OPCW

Scientific Advisory Board

Fifteenth Session
12 – 14 April 2010

SAB-15/1
14 April 2010
Original: ENGLISH

REPORT OF THE FIFTEENTH SESSION OF THE SCIENTIFIC ADVISORY BOARD

1. AGENDA ITEM ONE – Opening of the session

The Scientific Advisory Board (SAB) met for its Fifteenth Session from 12 to 14 April 2010 at the OPCW Headquarters in The Hague, the Netherlands. The session was opened by the Chairperson of the SAB, Philip Coleman of South Africa. Mahdi Balali-Mood of the Islamic Republic of Iran served as Vice-Chairperson. A list of participants appears as Annex 1 to this report.

2. AGENDA ITEM TWO – Election of the Chairperson and Vice-Chairperson of the Scientific Advisory Board[1]

In an informal meeting chaired by Herbert De Bisschop prior to the commencement of the formal session, the SAB members re-elected, by consensus, Philip Coleman as the Chairperson of the SAB and Mahdi Balali-Mood as Vice-Chairperson, each for a term of one year.

3. AGENDA ITEM THREE – Adoption of the agenda

The SAB adopted the following agenda for its Fifteenth Session:

1. Opening of the session
2. Election of the Chairperson and Vice-Chairperson of the Scientific Advisory Board
3. Adoption of the agenda
4. *Tour de table* to introduce Scientific Advisory Board Members
5. Welcome address by the Deputy Director-General

[1] In accordance with paragraph 1.1 of the rules of procedure for the SAB and the temporary working groups of scientific experts (EC-XIII/DG.2, dated 20 October 1998).

6. Overview of developments at the OPCW since the last session of the Scientific Advisory Board

7. Establishment of a drafting committee

8. Applications of nanomaterials and nanotechnology in drug delivery and their possible impact on the Chemical Weapons Convention

9. Introduction to molecularly imprinted polymers and their possible impact on the Chemical Weapons Convention

10. Methods of destruction for old chemical weapons

11. Scheduled chemicals, including ricin and saxitoxin

12. Future work of the Scientific Advisory Board

13. Any other business

14. Adoption of the report

15. Closure of the session

4. AGENDA ITEM FOUR – *Tour de table* to introduce Scientific Advisory Board members

The meeting was opened with the introduction of existing SAB members for the benefit of a new member, William Kane from the United States of America.

5. AGENDA ITEM FIVE – Welcome address by the Deputy Director-General

5.1 The Deputy Director-General, on behalf of the Director-General, welcomed the members of the SAB, in particular its new member. The Deputy Director-General conveyed to R. Vijayaraghavan of India and Zhiqiang Xia of China, who were completing their second term of office on the SAB, the deep appreciation of the Director-General for their commitment and contribution to the work of the Board.

5.2 The Deputy Director-General also thanked Professors Couvreur, Kumar, and Pernelle for sharing their knowledge and experience, and for their important contribution to the work of the SAB.

5.3 The Deputy Director-General indicated that the Director-General commended the work of the SAB on the modified version of the definition of ricin, which better clarifies the scope of this toxin that is relevant to the Chemical Weapons Convention (hereinafter "the Convention").

5.4 The SAB was informed that the Director-General has written to its Chairperson to seek his views on the extension of the SAB meeting from three to five days in order to increase the time for the SAB's deliberations. The Director-General is prepared to

make financing available from the Programme and Budget of the OPCW, upon approval of the Member States, for this purpose.

5.5 Since this was the last session of the SAB under the incumbent Director-General's tenure, the Deputy Director-General conveyed the deep appreciation of the Director-General for the dedication that the members of the SAB continue to show to their mission and their work. The appreciation of the Director-General stems from a full understanding of the importance of the mandate of the SAB, and of the unique expertise the SAB offers in respect of the effective implementation of the disarmament, non-proliferation, and verification regimes of the Convention.

6. AGENDA ITEM SIX – Overview of developments at the OPCW since the last session of the Scientific Advisory Board

The Secretary gave a presentation to the SAB on developments at the OPCW since the SAB's Fourteenth Session (which was held from 9 to 11 November 2009). The members were informed about the status of relevant aspects of the Convention, including destruction of Category 1 chemical weapons as at 28 February 2010, the status of membership of the Convention, and efforts made towards universality. The SAB was briefed on the financial status of its trust fund and noted that the current balance was insufficient to convene its planned meeting in November 2010.

7. AGENDA ITEM SEVEN – Establishment of a drafting committee

The SAB established a drafting committee, composed of Robin Black, Robert Mathews, and Stefan Mogl, to prepare a draft report of its Fifteenth Session.

8. AGENDA ITEM EIGHT – Applications of nanomaterials and nanotechnology in drug delivery and their possible impact on the Chemical Weapons Convention

8.1 At its Fourteenth Session, the SAB recommended that it should continue to maintain a close watch on developments in nanotechnology and nanomaterials. It also proposed that it should address the question of applications of nanomaterials and nanotechnology in drug delivery. The SAB therefore invited Professor Patrick Couvreur of the University of Paris-Sud, France, who is also holder of the chair of "Innovation Technologique" at the prestigious Collège de France, to give a presentation. Professor Couvreur is a leading figure in the field of medical nanotechnology, and has been practising nanotechnology since 1975. He was the first to develop nanometric capsules able to penetrate cells to deliver drugs and is now working on nanoparticles that can target cancerous cells directly. The SAB also invited Professor Ravi Kumar from India, who has been teaching drug delivery since January 2008 at the Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, United Kingdom of Great Britain and Northern Ireland, to give a presentation.

8.2 Professor Couvreur explained in his presentation how different types/architectures of nanocarriers have been developed for the transport of drugs to their target. These nanocarriers are generally delivered intravenously. He demonstrated the fact that these nanocarriers consist of an inner core and an outer corona, and that both can fulfil different functionalities. The core can be capsulated to protect the drug from the

body's defence system, and thus improve its pharmacokinetics. It can be tailored to simultaneously deliver more than one drug to different targets, and to release a drug in a controlled manner in response to an external stimulus. The corona consists of polyethylene glycol (PEG) chains containing different functional groups. It can be designed to give the nanocarriers a "stealth-type" effect to evade immunological recognition by the reticulo-endothelial system and to guide the drug to the desired biological target. Professor Couvreur also showed that by adding specific functionalities to the PEG chains of the corona, the nanocarriers can cross the blood-brain barrier and deliver drugs to the brain. He sees future challenges in designing new nanocarriers with higher drug load and reduced so-called burst release, the use of nanotechnologies for overcoming resistance mechanisms in cancers and infectious diseases, and the use of nanotechnologies for gene therapy with non-viral vectors as an alternative to the utilisation of viruses. The presentation given by Professor Couvreur appears as Annex 2.

8.3 Professor Kumar focussed his presentation on the oral delivery of drugs and the use of nanoparticles as carriers of drugs across the biological barriers. His research group uses specific preparation methods, polymers, surfactants, and solvents to engineer nanoparticles that can deliver drugs. He explained how nanocarriers are used to protect drugs from the pre-systemic gut and liver metabolism and to guide drugs to target the tissues and cells of interest. Using examples of different clinical studies, Professor Kumar demonstrated how oral delivery of drugs with nanocarriers allows a reduction in the amount of medication required to produce similar or enhanced therapeutic effects, with fewer undesirable side effects. The presentation given by Professor Kumar appears as Annex 3.

8.4 Following their presentations, the guest speakers provided detailed responses to the various questions and comments posed by members of the SAB. The SAB thanked the two excellent speakers for their presentations.

8.5 In its subsequent deliberations, the SAB considered that at the current state of development, delivery of agents with nanocarriers by intravenous or oral route would have only a limited application for offensive chemical weapons purposes. In the classic form of delivery of chemical agents, in excess of 99% of nanocarriers would not reach their primary target. However, the SAB considered that there may be applications of nanocarriers for improved drug delivery of medical countermeasures to chemical-warfare agents, for example therapeutic oximes as antidotes for nerve agents. The SAB also stated that nanocarriers may have applications in decontamination. Members of the SAB emphasised the fast pace of development in nanotechnology and stressed that the SAB should keep a watching brief on the development of nanocarriers. The SAB also considered nanotoxicology to be of relevance to the Convention and thus recommended that an expert in the toxicology of nanoparticles be invited to address the SAB during a future session.

9. AGENDA ITEM NINE – Introduction to molecularly imprinted polymers and their possible impact on the Chemical Weapons Convention

9.1 At its Fourteenth Session, the SAB proposed that the subject of molecularly imprinted polymers should be addressed at a future session. To that end, the SAB invited Professor Christine Pernelle from the Conservatoire National des Arts et Métiers,

France, where she is Head of the "Chaire de génie analytique", to speak at its Fifteenth Session.

9.2 Professor Pernelle provided an introduction to molecularly imprinted polymers (MIPs) and presented a selection of applications of MIPs related to chemical protection in the areas of detection, on-site monitoring, and decontamination. She demonstrated that there had been a large increase in the number of publications on this subject and in the number of patents for applications using MIPs related to the analysis and detection of chemical-warfare agents and toxic industrial chemicals in the past 10 years. Professor Pernelle explained imprinting methods for the creation of MIPs based on covalent and non-covalent imprinting, emphasising that the latter was now the predominant method used for producing imprinted receptors in synthetic polymers. She explained the advantages and disadvantages of both methods and provided examples of MIPs as sensors for detection, and for the selective extraction and pre-concentration of analytes of relevance to the Convention. Professor Pernelle provided an overview of different MIP-based sensors and of their capabilities and limitations. The presentation given by Professor Pernelle appears as Annex 4.

9.3 Professor Pernelle's excellent presentation stimulated an active discussion among the members of the SAB. The general view was that MIPs presented a very interesting field of chemistry and that laboratory experiments had shown very promising results. However, concerns were raised by a number of SAB members about the suitability for field use of sensors based on MIPs, in particular when exposed to different levels of humidity, dust, and other atmospheric contaminants. For analytical purposes, a limitation of first-generation MIPs was their lack of applicability directly to aqueous samples. Recent developments appear to have overcome this limitation. The SAB considered that MIPs may have applications in on-site sample preparation, and in sample preparation for more complex matrices in off-site analysis. It recommended that its temporary working group on sampling and analysis should evaluate the current status of MIPs for chemical weapons-related analysis and report its findings and recommendations to the SAB at its Sixteenth Session. Members of the SAB were also of the view that the developments in MIPs were very promising and that the SAB should keep a watching brief on these developments.

10. AGENDA ITEM TEN – Methods of destruction for old chemical weapons

10.1 Herbert De Bisschop presented an overview of the methodology used in Belgium to destroy old chemical weapons retrieved from World War I. Nowadays, about 200 metric tonnes of old munitions are found in Belgium annually during agricultural activities, roadworks, construction, and so on, of which some five to ten percent are identified as chemical weapons. The basic approach involves an identification of the contents to confirm the presence of chemical agent.

10.2 The essential steps are manual clean-up to remove earth and corroded parts, classification of type and calibre based upon exhaustive documentation obtained from the manufacturing countries, X-ray analysis to measure the internal dimensions, and neutron activation analysis to check for the presence of certain elements, such as chlorine, bromine, arsenic, and sulphur. Before dismantling, a sample is taken via a hole that has been drilled in the shell under remote control. Definitive identification is done by gas chromatography-mass spectrometry (GC-MS). If, during the drilling,

pressure builds up, the gas is evacuated via a scrubber. For shells with a liquid content, the head of the shell is milled off and the chemical and explosive components are separated. The explosive parts are destroyed by detonation. The chemical agent is destroyed through incineration; this process is outsourced.

10.3 However, the process described above cannot be applied to shells filled with Clark agent. This is a solid compound (diphenylchloroarsine or diphenylcyanoarsine) contained in a bottle and embedded in explosive material. For those shells, a method has been developed based upon contained detonation. In a series of trials, an existing detonation chamber from DeMil International Inc., United States of America, was adapted for destroying chemical agents. This system was continuously improved and finally replaced by a larger detonation chamber from Ch2m Hill, United States of America, which operated successfully until the end of 2005. However, the system was unable to handle larger calibres (155 mm upwards).

10.4 The Belgian authorities decided to start the procedure for acquiring a system capable of also handling larger calibres. In 2006, a more industrialised system was chosen, known as the Detonation of Ammunition in Vacuum Integrated Chamber (DA VINCH), developed by Kobe Steel Ltd., Japan. This system has been in operation since 2008. Over 60,000 shells unearthed since October 1981 have been processed by the identification system. Approximately 16,000 chemical shells have been destroyed using either dismantlement or detonation over a period of 10 years.

10.5 The SAB was grateful to Herbert De Bisschop for his excellent explanation of the destruction of old chemical weapons. In the discussion following the presentation, members asked Herbert De Bisschop about different technical considerations regarding the identification of old munitions, the operation of a detonation chamber, and the current and potential destruction capacity of the detonation chamber located in Belgium. The SAB agreed that it should continue to follow developments in respect of the destruction of old chemical weapons. The SAB also recommended organising a visit to the destruction site of Poelkapelle, Belgium, during a future session.

11. AGENDA ITEM ELEVEN – Scheduled chemicals, including ricin and saxitoxin

Subitem 11(a): Saxitoxin

11.1 The SAB received an update from Robert Mathews on the comments that had been received from Switzerland and the United States of America on the draft fact sheet that he had prepared and distributed at the Fourteenth Session of the SAB. Additional drafting suggestions were made by SAB members during the Fifteenth Session. It was agreed that the draft fact sheet should be finalised, based on comments received, at a future session of the SAB.

11.2 The SAB returned to the issue of whether saxitoxin should continue to be listed as a Schedule 1 chemical, or whether it might be more appropriate under Schedule 2; a preliminary discussion on this matter had already taken place during the Fourteenth Session of the SAB. The discussion paper prepared on this issue by the Spiez Laboratory, Switzerland (which has been posted on the SAB Port@l) was discussed by the SAB, and was considered to be very useful in demonstrating why saxitoxin should remain listed as a Schedule 1 chemical.

Subitem 11(b): Transfer provisions for saxitoxin and ricin

11.3 The Spiez Laboratory discussion paper also addressed the issue of the transfer provisions for saxitoxin and ricin, based on the difficulties that have been experienced in the transfers of samples containing saxitoxin and ricin for analytical purposes (including the recently conducted round-robin exercise on ricin analysis).

11.4 The SAB recommended that the exemption from the 30-day notification period currently in place for quantities of five milligrams or less of saxitoxin for medical/diagnostic purposes (paragraph 5bis of Part VI of the Verification Annex to the Convention) should be extended to cover analytical purposes for both saxitoxin and ricin.

11.5 Following further discussion, the SAB also recommended that retransfers to other States Parties of quantities of five milligrams or less of saxitoxin and ricin should be permitted for medical/diagnostic and analytical purposes, without being subject to a 30-day notification requirement.

11.6 The above recommendations would require the application of the relevant provisions of Article XV of the Convention by States Parties.

12. AGENDA ITEM TWELVE – Future work of the Scientific Advisory Board

12.1 The SAB recommended discussing the following topics at its next session, which could be held in the autumn of 2010, should voluntary contributions be made available by Member States[2]:

(a) nanotoxicology;

(b) incapacitating chemical agents;

(c) novel toxic compounds;

(d) plan for compiling the report of the SAB on developments in science and technology to be submitted by the Director-General to the Third Special Session of the Conference of the States Parties to Review the Operation of the Chemical Weapons Convention. The plan should include tentative dates for a possible IUPAC[3]/OPCW meeting on the impact of scientific developments on the Convention, such as the one held in Zagreb, Croatia, in April 2007; and

(e) consideration of the report of the fifth meeting of the temporary working group on sampling and analysis, to be held in November 2010.

12.2 The SAB also recommended that, subject to confirmation, its Sixteenth Session should be scheduled to take place directly after the above-mentioned fifth meeting of the temporary working group on sampling and analysis (see paragraph 12.1).

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[2] See the Note by the Secretariat entitled "Call for Voluntary Contributions to the Trust Fund of the Scientific Advisory Board" (S/818/2010, dated 1 March 2010).

[3] IUPAC = International Union of Pure and Applied Chemistry.

12.3 In addition, and with reference to the Note by the Director-General concerning possible ways of enhancing the interaction between the SAB and States Parties, as well as the policy-making organs, making best use of governmental experts (EC-58/DG.1, dated 22 July 2009), members of the SAB expressed an interest in informing Member States about the activities of the SAB during an informal meeting in the margins of a session of the Executive Council (hereinafter "the Council") or the Conference of the States Parties (hereinafter "the Conference").

13. AGENDA ITEM THIRTEEN – Any other business

Subitem 13(a): Riot control agents and incapacitating chemical agents

13.1 The SAB heard presentations on this subject by two of its members. Stefan Mogl reported on an expert meeting organised by the International Committee of the Red Cross, entitled "Incapacitating Chemical Agents: Implications for International Law", held from 24 to 26 March 2010 in Montreux, Switzerland. Robert Mathews presented a historical overview of the negotiations related to riot control agents and incapacitating chemical agents, emphasising the complexity of the issues, the differing interpretations of the Convention, and the different areas that may require further clarification, which include political, legal, and military input, as well as scientific input.

13.2 The SAB recognised the complexities presented by riot control agents and incapacitating chemical agents, and their treatment under the Convention. It recalled that both the SAB[4] itself and the Director-General[5] had made reference to the matter on several occasions. The SAB further recognised that it could be of assistance to the Director-General in categorising toxic chemicals that fall within the general definitions of riot control agents or incapacitants for law enforcement.

13.3 After a constructive debate, the SAB recommended that it start deliberations on riot control agents and incapacitating chemical agents by receiving briefings on the different technical, legal, law enforcement, military, and political aspects surrounding the subject in order to identify the technical areas in which it can be of most assistance.

Subitem 13(b): International cooperation

13.4 The SAB had requested that the Technical Secretariat's (hereinafter "the Secretariat") International Cooperation and Assistance Division (ICA) update it regularly about its

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[4] Paragraph 3.14 of the annex to the report of the SAB on developments in science and technology (RC-2/DG.1, dated 28 February 2008 and Corr.1, dated 5 March 2008).

[5] Paragraphs 2.3 and 3.7 of RC-2/DG.1 and Corr.1; subparagraph 2.49(b) of the Note by the Secretariat entitled "Review of the Operation of the Chemical Weapons Convention since the First Review Conference" (RC-2/S/1*, dated 31 March 2008); paragraph 57 of the opening statement of the Director-General to the Second Special Session of the Conference of the States Parties to Review the Operation of the Chemical Weapons Convention (RC-2/DG.2, dated 7 April 2008); paragraph 161 of the opening statement by the Director-General to the Conference at its Fourteenth Session (C-14/DG.13, dated 30 November 2009); and an interview with the Director-General in the magazine of the Arms Control Association of the United States of America, *Arms Control Today*, vol. 40, no. 1, January/February 2010.

activities. The SAB therefore highly appreciated the presentation from Kumaresh Misra, Head of the International Cooperation Branch (ICB), on current and planned activities. Mr Misra gave an overview of the ICB portfolio and programme focus in the areas of integrated chemicals management, chemicals knowledge promotion and exchange, enhancing laboratory capabilities, and industry outreach. Furthermore, he emphasised forthcoming special activities, such as the OPCW workshop on the full implementation of Article XI and the International Year of Chemistry in 2011, both of which offer further opportunities for the OPCW to build closer ties with the global chemical community.

13.5 The SAB thanked Mr Misra for his well-presented update of the activities of his Branch, and the SAB members emphasised the importance of and appreciation for the many important activities organised or supported by the ICA. The SAB is looking forward to future updates.

Subitem 13(c): First OPCW confidence-building exercise on biomedical samples

13.6 In 2007, the SAB recommended to the Director-General that a series of confidence-building exercises be held as a prelude to initiating a process for the development of a separate laboratory-designation system for biomedical samples (SAB-9/1, dated 14 February 2007). This recommendation was accepted by the Director-General. The intention of the Secretariat to proceed with the development of an OPCW capability for biomedical sample analysis was noted by the Council at its Forty-Fourth Session (paragraph 6.2 of EC-44/2, dated 17 March 2006).

13.7 The first OPCW confidence-building exercise on biomedical samples was held from November 2009 to January 2010, with 22 participating laboratories from 17 Member States. Samples were prepared by the TNO Defence, Security and Safety Laboratory, Rijswijk, the Netherlands, and the results were evaluated by the Defence Science and Technology Laboratory (Dstl), Chemical and Biological Systems, Porton Down, United Kingdom of Great Britain and Northern Ireland. Samples of commercial synthetic urine were spiked with urinary metabolites of nerve agents or sulfur mustard at concentrations of 100 or 10 ng/ml. For analysis, laboratories used liquid and gas chromatography combined with single-stage or tandem-mass spectrometry. The most sensitive and selective methods were provided by liquid chromatography coupled with tandem-mass spectrometry (LC-MS/MS) or gas chromatography with tandem-mass spectrometry (GC-MS/MS) in multiple reaction monitoring mode, or liquid chromatography-mass spectrometry (LC-MS) in high-resolution extracted ion mode. For alkyl methylphosphonic acids and thiodiglycol, perfluorinated derivatives using selective chemical ionisation gave greater selectivity and signal-to-noise ratio in comparison with silyl derivatives. The use of commercial synthetic urine caused some unexpected problems in the analysis of one of the sulfur mustard metabolites.

13.8 The results described above successfully demonstrated a broader capability for the analysis of urinary metabolites of Schedule 1 agents than had previously been shown. They have also provided a starting point for a discussion, initially by the temporary working group on sampling and analysis, on criteria for identification at trace levels that would be acceptable to the Secretariat, Member States, and the broader international community. Evidence of system or sample contamination was observed

in more than half of the laboratories, particularly for GC-MS and GC-MS/MS analysis. This is an important problem that needs addressing.

13.9 A meeting was held on 25 March 2010 between the Secretariat and most of the participating laboratories to discuss the results of the first OPCW confidence-building exercise. It was recommended that a second exercise and a workshop be held in 2011. The presentation on the first OPCW confidence-building exercise on biomedical samples appears as Annex 5.

Subitem 13(d): Activities of the temporary working group on sampling and analysis

13.10 An informal exercise on saxitoxin analysis is scheduled to be held from June to September 2010, coordinated by Martin Schär of the Spiez Laboratory. Interested laboratories will be asked to prepare their own samples in accordance with instructions, and submit analytical data to the Spiez Laboratory for evaluation. The results will be presented to the temporary working group on sampling and analysis at its next meeting.

13.11 As mentioned in paragraph 11.3, a round-robin exercise on ricin analysis was held recently by the Global Health Security Action Group. The results of this exercise will be provided to the temporary working group on sampling and analysis for discussion at its next meeting.

13.12 An ongoing activity of direct relevance to the temporary working group on sampling and analysis and the Secretariat is the updating of the Finnish "Blue Book" on the sampling and analysis of chemical warfare agents and their degradation products. This is being coordinated by the Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN), with contributions from several members of the temporary working group on sampling and analysis and other experts.

Subitem 13(e): Possible nomination of the OPCW for the Nobel Peace Prize

13.13 The year 2011 will be the International Year of Chemistry. Members of the SAB suggested that the OPCW should, on that occasion, be proposed as a candidate for the Nobel Peace Prize.

Subitem 13(f): Departure of two members of the Scientific Advisory Board

13.14 The Chairperson of the SAB bade farewell to the two members (see paragraph 5.1 above) who have completed their second term of office on the SAB. He thanked them for their invaluable contribution to the work of the SAB.

Subitem 13(g): Extension of the duration of SAB sessions

13.15 The Chairperson sought the opinions of the members of the SAB regarding the extension of the sessions of the SAB from three to five days (see paragraph 5.4 above), with a view to responding to the correspondence addressed to him by the Director-General.

14. AGENDA ITEM FOURTEEN – Adoption of the report

The SAB considered and adopted the report of its Fifteenth Session.

15. AGENDA ITEM FIFTEEN – Closure of the session

The Chairperson closed the session at 17:20 on 14 April 2010.

Annexes:

Annex 1: List of Participants in the Fifteenth Session of the Scientific Advisory Board

Annex 2 (English only, unedited): Presentation by Patrick Couvreur: "Smart" Nanocarriers for Drug Delivery and Targeting

Annex 3 (English only, unedited): Presentation by Ravi Kumar: Advanced Drug Delivery Group - Strathclyde

Annex 4 (English only, unedited): Presentation by Christine Pernelle: Introduction to Molecularly Imprinted Polymers: Consideration of their Impact on the Chemical Weapons Convention

Annex 5 (English only, unedited): First OPCW Confidence-Building Exercise on Biomedical Samples

Annex 1

LIST OF PARTICIPANTS IN THE FIFTEENTH SESSION OF THE SCIENTIFIC ADVISORY BOARD

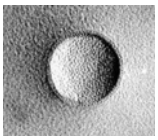
	Participant	State Party
1.	Djafer Benachour	Algeria
2.	Alejandra Graciela Suárez	Argentina
3.	Robert Mathews	Australia
4.	Herbert De Bisschop	Belgium
5.	Zhiqiang Xia	China
6.	Danko Škare	Croatia
7.	Jean-Claude Tabet	France
8.	Michael Geist	Germany
9.	László Halász	Hungary
10.	R. Vijayaraghavan	India
11.	Mahdi Balali-Mood	Iran (Islamic Republic of)
12.	Alberto Breccia Fratadocchi	Italy
13.	Shuzo Fujiwara	Japan
14.	Abdool Kader Jackaria	Mauritius
15.	José González Chávez	Mexico
16.	Godwin Ogbadu	Nigeria
17.	Muhammad Zafar-Uz-Zaman	Pakistan
18.	Titos Quibuyen	Philippines
19.	Igor V. Rybalchenko	Russian Federation
20.	Slavica Vučinić	Serbia
21.	Philip Coleman	South Africa
22.	Stefan Mogl	Switzerland
23.	Valery Kukhar	Ukraine
24.	Robin Black	United Kingdom of Great Britain and Northern Ireland
25.	William Kane	United States of America

Annex 2

PRESENTATION BY PATRICK COUVREUR: "SMART" NANOCARRIERS FOR DRUG DELIVERY AND TARGETING

Seminar for the « Organisation pour l'interdiction des armes chimiques »
April 12, 2010

« SMART » NANOCARRIERS FOR DRUG DELIVERY AND TARGETING

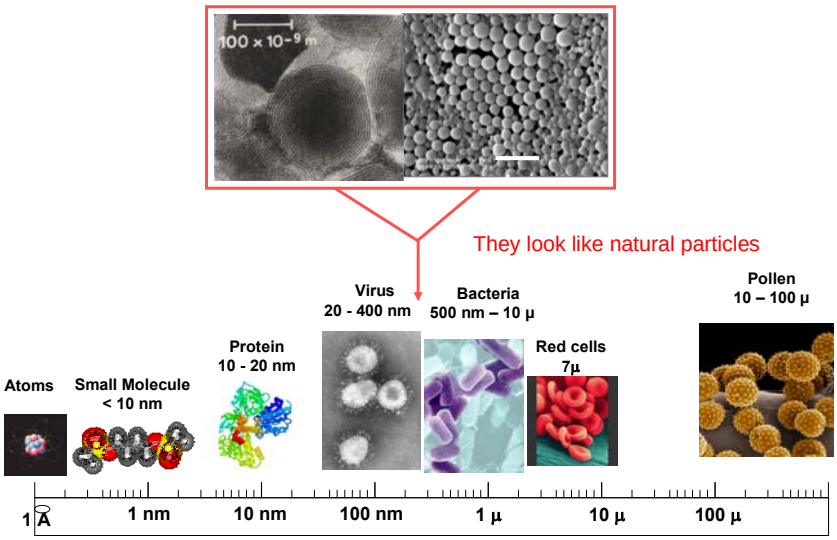


P.COUVREUR
Professor, Université Paris-Sud (France)
Chaire d'Innovation Technologique of the Collège de France

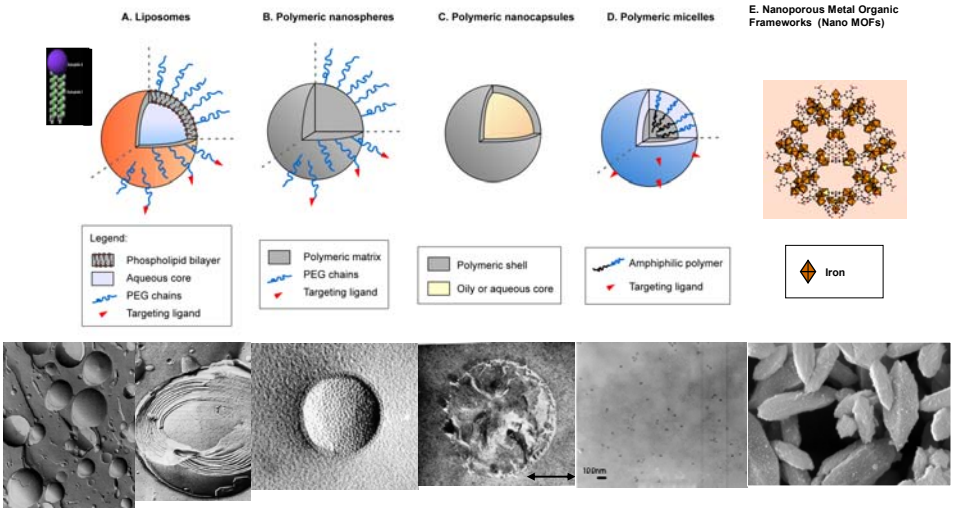
UMR CNRS 8612

UNIVERSITE PARIS-SUD

« NANOCARRIERS ARE SMART BECAUSE SMALL»



ARCHITECTURE OF THE NANOCARRIERS FOR DRUG TARGETING



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Al Khouri et al., Pharm Acta Helv. 61, 274-81(1986)

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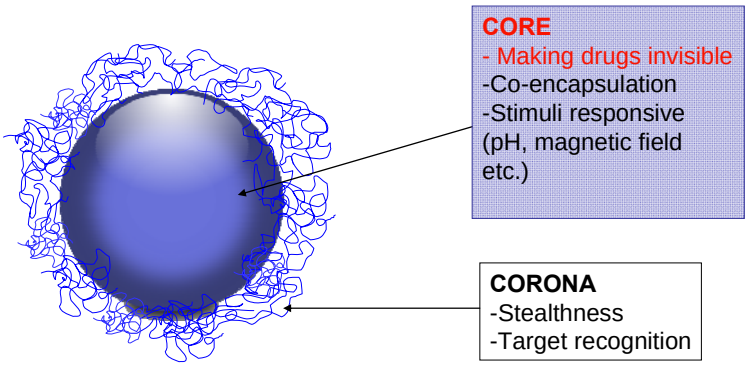
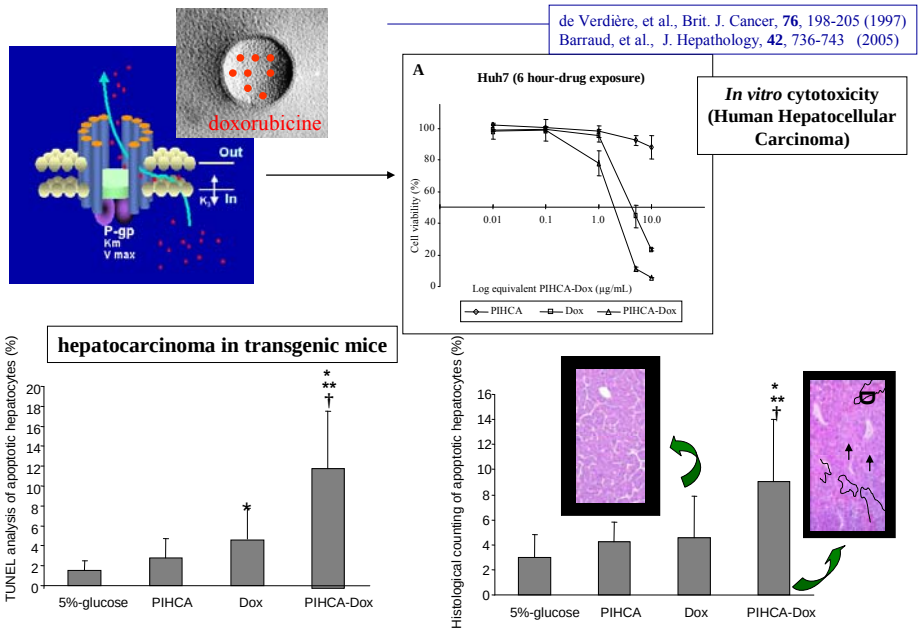
Horcajada P. et al. Nature Mater., DOI. 10.1038 (2010)

TWO MAIN COMPONENTS: THE « CORE » AND THE « CORONA »

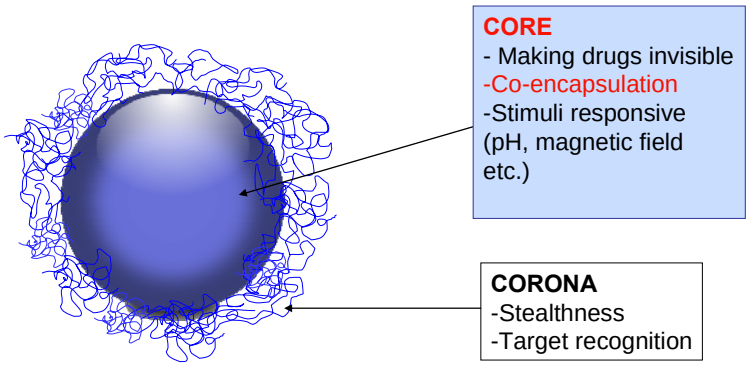


Drugs are protected from the organism detoxification processes

NANOPARTICLES MAKE DOXORUBICIN INVISIBLE FOR PgP IN MDR RESISTANT HEPATOCARCINOMA

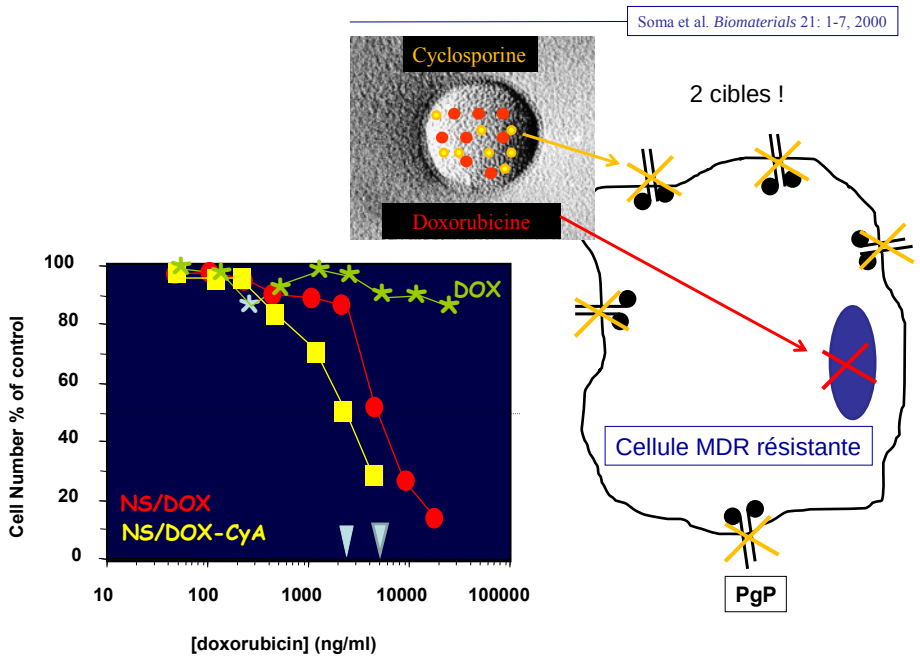


CONCEPTION OF NANOCARRIERS

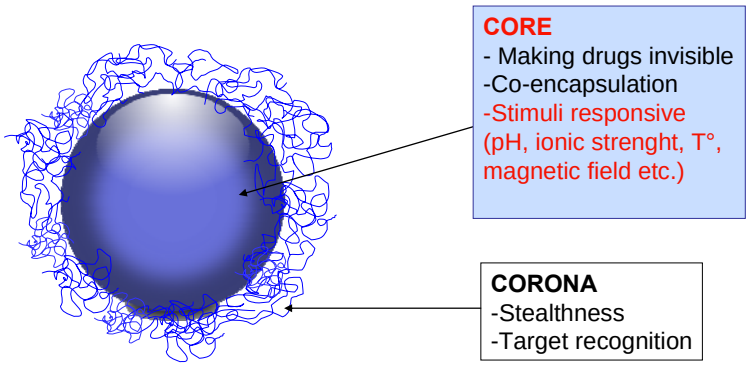


Co-encapsulation of two drugs acting on two different targets

CO-ENCAPSULATION DE DOXORUBICINE ET DE CYCLOSPORINE

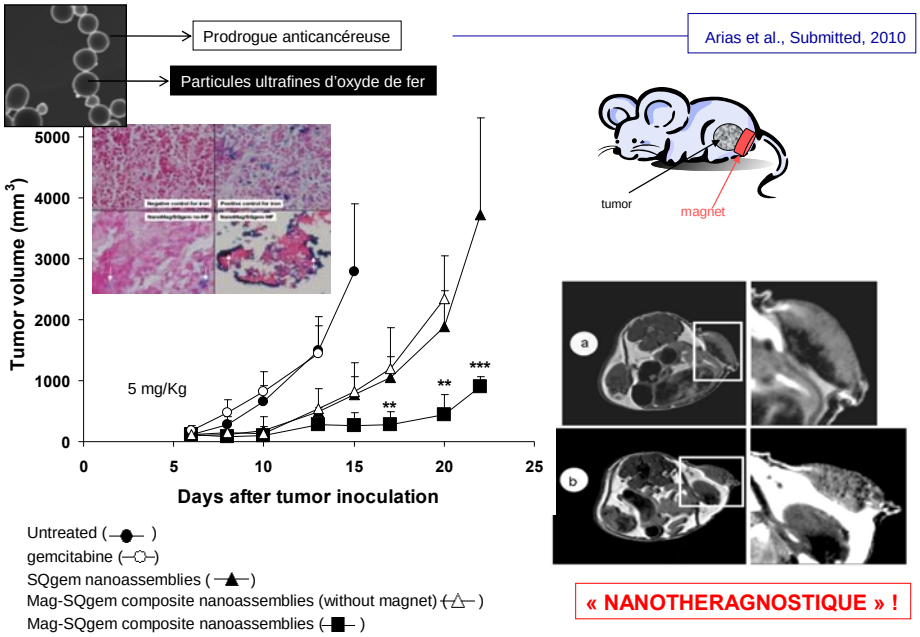


TWO MAIN COMPONENTS: THE « CORE » AND THE « CORONA »



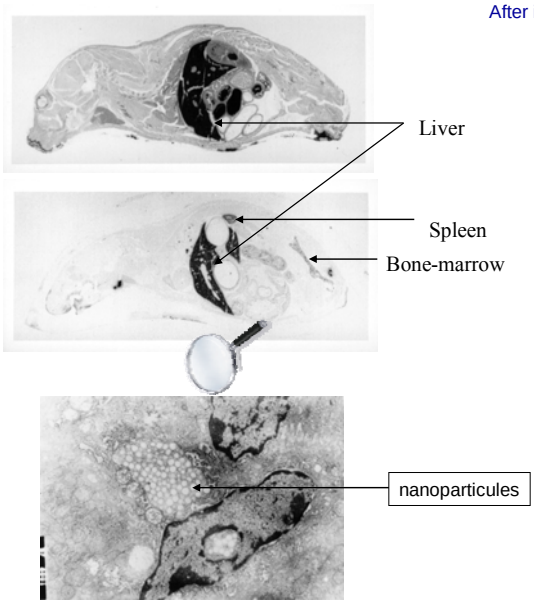
The (bio)material used to design the core allows the drug to be delivered as a response to an external stimulus

MAGNETICALLY GUIDED NANOPARTICLES
(Leucémie L1210 sc/iv)

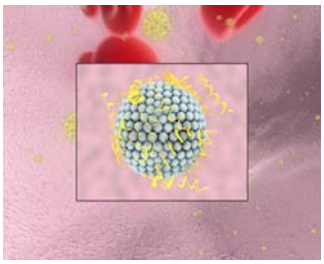


« NANOTHERAGNOSTIQUE » !

TISSUE DISTRIBUTION

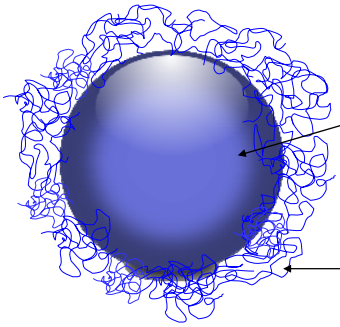


After iv administration nanoparticles are opsonized



…and recognized by liver and spleen macrophages

TWO MAIN COMPONENTS: THE « CORE » AND THE « CORONA »

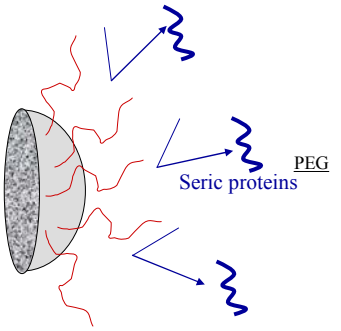


CORE
- Making drugs invisible
-Co-encapsulation
-Stimuli responsive (pH, magnetic field etc.)

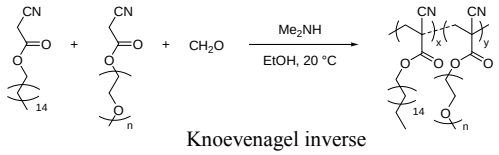
CORONA
-Stealthness
-Target recognition

PEG is able to make the core invisible for the recognition by the RES

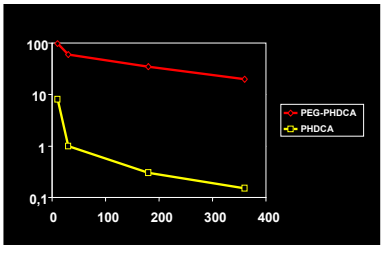
SURFACE FUNCTIONNALIZATION BY PEG



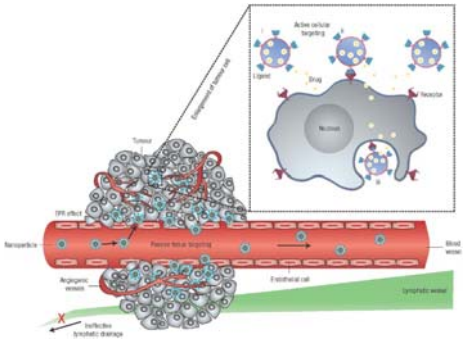
Perrachia et al. Macromolecules, **30**, 846-851 (1997)



Knoevenagel inverse



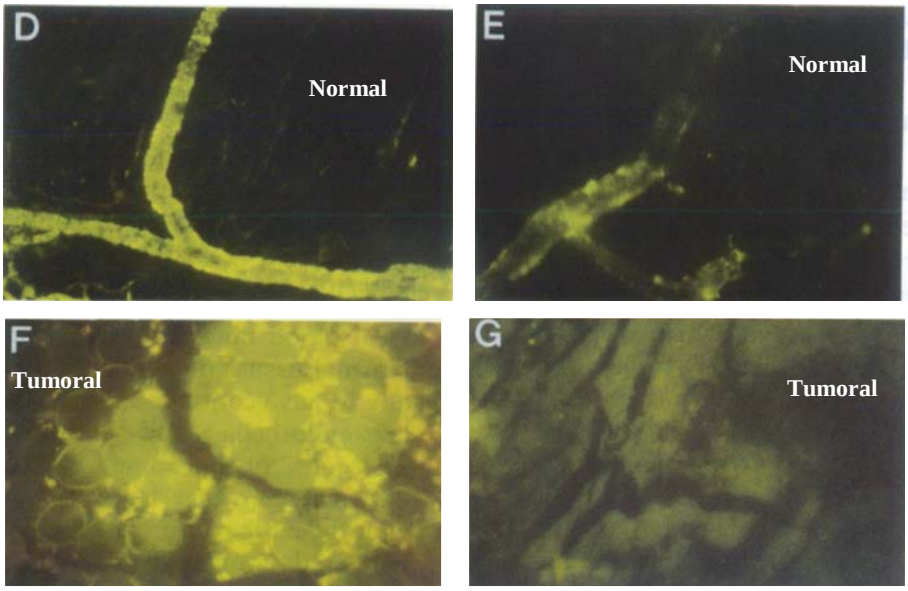
Perrachia et al., J. Control. Rel, **60**, 121-128 (1999)
Perrachia et al., Biomaterials, **20**, 1269-1275 (1999)



« EPR » effect

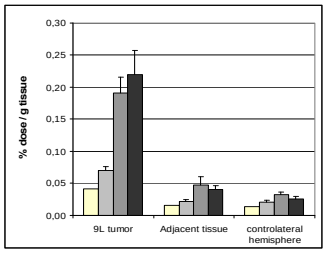
« EPR » EFFECT OF PEG-LIPOSOMES IN TUMORS

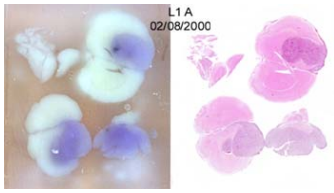
S. Unezaki et al., Int. J Pharm, 1996



BRAIN TRANSLOCATION OF PEG-PACA NANOSPHERES AND BHE DISRUPTION

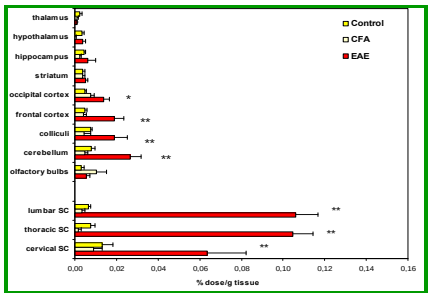
9L GLIOMA

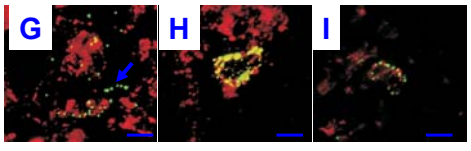




Brigger et al., J. Pharmacol. Exp. Ther., **303**, 928-936 (2002)

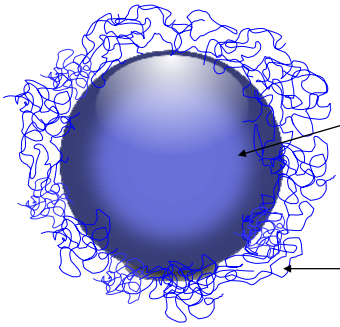
EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS





Calvo, et al., Europ. J Neurosc., **15**, 1317-1326 (2002)

TWO MAIN COMPONENTS: THE « CORE » AND THE « CORONA »



CORE
- Making drugs invisible
-Co-encapsulation
-Stimuli responsive (pH, magnetic field etc.)

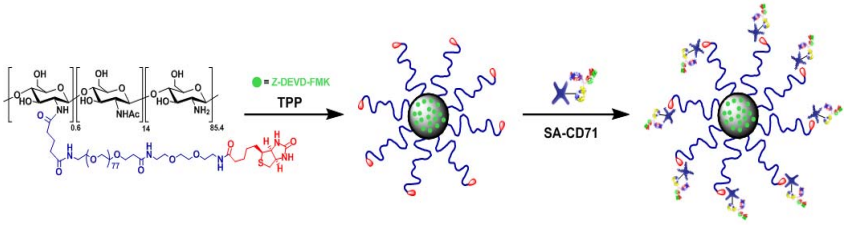
CORONA
-Stealthness
-Target recognition

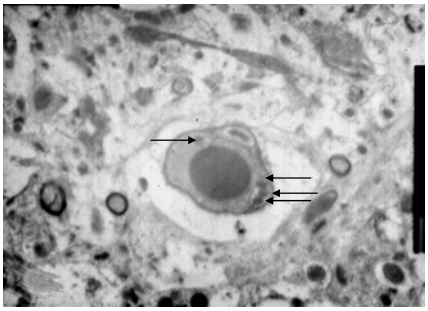
Molecular ligands are able to adress the nanocarriers to the diseased area

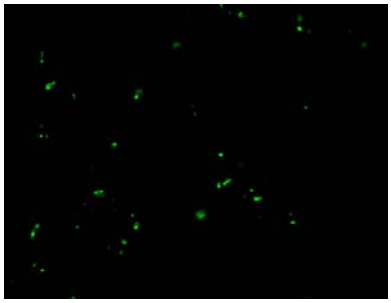
OX-26 PEGylated AND ADRESSED CHITOSAN NANOPARTICLES FOR BRAIN DELIVERY OF Z-DEVD-FMK

Y. Aktaş et al. , Bioconj. Chem., 16, 1503-1511 (2005)

N-benzyloxycarbonyl-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-fluoromethyl ketone (Z-DEVD-FMK)

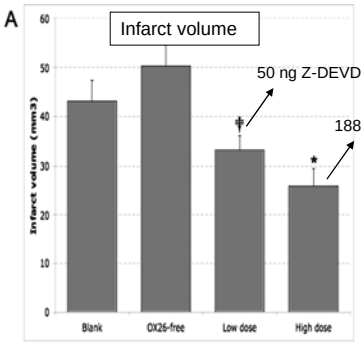




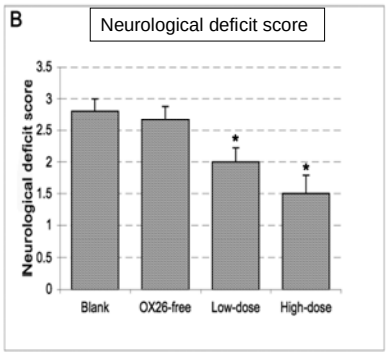


BRAIN DELIVERY BY OX 26 CHITOSAN NANOPARTICLES OF PEPTIDE CASPASE INHIBITOR ZDEVD FMK

Karatas H et al., J. Neurosci., **29**, 13761-13769, 2009

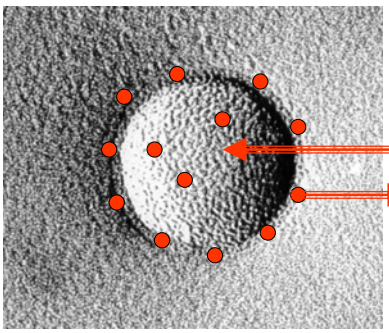


Ischemia induced by a nylon filament inserted into the common carotid artery (20 min + reperfusion 10 min)



0, no observable neurological deficits (normal); 1, failure to extend left forepaw on lifting the whole body by the tail (mild); 2, circling to the contralateral side (moderate); 3, leaning to the contralateral side at rest or no spontaneous motor activity (severe).

Various « smart » nanotechnologies are still available but major limitations still exist which explain the limited numbre of compounds on the market



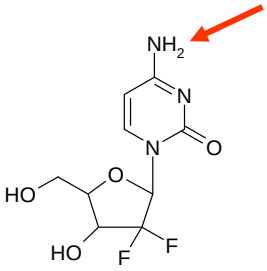
Thus, the amount of drug administered is insufficient
or
too high quantities of the carrier are needed

⇓

INEFFICACY OR TOXICITY

NUCLEOSIDES ANALOGUES: POTENT ANTICANCER AND ANTIVIRAL DRUGS

+ Interfering with DNA synthesis
+ Rapid metabolism → short plasma half-life
+ Poor diffusion through biological barriers
  - poor intracellular diffusion
  - poor absorption
+ Induction of resistances
+ Severe side effects

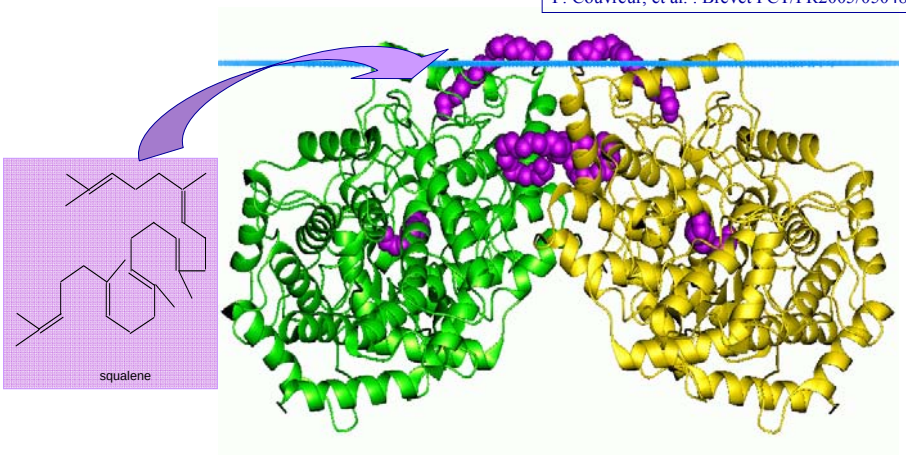


gemcitabine

Some attemps have been made to synthesize lipophilic derivatives of Nucleosides Analogues (ie. by coupling with fatty acids), but unsuccessfully due to their poor water solubility

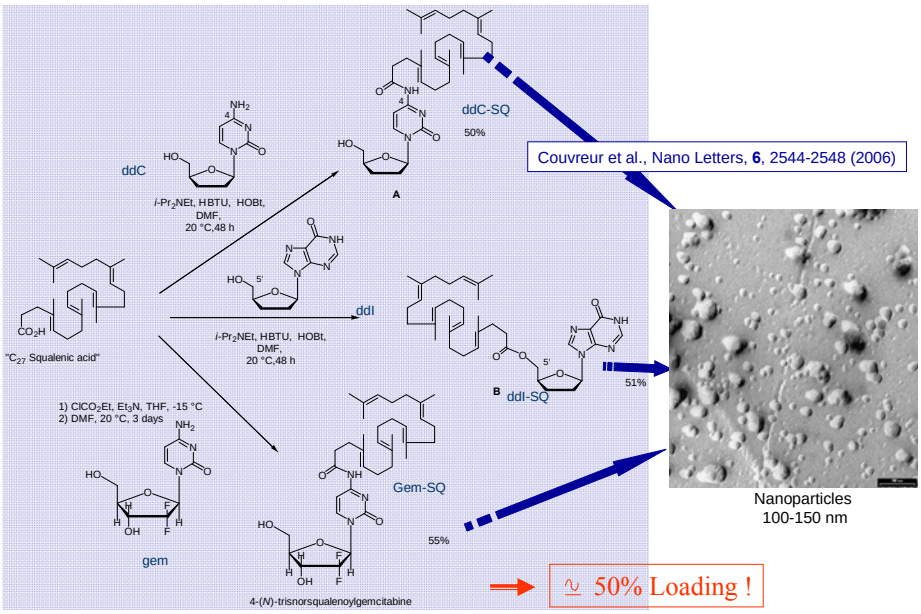
TO USE SQUALENE: A BIOMIMETIC APPROACH…

P. Couvreur, et al. . Brevet PCT/FR2005/050488



In an extraordinary way, SQUALENE a precursor of the CHOLESTEROL's biosynthesis, takes a dynamically folded conformation in aqueous solutions which helps it in reaching the hydrophobic pocket of the enzyme (i.e. oxidosqualene cyclase) in which the cyclization occurs (LANOSTEROL)

THE CONCEPT OF "*SQUALENIZATION*"

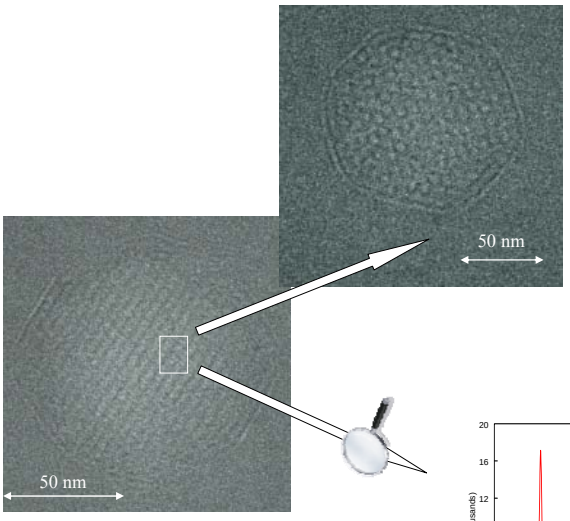


Couvreur et al., Nano Letters, **6**, 2544-2548 (2006)

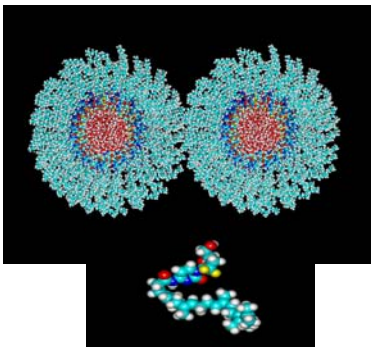
Nanoparticles
100-150 nm

≈ 50% Loading !

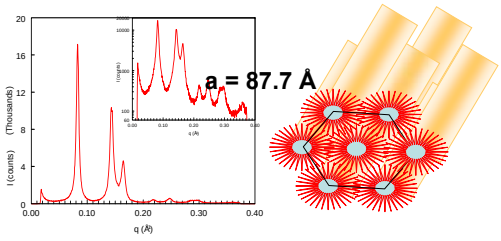
Also AZT, ARA-C, Thymidine…



Cryo-TEM



Molecular Modelling



Structural Analysis by SAX

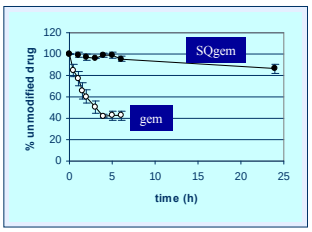
STRUCTURE OF SQUALENOYLATED GEMCITABINE

Couvreur et al., Small, **4**, 247-253 (2008)
Aoun et al., Adv Funct. Mater., **18**, 1-11 (2008)

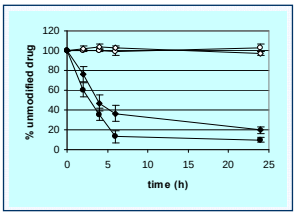
STABILITY IN PLASMA AND PHARMACOKINETICS OF GEMCITABINE-SQUALENE VERSUS GEMCITABINE FREE

Harivardahan et al., Drug Metab and Disposit, **36**, 1570-1577 (2008)
H. Khoury et al., J Chromatography B, **858**, 71-78 (2007)

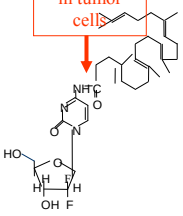
Stability in plasma (37°C)



Release in the presence of cathepsins B et D (37°C)

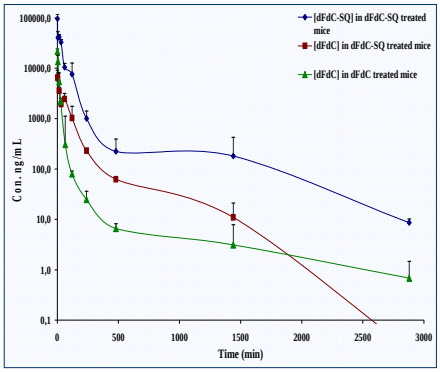


Gem release is tiggered by cathepsins hyper expressed in tumor cells



Squalenoylgemcitabine

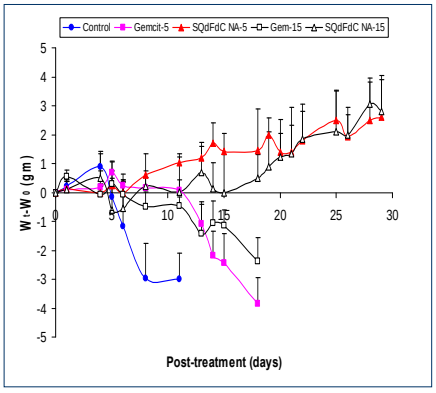
Pharmacokinetics
(IV administration 15 mg/Kg)

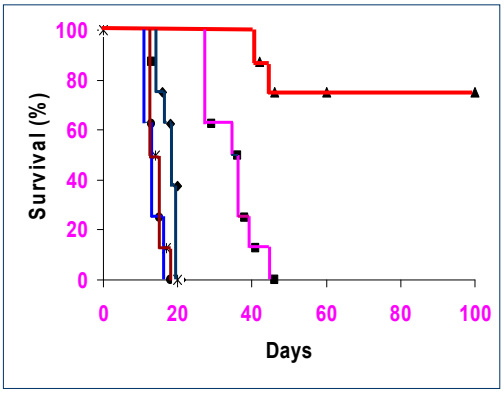


AUC dFdC/dFdC-SQ = 0.1288

IN VIVO ANTICANCER ACTIVITY AT MTD
(L1210 leukemia iv/iv

Harivardhan Reddy et al.,. J.Control. Rel., **124**,20-27 (2007)
Harivardahan Reddy et al., J. Pharmacol. Exp. Ther., **325**, 484-490 (2008)

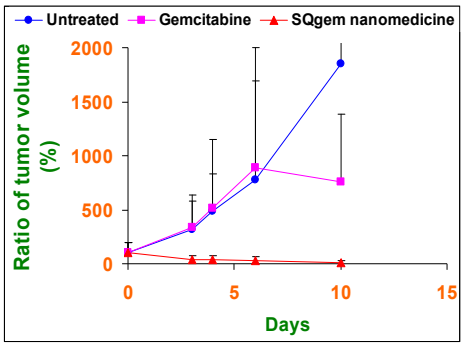


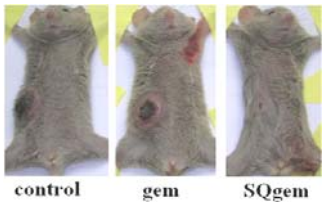


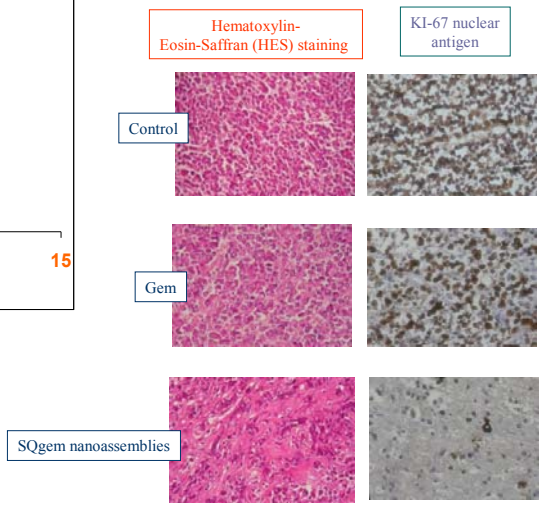
Untreated
Squalene 100mg/kg
Cytarabine 100mg/kg
Gemcitabine 100mg/kg
SQgem nanoassemblies 20mg/kg

IN VIVO ANTICANCER ACTIVITY AT MTD
(L1210 leukemia sc/iv)

Harivardhan et al., Mol. Pharm., ., **6**, 1526-1535, 2009







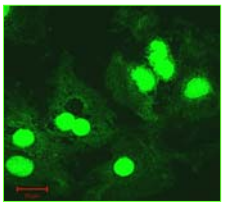
NANODEVICES FOR DRUG DELIVERY AND TARGETING ARE « SMART » BECAUSE THEY ARE ABLE…

- To camoufle and protect the drug from the biological environment
- To release the drug in a controlled manner in response to an external stimulus
- To escape from immunological recognition by the reticulo-endothelial system
- To address the drug to the desired biological target

→ but there is a need in the future for the discovery of new nanocarriers with higher drug loading and reduced « burst » release

FUTUR CHALLENGES…

- Use of nanotechnologies for overcoming resistance mechanisms (cancers or infectious diseases)
- Use of nanotechnologies for the design of « theragnostics »
- Use of nanotechnologies for gene therapy with non viral vectors as an alternative to the utilisation of viruses



ACKNOWLEGEMENTS

- **D. DESMAEL (BIOCIS, Chatenay)**
- **L. CATTEL (Univ. Turin, Italy)**
- **C. MALVY, L. MASSADE, A. PACI (CNRS, IGR)**
- **L BARRAUD, C. TREPO (INSERM, Lyon)**
- **JL ARIAS (Univ.Granada, Spain)**
- **Y.CAPAN and T.DALKARA (Univ. Hacettepe, Turkey)**

- **UMR CNRS 8612**
- **K. ANDRIEUX**
- **C. BOURGAUX**
- **I. BRIGGER**
- **P. CALVO**
- **H. CHACUN**
- **C. DUBERNET**
- **H. LAKKIREDDY**
- **R. GREF**
- **H. HILLAIREAU**
- **S. LEPETRE**
- **J. NICOLAS**
- **M. OLLIVON**
- **H. DE MARTIMPREY**
- **M. RENOIR**
- **C. VAUTHIER**
- **B. STELLA**

- **BIOALLIANCE**

Annex 3

PRESENTATION BY RAVI KUMAR:
ADVANCED DRUG DELIVERY GROUP - STRATHCLYDE

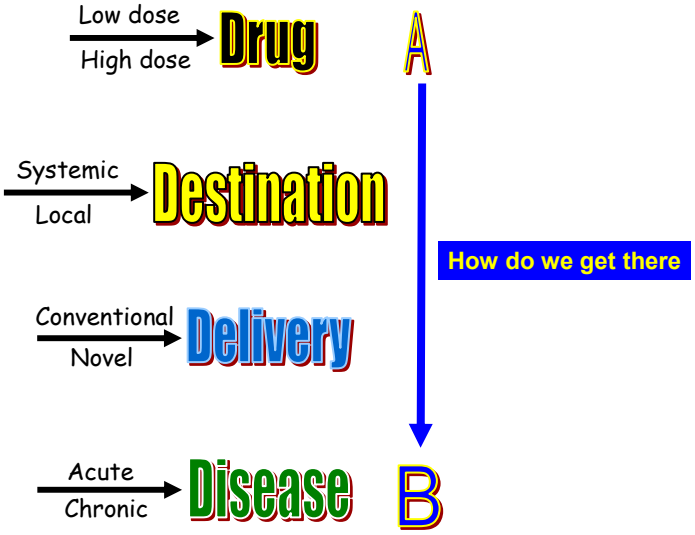
M N V Ravi Kumar
Professor of Drug Delivery
University of Strathclyde
Glasgow
E-mail: mnvrkumar@strath.ac.uk
http://www.advanceddrugdelivery.com

Advanced Drug Delivery Group-Strathclyde

We are a vibrant research group working on drug-delivery with a special emphasis on developing colloidal systems for oral administration.

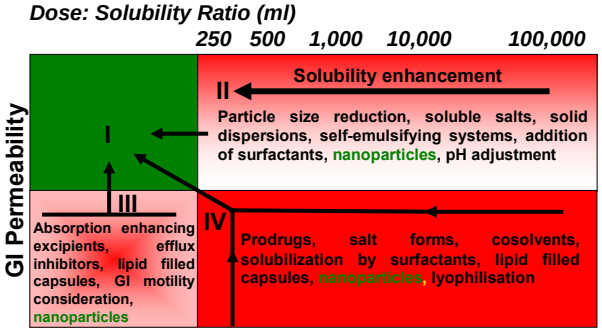
- Harness the maximum therapeutic potential of existing drug molecules & also possible new indications.
- Focus on alternative/traditional medicines. A lot of so called nutraceuticals and antioxidants are undergoing clinical trials these days, however, the success is limited due to their poor physicochemical/biopharmaceutical properties.
- We believe the future belongs to combination therapies involving a drug and phytochemicals/herbal medicines.

4-Ds in Medicines



Issues with oral route

Simplifying the concepts



Hurdles in Drug Delivery

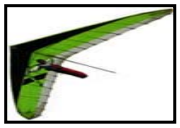
Other problems for low bioavailability include:

GIT metabolism
FPM
Rapid clearance

G. L. Amidon, H. Lennernas, V. P. Shah, J. R. Crison, Pharmaceutical Research. 12 (1995) 413-420.

Everything Changes with Time ☺

-A change is always need based

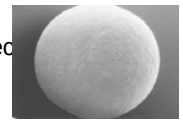


Stone age requirements (10,000 BC)

- ❑ Improve solubility, thereby bioavailability. (No Issues Straight forward)

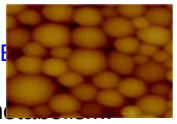
Polymers and composites age (1900's)

- ❑ Can prevent the GIT degradation of labile drug molec
- ❑ Can sustain the release over a period of time.

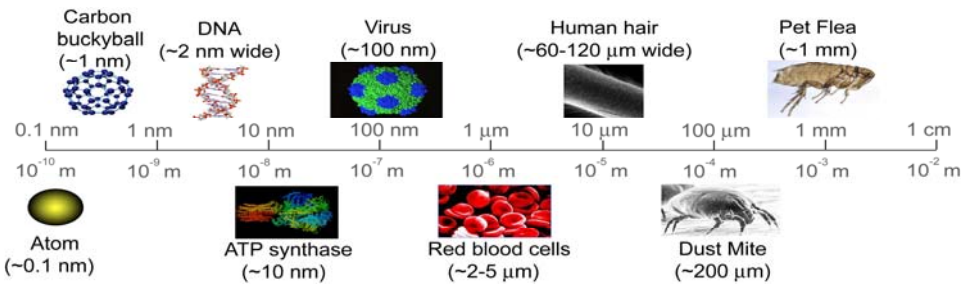


Modern era-Current

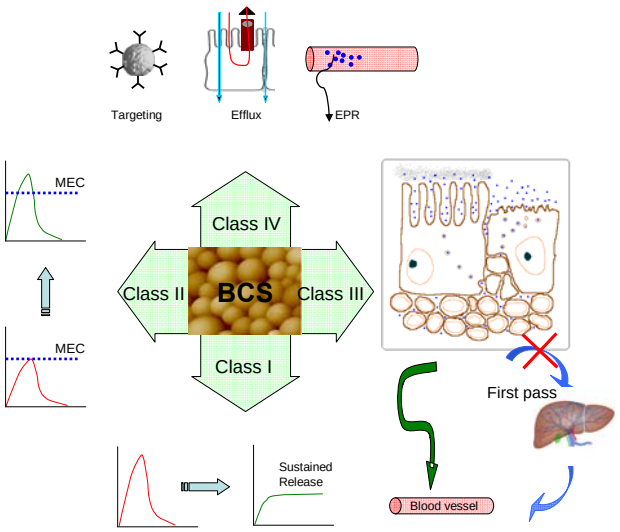
- ❑ Drug cargos (so called NANOPARTICLES) are getting across the biological barriers-CONCE REGULATORS.
- ❑ Protects drug from the pre-systemic (gut) and liver m
- ❑ Target drugs to tissue and cell of interest



On the Scale

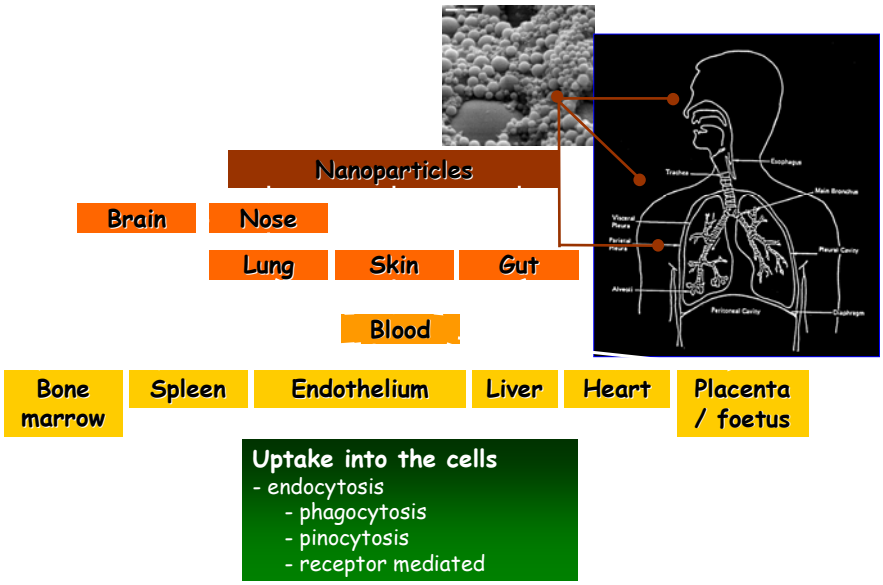


What makes NANO ☺ so exciting?



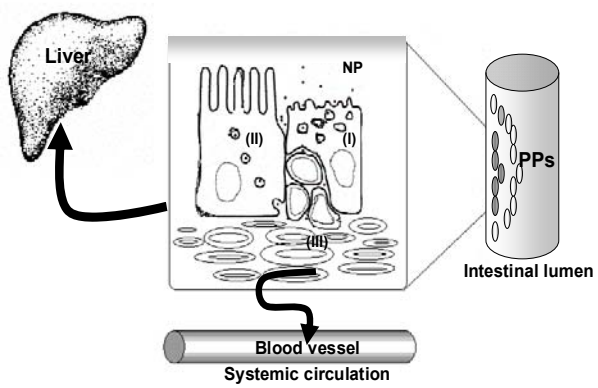
Cartoon not to scale..

Nanoparticle Port of Entry–*In vivo* effects



Occupational Medicine 2006 **56**; 307-311

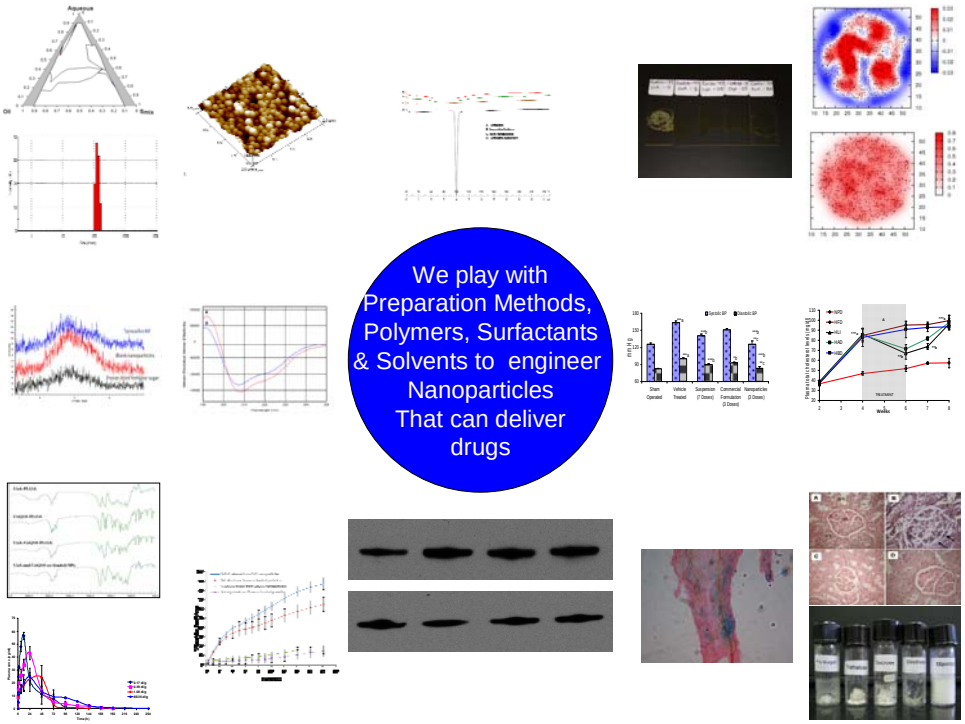
Lymphatic uptake of nanoparticles



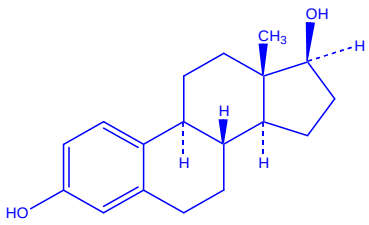
Mechanism of uptake of orally administered nanoparticles. NP: Nanoparticles PPs: Peyers patches, (I) M-cells of the Peyer's patches, (II) Enterocytes, (III) Gut associated lymphoid tissue (GALT)

Schematic representation of the uptake of nanoparticles upon oral administration. The direct uptake of nanoparticles through the lymph into the systemic circulation bypassing the liver reduces the first pass metabolism; thus improving bioavailability.

Bhardwaj et, al. Pharmaceutical Aspects of Polymeric Nanoparticles for Oral Delivery, Journal of Biomedical Nanotechnology (2005)



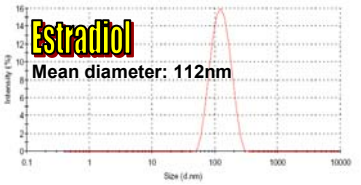
Estradiol



Molecular Weight	272.4 Da
Presystemic metabolism	Extensive
Oral bioavailability	Low (< 10%)
Endogenous Levels	300-800 pmol/l
Plasma protein binding	97-99%
Plasma half life	20-70 min.

Traditionally used to prevent hot flashes can be used for new indications if delivered efficiently:

Osteoporosis
Cardiovascular disease
Colorectal cancer
Alzheimer's disease (AD)
Stroke

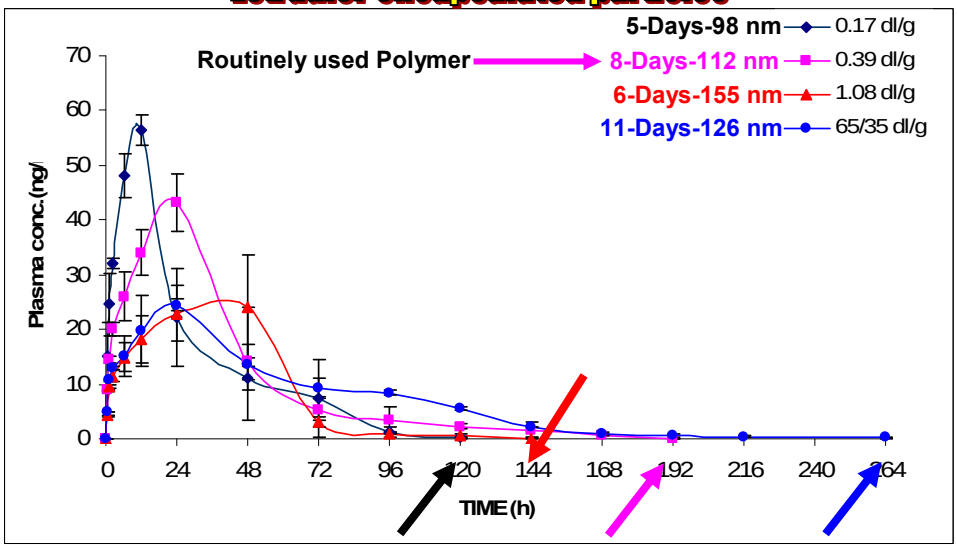


Why is it IMPORTANT administer minimum THERAPEUTIC DOSE?: Lobo RA (1995). Benefits and risks of estrogen replacement therapy. Am J Obstet Gynecol 173: 982-989. Yager JD, Liehr JG (1996). Molecular mechanism of estrogen carcinogenesis. Annu Rev Pharmacol Toxicol 36:203-232.

Pharmacokinetics with different particle sizes

Influence of Polymer molecular weight and copolymer composition on release

Estradiol-encapsulated particles



Mittal et al., Journal of Controlled Release, 2007

Dose Dependent Kinetics

Pharmacokinetic parameters obtained after oral administration of drug suspension and drug loaded PLGA nanoparticles at 3 different doses. (n=5)

Dose & from µg/kg	C_{max} (pg/ml)	T_{max} (h)	AUC_{0-inf} (pg.h/ml)	Absolute BA (%)	Relative BA (%)	K_a (h^{-1})	K_{el} (h^{-1})	$T_{1/2}$ (h)	MRT (h)	MAT (h)
100 DS	101.90	4	1289.50	11.43	-	1.099	0.087	7.98	10.60	8.69
200 DS	193.10	4	2473.02	11.46	-	1.188	0.089	7.74	12.35	10.31
500 DS	757.03	4	11339.20	20.35	-	0.839	0.127	5.46	12.20	10.45
100 NP	85.30	24	6578.90	58.32	510.19	0.067	0.056	12.32	48.57	46.66
200 NP	164.54	24	12443.17	57.67	503.16	0.066	0.055	12.71	47.05	45.00
500 NP	476.02	18	34212.08	61.39	301.72	0.071	0.059	11.84	46.47	44.72

DS: Drug suspension; NP: Nanoparticles; Plasma profiles were fit in one compartment model; Relative bioavailability (%) refers to AUC0-inf NP/AUC0-inf DS (%); AUC 0-inf was calculated by linear trapezoidal rule; Kel was calculated by log linear regression; Ka was calculated by back stripping of curve; MRT was calculated by area under first moment curve (AUMC0-inf)/area under curve (AUC0-inf); MAT was calculated by MRToral - MRTi.v.

Mittal and Kumar, JPS (2009)

Postmenopausal dyslipidaemia

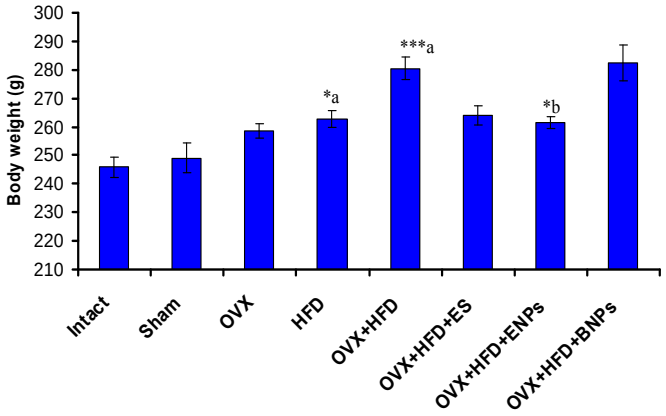
- Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in postmenopausal women.
- CVD - Menopausal metabolic syndrome (obesity, dyslipidaemia, insulin resistance, hypertension).
- Estrogen deficiency due to loss of ovarian function at menopause is primarily responsible.
- Oxidative stress has also been implicated as a risk factor in the pathogenesis of cardiovascular disease.
- Postmenopausal estradiol treatment reduces the cardiovascular disease risk up to 50%.

Study Design

Groups	Weeks after OVX					
	1	2	3	4	5	6
					TREATMENT	
Intact						
Sham operated						
OVX (No HFD)						
HFD (no OVX)						
OVX+HFD	High Fat Diet (HFD) for 4 weeks after OVX to induce hyperlipidaemic condition.					
OVX+HFD+ES					200 µg/kg daily	
OVX+HFD+ENPs					200 µg/kg once in 3 days	
OVX+HFD+BNPs						

Mittal et al., Pharmaceutical Research, 26 (2009) 218-223

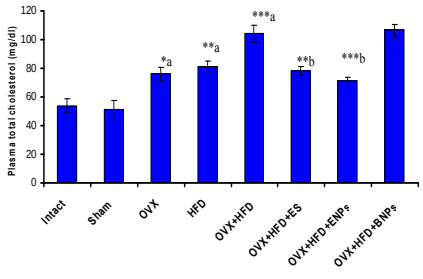
Body Weight



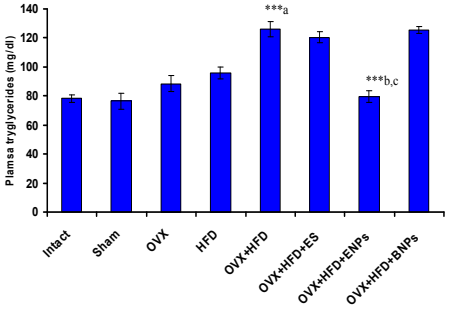
Effect of estradiol treatment on body weight of estrogen deficient hyperlipidemic rats. Each data point is represented as Mean ± SEM (n=6). ***p<0.001, ** p<0.01, *p<0.05; a Vs intact and b Vs OVX+HFD.

Plasma Lipid Levels

(A) Total Cholesterol



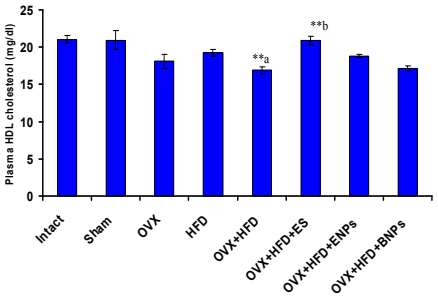
(B) Triglycerides



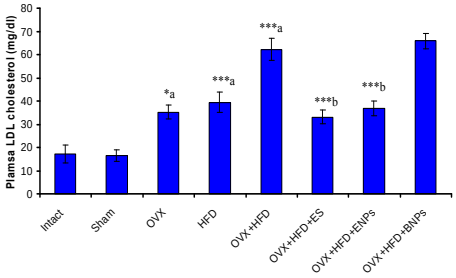
Plasma levels of (A) total cholesterol (TC), and (B) triglycerides (TG), after 6 weeks of study. Each data point is represented as Mean ± SEM (n=6). ***p<0.001, ** p<0.01, *p<0.05; a Vs intact, b Vs OVX+HFD and c Vs OVX+HFD+ES.

Contd...

(C) HDL Cholesterol

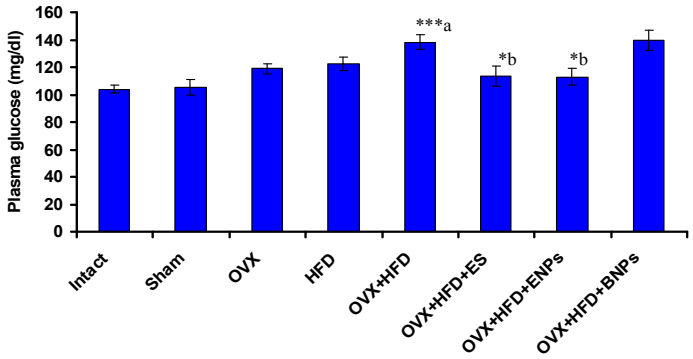


(D) LDL Cholesterol



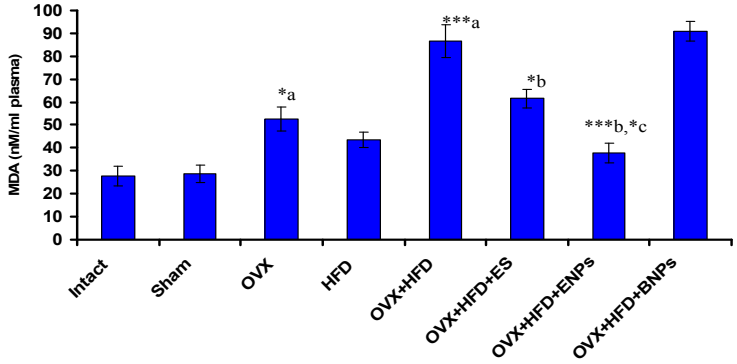
Plasma levels of (C) high density lipoproteins cholesterol (HDL-C), and (D) low density lipoproteins cholesterol (LDL-C), after 6 weeks of study. Each data point is represented as Mean ± SEM (n=6). ***p<0.001, ** p<0.01, *p<0.05; a Vs intact, b Vs OVX+HFD and c Vs OVX+HFD+ES.

Plasma Glucose Levels



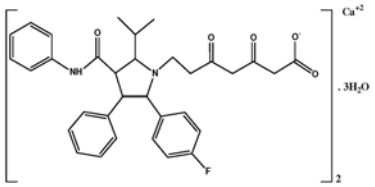
Plasma Glucose levels after 6 weeks of study. Each data point is represented as Mean ± SEM (n=6). ***p<0.001, ** p<0.01, *p<0.05; a Vs intact and b Vs OVX+HFD.

Plasma MDA Levels



Effect of estradiol treatment on plasma lipid peroxidation in estrogen deficient hyperlipidemic rats. Each data point is represented as Mean ± SEM (n=6). ***p<0.001, ** p<0.01, *p<0.05; a Vs intact, b Vs OVX+HFD and c Vs OVX+HFD+ES.

Atorvastatin



Atorvastatin

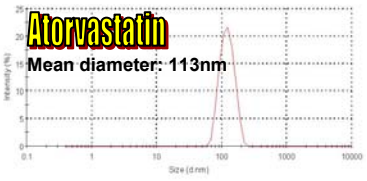
Molecular weight: **568.64 Da**
Poor solubility
Half life 14 hours
Oral BA 12%

2008 Sales $12.4 billion

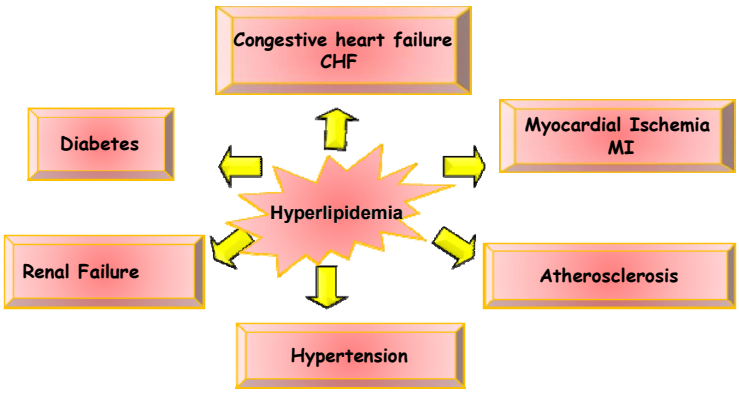
Mechanism of action: Main role of atorvastatin is to inhibit HMG-CoA reductase lowering LDL, also stabilizes plaques and prevents stroke through anti-inflammatory and other mechanisms.

Other indications/benefits of Atorvastatin include:

- ❑ Cancer
- ❑ Pulmonary Hypertension
- ❑ Alzheimer's disease
- ❑ Rheumatoid arthritis
- ❑ Multiple Sclerosis
- ❑ Psoriasis



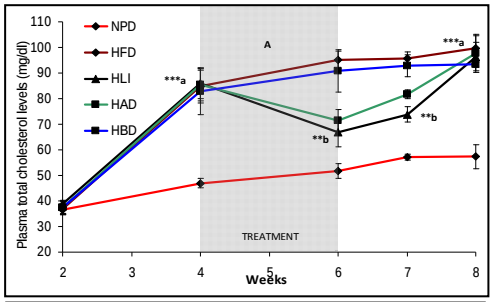
What Hyperlipidemia can lead to?

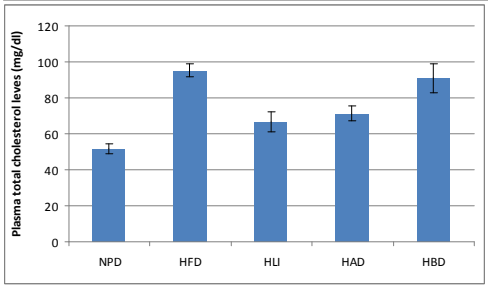


- **Mortality is twice as compared to cancer**
- **Ten times as compared to other casualties and diseases**

Walsh, Time 164 (2004), 21. & NCEP. Circulation 89(1994) 1329-1445

Efficacy of Atorvastatin-NP compared to Lipicure in high fat diet model

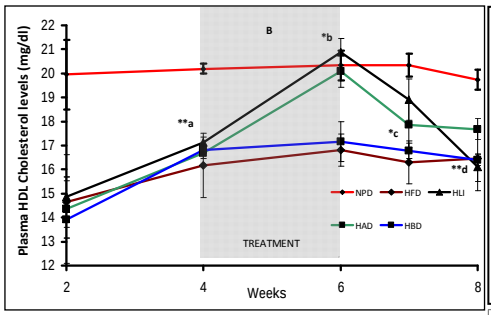


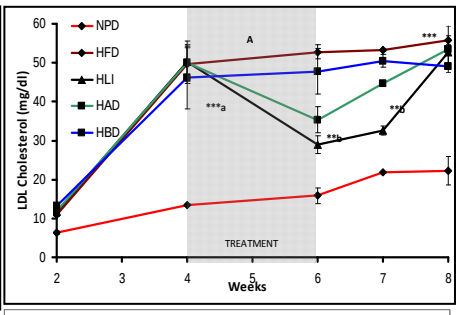


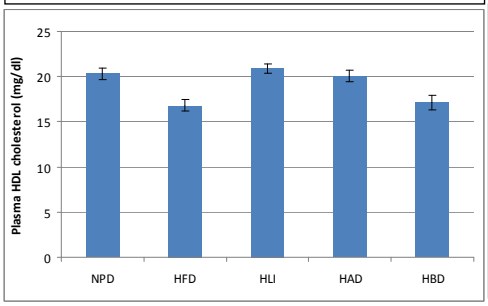
Dose: Lipicure 3 mg/Kg daily (Total dose 11.25 mg);
Nanoparticles 3 mg/Kg once in 3 days (Total dose 3.75 mg)

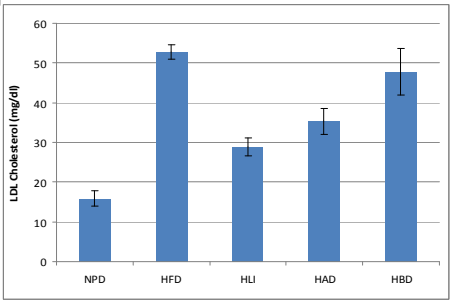
Meena et al., Lipids 2008

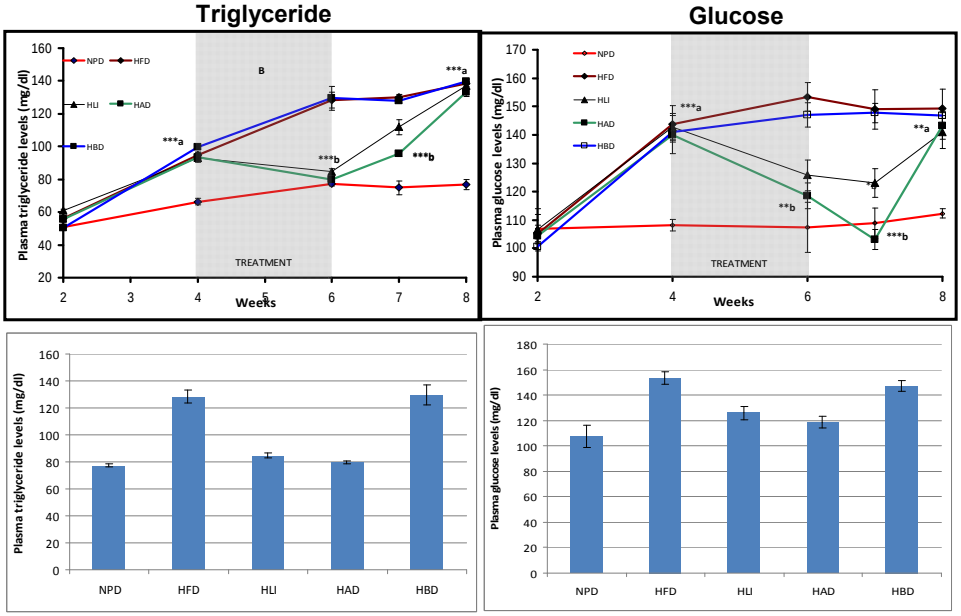
HDL **LDL**



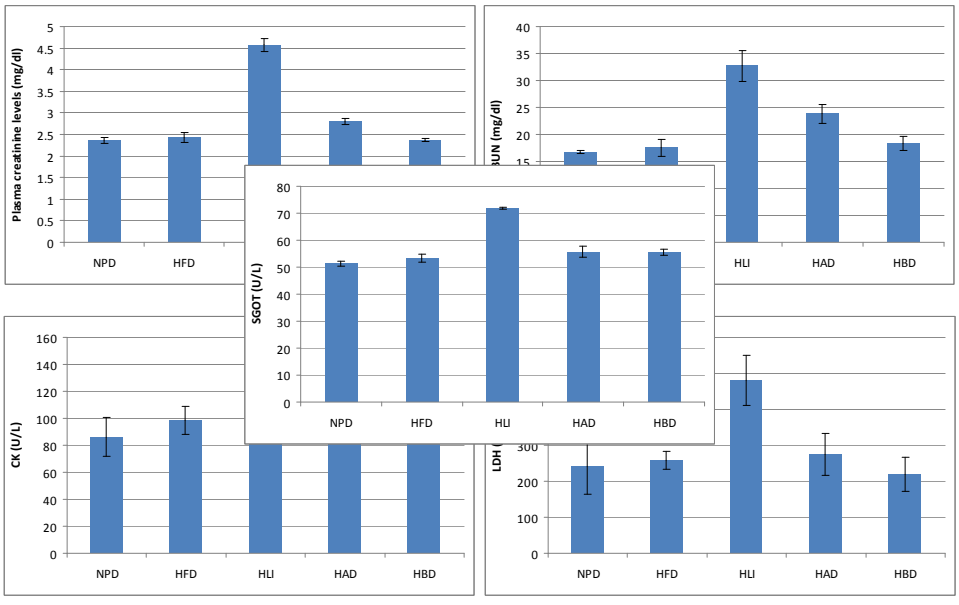




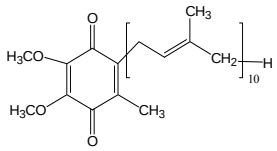




Safety of Atorvastatin-NP compared to Lipicure in high fat diet model



Coenzyme Q10

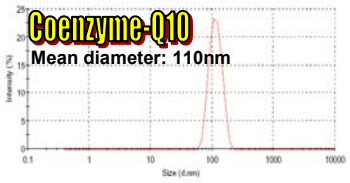


Molecular weight 863 Da
Poor solubility
Poor permeability

The CoQ10 is used as nutritional supplement, however, if delivered efficiently it can be used to prevent/treat many diseases

Benefits of Coenzyme Q10 include:

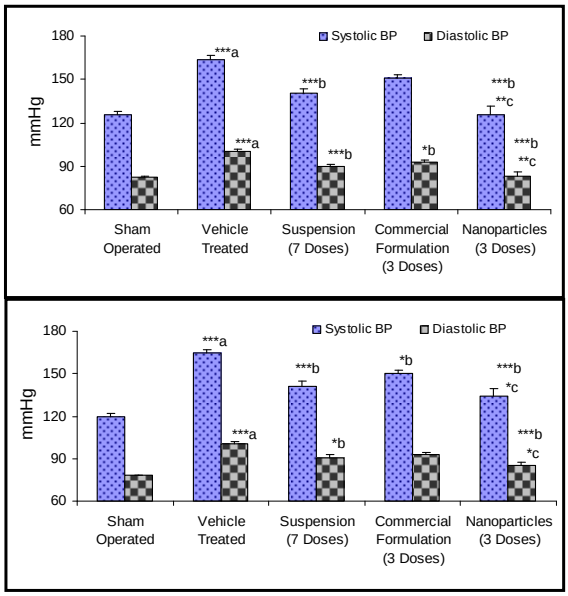
- ❑ Alzheimer's
- ❑ Parkinson's
- ❑ Cardiovascular
- ❑ Cancer
- ❑ Diabetes
- ❑ Dental
- ❑ Dermatology
- ❑ Aging



Coenzyme Q10 in Managing Hypertension

Groups	Day after surgery									
	SURGERY	1	2	3	4	5	6	7	12	15
Sham Operated (no clipping)		----------							BP RECORDING	BP Recording and Lipid peroxidation measurements in plasma
Vehicle treated (0.5%NaCMC,1ml/Kg)		V	V	V	V	V	V	V		
CoQ10 as Suspension in CMC (100 mg/Kg bw)		D	D	D	D	D	D	D		
Li-Q-Sorb (Marketed) (100 mg/Kg bw)		D			D			D		
CoQ10 Nanoparticles (Developed formulation) (100 mg/Kg bw)		D			D			D		

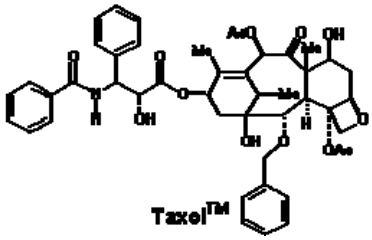
Blood Pressure



Effect of different CoQ10 formulations on systolic and diastolic blood pressure on 12th day after surgery in Goldblatt hypertensive rats ***p<0.001, **p<0.01 & *p<0.05; a Vs sham operated group; b Vs vehicle treated group; c Vs commercial formulation (n=5-7)

Effect of different CoQ10 formulations on systolic and diastolic blood pressure on 15th day after surgery in Goldblatt hypertensive rats. ***p<0.001, *p<0.05; a Vs sham operated group; b Vs vehicle treated group; c Vs commercial formulation (n=5-7)

Paclitaxel



Paclitaxel

Molecular weight: 853.9 Da
Poor solubility
Poor permeability
Oral BA 6.5%

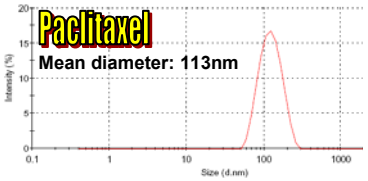
Dose: 175 mg/m²
Route: IV infusion over 03-24 h

Cytotoxics market $ 13.6 2008
Abraxane $ 500 million

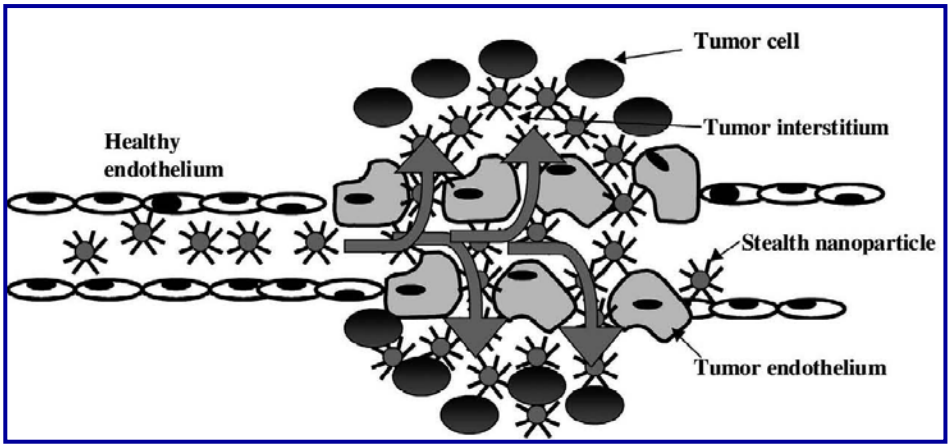
Unique mechanism of action: Promotes assembly of microtubules, stabilizes them against depolymerization and inhibits cell replication.

Other indications/benefits of Paclitaxel include:

- ❑ Alzheimer's (http://www.angiotech.com)
- ❑ Parkinson's (http://www.angiotech.com)
- ❑ Polycystic Kidney Syndrome
- ❑ Rheumatoid arthritis
- ❑ Multiple Sclerosis
- ❑ Psoriasis



Enhanced Permeation & Retention Effect (EPR)

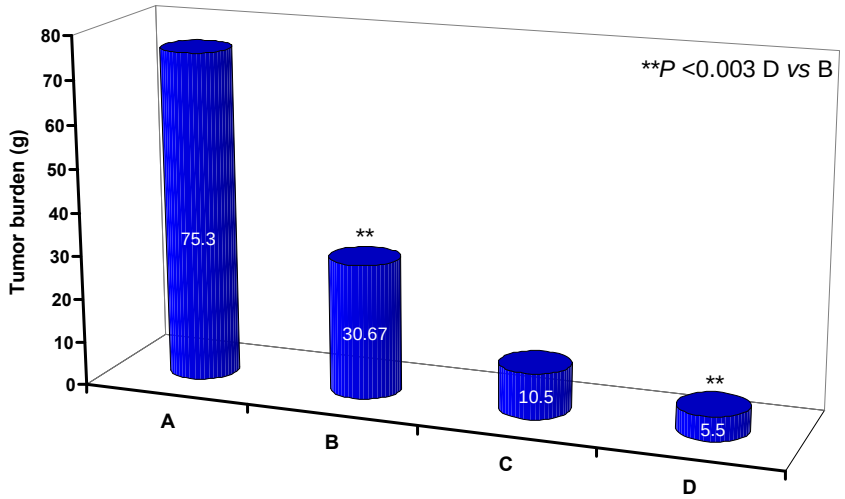


Extravasation of long-circulating (Stealth™) nanoparticles in the tumor interstitium by passive diffusion or convection across the altered and hyperpermeable neoplastic endothelium.

I. Brigger, C. Dubernet, P. Couvreur, Advanced Drug Delivery Reviews 54 (2002) 631–651

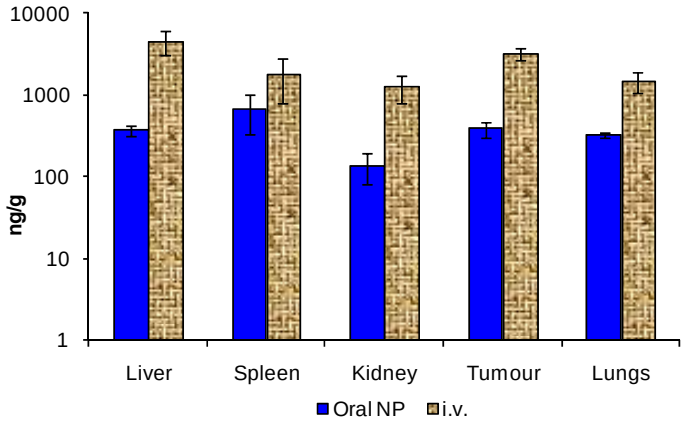
Tumor burden

(A) Untreated; (B) Taxol-Cremophore oral (7.5 mg/Kg once in 3 weeks for 9 weeks) (C) Taxol-Cremophore IV (7.5 mg/Kg once in 3 weeks for 9 weeks) (D) Taxol NP oral (3.75 mg/Kg once in 3 weeks for 9 weeks).



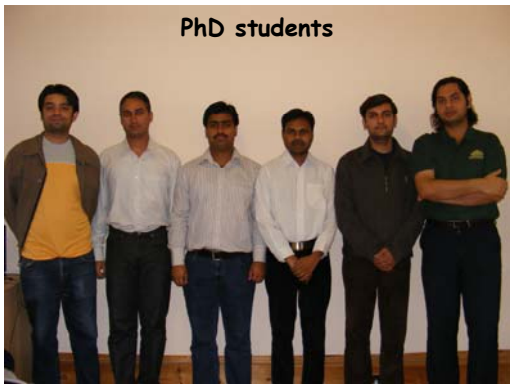
Female Sprague-Dawley Rats: Oral administration of DMBA (7,12-Dimethyl benzanthracene) @ 100mg/kg body wt in vegetable oil, Single dose at 47-50 days of age (100% tumors in 13 weeks)

Distribution Profile in Tumor bearing animals



Tissue distribution of paclitaxel in rats (n=3) bearing mammary tumours 24 hours after dose. Oral NP group received 3.75 mg/kg and i.v. group received 7.5 mg/kg paclitaxel. Concentration of drug is denoted as nanogram of drug per gram of tissue. Error bars denote standard error of mean

Acknowledgement



Annex 4

**PRESENTATION BY CHRISTINE PERNELLE:
INTRODUCTION TO MOLECULARLY IMPRINTED POLYMERS:
CONSIDERATION OF THEIR IMPACT ON THE CHEMICAL WEAPONS CONVENTION**

Introduction to Molecularly Imprinted Polymers

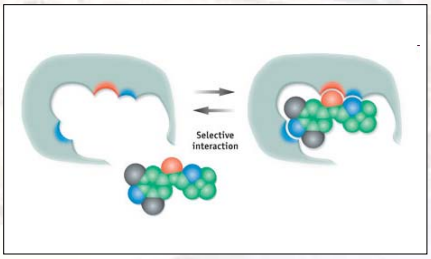
Consideration of their Impact on the Chemical Weapons Convention

Pr Christine PERNELLE
christine.pernelle@cnam.fr
April 13th, 2010

Introduction to Molecularly Imprinted Polymers (MIPs)

- **Introduction to MIPs applied to CWAs**
- **Selection of Applications in the CW field**
  - **Detection/protection: Chemical sensors**
  - **On-site monitoring and sampling of the presence of CW**
  - **Decontamination**
- **MIP Technology: what Potentials for CW/TICs**
- **MIP Technologies: Methodologies and Future Developments**

Molecularly Imprinted Polymers: an Opportunity for CW Detection



Molecular imprinting is a process for making binding sites in synthetic polymers

Molecular imprinting provides functional materials able to recognize biological and chemical agents of interest

MIP is a growing field of research.

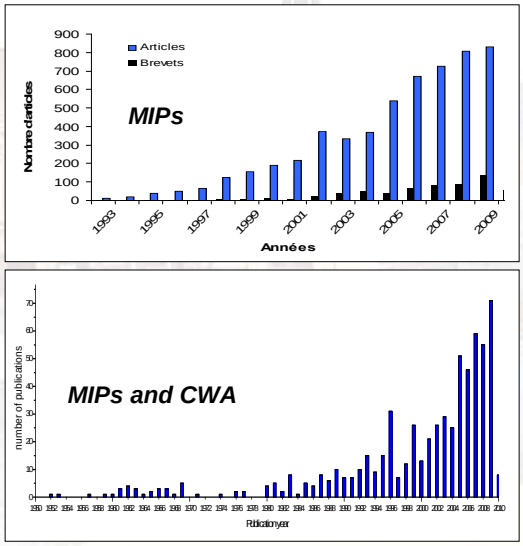
70 Years of MIP R&D

1940: Dickey was inspired by the hypothetical 'Lock and Key' relationship between enzyme and substrate to create affinity for dye molecules in silica gel

1970: First apparition of imprinting in organic polymers (covalent imprinting in vinyl polymers)

1980: Non-covalent imprinting polymers in the form known today

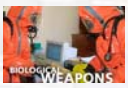
MIPs : Increasing Number of Publications and Patents during the Last Ten Years

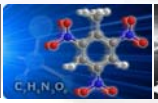


Detection and Identification of the Main Sources of Danger
The CBRNE Threat Concept

The CBNRE threat concept includes five categories of weapons of massive destruction usuable by terrorist groups:

- **Chemical Weapons and Industrial toxins**
- **Biological threats**
- **Radiological and nuclear weapons**
- **Explosives**
- **Narcotics**

Detection and Identification of Additionnal Chemical Substances Related to the Main Categories of Weapons

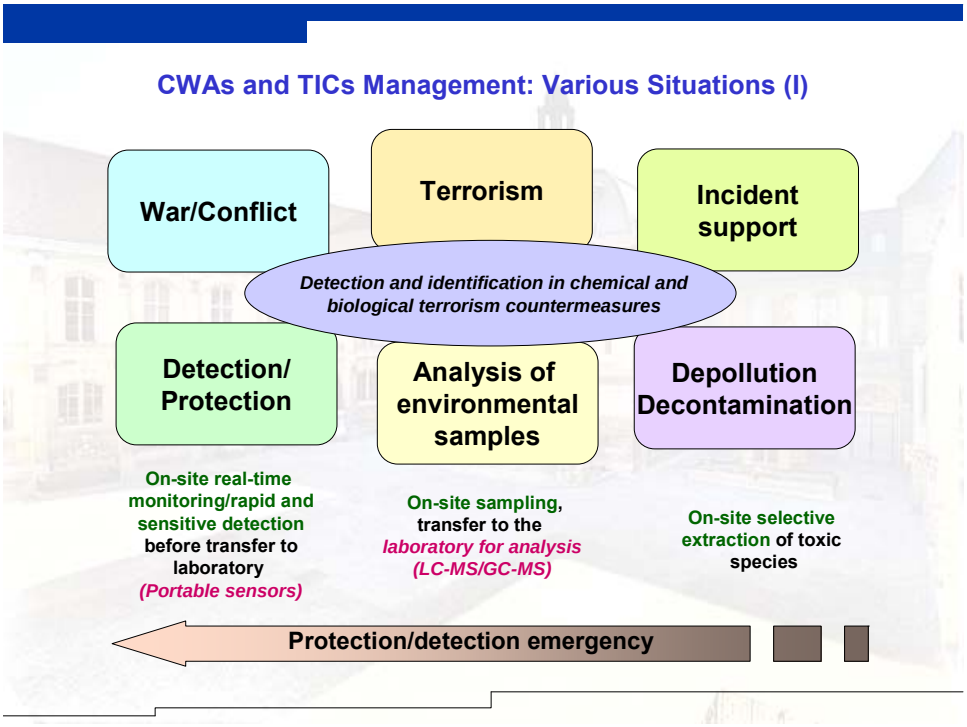
The manufacture of massive destruction weapons involves the use of various chemical substances

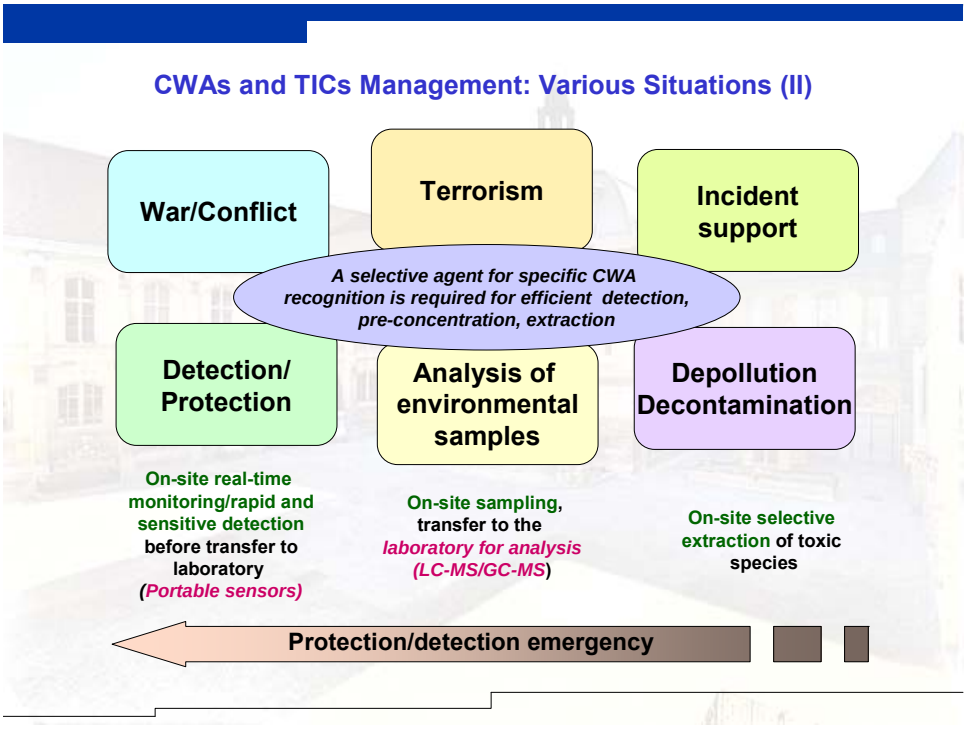
Either in the case of preventing terrorist actions, or applying rescue procedures	
Rapid sensitive and reliable identification of CW, their captive intermediates and their degradation products is crucial	Search for traces, in particular in environmental samples allows to identify chemical indices or to detect the use of toxic substances

Some Chemical Warfare Agents and Industrials Toxic Agents

Category	Name
Nerve agent	Tabun, Sarin, Soman, Cyclosarin, VX, Novichok agents
Choking agents	Chloropicrin, chlorine, phosgene, diphosgene
Blister agent	Sulphur mustard, Nitrogen mustard, Lewsite, Phosgene oxime
Cytotoxic proteins	Ricin, Abrin
Industrial toxic agents (TICs)	Phosogene , Hydrogen cyanide, Nitrous oxide, Carbon monoxide, Hydrogen Chloride, Methyl isocynate, Mercury, Lead, Benzene hexachloride, 1,3,5 trichlorobeneze , Dichloromethane, Chloroform

Adapted from Kshitij Aditeya Singh, Organisation – Institute of Nanotechnology May, 2009


CWAs and TICs Management: Various Situations (I)
War/Conflict
Terrorism
Incident support
Detection and identification in chemical and biological terrorism countermeasures
Detection/ Protection
Analysis of environmental samples
Depollution Decontamination
On-site real-time monitoring/rapid and sensitive detection before transfer to laboratory (Portable sensors)
On-site sampling, transfer to the laboratory for analysis (LC-MS/GC-MS)
On-site selective extraction of toxic species
Protection/detection emergency


CWAs and TICs Management: Various Situations (II)
War/Conflict
Terrorism
Incident support
A selective agent for specific CWA recognition is required for efficient detection, pre-concentration, extraction
Detection/ Protection
Analysis of environmental samples
Depollution Decontamination
On-site real-time monitoring/rapid and sensitive detection before transfer to laboratory (Portable sensors)
On-site sampling, transfer to the laboratory for analysis (LC-MS/GC-MS)
On-site selective extraction of toxic species
Protection/detection emergency

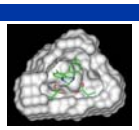
Current Selective Agents for Specific Molecular Recognition are Biological Receptors

Enzymes, Antibodies, DNA, Aptamers

- **Detection/ Protection**
- **Analysis of environmental samples**
- **Depollution Decontamination**

- Due to their biological origin, these biomolecules may suffer for:
  - Instability during manufacturing
  - Instability in a non biological environment
  - Problems associated with the sterilisation process
- They often suffer for a high manufacturing cost
- Detecting small molecules is still a challenge

Molecularly Imprinted Polymers: A Promising Route to Overcome these Issues



MIPs

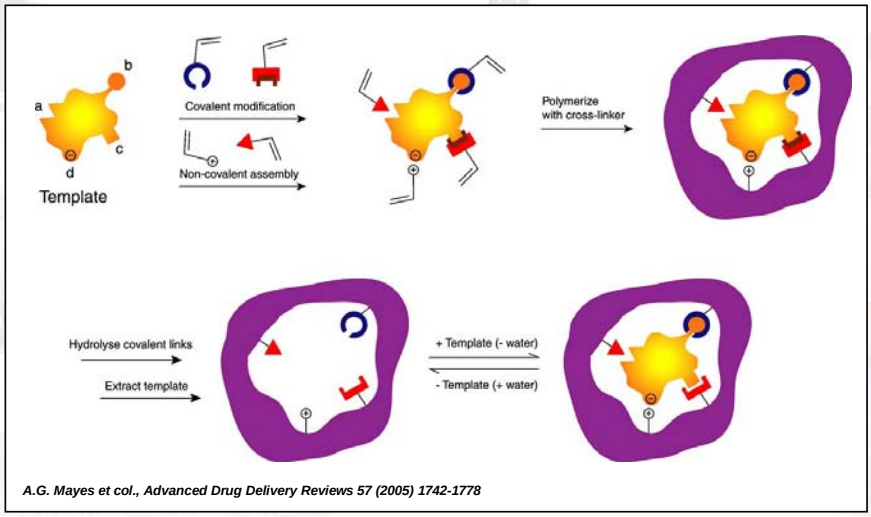
- **Detection/ Protection**
- **Analysis of environmental samples**
- **Depollution Decontamination**

- Molecular imprinting is a versatile technique providing functional synthetic materials able to recognize biological and chemical agents
- In contrast to biological receptors, MIPs are obtained by template-directed synthesis

Advantages of MIP-based materials include:

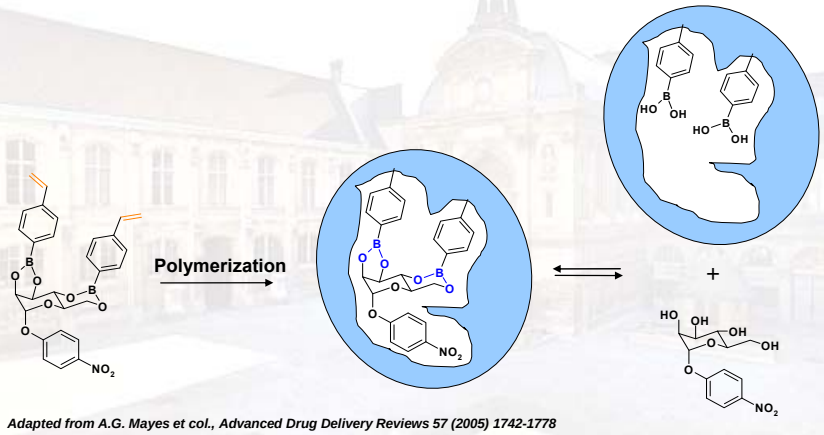
- Binding affinities comparable to a biological recognition element
- Robustness and stability under a wide range of chemical and physical conditions
- Ability to easily design recognition sites for analytes that lack suitable biorecognition elements

Representation of the Imprinting Process Showing some of the Interactions used in Creating Affinity in the Binding Site for Template



A.G. Mayes et col., Advanced Drug Delivery Reviews 57 (2005) 1742-1778

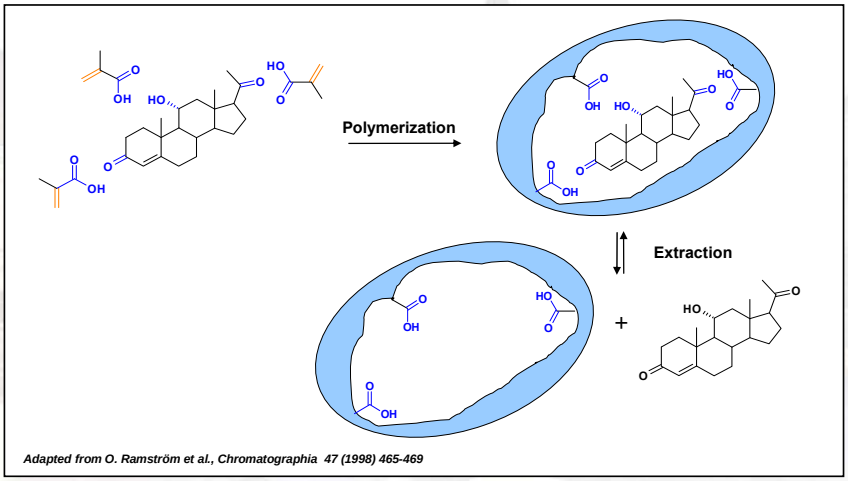
An Example of Covalent Imprinting: 4-Nitrophenyl-α-D-manopyranoside-2,3:4,6-di-O-(4-vinylphenylboronate)



Adapted from A.G. Mayes et col., Advanced Drug Delivery Reviews 57 (2005) 1742-1778

Classical methods of covalent imprinting involve readily reversible condensation reactions such as boronate ester, ketal/acetel and Schiff's base formation to prepare template-monomers .

An Example of Non-Covalent Imprinting: 11-α-Hydroxyprogesterone



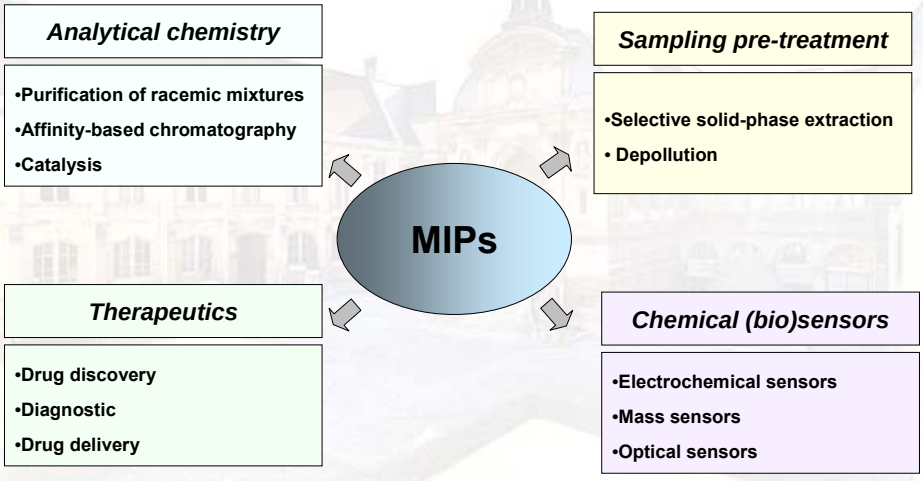
Adapted from O. Ramström et al., Chromatographia 47 (1998) 465-469

- Today, non-covalent imprinting is the predominant method used for producing imprinted receptors in synthetic polymers.
- It offers much more flexibility in terms of the fonctionalities on a template that can be targeted than the covalent imprinting process

Pros and Cons of Covalent vs Non-covalent Imprinting Methods

	Covalent Imprinting	*Non-covalent Imprinting*
Pros	•Stochiometric nature of the covalent imprinting step, lowering non-specific interactions •Compatibility of semi-covalent methods with a wider range of polymerization conditions	• Little or no synthetic chemistry needed • High range of functionalities that can be targeted
Cons	• Template monomers: •Requirement for synthesis •Sensitivity to the presence of water • Low to moderate template recovery	• Heterogeneity of the receptor sites produced • Non-specific single point interactions between analyte molecules and the polymer •Very low yield of functional high-affinity receptor sites

Applications of the MIP Technology



Selection of Applications in the CW Field

Detection/protection: Chemical sensors

- Generic sensor definition
- MIP-based sensors applied to chemical weapons

On-site monitoring of the presence of CW and their sampling

- selective extraction
- pre-concentration

Decontamination

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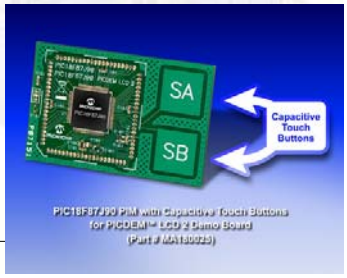
- **pre-concentration**

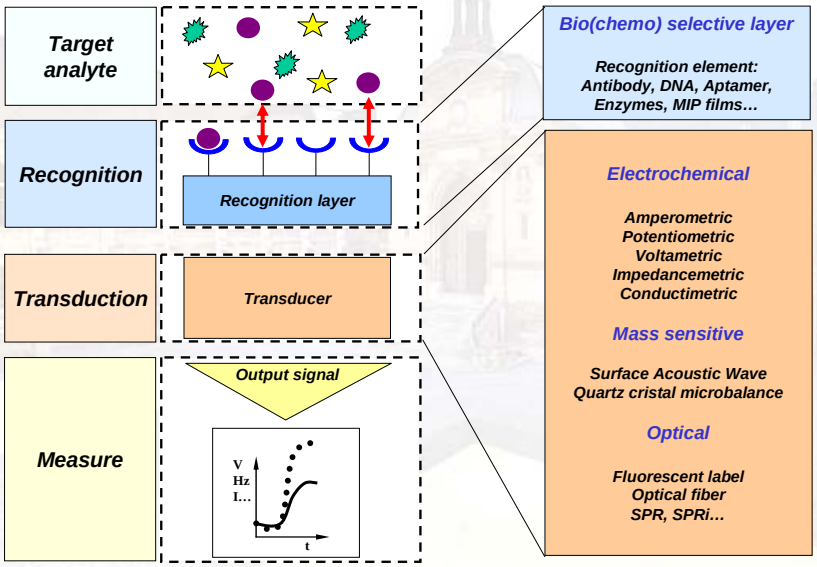
Decontamination

Highly Selective Sensors to CWA: Required Performances

- **Heightened sensitivity for detection at relevant concentration**
- **Substantial selectivity to rapidly extract the pertinent information about the presence or absence of a CWA, their captive intermediates and of their degradation products under myriad possible background conditions**
- **Wide dynamic range**
- **On site measurement and field deployable**
- **Real time monitoring of the detection and fast response**
- **Simultaneous multicomponent detection**
- **Miniaturized, robust microchip devices**
- **Low cost**



Schematic Representaion of a Generic Sensor



Design and Application Examples of MIP-based Sensors

Target analyte(s)	Template	Polymer material(s)	Transduction method	Detection limit
Atrazine *Prasad K. 2007*	Atrazine	Methacrylic acid	Electrochemical	0,5 µM
L-Histidine *Zhang Z. 2005*	L-Histidine	Phenyl and Methyltrimethoxy silane	Electrochemical	25 nM
Parathion *LI C. 2005*	Parathion	*p-tert*-Butylcalix(6)-1,4-crown-4 TetraethoxysilanePoly (methylhydrosiloxane)	Electrochemical	1 nM
Hydrolysis product of Soman *Jenkins A.L. 1999*	Hydrolysis product of Soman	$Eur(R)_2(NO_3)_3$ (R=pinacolylmethyl phosphonate or divinyl-methyl benzoate)	Optical	4 pM
Pinacolyl methylphosphonate *Vishnuvardhan K.P. 2007*	Pinacolyl methylphosphonate	Methacrylic acid Ethylenglycoldimethacrylate Modified vinylphenol	Electrochemical	40 nM
Fluorene *Carlson C.A.2006*	*N*-(9-Fuorenyl methoxycarbonyl-β-phenyl-D-phenylalaninol)	Bis(trimethoxysilyl) benzene	Optical	10 nM
Dopamine *Jianping Li 2009*	Dopamine	O-aminophenol (MIP-PC)	Electrochemical	2 nM

E.L. Holthoff, F.V. Bright / Analytica Chimica Acta 594 (2007) 147–161

(Bio)Chemical Sensors: Some Comparative Criteria

Biosensors (ADN and polymer conductor layer)				
Substrats	**Size criteria (diameter)**	**Detection Systems**	**Sensitivity**	**Detection**
Ultra Microelectrode Array	3 µm	Fluorescence labellling	0.1 - 1 pM	End point
Microelectrode Array	50 µm	Fluorescence labelling	0.1 - 10 pM	End point
Homogeneous Gold layer	500 µm	Fluorescence labelling	0.1 - 10 pM	End point
Homogeneous Gold layer	500 µm	SPRi	1- 10 nM	Real time
Quartz cristal	5000 µm	Mass Detection	250 - 1000 nM	Real time
Microelectrode Array	100 µm	Electrochemistry	1 mM - 10 pM	Real time
Chemical sensor (small molecules and MIP)				
Microelectrode Array	100 µm	Electrochemistry	1 µM- 10 pM	Real time
Optical fibers	-	Fluorescence MIP labelling	1 nM - 5 pM	End point

Efficacy in terms selectivity, specificity and miniaturization feasability of (bio)chemical sensors relay on the:

- *Recognition of the analyte by the (bio)chemical selective layer*
- *Efficient integration of the (bio)chemical layer to the sensor*
- *Intrinsic properties of the transducer*

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Detection/protection: Chemical sensors

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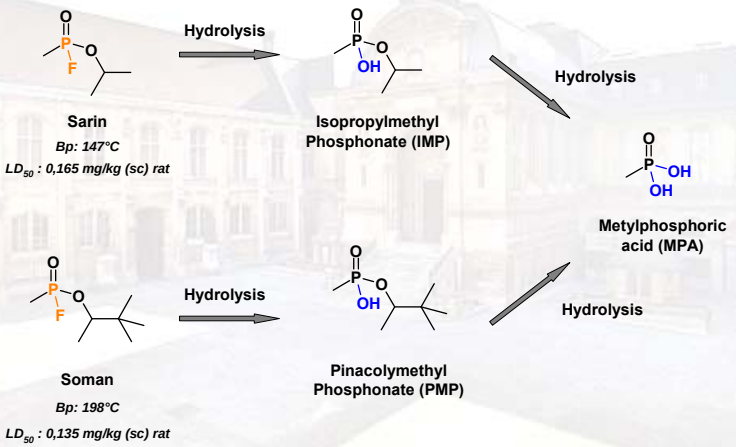
- Selective extraction
- Pre-concentration

Decontamination

Mip Based Sensors Applied to the Detection of Toxic Compounds Containing Organophosphorous Moiety (*i.e.* Nerve Agents)

MIP-based sensors are used for the detection of organophosphorous compounds	Detection of nerve agents is currently based on breakdown end products	MIP labile covalent bonding imprinting approach has been investigated
e.g: parathion	*e.g:* sarin & soman (mimics)	

The Fate of Organophosphorous Nerve Agents in Natural Medium



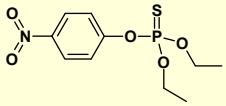
Two Examples of the Detection of Organophosphorous Nerve Agents Based on their Degradation Products

Analysis of ground water spiked with methylphosphonic acid in the presence of inferents (NaH_2PO_4, H_3PO_4, dichlorovos, parathion)[a]		Routine analysis of ground/tap waters spiked with pinacolylmethyl phosphonate (PMP), a degradation product of Soman [b]
Methacrylic acid/ vinylpyridine/ ethyleneglycol dimethacrylate + MPA	MIP composition	Methylmethacrylate/ethyleneglycoldimethacrylate (bulk) + PMP
Potentiometric detection	Measurement	Potentiometric detection
From 5 10^{-8} to 1 10^{-4} M and 1 10^{-3} to 1 10^{-1} M	Linear response	From 4 10^{-8} to 1 10^{-5} M and 1 10^{-5} to 1 10^{-3} M
5 10^{-8} M	Detection limit	4 10^{-8} M (7.2 ppb)
2 to 5 min. in the concentration range 10^{-6} to 10^{-2} M	Response time	5 min.
Not given	Reproducibility	Not given
Stable for 2 months - Reusable > 20 times	Stability/reusability	Stable for 2 months - Reusable ~15 times
Demonstrated for 4 phosphorous related compounds in concentration the range from 10^{-5} to 10^{-4} M	Selectivity	Established for 8 phosphorous related compounds

(a) K. P. Parthish et al Talanta (2007) 71, 1976-1980
(b) V. Vishnurdhan et al Electrochim. Acta (2007) 52, 6922-28

MIP-based sensors allow specific detection of organophosphorous neurotoxic molecules but via their degradation end-products

Determination of Parathion in Vegetables by Electrochemical Sensor Based on Molecular Imprinted Polyethylene/silica gel films[a] (I)



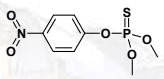
(Ethyl)parathion

Half-life[b] : 2 days (ground water)
3 days (river)

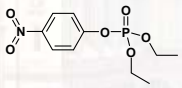
European Union Legislation:

< 0,05 mg kg^{-1} for vegetable

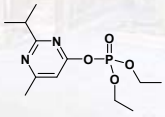
< 0,1 µg L^{-1} in drinking water



Methylparathion



Paraoxon



Diazinon

(a) Q. Yang et al J. Agric. Food Chem. (2009) 57, 6558-6563
(b) : M. Castillo et al. Anal. Chim. Acta (1997) 353, 133-142

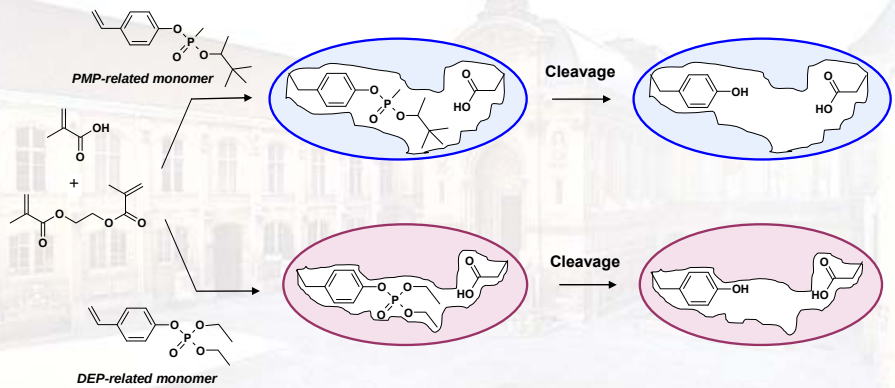
Determination of Parathion in Vegetables by Electrochemical Sensor Based on Molecular Imprinted Polyethylene/silica gel films[(a)] (II)

Quantification of parathion in spiked vegetables	
MIP composition	Polyethyleneimine (PEI)/ ethylenglycoldimethacrylate (EGDMA)
Measurement	Electrochemical (cyclic voltammetry)
Linear response	from 0,015 to 15 mg/kg
Detection limit	0,003 mg/kg
Response time	~5-10 min @ 3 mg/kg – Saturation after 50 min
Reproducibility	RSD : 5,4 % (n = 8)
Stability/reusability	Storage in PBS @ 4°C for 10 days – reused ~30 times
Selectivity	Demonstrated for 4 organophosphate related pesticides (parathion, methylparathion, paraoxon, diazinon)

Adapted from Q. Yang et al J. Agric. Food Chem. (2009) 57, 6558-6563

However MIP-based sensors have proven their ability to detect molecules containing organophosphorous moieties in their unaffected form in case their chemical stability is high enough

Pinacolmethylphosphonate (PMP) Detection by Molecularly Imprinted Polymers: a Labile Covalent Binding Approach [(a)]



Improvement of selectivity via better molecular recognition induced by a more precise arrangment of functionnal groups within the pores of the MIP and the size of the cavity

(a) P. Taranekar et al Polymer (2006) 47, 6485-90

Selection of Applications in the CW Field

Detection/protection: Chemical sensors

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On-site monitoring of the presence of CW and their sampling

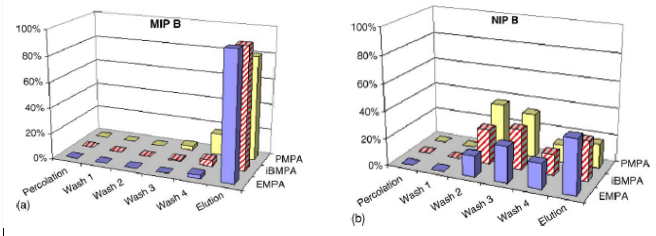
- **selective extraction**
- **pre-concentration**

Decontamination

Verification/Monitoring for the Presence of CW

Selective Extraction of Organophosphorus Nerve Agent Degradation Products by MIP- SPE

- **Template: pinacolyl methylphosphonic acid (PMPA)**
- **Monomer: MAA**
- **Cross-linker: TRIM**
- **MIP capacity: 97 µg/g of MIP**
- **MIP recovery for EMPA: 97%**



Behaviour of MIP-SPE for alkyl methylphosphonic acids: step elution of PMPA, EMPA, iBMPA from MIP/NIP

MIP-SPE represents an advance versus conventional SPE methods only based on non-specific retention

S. Le Moullec et al. J. Chrom. A, 1108 (2006) 7-13

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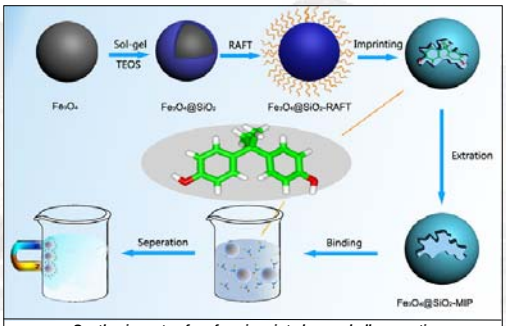
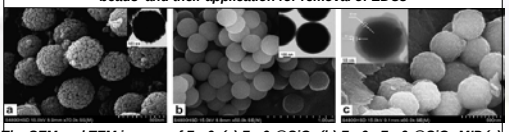
Decontamination

Verification/monitoring for the Presence of CW Decontamination

Fast and Selective removal of endocrine Disrupting Chemicals from water by using Core-shell Magnetic MIPs



Synthesis route of surface-imprinted core-shell magnetic beads and their application for removal of EDCs

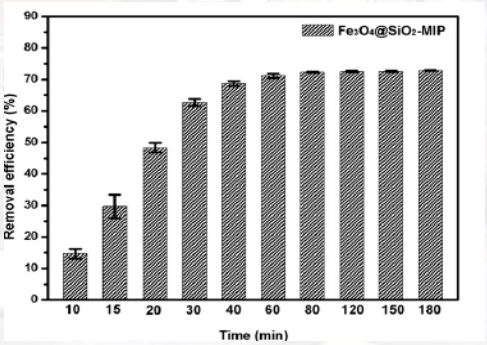


The SEM and TEM images of Fe_3O_4 (a) Fe_3O_4@SiO_2 (b) Fe_3O_4 Fe_3O_4@SiO_2-MIP (c)

Y. Li et al. Environmental Pollution (2010) 1-7

Verification/monitoring for the Presence of CW Decontamination

Fast and Selective removal of endocrine Disrupting Chemicals from water by using Core-shell Magnetic MIPs



Applicability of Fe_3O_4-SiO_2 MIP to a pratical water treatment.
20 ml real water sample.In Biphenol A concentration, 0,1 mmol L^{-1}

Y. Li et al. Environmental Pollution (2010) 1-7

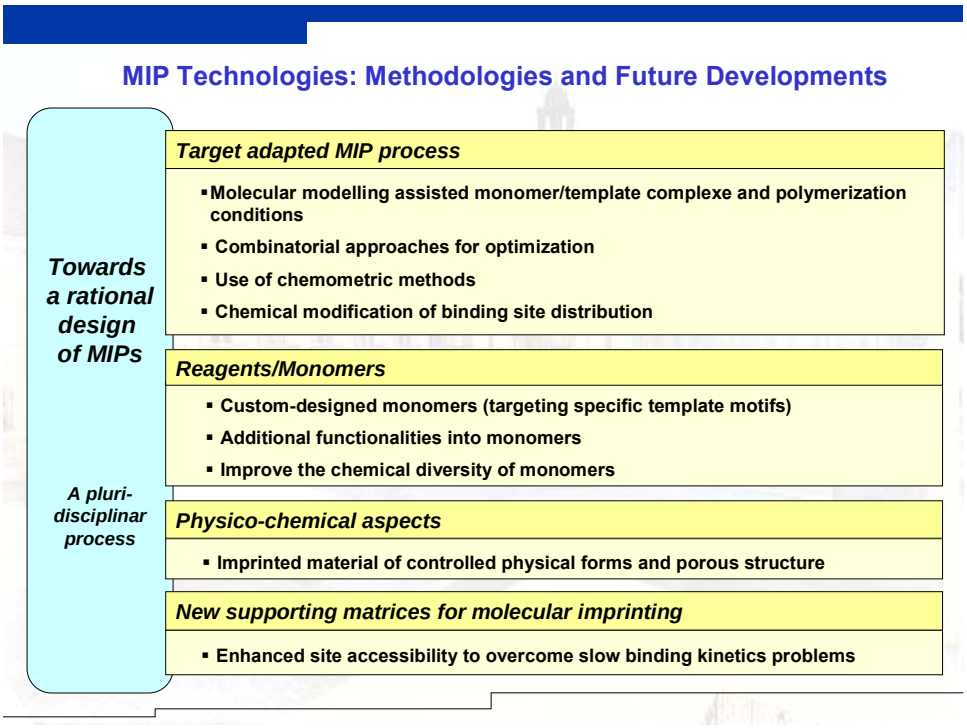
MIP Technology: what Potentials for CW/TICs

Can be applied to multiple detection constraints linked to CW and TICs :

- **Fast and real-time on site monitoring detection**
- **Selective extraction/pre-concentration**
- **Efficient decontamination/depollution**

Major specific strengths

- **Ability to easily and selectivly detect small molecules**
- **Binding affinities comparable to a biological recognition element**
- **Robustness and stability under a wide range of chemical and physical conditions**
- **Accessible by a simple and generic cost effective process**
- **Ability to be integrated with a various range of transducers**


MIP Technologies: Methodologies and Future Developments
Towards a rational design of MIPs
A pluri-disciplinar process
Target adapted MIP process
Molecular modelling assisted monomer/template complexe and polymerization conditions
Combinatorial approaches for optimization
Use of chemometric methods
Chemical modification of binding site distribution
Reagents/Monomers
Custom-designed monomers (targeting specific template motifs)
Additional functionalities into monomers
Improve the chemical diversity of monomers
Physico-chemical aspects
Imprinted material of controlled physical forms and porous structure
New supporting matrices for molecular imprinting
Enhanced site accessibility to overcome slow binding kinetics problems

Annex 5

FIRST OPCW CONFIDENCE-BUILDING EXERCISE ON BIOMEDICAL SAMPLES

The First OPCW Confidence Building Exercise on Biomedical Sample Analysis November 2009 – January 2010

Robin Black

ORGANISATION FOR THE PROHIBITION OF CHEMICAL WEAPONS

OPCW requirement for biomedical sample analysis

- The CWC provides for the collection and analysis of environmental and biomedical samples (BMS) in investigations of alleged use of chemical weapons
- DG requested the SAB to review scientific aspects of BMS & consider how the OPCW could develop such a capability
- TWG convened in 2004, 3 meetings held, final report endorsed by SAB Feb 2007 and accepted by DG
- The Executive Council (March 2006) noted the TS intention to proceed with developing an OPCW capability for BMS

2

Recommendation of SAB TWG on Biomedical Samples

- The next stage in building a capability should be coordinated by the OPCW laboratory with assistance from member states
- A progression recommended:

collation/dissemination of knowledge
⇒ confidence building exercises
⇒ validated methods
⇒ proficiency tests
⇒ designation

3

Differences in requirements from OPCW analysis of chemical/environmental samples

- BMS in most cases requires trace analysis (low ppb)
  - in complex matrices, primarily blood and urine
  - analyte or class targeted analysis as opposed to generic
- Other than simple hydrolysis products, biological markers are quite different from environmental markers
  - free metabolites in urine and blood
  - covalent adducts with blood proteins and DNA

4

Objectives of the first confidence building exercise

- To broaden the capability for biomedical sample analysis across member states
- To assess advantages & disadvantages of different methods
- To commence a discussion on criteria for identification at trace levels
- Identification is the main requirement but laboratories encouraged to report quantitative results if obtained

5

Overview

- Samples and standards prepared by TNO Defence, Security & Safety, NLD
- Dispatched 6 November 2009 by OPCW laboratory
- Details of selected methods provided
  - but laboratories free to use any method
- Submission of reports by 15 January 2010
  - with some flexibility
- Results evaluated by Dstl, Porton Down, UK
  - but not on the lines of a proficiency test, no scoring
- Meeting to discuss results 25 March 2010

22 April 2010

Samples

- Commercial synthetic urine selected as the matrix
  - to avoid problems of transport of biological materials
- Urinary metabolites of nerve agents and sulfur mustard as spiking chemicals
- Six spiked samples and one labelled blank
- Spiking levels:

s1 : blank synthetic urine	
s2 : ethyl methylphosphonic acid	100 ng/ml
s3 : isopropyl methylphosphonic acid	100 ng/ml
s4 : thiodiglycol	100 ng/ml
s5 : sulfur mustard β-lyase metabolite	100 ng/ml
s6 : isopropyl methylphosphonic acid	10 ng/ml
s7 : thiodiglycol	100 ng/ml
β-lyase metabolite	10 ng/ml

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Overview of results

- 22 laboratories from 17 member states submitted reports (1 no results)
- 6 laboratories reported all spiking chemicals in all samples
- 5 laboratories did not analyse for thiodiglycol
  - 4 of these reported all other spiking chemicals
- 6 laboratories reported false positives
  - mostly alkyl methylphosphonic acids
- More than half the laboratories reported ‘system’ and/or urine blanks with traces of analyte or interferents

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Instrumentation

	Number of labs			
	Triple quad	Ion trap	Single quad	Other
LC-MS/MS*	11			1 Orbitrap
LC-MS			1	1 Q-TOF (HR)
GC-MS/MS	5	3		1 linear ion trap
GC-MS			14	
GC/GC-MS				1 TOF
GC-FPD				2

* 4 labs used LC-MS/MS as the only technique

Negative controls (blanks)

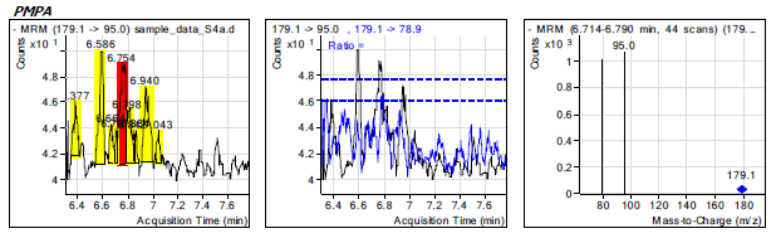
- Laboratories were asked to provide chromatograms for system blanks and urine blanks (sample s1)
- System blanks varied from simply injecting solvent, injecting derivatising mixture, to taking a sample of water through the entire procedure.
- If the GC injector is contaminated, e.g. with underivatised analyte, simply injecting solvent will not detect this contamination.
- Taking a sample of water through the entire procedure is recommended.

System contamination

- Data presented by > half the laboratories showed evidence of system contamination
- The more selective the method the more likely that peaks in the retention window of an analyte represent contamination rather than interferents
- Problem much greater with GC-MS(MS) where derivatisation required
  - particularly very sensitive –ve CI methods, and silyl derivatives
- Common sources of contamination are underivatised agent in the GC injector (from incomplete derivatisation or thermal degradation), the SPE vacuum manifold, syringe in automated methods
- Very important that this problem is addressed

Need to define what is a significant peak

- This sample was reported as containing pinacolyl methylphosphonic acid
  - reflects either trace system contamination from calibrations or an interfering peak
  - in Proficiency Tests peaks with S/N < 5:1, or < 1% of analyte intensity are not deemed to be significant



Sample preparation

- Most labs followed literature procedures, sometimes with minor modifications
- Omitting sample clean-up, or simple lyophilisation, for LC-MS/MS OK for clean, high concentration samples but not recommended for real samples
- Some labs used liquid-liquid extraction for removing extraneous materials or for extracting analytes
  - solid phase extraction (SPE) would probably have been easier & more efficient
- SPE methods mostly based on polymeric materials (e.g. Oasis HLB, ENV+), SAX ion exchange, or silica

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Methods: alkyl methylphosphonic acids

Technique	Derivative	Ionisation	Mode
LC-MS/MS	none	-ve ESI +ve ESI (1 lab)	MRM, full product ion scan
LC-MS	none	-ve ESI	SIM
LC-HRMS (1 lab)	none	-ve ESI	full scan, HR extracted ion
GC-MS/MS	PFB	-ve CI (CH_4, NH_3)	MRM
	TMS	EI	MRM
	TBDMS	EI, +ve CI	MRM
GC-MS	PFB	-ve CI (CH_4, iBu), EI	SIM, full scan
	TMS	EI, +ve CI	SIM, full scan
	TBDMS	EI	SIM
	Me ester	EI, +ve CI (CH_3CN)	full scan, extracted ion

Sample s2: ethyl methylphosphonic acid (EMPA) 100 ng/ml

- 20 laboratories reported EMPA in sample s2
  - 3 also reported EMPA as a false +ve in other samples
  - 4 laboratories reported other analytes (IMPA, PMPA, TDG) in s2
  - 2 laboratories reported MPA

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Sample s2: EMPA by LC-MS/MS, -ve ESI

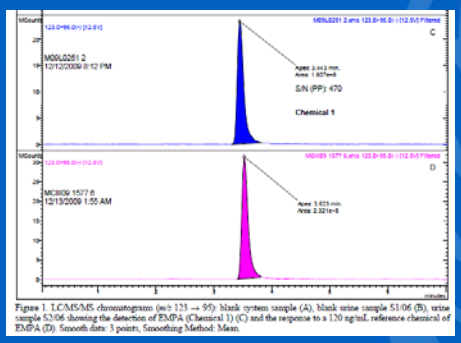

Figure 1. LC/MS/MS chromatograms (m/z 123 → 95): blank system sample (A), blank urine sample 51/06 (B), urine sample 52/06 showing the detection of EMPA (Chemical 1) (C) and the response to a 120 ng/mL reference chemical of EMPA (D). Smooth data: 5 points, Smoothing Method: Mean.

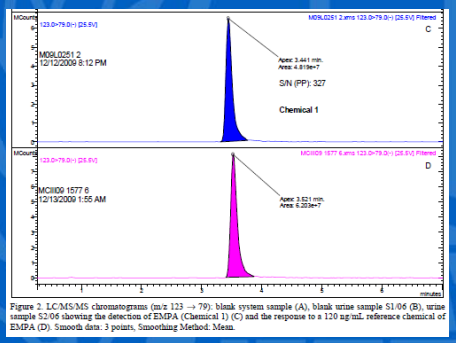

Figure 2. LC/MS/MS chromatograms (m/z 123 → 79): blank system sample (A), blank urine sample 51/06 (B), urine sample 52/06 showing the detection of EMPA (Chemical 1) (C) and the response to a 120 ng/mL reference chemical of EMPA (D). Smooth data: 3 points, Smoothing Method: Mean.

m/z 123 → 95

m/z 123 → 79

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Sample s2: EMPA by GC-MS/MS, PFB deriv, -ve CI

S/N > LC-MS/MS

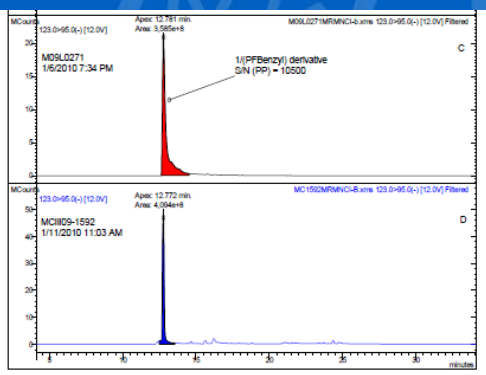


Figure 5. GC/MS/MS NCI chromatograms (*m/z* 123 → 95): Blank system (acetonitrile + pentafluorobenzyl bromide) (A), blank urine sample S1/06 derivatized with pentafluorobenzyl bromide (B), urine sample S2/06 derivatized with pentafluorobenzyl bromide, showing the detection of EMPA/(PFBenzyl) derivative (C) and the response to a 100 ng/mL reference chemical EMPA/(PFBenzyl) derivative (D). Smooth data: 3 points, smoothing method: Mean.

Sample s2: EMPA by GC-MS, TMS deriv, EI

S/N << LC-MS/MS or
GC-MS/MS, PFB deriv, -ve CI

Selectivity lower as indicated by number of additional peaks across chromatogram

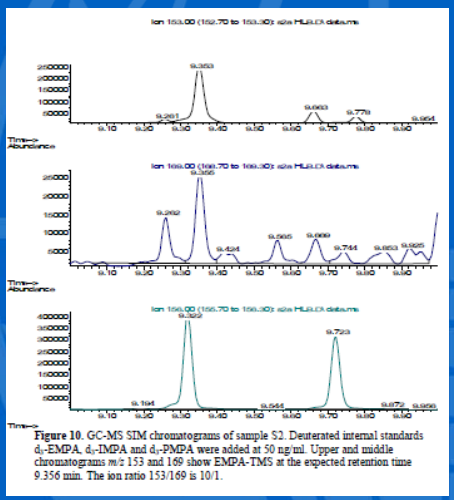


Figure 10. GC-MS SIM chromatograms of sample S2. Deuterated internal standards d_3-EMPA, d_3-IMPA and d_3-PMPA were added at 50 ng/ml. Upper and middle chromatograms *m/z* 153 and 169 show EMPA-TMS at the expected retention time 9.356 min. The ion ratio 153/169 is 10/1.

Sample s2: EMPA by GC-MS, TBDMS deriv, EI

S/N << LC-MS/MS,
or GC-MS/MS, PFB deriv,
-ve CI

Selectivity lower as indicated by number of additional peaks across chromatogram

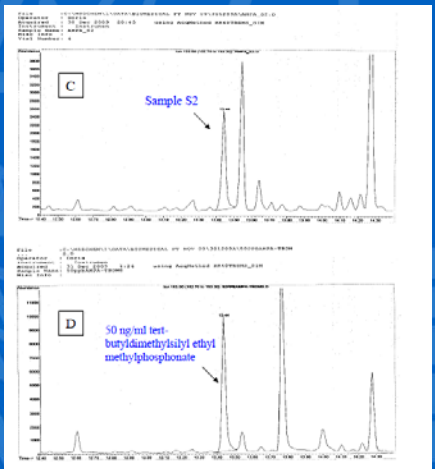


Figure 1: GC-MS SIM chromatograms (EIC of m/z 153): Urine sample S2 extracted onto a ENV+ polymeric SPE cartridge, showing the detection of TBDMS derivative showed in [C], with a system blank [A]; S1 urine blank [B]; and the response to 50 ng/ml reference chemical (r1) TBDMS derivative [D]

Samples s3 & s6: isopropyl methylphosphonic acid (IMPA) 100 & 10 ng/ml

- 21 laboratories reported IMPA in sample s3
  - 3 also reported IMPA as a false +ve in other samples
  - 3 laboratories reported other analytes in s3 (EMPA, PMPA)
  - 1 laboratory reported MPA
- 14 laboratories reported IMPA in sample s6
  - 2 laboratories reported other analytes in s6 (EMPA, PMPA)
- Of the 6 labs that detected IMPA in s3 but not s6, 2 used LC-MS/MS, 5 used GC-MS, 1 used GC-FPD
- All labs that used GC-MS/MS detected IMPA at 10 ng/ml

Sample s6: IMPA (10 ng/ml) by LC-MS/MS and GC-MS/MS

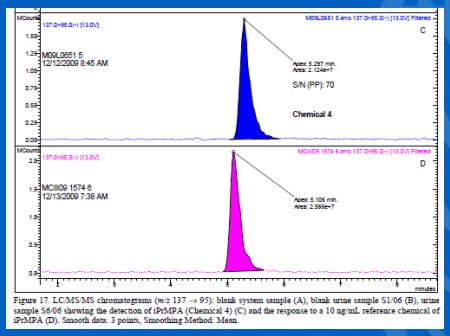


Figure 17. LC/MS/MS chromatograms (m/z 137 → 95): blank system sample (A), blank urine sample S1/06 (B), urine sample S6/06 showing the detection of iPrMPA (Chemical 4) (C) and the response to a 10 ng/mL reference chemical of iPrMPA (D). Smooth data: 3 points, Smoothing Method: Mean.

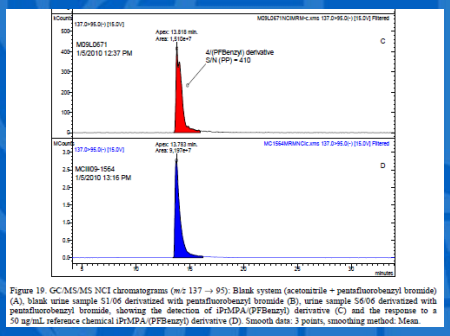


Figure 19. GC/MS/MS NCI chromatograms (m/z 137 → 95): Blank system (acetonitrile + pentafluorobenzyl bromide) (A), blank urine sample S1/06 derivatized with pentafluorobenzyl bromide (B), urine sample S6/06 derivatized with pentafluorobenzyl bromide, showing the detection of iPrMPA/(PFBenzyl) derivative (C) and the response to a 50 ng/mL reference chemical iPrMPA/(PFBenzyl) derivative (D). Smooth data: 3 points, smoothing method: Mean.

LC-MS/MS, -ve ESI

GC-MS/MS, PFB, -ve CI

Sample s6: IMPA (10 ng/ml) by LC-HRMS (Q-TOF)

- Full scan LC-MS using a Q-TOF instrument & HR (>10,000) extracted ion (m/z 137.0373) gave impressive results

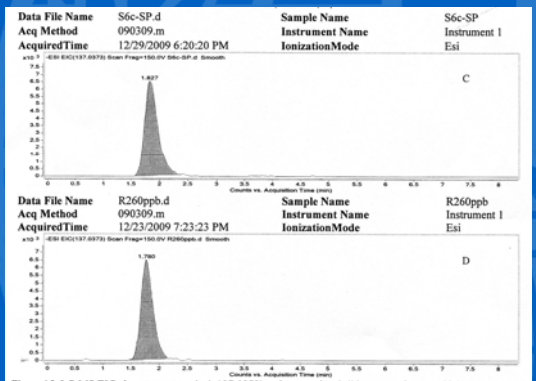
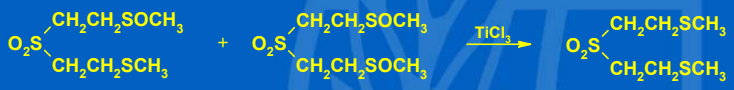


Figure 15. LC-MS EIC chromatograms (*m/z* 137.0373): urine sample s6c/03 extracted onto a Si-SAX (500mg,3mL,Agilent) SPE cartridge, showing the detection of Isopropyl methylphosphonate (C), system blank (A), urine blank(B)and the reference chemical of **Isopropyl methylphosphonate** (D).

Sample s5: problems with $TiCl_3$ & Surine

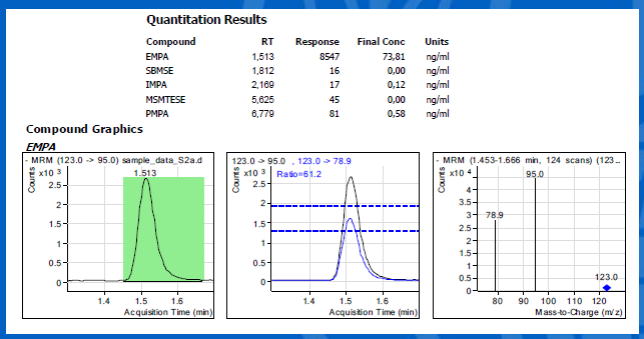
- 2 β-lyase metabolites reduced to a single analyte, SBMTE



- Initially developed at Dstl, and has always been robust
  - used for confirming SM exposure in Iranian & Kurdish CW casualties
- 2 labs could not detect SBMTE by GC-MS/MS, one lab detected it only after diluting sample s5 with real urine.
- 1 lab could not detect the reduced analyte SBMTE by LC-MS/MS
  - nor the reduced internal std $^{13}C_4$-SBMTE, added before $TiCl_3$ reduction

Identification

- Identification was based on retention time, & selected ions or MS/MS transitions, and in a few cases full scan spectra
- Approx one third of the labs reported ion ratios for MRM or SIM
  - and within 10-20% of ratios in reference chemical



LC-MS/MS MRM ion ratios for EMPA in sample s2

Quantitation

- Where reported was generally good
- Ranged from estimates by comparison of samples with one or two standard solutions to comparison with a multi-point calibration curve
- Most rigorous procedures compared peak areas of the analyte with isotopically labelled internal standard, against a multipoint calibration curve in Surine (sample s1)
- Use of internal standards aids quantitation and increases confidence in the performance of the method

Conclusions

- Broader capability for biomedical sample analysis demonstrated
- Levels of identification & limits of detection dependent on instrumentation
- Triple quadrupoles, other MS/MS instruments, and high resolution TOF provided best quality data
  - broader application of TOFs expected in the future?
- LC-MS/MS very sensitive for alkyl methylphosphonic acids & β-lyase metabolites
  - and generally less prone to system contamination than GC-MS(MS)
- Perfluorinated derivatives with –ve CI provided the most sensitive GC-MS(MS)
  - but number of ions for monitoring may be less than with silyl derivs
- System contamination was a significant problem

Recommendations

- Request TWG on S&A to discuss report, particularly with regard to drafting criteria for identification
- Need criteria for what is a reportable peak, particularly in system and matrix blanks
- Efforts should be made to reduce the sources and occurrence of system contamination, particularly with some of the GC-MS methods
- Broader use of isotopically labelled internal standards should be encouraged
- Stricter format for reporting will be used for next exercise
- Workshop to be held in 2011?
- Second exercise in 2011?

Update on activities of TWG on sampling & analysis

- Report of round robin exercise on ricin analysis, organised by Global Health Security Action Group, not yet received
- Plan to hold simple exercise on saxitoxin analysis June-September 2010
  - organised by Martin Schar, Spiez Laboratory, Switzerland
  - laboratories to prepare their own samples as instructed
  - data to be evaluated by Spiez Laboratory
- New version of Finnish 'Blue Book' (on analytical methods) is being produced, coordinated by VERIFIN