よる浄化能を調べることとした。これまで、行われた河川微生物によるLASの浄化能の定量化に関する検討は、Boeijeら[9]のポリプロピレン担体を用いた水路実験や、Leeら[10]の下水処理放流口の上下流での反応速度定数比較が報告されているものの、いずれも季節による違いや河川の汚染度による違いを十分に検討していない。そこで、本研究では2006年8月から2007年1月にかけて徳島市周辺の下水道未普及地域を流域とする6河川を対象に、3度の季節で相対的比較を主な目的とした。それに加えてBOD、TOC、河川中LAS濃度、NH_4^+-N等の水質項目を生物膜採取中に測定し、LAS浄化能との相関を調べた。また、各河川におけるLAS負荷量をPRTR推計値や流域人口、流量、浄化槽普及率等をもとに推計するとともに、実験室内実験結果をもとに河川での単純なLAS浄化モデルに当てはめ、浄化率を算出した。

2. 河川水質調査及び実験方法

(1) 選定河川

対象は下水道未普及地域を流域とする河川に限定した。また家庭用界面活性剤が直接排出されているおそれの高い流域人口の比較的多い河川と、流域人口が比較的少ない河川の両方を選定することにした。各河川のサンプリングポイントの水深、川幅、流量は冬季に測定しており、それぞれ田宮川が20 cm、2.7 m、0.28 m^3/s、冷田川が25 cm、6.5 m、0.18 m^3/s、多々羅川が60 cm、8.3 m、0.18 m^3/s、打樋川が5 cm、5.3 m、0.027 m^3/s、芝生川が60 cm、10 m、1.2 m^3/s、正法寺川が75 cm、14 m、1.8 m^3/sであった。各河川の位置を図-2に示す。

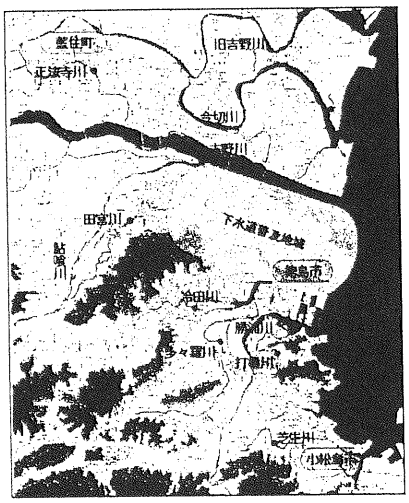

図-2 対象河川と採取地点の周辺地図

(2) 生物膜回収装置

試験管立てに5 mm×20 mm×75 mmのスライドガラスを設置し、漂流物や河川に生息する大型の動物に影響を受けないよう防護ネットで覆った。スライドガラスの向きが河川の流れと平行になるように約3週間川岸から約30cm離れた川底に重石を付けて沈設し、生物膜を回収した。

(3) 添加回収実験

100 mL三角フラスコに試験水を入れ、図-1に示すLASのうちその主成分として知られるC_{12}-LAS（東京化成製標準品）を初期濃度1.0 mg/Lになるように添加した。試験水の量は超純水のみ、河川水のみ、河川水をそれぞれスライドガラスが完全に浸るように130 mLとした。超純水のみ、河川水のみ、河川水に採取した生物膜付着スライドガラスを1枚入れたものの3種類を2連ずつ用意し、河川水はOECDテストガイドラインにおける生分解試験[11]を参考にメンブレンフィルター(孔径=3 μm)でろ過したものを用いた。夏季25 ℃、秋季20 ℃、冬季15 ℃に設定、90 rpmで往復振とうして0、2、6、24、48、72、120時間に蛍光・吸光検出器付HPLCで溶液中のC_{12}-LAS濃度を測定した。また、実際の河川では光分解の影響は生分解に比べて影響は小さいと考えられること[12]、さらに実験系の単純化のために光分解の影響を避け、暗所で振とうした。なお、付着した生物膜量は各季節、各河川ごとに別のスライドガラスを3枚用意し、1枚当たりの乾燥重量を測定した。

(4) 水質項目測定

各季節ごとの生物膜回収装置の沈設期間中で、降雨による影響を大きく受けないと考えられる日を選び、2回ずつ水質を測定した。以下にそれぞれの測定方法を示す。

a) 河川水中のC_{12}-LAS濃度測定[13]

2006/8/17および8/25（夏季）、10/20および11/8（秋季）、12/18および2007/1/11（冬季）の計6回測定した。河川水は採取後4℃で保存しながら速やかに実験室内に持ち帰った。ガラス繊維ろ紙(孔径=1.0 μm)でろ過後、Scp-Pak Plus C18カートリッジに通水したものをメタノールで溶出し、蛍光・吸光検出器付HPLC（島津製作所製LC-10ADVP）で測定した。

b) BOD測定[14]

2006/8/1および8/28（夏季）、10/20および11/9（秋季）、12/18および2007/1/11（冬季）の計6回測定した。採取後速やかに実験室内に持ち帰った河川水に緩衝液、硫酸マグネシウム溶液、塩化カルシウム溶液、塩化鉄溶液を加え、101 mLふらん瓶3連にわけた。一つは15分後、残り

は、5日後にDO計（堀場製作所製）によってDOを測定し、その差をBODとした。

c) TOC測定

2006/8/1および8/28（夏季）、10/20および11/9（秋季）、12/18および2007/1/11（冬季）の計6回測定した。採取した河川水にアジ化ナトリウムを入れ、有機物の分解を阻害し、速やかに実験室内に持ち帰った。これをガラス繊維ろ紙(孔径=0.7 μm)でろ過し、島津製作所社製TOC-5000で測定した。

d) NH_4^+-N測定[14]

2006/10/20および11/9（秋季）、12/18および2007/1/11（冬季）の計4回測定した。採取後速やかに実験室内に持ち帰った河川水をガラス繊維ろ紙(孔径=1.0 μm)でろ過し、インドフェノール青法を用いて測定した。

3. LAS浄化モデル

(1) 河川水や付着微生物膜によるLAS浄化作用の定量化

添加回収実験における河川水中の浮遊微生物やスライドガラス表面への付着微生物膜の量は、実験期間内は一定であると仮定した。この仮定は理論的には正確性に欠けるものの、採取した微生物膜量が基質であると考えられるC_{12}-LASなどの量より過剰であること、実験実施中に生物膜量の時間変化を調べるのが困難であること、この単純化により溶液中のC_{12}-LAS減少を擬似的に下式のように一次反応に当てはめることができるなどの理由による。

$$C/C_o = \exp(-kt) \qquad (1)$$

ここで、C_oはC_{12}-LASの初期濃度、Cは時間t(h)後のC_{12}-LAS濃度、k(1/h)は一次反応速度定数である。本研究ではこのC_{12}-LAS除去の反応速度定数kを算出することで、各河川の各季節でのLAS浄化作用を相対的に比較することとした。本研究では河川水のみのk_wと河川水＋生物膜のk_tの両方を算出した。

(2) 反応速度定数を用いた河川中LAS浄化モデル

まずはじめに、単純に河川水＋生物膜の系での反応速度定数k_tから河川水のみの系での反応速度定数k_wを差し引くことで、生物膜のみによる反応速度定数を求めた。さらに、実験系での河川水量(V_L=130 mL)と生物膜付着面積（スライドガラスの表面積A_L≈16 cm^2）の比率（V_L/A_L≈8.1 cm）を計算し、実際の河川での実測した水深Dおよび川幅Wから河床が平面であると仮定して得られた水量（V_R）と河床面積（A_R）の比（V_R/A_R≈DW/(2D+W)）を計算した。この両者の比によって実験系での付着生物膜のみによる反応速度定数k_bを実際の河川での付着生物膜のみによる反応速度定数k_{br}に換算した。

$$k_{br} = k_b \frac{V_L A_R}{A_L V_R} \qquad (2)$$

そして、河川水のみの反応速度定数k_wとこのk_{br}をもとに、流量や流速など実測データと合わせて生物膜採取地点から1 km先までの浄化率を試算した。ここでは、追加的なLAS負荷や流量・流速の変化、生物膜量（および質）の時間的変化がなく、速度定数k_wとk_w+k_{br}による分解のみを考慮した簡易な押し出し流れモデル(拡散なし)を仮定した。つまり、完全混合槽と考えられる微小区間でのマスバランスは以下のように仮定した。

$$V\frac{dC_{out}}{dx} = (C_{in} - C_{out})Q - kC_{out}V \qquad (3)$$

なお、ここでVは微小区間の体積、C_{in}とC_{out}はそれぞれC_{12}-LAS流入および流出濃度、Qは流量、kはk_wもしくはk_w+k_{br}である。

4. 結果・考察

(1) 各河川・各季節のLAS浄化作用の相対比較

最初に添加回収実験における代表的なC_{12}-LAS濃度の時間変化の例を図-3に示す。図に示すように、初期濃度に対する相対濃度C/C_oは超純水のみのブランクでは減少は緩やかで120時間後でも初期濃度の約7割程度が残存していたのに対して、河川水を入れると120時間後には初期濃度の10%未満に、さらに生物膜付スライドガラスを入れると48時間後までに初期濃度の10%未満になった。

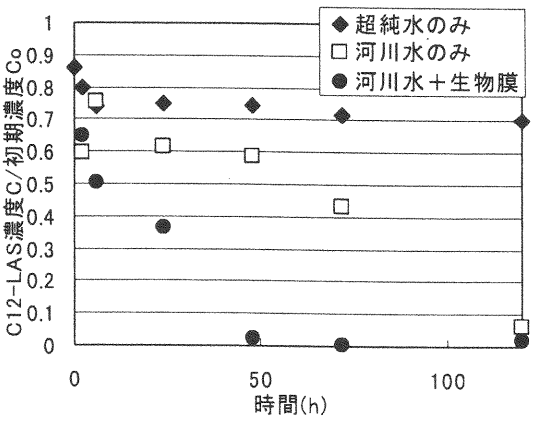

図-3 C_{12}-LAS濃度時間変化(冬季　田宮川)

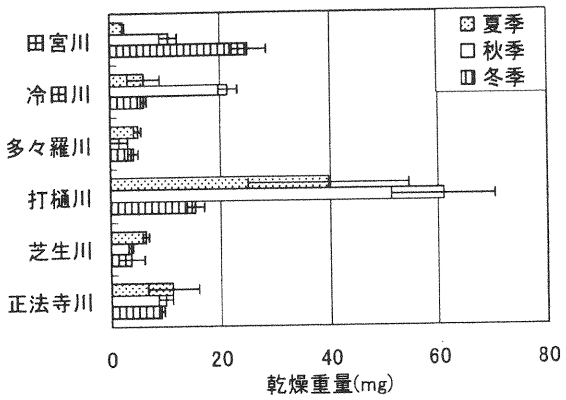

図-4 採取した生物膜量の平均値

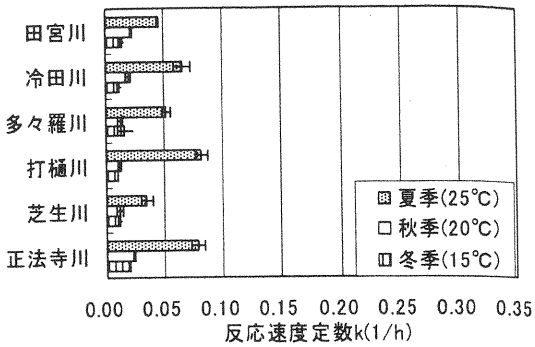

図-5 C_{12}-LAS減少の一次反応速度定数 k_w
n=2(河川水のみ)

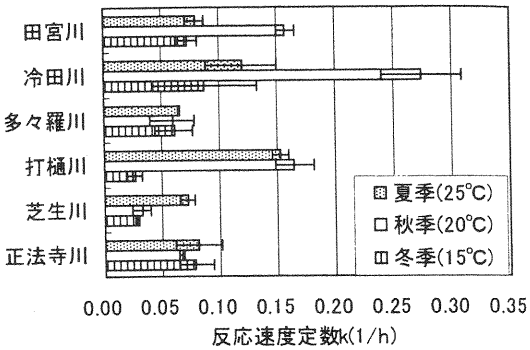

図-6 C_{12}-LAS減少の一次反応速度定数 k_t
n=2(河川水＋生物膜)

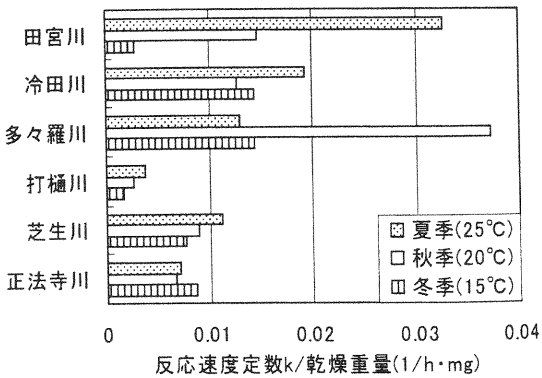

図-7 各河川の生物膜量あたりの反応速度定数

そのため、この時間変化を元に一次反応速度定数であるk_wやk_tをフィッティングにより推定した。なお、生物膜を導入した場合の濃度減少は早く、2、6、24時間での測定では反応速度定数が不正確になることも推測される。しかし、2連で複数河川を同時に実施している上、HPLCでの測定を速やかに実施するにはこれ以上測定を増やすことはできないので、本研究では複数河川、季節の相対比較が主な目的であることから全て同様の測定時間とすることにした。

各河川で回収したスライドガラスの乾燥重量による生物膜量の平均値(n=3)を図-4に示す。ここで、エラーバーは標準偏差を表している。図からわかるように、打樋川を除き各スライドガラスあたり約10 mg程度の生物膜が付着しており、河川、季節ごとに多少のバラつきがあることがわかる。やはり、河川による気候条件、流量や水深などの違いにより、付着微生物膜の量や質にも変化があることが示唆される。また、相対標準偏差も50%程度あるものもあり、設置場所する場所のわずかな違いによって生物膜付着量が異なることから、添加回収実験に使用するスライドガラス（2連で実施）に対する生物膜量にも多少のバラつきがあり、結果の解析を慎重に行うことが求められる。

次に、図-3のような溶液中のC_{12}-LAS濃度の減少から算出した一次反応速度定数k(各2連)の平均値と標準偏差（エラーバー）を図-5、6に示す。河川水のみの図-5では概して温度が高い夏季の方が秋季や冬季よりも速度定数が大きく、LASの分解に関わる河川水中の浮遊微生物の活動も高いと考えられる。一方、生物膜を加えた図-6では秋季が最も大きかった。これは生物膜が多く付着したことに起因すると考えられるため、速度定数を各スライドガラスに付着した微生物の乾燥重量(図-4)で除した値を比較した(図-7)。

図-7では図-5の河川水のみの場合と同様に非常に汚染度が高く、下流部の水門の開閉によって水深の変化が激しかった打樋川を除いて夏季＞秋季＞冬季と温度が高い方が浄化が進みやすい傾向が見られ、これまでの様々な研究結果[5)15)]と一致する。そのため、浮遊微生物同様に付着微生物についても温度が高いほどLASの分解に関わる活動が活発になるものと考えられる。また、表-1に示す河川水質測定値において、汚染度の高い田宮川、冷田川、

打樋川では速度定数は大きく、汚染度の低い多々羅川、芝生川では小さい値となった。

なお、上述したように、本研究では複数河川の同時測定による相対比較を優先したため、0、2、6、24時間で測定するのが技術上限界であり、そのため比較的除去が速いk=0.2を越えるような場合は正確性に欠けるという問題点があった。今後、より正確な値を求める場合は特に除去速度が速い場合は測定間隔を狭める工夫が必要である。また、初期濃度で設定した1 mg/Lという値は、実際の河川中の濃度影響を避けることと、測定感度上の問題から実際に存在する濃度よりもかなり高かった。そのため、実環境中に近い低濃度にそのまま当てはめることはできないことを留意する必要がある。さらに、暗所での実験だったが光の影響についても検討する必要があるなど、今後より詳細な実験条件の検討も課題として残る。

(2) 生物膜量や各水質指標と反応速度定数との相関

表-1に対象河川の各水質項目の各季節を通して測定した値の最大値、最小値、ならびに中央値を示す。概してLAS濃度は流域面積に対して人口が多い河川で濃度が高く、LAS濃度が高い河川ではBODやTOC、NH_4^+-Nの値も高かった。なお、本研究で測定されたC_{12}-LAS濃度はあくまでも蛍光検出器付HPLCでの測定であり、詳細な同定作業を経ていない予備的なデータである。しかしながら、試行的に最近の全国一級河川でのLAS検出濃度報告[3]と比較すると、芝生川、多々羅川、正法寺川では数μg/Lで淀川・多摩川などと同程度、冷田川、田宮川、打樋川では菊川や鶴見川と同程度か1から2オーダー程度高い値であることがわかった。LAS濃度とBOD、TOC、NH_4^+-N濃度を総合して考えると、田宮川、冷田川、打樋川の3 河川はともに汚染度が高く、次いで正法寺川、多々羅川、芝生川という順であった。

図-7とは視点を変えて、各季節における付着微生物膜量の平均値と生物膜付スライドグラスを導入した際の反応速度定数との相関を調べた（図-8）。図-8に示すように、打樋川はやや例外であったが、その他の5河川については正の相関(r=0.93)が見られ、付着微生物膜量がLAS浄化作用の大小に大きく影響を与えることが示唆される。

次に、秋季2回の測定の河川中LAS濃度、BOD、TOC、NH_4^+-Nの平均値と河川水のみの反応速度定数k_w、ならびに生物膜を加えた際の反応速度定数k_tとの相関を調べた。

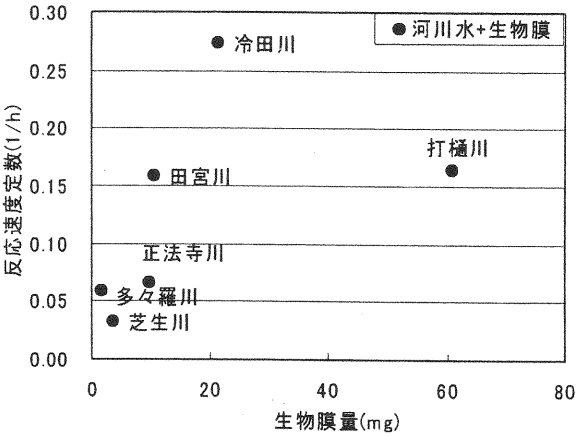

図-8 付着生物膜量と反応速度定数 k_t の相関(秋季)

表-1 河川の水質測定結果と流域人口・面積・汚水処理普及率

	田宮川	冷田川	多々羅川	打樋川	芝生川	正法寺川
LAS(mg/L)	0.0023-0.16 〔0.080〕	0.015-0.084 〔0.035〕	N.D.-0.010 〔0.0046〕	0.12-0.49 〔0.25〕	N.D.-0.0094 〔0.0017〕	0.0060-0.0815 〔0.0089〕
BOD(mgO_2/L)	1.5-4.7 〔3.7〕	0.92-3.0 〔2.2〕	N.D.-1.1 〔0.75〕	1.8-4.1 〔3.6〕	N.D.-1.1 〔0.20〕	1.7-6.8 〔2.2〕
TOC(mgC/L)	0.96-8.6 〔4.9〕	1.2-5.9 〔4.3〕	0.43-3.6 〔2.5〕	4.5-10 〔7.1〕	1.8-4.5 〔2.2〕	2.6-6.3 〔3.7〕
NH_4^+-N(mgN/L)	1.6-3.8 〔2.9〕	0.44-1.3 〔0.84〕	0.11-0.25 〔0.18〕	2.8-5.5 〔3.7〕	N.D.-0.46 〔0.095〕	0.57-1.01 〔0.74〕
推定流域面積(km^2)	2.7	2.7	5.5	1.0	1.6	3.0
推定流域人口(人)	8,400	12,000	5,000	2,000	2,400	5,200
流域合併浄化槽普及率[a]	31.8%	31.8%	31.8%	31.8%	16.8%	29.7%
流量(m^3/s)[b]	0.28	0.18	0.18	0.027	1.2	1.8

〔〕内は中央値、N.D.: 検出限界 (LAS 0.0012 mg/L、BOD DO変化率10%未満、NH_4^+-N 0.08 mgN/L)未満
[a]合併浄化槽普及率は環境省の水洗化人口資料[10]をもとに正法寺川は藍住町、芝生川は小松島市、その他4河川は徳島市の下水道未普及地域の平均値を採用、[b]流量は冬季に2回測定した平均値

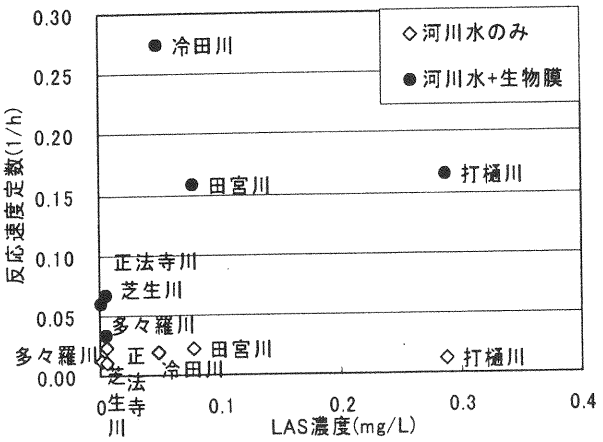

図-9 LAS濃度と反応速度定数の相関(秋季)

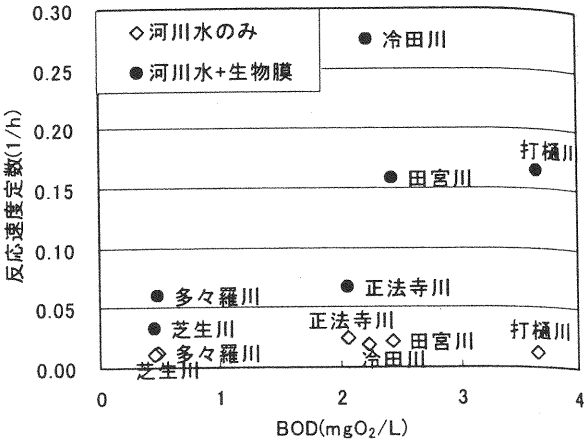

図-10 BODと反応速度定数の相関(秋季)

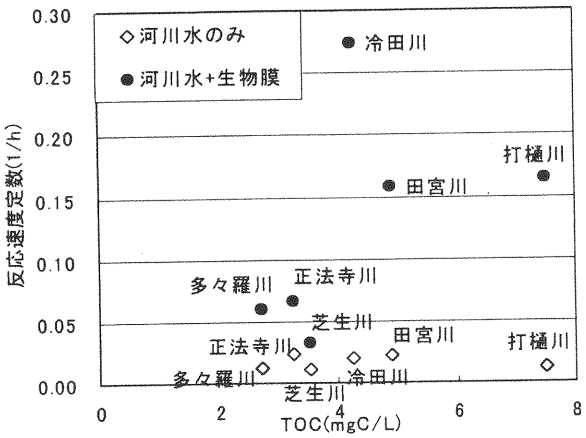

図-11 TOCと反応速度定数の相関(秋季)

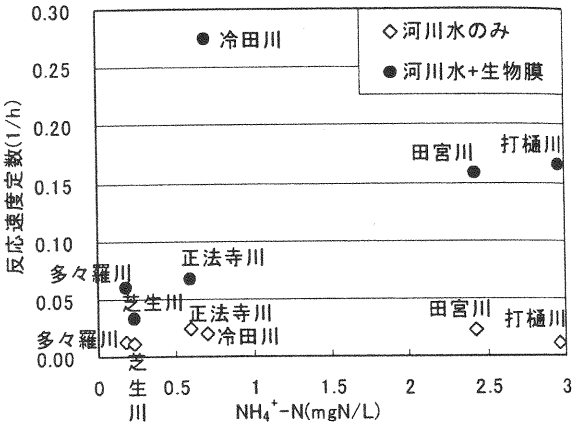

図-12 NH_4^+-Nと反応速度定数の相関(秋季)

その結果をそれぞれ図-9から12に示す。

図-9より汚染度の特に高い打樋川を除いてはLAS濃度と反応速度定数に弱いながら正の相関(r=0.73)が見られた。LAS濃度が高い河川では微生物群が馴化されることでLASを分解する微生物種が増殖しやすい状態になっていると考えられ、これまでの研究結果[10]とほぼ一致している。図-10、11に関しても、打樋川を除いて弱い正の相関(r=0.70、0.68)が見られた。図-12については汚染度の高い打樋川、田宮川を除き相関(r=0.74)が見られた。これは一定量のNH_4^+-NはLASの分解を促進するという報告[17]とほぼ一致した。LASが微生物の炭素(エネルギー)源としてはたらく一方で、ある程度のNH_4^+-Nは窒素源としてLASの分解に不可欠であると考えられる。

いずれにせよ、本研究では各水質項目との間に弱い相関が見られたものの、各季節の測定回数は2回に過ぎず、生物膜回収装置を沈設している3週間絶えず変化している水質を代表しているとは限らない。また、河川水のみと生物膜を加えたものを同時に解析するために、生物膜量あたりの反応速度定数に換算するべきであるという考え方もある。さらに、3週間の中には降水の影響で生物膜が剥離して質的にも量的にも変化した可能性もある。以上の理由から、これらの水質指標や乾燥重量と反応速度定数の相関関係を深く議論するには十分な結果とは言えず、今後経時的なきめ細かい水質測定や生物膜の質・量的な把握など詳細な検討を要する。

(3) PRTR推計値によるLAS負荷推定

次に、対象とした河川の流域が下水道未普及であることから、PRTRの水系への排出量推計値から下水道や合併浄化槽普及地域では処理によって排出量がゼロと仮定して未普及地域一人当たりの排出量を算出した。この値に表-1に示した合併浄化槽普及率、炭素鎖の違う同族体のうちC_{12}の比率を30%[18]と仮定し、それぞれの河川へのC_{12}-LAS負荷量を算出した。2007年1月に実測した流量を合わせて家庭等から排出後に分解がないと仮定してC_{12}-LAS濃度推計値を求め、冬季の実測値と比較した(表-2)。

表-2 河川中LAS濃度の実測値と推計値の比較(冬季)

	C_{12}-LAS 実測値(mg/L)	C_{12}-LAS 推計値(mg/L)
田宮川	0.0023-0.16	0.75
冷田川	0.038-0.071	0.17
多々羅川	0.0050-0.010	0.072
打樋川	0.22-0.49	0.18
芝生川	0.0019.-0.0028	0.0081
正法寺川	0.022-0.081	0.011

表-3 採取地点から1 km流下する際の推計LAS浄化率(冬季)

	河川水+生物膜	河川水のみ
田宮川	24%	13%
冷田川	6.2%	2.7%
多々羅川	14%	11%
打樋川	6.7%	2.7%
芝生川	1.7%	1.5%
正法寺川	3.8%	3.2%

その結果、打樋川を除くすべての河川で推計値の方が高かった。これは各家庭等から排出後に河川を流下する過程でC_{12}-LASの一部が分解を受けていることが主な原因であり当然の結果と考えられる。また、同じ流域面積であるが流域人口が多い冷田川の方が田宮川よりLAS濃度が低いのは、上流部で別の河川から取水しており流量が多い冷田川の方が希釈効果が大きいためであると推察される。

(4) 反応速度定数による河川中でのLAS浄化率推定

最後に、最も浄化率が低く汚染が深刻であると考えられる冬季の反応速度定数kを用いて、上述したLAS浄化モデルに当てはめて試行的に算出した浄化率を表-3に示す。汚染度の高い田宮川、打樋川、冷田川で浄化率はそれぞれ24%、6.7%、6.2%と比較的高く、生物膜を考慮すると河川水のみの2倍以上になった。河川でのLASの分解には浮遊微生物ではなく付着微生物の作用の方が大きいことが示唆される。しかしこの3河川は他の河川に比べ、連続的なLAS負荷量が多いと考えられるため、この浄化率では十分であるとはいえない。また、このモデルは非常に単純で河川形状や付着生物膜量の変化を考慮していないため正確とはいえない。今後、実測値との比較やより実際に近づけるためのパラメーター設定などが必要であることは言うまでもない。

5 結論

汚濁負荷が高いと考えられる田宮川、冷田川、打樋川でC_{12}-LAS分解の一次反応速度定数が大きく、汚濁負荷の少ない多々羅川、芝生川では反応速度は小さかった。河川水のみでも河川水に生物膜を加えたものでも、概して温度が高いほうが反応速度が大きい傾向が見られた。河川水中の浮遊微生物に加え、付着微生物もC_{12}-LASの分解に対する寄与率は高いと考えられる。生物膜量、河川中LAS濃度、TOC、BOD、NH_4^+-Nにはそれぞれ反応速度定数と弱い正の相関が見られた。

謝辞:本研究は徳島大学研究連携推進機構に対する徳島大学学長裁量経費の助成を受けて実施いたしました。また、TOC測定等でご協力いただいた徳島大学ソシオテクノサイエンス研究部上月康則教授にもこの場を借りて感謝申し上げます.

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(2007.5.25 受付)

Biodegradation of linearalkylbenzensulfonate by riverine biofilm in the area of no sewage service coverage

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Although the recent sewage service coverage in Japan is approximately 70% based on national population, regional variance is still large and water pollution is still public concern in small rivers and streams in no sewage service coverage area. Since details of biodegradation in urban streams have long been focused on indirect water quality indices such as T-N, T-P, and BOD, few studies have been focused on individual pollutants. In this research, therefore, linear alkylbenzensulfonate (LAS), a popular anionic surfactant, was selected as a target compound. Tokushima Prefecture is known as the lowest sewage coverage in Japan, and we selected six streams of suburban Tokushima city with little sewage coverage, and collected biofilm in three seasons, and the biodegradation rate was investigated. As results, the riverine biofilm sampled from the highly LAS- or NH_4^+-N contaminated streams showed relatively higher biodegradation rate.. In addition, our estimation of the contribution of biofilm to the biodegradation of LAS is relatively higher than suspended bacteria, which suggests the biofilm plays important role in the degradation of LAS in the highly contaminated urban streams with no sewage service coverage.

ES 659

Initial Ecological Risk Assessment of Eight Selected Human Pharmaceuticals in Japan

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(Received December 18, 2006; accepted May 14, 2007)

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Key words: pharmaceuticals, initial risk assessment, aquatic organisms, predicted no effect concentration

Eight pharmaceuticals were selected on the basis of their domestic consumption in Japan, the excretion ratio of the parent compound and the frequency of detection in the aquatic environment or wastewater treatment plant effluent. Toxicity tests on these pharmaceuticals were conducted using Japanese medaka (*Oryzias latipes*), daphnia (*Daphnia magna*), and green algae (*Psuedokirchneriella subcapitata*). Predicted no effect concentration (PNEC) was calculated using lethal or effect concentration 50 (LC_{50} or EC_{50}) values and no effect concentration (NOEC) obtained in the toxicity tests for these compounds. Predicted environmental concentration (PEC) was also calculated from annual consumption, the excretion rate of the parent compound, and removal rate in the preliminary batch activated sludge treatment performed in this study. Maximum concentrations found in the aquatic environment or sewage effluent in Japan or foreign countries were also used for another calculation of PEC. Initial risk assessment on the selected pharmaceuticals was performed using the PEC/PNEC ratio. The results of initial risk assessment on the eight selected pharmaceuticals suggest neither urgent nor severe concern for the ecological risk of these compounds, but further study needs to be conducted using chronic toxicity tests, including reproduction inhibition and endocrine disruption assessments.

1. Introduction

While the consumption of human pharmaceuticals has been continuously growing together with the rapid aging in developed countries including Japan,[1] these chemical compounds have started to attract attention as aquatic micropollutants that might have been affecting the ecological system since the late 1990s.[2–4] Pharmaceuticals are designed and manufactured to have certain physiological effects on humans (and veterinaries) and safety on mammals (and other animals) should be confirmed before they

can become commercially available. These pharmaceuticals are excreted through urine as an unaltered or altered form with or without metabolism, and later released into the aquatic environment. However, the effects of these released contaminants on aquatic organisms have been investigated only from the late 1990s. In the EU, the monitoring of pharmaceuticals in the aquatic environment and the removal rate of these compounds in drinking and sewage water processes has been extensively conducted under the EU-POSEIDON project,[5] whereas the European Medical European Agency for the Evaluation of Medical Products (EMEA) has started to assess the environmental risk of some human pharmaceuticals.[6,7] In the US, the results of a large-scale monitoring of pharmaceuticals in 139 sites by the USGS[8] and the detection of an antidepressant fluoxetine from fish[9] provoked public concern, and thus, a large-scale project on pharmaceuticals deposited in the environment had been directed by the USEPA.[10] The conventional acute and chronic toxicities of pharmaceuticals have also been examined by several researchers and a review has recently been published.[11] From the results of these toxicity tests, detections from the effluent of a wastewater treatment plant (WWTP) or surface water, predicted no effect concentration (PNEC) and predicted environmental concentration (PEC) were determined for several pharmaceutical compounds. Consequently, the initial ecological risk of some pharmaceuticals has been assessed by calculating the PEC/PNEC ratio by several researchers in Europe[12–15] and a low human risk of pharmaceuticals through drinking water and fish consumption was suggested by American researchers of pharmaceutical companies.[16]

In Japan, pharmaceuticals in the aquatic environment have recently started to attract researchers, and the Journal of the Japan Society on Water Environment published a special edition on this topic[17] in April 2006. According to the articles in the issue and other proceedings, the monitoring of pharmaceuticals in the aquatic environment has been started by several researchers mainly around the Tokyo metropolitan area since 2001, and the maximum detected concentration from sewage effluent and river water is approximately 0.3 μg L^{-1}.[18,19] On the other hand, few researchers have investigated the toxic effects of these compounds except for those of triclosan[20] and other antibiotics.[21] Overall, initial risk assessment and screening were conducted by Iwane *et al.*[22] for 87 human pharmaceuticals with large domestic consumption. They concluded that eight compounds should be further investigated for their environmental risk on the basis of their persistence in the environment and the comparison of their PEC/PNEC ratios. Experimental toxicity data for these compounds were, however, not sufficient at the time of their screening test and they mainly used ecological structure activity relationship (ECOSAR) to estimate the acute/chronic toxicity of the pharmaceuticals. For those with available experimental toxicity data, they found that some chemicals showed more than one order of magnitude difference between the estimated and experimental values. Additionally, they estimated the bioconcentration factor (BCF) using the software BCFWIN, which apparently fails to estimate precisely the BCF values for human pharmaceuticals usually ionized at neutral pH.[23,24] As far as PEC is concerned, they used two methods, the estimation from domestic consumption and the excretion ratio of the parent compounds and that from the concentration detected outside Japan. Furthermore, the removal efficiency in a WWTP was not taken into consideration by assuming the worst-case scenario, which could cause an overestimation of the risk.

Based on the background described above, we selected eight human pharmaceuticals with high domestic consumption, high excretion ratio of an unaltered form, the number/maximum detected concentration reported by other researchers, the high PEC/PNEC ratios reported by other researchers outside Japan, and the relatively higher PEC/PNEC ratios obtained by Iwane *et al.*[22] Acute toxicities for fish (*Oryzias latipes*), daphnia (*Daphnia magna*), and green algae (*Pseudokirchneriella subcapitata*) were examined using international standard methods (i.e., Organisation for Eco-

nomic Co-operation and Development (OECD) guidelines for testing of chemicals) and PNEC was recalculated. The bioconcentrations of the selected pharmaceuticals were reevaluated using the synthetic membrane vesicles (liposomes)/water system, which has been proven to better simulate the bioconcentration system. Additionally, the removal efficiency of the selected pharmaceuticals in the WWTP was roughly estimated by batch activated sludge treatment, and PEC was also recalculated. By using the recently detected concentrations of the selected pharmaceuticals, initial ecological risk was more accurately assessed in this study.

2. Materials and Methods

2.1 *Materials*

The pharmaceuticals used in this study include three nonsteroidal anti-inflammatory drugs (NSAIDs), ibuprofen, indomethacin, and mefenamic acid; an analgesic agent, acetaminophen; two β-blockers (antihypertension drugs), atenolol and propranolol; an N-methyl-D-aspartate (NMDA) receptor antagonist, ifenprodil, and an antiepileptic, carbamazepine. Acetaminophen (97%), atenolol (98%), ibuprofen (98.5%), ifeprodil tartate (98.5%), indomethacin (98%), and mefenamic acid (99%) were purchased from Wako Pure Chemical Co. (Osaka, Japan), whereas carbamazepine and propranolol (99%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Palmitoyl-oleoyl phosphatidylcholine (POPC), a phospholipid used to make liposomes described below, was purchased from Nippon Fine Chemical Co. (Osaka, Japan).

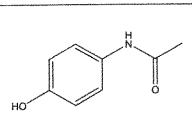
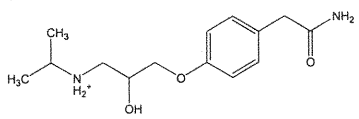
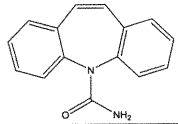
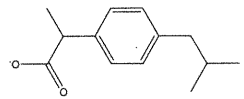
Chemical structure, domestic consumption in Japan, the excretion ratio of an unaltered form, octanol-water distribution constant (log D_{ow}), acidity constant (pK_a), and the PEC/PNEC ratio reported by Iwane *et al.*[22] are shown in Table 1. Domestic consumption was obtained from the "Survey of Pharmaceutical Industry Productions"[25] edited by the Japan Ministry of Health, Labor and Welfare if available. Otherwise, annual sales listed in "Pharma Japan 2006 Handbook"[1] were divided by drug price listed in "Today's Drug 2005"[26] to determine the annual domestic consumption in Japan. The excretion ratio of an unaltered form was from the attachment form of each pharmaceutical company. Log D_{ow} and pK_a values were estimated using the ACD software log D suite.

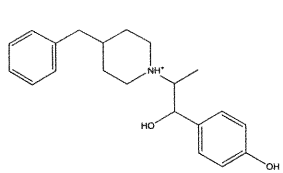
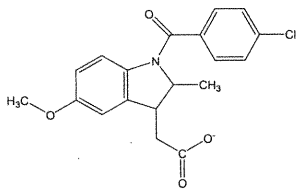
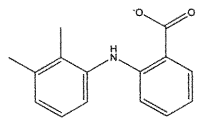
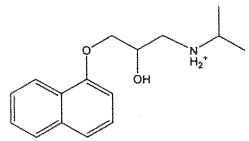
2.2 *Procedure of batch activated sludge treatment*[27]

The preliminary batch activated sludge treatment test was conducted in a 100-ml Erlenmeyer flask capped with parafilm. Activated sludge was sampled from a WWTP using the conventional activated sludge process and used for the batch experiments immediately after sampling. The concentration of the mixed liquor suspended solid (MLSS) was set at 2,000 mg L^{-1}, and the initial concentration of each pharmaceutical was set at 100 μg L^{-1} in a total volume of 100 ml. The mixed liquor was aerated by an aeration pump at an air flow rate of 2.0 L min^{-1} in the dark at 25°C for a typical hydraulic retention time of 6 h. No additional food was added during the batch experiments.

After the reaction time of 6 h, the mixed liquor was centrifuged at 2,500 rpm in 10-ml amber glass centrifuge tubes. The supernatant was filtered through a 0.2 μm pore size membrane filter (OMNIPORE membrane, Millipore Co., Billerica, MA) and analyzed by high-performance liquid chromatography (HPLC), as described below. For the residue after the centrifugation, the supernatant was carefully decanted and acetonitrile was added. After 5 min of sonication, the extract was filtered through a membrane filter, and diluted with Milli Q water 10 times before the analysis by HPLC. Samples with instantaneous contact of pharmaceuticals with activated sludge were prepared for all selected compounds, and the fraction sorbed to the activated sludge at 6 h was corrected by these recoveries.

Table 1
Pharmaceuticals selected in this study.

Pharmaceutical	Acetaminophen	Atenolol	Carbamazepine	Ibuprofen
Domestic consumption	1027 t[(a)]	5.4 t[(b)]	45 t[(a)]	107 t[(a)]
Excretion ratio of unaltered form[(c)]	0.9–2.7%	90%	2–3%	<1%
Chemical structure				
log D_{ow}[(d)] (at pH 7)	0.34	–2.02	2.67	1.16
pK_a[(d)]	1.72, 9.86	9.16, 13.88	–0.49, 13.94	4.41
Reported PEC/NEC	0.057[(e)]	0.7[(f)]	0.23[(e)]	0.043[(e)]

Pharmaceutical	Ifenprodil	Indomethacin	Mefenamic acid	Propranolol
Domestic consumption	8.6 t[(a)]	86 t[(a)]	41 t[(a)]	1.3 t[(a)]
Excretion ratio of unaltered form[(c)]	20–30%	64%[(g)] 0.08–0.1%[(h)]	74%	<1%
Chemical structure				
log D_{ow}[(d)] (at pH 7)	1.97	0.14	2.42	1.00
pK_a[(d)]	9.34, 9.99	3.96	–1.31, 3.73	9.14, 13.84
Reported PEC/PNEC	0.20[(e)]	0.28[(e)]	0.65[(e)]	0.08[(f)]

[(a)]From ref. 25, [(b)]calculated from annual sales shown in ref. 1 and drug price shown in ref. 26, [(c)]cited from attachment form of each pharmaceutical compound issued by pharmaceutical industry, [(d)]predicted by log D Suite (ACD software), [(e)]from ref. 22, [(f)]from ref. 53, [(g)]used as oral medicine, [(h)]used as topical medicine.

The concentration of the pharmaceuticals was determined by HPLC. The HPLC system used was an LC-10AD VP series (Shimadzu, Kyoto, Japan) equipped with an ODS column (Shimpack VP-ODS, Shimadzu, Kyoto, Japan) and both a fluorescence (RF-10A XL, Shimadzu, Kyoto, Japan) detector and a UV/visible absorbance (SPD-10A VP, Shimadzu, Kyoto, Japan) detector. In order to avoid preventive peaks and noise originating from activated sludge, sludge blanks with no pharmaceuticals were prepared for all HPLC analytical conditions and no preventive peak/noise near the retention time of the selected pharmaceuticals was confirmed.

2.3 *Procedure of toxicity tests*

2.3.1 *Fish acute toxicity test*

Fish acute toxicity tests were conducted using Japanese medaka (*Oryzias latipes*) bred at the National Institute for Environmental Studies (Tsukuba, Japan) and acclimated in a laboratory of The University of Tokushima for at least two months. Tests were conducted in conformity with the "OECD Test Guidelines for Testing of Chemicals No. 203"[28] and the Test Guideline for Chemicals by the Japan Ministry of the Environment.[29] Briefly, 10 approximately 10-day-old fish were exposed to at least six different concentrations of the pharmaceuticals in a 100-ml beaker. Half of the pharmaceutical solution was replaced every 24 h (semistatic test), and lethal concentration 50 (LC_{50}) was determined.

2.3.2 *Daphnia acute immobilization test*

Daphnia magna provided by the National Institute for Environmental Studies (Tsukuba, Japan) was used for the acute immobilization tests after at least two months of acclimation in a laboratory of The University of Tokushima. Tests were conducted in conformity with the "OECD Test Guidelines for Testing of Chemicals No. 202"[30] and the Test Guideline for Chemicals by the Japan Ministry of the Environment.[29] Briefly, 20 less than 24-h-old daphnid larvae (five larvae per beaker) were exposed to at least six different concentrations of the pharmaceuticals in 50-ml beakers. The number of immobilized bodies was counted after 48 h of exposure and the effect of median effective concentration 50 (EC_{50}) was determined.

2.3.3 *Algal growth inhibition test*

Pseudokirchneriella subcaptata was purchased from the National Institute for Environmental Studies (Tsukuba, Japan) (NIES-35) and was acclimated for at least one month in a laboratory of The University of Tokushima before the exposure tests. Tests were conducted in conformity with the "OECD Test Guideline for Testing of Chemicals No. 201"[31] and the Test Guideline for Chemicals by the Japan Ministry of the Environment.[29] Briefly, a preincubated algal suspension was exposed to at least five different concentrations of the pharmaceuticals in 100-ml Erlenmeyer flasks in AAP medium[29] at 24°C with illumination controlled at 5,000 Lux. The number of algae was measured every 24 h during the 96-h exposure using a UV/visible spectrophotometer at 450 nm after calibration with algal counts. EC_{50} was calculated by linear correlation at a log-normalized plot. Maximum no effect concentration (NOEC) was also determined for the selected pharmaceuticals.

2.3.4 *Prediction using ECOSAR*

The acute and chronic toxicities of the chemical compounds can be predicted using the software ECOSAR v0.99h as part of the EPI suite, freely downloadable from the USEPA.[32] Fish 96-h LC_{50}, Daphnia 48-h EC_{50}, and Algal 96-h EC_{50} and the arithmetic mean of NOEC and the lowest effect concentration (ChV) for the eight selected pharmaceuticals were all estimated with the mode of "The Other Compounds" which excluded inorganic compounds, organometallics, dyes, polymers, and surfactants.

2.4 *Procedure of measuring liposome/water partition coefficient*

The partition coefficient of the pharmaceuticals between liposomes and water was determined using equilibrium dialysis developed by Escher and Schwarzenbach,[23] later modified by Yamamoto and Liljestrand.[33] A liposome suspension was prepared from POPC using thin film hydration,[34] followed by an extrusion process.[35] The final aqueous concentration of the pharmaceuticals was determined by HPLC, and the partition coefficient was determined using initial and final pharmaceutical concentrations, and the concentration of liposome. The total organic carbon (TOC) concentration of the membrane vesicle suspension was measured using a TOC analyzer (TOC-5000, Shimadzu, Kyoto, Japan).

2.5 *Procedure of initial ecological risk assessment*

PNEC was calculated on the basis of experimental EC_{50} and LC_{50} for three acute tests and NOEC for the algal growth inhibition test assumed as a chronic test. $PNEC_{exp}$ was determined by dividing these experimental EC_{50}/LC_{50} or NOEC values by an assessment factor as follows:

$$PNEC_{exp} = \text{minimum of } \frac{\text{minimum of } EC_{50}/LC_{50} \text{ in three acute tests}}{AF_{acute\text{-}3}} \quad \text{and} \quad \frac{\text{algal NOEC}}{AF_{chronic\text{-}1}}, \tag{1}$$

where $AF_{acute\text{-}3}$ is the assessment factor for at least one acute EC_{50}/LC_{50} from each of three trophic levels of the base set (fish, daphnia, and algae), and $AF_{chronic\text{-}1}$ is that for one chronic NOEC. Several sets of assessment factors have been proposed by several organizations (Table 2).[36] In the present study, an assessment factor of 100 was used for both the minimum of the acute results ($AF_{acute\text{-}3}$) and NOEC of the algal growth inhibition test ($AF_{chronic\text{-}1}$) on the basis of the "Initial Ecological Risk Assessment Guidelines for Chemicals,"[37] as directed by the Japan Ministry of the Environment.

Table 2
Proposed assessment factors for application to aquatic toxicity data for estimating PNEC.[36,37]

Available information applied	Assessment factor applied to lowest value			
	Japan Ministry of Environment	OECD Workshop	EU Technical Guidance Document	ECETOC Proposal
One acute LC_{50}/EC_{50} for acute toxicity from one trophic level	1000	1000	—	—
Two acute LC_{50}/EC_{50} from species representing two trophic levels (two of fish, daphnia and algae)		—	—	—
One acute LC_{50}/EC_{50} from each of three trophic levels (two of fish, daphnia and algae) ($AF_{acute\text{-}3}$)	100	100	1000	200
One chronic NOEC ($AF_{chronic\text{-}1}$)	100	—	100	—
Two chronic NOECs from species representing two trophic levels (two of fish, daphnia and algae)		—	50	5
Chronic NOECs from at least three species (normally fish, daphnia, and algae) representing three trophic levels ($AF_{chronic\text{-}3}$)	10	10	10	

ECETOC: European Centre for Ecotoxicology and Toxicology of Chemicals

$PNEC_{ECOSAR}$ was also determined from the ECOSAR prediction as follows:

$$PNEC_{ECOSAR} = \text{minimum of } \frac{\text{minimum of three } EC_{50}/LC_{50} \text{ predictions}}{AF_{acute\text{-}3}}$$

$$\text{and } \frac{\text{minimum of three NOEC/ChV predictions}}{AF_{chronic\text{-}s}}, \quad (2)$$

where $AF_{chronic\text{-}3}$ is the assessment factor for at least one chronic NOEC/ChV from each of the three trophic levels of the base set (fish, daphnia, and algae). As for $PNEC_{exp}$, 100 and 10 were used for $AF_{acute\text{-}3}$ and $AF_{chronic\text{-}3}$, respectively, on the basis of the "Initial Ecological Risk Assessment Guidelines for Chemicals,"[37] as directed by the Japan Ministry of the Environment (Table 2).

Firstly, PEC for WWTP effluent (PEC_{eff}) and that for surface water (PEC_{sw}) were each estimated using three different procedures. One ($PEC_{conseff}$ or PEC_{conssw}) is the estimation from domestic consumption in Japan, the excretion ratio of an unaltered form (assumed as 1% for those with less than 1%), the total volume of domestic wastewater in Japan, and the removal rate in the activated sludge treatment (assumed as 1% for those with less than 1%) obtained in this study as follows:

$$PEC_{cons} = \frac{A_{dom} \times U \times R}{V_{dom} \times D}, \quad (3)$$

where A_{dom} is the annual consumption of the pharmaceutical in Japan (kg $year^{-1}$), U is the unaltered excretion ratio (%) (all indomethacin was assumed as oral intake), R is the measured removal efficiency in the activated sludge treatment (%), Vdom is the volume of wastewater per year in Japan (m^3 $year^{-1}$), and D is the dilution factor. The dilution factor was 1 for WWTP effluent ($PEC_{conseff}$) and 10 for surface water (PEC_{conssw}), which is recommended by EMEA[7] and is also widely accepted as the relationship between environmental standard and effluent standard in Japan.

Secondly, the estimation from the concentration detected outside Japan was carried out using the equation as follows:

$$PEC_{inteff} = \text{maximum of } \frac{C_{inteff} \times A_{dom} \times P_{int}}{A_{int} \times P_{dom}}, \quad (4)$$

$$PEC_{intsw} = \text{maximum of } \frac{C_{intsw} \times A_{dom} \times P_{int}}{A_{int} \times P_{dom}} \text{ and } \frac{C_{inteff} \times A_{dom} \times P_{int}}{A_{int} \times P_{dom} \times D}, \quad (5)$$

where C_{inteff} and C_{intsw} are the maximum concentrations of the pharmaceutical detected in WWTP effluent and surface water, respectively, outside Japan,[2–4,8,12,13,38–43] A_{int} is the annual consumption of the pharmaceutical in the country where it was detected (kg $year^{-1}$), and P_{dom} and P_{int} are the populations in Japan and the country where it was detected. If A_{int} is unavailable, C_{inteff} or C_{intsw} is directly used as PEC_{inteff} or PEC_{intsw}, respectively. A dilution factor of 10 is used for eq. (5).

Thirdly, the estimation from the concentration detected in Japan was carried out as follows:

$$PEC_{domeff} = \text{maximum of } C_{domeff} \text{ and } (C_{dominf} \times R), \quad (6)$$

$$PEC_{domsw} = \text{maximum of } C_{domsw} \text{ and } (C_{domeff} \div D) \text{ and } (C_{dominf} \times R \div D), \quad (7)$$

where C_{domeff}, C_{dominf}, and C_{domsw} are the maximum concentrations of the pharmaceuti-

cal detected in WWTP effluent,[18,19,44] raw wastewater (or WWTP influent),[45] and surface water,[18] respectively, in Japan. Again, a dilution factor of 10 is used for eq. (7).

These PEC/PNEC ratios were used to assess the ecological risk of the selected pharmaceuticals. According to the Japan Ministry of Environment's "Initial Ecological Risk Assessment Guidelines for Chemicals,"[37] if the PEC/PNEC ratio is larger than 0.1, further environmental risk assessment is necessary. As far as bioaccumulation is concerned, those compounds with a liposome/water partition coefficient larger than 1000 can have a bioconcentration factor larger than 1000, which is used as a criterion under the Chemical Substances Control Law for newly registered chemicals other than pesticides and pharmaceuticals in Japan.

3. Results

3.1 *Efficiency of removal in batch activated sludge treatment*

The results of the 6-h preliminary batch activated sludge treatment are summarized and shown in Table 3. Removal efficiency is equal to the summation of the sludge phase (i.e., sorbed fraction) and the unknown fraction (i.e., transformed or unextractable).

As can be seen from Table 3, the removal efficiencies of acetaminophen and ibuprofen were both relatively high and approximately 96%. The sludge phase was under the detection limit for both compounds, and the unknown fraction was nearly 95%, which is possibly biologically transformed. This trend is in agreement with our previous results[27] conducted under slightly different experimental conditions in terms of MLSS concentration and aeration. Moreover, the results are in good agreement with those of other researchers who compared the concentration of these pharmaceuticals in the influent and WWTP effluent.[3,14,19,44,46] These results suggest that environmental loading is significantly decreased by wastewater treatment for both acetaminophen and ibuprofen.

The removal efficiency of ifenprodil and mefenamic acid was nearly 80%. Their sorbed fractions by activated sludge were relatively low, 8.2 and 17%, and the unknown fractions were 69 and 59% for ifenprodil and mefenamic acid, respectively. No report by other researchers is available for ifenprodil, whereas the efficiency of removal in Swiss WWTP[14] was slightly lower than our experimental results for mefenamic acid. Differences in experimental conditions such as temperature, MLSS concentration, treatment system, and effects of metabolites such as conjugates are the possible reasons behind the higher efficiency shown in this study. Our preliminary results suggest that as high as 20% each of these two compounds in the wastewater influent is possibly released into the environment, but further investigation is necessary in the case of large-scale WWTP with additional treatment such as chlorination.

Table 3
Results of batch activated sludge treatment.

	Aqueous phase	Sludge phase	Unknown	Removal efficiency	Literature value
Acetaminophen	3.6%	<0.3%	96.1%<	96.4%	98%[3]
Atenolol	78%	<0.2%	21.8%<	22%	<10%[46]/0–56%[47]
Carbamazepine	99%<	<0.1%	<0.1%	<0.1%	7%[3]/0–83%[19]
Ibuprofen	4.5%	<0.5%	95.0%<	95.5%	90%[3]/ 83–100%[19]
Ifenprodil	25%	8.2%	69%	77%	NA
Indomethacin	69%	3.0%	28%	31%	75%[3]
Mefenamic acid	24%	17%	59%	76%	41–50%[14]/20–50%[45]
Propranolol	40%	37%	23%	60%	96%[3]

NA: not available

The poor removal efficiency of less than 22% for atenolol and carbamazepine is in agreement with the results obtained by European researchers.[3,47] Despite additional removal by chlorination or ozonation, significant fractions of these two pharmaceuticals collected into WWTPs are released into the aquatic environment. The low removal efficiency of carbamazepine is also in agreement with the comparison of influent and effluent for Japanese WWTP.[19] Propranolol and indomethacine were moderately removed and the experimental removal efficiency was lower than those in the literature.[3] The reactivities of these compounds with disinfectants such as hypochlorite and ozone in addition to the difference in the conditions used for the secondary treatment are the possible reasons behind the lower removal efficiency in this study. Since almost all pharmaceuticals are weak acids or bases, the sorption/biodegradation of these compounds by activated sludge is highly affected by pH,[48] operational conditions of the secondary treatment and the characteristics of influents. Particularly for atenolol and carbamazepine, both of which are reported to be poorly removable, the mass balance in the WWTP needs to be experimentally investigated using a large-scale pilot plant or by performing batch experiments using radiolabeled compounds.

3.2 *Results of toxicity tests for aquatic organisms*

The results of toxicity tests using fish (*Oryzias latipes*), daphnia (*Daphnia magna*), and green algae (*Pseudokirchneriella subcapitata*) are shown in Table 4. The ECOSAR predictions are also added in Table 4. Although there are slight differences in endpoints for daphnia and green algae, a difference larger than one order of magnitude was not found between the measured and predicted values for any compounds except for the 96-h NOEC of propranolol for green algae. A preliminary comparison between measured and ECOSAR predictions was conducted by the Japan Ministry of the Environment for 35 compounds for fish 96-h LC_{50}, daphnia 48-h EC_{50} or 48-h LC_{50}, and green algae 96-h EC_{50}. Discrepancies larger than one order of magnitude were found for approximately 10, 20, and 30% of all compounds for fish 96-h LC_{50}, daphnia 48-h EC_{50} or 48-h LC_{50}, and green algae 96-h EC_{50}, respectively.[49] The results obtained in this study were slightly better than the preliminary comparison results.

Table 4
Results of measured toxicity for fish, daphnia, and green algae and ECOSAR predictions (mg L^{-1}).

Endpoint:	Fish [*Oryzias latipes*]		Daphnia [*Daphnia magna*]		Green algae [*Pseudokirchneriella subcapitata*]			
	Measured	Predicted	Measured	Predicted	Measured	Predicted	Measured	Predicted
	96-h LC_{50}	96-h LC_{50}	48-h EC_{50}	48-h LC_{50}	96-h EC_{50}	96-h EC_{50}	96-h NOEC	96-h ChV
Acetaminophen	*800* (630–960)	260	17 (15–19)	41	2300 (1100–4500)	2600	*550*	94
Atenolol	1800 (1600–1900)	1500	*180* (150–210)	83	110 (51–230)	78	10	11
Carbamazepine	**20** (20–20)	100	55 (50–59)	110	64 (35–120)	70	6.4	8.1
Ibuprofen	*89*[a] (84–95)	32	31 (29–33)	39	*360*[a] (22–6000)	27	**2.0**	7.5
Ifenprodil	4.4 (4.0–5.2)	3.2	4.1 (3.7–4.8)	2.9	1.9 (1.2–3.0)	3.4	**0.18**	1.0
Indomethacin	*44* (41–47)	21	22 (19–26)	27	*39* (2.9–520)	19	**2.9**	6.9
Mefenamic acid	*3.4* (3.3–3.5)	1.5	*10* (7.6–13)	2.0	*18* (12.2–26.3)	1.5	*2.5*	1.0
Propranolol	**9.0** (7.9–10)	30	**0.46** (0.31–0.61)	2.3	**0.66** (0.24–1.4)	5.5	**0.10**	1.4

95% confidence interval for measured values are within the parentheses; ChV is the arithmetic mean of NOEC and LOEC; **Bold letters** are those for Measured/Predicted<0.5; *Italic letters* are those for Measured/Predicted>2; [a]larger than aqueous solubility limit.

In the comparison of the toxicities of the selected pharmaceuticals, it was found that the toxicities for three compounds, ifenprodil, mefenamic acid, and propranolol, were relatively stronger than those of the other five compounds. In particular, for green algae 96-h EC_{50}, a strong toxicity of less than 1.9 mg L^{-1} was found for ifenprodil and propranolol; for daphnia 48-h EC_{50}, a toxity of less than 4.1 mg L^{-1} was found for ifenprodil and propranolol; for fish 96-h LC_{50}, a toxicity of less than 4.4 mg L^{-1} was found for mefenamic acid and ifenprodil. Among the five compounds of relatively weak toxicity, the LC_{50} or EC_{50} of acetaminophen and atenolol was as high as 100 mg L^{-1}. For ibuprofen, LC_{50} (or EC_{50}) was not accurately determined for fish and green algae because of the aqueous solubility limit.

Several researchers have examined the acute/chronic toxicity of carbamazepine, ibuprofen, and propranolol on aquatic organisms, and the results shown in the review paper[11] were all similar to our results. However, Huggett *et al.*[50] reported that the reproduction of fish was significantly inhibited by a very low concentration (0.1 μg g^{-1}) of the β-blocker propranolol. Other chronic tests such fish reproduction/ early-stage development test (*e.g.*, OECD test guideline 210) and daphnia reproduction test (*e.g.*, OECD test guideline 211) are necessary for those compounds with a relatively strong acute toxicity. Moreover, pharmaceuticals are designed and manufactured to have a specific physiological function such as nuclear receptor agonist or antagonist, so that the grouping of compounds with a similar pharmacological function and the investigation of the specific endpoint of these grouped compounds need to be examined in addition to genetic approaches using tools such as DNA microarrays.

3.3 *Evaluation of bioaccumulation using liposome/water system*

The relationship between logarithm of liposome/water distribution coefficient (log D_{lipw}) and that of octanol/water distribution coefficient (log D_{ow}) predicted by ACD software (at pH 7) is shown in Fig. 1. A moderate linear relationship has been found for moderately hydrophobic estrogenic compounds that are nonionized at the testing condition of pH 7,[33] but a poor relationship was found for the selected pharmaceuticals, most of which are ionized at pH 7. Various substituted phenols[23] and the secondary amine fluoxetine,[24] which both exist in ionized form at the tested pH, were found to have an octanol-water partition coefficient of as low as two to three orders of magnitude lower than that in the nonionized form, whereas the BCF and liposome water partition coefficient were also lower for the nonionized form but the

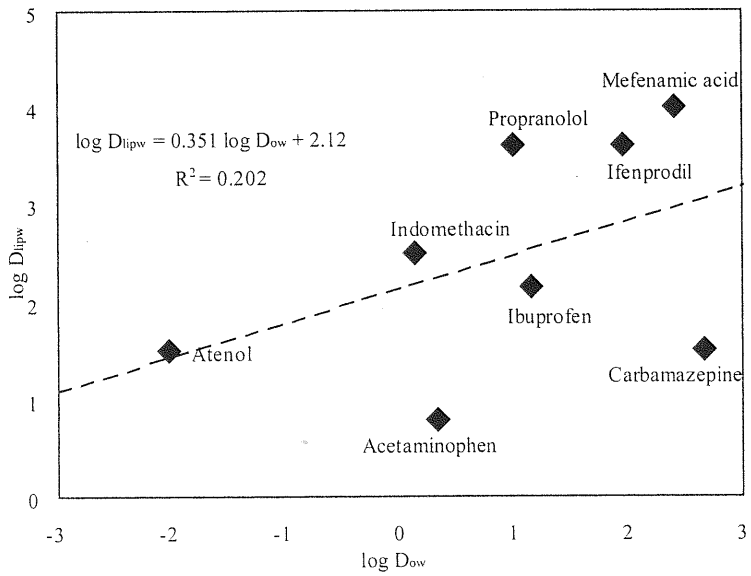


Fig. 1. Relationship between log D_{lipw} and log D_{ow}.

difference was as low as one order of magnitude. These results suggest that the liposome/water system seems to be a much better model of bioaccumulation than the octanol/water system, and our results may be a better estimate than the BCF values calculated from log K_{ow}.

D_{lipw} values larger than 5,000 (log $D_{lipw} > 3.7$) were found for three selected pharmaceuticals, ifenprodil, mefenamic acid, and propranolol. These values are similar to those obtained for 17β-estradiol and 17α-ethynylestradiol.[33] For the other five compounds, D_{lipw} values were less than 300. The BCF values are probably smaller than the D_{lipw} values because of the metabolic pathway in the aquatic organisms and the existence of more hydrophilic nonlipid constituents of cells; however, these three compounds were identical to those with a relatively stronger toxicity, as presented in the previous section. Further investigation such as bioconcentration factor measurement is necessary for these three compounds.

4. Initial Ecological Risk Assessment

Initial ecological risk was assessed for the selected pharmaceuticals on the basis of the PNEC and PEC determined in Japan. As presented above, these concentrations were calculated from the measured or predicted toxicity, estimated removal efficiency at WWTP, and the detection from surface water and WWTP effluent inside or outside Japan. First, the predicted environmental concentrations were calculated by three different methods for WWTP effluent and surface water, as presented above. These results are shown in Table 5.

As can be seen from Table 5, the PEC for WWTP effluent (PEC_{eff}) from the detected concentration outside Japan (PEC_{inteff}) is the highest followed by that from annual consumption (PEC_{domeff}), except for a few cases. PEC_{inteff} values could highly overestimate PEC, particularly those without correction by annual consumption in Japan and the country of detection (*e.g.*, mefenamic acid and propranolol). The difference in removal efficiency in WWTP attributed to the difference in the treatment system and temperature between Japan and other countries, and the smaller number of available data in Japan all possibly resulted in the lower concentration detected in Japan. $PEC_{domeff}/PEC_{conseff}$ ratios, often abbreviated as measured environmental concentration (MEC)/PEC ratio by other researchers,[14,51] were higher than 40 for ibuprofen and propranolol, and higher than 4 for carbamazepine. The possible reasons behind these significant underestimations were the use of maximum detected concentration (rather than median or mean), no uniformity in drug consumption throughout the country/season, and the alteration of removal efficiency in WWTP (i.e., highly temperature-dependent) in addition to the metabolites, retransformation back to the parent compounds from metabolites such as conjugates in the WWTP. For other compounds, the ratios were less than 1, which is due to the unused drugs and decomposition between the discharge of urine into wastewater and WWTP.

Table 5
PEC of selected pharmaceuticals in Japan (μg L^{-1}).

	WWTP effluent (PEC_{eff})			Surface water (PEC_{sw})		
	$PEC_{conceff}$	PEC_{inteff}	PEC_{domeff}	PEC_{concsw}	PEC_{intsw}	PEC_{domsw}
Acetaminophen	0.071	6.2	0.025	0.0071	1.3	0.0025
Atenolol	0.27	ND	0.00078	0.027	0.027	0.000078
Carbamazepine	0.10	2.1	0.45	0.0096	0.36	0.050
Ibuprofen	0.0034	1.3	0.18	0.00034	0.29	0.018
Ifenprodil	0.042	ND	0.0021	0.0042	ND	0.00021
Indomethacin	1.4	0.60	0.19	0.14	0.20	0.019
Mefenamic acid	0.52	4.5	0.35	0.052	0.45	0.035
Propranolol	0.00037	0.37	0.016	0.000037	0.10	0.0093

ND: not detected

As far as the predicted PEC_{sw} is concerned, PEC_{sw} values were clearly smaller than PEC_{eff} because of dilution. Ternes[3] reported higher propranolol concentration in river water than in WWTP effluent possibly due to the formation of parent compounds from conjugates. Otherwise, the trend of PEC_{sw} was similar to that of PEC_{eff}. Further investigation is necessary to extensively measure the concentration of these pharmaceuticals in the aquatic environment, including WWTP effluents and river waters in several seasons and locations, to more accurately determine PECs. Once sufficient data are collected for river waters and WWTP effluents, a 75 or 90 percentile value rather than the maximum concentration needs to be used to more accurately and realistically evaluate the ecological risk.

The PNEC values predicted using ECOSAR ($PNEC_{ECOSAR}$) and those calculated from toxicity tests conducted in this study ($PNEC_{exp}$) are shown in Table 6. As can be seen from Table 6, the $PNEC_{exp}/PNEC_{ECOSAR}$ ratio becomes less than 0.5 for five compounds, carbamazepine, ibuprofen, ifenprodil, indomethacin, and propranolol, partly because of the underestimation of algal toxicity by ECOSAR. Another reason is the assessment factor for chronic toxicity of 100 for this study due to the test using only one species, whereas the factor was 10 for ECOSAR because the chronic values are available for all three species. For these compounds, chronic toxicities for fish and daphnia need to be determined to more accurately calculate PNEC. $PNEC_{exp}$ was as low as 1.8 μg L^{-1} for ifenprodil and propranolol whose algal NOEC was lower than 0.18 mg L^{-1}. $PNEC_{exp}$ was used for initial ecological risk assessment.

Finally, three PEC values each for WWTP effluent and surface water shown in Table 5 and the $PNEC_{exp}$ values shown in Table 6 were compared in Figs. 2 and 3 on the basis of the criteria presented by the Japan Ministry of Environment for initial ecological risk assessment for chemicals (Table 7). The PEC/PNEC ratios determined in this study for selected pharmaceuticals were all less than 1 and these results suggest no prominent effects on aquatic organisms such as fish, daphnia, and algae. The PEC/PNEC ratio for propranolol was the highest and as high as 0.37 and 0.10 for WWTP effluent and surface water, respectively, followed by mefenamic acid, which became as high as 0.18 and 0.018 for WWTP effluent and surface water, respectively. However, these extremely high PEC/PNEC ratios resulted from the possible overestimation of PEC_{int}. Since the risk assessment is targeted for Japan, the other two PEC predictions are more reliable. For the PEC/PNEC values originating from PEC_{cons} and PEC_{dom}, the highest values were 0.047 for indomethacin, followed by 0.023 for ifenprodil and 0.021 for mefenamic acid, all of which were less than 0.1.

Compared with the results obtained by other researchers, Stuer-Lauridsen *et al.*[12] first conducted an initial ecological risk assessment for the top 25 pharmaceuticals sold in Denmark using ECOSAR predictions and reported the toxicity data to determine PNEC. They found that the MEC/PNEC ratio was 0.68 for ibuprofen, and the PEC/PNEC ratios were 7.1 for acetaminophen and 1.8 for ibuprofen. These values are probably overestimated because of the use of an assessment factor of 1000 to de-

Table 6
PNEC of selected pharmaceuticals determined in this study and ECOSAR prediction (μg L^{-1}).

	$PNEC_{exp}$	$PNEC_{ECOSAR}$	$PNEC_{exp}/PNEC_{ECOSAR}$
Acetaminophen	170	110	1.5
Atenolol	100	110	0.91
Carbamazepine	64	640	0.10
Ibuprofen	20	270	0.074
Ifenprodil	1.8	5.5	0.33
Indomethacin	29	190	0.15
Mefenamic acid	25	15	1.7
Propranolol	1.0	14	0.071

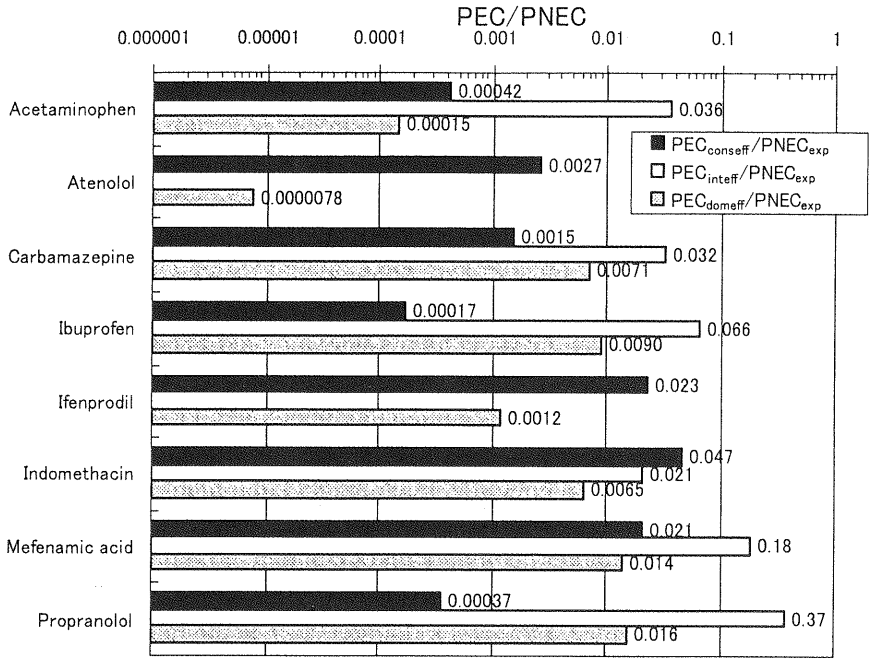


Fig. 2. PEC/PNEC ratio for WWTP effluent PEC (PEC_{eff}).

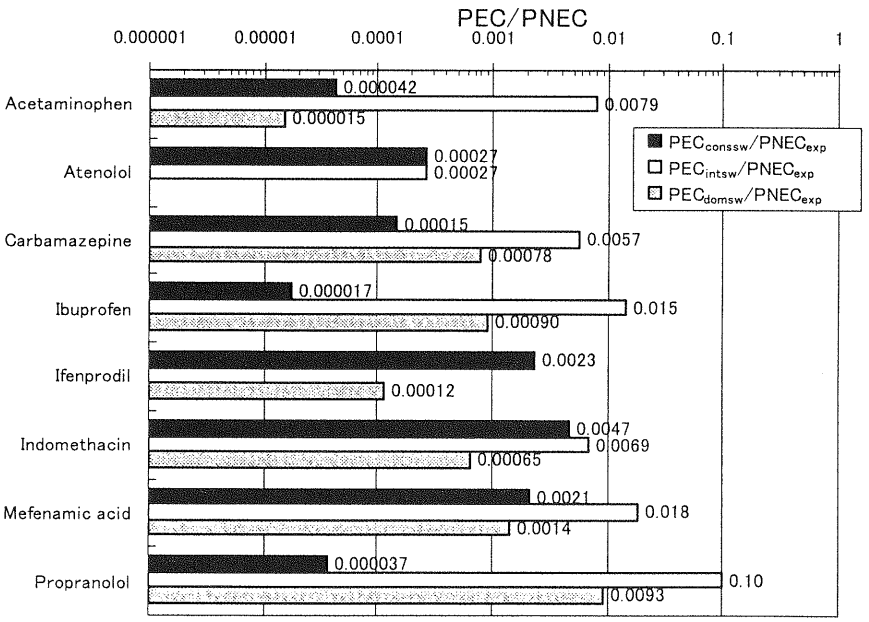


Fig. 3. PEC/PNEC ratio for surface water PEC (PEC_{sw}).

Table 7
Criteria for initial ecological risk assessment for chemical compounds proposed by Japan Ministry of Environment.[20]

PEC/PNEC value	Evaluation
PEC/PNEC<0.1	No more investigation is necessary
0.1≤PEC/PNEC<1	Need to collect further information
1≤PEC/PNEC	Considered as candidate for further detailed evaluation