染物質排出移動登録)法の第一種指定化学物質に分類されており、平成16年度の推計値によれば、水域への排出量が第一位の約19,000 tと非常に多く[2]、その使用量や水環境中への排出量の多さから様々な調査が行われてきた。最近、わが国でおこなわれた例としては、真名垣らが冬季に全国18の一級河川中のLASの濃度をLC/MS/MSを用いて測定し、淀川や多摩川、石狩川などでは最大で6 μg/L程度であるのに対して、鶴見川や菊川などで最大60 μg/Lという比較的高濃度で検出されており[3]、水棲生物への影響が見られる濃度レベルとも近い[4]ため, その影響が懸念される。周辺の下水道整備状況や流量等さまざまな要素が関連している。また、河川中のLAS濃度も微生物の活動に伴う自浄作用が盛んであると考えられる夏季は低く、逆に低温で活動が弱いと考えられる冬季の方が有意に高いという報告もある[5]。さらに、兵庫県南東部を対象にした調査では、下水道普及率が高いほど河川中の陰イオン界面活性剤濃度が低くなるという調査結果も報告されている[6]。

一方、徳島県は平成18年3月現在、下水道人口普及率が全国平均の70%を大きく下回る11.5%[7]、合併浄化槽等普及率を合わせても38.4%とともに全国最下位である[8]ことから、LASを含む家庭雑排水の一部が河川に未処理のまま放流されている可能性が高い。本研究では、LASの主成分C_{12}-LASを対象物質として、河川微生物に

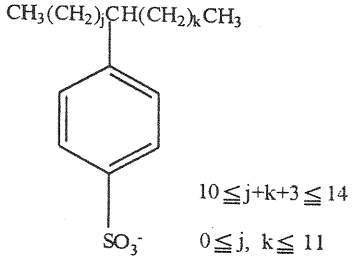

図-1 LASの化学構造