算定のための定量的評価にも実質的には使用されていない．

以上のことから，TDI算定の出発点となる最低の毒性発現体内負荷量の算定のためにFaqiら[8]やOhsakoら[11]の両報告結果を用いる積極的な理由は現在のところなく，1999年の我が国のTDI再評価に用いたGrayら[22,26]およびGehrsら[30]の報告で母動物にTCDDを投与した場合に児動物に見られる開眼促進，精巣上体精子数の減少，雌性生殖器形態異常，遅延型過敏症の抑制最低の毒性発現体内負荷量：86ng/kgを出発点としてTDIを算定することは現在でも妥当であると考えられる．

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OECD化学物質対策の動向（第5報）
第12回及び第13回OECD高生産量化学物質初期評価会議(2001年)

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Progress on OECD Chemicals Programme

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The twelfth SIDS, the Screening Information Data Set, Initial Assessment Meeting (SIAM 12) was held at the Organisation for Economic Co-operation and Development (OECD) headquarters in Paris, France and SIAM 13 was held in Bern, Switzerland, hosted by the European Commission. Two substances at SIAM 12 (CAS No:91-15-6, 123-77-3) and 4 substances at SIAM 13 (CAS No:91-76-9, 112-85-6, 868-77-9, 1477-55-0)were submitted by the Japanese Government and/or International Council of Chemical Associations (ICCA). These substances were agreed at the meetings. In this report, the human health effects of 6 substances above-mentioned are introduced.

Keywords: OECD, HPV program, SIAM, SIDS Initial Assessment Meeting

はじめに

経済協力開発機構（Organization for Economic Cooperation and Development：OECD）加盟各国における高生産量化学物質（High Production Volume Chemical：HPV）の安全性は，1992年に始まったOECD高生産量化学物質点検プログラム（HPV program）によって評価されている[1]．加盟各国での生産量・既存の毒性データ量に基づいてOECD HPV Chemicals Listの作成及び評価の優先順位付けが行われた[1]．現在は，加盟各国と企業が，生産した化学物質に関する情報収集や試験を行って評価文書を完成させ，順次，それらの文書が初期評価会議（SIAM：SIDS, Screening Information Data Set, Initial Assessment Meeting）で討議されている．日本政府は初回より評価文書を提出しており，第6回までに27物質の評価文書について合意を得た．第7回から第11回のSIAMにおいて日本政府が担当し，結論及び勧告が合意された化学物質の初期評価文書の健康影響部分については既に紹介された[2)3)4)]．

SIAMで評価された物質数は2000年までは年間20程度（最多31，最少8）であったが，SIAM 11（2001年）から始まったICCA（International Council of Chemical Associations，国際化学工業協会協議会）による評価文書の提出に伴い，2001年には年間79（SIAM 11-13）と飛躍的に評価物質が増加した．

本稿では，SIAM 12及び13で合意に至った化学物質名と日本担当物質の初期評価要旨の健康影響部分について紹介する．

SIAMにおける合意はFWまたはLPとして示される（FW = The substance is a candidate for further work. LP = The substance is currently of low priority for further work.）．FWは「今後も追加の調査研究作業が必要である」ということを意味する．LPは「現状の使用状況においては追加作業の必要はない」ということを意味し，状況によっては追加作業が必要となる可能性を含む．現在，SIAMでFWとされたのは約100物質，LPは約300物質である．

SIAM 12及び13で合意された化学物質名と日本担当物質の初期評価内容

SIAM 12は2001年6月にフランス（パリ）で開催され，化学物質の初期評価文書が検討され，表1に示す14物質の初期評価結果及び勧告が合意された．SIAM 13は2001年11月にスイス（ベルン）で開催され，化学物質の初期評価文書が検討され，表2に示す36物質の初期評価結果及び勧告が合意された．日本政府が担当した化学物質の初期評価報告書のヒトへの健康影響について，概要を以下に示す．

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Table 1. Chemical substances discussed at SIAM12 and their outcomes

CAS No.	Name of substance	Sponsor country	Outcome
75-68-3	1-Chloro-1,1-difluoroethane	FR/ICCA	LP
79-06-1	Acrylamide	UK:eu	FW
84-74-2	Dibutyl phthalate	NL:eu	FW
91-15-6	*o*-Phthalodinitrile	JP+DE/ICCA	LP
100-21-0	Terephthalic acid	US+IT	LP
105-60-2	Epsilon-Caprolactam	DE/ICCA	LP
123-77-3	Azodicarboxamide	DE+JP	LP
126-73-8	Tributyl phosphate	US	LP
141-97-9	Ethyl acetoacetate	DE:eu	LP
822-06-0	1,6-Hexamethylene diisocyanate	DE/ICCA	FW
1717-00-6	1,1-Dichloro-1-fluoroethane	US/ICCA	LP
25154-52-3	Nonyl phenol	UK:eu	FW
34590-94-8	Dipropylene glycol methyl ether	US/ICCA	LP
84852-15-3	Phenol, 4-nonyl-, branched	UK:eu	FW

Note. Abbreviations show DE: Germany, FR: France, IT: Italy, JP: Japan, NL: Netherlands, UK: United Kingdom, and US: United States of America. ":eu" indicates the document was based on the risk assesment in European Communities.

Table 2. Chemical substances discussed at SIAM13 and their outcomes

CAS No.	Name of substance	Sponsor country	Outcome
58-55-9	Theophylline	DE/ICCA	LP
65-85-0	Benzoic acid	NL/ICCA	LP
68-12-2	N,N-Dimethylformamide	DE/ICCA	FW
71-36-3	*n*-Butyl alcohol	US/ICCA	LP
74-83-9	Methyl bromide	US/ICCA	LP
75-01-4	Vinyl chloride	US/ICCA	LP
75-38-7	Vinylidene fluoride	US/ICCA	LP
75-56-9	Methyl oxirane	UK:eu	FW
79-10-7	Acrylic acid	DE:eu	FW
79-20-9	Methyl acetate	DE:eu	FW
88-73-3	1-Chloro-2-nitrobenzene	DE/ICCA	FW
88-74-4	2-Nitroaniline	FR/ICCA	LP
91-76-9	2,4-Diamino-6-phenyl-1,3,5-triazine	JP/ICCA	LP
95-50-1	1,2-Dichlorobenzene	Aus	FW
100-51-6	Benzyl alcohol	NL/ICCA	LP
103-84-4	Acetanilide	KO	LP
107-15-3	Ethylenediamine	US/ICCA	LP
107-41-5	Hexylene glycol	UK/ICCA	LP
108-77-0	Cyanuric chloride	CH/ICCA	LP
109-66-0	*n*-Pentane NO:eu		LP
112-57-2	Tetraethylenepentamine	US/ICCA	LP
112-85-6	Docosanoic acid	JP/ICCA	LP
123-54-6	2,4-Pentanedione	DE/ICCA	FW
123-86-4	*n*-Butyl acetate	US/ICCA	LP
127-19-5	N,N-Dimethylacetamide	IT	LP
532-32-1	Sodium benzoate	NL/ICCA	LP
582-25-2	Potassium benzoate	NL/ICCA	LP
616-38-6	Dimethyl carbonate	IT/ICCA	Not finalized
868-77-9	2-Hydroxyethyl methacrylate	JP/ICCA	LP
1310-58-3	Potassium hydroxide	BE/ICCA	LP
1477-55-0	1,3-Bis(aminomethyl)benzene	JP/ICCA	LP
5392-40-5	Citral	JP	LP
6386-38-5	Metilox	CH	LP
6864-37-5	2,2'-Dimethyl-4,4'-methylenebis (cyclohexylamine)	DE/ICCA	LP
7447-40-7	Potassium chloride	NO/ICCA	LP
7681-57-4	Disodium disulphite	KO/ICCA	LP
16470-24-9	Fluorescent Brightener 220	DE/ICCA	FW

Note. Additional abbreviations to table 1, Aus: Australia, CH: Switzerland, NO: Norway, KO: Korea, and BE: Belgium. Health effects of citral have already described in SIAM11.

o-Phthalodinitrile (91-15-6) (ICCA 日本及びドイツ企業作成) (SIAM 12)

本化学物質はフタロシアニン系染料，顔料の原料として用いられている．

単回経口投与毒性試験（OECD TG 401）では，ラットの雌雄ともに60 mg/kg以上の投与で死亡，痙攣，口周囲の汚れがみられ，240 mg/kg以上の投与で自発運動低下，腹臥位，チアノーゼ等が観察された．経口LD_{50}は85 mg/kgであった．

吸入毒性試験では，20℃で8時間の飽和蒸気圧に曝露させたラットの死亡はみられなかった．

皮膚及び眼に対する刺激性はみられなかった．皮膚感作性に関する情報はなかった．

反復投与毒性・生殖発生毒性併合試験（OECD TG 422）では，0, 1, 6, 30 mg/kg/dayを雌雄のラットに少なくとも42日間強制経口投与した．30 mg/kg/dayの雌雄で体重増加抑制及び摂餌量の減少，雄では総コレステロール及び総蛋白の増加，血清尿窒素の減少，肝臓，腎臓及び精巣重量の増加，精巣上体重量の減少，雌では全例が妊娠末期に痙攣を伴い死亡した．また，30 mg/kg/dayの雄において肝臓の小葉中心性肝細胞肥大，腎臓の近位尿細管上皮における硝子滴沈着，精細管萎縮及び精巣上体の管腔内の細胞残屑出現と精子数の減少が観察された．6 mg/kg/day投与では雌雄とも毒性所見はみられなかったので，この試験における反復投与毒性の無毒性量（NOAEL）は6 mg/kg/dayであった．

90日間反復経口投与毒性試験（OECD TG 408）及び米国EPA神経毒性試験ガイドラインに従い，雄ラットに0, 3, 8, 25 mg/kg/day，雌ラットに0, 3, 10, 30 mg/kg/dayの用量を13週間混餌投与したところ，自発運動量の増加がみられたが，中枢及び末梢神経毒性に関連した症状及び神経病理学的変化は観察されなかった．雄は25 mg/kg/day，雌は10 mg/kg/dayで体重減少がみられたことから，この試験における反復経口投与のNOAELは雄ラットでは8 mg/kg/day，雌ラットでは3 mg/kg/dayであった．

これらの結果から，反復経口投与毒性のNOAELは3 mg/kg/dayと考えられた．

上述の反復投与毒性・生殖発生毒性併合試験において，雄ラットには交配前後の14日間ずつを含む少なくとも42日間，雌ラットには交配前14日間から哺育3日まで，0, 1, 6, 30 mg/kg/dayを強制経口投与した．30 mg/kg/dayにおいて，雄で精巣毒性がみられ，さらに，すべての妊娠ラットが死亡したため分娩児のデータは得られなかった．6 mg/kg/day以下の用量では生殖発生に対する影響がみられなかったことから，生殖発生毒性のNOAELは6 mg/kg/dayと考えられた．

細菌を用いた復帰突然変異試験の結果はS9mix存在下及び非存在下のいずれでも陰性であった．チャイニーズ・ハムスター培養細胞を用いた染色体異常試験では，いずれの連続処理でも染色体の構造異常は認められなかったが，中濃度（0.40 mg/mL）及び高濃度において倍数体が誘発された．また，S9mix存在下及び非存在下のいずれの短時間処理でも染色体の構造異常は認められなかったが，S9mix存在下及び非存在下のいずれの短時間処理でも倍数体が誘発され，これらの結果より陽性と判断された．しかしながら，*in vivo*でのマウスの小核試験で投与可能な最高投与量20 mg/kgの結果が陰性であったことから，本化学物質は*in vivo*では遺伝毒性を発現しないと考えられた．

発がん性に関する有効な情報はなかった．

Azodicarboxamide（123-77-3)（ドイツ政府及び日本政府作成）(SIAM 12)

本化学物質は，プラスティックやゴム製品の発泡剤，米国の食品添加物（膨張剤）として用いられている．

雄ラットへの吸入暴露で本化学物質の約34%が，経口投与で10-33%が吸収されるが，本化学物質のかなりの量は胃腸管で吸収されず，糞とともに排泄される．本化学物質は吸収後速やかにbiurea（CAS 110-21-4)に代謝物され，72時間以内に主に尿中に排泄される．

急性経口毒性は弱く，雄ラットでの2つの試験では2,500 mg/kgで毒性発現はみられず，別の試験ではLD_{50}は4,000 mg/kg以上（雌雄Alderly Parkラット），5,000 mg/kg以上（雄Wistarラット）であった．

雌雄ラット（1群5匹）を用いた急性吸入毒性試験では，LC_{50}は6,100 mg/m³以上（4時間暴露，粒子サイズ5.8 μm）であった．4時間暴露後，6,100 mg/m³で10例中8例が呼吸困難を示したが，死亡はみられなかった．暴露後14日での病理組織検査では影響はみられなかった．

モルモットでの吸入刺激試験では，肺機能に影響はみられず，97 mg/m³（1時間）までの濃度において吸入刺激は極軽微であった．

急性皮膚毒性試験が5匹の雄ラットに500 mg/kgを塗布して行われ，毒性徴候や死亡はみられなかった．

1匹の雌ウサギに2,000 mg/kgを塗布したスクリーニング試験でも毒性徴候はみられなかった．

ウサギの皮膚への刺激性はみられず，眼に対しては可逆的な角膜の発赤や腫脹がみられた．ヒトの皮膚を用いたパッチテストが陽性であったことから，皮膚感作性の可能性が示された．

ラットに100, 500, 2,500 mg/kg/day（雄），200, 1,000, 5,000 mg/kg/day（雌）を強制経口投与した90日間反復経口投与毒性試験では，雄の2,500 mg/kg/dayと雌の5,000 mg/kg/day（雌）で死亡がみられたが，一般毒性の徴候はみられず，病理組織学的検査で腎盂腎炎等がみられた．NOAELは500 mg/kg/day（雄），1,000 mg/kg/day（雌）であった．

また，マウスに0, 78, 156, 312, 625, 1,250 mg/kg/day（雄），0, 156, 312, 625, 1,250, 2,500 mg/kg/day（雌）を強制経口投与した90日間反復経口投与毒性試験では，最高用量でも投与に関連した影響はみられなかった．

一世代生殖毒性試験（OECD TG 415）では，ラットに0, 100, 300, 1,000 mg/kg/dayの用量で強制経口投与した．雄親ラットには最高用量でも影響はみられなかったが，雌親ラットでは1,000 mg/kg/dayで腎盂の拡張，間質のリンパ球浸潤等の腎臓への影響がみられた．NOAELは300 mg/kg/dayであった．

これらの試験の結果から，反復経口投与のNOAELは500 mg/kg/day（雄），300 mg/kg/day（雌）とされた．

13週間吸入試験において，ラット及びマウスに0, 50, 10, 200 mg/m³の濃度を1日6時間（週5日）で暴露させたところ，最高濃度の200 mg/m³でも有意な毒性影響はみられなかった．13週間暴露試験に基づき，吸入反復暴露のNOAELは200 mg/m³（ラット，マウス）であった．

上述の一世代生殖毒性試験では，雄ラットには98日間，雌ラットには交配前14日間から哺育20日まで，それぞれ0, 100, 300, 1,000 mg/kg/dayを強制経口投与した．最高投与量の1,000 mg/kg/dayでも生殖発生への毒性影響はみられず，生殖発生毒性のNOAELは1,000 mg/kg/dayと考えられた．ラットとマウスを200 mg/m³の濃度まで暴露した上述の13週間吸入試験においても生殖器系や精子の形態，発情周期に影響は認められなかった．

細菌を用いた復帰突然変異試験の結果はS9mix存在下及び非存在下のいずれでも陰性であった．チャイニーズ・ハムスター培養細胞を用いた染色体異常試験では，いずれの連続処理及びS9mix存在下及び非存在下のいずれの短時間処理でも染色体の構造異常及び倍数体の誘発は観察されなかったことから，染色体異常試験は陰性と判断された．

発がん性に関する有効な情報はなかった．

Docosanoic acid（112-85-6)（ICCA日本企業作成）(SIAM 13)

本化学物質は近年化粧品の原料として用いられている．

トキシコキネティクスに関する情報はなかった．

単回経口投与毒性試験（OECD TG 401）では，最高投与量の2,000 mg/kgでもラットの死亡はみられず，また，一般状態，投与後の体重の推移及び剖検所見のいずれにも投与に起因すると考えられる変化は観察されず，経口LD_{50}は2000 mg/kg以上であった．

皮膚及び眼に対する刺激性，皮膚感作性に関する情報はなかった．

反復投与毒性・生殖発生毒性併合試験（OECD TG 422）では，雄ラットには42日間，雌ラットには交配前14日間から哺育3日まで，それぞれ0, 100, 300, 1,000 mg/kg/dayを強制経口投与した．雄ではいずれの投与群においても死亡及び一般状態の異常は観察されなかった．また，42日間反復投与後の剖検，病理組織学的検査，血液学検査及び血液生化学検査でも，投与の影響を示唆する所見または異常値は認められなかった．雌でもいずれの投与群においても死亡及び一般状態の異常は観察されなかった．分娩後4日の剖検及び病理組織学的検査においても投与の影響を示唆する所見は認められなかった．反復経口投与のNOAELは1,000 mg/kg/dayと考えられた．

上述の反復投与毒性・生殖発生毒性併合試験において，最高投与量の1,000 mg/kg/dayでも交尾能及び受胎率に影響はみられなかった．また，母動物の妊娠期間，出産率，分娩状態及び哺育状態に投与の影響を示唆する変化は認められなかった．出生児の性比，体重及び生存率に，投与の影響を示唆する変化は認められなかった．また，出生児の形態異常は

いずれの投与群にも観察されなかった．生殖発生毒性のNOAELは1,000 mg/kg/dayと考えられた．

細菌を用いた復帰突然変異試験の結果はS9mix存在下及び非存在下のいずれでも陰性であった．チャイニーズ・ハムスター培養細胞を用いた染色体異常試験では，いずれの連続処理及びS9mix存在下及び非存在下のいずれの短時間処理でも染色体の構造異常及び倍数体の誘発傾向は観察されなかったので，染色体異常試験は陰性と判断された．

発がん性に関する情報はなかった．

2,4-Diamino-6-phenyl-1,3,5-triazine (91-76-9) (ICCA日本企業作成) (SIAM 13)

本化学物質はベンゾグアナミンーホルムアルデヒドの中間体として使用される．

トキシコキネティクスに関する情報はなかった．

単回経口投与毒性試験（OECD TG 401）では0, 250, 500, 1,000, 2,000 mg/kgを投与したところ，雌雄とも1,000 mg/kg以上の投与で死亡がみられた．死亡例では，前胃で肉眼的に粘膜の肥厚，病理組織学的に粘膜下組織の浮腫がみられ，脾臓及び胸腺では肉眼的及び病理組織学的に萎縮がみられた．また，膀胱では濃緑色尿の貯留がみられた．生存例では，前胃に肉眼的に粘膜の白色点がみられ，病理組織学的には粘膜に扁平上皮の過形成がみられた．これらより，ラットでの経口LD_{50}は雄で933 mg/kg，雌で1,231 mg/kgであった．

吸入毒性試験（OECD TG 403）では，ラットでの吸入LC_{50}は2.932mg/L（4時間）であった．

ウサギにおいて，皮膚への刺激性はなく，眼への刺激性は軽度であった．皮膚感作性に関する情報はなかった．

反復投与毒性・生殖発生毒性併合試験（OECD TG 422）では，雌雄ラットに少なくとも39日間，それぞれ0, 4, 20, 100 mg/kg/dayを強制経口投与した．雌雄の100 mg/kg/dayで1例ずつの死亡がみられた．雄の血液学検査では，100 mg/kg/dayの赤血球数及びヘマトクリット値の減少及び網状赤血球率の増加が認められた．雄の血液生化学検査では，100 mg/kg/dayでアルブミン，A/G比，GOT，GPT，総コレステロール，リン脂質及び総ビリルビンの増加ならびにトリグリセライド，ナトリウム及びカリウムの減少が認められた．100 mg/kg/dayの雄で肝臓重量の増加がみられ，病理組織学的には雌雄で小葉中心性肝細胞肥大が認められた．また，死亡例では，100 mg/kg/dayの雄1例で回腸の粘膜固有層から漿膜にかけて好中球性の細胞浸潤及び肉芽形成がみられたほか，腺胃のびらん，肺の水腫，脾臓の萎縮，胸腺の萎縮及び出血がみられ，同群の雌1例で腺胃のびらん，肺の水腫，脾臓の萎縮及び副腎の壊死が認められた．これらに結果から，ラットにおける反復経口投与のNOAELは20 mg/kg/dayと考えられた．

90日間反復経口投与毒性試験（OECD TG 408）に従い，雄ラットに90日間0, 1.9, 19.0, 173.0 mg/kg/dayを混餌投与した反復投与毒性試験では，死亡はみられず，173.0 mg/kg/dayにおいて，雌雄ラットで弓なり姿勢，立毛，体重増加量の減少，摂餌量の減少がみられた．血液化学では，雌雄ラットでGPT及びビリルビンが増加し，雌の肝臓重量が増加した．病理組織学的検査では，小葉中心性肝細胞肥大，脾髄外造血の亢進，副腎球状帯細胞の異常発達及び空胞形成，炎症細胞の浸潤を伴う膵臓外分泌細胞の退化が観察された．また，ヘモジデリン色素沈着の増加が雌雄ラットの腎臓及び脾臓で観察された．19 mg/kg/dayの雄では脾臓のヘモジデリン色素沈着の増加が投与に関連した唯一の病理組織学的変化として認められたが，この変化は穏やかであり，他の影響は観察されなかった．これらの結果にもとづき，混餌による反復経口投与のNOAELは19 mg/kg/dayと考えられた．

反復投与毒性・生殖発生毒性併合試験（OECD TG 422）では，雄ラットには交配の14日前から49日間，雌ラットには交配前14日間から哺育3日まで，それぞれ0, 4, 20, 100 mg/kg/dayを強制経口投与した．親動物の生殖機能に関しては，性周期，黄体数，交尾率，着床痕数，授（受）胎率及び交尾所要日数に投与の影響は認められなかった．分娩時の検査では，100 mg/kg/dayの2例で分娩直後の児の回集及び保温の不良などが認められた．さらに，100 mg/kg/dayで死産率の増加及びそれに伴う出生率の減少，雌雄新生児体重の減少が認められた．各群とも妊娠期間，出産児数，出産率，新生児数及び新生児の性比では投与の影響はみられず，新生児の外表検査においても，異常は認められなかった．哺育期の検査では，20 mg/kg/dayの2例及び100 mg/kg/dayの7例で児の回集，授乳，保温などの哺育行動の不良がみられ，これらの母動物では全児が死亡した．また，20 mg/kg/day以上では母動物の哺育行動の不良に起因した新生児の4日の生存率の減少が認められた．さらに，100 mg/kg/dayでは新生児の哺育4日の体重に低値が認められた．これらより，雄では100 mg/kg/dayで影響がみられず，雌では20 mg/kg/dayで哺育行動の異常がみられた．最高用量でも児に形態異常はみられなかった．これらの結果から，生殖毒性のNOAELは100 mg/kg/day（雄），4 mg/kg/day（雌）であった．また，100 mg/kg/dayで児の体重減少がみられたので，発生毒性のNOAELは20 mg/kg/dayであった．

細菌を用いた復帰突然変異試験の結果はS9mix存在下及び非存在下のいずれでも陰性であった．チャイニーズ・ハムスター培養細胞を用いた染色体異常試験では，連続処理法の48時間処理及びS9mix存在下の短時間処理で染色体構造異常の誘発作用が認められた．また，連続処理法の48時間処理による試験では，倍数体の誘発作用が認められた．連続処理法の24時間処理による試験では，用量に依存した染色体構造異常の誘発作用が認められた．ヒト・リンパ培養細胞を用いた染色体異常試験では，S9mix存在下及び非存在下のいずれでも本化学物質の溶解限度内では染色体構造異常の誘発作用は認められず，S9mix非存在下で溶解限度を超え

ると染色体構造異常の誘発作用が認められた．マウス・リンフォーマ培養細胞を用いた遺伝毒性試験では，結果は陰性であったが，S9mix存在下で溶解限度を超えると遺伝毒性の誘発作用が認められた．*In vivo*でのマウスの小核試験で投与可能な最高投与量での結果が陰性であったことから，本化学物質は*in vivo*では遺伝毒性を発現しないと考えられた．

発がん性に関する有効な情報はなかった．

2-Hydroxyethyl methacrylate（HEMA）（868-77-9）(ICCA日本企業作成)(SIAM 13)

本化学物質は塗料，接着剤，コーティング剤，歯科用接着剤等に含まれる合成ポリマーのモノマーとして使用される．*In vitro*では1日で80%以上が加水分解される．*In vivo*でのトキシコキネティクスに関する情報はない．

急性毒性は弱く，急性毒性試験では，経口LD_{50}は4,000 mg/kg以上，経皮LD_{50}は3,000 mg/kg以上であった．

ウサギの皮膚に対して弱い刺激性がみられた．ウサギの眼に対して中程度の刺激性がみられ，モルモットの皮膚に対して弱い感作性を示したが，フロイント・アジュバントの注入でのみ陽性を示し，本化学物質の単独塗布では感作性は認められなかった．ヒトの皮膚でのパッチテストで感作性が示され，他のアクリル酸塩との交差反応の可能性が考えられた．

本化学物質はメタクリル酸とエチレングリコールに加水分解される．他のアクリル酸塩及びメタクリル酸塩では，methylacrylic acid（MAA）(SIAM 11で議論）へ加水分解された後，吸入による鼻腔の損傷が報告されている．この影響はHEMAについては調べられていない．

反復投与毒性・生殖発生毒性併合試験（OECD TG 422）では，雄ラットには交配の14日前から49日間，雌ラットには交配前14日間から哺育3日まで，それぞれ0, 30, 100, 300, 1,000 mg/kg/dayを強制経口投与した．雄では1,000 mg/kg/dayで流涎がみられ，1/12が死亡した．1,000 mg/kg/dayで体重増加抑制，摂餌量減少がみられた．すべての投与群で血液学検査の各検査項目に投与による影響はみられなかった．血液生化学検査では30 mg/kg/day以上で尿素窒素の高値あるいはその傾向，1,000 mg/kg/dayでカリウム，塩素及び無機リンの高値及びトリグリセライドの低値がみられた．剖検では各投与群とも投与による影響はみられなかった．死亡例では胸腺及び肺の暗赤色化，副腎の肥大がみられた．100 mg/kg/day以上で腎臓の相対重量の高値，1,000 mg/kg/dayで肝臓の相対重量の高値がみられた．病理組織学的検査では，1,000 mg/kg/dayで腎臓に尿細管拡張及び集合管拡張がみられた．雌では1,000 mg/kg/dayで流涎，自発運動の低下，腹臥位，流涙，被毛の汚れ，表皮温下降及び呼吸緩徐がみられ，6/12が死亡した．1,000 mg/kg/dayで交配前投与期間の体重増加抑制及び摂餌量減少がみられた．剖検所見では1,000 mg/kg/dayで胸腺の萎縮及び副腎の肥大がみられた．100 mg/kg/day以上で腎臓の絶対重量の高値あるいはその傾向，1,000 mg/kg/dayで腎臓の絶対・相対重量の高値がみられた．病理組織学的検査では1,000 mg/kg/dayで腎臓の髄質及び乳頭部への好中球浸潤，延髄の広範な軟化がみられた．雄での100 mg/kg/dayにおける腎臓の相対重量の増加，雌での100 mg/kg/dayにおける腎臓の絶対重量の増加を根拠に，ラットにおける反復経口投与のNOAELは30 mg/kg/dayと考えられた．

上述の反復投与毒性・生殖発生毒性併合試験においては生殖発生毒性に関するいずれの指標にも本化学物質投与の影響は認められなかったことから，ラットにおける生殖発生毒性のNOAELは1,000 mg/kg/dayと判断された．

細菌を用いた復帰突然変異試験の結果はS9mix存在下及び非存在下のいずれでも陰性であった．チャイニーズ・ハムスター培養細胞を用いた染色体異常試験は代謝活性の存在下及び非存在下で陽性であった．*In vivo*でのラットの小核試験で投与可能な最高投与量での結果が陰性であったことから，本化学物質は*in vivo*では遺伝毒性を発現しないと考えられた．

発がん性に関する情報はなかった．

1, 3-Bis(aminomethyl) benzene（1477-55-0）(ICCA日本企業作成)(SIAM 13)

本化学物質は主に，エポキシ樹脂硬化剤，ポリウレタン，有機合成などに使用される．

トキシコキネティクスに関する情報はなかった．溶液はアルカリ性で，腐食性がある．

単回経口投与毒性試験（OECD TG 401）では，ラットでの経口LD_{50}は1,090 mg/kg（雄），980 mg/kg（雌），マウスでの経口LD_{50}は1,180 mg/kgであった．ラットでの急性吸入毒性のLC_{50}（4 h）は1.42 mg/L以上（雄），0.8 mg/L（雌）であった．毒性は接触部位における腐食性によるもので，経口／吸入毒性では消化管／呼吸器系障害であった．

皮膚腐食性（ラットとマウス）及び感作性（モルモット）が認められた．

28日間反復経口投与毒性試験（OECD TG 407）に準拠した化審法ガイドラインの28日間反復経口投与毒性試験に従い，雌雄ラットに28日間，それぞれ0, 10, 40, 150, 600 mg/kg/dayの用量を強制経口投与した．動物数は1群雌雄各6匹とし，7群を設け，5群は投与終了後屠殺群，2群は対照及び600 mg/kg/dayの14日間回復群とした．10, 40, 150 mg/kg/dayでは被検物質の投与に起因する変化は認められなかった．600 mg/kg/dayでは，流涎，自発運動の低下，立毛，腹部膨満などの症状が雌雄に，摂餌量の減少及び体重増加の抑制が雄に認められ，雄の1例及び雌の4例が死亡した．さらに，胃の前胃粘膜に潰瘍及び角化亢進を伴う上皮の過形成，骨髄に顆粒球系造血細胞の増加，副腎に皮質細胞の肥大・空胞化，盲腸の拡張が雌雄に，白血球好中球比，尿タンパク及び血清無機リンの増加，血色素量及びヘマトクリット

値の減少，プロトロンビン時間の延長，活性化部分トロンボプラスチン時間の短縮が雄に，トリグリセライドの増加が雌に認められた．回復期間後には，前胃部粘膜の変化は回復傾向を示し，その他の変化はいずれも回復した．この28日間投与試験からはNOAELは150 mg/kg/dayと考えられた．

雌雄ラットに少なくとも41日間，それぞれ0, 50, 150, 450 mg/kg/dayの用量を強制経口投与した簡易生殖毒性試験（OECD TG 421）によると，150 mg/kg/dayの雄1例及び450 mg/kg/dayの雄3例，雌1例が死亡した．被検物質投与による症状として，150 mg/kg/dayの雄及び450 mg/kg/dayの雌雄で流涎，鼻音，不整呼吸，腹部膨満及び鼻分泌物が認められた．また，450 mg/kg/dayの雌雄で体重増加抑制及び摂餌量の減少が認められた．病理組織学的検査では投与の直接作用によると考えられる前胃の潰瘍，角化亢進を伴った扁平上皮増生等が450 mg/kg/dayの雌雄に認められた．なお，生殖器系には投与に起因する所見は観察されなかった．

経口投与簡易生殖毒性試験（OECD TG 421）によるNOAELのほうが低いことから，ラットにおける反復経口投与のNOAELは50 mg/kg/dayとされた．

上述の経口投与簡易生殖毒性試験では，生殖発生毒性に関するいずれの指標にも本化学物質投与の影響は認められなかったことから，ラットにおける生殖発生毒性のNOAELは450 mg/kg/dayと考えられた．

細菌を用いた復帰突然変異試験の結果はS9mix存在下及び非存在下のいずれでも陰性であった．チャイニーズ・ハムスター培養細胞を用いた染色体異常試験では，いずれの連続処理及びS9mix存在下及び非存在下のいずれの短時間処理でも染色体の構造異常及び倍数体の誘発は観察されなかったので，染色体異常試験は陰性と判断された．

発がん性に関する情報はなかった．

製造工場の労働者における臨床観察の結果，この物質は胃腸への刺激性を持ち，また，0.1 mg/m³（米国の職業閾値限界）以下の濃度で労働者に接触感作反応を引き起こすことが示されている．

おわりに

本稿では，第12回及び第13回初期評価会議で合意された日本担当の6物質についての，ヒトへの健康影響部分を紹介した．SIAMで合意された物質については，初期評価文書が出版されたのち，インターネットのOECD webサイト（http://cs3-hq.oecd.org/scripts/hpv/）で報告書の入手が可能である．

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Aryl hydrocarbon receptor-mediated suppression of GH receptor and Janus kinase 2 expression in mice

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Abstract **Differential mRNA display revealed that a cDNA encoding the major urinary protein 2 (MUP2) that belongs to the lipocalin superfamily was absent in livers of mice treated with 3-methylcholanthrene (MC). The expression of MUP2 is known to be stimulated by growth hormone (GH), through the GH receptor (GHR), Janus kinase 2 (JAK2) and signal transducer and activator of transcription 5 (STAT5) signal transduction pathway. Since MC is an aryl hydrocarbon receptor (AhR) ligand, the effects of MC treatment on the expression of GHR, JAK2 or STAT5 in the livers of wild-type or AhR-null mice were examined. The result indicated that the expression of GHR and JAK2 mRNA was greatly decreased by MC in wild-type mice but not in AhR-null mice. In addition, the binding activity of STAT5 bound to STAT5-binding element was reduced after MC treatment in wild-type mice but not in AhR-null mice. Based on these results, we conclude that the suppression of MUP2 mRNA expression by MC is caused by the AhR-mediated disruption of the GH signaling pathway. Possible mechanism(s) by which exposure to aromatic hydrocarbons causes a decrease in the body weight of mice, which has been referred to as wasting syndrome, will also be discussed.**

Key words: Aryl hydrocarbon receptor; Growth hormone receptor; 3-Methylcholanthrene; Janus kinase 2; Major urinary protein; Signal transducer and activator of transcription 5; Wasting syndrome

1. Introduction

Exposure to halogenated aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related polycyclic aromatic hydrocarbons (PAHs) results in a variety of biological responses including wasting syndrome, epithelial hyperplasia, teratogenesis, tumor promotion and the induction of enzymes responsible for the metabolism of xenobiotics, such as cytochrome P450 (CYP) [1–5]. It is the consensus view that most of the biological responses to TCDD and related PAHs are mediated by a cytosolic protein designated the aryl hydrocarbon receptor (AhR), which exists in the cytosol as a part of a complex that has a molecular mass of about 280 kDa [6,7]. Upon binding of TCDD with AhR, AhR dissociates from the above complex and translocates to the nucleus where it heterodimerizes with a structurally related protein, called the AhR nuclear translocator (Arnt) [8]. The heterodimeric AhR/Arnt complex binds to a 5-bp sequence, named xenobiotic-responsive element (XRE) (5′-GCGTG-3′), located within the 5′-flanking region of the AhR target genes, *CYP1A1*, *CYP1A2* and *CYP1B1* [9].

A phenomenon known as wasting syndrome is observed in wild-type (WT) mice treated with TCDD but not in AhR-null mice treated with TCDD [10]. In addition, teratogenesis such as cleft palate and hydronephrosis is seen upon treatment of dams with TCDD [4,10], while AhR-null mice do not exhibit teratogenesis after treatment with this agent [10]. These findings suggest that the toxicities seen with TCDD are mediated by AhR. However, the AhR target genes involved in the wasting syndrome and teratogenesis have not yet been elucidated.

To determine causal genes for the toxicity of dioxins and PAHs, we examined molecular changes in gene expression profiles caused by treatment of mice with 3-methylcholanthrene (MC) by using differential mRNA display. We found a cDNA that disappeared upon treatment of mice with MC. This cDNA encoded major urinary protein 2 (MUP2), which is a group of closely related proteins secreted into mouse urine [11] and known to be a member of the lipocalin superfamily of proteins [12]. The expression of MUP2 is thought to be mediated by the growth hormone (GH) [13,14]. Signal transduction pathway binding of GH to the GH receptor (GHR) promotes the association of the GHR with the Janus kinase 2 (JAK2) and tyrosyl phosphorylation of JAK2. Then activated JAK2 phosphorylates the tyrosine residues of a signal transducer and activator transcription (STAT) protein. The homodimer of the STAT or heterodimer of STAT protein with other factor(s) is found in the cytoplasm. The complex then translocates to the nucleus, and then binds to target sequences. Supporting this idea, STAT5 has been reported to participate in the GH-induced expression of MUP2, cytokine-inducible SH2-containing protein (CIS) or α-whey acidic protein (WAP) [15–17].

In the present study, we found that the expression of GHR

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Abbreviations: AhR, aryl hydrocarbon receptor; Arnt, AhR nuclear translocator; CIS, cytokine-inducible SH2-containing protein; CYP, cytochrome P450; EMSA, electrophoretic mobility shift assay; GAS, γ-interferon-activated site; GH, growth hormone; GHR, growth hormone receptor; JAK2, Janus kinase 2; MC, 3-methylcholanthrene; MUP, major urinary protein; PAH, polycyclic aromatic hydrocarbon; PCR, polymerase chain reaction; RT, reverse transcription; STAT, signal transducer and activator of transcription; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; WAP, α-whey acidic protein; WT, wild-type; XRE, xenobiotic-responsive element

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and JAK2 mRNAs was inhibited by MC, resulting in a decrease in the binding activity of STAT5 for the STAT5-binding site. We discuss the possible mechanism(s) by which exposure to PAHs leads to a decrease in body weight, which is known as the wasting syndrome.

2. Materials and methods

2.1. Animal treatment

Male 7-week-old C57BL/6J mice (Sankyo Experimental Animals, Tokyo, Japan) and AhR-null mice [18] were treated with MC (Sigma, St. Louis, MO, USA) dissolved in corn oil at a dose of 80 mg/kg/day intraperitoneally once daily for 2 days. Twenty-four hours after the last administration, the mice were killed, and the livers were removed and immediately used for the following experiments.

2.2. Differential mRNA display

Total RNAs were prepared from the livers of mice by the guanidinium/cesium chloride method [19]. Reverse transcription polymerase chain reaction (RT-PCR)-based differential mRNA display was performed by using a fluorescence differential display kit (Takara, Kyoto, Japan) essentially according to the manufacturer's instructions [20] with minor modifications. Briefly, total RNA (0.5 μg) and the two-base-anchored 5′-fluorescein-labeled oligo(dT) primer (5′-T_{13-16}AC-3′) were used for RT reaction. cDNA was synthesized by means of PCR using an arbitrary primer (5′-CTGCTTGATG-3′). The PCR products were separated on a 6% DNA sequencing gel and analyzed by autofluorography (FLA2000, Fuji Film, Tokyo, Japan). Differentially expressed cDNAs were recovered from the gel, and then amplified using the same PCR primers. Amplified cDNAs were subcloned into the pGEM-T® Easy Vector (Promega, Madison, WI, USA) and transformed into competent *Escherichia coli* cells. Plasmids which contained inserts were subjected to sequencing. Using a Bigdye primer cycle sequencing kit (PE Applied Biosystems, Foster City, CA, USA) on an ABI 310 automated sequencer (PE Applied Biosystems), a sequence similar to the isolated cDNA was sought by using the BLAST 2.1 program.

2.3. Northern blot analysis

Total RNAs were prepared from the livers of WT mice or WT mice treated with MC as previously reported [20]. A part of MUP2 cDNA [11] (593–774, relative to the initiation codon) was used as a probe. The entire coding regions of murine CIS and WAP cDNAs were obtained by RT-PCR as described elsewhere [21,22]. Total RNA (20 μg) was electrophoresed in a 0.8% agarose gel containing 18% formaldehyde and was transferred to a nylon membrane (Nytran N, Schleicher and Schuell, Dassel, Germany). The membrane was hybridized with ^{32}P-labeled cDNA by using DNA labeling system (Nippon Gene, Tokyo, Japan). Hybridization was carried out by the method as previously described [23]. The membrane was washed twice with 1×saline sodium citrate containing 0.2% sodium dodecyl sulfate at 50°C for 30 min.

2.4. RT-PCR

Determining the expression levels of mRNAs for GHR, JAK2 and STAT5, RT-PCR using total RNAs prepared from the livers of mice was carried out as follows. Briefly, total RNA (3 μg) was mixed with 50 μl of RNA–primer mixture (oligodeoxythymidylic acid primer (0.5 μg), a Moloney murine leukemia virus reverse transcriptase (20 U) (Toyobo, Tokyo, Japan), RNase inhibitor (20 U) (Takara, Tokyo, Japan), 0.5 mM each of four deoxynucleoside triphosphates), and incubated at 37°C for 60 min. PCR was performed in 50 μl reaction mixture (1.5 mM $MgCl_2$, 0.2 mM each of four deoxynucleoside triphosphates, each primer (50 pmol), AmpliTaq Gold polymerase (2.5 U) (Perkin Elmer, Norwalk, CT, USA), 10×AmpliTaq reaction buffer (5 μl) (Perkin Elmer)) containing cDNA synthesized by RT (1 μl). The reaction mixture was incubated at 94°C for 12 min. The reaction was performed in 30–35 cycles at 94°C for 1 min, at 55°C for 1 min 10 s, and at 72°C for 1 min 30 s. The PCR products were subjected to a 2% agarose gel, and then visualized by ethidium bromide staining. The sequences of oligonucleotide primers were as follows. Forward primers for murine STAT5, GHR, JAK2 and β-actin were 5′-GATCGGAATTCCCAGAAGGAT-3′, 5′-AATGCAGATGTTCTGAAGGGA-3′, 5′-GACGTACAGTTATATTGTGAT-3′, and 5′-CAACTGGGACGACATGGAGAA-3′, respectively [24]. Reverse primers for murine STAT5, GHR, JAK2 and β-actin were 5′-TGCTGTTGTAGTCCTCGAGG-3′, 5′-ATACTTGCTGTCTCAGACATCT-3′, 5′-AACTGTAATGCTAATGCCAGG-3′, and 5′-CATCTCCTGCTCGAAGTCTAG-3′, respectively [24].

2.5. Preparation of liver homogenates and electrophoretic mobility shift assay (EMSA)

Livers (1 g) were homogenized with three strokes in 10 ml of homogenizing buffer (10 mM Tris–HCl (pH 7.4), 1 mM EDTA, 250 mM sucrose, 1 mM sodium orthovanadate, 10 mM sodium fluoride and 100 μM phenylmethylsulfonyl fluoride) and centrifuged at 9000×*g* for 15 min. The supernatant was used as liver homogenates for the following experiments. Protein concentration was determined using a protein assay kit (Bio-Rad, Hercules, CA, USA) [25]. EMSA was performed with 20 μl of a reaction mixture containing 25 mM HEPES (pH 7.9), 4% Ficoll, 40 mM KCl, 0.5 mM dithiothreitol, 0.1 mM EGTA, 1 mM $MgCl_2$, poly[dI-dC] (1 μg), carrier DNA (1 μg), 5% glycerol, liver homogenates (15 μg) and ^{32}P-labeled probe DNA (5×10^4 cpm). The mixture was incubated at room temperature for 20 min, and then further incubated for 10 min on ice. The DNA-binding complex was electrophoresed in a 4% polyacrylamide gel. Oligonucleotide primers used as probes are as follows: STAT5 consensus sequence (rat β-casein γ-interferon-activated site (GAS) element) [26], 5′-GATCAGATTTCTAGGAATTCAATCC-3′ and 5′-GATCGGATTGAATTCCTAGAAATCT-3′.

2.6. Antibodies

Antibodies against STAT5a or STAT5b proteins were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). A supershift assay was performed using these antibodies as follows. After incubation of probe DNA with liver homogenates as described above, antibodies were added to the reaction mixture and incubated at room temperature for 10 min. The mixture was then incubated for 10 min on ice and the products subjected to EMSA.

3. Results and discussion

To monitor molecular alterations caused by treatment of mice with MC, differential mRNA display using total RNAs prepared from both the livers of WT mice and WT mice treated with MC was performed (Fig. 1). A cDNA band with the size of 1.35 kb (designated A45) was present in untreated mice but not in MC-treated mice. This band was recovered from a gel and subjected to sequence analysis. A data base search using the BLAST 2.1 program revealed that the

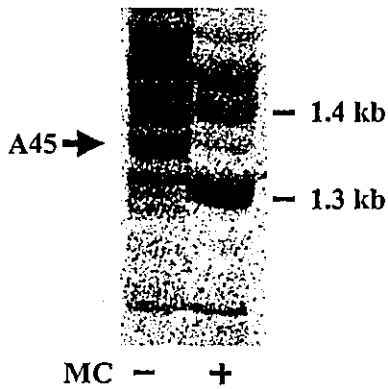

Fig. 1. Loss of a cDNA band by treatment of mice with MC as detected by differential mRNA display. Male C57BL/6J mice were treated with MC dissolved in corn oil at a dose of 80 mg/kg/day intraperitoneally once daily for 2 days. Twenty-four hours after the last injection, these mice were killed, and the livers were removed to prepare RNAs. Total RNA (0.5 μg) was converted to cDNA and differential display was performed. Samples were run on a 6% polyacrylamide/8 M urea gel.

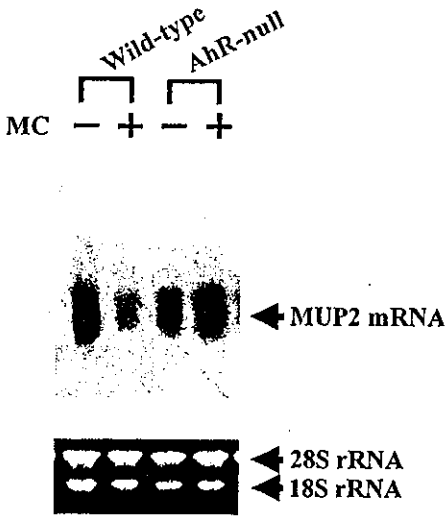

Fig. 2. Suppression of MUP2 expression by MC. WT and AhR-null mice were treated with MC and a portion (20 μg) of total RNA was subjected to Northern blot analysis. The position of migration of MUP2 mRNA is indicated by an arrow (upper panel). An identical blot was stained with ethidium bromide to verify equal RNA loading (lower panel).

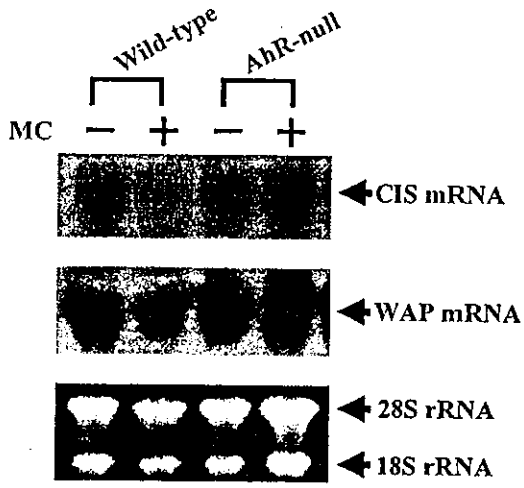

Fig. 3. Down-regulation of the expression of CIS and WAP by treatment of mice with MC. Total RNA (20 μg) was subjected to Northern blot analysis. The mRNAs encoding CIS or WAP are indicated by arrows (upper and middle panels). An identical blot was strained with ethidium bromide to verify the amounts of RNA loaded (lower panel).

sequence of clone A45 was identical to that of the murine MUP2 cDNA (data not shown). MUP2 is a member of the major urinary protein (MUP) gene family [11]. MUPs are a group of closely related pheromone carriers secreted into mouse urine [27]. MUPs are a class of lipocalin proteins encoded by a family of about 35–40 genes that exhibit sequence conservation of at least 85%, both in the transcribed and in the flanking sequences [12,28]. In male liver, most MUPs belong to the group 1 MUP gene family. MUP2 is a major isoform of group 1 MUP gene product in C57BL/6 mice [11] and is reported to be induced by GH [14].

To further confirm that the expression of MUP2 mRNA was suppressed by MC, we carried out Northern blot analysis using murine MUP2 cDNA as a probe and total RNAs prepared from the livers of untreated and MC-treated mice (Fig. 2). Consistent with the results shown in Fig. 1, the expression of MUP2 mRNA was clearly decreased by MC. In addition, to examine whether or not the decreased expression of MUP2 mRNA by MC was mediated by AhR, AhR-null mice were also treated with MC (Fig. 2). The result showed that the expression level of MUP2 mRNA was not reduced by treatment of AhR-null mice with MC. Thus, it appeared that the suppression of the expression of MUP2 mRNA by MC was dependent on AhR. Since there is a possibility that the MUP2 cDNA probe can be hybridized with other group 1 MUP gene products due to the high degree of homology, the band detected using the MUP2 cDNA probe may contain other MUP isoforms [11,12].

It is of particular interest to note that expression of the MUP2 gene is stimulated by the GH signal transduction pathway, through GHR, JAK2 and STAT5 [1,14]. GH is known to induce the expression of CIS and WAP, which encodes a negative regulator of signaling through cytokine receptor and a group of milk proteins related to mammary gland development, in addition to MUP2 [15,16,21]. If the GH signal is affected by MC, then expression of other GH-regulated genes, such as CIS and WAP, should be down-regulated by MC. To explore this possibility, we compared the expression levels of CIS and WAP mRNAs in livers from untreated and MC-treated mice (Fig. 3). As noted in the expression of MUP2 mRNA, the expression of mRNA for CIS and WAP was also down-regulated by MC. Due to the possibility that down-regulation of expression of mRNAs encoding CIS and WAP by MC was mediated by AhR, the same experiment was performed with AhR-null mice (Fig. 3). Indeed, the expression of CIS and WAP mRNAs was unaffected by MC in AhR-null mice. These results suggest that GH signal transduction may be disrupted by MC dependent on AhR.

As noted above, JAK2 and STAT5, through the GHR, mediate the stimulation of the MUP2, CIS and WAP genes by GH. Thus, we examined the effects of MC treatment on

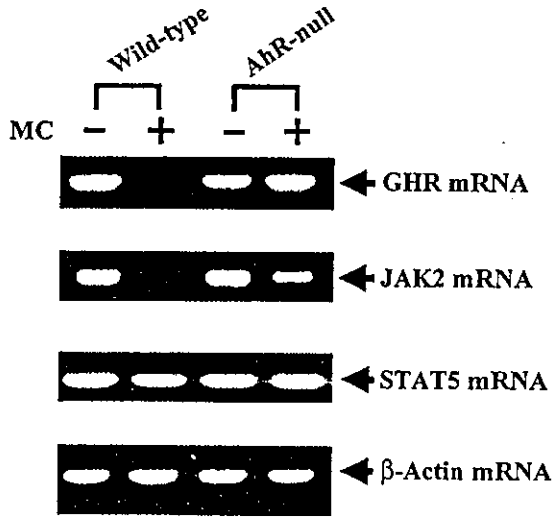

Fig. 4. Effects of MC on the expression of mRNAs for GHR, JAK2 and STAT5. Total RNA (3 μg) was subjected to RT-PCR. The mRNAs encoding GHR, JAK2 and STAT5 are indicated by arrows. RT-PCR for β-actin was used to check equal loading of cDNA samples.

the expression levels of mRNAs encoding GHR, JAK2 and STAT5. As shown in Fig. 4, the expression of both GHR and JAK2 mRNAs as determined by RT-PCR was inhibited by MC, although the expression of STAT5 was unaffected. These alterations in the expression of GHR and JAK2 mRNAs were not seen in AhR-null mice. Thus, it appeared that down-regulation of the expression of GHR and JAK2 mRNA by MC was also mediated by AhR.

It was reported that STAT5 activated by JAK2 mediated tyrosyl phosphorylation to bind to a STAT5-binding site. As shown in Fig. 4, we found that the expression of STAT5 was not altered by MC. From these lines of evidence, it was expected that the amounts of STAT5 bound to the STAT5-binding site were reduced by MC. Thus, we performed EMSA using the STAT5 consensus sequence [26] as a probe and liver homogenates from untreated and MC-treated mice (Fig. 5). A band appeared using the STAT5 consensus sequence and liver homogenates from untreated mice. This band was supershifted by the presence of antibodies to STAT5a or STAT5b, indicating that the band is derived, at least in part, from the STAT5a/STAT5b heterodimer. The binding of the homodimer or heterodimer of STAT5a and/or STAT5b to the STAT5 consensus sequence was diminished by MC. To further confirm that the reduced binding of STAT5 caused by MC was mediated by AhR, liver homogenates from AhR-null mice or AhR-null mice treated with MC were applied to EMSA (Fig. 5). As expected, the binding of STAT5 to STAT5-binding element was not altered in AhR-null mice. Based on these results, we confirmed that the reduction of the expression of GHR and JAK2 mRNAs by MC attenuates the binding activity of STAT5 for the STAT5-binding element, thus resulting in down-regulation of the expression of MUP2, CIS and WAP mRNAs.

MUP plays important roles in individual recognition, territorial marking and sex behavior [29]. Since it is reported that cognitive function and reproductive behavior are disrupted by polychlorinated biphenyls and TCDD in humans and laboratory animals [30–32], the suppression of MUP expression by

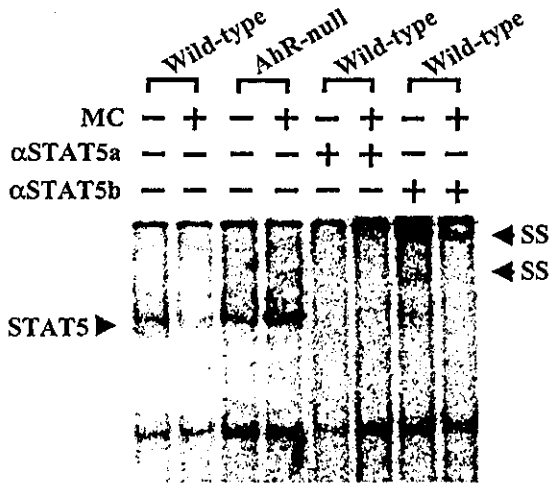

Fig. 5. Decreased binding of STAT5 to STAT5-binding element by treatment of mice with MC. A ^{32}P-labeled double-stranded STAT5 consensus sequence (rat β-casein GAS element) [28] was incubated with liver homogenates (15 μg) prepared from WT or AhR-null mice in the presence or absence of antibodies against STAT5a (αSTAT5a) or STAT5b (αSTAT5b). SS, supershifted band.

PAHs may occur in the behavioral changes. The PAH-induced repression of WAP may influence the process of mammary gland development, because it is known that the development and proliferation of terminal end buds is impaired by exposure to AhR ligands in rodents [33,34]. CIS encodes a negative regulator of signaling through cytokine receptor. Thus, the down-regulation of CIS expression may lead to disruption of cytokine signaling.

The suppression of MUP2 expression by MC was apparently mediated by AhR. However, there are no apparent XREs within the promoter region up to 869 bp of the murine MUP2 gene [11]. In addition, XREs were also absent in the promoter region of the CIS [17] and WAP genes (GenBank accession number U38816). Thus, these observations suggest that the suppression of MUP2, CIS and WAP expression by MC is due to the suppression of the expression of GHR and JAK2 mRNAs.

Searching for a possible XRE sequence(s), we found several possible XREs in the promoter regions (L1–L4) of the murine GHR gene [35,36]. A possible XRE sequence was also located within a V1 promoter region necessary for the liver-specific expression of the human GHR gene [37,38]. Thus, AhR may affect the liver-specific transcription of the human GHR gene through XREs. The mechanism for the repression of GHR expression is currently under examination. Unlike GHR, the promoter region of the JAK2 gene [39] did not contain any obvious XREs. Thus, the expression of JAK2 may be indirectly regulated by AhR on the GHR. We identified possible XREs in the 5′-upstream region of the STAT5a and STAT5b genes. However, these XREs may not function as *cis*-acting elements to modulate the expression of STAT5 genes, since the expression of STAT5 was unaffected by MC.

The amounts of binding of STAT5a/STAT5b homo- or heterodimer were reduced by MC. This context should be noted in that STAT5a- and STAT5b-null mice showed a slower growth rate than WT mice, and were smaller in size [15,40,41]. It was reported that exposure to PAHs causes a decrease in body weight, which is called the wasting syndrome [4]. Thus, it is tempting to speculate that the decrease in body weight by exposure to PAHs may be explained, at least in part, by the AhR-mediated disruption of the GH signaling pathway. The GH signaling pathway also plays an important role in skeletal growth [42]. Interestingly, it was reported that PAHs and TCDD inhibited GH-induced proliferation and differentiation of osteoblasts via AhR [43,44]. Therefore, AhR-mediated suppression of the GH signaling pathway may also account for the abnormality of bone formation induced by PAHs and TCDD.

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In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) affects tooth development in rhesus monkeys

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Introduction

The current tolerable daily intake (TDI) of dioxin and dioxin related compounds has been set at 4 pg TEQ/kg/day in Japan. This value was calculated from the lowest-observed-adverse-effect level (LOAEL) in experimental animals, mostly rodents. Gray *et al.* reported that a single oral dose of 200 ng/kg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to pregnant rats on day 15 of gestation resulted in abnormalities of reproductive organs in the offspring[1]. The maternal body burden at this dose was measured to be 86 ng/kg. To attain this body burden level, human daily intake was calculated to be 43.6 pg/kg/day. An uncertainty factor of 10 was applied to this value, and the human TDI was established. However, due to great differences in the biological half life of TCDD between human and rodents, the validity of this calculation is questioned. To obtain more reliable LOAEL in the second generation, we initiated a long-term study in rhesus monkeys in 1999.

In rodents, teeth are known to be targets of developmental toxicity of dioxin. *In utero* and lactational TCDD exposure affects rat incisor and molar developmet[2,3]. In humans also tooth abnormalities were reported among populations exposed to dioxins[4]. In our monkey experiment, some young were stillborn or died neonatally. These animals provided us with a unique opportunity to study tooth development in primate young exposed to TCDD *in utero* and lactationally. By macroscopic observation we found some tooth abnormalities among died young exposed to TCDD[5]. This prompted us to examine surviving young by radiography. This is an interim report of our findings in these young.

Methods and Materials

Animals: Adult female rhesus monkeys at the age of 5-7 years and weighing 4-6 kg purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijin, China) were used. Details of breeding conditions were given elsewhere[6]. Female monkeys were allowed to cohabit with males for three days on days 12, 13, and 14 of the menstrual cycle. When copulation was confirmed visually, the median day of the mating period was disignated as day 0 of gestation (GD 0). On GD18 or 19, pregnancy was confirmed by an ultrasound device. Pregnant monkeys were divided into three groups each consisting of approximately 20 animals and allowed to deliver naturally. The day on which delivery was detected was designated as postnatal day 0 (PD0).

Administration of TCDD: TCDD was dissolved in a mixture of toluene/DMSO (1:2, v/v) at a concentration of 300 ng/ml. Pregnant females were given TCDD subcutaneously into the back region on day 20 of gestation at an initial dose level of 30 or 300 ng/kg. The control animals received the vehicle in a volume of 1 ml/kg. For maintenance of a certain body burden, 5% of the initial dose, i.e. 0.6 or 6 ng/kg, was given to dams every 30 days during pregnancy and lactation until day 90 after birth.

Measurement of TCDD in maternal serum: Approximately 20 ml of blood was taken from the femoral vein of the dams on day 80 of pregnancy, and centrifuged. The obtained serum was subjected to high resolution gas chromatography (HRGC)/high resolution mass spectrometry (HRMS) by the method of Patterson *et al.*[7]

Observation of teeth of the young: Stillborn and postnatally died young were autopsied, and the upper and lower jaws were dissected for detailed observation. Surviving young were anesthesized by intramuscular injection of ketamine at 10 mg/kg into the thigh before examination. Photographs were taken by an intraoral digital camera (Crystal Cam II, GC Co., Ltd., Tokyo). Conventional intraoral radiographs were taken by a portable X-ray apparatus (KX-60, Asahi Roentgen Ind. Co., Ltd., Kyoto) with a charge coupled device (CCD) (Gendex Visualix, Dentsply International Inc., York, PA, USA).

Results and Discussion

Pregnancy outcome and postnatal development of the young: Table 1 summarizes the pregnancy outcome and postnatal mortality of the young. Abortions, stillborns, and postnatal deaths occurred fairly frequently even in the control group. To increase the number of surviving young in the 300 ng/kg, we added 9 dams to the group approximately 2 years after the initiation of the experiment. However, only two young survived more than a year due to a high incidence of abortions. No significant differences were noted in the gestation length and birth weight among the control and TCDD-treated groups, indicating the body burden of TCDD at 300 ng/kg did not affected general growth of the young.

Table 1: Pregnancy outcome and postnatal mortality of rhesus monkeys exposed to TCDD.

Group	No. of dams	No. of abortions	No. of stillborns	No. of live borns	No. of postnatal deaths	Gestation length (days)	Birth weight (g)
Control	23	2	3	18	5	161.8±7.8	426.1±58.6
30 ng/kg	20	0	5	15	3	163.8±5.9	426.8±56.9
300 ng/kg	20	2	2	16	8	164.9±9.7	408.6±63.7
300 ng/kg[1)]	9	5	1	3	1	165.0±3.0	466.0±87.1

1) Newly added group

Tooth abnormalities in the young: The incidence of tooth abnormalities in the young was shown in Table 2. Tooth abnormalities in the stillborn and postnatally died young were described previously[5]. No abnormalities were detected in the control and 30 ng/kg groups, whereas more than half of the young in the 300 ng/kg had tooth abnormalities as listed in Table 3. The upper permanent lateral incisors were most frequently affected. In contrast, among the deciduous teeth, the central incisors seemed to be most sensitive targets of developmental toxicity of TCDD. The permanent premolars were also affected frequently, while the canine and the first molar were resistant to the adverse effect of TCDD. Probably these larger teeth have become resistant to odontotoxic chemicals during the course of evolution.

Table 2: Incidence of tooth abnormalities among F1 exposed to TCDD.

Group	Stillborns and postnatally died young			Surviving young		
	No. of specimens	No. of specimens with tooth abnormalities (%)		No. of young	No. of young with tooth abnormalities (%)	
Control	4	0	(0)	13	0	(0)
30 ng/kg	5	0	(0)	12	0	(0)
300 ng/kg	8	3	(38)	8	6	(75)
300 ng/kg[1)]	2	0	(0)	2	1	(50)

1) Newly added group

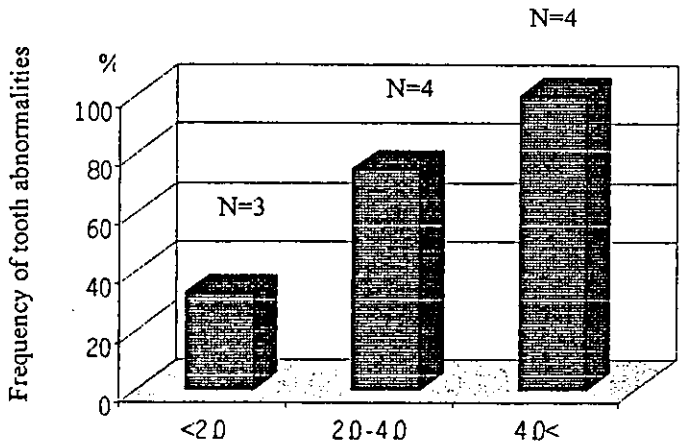
Relationship between maternal serum TCDD concentration and occurrence of tooth abnormalities: In the control maternal serum, the TCDD levels were below the detection limit. In the 30 ng/kg group, the levels were fairly constant, ranging from 0.19 to 0.21 pg/g wet weight. In contrast, the levels varied largely in the 300 ng/kg group, ranging from 1.1 to 8.9 pg/g wet weight. The average of those without tooth abnormalities in their young was 1.4 ± 0.6 pg/g wet weight, whereas that with tooth abnormalities was 4.3 ± 2.4 pg/g wet weight. The concentration-response relationship is shown in Fig. 1.

Table 3: Tooth abnormalities detected in the young exposed to TCDD at 300 ng/kg.

Young	Sex	Age (days)[2)]	Abnormal Findings
31	♀	1430	542|24 missing |5 conical
39	♂	1410	542|245 missing
42	♀	1415	5|5 missing |4 conical
44	♂	1415	54 |45 missing |5 conical
60	♂	1388	542|245 5|5 missing
66	♂	1338	52|2 1 |1 missing
			54 |45 malaligned |45 conical
106[1)]	♀	688	A |A 4 |24 missing

1) Newly added group
2) Age at X-ray examination

Figure 1: Maternal serum concentration of TCDD and the incidence of tooth abnormalities.



Maternal serum concentration of TCDD (pg/g wet weight)
N = number of specimens in each concentration category

Validity of the current TDI: The above results indicate that the LOAEL body burden for induction of tooth abnormalities in the rhesus monkey is at a certain level between 30 ng/kg and 300 ng/kg, probably not much different from the LOAEL body burden for rodents, 86 ng/kg. Hence it is reasonable to conclude that the current TDI of dioxins in Japan needs no immediate modification.

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NO EFFECTS OF DIOXIN SINGLY ON LIMB MALFORMATIONS IN MACAQUE MONKEYS THROUGH EPIDEMIOLOGICAL AND TREATED STUDIES

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Introduction

Human populations exposed with highly dioxin were suspected to be caused immunological dysfunctions, carcinogenesis, and developmental and reproductive dysfunctions. Because of species resemblances, the dioxin effects have been investigating using monkeys as a model for assessment of dioxin exposure on human health [1-6]. Since 1957 the limb malformations of monkeys in Japan have been reported [7-8]. The higher frequency of them was found in provisional groups of monkeys who were given the same kind of food for human. The chromosomal abnormalities are excluded from the factor for the congenital limb malformations [9] that are still producing in Japan. In this study, the relations between dioxin and the limb malformations of macaque monkeys were estimated by the epidemiological and administered researches. The dioxin levels in monkeys were measured at two districts that one has the provisional groups including monkeys with limb malformations and the other has breeding groups never seeing the malformations for a long time. TEQ was calculated by the levels of dioxin isomers in the monkeys and the values show no difference between the two places and between the individuals with and without the limb malformations. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was administered via subcutaneous to pregnant rhesus monkeys from the day 20 of gestation to the day 90 after birth. The exposed babies, including the offspring and died in neonatal, had observed normal limbs in the range of 30-300 ng TCDD /kg of body weight.

Methods and Materials

Chemicals. 2, 3,7,8-TCDD dissolved in toluene and DMSO (1:2, V/V) was purchased from Daiichi Pure Chemicals Co., Ltd. Tokyo, Japan.

Animals. For epidemiological studies, blood samples were collected from Japanese monkeys living in two different districts, provisional groups of semi-wild monkeys and breeding monkeys. Some in the former are born with limb malformations and in the later no records of birth with malformation. The monkeys captured and treated according to the animal control program and the guide for the care and use of laboratory primates. Four samples including two abnormal monkeys were obtained from the provisional group of monkeys at Arashiyama, Kyoto Japan. The other four samples were obtained from the breeding facility at Primate Research Institute, Kyoto University, Inuyama, Aichi, Japan. The ages of all the monkeys tested were conformed by records of birth. The samples were collected after anaesthetization with Ketaral and kept in freezing until used. For exposure studies, rhesus monkeys purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijing, China). The monkeys (6-9 years old and 4.5-6.5 kg in body weight) were kept in Shin Nippon Biomedical Laboratories, Ltd, Kagoshima, Japan. The breeding conditions were described previously [10]. The rhesus monkeys were mated, and the pregnancies were administered 2, 3,7,8-TCDD (30-300 ng/kg of body weight) via subcutaneous on day 20 of their gestation. Every 30 days interval, 5% of the initial dose of TCDD was given to the pregnancies until day 90 after birth for maintaining the body burden. Controls were given the vehicle.

Measurement of dioxin isomers in blood. The isomers of dioxins in 10 ml of blood samples from the monkeys was measured using a high resolution mass spectrometer by the methods of the provisional manual for analyzing the dioxin in blood by the Ministry of Health, Labour and Welfare (22 Dec, 2000).

Results and Discussion

Epidemiological analysis – In total four monkeys at the provisional groups, two monkeys had limb malformations that were split hands as shown in figure 1. This type categorized in absence deformities is the most frequently observed in the limb malformations that occurred under 5% of the groups at Arashiyama in 1972-1979 [8]. The other monkeys including breeding monkeys had normal llimbs. Among the measured dioxin isomers, polychlorinated dibenzofran and coplanar polychlorobiphenyl were detected in major, and polychrolodibenzo-p-dioxine was detected in minor in the bloods of monkeys living at both semi-wild and breeding. TEQ was calculated by the amount of the dioxin isomers among the monkeys. Figure 2 show the values in the bloods of the two malformation monkeys were pointed in the range of the values of the other normal monkeys. The difference of TEQ between the two districts also was not detected in the values of their averages.

Exposure analysis – TCDD is the most toxic in dioxin isomers. In utero and lactational exposure, TCDD affects morphological abnormalities that are reported on teeth development especially at incisor and molar in rodents[11-12] and monkeys[6]. But in the case of TCDD administration in the range of 30-300 ng /kg of body weight, the babies exposed with TCDD had observed normal limbs including the offspring and died in neonatal. The TCDD administration was started on day 20 of gestation that is enough, if TCDD is teratogic on the limbs, to effect on the limb malformation in