

ΙΔΙΟΚΤΗΣΙΑ ΚΑΙ ΣΥΝΤΑΞΗ - ΕΚΔΟΤΗΣ:

ΕΛΛΗΝΙΚΗ ΑΝΔΡΟΛΟΓΙΚΗ ΕΤΑΙΡΕΙΑ

Ενδοκρινολογικό Τμήμα Νοσοκομείου "ΕΛΕΝΑ ΒΕΝΙΖΕΛΟΥ"

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ΟΔΗΓΙΕΣ ΠΡΟΣ ΣΥΓΓΡΑΦΕΙΣ

Το περιοδικό ANHP, έκδοση της Ελληνικής Ανδολογικής Εταιρείας έχει στόχο τη συνεχή επιμόρφωση των ασχολούμενων στο χώρο της Ανδρολογίας και την προαγωγή του γνωστικού αντικείμενου της στον ελληνικό χώρο. Για την πραγμάτωση αυτού του σκοπού δημοσιεύονται στο περιοδικό:

1. Άρθρα Σύνταξης. Σύντομες ανασκοπήσεις σε επίκαιρα και αμφιλεγόμενα θέματα, που γράφονται με προτροπή της συντακτικής επιτροπής. Όταν εκφράζουν συλλογικά τη Σύνταξη του περιοδικού, είναι ανυπόγραφα. Στις άλλες περιπτώσεις είναι ανυπόγραφα.

2. Γενικά θέματα. Σχετιζόμενα με την Ανδρολογία

3. Ανασκοπήσεις. Ολοκληρωμένες αναλύσεις ιατρικών θεμάτων, στις οποίες υπογραμμίζονται οι σύγχρονες απόψεις. Γίνονται δεκτές ανασκοπήσεις μέχρι δύο συγγραφέων.

4. Ερευνητικές εργασίες. Κλινικές δοκιμές ή μη πειραματικές έρευνες προοπτικού ή αναδρομικού χαρακτήρα, που πραγματοποιήθηκαν με βάση ερευνητικό πρωτόκολλο, το οποίο να περιγράφεται αναλυτικά στη μεθοδολογία. Περιέχουν πρωτοδημοσιευμένα αποτελέσματα.

5. Ενδιαφέρουσες περιπτώσεις. Γίνονται δεκτά άρθρα εφόσον αφορούν νέα και πολύ σπάνια νοσήματα ή νοσήματα εμφανίζοντα ιδιαίτερες ως προς την κλινική τους εκδήλωση ή την διερευνητική τους προσπέλαση ή έχει ακολουθηθεί νέα θεραπευτική μεθόδευση με ελεγχόμενο το αποτέλεσμα. Επίσης στα άρθρα αυτά μπορούν να παρουσιασθούν πρωτότυπες περιπτώσεις προς συζήτηση με τους αναγνώστες του περιοδικού.

6. Επίκαιρα θέματα. Σύντομη περιγραφή των τελευταίων απόψεων σε συγκεκριμένα θέματα.

7. Πρακτικά από σεμινάρια και στρογγυλά τραπέζια ή κείμενα από διαλέξεις.

8. Περίληψη άρθρων της διεθνούς βιβλιογραφίας συνοδευόμενη από σύντομο σχόλιο. Δημοσιεύονται ανυπόγραφα.

9. Γράμματα προς τη Σύνταξη. Περιέχουν κρίσεις για δημοσιευμένα άρθρα, πρόδρομα αποτελέσματα εργασιών, παρατηρήσεις για ανεπιθύμητες ενέργειες, κρίσεις για το περιοδικό κ.λ.π. Δημοσιεύονται ανυπογράφως.

Προηγούμενη ταυτόχρονη δημοσίευση. Τα άρθρα που υποβάλλονται στο περιοδικό ANHP δεν μπορεί να έχουν υποβληθεί ταυτόχρονα για δημοσίευση σε άλλα Ελληνικά περιοδικά. Το γεγονός πρέπει να βεβαιώνεται από επιστολή - δήλωση του πρώτου συγγραφέα προς τον Διευθυντή Σύνταξης. Όμως επιτρέπεται η υποβολή εργασιών μέρος των οποίων έχει δημοσιευθεί ή παρουσιασθεί με μορφή περίληψης σε Ελληνικό ή Διεθνές Συνέδριο.

Όλα τα χειρόγραφα συνοδεύονται από επιστολή που υπογράφεται από τον υπεύθυνο για την αλληλογραφία συγγραφέα. Η συνοδευτική επιστολή πρέπει να περιλαμβάνει δήλωση ότι τα χειρόγραφα έχουν εγκριθεί και από όλους τους υπόλοιπους συγγραφείς οι οποίοι και συνυπογράφουν την επιστολή.

Προετοιμασία του χειρόγραφου. Η γλωσσική ομοιομορφία των άρθρων είναι απαραίτητη. Τα άρθρα που υποβάλλονται για δημοσίευση πρέπει να είναι γραμμένα στη δημοτική και με το μονοτονικό σύστημα.

Το περιοδικό ANHP έχει αποδεχθεί το σύστημα Vancouver και εφαρμόζει το ελληνικό πρότυπο γραφής βιοιατρικών κειμένων.

Τα άρθρα πρέπει να είναι δακτυλογραφημένα με διπλό διάστημα σε λευκό χαρτί, από τη μια πλευρά των σελίδων, με περιθώρια τουλάχιστον 2,5 cm. Τα εξής κεφάλαια αρχίζουν σε ιδιαίτερη σελίδα: η σελίδα με τον τίτλο, η περίληψη και οι λέξεις ευρετηρίου, το κείμενο, οι ευχα-

ριστίες, η αγγλική περίληψη, οι βιβλιογραφικές παραπομπές, οι πίνακες, οι εικόνες και οι λεζάντες των εικόνων. Όλες οι σελίδες αριθμούνται, αρχίζοντας από τη σελίδα τίτλου.

Σελίδα τίτλου. Περιλαμβάνει (α) τον τίτλο του άρθρου, ο οποίος πρέπει να είναι σύντομος (μέχρι 12 λέξεις), (β) το όνομα και τον τίτλο του συγγραφέα (-ων), (γ) το ίδρυμα ή το εργαστήριο, από το οποίο προέρχεται η εργασία και η προέλευση του συγγραφέα, (δ) το όνομα, τη διεύθυνση και το τηλέφωνο του συγγραφέα για αλληλογραφία και ανάτυπα, (ε) πηγές που ενδεχομένως ενίσχυσαν και βοήθησαν στην πραγματοποίηση της εργασίας, (στ) αν υπάρχουν διαφωνούντες με την εργασία.

Περίληψη και λέξεις ευρετηρίου. Η περίληψη δεν πρέπει να υπερβαίνει τις 200 - 300 λέξεις, ενώ για τα επίκαιρα θέματα και τις περιγραφές περιπτώσεων ασθενών τις 150 - 200 λέξεις. Για τις ανασκοπήσεις πρέπει να εφαρμόζονται οι περιγραφικές περιλήψεις (descriptive), που αναφέρουν συνοπτικά όλα τα κεφάλαια που περιέχει το άρθρο και σημαντικά συμπεράσματα. Οι περιλήψεις των ερευνητικών εργασιών πρέπει να χωρίζονται σε τέσσερις παραγράφους, οι οποίες φέρουν κατά σειρά την ακόλουθη επικεφαλίδα. Σκοπός, Υλικό Μέθοδος, Αποτελέσματα, Συμπεράσματα. Μετά την περίληψη παρατίθενται 3 - 10 λέξεις κλειδιά. Οι λέξεις αυτές πρέπει να αντιστοιχούν στους διεθνείς όρους που χρησιμοποιεί το Index Medicus.

Κείμενο. Οι ερευνητικές εργασίες αποτελούνται συνήθως από την Εισαγωγή, Υλικό και μέγεθος, Αποτελέσματα και Συζήτηση. Η εισαγωγή περιλαμβάνει τις απαραίτητες βιβλιογραφικές παραπομπές και αναφέρει το λόγο για τον οποίο πραγματοποιήθηκε η εργασία.

Στη μεθοδολογία περιγράφεται το πρωτόκολλο, με βάση το οποίο εξελίχθηκε η έρευνα. Αναφέρονται λεπτομερώς ο τρόπος επιλογής ασθενών ή οποιουδήποτε υλικού, καθώς και η μέθοδος που εφαρμόστηκε, ώστε η ίδια έρευνα να μπορεί να αναπαραχθεί από μελλοντικούς ερευνητές. Στην περίπτωση ερευνών που αφορούν ανθρώπους, πρέπει να τονίζεται ότι η έρευνα πραγματοποιήθηκε με βάση την Υπουργική απόφαση Αριθ. Α6/10983/1 {ΦΕΚ 886/Β 20-12-84} για τη "Διεξαγωγή Κλινικών Δοκιμών φαρμάκων και την προστασία του ανθρώπου" και η οποία παραπέμπει στη Διακήρυξη του Ελσίνκι (1975). Οι φαρμακευτικές ουσίες που χρησιμοποιήθηκαν στη μελέτη πρέπει να αναφέρονται με την κοινόχρηστη ονομασία τους. Περιγράφεται το υλικό που αξιολογήθηκε κατά τη διάρκεια της μελέτης και το κεφάλαιο ολοκληρώνεται με τα στατιστικά κριτήρια που χρησιμοποιήθηκαν.

Τα αποτελέσματα παρουσιάζονται ολοκληρωμένα και σύντομα. Όσα αναφέρονται σε πίνακες, δεν επαναλαμβάνονται στο κείμενο.

Στη συζήτηση περιγράφονται οι προοπτικές που διανοίγονται με τα αποτελέσματα της μελέτης, καθώς και τα τελικά συμπεράσματα. Δεν επαναλαμβάνονται όσα έχουν αναφερθεί στα αποτελέσματα. Επίσης, μπορεί να γίνει σύγκριση με τα αποτελέσματα άλλων ομοειδών εργασιών. Συνδέονται τα αποτελέσματα με τους στόχους της μελέτης, αποφεύγονται όμως αυθαίρετα συμπεράσματα, που δεν προκύπτουν από τα αποτελέσματα της εργασίας.

Ευχαριστίες. Απευθύνονται μόνο προς τα άτομα, που έχουν βοηθήσει ουσιαστικά.

Στα υπόλοιπα είδη άρθρων, το κείμενο διαμορφώνεται ανάλογα με τις απαιτήσεις και τους στόχους του συγγραφέα. Στις ενδιαφέρουσες περιπτώσεις ασθενών προηγείται η εισαγωγή και ακολουθούν η περιγραφή της περιπτώσεως και η συζήτηση.

Βιβλιογραφικές παραπομπές. Αριθμούνται στο κείμενο με αύξοντα αριθμό, ανάλογα με τη σειρά που εμφανίζονται. Σε περίπτωση αναφοράς σε ονόματα συγγραφέων στο κείμενο, εφόσον είναι ξένοι, μετά το επώνυμο του πρώτου συγγραφέα ακολουθεί η συντομογραφία et al, ενώ στους Έλληνες συγγραφείς "και συν.". Εφόσον οι συγγρα-

φείς είναι δύο, μεταξύ των επωνύμων τοποθετείται η λέξη "και". Όλες οι βιβλιογραφικές παραπομπές του κειμένου - και μόνον αυτές - πρέπει να υπάρχουν στο βιβλιογραφικό κατάλογο.

Ο αριθμός των βιβλιογραφικών παραπομπών πρέπει να περιορίζεται στον τελειώς απαραίτητο. Στις ανασκοπήσεις, οι βιβλιογραφικές παραπομπές δεν πρέπει να είναι περισσότερες από 100. Στα άρθρα επικαιρότητας (επίκαιρα θέματα, άρθρα Σύνταξης) θα πρέπει να αναφέρονται μόνο 5-6 άρθρα ή μονογραφίες, για τα οποία ο συγγραφέας πιστεύει ότι είναι απαραίτητα για την ολοκληρωμένη πληροφόρηση του αναγνώστη στο θέμα.

Η σύνταξη του βιβλιογραφικού καταλόγου γίνεται αριθμητικώς, με βάση τον αύξοντα αριθμό και τη σειρά των βιβλιογραφικών παραπομπών στο κείμενο. Αναφέρονται τα επώνυμα και τα αρχικά των ονομάτων όλων των συγγραφέων μέχρι έξι (όταν είναι περισσότεροι ακολουθεί η ένδειξη et al), ο τίτλος της εργασίας, η συντομογραφία του τίτλου του περιοδικού, το έτος, ο τόμος, η πρώτη και η τελευταία σελίδα της δημοσίευσης π.χ. You CH, Lee KY, Chey WY, Menguy R. Electrogastrographic study of patients with unexplained nausea. *Gastroenterology* 1980, 79:311 - 314.

Σε περίπτωση που δεν αναφέρεται όνομα συγγραφέως, σημειώνεται η λέξη Ανώνυμος (για ελληνική δημοσίευση) ή Anonymous Π.χ. Anonymous. Coffe drinking and cancer of the pancreas (Editorial). *Br Med J* 1981, 283:628.

Παραπομπές που αναφέρονται σε εργασίες που δημοσιεύονται σε συμπληρώματα (supplements) εκδόσεων, πρέπει να συνοδεύονται με τον αριθμό του συμπληρώματος, που σημειώνεται σε παρένθεση, μετά τον τόμο. Π.χ. *Blood*, 54 (Suppl 1):26. Οι συντμήσεις των τίτλων των περιοδικών πρέπει να γίνονται με βάση το *Index Medicus*. Δεν τοποθετούνται τελείες στα ακρώνυμα των συγγραφέων και στις συντμήσεις των περιοδικών. Στη βιβλιογραφία των επίκαιρων θεμάτων, παραλείπονται οι τίτλοι των εργασιών. Για την καταχώρηση συγγραμμάτων ή μονογραφιών στο βιβλιογραφικό κατάλογο, αναφέρονται στη σειρά τα επώνυμα και τα αρχικά των συγγραφέων, ο τίτλος, ο αριθμός εκδόσεως, ο εκδότης, η πόλη εκδόσεως, το έτος και οι σελίδες της αναφοράς. Η αναφορά σε κεφάλαιο βιβλίου πρέπει να γίνεται με τον ακόλουθο τρόπο: Παπαβασιλείου ΙΘ. Πρωτόζωα. Στο: Παθολογία μύκητες και παράσιτα. ΒΗΤΑ, Αθήνα, 1983:67 - 113.

Αν η βιβλιογραφική παραπομπή αποτελεί κεφάλαιο συγγραμματος που έχει γραφτεί από άλλον συγγραφέα, η αναφορά γίνεται ως εξής: Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. In: (Στο): Sodeman WA ed (:h eds ;h Συντ.) *Pathologic Physiology*. Saunders, Philadelphia, 1987: 457-472.

Μη δημοσιευμένες εργασίες καθώς και "προσωπικές επικοινωνίες" δεν χρησιμοποιούνται ως βιβλιογραφικές παραπομπές. Άρθρα, που έχουν γίνει δεκτά για δημοσίευση, μπορούν να περιληφθούν στη βιβλιογραφία. Στην τελευταία περίπτωση, μετά τη συντομογραφία του περιοδικού σημειώνεται η ένδειξη "υπό δημοσίευση".

Αγγλική περίληψη. Περιλαμβάνει τα ονόματα των συγγραφέων και την ιδιότητά τους, τον τίτλο της εργασίας και το ίδρυμα ή το εργαστήριο από το οποίο προέρχεται η εργασία. Η περίληψη δεν πρέπει να υπερβαίνει τις 200 - 300 λέξεις, ενώ για τα επίκαιρα θέματα και τις περιγραφές περιπτώσεων ασθενών τις 150 - 200 λέξεις. Για τις ανασκοπήσεις πρέπει να εφαρμόζονται οι περιγραφικές περιλήψεις (descriptive), που αναφέρουν συνοπτικά όλα τα κεφάλαια που περιέχει το άρθρο και σημαντικά συμπεράσματα. Οι περιλήψεις των ερευνητικών εργασιών πρέπει να χωρίζονται σε τέσσερις παραγράφους, οι οποίες φέρουν κατά σειρά την ακόλουθη επικεφαλίδα. Aim, Material, Methods, Results, Conclusions. Μετά την περίληψη παρατίθενται 3-10 λέξεις, απαραίτητες για τη σύνταξη των ευρετηρίων του περιοδικού (Key words).

Η ποιότητα των αγγλικών περιλήψεων πρέπει να είναι αρκετά ικανοποιητική, επειδή αποτελεί σημαντικό κριτήριο αποδοχής του περιοδικού στους διεθνείς καταλόγους βιοιατρικών περιοδικών (*Index Medicus*).

Αρίθμηση κεφαλαίων σε ανασκοπήσεις, επίκαιρα θέματα. Όλα τα κεφάλαια αριθμούνται με αραβικούς αριθμούς: 1,2,3 κλπ. Τα υποκεφάλαια φέρουν τον αριθμό του αρχικού κεφαλαίου, τελεία και ακολουθεί ο αριθμός του υποκεφαλαίου: 1.1., 1.2 ή 1.1.1., 1.2.1. κ.ο.κ.

Πίνακες. Δακτυλογραφούνται με διπλό διάστημα, σε χωριστή σελίδα. Αριθμούνται με τη σειρά που εμφανίζονται στο κείμενο, με αραβικούς αριθμούς. Πρέπει να φέρουν περιεκτική και σύντομη επεξήγηση, ώστε για την κατανόησή τους να μην είναι απαραίτητο να καταφύγει ο αναγνώστη στο κείμενο. Κάθε στήλη φέρει επεξηγηματική και σύντομη επικεφαλίδα. Οι επεξηγήσεις των συντομογραφιών καθώς και οι λοιπές διευκρινίσεις γίνονται στο τέλος του πίνακα.

Εικόνες. Τα σχήματα, σχεδιασμένα με σιλική μελάνη, και οι φωτογραφίες πρέπει να στέλνονται στο πρωτότυπο, ώστε να είναι κατάλληλα για άμεση φωτογραφική αναπαραγωγή και εκτύπωση. Στο πίσω μέρος τους να γράφονται με μολύβι ο αριθμός της εικόνας, ένα βέλος που να δείχνει το άνω μέρος και οι συγγραφείς. Τοποθετούνται σε φάκελο, ανάμεσα σε δύο σκληρά χαρτόνια, για να μην τσακιστούν στη μεταφορά. Οι τίτλοι των εικόνων πρέπει να αναγράφονται με τον αριθμό που αντιστοιχεί στην εικόνα σε χωριστό χαρτί. Επεξηγήσεις σχετικές με τις εικόνες μπορούν να αναφερθούν στον τίτλο. Για το μέγεθος των εικόνων συμβουλευθείτε το σχήμα του περιοδικού. Εφόσον χρησιμοποιούνται φωτογραφίες ασθενών, το πρόσωπο δεν πρέπει να φαίνεται. Στην αντίθετη περίπτωση επιβάλλεται έγγραφη συγκατάθεση του ασθενούς για τη δημοσίευση της φωτογραφίας. Όλες οι εικόνες αναφέρονται στο κείμενο και αριθμούνται με αραβικούς αριθμούς.

Ονοματολογία. Οι συγγραφείς πρέπει να χρησιμοποιούν τους παγκοσμίως παραδεκτούς τίτλους. Για την επιλογή των όρων και των ονομάτων (ουσιών, οντοτήτων, οργανισμών, νοσημάτων κ.λ.π.), κρίνεται σκόπιμο οι συγγραφείς να συμβουλευθούν το Λεξιλόγιο Βιοιατρικής Ορολογίας, MeSH-ΕΛΛΑΣ. Εκδοση ΙΑΤΡΟΤΕΚ, Αθήνα, 1991.

Μετρήσεις. Μετρήσεις μήκους, ύψους, βάρους και όγκου πρέπει να αναφέρονται σε μετρικές μονάδες (μέτρο, χιλ., λίτρο) ή στις υποδιαιρέσεις τους. Οι θερμοκρασίες πρέπει να δίνονται σε βαθμούς Κελσίου. Οι αρτηριακές πιέσεις πρέπει να δίνονται σε χιλιοστά στήλης υδραργύρου.

Διόρθωση τυπογραφικών δοκιμών. Πραγματοποιείται μία φορά από τους συγγραφείς. Εκτεταμένες μεταβολές δεν γίνονται δεκτές.

Ανάτυπα. Απαγορεύεται η φωτοτυπική αναπαραγωγή των δημοσιευμένων εργασιών. Η προμήθεια από τους συγγραφείς ανατύπων γίνεται αποκλειστικά από την εταιρεία MEDLINE. Οι συγγραφείς επιβαρύνονται με το κόστος τους. Τα ανάτυπα παραγγέλλονται κατά τη διόρθωση των δοκιμών.

Χειρόγραφα εργασιών που δημοσιεύονται, δεν επιστρέφονται στους συγγραφείς.

Υποβολή χειρογράφου: Τα χειρόγραφα αποστέλλονται στη διεύθυνση: Δ.Α. ΑΔΑΜΟΠΟΥΛΟΣ

ΠΕΡΙΟΔΙΚΟ ΑΝΗΡ

ΕΝΔΟΚΡΙΝΟΛΟΓΙΚΟ ΤΜΗΜΑ-ΠΓΝ ΜΑΙΕΥΤΗΡΙΟ "ΕΛΕΝΑ ΒΕΝΙΖΕΛΟΥ"

Πλ. Ε. ΒΕΝΙΖΕΛΟΥ 2 -115 21 ΑΘΗΝΑ

Η εργασία ταχυδρομείται σε φάκελο από χοντρό χαρτί, εσωκλείοντας τις φωτογραφίες και τη δισκέτα (εφ' όσον υπάρχει) μέσα σε σκληρό χαρτόνι. Εάν η αποστολή γίνεται μέσω των Ελληνικών Ταχυδρομείων να μην ακολουθείται συστημένη διαδικασία.

ΠΕΡΙΕΧΟΜΕΝΑ

CONTENTS

- 54** Σημείωμα Σύνταξης
Editorial Note
- 55** Πρόλογος προσκεκλημένου εκδότη, Π. Νικολοπούλου-Σταμάτη
Forward of the Invited Editor: Special Issue of Andrology on Endocrine Disrupters, *Polyxeni Nicolopoulou-Stamati*
- 57** Ενδοκρινικοί Διαταράκτες: Τι ακριβώς είναι, Π. Νικολοπούλου-Σταμάτη, C.V. Howard, E. Πατσούρης
Endocrine Disrupters, What are they, *P. Nicolopoulou-Stamati, C.V. Howard, E. Patsouris*
- 73** Εστιάζοντας στις Επιδράσεις των Ενδοκρινικών Διαταρακτών στην Ανθρώπινη Υγεία, Α. Λάζαρης, Π. Νικολοπούλου-Σταμάτη
Focusing on Human Health Effects of Endocrine Disrupters, *A. Lazaris, P. Nicolopoulou-Stamati*
- 79** Επίδραση των Ενδοκρινικών Διαταρακτών στην Ενδομήτρια Ζωή, Α. Ε. Κωνσταντινίδου, Ε. Πατσούρης, Π. Νικολοπούλου-Σταμάτη
Intra Uterus Impact of Endocrine Disrupters, *A. E. Konstantinidou, E. Patsouris, P. Nicolopoulou-Stamati*
- 84** Θυρεοειδής και Ενδοκρινικοί Διαταράκτες: Μια ενδιαφέρουσα ιστορία, Ν. Ι. Λέλος, Π. Νικολοπούλου-Σταμάτη
Thyroid and Endocrine Disruption: a fascinating theory, *N.J. Lelos, P. Nicolopoulou-Stamati*
- 87** Ενδοκρινικοί Διαταράκτες στην Αγρία Ζωή, Ν. Ι. Λέλος, Π. Νικολοπούλου-Σταμάτη
Endocrine Disrupters and the Wildlife, *N.J. Lelos, P. Nicolopoulou-Stamati*
- 93** Μετεκπαιδευτικό Σεμινάριο: “Στυτική Δυσλειτουργία”
Postgraduate Course on Erectile Dysfunction

Προσεχές Τεύχος
Πολυθεματικό τεύχος

Next Issue
Multi-topic reviews

June, 2005

The present issue of "ANIR" deals with a topic of universal importance, which exceeds the usually defined and well-limited scopes of a medical review. In fact, it touches upon an issue related to the impact of the environment on the reproductive health of animal species and the human, with particular emphasis placed on its effect on the male.

For a number of decades, some pioneers in the field of reproduction and toxicology have voiced their concerns about the deleterious effects of some chemicals in the environment on human health at large and reproduction in particular. With time passing some alarming messages were aired and proposals for remedies were put forward. Most of these concerns were faced with scepticism or even disbelief at the beginning until evidence from clinical observations started accumulating and the attitudes of the scientific community has completely changed. It was also in those days that a number of distinguished workers from all walks of biological, biochemical and medical research voiced their concerns in the classic, now, manifesto "A blueprint for survival". It was in those days that the first "Centre for Human Ecology" was established in Edimburgh and I had the luck, as a young trainee, to witness the writing of an important contribution by my teacher and mentor, the late John Loraine, entitled "The death of tomorrow". This book was granted the blessing of the Duke of the Edinburgh, Prince Philip, with an extensive introduction and I had the chance to be the delivery boy from London to Edimburgh!

Over the years, in our part of the world, a definite deterioration of the local environment and marked changes in living conditions, life-style and dietary habits have been recorded. These changes occurred in parallel with important effects observed in a number of parameters in reproductive health in this country. These effects have been properly documented and presented in peer-reviewed international journals and included changes in sperm quantity, increased incidence of premature menopause cases, a drop of the relative number of newborn male babies and deterioration in other aspects of reproductive health (for those interested a review article by Adamopoulos and Koukku will be soon available in a book edited by our invited editor).

In this field, substances interfering with the normal endocrine response for which the term "endocrine disrupters" was coined, have been associated with important deleterious effects on human reproductive health. The present issue has a particular interest for this country since its population is declining with an annual growth rate well below the replacement level. To address the topic we have engaged a distinguished research worker, an able organizer and a well-known persona in the field, Prof. Polyxeni Nicolopoulou-Stamati, as a guest editor. In addition to her original work she has produced a number of excellent reviews and books on this and related topics.

Enjoy the issue.

D.A. Adamopoulos
Editor - in - Chief

Ιούνιος, 2005

Το παρόν τεύχος του "ΑΝΗΡ" παρουσιάζει ένα θέμα ευρύτερης σημασίας αφού ξεπερνά τα στενά όρια μιάς συνήθους ιατρικής ανασκόπησης. Πράγματι ασχολείται με τις επιπτώσεις του ευρύτερου περιβάλλοντος στην αναπαραγωγική υγεία τόσο των κατωτέρων οργανισμών όσο και του ανθρώπου, με ιδιαίτερη έμφαση στο άρρεν.

Από δεκαετίες, αρκετοί πρωτοπόροι στην αναπαραγωγή και τοξικολογία δημοσίευσαν τις επιφυλάξεις τους για τα βλαπτικά αποτελέσματα διαφόρων χημικών ουσιών στην αναπαραγωγική λειτουργία. Με την πάροδο του χρόνου παρουσιάστηκαν κάποια επί πλέον στοιχεία και ακολούθησαν προτάσεις αντιμετώπισης, που αρχικά, έγιναν δεκτές με σκεπτικισμό και επιφύλαξη μέχρις ότου περαιτέρω στοιχεία άρχισαν να αθροίζονται από κλινικές παρατηρήσεις με συνέπεια τη μεταβολή των επιφυλάξεων. Εκείνη την εποχή, μια ομάδα διακεκριμένων επιστημόνων από όλους τους κλάδους της βιολογίας, βιοχημείας και ιατρικής διατύπωσαν τις ανησυχίες τους στο κλασικό, πια, μανιφέστο "Blueprint for survival". Τότε ιδρύθηκε και το Κέντρο Ανθρώπινης Οικολογίας στο Εδιμβούργο και προσωπικά ο ίδιος είχα την ευκαιρία να παρακολουθήσω την προετοιμασία μιας σημαντικής προσφοράς από το δάσκαλο και μέντορά μου, αείμνηστο John Loraine, του βιβλίου "The death of tomorrow". Το βιβλίο μάλιστα είχε εκτενή πρόλογο από τον Δούκα του Εδιμβούργου, Πρίγκιπα Φίλιππο.

Τις τελευταίες δεκαετίες στη χώρα μας έχει καταγραφεί μια επιδείνωση των συνθηκών στο τοπικό περιβάλλον, παράλληλα με σημαντικές αλλαγές στις συνθήκες ζωής, εργασίας και διατροφής. Στην ίδια περίοδο έχουν παρατηρηθεί σημαντικές επιπτώσεις στην αναπαραγωγική υγεία των Ελλήνων. Οι συνέπειες αυτές έχουν παρουσιασθεί στο διεθνή επιστημονικό τύπο και περιλαμβάνουν πτωτικές τάσεις στην ποσότητα του σπέρματος, αυξημένη επίπτωση πρώιμης εμμηνοπάυσης στις Ελληνίδες, ελάττωση του σχετικού αριθμού γεννήσεων αγοριών και άλλες επιπτώσεις (οι ενδιαφερόμενοι μπορούν να αναζητήσουν την ανασκόπηση που θα παρουσιασθεί σύντομα από τους Adamopoulos and Koukku).

Στο πεδίο αυτό, ξένες ουσίες που επεμβαίνουν στη φυσιολογική ενδοκρινική ανταπόκριση, γνωστές σαν "ενδοκρινικοί διαταράκτες", έχουν συνδεθεί με αρνητικές επιπτώσεις στην αναπαραγωγική υγεία των ανθρώπων. Το παρόν τεύχος έχει ιδιαίτερο ενδιαφέρον για τους Έλληνες αφού ο πληθυσμός της χώρας παρουσιάζει σταθερή καθοδική πορεία με ελάττωση του ετήσιου ρυθμού γεννήσεων σε επίπεδα κάτω από το όριο αναπλήρωσης. Για την ανάδειξη του θέματος ζητήσαμε βοήθεια από μια εξέχουσα ερευνητρια, διακεκριμένη Πανεπιστημιακή, ικανότατη οργανώτρια και προσωπικότητα στο χώρο, την Καθηγήτρια Πολυξένη Νικολοπούλου-Σταμάτη, σαν προσκεκλημένη εκδότρια. Η κ. Νικολοπούλου-Σταμάτη έχει δημοσιεύσει ένα μεγάλο αριθμό ερευνητικών εργασιών και έχει εκδόσει σχετικές μονογραφίες και βιβλία στο συγκεκριμένο θέμα.

Καλή ανάγνωση

Δ.Α. Αδαμόπουλος
Υπεύθυνος Σύνταξης

FORWARD OF THE INVITED EDITOR: SPECIAL ISSUE OF ANDROLOGY ON ENDOCRINE DISRUPTERS

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Πρόλογος της προσκεκλημένης εκδότριας

Η ικανότητα της αναπαραγωγής είναι ουσιαστική όχι μόνο για τον άνθρωπο αλλά και για όλα τα πλάσματα της φύσης. Η παρουσία ανθρωπογενών χημικών ουσιών τα τελευταία 50 χρόνια, που εμφανίζουν την ιδιότητα να παρεμβάλλονται στις ορμονικές λειτουργίες, ιδιαίτερα της αναπαραγωγής και ονομάζονται Ενδοκρινικοί Διαταράκτες, παρουσιάζει μεγάλο ιατρικό ενδιαφέρον.

Είναι σημαντικό επίσης το γεγονός ότι οι ουσίες αυτές διαπερνούν τον πλακούντα και προκαλούν άμεσα και έμμεσα αποτελέσματα.

Διαταραχές της γονιμότητας, άμεσες και έμμεσες επιδράσεις στο έμβρυο και καρκινογένεση συνδέονται με τους Ενδοκρινικούς Διαταράκτες.

Το τεύχος αυτό του περιοδικού ANHP είναι αφιερωμένο στην παρουσίαση των διαφόρων επιστημονικών πτυχών του πολύπλοκου ιατρικού προβλήματος, που αναγνωρίζεται ότι απαιτεί επαναξιολόγηση των ερευνητικών προτεραιοτήτων και την προσεκτική κλινική προσέγγισή του, δεδομένου ότι η επίδραση του περιβάλλοντος στην Υγεία είναι αδιαμφισβήτητα μια καινούργια ιατρική κατεύθυνση.

The ability of any animal to reproduce and successfully nurture its young is essential for the survival of a species. There are however a number of things which may adversely influence the development of young. In both humans and mammals alike, toxic substances which are present in the mother's body may affect the developing foetus in the womb. For instance, it is common knowledge that smoking cigarettes during pregnancy is detrimental to the unborn child. Not only does it retard foetal growth, but once the child is born, it may also increase the chance of the young child getting respiratory diseases. This demonstrates a very important point, namely that the placenta does not protect the developing foetus from toxic substances which are circulating inside the mothers' body. In fact, numerous chemicals and many pharmaceutical drugs can pass directly across the placenta to the developing foetus. Similarly, if these substances are present in the body, they may also contaminate a mother's breastmilk and pass to her nursing infant.

There is now a growing concern that certain man-made toxic pollutants which are widespread in the environment, may be affecting development of the young in humans and in wildlife. Initial warnings of this came, following observations that some wildlife populations were declining because of problems with development of their young. These problems were occurring in many different species which inhabited heavily polluted areas, such as the Great Lakes, but were also evident in species in numerous other regions of the world. The effects on wildlife which were seen included decreased hatching success in fish, birds and turtles, reproductive problems and decreased fertility in fish, birds, reptiles and mammals, behavioural abnormalities in birds and

compromised immune systems in mammals. In 1991, a small group of scientists concluded that the damaging effects to the health of these wildlife species, was due to certain man-made chemicals which are able to upset the balance of the body's hormones. These so called hormone-disrupting chemicals, can alter the delicate balance of hormones which control many bodily functions, including the regulation of the development of tissues and organs in the early stages of life. Since the hormones which regulate processes of development in animals are essentially the same as those in humans, it is possible that hormone-disrupting chemicals may be affecting human development as well.

Chemicals which are known to disrupt the body's hormone systems have a diverse range of uses including pesticides, industrial chemicals, plastics, detergents, paints and cosmetics. As a result of man's use of vast quantities of such chemicals over the past 50 years, many have become ubiquitous throughout the world, from the deep ocean bed to the high stratosphere and from the Arctic to the Antarctic. Consequently, humans and wildlife are continually exposed to hormone-disrupting chemicals. Many of them are resistant to being degraded by natural processes and persist in the environment for years. They also accumulate in the fatty tissues of animals, reaching the highest levels in predatory animals at the top of food chains, including humans. Presently, humans have no choice in whether they are exposed to these chemicals, because some of the highest levels are present in the food we eat. Some hormone-disrupting chemicals are now present in the general population at levels (parts per trillion or parts per million) which could affect the body's hormone systems. The greatest worry is the effects they may cause on the health of the foetus and infant since they can cross the placenta and are present in breastmilk. It is possible that the effects hormone-disrupting chemicals have on development, may be compromising human fertility and intelligence.

It is likely that most diseases and disorders result from alterations in signals crucial to all stages of life: molecular, cellular, extracellular and ecosystem signals. Many human diseases including cancer have been associated with genetic alterations that occur spontaneously or are expressed once triggered by environmental factors. While the etiology of disease is a critical area for investigation perhaps only a small percentage of human diseases have a purely genetic base, while the larger part is due to man related activities in the environment.

This issue of *Andrology* is devoted to bring the state of the art on Endocrine Disrupters aiming at providing the scientific information needed for the understanding of this major problem that has occurred and is becoming a research priority in the actual Environmental – Health context,

demanding efficient monitoring and careful surveillance for the benefit not only of public health but also of the ecosystem.

ENDOCRINE DISRUPTERS, WHAT ARE THEY

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Ενδοκρινικοί Διαταράκτες. Τι ακριβώς είναι

Οι Ενδοκρινικοί Διαταράκτες είναι χημικές ουσίες που δημιουργήθηκαν από ανθρωπογενείς δραστηριότητες και που εκτεθειμένος ο άνθρωπος σε όλες τις φάσεις της ζωής του ακόμα και ενδομητρίως, αλλά και όλα τα πλάσματα της φύσης υφίστανται την επίδρασή τους που είναι η παρεμβολή στις ορμονικές λειτουργίες.

Οι πηγές ρύπανσης, η τύχη τους μετά την έκκλυσή τους στο περιβάλλον, η ανθρώπινη έκθεση, η επίδρασή τους στην υγεία και ιδιαίτερα στην αναπαραγωγή αναλύονται εκτενώς. Επιπλέον σκιαγραφείται ο προβληματισμός της διαχείρισής τους.

Introduction

It is well known that human beings and not only need a well balanced hormonal activity to experience living. This story has been going for thousands, perhaps million of years. The regulation mechanisms have evolved reaching specific complex levels of interaction. This evolutionary process was during the years free from fraud interventions.

The interaction of hormones and target organs was a "clean story", meaning that a specific hormone targeted at a specific receptor with a specific result, able through again a specific loop-feedback to restore the starting point of the process. This was the situation until man made substances mimicking hormones were introduced in our every day life, mainly chemicals that are acting as hormones and are called Endocrine Disrupters (ED's).

Scientific evidence suggesting that ED's are implicated in declining sperm count, congenital malformations, cancer, retarded neurobehavioral disorders, retarded sexual development pose major concern and need to be carefully

attended. In view of the Hippocratic Oath statement "do no harm" it is essential for the medical doctor to be aware of the ED's threat from every aspect.

HORMONE SYSTEMS IN THE BODY

The role of hormones is to act as messengers, providing means of communication between different parts of the body. They travel from one part of the body to another in the bloodstream, sending essential signals and instructions which regulate the body's cells, tissues and organs.

Different types of hormones are produced by different organs in the body, the endocrine glands. These include the testicles, the ovaries, the pancreas, the adrenal glands, the thyroid and the pituitary gland in the brain.

A specific type of hormone will have specific roles to play in the body. For example, the female sex hormone estrogen has a number functions, including the development and maintenance of the female reproductive system.

For messages which hormones carry to be interpreted, the hormones must bind to molecules in cells, the receptors. The hormone and receptor have a precise fit, like a lock and key. Once the bond has been formed, it will trigger a particular biological effect in the cell. Thus by binding to receptors, hormones cause biological effects which can lead to changes in the functioning of cells, tissues and organs. Specific types of hormone are only able bind with specific types of receptors, so for example, estrogen can only bind to estrogen receptors to convey its messages.

Estrogen belongs to a particular group of hormones, the steroid hormones. Other hormones in this group include the male sex hormone testosterone, another female sex hormone, the progesterone, and some hormones produced by the adrenal glands. Steroid hormones, as well as other

hormones such as thyroid hormones, are found in all backboned animals, including fish, amphibians, reptiles, birds and mammals. The structure of the hormones is almost identical in all of these animals, and they serve similar functions. It therefore seems that throughout evolution these hormones have remained a successful way of regulating processes in the bodies of animals and humans alike.

Why Hormone-Disrupting Chemicals are Toxic

A wide range of man-made chemicals have been found which can disrupt the delicate balance of the body's hormone systems. These hormone-disrupting chemicals have been found to especially affect steroid hormones and thyroid hormones.

There is variety of ways in which these chemicals can disrupt hormone systems, such as:

They may mimic a hormone by binding to its receptor, and producing the same response in the body that the hormone would. Conversely, they may bind to a receptor and block it, so that the real hormone cannot bind to it. Alternatively, they may alter the production or the metabolism of a hormone. While some chemicals may only act in one of these ways, others may be able to act in several ways.

About 30 man-made chemicals are now known which can mimic the female sex hormone estrogen. These are called estrogenic chemicals. Chemicals have been found also that block the male sex hormone (androgen) receptor.

Hormones regulate many different bodily functions. Any upset in the balance of a hormone would affect the body functions it regulates. Ultimately, this could lead to undesirable effects on health. Since hormone-disrupting chemicals can upset the balance of hormones in the body, they could lead to adverse health effects.

This is true in the case of adults, but is especially worrisome in the case of the developing foetus in the womb and the young infant. In these early stages of life, the development of many tissues and organs is controlled by steroid hormones as well as thyroid hormones. For example, these hormones play a critical role in the development of the brain and nervous system, the reproductive system and the immune system. If hormone-disrupting chemicals upset the balance of hormones while these body systems are developing and growing, it can result in permanent detrimental health effects on the foetus or infant. While some effects on health could be apparent in the infant, others may not be evident until later on in life. Observations of wildlife populations, in laboratory animals, and in some cases humans, have shown that if the developing young are

exposed to hormone-disrupting chemicals, then a wide range of adverse health effects do occur.

In addition to disrupting hormone systems, man-made chemicals that are toxic to the development of the foetus and infant may also cause effects in other ways, such as by altering the levels of enzymes, or the levels of natural chemicals which control the transmission of nerve impulses. One class of chemicals, the dioxins, is very toxic to the developing young. These chemicals do not interfere with hormone receptors, but with a different sort of receptor, called the Ah receptor. In so doing, they elicit a broad range of biochemical effects, including disrupting steroid hormones (*Safe and Krishnan 1995*).

IDENTIFICATION OF HORMONE DISRUPTING CHEMICALS

About 30 man-made chemicals have been tested in the laboratory and identified as being estrogenic chemicals. In addition to these, another 20 or so chemicals have been listed as hormone-disrupting chemicals. Some of these have been shown to interfere with hormone systems in laboratory experiments, whilst others are known to affect the reproductive systems of wildlife populations.

SOURCES OF HORMONE-DISRUPTING CHEMICALS

Many pesticides (including herbicides, fungicides or insecticides) have been identified as being hormone-disrupters. Pesticides represent the highest volume chemicals deliberately released into the environment, not only by farmers but by homeowners and professionals alike. A number of industrial chemicals have also been identified as being hormone-disrupters. These chemicals have a diverse range of uses, including those used in the manufacture of plastics eg. phthalates, surfactants eg. alkylphenols, and solvents, as well as some heavy metals, eg. mercury, lead and cadmium.

Organochlorine Chemicals

Organochlorine chemicals are produced by industry by reacting chlorine gas with organic matter. Most chlorine gas is combined with petrochemicals to produce pesticides, solvents, plastics and other chemicals. About a third is used to make the plastic polyvinyl chloride (PVC). Two well known classes of Organochlorine chemicals are the polychlorinated biphenyls (PCBs) and dioxins. Many pesticides, for instance DDT, toxaphene, atrazine, methoxychlor and endosulphan are also organochlorines.

Polychlorinated Biphenyls

The PCBs are a group of 209 compounds, which were marketed commercially in a variety of different mixtures. These industrial chemicals were used in numerous products including dielectric fluids for transformers and capacitors, cutting oils, flame retardants, heat transfer fluids, sealants, adhesives and plasticisers. They have been used since 1929, and world production since then has been estimated to be between 1 to 2 million tons. Peak production came at the end of the 1960's, but a voluntary production ban was enacted by US and UK manufacturers in 1977. Production continued until at least 1987 in France and Spain and may still be continuing in some areas.

The use of PCBs led to their release into the environment, in particular from careless disposal practices, accidents, and leakages from industrial facilities and waste disposal sites. It has been estimated that around 35% of the total PCBs produced worldwide have entered the environment. A further 65% remains in waste dumps and capacitor applications. Many of these applications will come to the end of their useful lives over the next 10-15 years. Hence, future losses of PCBs into the environment could theoretically be double the releases to date.

Despite widespread bans on production, regulatory measures have not caused significant reductions of levels PCBs in the environment. Although there have been falls in the levels of PCBs in some areas, in general, levels have now stabilised. This is not surprising given the persistent nature of PCBs and the fact that PCB inputs to the environment have not halted due to leakages from waste dumps and careless disposal practices. Moreover, there is still no large scale comprehensive plan for adequate disposal of stocks or the clean up of waste sites.

Dioxins

Dioxins are a group of chemicals comprising of 75 different polychlorinated dibenzo-p-dioxins (PCDD) and 135 polychlorinated dibenzofurans (PCDF). These chlorinated chemicals have never been produced intentionally for any industrial use. Instead, they are produced as unintentional by-products in many processes in which chlorine and chlorine derived chemicals are produced, used and disposed of. Combustion and thermal processes such as waste incineration account for a major source of dioxins and a second source is chemical industrial processes. Extremely small amounts of dioxins may be formed during natural fires, but the major sources come from industrial practices which began around the 1920's.

Today, the main source of dioxins is from incinerators, in particular municipal waste incinerators. Hazardous waste and

medical waste incinerators and cement kilns also emit dioxins into the atmosphere. Another important source in which dioxins are released into air are from several processes in the metal industry, such as smelting. Other sources include industrial and domestic burning of coal and wood, and petrol combustion by vehicles. Less significant sources on a global scale appear to accidental fires involving the burning of plastics or PCBs and forest fires.

Dioxins can also be formed in the manufacture and use of organochlorine chemicals. A major source used to be the production and spraying of chlorinated phenol-based pesticides such as pentachlorophenol, although their use has become restricted since the 1980's. The manufacture of PVC has been reported to produce dioxins. Besides the production of chlorinated chemicals, dioxins are released into watercourses by the pulp and paper industry as a result of chlorine bleaching.

With improving technologies for municipal waste incinerators, stricter regulations being enforced on dioxin emissions at national levels, and decreased production of chlorinated phenol chemicals, the industrial input of dioxins into the environment and levels in the environment may be declining. However, considerable deposits of dioxins remain in sediments and soils and these may recirculate in the environment. In addition, dioxins present in sewage sludge, re-enter the environment when the sludge is applied onto agricultural land. Given this and the persistent nature of dioxins, despite efforts to reduce them it is likely that a sizable fall in human exposure to dioxins will take many years.

Pesticides

Pesticides are still widely used throughout the world, such as atrazine, endosulfan and 2,4-D. Several others have restricted use or are banned in some countries, but are still used in others, notably developing countries. For example, DDT which has been used since the 1940's, was banned or severely restricted from use in many countries in the 1970s. However, it is still produced and sprayed in some developing countries such as India and Mexico, mainly for killing mosquitoes in an attempt to combat malaria. Currently, the annual global usage of DDT is estimated to be 550,000 tones.

Some countries which have banned or restricted the use of a pesticide may still produce and export to countries where the use is permitted. For example, the US has discontinued the use of chlordane, heptachlor and mirex, but exports them to other countries.

It is difficult to estimate the global usage of pesticides because some countries do not appear to hold records on pesticides, whereas in others information on production and

distribution is kept secret for marketing purposes. Overall, though it appears that the use of pesticides is increasing on a global basis. Without international co-operation, changes in regulations and improved record keeping by governments, it will not be possible to restrict pesticide usage or improve estimates of global usage.

Alkylphenol Compounds

In the 1940's, chemicals called alkylphenol ethoxylates (APE) were introduced for a number of industrial uses. Recent studies have shown that these chemicals are broken down in the environment to form other chemicals, such as alkylphenols (AP), which are estrogenic and are also more persistent and bioaccumulative than the original chemicals.

APEs, which are surfactants, have widespread industrial uses, for example, industrial cleaning, paint, agrochemicals, emulsion polymers, textiles, metal finishing, certain plastics, and lubricating oils. A recent survey in the UK estimated that 83% of the APEs used in 1992 entered the environment. In some cases, levels have been found in sewage effluent and UK rivers that are high enough to cause estrogenic effects in fish. Alternatives for current industrial uses of APEs are available and efforts to phase out their use are being implemented.

Phthalates

Phthalates are used as plasticisers to make plastics more flexible and improve their workability. Some phthalates, butyl benzyl phthalate (BBP), di-n-butyl phthalate (DBP), and to a lesser extent di-ethylhexyl phthalate (DEHP) have been found to be estrogenic. In Western Europe about 90% of all plasticisers and especially DEHP, are used in the manufacture of PVC. There are numerous uses of PVC including flooring, water pipes, cables, tubing, furniture and upholstery, children's toys, pharmaceutical packaging including blood bags, and different types of food packaging. In addition to PVC, the above mentioned phthalates are used to produce other plastics such as polyvinyl acetate and polyurethane and have non-plastic uses such as in paints, pesticides, inks, hairspray and insect repellents.

In 1985 it was estimated that the global production of phthalates was 2.7 million tonnes per year, of which DEHP accounted for 50%. Phthalates may enter the environment directly into water or landfill sites from industrial discharges or waste, or into air from burning plastics. Phthalates leach out of plasticised products into the surrounding air or into other substances which are in contact such as food, soil or water.

Metals

Lead, cadmium and mercury are naturally occurring substances. Although metals have been used by humans since ancient times, the use of these three metals for industrial purposes increased dramatically during the last century. The main use of lead is the manufacture of batteries, but it is also used in the production of chemicals including paint, petrol additives, various metal products and ammunition. It has been estimated that 4 to 5 million tonnes of lead have been discharged into the environment since the 1920's in the US alone. The amount of lead in many products has been reduced in recent years because of its toxicity, but the world demand for lead continues to rise. Some lead is recycled for reuse, but much is dumped in landfill. The phasing out of lead additives in petrol has resulted in a marked reduction in the amount present in air and consequently in humans. However, the declines in environmental levels are relatively small and with the continuing use of lead, further reductions will be increasingly difficult to achieve. Lead in human tissue is mainly stored in bone, and present levels in human bones are 500-fold greater than in prehistoric times.

In its metal form, cadmium is used mainly in the production of batteries and metal plating but also for pigments, plastics and synthetics. Smelting and industrial combustion leads to atmospheric releases of cadmium. Although recent regulations have led to a reduction in certain uses of cadmium, its industrial use is increasing globally, largely due to the manufacture of batteries. Cadmium is disposed of principally in landfills, but it can be recycled. Over the last 10 years or so levels in the environment appear to have decreased. Plants, including tobacco plants take up cadmium in a mineral form from the soil. Consequently cadmium is present in cigarette smoke, and people who smoke have significantly more cadmium in their bodies.

Mercury is used in industry in the production of chlorine gas, electrical equipment, batteries, thermometers, dental fillings, and as a by-product of gold mining. Municipal waste incineration and other combustion processes can lead to atmospheric emissions of mercury. Because of its toxicity, several uses have declined in the last two decades, such as in medicinal products, in mercury containing fungicides which are now banned, and a reduction in by-products of the chemical industry. Recovery and recycling of mercury from industry has recently become more commonplace. Mercury can combine with carbon containing products to form organic mercury. Although all forms of mercury are poisonous, one organic form known as methylmercury is of particular concern because it can bioaccumulate in animals, especially fish.

Estrogenic chemicals have been identified in the laboratory, either by using cell culture techniques, or by

monitoring the effects they have in animals. Cell culture tests are now available which can identify whether a chemical is estrogenic in different kinds of animals. For example, a test which uses fish cells has been developed to test whether chemicals are estrogenic to fish in the aquatic environment (*Sumpter and Jobling 1993*). A test using human cells, has also been developed, and may be used to predict whether a chemical would be estrogenic in humans (*Soto et al. 1995*).

Where chemicals have been found to be estrogenic in fish or in fish cells, they have also been found to be estrogenic in mammals or in human cells (*Sumpter and Jobling 1995*). This suggests that the estrogenic chemicals would be capable of having estrogenic effects in all backboneed animals. This is not surprising because the structure of the estrogen hormone and its receptor are virtually identical in these animals.

No systematic screening of chemicals for estrogenic or other specific hormone-disrupting effects has ever been used to test chemicals which are released onto the market for commercial use. In fact, it was discovered by accident rather than intent that some chemicals were estrogenic. It is therefore very likely that there are other estrogenic or hormone-disrupting chemicals which are currently produced and used, but remain unidentified. Even where chemicals are known to be estrogenic, this has currently not resulted in their withdrawal from use.

FATE OF HORMONE-DISRUPTING CHEMICALS IN THE ENVIRONMENT

A number of industrial chemicals which have a wide range of uses, and many pesticides have been identified as hormone-disrupting chemicals. Through these uses, most have become widespread environmental contaminants. The sources of these chemicals and their levels in the environment pose a major concern.

Transport of Chemicals in the Environment

Chemicals do not necessarily remain where they are released into the environment but may be transported in water or on air currents. Many chemicals become airborne, either by direct emission, for example from combustion processes, or by volatilising from the ground, from the leaves of sprayed crops, or from water. Once airborne, they can be transported for long distances around the planet before falling again to the surface. In this way, many hormone-disrupting chemicals have become ubiquitous contaminants throughout the global environment. These include many pesticides, PCBs, dioxins, phthalates, lead, mercury and cadmium (*Loganathan and Kannan 1994*).

A further problem is that a number of these chemicals are resistant to being degraded and broken down by natural

processes. Some take years to degrade, others tens of years or even centuries. These chemicals are called persistent pollutants because they remain in the environment for so long.

It has been found that persistent chlorine-derived chemicals (organochlorines), such as PCBs, dioxins, DDT, toxaphene and other pesticides, are preferentially transported on air currents to colder regions such as polar and sub-polar regions. This "global distillation" process has resulted in particularly high organochlorine levels in air, seawater and wildlife in arctic regions (*Iwata et al. 1993*).

The Food Web

Many of the hormone-disrupting chemicals which are persistent, are also soluble in fats. As a result they build up (bioaccumulate) in the fatty tissues of animals. Even very low environmental levels of these chemicals can lead to high levels in the body tissues of animals. The levels increase more as one animal eats another, such that the highest levels are found in animals at the top of food chains. An example, of how this occurs is illustrated by what could happen to PCBs in the environment:

Small aquatic organisms like algae, accumulate PCBs at concentrations hundreds of times higher than levels in the surrounding water; small fish eat the algae and concentrate the PCBs in their tissues to even higher levels; still higher levels are accumulated by bigger fish which eat the small ones; finally near the top of the food web, predatory birds which eat the big fish, such as herring gulls, accumulate the PCBs to higher levels. The concentrations in their eggs can be 25 million times greater than the original level in the water.

HUMAN EXPOSURE TO HORMONE-DISRUPTING CHEMICALS

Human exposure to hormone-disrupting chemicals can occur in a variety of ways, including by ingestion of food and water, inhalation of air and by skin absorption. For the majority of these chemicals however, the major source of exposure is via food.

Everyday when we eat food, we will also be taking in hormone-disrupting chemicals which are present in the food. Being exposed to such chemicals is therefore completely unavoidable. Because many of these chemicals are found everywhere in the world and they build up through food chains, all food sources are contaminated by some of them. For those chemicals which are persistent and bioaccumulative, by far the highest levels are found in meat, fish and dairy products.

Apart from the unavoidable hormone-disrupting chemicals in our everyday food, there are also other reasons why food contains certain hormone-disrupting chemicals. For example, pesticide residues may remain on the vegetables and fruit we consume, and other chemicals may leach from food packaging into the food.

Pesticide residues in food and water

Spraying pesticides on crops, means that a proportion of them may remain on the crops by the time they are harvested. According to the US food and drugs administration, about 40% of foods purchased have detectable pesticide residues. The contamination rate with pesticides is highest in vegetables, and especially fruit. Testing foods for pesticide residues in the U.S. showed that 1 to 3% of foods have residue levels which are above the legal level. However, the instances where residue levels are above legal limits may be higher because only about one third of pesticides which are used on crops are tested for (*Pimentel 1992*).

Drinking water may also be contaminated with very low levels of some pesticides. Contamination of drinking water by pesticides occurs as a result of their application to crops, from where certain pesticides eventually end up in groundwater and surface waters. The most common pesticides found to be present in the US drinking water were atrazine, alachlor and aldicarb all of which are hormone-disrupting chemicals (*Pimentel et al. 1992*). In northern Europe atrazine was also the most common pesticide found. Drinking water standards do permit very small quantities of pesticides (usually in ug/l quantities) and metals to be present. If breaches in regulation limits occur for drinking water, pesticides are the most common culprit. In fact, two government bodies in the UK recently concluded that the best way to control pesticides leaching into potential water supplies was to reduce their application on crops. This was also found to be cheaper than removing them during water treatment.

Food Packaging and Processing

A few estrogenic chemicals are known to be commonly used to make food packaging materials. These are chemicals called phthalates and a chemical called bisphenol-A. These chemicals have been found to leach out of the packaging into foods that they are in contact with.

Bisphenol-A has been found in the lacquer coating on the inside of food cans. The liquid surrounding vegetables in some food cans was found to contain this chemical (*Brotons et al. 1995*). The levels of bisphenol-A which had migrated into the liquid (highest level 80ug/kg) were well within EC limits (3mg/kg), but this limit was set before it was

discovered that bisphenol-A was an estrogenic chemical. Bisphenol-A is also present in polycarbonate plastic which is widely used for the packaging of food and beverages. Whether it leaches from this plastic into foodstuffs is yet to be tested (*Feldman and Krishnan 1995*).

The phthalates (DBP, BBP and DEHP) are present in different types of food packaging, such as cellophane and aluminium paper-foil laminate, and they are also in inks used to print on plastic and paper board food wrappings. These chemicals have been found to leach from food wrappings and inks into a variety of foods including butter, cheese, sandwiches, meat pies, chocolate, crisps and cakes. The highest levels (ppm quantities, or ten's of mg/kg) have been found in fatty foods. It is highly possible that a person could exceed the EC safety limits (called tolerable daily intakes) set for the intake of phthalates by eating a lot of packaged foods.

Phthalates have also been reported to contaminate food during food processing. For example, DEHP was reported to leach into milk from PVC tubing used in milking equipment (*Castle et al. 1990*). Formula baby milk in the UK was also found to contain DEHP and other phthalates (at levels 1.2-10.2mg/kg). For a baby's average intake of milk, such levels of phthalates would result in exceeding the EC precautionary limit by about double.

FATE OF HORMONE-DISRUPTING CHEMICALS IN THE BODY

Persistent hormone-disrupting chemicals are not easily broken down or detoxified by the body and they become stored in fatty tissues. They are only released and excreted from the body very slowly (*Hall 1999*). The only times that the levels of these chemicals in the body are significantly reduced is during starvation when fat stores are broken down, or during lactation in women, when the chemicals are passed via breastmilk to the infant.

Some hormone-disrupting chemicals, such as phthalates, are more easily broken down and excreted from the body, so they do not accumulate in body tissues to a great extent. However, because phthalates are so ubiquitous in the environment, people are continually exposed to them.

The levels of hormone-disrupting chemicals in people's bodies do vary in different countries and different areas. For example, people living in industrialised regions have higher body burdens of industrialised chemicals such as PCBs and dioxins. Some populations are particularly highly exposed to certain chemicals because they consume a diet which is rich in fish. For example, Arctic indigenous people rely on a diet consisting of fish and sea mammals which contains very high levels of PCBs, dioxins, methylmercury and chlorinated

pesticides like DDT and toxaphene (Kuhnlein 1995). Consequently, the levels of these chemicals in their bodies are high, and in the women's breastmilk, levels are 3 to 7 times higher than average (Dewailly *et al.* 1994).

Exposure of the Foetus

Chemicals in the mother's body can be transferred to the foetus via the placenta. Many hormone-disrupting chemicals are likely to be passed to the foetus in this way. Those which have been identified in placental tissue or in blood from the umbilical cord, include DDT, DDE, hexachlorobenzene, PCBs and dioxins. Cadmium, lead and methylmercury are also known to cross the placenta (Ando *et al.* 1986, Kanja *et al.* 1992, Koopman-Esseboom *et al.* 1994).

Exposure of the Nursing Infant

Any chemical in a mother's body which circulates in her blood may contaminate her breast milk and pass to her nursing infant. Breast-feeding is highly recommended for the health of the child, but there is now concern about the amount of chemicals which are present in human breast milk. Hormone-disrupting chemicals which are commonly found in breast milk (in ppt or ppm levels) include PCBs, dioxins, and the pesticides DDT, DDE, dieldrin, hexachlorobenzene, hexachlorohexane, heptachlor, and chlordane.

During breast-feeding, the levels of persistent chemicals in the mother's body which have accumulated during her lifetime are reduced, because they pass into the breastmilk. For example, in the case of dioxins, it has been estimated that the baby will receive 4-12% of the dioxins it will accumulate during its whole life, if it is breast fed for one year. However, recent estimates suggest that the amount of dioxins transferred in the breastmilk could be much higher. An approximation using data from 3 women, showed that if a mother breast feeds for just 6 months, she will reduce the level of dioxins in her own body by about 50%. Nearly all of these dioxins will be passed to her baby in the breastmilk. This means that after 6 months of breast feeding, the infant may have about the same body level of dioxins as its mother.

There are regulations which concern the amount of chemicals present in commercial foods, but of course, breast milk which is fed to babies cannot be regulated. Banning and restricting the production of some persistent organochlorines, such as dioxins has led to reduced levels in breast milk in some countries. However, in the case of DDE and PCBs, levels decreased initially following bans but have now stopped declining to any extent (Huisman 1995).

HEALTH EFFECTS OF HORMONE-DISRUPTING CHEMICALS

To prove scientifically that a specific chemical causes a particular health effect in humans, direct evidence from studies on humans is required. However, there are many difficulties in conducting human studies. For instance, it is difficult to assess whether chemicals cause effects on human development, because of the comparatively low levels of chemicals in the environment, the complexities of estimating exposure to such chemicals, and numerous other factors which may be involved in causing developmental effects. On the other hand, it is far easier to conduct studies using laboratory animals without such factors being a problem- and to prove whether a chemical causes a particular effect. Consequently, animal studies have played a major role in predicting whether chemicals may represent a health hazard to humans. Other information on effects of chemicals may come from observing effects in wildlife populations and using other laboratory techniques such as cell culture.

The idea that a number of chemical pollutants in the environment may be acting as hormone-disrupters, initially came from observations of harmful reproductive and developmental effects which had occurred in many wildlife populations. Putting together this information with other studies on humans and laboratory animals, led to the hypothesis that hormone-disrupting chemicals, which were widespread in the environment, may cause adverse effects on reproduction and development in humans.

Since hormones play an essential role in regulating development of the foetus and infant in humans, the presence of hormone-disrupting chemicals during development could cause adverse effects on health. Evidence from human studies on a few pollutants, namely PCBs, dioxins, lead and methylmercury, strongly suggests that this is indeed the case. Other evidence for some hormone-disrupting chemicals is available from animal studies. Most evidence from animal studies comes from specific scientific studies, rather than from routine tests on animals which are carried out on commercial chemicals to find out whether they are harmful to human health. This is because routine testing of developmental health effects has focused mainly on birth defects which are visible to the naked eye. However, hormone-disrupting chemicals cause other effects such as toxicity to the nervous and immune systems which would not be detected in such routine tests. Consequently, for some chemicals which are known to be hormone-disrupters, there are presently few studies on the developmental effects they cause in animals.

Evidence on effects caused by hormone-disrupting chemicals in laboratory animals is very relevant to effects

which could occur in humans. This is because hormone-disrupting chemicals typically affect steroid hormones, and these hormones are extremely similar in animals and humans. The processes they regulate in the body are also similar. For example, the basic mechanisms of sexual development are common to all mammals, and they are regulated by the same hormones such as estrogen and testosterone. Therefore, the effects that hormone-disrupting chemicals have on estrogen and testosterone in animals, and the resulting effects on sexual development, would be expected to be similar in humans (Nicolopoulou et al. 2000, Nicolopoulou et al. 2001)

Ability of Hormone-Disrupting Chemicals to Cause Effects on Health

Estrogenic or other hormone-disrupting chemicals are known to cause a wide range of effects on development in laboratory animals, wildlife and in some cases humans, including effects on reproductive system, the immune system, the nervous system and brain. However, it has been shown using cell culture techniques that estrogenic chemicals are 1000 to 100,000 times less efficient at binding to the estrogen receptor than the body's own natural estrogen. They are therefore classed as being only very weak estrogens. This makes it difficult to see how estrogenic chemicals could possibly have effects on the body which are of any consequence. Yet when they are tested in animals, they bind to estrogen receptors much more efficiently and do cause effects on health. There are several reasons which explain why this may occur:

Firstly, many hormone-disrupting chemicals bioaccumulate in the fatty tissue of animals, reaching levels which can be much higher than levels in the environment. Some of these chemicals have now reached levels in people's bodies which are millions of times higher than the levels of natural hormones in the body. Consequently, they have an increased chance of binding to estrogen receptors and causing biological effects.

Secondly, over 90% of natural estrogen in the body becomes bound to special proteins in the blood, so that it is no longer free to bind to estrogen receptors. However, estrogenic chemicals do not become bound to these proteins, and remain free to bind to estrogen receptors in the body.

Thirdly, in the real world, humans and animals are exposed to mixtures of numerous chemicals. It has been found when some estrogenic chemicals are mixed together they produce additive effects or even synergistic effects. For example, when the estrogenic pesticides endosulphan and toxaphene were mixed together, they were 1000 times more potently estrogenic than used alone at the same concentration. Furthermore, when the pesticide chlordane,

which is not estrogenic, was mixed with an estrogenic pesticide, the mixture became more potently estrogenic. Since people are exposed to a wide range of estrogenic and other chemicals, it is thus very likely that their effects on the body could be cumulative.

Developmental Health Effects

It is thought that every pregnant woman in the world has hormone-disrupting chemicals in her body that can be transferred to the foetus or via breastmilk to the young infant. These early life stages are exquisitely sensitive to toxic effects of chemicals, and often chemicals which cause little problem to the mother, cause harmful effects to the foetus. This is because mechanisms which provide some protection against toxic chemicals in the adult are not fully developed in the foetus. The early stages of life are also particularly vulnerable, because it is the time when the body organs and tissues are developing. Exposure to hormone-disrupting chemicals at critical periods when an organ or body system is developing can result in permanent effects on health. A broad range of health effects have now been associated with exposure to hormone-disrupting chemicals during the development. Some of the evidence for these effects is discussed in following sections.

MALE REPRODUCTION

Over the last 50 years or so, there has been a dramatic increase in several male reproductive disorders:

- There has been a worldwide increase in cancer of the testicles, with the incidence rising by as much as 4-fold in some countries. The disease is most prevalent in men aged between 20 and 45, and in some countries it is now the most common cancer in men.

- Sperm count has decreased in men from several European countries (Adamopoulos 1996, Irvine 1996). Studies show that it has declined over the past 20 years at a rate of about 2% per year. The studies found that the year of a man's birth influenced sperm count, for example, a 20 year old man's sperm count today is lower than a 20 year old's sperm count would have been ten years ago (Auger 1995).

- Sperm count does vary geographically and it is not yet clear if it has decreased worldwide.

- The incidence of testicular maldescent appears to be increased in many countries.

- The incidence of boy's born with genital abnormalities appears to have increased in many countries (Toppari 2003).

- Prostate cancer is on the increase in a number of countries, particularly northern Europe and North America.

The male reproductive disorders listed above have increased in many countries over a relatively short time period. It thus seems likely that they have been caused by some common environmental factor. To answer the question "what could this factor be?", scientists have looked at what causes the disorders. They believe that all of these male reproductive disorders have their beginnings during foetal and early life. Too much estrogen at a critical time when the male reproductive system is developing in the womb seems to cause these disorders. Following this line of thought, scientists have hypothesised that if too much estrogen has these consequences, exposure to estrogenic chemicals in the womb may cause the same problems (*Sharpe 2003*).

Evidence is mounting from laboratory and wildlife studies which shows that this could be the case, and that estrogenic chemicals in the environment may be partly or wholly to blame for rising male reproductive problems. Other hormone-disrupting chemicals which affect the male sex hormone testosterone, during development may also share some of the blame.

A critical piece of evidence in reaching the conclusion that estrogenic chemicals may be causing male reproductive disorders, came from an unfortunate medical mistake. Between 1945 and 1971, about 5 million women were given a synthetic estrogen drug called diethylstilbestrol (DES), during their pregnancies, because it was thought to reduce the risk of miscarriage and pregnancy complications. Unfortunately, it did not have this effect. Instead it was found that among boys who were born to these women, there was an increased incidence of urethral abnormalities, testicular maldescent, and when they reached adulthood their sperm count was reduced. The results of this experience clearly showed that exposure to extra estrogen in the womb can result in increasing male reproductive disorders. The same effects have also been found to occur in laboratory animals which are exposed to DES during development.

Evidence which is available from studies of wildlife and laboratory animals is supportive of the concept that hormone-disrupting chemicals can increase male reproductive disorders:

Wildlife Studies

Lake Apopka in Florida, USA was heavily polluted in 1980 by a chemical spill of the estrogenic pesticides dicofol and DDT. Since that time, the population of alligators which inhabit the lake have continually declined. High levels of the chemicals have accumulated in the animals, and particularly high amounts of DDE, the breakdown product of DDT, are found in their eggs. Scientists think that the most likely reason for the decline in population is that the chemicals alter the sexual development of the alligators whilst inside the egg,

causing reproductive problems in the adult alligators. For example, male alligators have small phalli and abnormalities in their testes. The hormone-disrupting effects of dicofol and DDE are thought to be responsible (*Guillette et al. 1994*).

Laboratory Studies

Unfortunately, routine testing of chemicals before they are marketed is only done using relatively high doses of a chemical which are not representative of environmental levels. Furthermore, testing for effects on male reproduction has often only been carried out on adult animals. Where testing of chemicals has been done on developing animals, such as those chemicals registered for use by the EC since the 1980's, there are still inadequacies. This is because the tests use insensitive endpoints, such as fertility, rather than using more sensitive endpoints such as sperm count which should be used to monitor effects on male reproduction. As a consequence of such inadequate chemical testing, there could be a multitude of chemicals on the market which may cause effects on male reproduction which have not been identified.

Experiments using rats or mice, have provided evidence that exposing animals to relatively high levels of hormone-disrupting chemicals in the womb or in very early life can cause undesirable effects on the male reproductive system. For example, chemicals which produced effects such as decreased sperm count or reduced testes weight include DDT, DDE, methoxychlor, chlordecone, hexachlorohexane, vinclozolin and PCBs.

The only hormone-disrupting chemicals which have been tested using appropriate sensitive endpoints, and at levels close to those found in the environment are dioxin, an alkylphenol compound called octylphenol and the phthalate BBP. These chemicals all caused marked decreases in sperm count and other male reproductive effects. For BBP, the level of exposure which caused effects approached levels to which humans are exposed in everyday life (*Gray et al. 1995*).

Human Studies

There is little direct evidence in humans that hormone-disrupting chemicals causes male reproductive problems. There is some evidence from an accident which occurred in Taiwan in 1979 in which many people consumed rice which was contaminated with PCBs and dioxins. Women who were pregnant at the time of the incident, gave birth to boys who had slightly shorter penises at ages 11-14 (*Guo et al. 1995*).

Recently, a report was published about the pesticide chlorpyrifos which is used to treat homes for pest control. Two women whose homes had been treated with chlorpyrifos during their early pregnancy, and one woman

who was exposed to the chemical at work, gave birth to four children who had multiple birth defects. The two boys had testicular maldescent and one had a tiny penis. Scientists suggested that these effects were caused by hormone-disrupting effects of chlorpyrifos in the womb.

Finally, there are things in adult life which can reduce sperm count, such as drinking alcohol to excess, sexually transmitted diseases, and a number of commonly prescribed drugs. However, it is unlikely that these things can account for the extent to which sperm count seems to have decreased in some countries. More importantly, they cannot account for the rise in testicular cancer, urethral abnormalities and testicular maldescent over the past 50 years. Considering the available evidence, it is highly possible that exposure to hormone-disrupting chemicals during the developmental stages of life may be responsible for increasing reproductive disorders in men.

Further to the reproductive problems imposed by ED's another major medical field that is involved is the one of cancer. Hormonal related cancers as breast, prostate and testicular cancer, are increasing, calling for an urgent need for considering in research protocols the ED's. Cancer can be also seen as an environmental disease (Nicolopoulou et al. 2004)

FEMALE REPRODUCTION

It is not clear whether exposure to current environmental levels of hormone-disrupting chemicals during the early stages of life, is having an impact on the reproductive system of women. Data from animal experiments certainly shows that exposure to some estrogenic chemicals causes adverse effects on the reproductive system, but there is very little evidence in humans.

The age when puberty is reached in humans and mammals is predetermined during the developmental stages of life. Women in industrialised countries now reach puberty at a younger age. It has been suggested that the declining age of puberty in women maybe due to exposure to estrogenic chemicals in the womb. The evidence for this idea comes from studying human records on the subject, and from animal experiments which show that exposure to increased levels of estrogens in the womb causes an earlier onset of puberty.

Studies on female rodents have investigated what happens to the reproductive system if they are exposed to estrogenic chemicals whilst in the womb. It has been found that exposure to the synthetic estrogen DES, and to high levels of estrogenic chemicals such as DDT, methoxychlor, and chlordecone, caused reduced fertility in the females and structural abnormalities of their reproductive systems. Similar

effects were seen when female rats were exposed to very low levels of dioxins in the womb.

It is not known what the effects of such hormone-disrupting chemicals would be on humans, but there is some evidence which suggests that effects would be similar. This comes from an unfortunate experience in the 1950's and 60's, when women were inappropriately given the synthetic estrogen drug, DES, during their pregnancies. Many of the girls born to these women suffered from vaginal cancer in their teens. They also suffered from reduced fertility and a high incidence of structural abnormalities of the reproductive system.

Studies with laboratory rats showed that exposure to DES in the womb caused the same reproductive effects as in women. This indicates that evidence from animal experiments on the female reproductive system is very relevant to humans. However, the animal studies have generally only tested high doses of chemicals which do not represent present levels in the environment, so it is not possible to estimate whether current environmental levels are affecting the reproductive system of women (Nicolopoulou and Pitsos 2001).

INTELLIGENCE AND BEHAVIOUR

There is much evidence from human and laboratory animal studies on lead, methylmercury and to a lesser extent on PCBs and dioxins, which shows they are toxic to the developing nervous system. In some cases, current levels of these chemicals present in women of the general population may be sufficient to result in subtle effects on behaviour and intelligence in their children. However, information for other chemicals which may have similar effects, such as toxaphene and hexachlorobenzene, is not available.

Lead

In the last 15 years it has become clear that exposure to lead, either in the womb or during childhood, at levels which are presently typical of humans in industrialised countries, results in decreases in intelligence quotient (IQ) and other behavioural problems. The evidence for this has been provided by many human studies with consistent results, and has been backed up by a large body of data from animal studies.

Human studies have generally looked at the effects of lead levels in middle class children from industrialised countries. Several studies have shown that children with higher blood levels score lower values on IQ tests and are prone to other behavioural problems such as poor attention or hyperactivity. Further studies investigated whether exposure to lead in the womb causes effects on behaviour.

These studies measured lead levels in the mother's umbilical cord and followed up the children from birth to several years of age. The studies provide convincing evidence that exposure to higher lead levels during development in the womb results in lower IQ scores in the children. Moreover, there seems to be no safe level of lead which does not affect the mental ability of children.

All of the studies described above were carried out on children from the general population. It therefore seems that levels of lead in humans are already in the range where deficits in IQ and behaviour are probable. Although the effects are subtle, with for example changes of 5-7 points in IQ, such changes may have an impact on a child's ability in school. Furthermore, at a population level, a drop in IQ by 5 points could have consequences for human society. The generation of women exposed to the most lead since ancient times are currently at reproductive age, so this represents an important problem for years to come despite the fact that environmental levels of lead are declining.

Methylmercury

In the late 1950's in Minamata Bay, Japan, many people were poisoned by methylmercury after a factory dumped it into the nearby sea. The methylmercury became accumulated to high levels in fish in the bay, which were caught and eaten by local residents leading to the poisoning incident. A further poisoning outbreak occurred in Iraq in 1971-2 when grain treated with a mercury fungicide was made into bread.

Women who were pregnant at the time of these incidents gave birth to children who had cerebral palsy, mental retardation or delayed walking and speech. Sight and hearing were also damaged. The degree to which children were affected depended on the amount of exposure in the womb, with lower levels resulting in less severe effects. For example, relatively low levels of methylmercury in the mother have been associated with producing small deficits in intelligence and behaviour in their children.

Today, the largest source of methylmercury comes from fish. Communities who consume high levels of sea mammals or fish in their diet such as the Inuit of North America, or the people of the Faroe Islands, have the highest body levels of methylmercury. People who eat fish from the Great Lakes also consume high amounts of methylmercury. For women, it has been estimated such high consumption of fish represents a potential hazard to the mental health of her offspring.

PCBs and Dioxins

The development of the nervous system and brain in mammals and humans is partly under the control of thyroid hormones and reproductive hormones. It is thought that

PCBs and dioxins may cause damage to the developing brain and nervous system by disturbing the balance of these hormones. PCBs may also be toxic to the developing nervous system because they alter the level of a chemical in the brain called dopamine which is essential for transmission of nerve signals (*Jacobson 1997*).

Recently, studies in the Netherlands have investigated whether levels of dioxins and PCBs which are present in pregnant women from the general population are affecting development of their babies. It was found that higher levels of PCBs and dioxins in the mother were linked with altered thyroid hormone levels in their babies. Therefore, present PCB and dioxin levels in some women may already be sufficient to affect the thyroid hormone system in their offspring (*Koppe et al. 2001*).

It is certain that exposure to high doses of PCBs and dioxins in the womb can result in reduced intelligence and behavioural problems. Evidence for this comes from accidents which occurred in the late 1970's in Japan and Taiwan when people consumed rice which was heavily contaminated with PCBs and dioxins. The children of women who were pregnant at the time of the incidents have slightly lower intelligence and behavioural problems which have persisted as they grow up. Other studies have suggested that exposure to lower levels of PCBs in the womb may produce similar problems. For example, a study was made on women who had eaten moderate amounts of fish from Lake Michigan before getting pregnant. Results suggested that subtle behavioural and intelligence problems found in their children up to age 4, were associated with eating the fish, and especially with PCBs found in the fish.

Finally, studies in the Netherlands on healthy women and their babies mentioned above, found that infants who were exposed to higher PCB and dioxin levels in the womb had slightly lower scores in neurological tests which assessed grasping, sitting, crawling, standing and walking (*Huisman et al. 1995*). They also had a slightly lower quality of muscle tone, which means the ability to use their muscles was reduced.

It is not yet clear whether exposure in the womb to current levels of PCBs and dioxins is causing subtle effects on the intelligence of children. However, studies on monkeys suggest that this could indeed be the case. For example, 10% of the population of industrialised countries have body levels of dioxins and PCBs which are the same as the levels that cause small intelligence problems in the offspring of monkeys experimentally exposed to these chemicals.

Sexual Behaviour

In mammals and humans, there are some differences in

the structure and function of the brain between males and females. This partly accounts for differences in sexual behaviour between the sexes. The development of these sexually different areas of the brain in the foetus and infant are partly controlled by reproductive hormones.

Studies in rats and mice have shown that slight disturbances in the levels of these hormones can result in changes in sexual behaviour. For example, exposure of female rat embryos to a synthetic estrogen (DES), or to estrogenic pesticides such as DDT and chlordecone, causes them to behave more like males when they grow up. Conversely, if male rat embryos are exposed to low levels of dioxins, they behave more like females when they become mature.

In humans, there is only very limited evidence on possible changes in sexual behaviour, from women who were exposed to the synthetic estrogen DES in the womb. These women were found to have less well established sexual partners and a lower sexual desire. It is therefore not certain whether current levels of hormone-disrupting chemicals in the environment affect sexual behaviour in humans.

THE IMMUNE SYSTEM

The immune system consists of a variety of specialised cells which circulate in the body and function to prevent infection and disease. Toxic chemicals may alter the levels of these cells which can cause an increase in disease such as infectious diseases and cancer. Presently, there is only limited data available on the effects of hormone-disrupting chemicals on the immune system. However, studies on dioxins and PCBs do suggest that some women of the general population have body levels of these chemicals which may affect the immune system of their babies.

Studies on mice have shown that exposure during pregnancy to the synthetic estrogen DES, or to the estrogenic pesticide DDT, caused detrimental effects to the immune system of their offspring. In humans, women who were exposed in the womb to the synthetic estrogen DES had an increased incidence of certain diseases throughout their lives.

Other studies have found that high levels of PCBs and dioxins in women as a result of a diet rich in fish, are related to increased incidence of infectious diseases in their babies. For example, high levels of PCBs and dioxins in the breastmilk of Inuit women have been linked to more episodes of ear infections in their infants (*Dewailly et al. 1994*). Another study showed that high levels of PCBs in women who consumed large amounts of fish from the Great Lakes, were related to a higher incidence of bacterial infections in their babies (*Bemier et al. 1995*). In both cases, the increase in infections in the infants could be due to deficiencies in the immune system as a result of exposure to dioxins and PCBs.

In the 1970's at Times Beach in Missouri, USA, residents were accidentally exposed to high levels of dioxins after contaminated waste oil was sprayed on roads for dust control. Children born to women who lived in the area during the incident were found to have significant changes in the number of several types of cells in their immune systems.

Recently, a study was made of healthy women and their babies from the general population in the Netherlands. Some of the women had higher levels of PCBs in their bodies and breastmilk than others. Those infants who were exposed to higher levels of dioxins and PCBs from their mothers, both in the womb and via breast feeding, were found to have altered numbers of certain cells of the immune system. It is not known what effect this could have on their health (*Nicolopoulou et al. 2000*).

GROWTH RETARDATION

Many things may affect the growth rate of a foetus in the womb, including some chemicals. If growth is slowed, the baby will have a lower birthweight as a consequence. Studies on growth retarded babies have found they have significantly less cells in several organs such as the brain, kidneys and lungs. It has also been discovered that babies which are growth retarded are more likely to die from cot death than other babies, and are more likely to die from heart disease in adult life.

Studies on laboratory animals have shown that exposure to PCBs during pregnancy, causes a lower birthweight in the offspring. This is in agreement with data from human studies which suggests that slight reductions in birthweight may be caused by the current body levels of PCBs in some members of the general population.

An accident in 1979 occurred in Taiwan, in which people consumed rice which was contaminated with high levels of PCBs and dioxins. Women who were pregnant at the time of the incident, or who became pregnant shortly afterwards, gave birth to growth retarded babies. The babies had a lower birthweight (by 0.5kg), and as they grew up they remained slightly shorter than the other children (3cm).

Other studies in the U.S. have been conducted on women from the general population who had eaten moderate amounts of fish from Lake Michigan. These women had relatively high tissue levels of PCBs as a consequence of eating the fish. The PCB levels in the women were found to be related to slightly reduced birthweights (0.16-0.19kg) of their babies. Finally, a recent study in Sweden looked at the effect of consuming fish from the Baltic sea, because it is known to contain high levels of PCBs and other persistent pollutants. It was found that women who ate high amounts of fish from the Baltic Sea, gave birth to babies with lower

birthweights than babies born to women who did not eat this fish (Hagmar *et al.* 1996, Brower 1998).

NATURALLY OCCURRING SUBSTANCES IN PLANTS WHICH AFFECT HORMONE SYSTEMS

About 300 different plants are known to produce and contain a number of natural substances called phytoestrogens. These are thought to serve a variety of functions such as acting as fungicides, regulating plant hormones, deterring herbivores from eating them, and a protection against ultraviolet radiation from the sun. Plants which contain phytoestrogens include many in our diet such as whole cereal grains, seeds, soya, cabbage, beet, broccoli and peas (Barrett 1996).

When eaten by humans, phytoestrogens are broken down in the gut to form hormone-like compounds, which are able to bind to estrogen receptors. These compounds may be immediately excreted from the body, or they may be absorbed from the gut into the bloodstream. They may then affect hormone systems. Their actions can result in lowering the activity of sex hormones in the body. They also have antioxidant properties (Adiercreutz 1995). All of this begs the question, are these phytoestrogens good or bad for our health?

To investigate the impact of eating phytoestrogens, scientists have studied populations who consume high, medium or low amounts of phytoestrogens in their diet. It appears from these studies that eating a diet rich in grains and vegetables with high quantities of phytoestrogens, has a protective effect against many diseases including estrogen-related cancers, such as breast cancer and prostate cancer, some other cancers, osteoporosis and cardiovascular diseases. Thus, people who consume high amounts of phytoestrogens have a lower incidence of these diseases. Such preventative effects of phytoestrogens on disease have also been demonstrated in laboratory animals and using cell culture techniques.

Harmful effects of phytoestrogens have also been found. For example, in Australia, female sheep which were grazed for prolonged periods on a species of clover, which contained phytoestrogens, suffered sharp declines in fertility. A possible explanation might be that phytoestrogens may act as a defense mechanism, to deter herbivores from heavy predation of a plant species.

If phytoestrogens can affect the fertility of sheep, they may be also be able to affect human fertility. This is indeed the case for a few plants which have been known to herbal medicine for centuries and used as contraceptives. However, eating a variety of plants in a normal diet does not seem to affect human fertility. For example, Asians consume very high

levels of phytoestrogens without affecting their fertility. One explanation might be that humans and animals have evolved alongside plants and any harmful effects on fertility that their normal diet may cause could have been selectively bred out of the populations long ago (Barrett 1996). Some questions still remain however, such as would there be beneficial or harmful effects to a developing infant fed on soya-based milks which are known to contain large quantities of phytoestrogens.

There are major differences between phytoestrogens and man-made endocrine-disrupting chemicals. Phytoestrogens are easily broken down and readily excreted from the body.

Consequently they spend very little time inside the body. The situation with man-made chemicals is different. Humans and animals have not evolved alongside vast quantities of man-made endocrine-disrupting chemicals in the environment. Many are persistent and cannot be broken down or detoxified, and they bioaccumulate in body fat. Therefore, unlike phytoestrogens, persistent chemicals remain in our bodies for long periods of time, with their levels gradually building up in body tissues throughout our lives. They are also known to have a wide spectrum of adverse health effects (Adiercreutz 1995).

REGULATION OF CHEMICALS

At present, governments employ two basic strategies for protecting people's health from the harmful effects of man-made chemicals. These are prevention and regulation.

Prevention

Prevention from harmful effects of man-made chemicals involves taking measures to ban the production and use of chemicals in a country. For instance, several persistent organochlorine pesticides, (eg. DDT, toxaphene, HCB), have been banned from use in many developed countries.

Banning harmful chemicals like these, has successfully stopped their release into the environment in countries where governments have enforced a ban. However, there is one major problem with the present system of banning chemicals - the bans are not implemented on a worldwide scale. So, while some countries have banned chemicals such as DDT and toxaphene, other countries have not and continue to use them. Another problem arising from this is that chemical companies in countries which have banned specific chemical, can still export the chemical to countries where it is not banned. Alternatively, they may export the technology to make the chemical to a country where it is not banned. Some developing countries may lack the resources or technology to properly monitor and control exposure levels to such imported chemicals.

Regulation

To regulate the production and use of chemicals, governments or regulatory authorities set limits which define allowable rates of release of chemicals into the environment. In order to calculate the allowable rates of release for a chemical, a process called risk assessment is used.

Risk assessment is a method which attempts to provide a numerical measure of human health impacts caused by a specific pollutant. It is a complex process which involves a whole series of calculations which estimate human exposure from the release of a chemical into the environment, and the health impact that such exposure would cause.

In risk assessment, the health effects that a chemical could cause in humans, are estimated by testing the chemical in laboratory animals. From these animal experiments, a "safe" level of the chemical is calculated, which humans could be exposed to without damaging their health. This "safe" level is determined, by finding the level of the chemical at which there are no harmful health effects in animals. Because this level is determined from animal experiments, an extra safety factor is then added to the level, to account for differences there may be between humans and animals. This gives the final result of the "safe" level of the chemical which should not harm human health.

However, there are major problems and limitations with the risk assessment process. Some of these problems occur in the animal tests described above, which are used to set "safe" levels of chemicals. These problems can be illustrated by considering how risk assessment is performed in the USA:

To estimate a safe level for human health effects which could be caused by a chemical, the animal experiments are based entirely on one endpoint - the risk of developing cancer. However, many chemicals cause other toxic effects on health such as on development, reproduction, or on the nervous system, at much lower levels than they cause cancer. In fact, some chemicals may not cause cancer at all, but still produce other adverse health effects. None of these other effects are taken into consideration. Therefore, as a consequence of using development of cancer as the sole endpoint in animal experiments, risk assessment may greatly under-estimate the true impacts of a chemical on human health.

In European countries the methods of risk assessment are somewhat different to the US system. For chemicals that will come into contact with food such as pesticides, or for chemicals which will be used in large quantities, more extensive animal testing is carried out. This testing includes examining the effects caused by a chemical on the development of an animal. For example, to investigate the effects a chemical could have on reproduction, pregnant

animals are exposed to the chemical, and their offspring are then monitored to find out if they fertile, by checking whether they are able to reproduce. The problem with this is that other effects on male reproduction could occur, such as a substantial decrease in sperm count, which would probably not be detected by just monitoring fertility. The problems are thus similar to the process used by the US for risk assessment - health effects can occur but may be undetected.

The problems with the animal experiments used for risk assessment purposes could be summed up by saying that inappropriate endpoints are used, which may not be sensitive enough to detect the harmful health effects that chemicals may cause. In simple terms this means that risk assessment asks the wrong questions about health effects - and so it gets the wrong answer. Even though information about hormone-disrupting chemicals and the effects they can cause is growing, no risk assessment process used to date has attempted to screen chemicals for these properties.

A further example of the "wrong question - wrong answer problem" in the animal testing procedures is that only a single chemical is tested at a time. However, in real life people are exposed to mixtures of thousands of chemicals in the environment. It is known that when some chemicals are mixed together the harmful effects they produce are additive or even synergistic (bigger than additive). Risk assessment does not take mixtures of chemicals into consideration. In fact, it would be impossible to test the multitude of different chemicals that people are exposed to in everyday life.

There are many chemicals which came onto the market years ago which underwent little or no testing for toxic effects. Such chemicals which still remain on the market are essentially regulated as though they are safe.

Finally, although risk assessment is a scientific process, in practice it depends upon highly uncertain and subjective assumptions at every stage of the process where numerical estimations are made. It is inevitably subject to the personal and political predilections of the people who interpret the data. A former director of the US Environmental Protection Agency warned of the subjective nature of the process when describing risk assessment: *"We should remember that risk assessment data can be like a captured spy: if you torture it long enough, it will tell you anything you want to know"*.

Regulation of chemicals using the risk assessment process has doubtlessly stopped the use and release of numerous harmful chemicals into the environment. However, as described above, the process is fraught with uncertainties and limitations and is highly subjective and open to manipulation. Consequently, many chemicals which could be detrimental to human health and to wildlife, such as hormone-disrupting chemicals, have been widely used and are now widespread in

the environment. It can be said therefore that current regulatory strategies, which set legally acceptable releases of chemicals into the environment based the assumption that such releases are harmless, are not protective of human health.

Problems concerning the regulation of hormone-disrupting chemicals were documented at a meeting of scientific experts on these chemicals at Erice, Sicily in November 1995: *Our judgment is that: A trivial amount of governmental resources is devoted to monitoring environmental chemicals and health effects. The public is unaware of this and believes that they are adequately protected. The message that endocrine-disrupters are present in the environment and have the potential to affect many people over a life span has not effectively reached the general public, the scientific community, regulators, or policy makers. Although this message is difficult to reduce to simple statements without over- or under stating the problem, the potential risks to human health are so widespread and far-reaching that any policy based on continued ignorance of the facts would be unconscionable* (Erice Sicily 1995).

Further faults with current regulation were also pointed out at the meeting. This involved present trade secret laws which afford companies confidentiality so that they do not have to reveal the names of chemicals they use in commercial products, either to consumers, or to regulatory authorities. The following was proposed:

Our judgment is that: "Those responsible for producing man-made chemicals must assure product safety beyond a reasonable doubt. Manufacturers should be required to release the names of all chemicals used in their products with the appropriate evidence that the products pose no developmental health hazard.

The Precautionary Principle

The problems of regulating chemicals using risk assessment can be avoided by adopting an alternative approach - the precautionary principle. The precautionary principle requires that chemicals are not discharged into the environment until they are proven to be harmless. This is in contrast to risk assessment, which assumes that chemicals are harmless until proven harmful. The precautionary approach avoids problems that may arise from limitations of our understanding of the toxic effects of chemicals on health, by removing the assumption that a safe level of a particular compound can be estimated. The precautionary principle is now gaining acceptance internationally, as a foundation for strategies to protect the environment and human health (Wingspread Conference 1998).

In line with the precautionary principle, some international political fora have agreed to phase out releases of persistent and bioaccumulative chemicals, particularly organochlorine chemicals, by several years time. No specific action has yet been taken on hormone-disrupting chemicals. Since these chemicals, and other persistent bioaccumulative chemicals are a global problem, the only way to successfully implement a phase out would be a legally-binding global phase out.

Phasing out chemicals requires careful planning to take into account the many factors involved. For instance, the International Joint Commission on the Great Lakes has recommended to the governments of Canada and the US, that organochlorine chemicals should be phased out because of the harm they could be causing to the environment and human health. They have proposed steps that could be taken to cope with the phasing out, such as: *"Consult with industry and other interests to develop timetables to sunset the use of chlorine and chlorine containing compounds as industrial feedstocks, and examine the means of reducing and eliminating other uses, recognising that socioeconomic considerations must be taken into account in developing the strategies and timetables"* (International Joint Commission on the Great Lakes 1978).

Phasing out hormone-disrupting or other toxic chemicals is only an initial step. It is important that other chemicals are not simply used to replace chemicals which are phased out of use. Instead, it has been suggested that alternative technologies are used where they are available and others should be developed, which use far less chemicals so those that are used can be controlled. In addition technologies should be able to re-use their waste products. Such technologies are not unattainable and some are already in existence. Similarly, other measures such as drastically reducing the use of pesticides in agriculture and adopting more traditional or organic methods, have been proposed. It is unlikely that the manufacture of man-made chemicals will ever stop completely, but at least a goal can be set to keep human and environmental exposure to an absolute minimum. The task ahead is clearly one of redesign - of industrial and agricultural institutions, which were spawned by the chemical age over the last 50 years (Communication from the Commission 2000).

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FOCUSING ON HUMAN HEALTH EFFECTS OF ENDOCRINE DISRUPTERS

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Εστιάζοντας στις Επιδράσεις των Ενδοκρινικών Διαταρακτών στην Ανθρώπινη Υγεία

Ο συσχετισμός των Ενδοκρινικών Διαταρακτών με την αύξηση της συχνότητας του καρκίνου του μαστού, του προστάτη και των όρχεων, αλλά και με την επίδραση στη γονιμότητα αναλύονται με στοιχεία τεκμηριωμένης διεθνούς βιβλιογραφίας.

INTRODUCTION

Endocrine disrupters (EDs) as exogenous environmental molecules, are considered to potentially affect synthesis, secretion, transport, metabolism, binding, action and catabolism of natural hormones of the body. EDs are likely to interact with the endocrine system of animals and humans and can exert their effects even when they are present in very small amounts.

Direct evidence from human studies is required in order to show that a specific chemical causes a particular health effect. Since hormones play an essential role in regulating the development of human fetus and infant, the presence of hormone-disrupting chemicals during development potentially has an adverse effect on health. So far, several pollutants [e.g. polychlorinated biphenyls (PCBs), dioxins, lead and methylmercury] have been investigated (1). Hormone-disrupting chemicals typically affect steroid hormones which regulate various processes including sexual development (2).

Many hormone-disrupting chemicals bioaccumulate in the adipose tissue of animals, where they can reach a higher level than that observed in the environment. Over 90% of natural estrogen in the body binds to special proteins in the blood, consequently only a small quantity becomes bound to estrogen receptors. Nevertheless, estrogenic chemicals do

not bind to these proteins and remain unbound to estrogen receptors in the body. Moreover, it has been found that when some estrogenic chemicals are mixed together they produce additive effects or even synergistic effects. Since people are exposed to a wide range of estrogenic and other chemicals, it is very likely that these chemicals can cause a cumulative effect on the body.

It is widely accepted that hormone-disrupting chemicals can be transferred from a pregnant woman either to the fetus or, via breast-milk, to the infant. These hormone disrupting chemicals can be harmful to the fetus, as mechanisms which are responsible for providing protection against toxic chemicals in the adult, are not fully developed in the fetus. Exposure to hormone-disrupting chemicals at critical periods when an organ or body system is developing, can result in permanent effects on health.

As far as the male reproductive system is concerned, an increase in male reproductive disorders has been reported (3). An excess of estrogen at a critical time when the male reproductive system is developing in the uterus seems to have a causative relation to these disorders. Other hormone disrupting chemicals which affect testosterone (the male sex hormone) during development may be also involved. It is known that after accidental consumption of rice, which was contaminated with PCBs and dioxins, by women who were pregnant at the time of the incident, they gave birth to boys who had slightly shorter penises at ages 11-14.

As evidenced by human and laboratory animal studies on lead, methylmercury and to a lesser extent on PCBs and dioxins, these are toxic to the developing nervous system (1). In some cases, current levels of these chemicals present in women of the general population may be in sufficient quantity to result in subtle effects on behavior and intelligence of their children. Several studies have shown that

children with higher lead blood levels score lower values on IQ tests and are prone to other behavioral problems such as poor attention or hyperactivity; there seems to be no safe level of lead which does not affect the mental ability of children. Women who were pregnant at the time of poisoning by methylmercury gave birth to children who had cerebral palsy, mental retardation or delayed walking and speech; sight and hearing were also damaged. Today, the largest source of methylmercury comes from fish. The development of the nervous system and brain in mammals and humans is partly under control of thyroid hormones and reproductive hormones. It is thought that PCBs and dioxins may cause damage to the developing brain and nervous system by disturbing the balance of these hormones. PCBs may also be toxic to the developing nervous system because they alter the level of a chemical in the brain, called dopamine, which is essential for transmission of nerve signals.

The immune system consists of a variety of specialized cells which circulate in the body and function so as to prevent infection and disease. Toxic chemicals may alter the levels of these cells and thus cause an increase in infectious diseases and cancer. High levels of PCBs and dioxins in women as a result of a diet rich in fish, are related to increased incidence of infectious diseases in their babies.

ENDOCRINE DISRUPTERS AND CANCER

For approximately the last 10 years it has been widely suspected that *exogenous hormonally-active agents* (HAAs), or endocrine disrupters (EDs), may influence the development of cancer. A wide variety of chemicals act as EDs and they do so through a variety of different pathways. The effects that a particular HAA has on an individual is not readily predicted by its chemical structure, as timing, dosage of exposure and underlying mechanisms of action are crucial to the outcome. Links between hormone disruption and cancer raise major scientific problems.

The incidence of breast, testicular and prostate cancer is being investigated in regard to exposure to EDs (1,3).

The effects of exogenous hormones on the development of human cancers have been identified. The relationship between EDs and breast cancer has been described (4-27). Their contribution focuses on the *in vivo* action of xenoestrogens; a large proportion of the natural hormones bind to carrier proteins and become biologically inactive, in contrast to xenoestrogens which remain unbound and active. This latter group are suspected of eliciting biological effects at low concentrations of weak xenoestrogens. Low-dose effects may be inverted at high doses. Xenoestrogens can also alter the metabolic pathways, producing more carcinogenic metabolites, while their non threshold-influence in the receptor mediated mechanism of carcinogenesis is usually

ignored. Xenoestrogens are also known to alter gap-junctional intercellular communication and DNA methylation; conformational changes in the receptor imposed by the shape of the xenoestrogen lead to functional changes: the oestrogen receptor in the plasma membrane leading to the production of prolactin, the induction of growth factor genes as well as the genes for protein kinase C and other second messengers. Based on these complex action mechanisms of xenoestrogens, the hypothesis on how they could increase the risk for breast cancer is developed. A dramatic increase in the incidence of breast cancer, especially among postmenopausal women, exists world-wide. Epidemiological studies have shown a positive correlation between organochlorine concentrations in adipose tissue and the development of breast cancer (3).

As far as the various hormonal compounds are concerned, they are classified in the following groups. In group 1 (carcinogenic to humans), one finds diethylstilbestrol (DES), postmenopausal estrogen therapy, nonsteroidal estrogens, steroidal estrogens, combined oral contraceptives, sequential oral contraceptives and tamoxifen. In group 2A (probably carcinogenic to humans), one finds androgenic anabolic steroids. In group 2B (possibly carcinogenic to humans) we have me-droxyprogesterone acetate, progestins, postmenopausal estrogen-progesterone therapy, progestin-only oral contraceptives. Finally, in group 3 (not classifiable as to carcinogenicity to humans), one finds clomiphene citrate, estradiol mustard, toremifene and droloxifene (1).

Some data implicate a polymorphism in catechol-o-methyl transferase (COMT) in breast cancer risk. Methylation by COMT is an important pathway for inactivation of catechol estrogens. The allele encoding low-activity COMT may be a contributor in particular to post-menopausal breast cancer (3).

Among xenoestrogens, the example of diethylstilboestrol (DES) is very well known. This xenoestrogen was prescribed during pregnancy in order to prevent complications. However, after years of prescription, the increased risk of cervicovaginal clear cell adenocarcinoma, a rare tumour, was reported, and the use of DES was banned. Even after years of studies the mechanism of carcinogenesis remains unknown. DES is not mutagenic in the Ames test, although chromosomal aberrations have been observed in experimental animals. Many more anomalies have been observed in DES-mothers and offspring. DES-mothers exhibited increased risk of developing breast cancer. DES is also suspected of having many more adverse effects including testicular cancer in DES-sons (28).

Increase in the incidence of testicular cancer, especially in Nor-dic and Baltic countries (29) are attributed to prenatal exposure to EDs (30). Markers of exposure should be

measured at the appropriate period of vulnerability.

The great majority of testicular cancer, particularly before the age of 60 years, are germ cell tumours. The origin of these cancers is likely to be the population of primordial germ cells and testicular cancers express markers that are similar to fetal germ cells. Testicular cancer is initiated in fetal life before the normal differentiation of the primordial germ cells into spermatogonia, which coincides with the differentiation of the male sexual phenotype. A role of hormones, particularly maternal endogenous oestrogens and environmental exposure to substances with oestrogen-like action has been postulated. More recently, the interest has been expanded to include also substances with anti-androgenic potential. This line of causation of testicular cancer may account for the established association between testicular cancer and cryptorchidism and the possible association with hypospadias, both of which may represent imperfect differentiation of the male sexual phenotype (1,3).

The cohort pattern of testicular cancer incidence strongly suggests a role of exposures in everyday life that act through exposure of the developing male embryo, and which may change quite rapidly. A role of dietary habits particularly of pregnant women is being investigated (31).

Next to testicular cancer, there is a definite role of androgens in the development and progression of prostate cancer. Data from the Danish Cancer Registry show that the age adjusted incidence of prostate cancer increased over time at around 1.6 per cent per year. Any causative role for xenoestrogenic chemicals in the development of prostate cancer has not been established. There is a definite role of androgens in the progression of prostate cancer, which may be treated (but not cured) by administration of anti-androgens (1,3).

In view of the vast array of possible carcinogens that humans are exposed to it is important to increase research on effects, mechanisms and risk assessments of real life multiple exposures. Existing assessments are based on models that are too simple to reflect reality. Also timing and dosage of exposure are most critical in determining individual response and are not always considered. Although it is recognized that more research is needed, this should not prohibit the invocation of the Precautionary Principle, which ensures the fundamental human right – the right to implement prevention, where ever there is suggestive evidence of harm to health (50,51).

THE IMPACT OF ENDOCRINE DISRUPTERS ON THE FEMALE AND MALE REPRODUCTIVE SYSTEM

The development and the function of the female reproductive tract depends upon hormone concentrations

and balance. Many abnormalities may result from modulation of the concentrations of oestrogens, thecal androgens and thyroid hormones. As EDs have the ability to modulate these hormones, it is vital to establish whether they can affect female genital function.

Endocrine disrupting chemicals may affect the function of oestrogen and progesterone and/or the hypothalamic-hypophysial axes and may alter the natural menstrual cycle, ovulation and fertility. An epidemiological study of women who consumed fish from Lake Ontario showed a link between fish consumption, PCB exposure and a reduction in menstrual cycle length, indicating the possible impact of PCBs through food on menstrual cycle (1,3).

Lindane (γ -hexachlorocyclohexane) is a widely distributed organochlorine pesticide. This pesticide intercalates into the sperm membrane and alters the molecular dynamics of the lipid bilayer. Lindane in doses as high as that found in female genital tract secretions may inhibit sperm responsiveness to progesterone in vitro, which induces the acrosome reaction at the site of fertilization. This could be a cause of infertility in women exposed to lindane.

The effect of pentachlorophenol in women with endocrine dysfunction may be at the hypothalamic or suprathalamic level, causing mild ovarian or adrenal insufficiency (1,3).

The organochlorine compounds entering the embryonic circulation through the placenta could affect the pregnancy outcome resulting in many congenital disorders, in spontaneous abortion but also in in-uterus growth retardation.

Endometriosis is an oestrogen related disease and some EDs in the human body mimic oestrogen; so the link between endometriosis and EDs should be considered. In Belgium, a country heavily polluted with dioxin the incidence of endometriosis is high. Chronic exposure to dioxin is directly correlated with a significant increase in the incidence of the development of endometriosis. It is known that endometriosis is a major factor in female infertility and that 18% of women with endometriosis have measurable concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin TCDD in their blood, while only 3% of women with tubal infertility have measurable concentrations of TCDD (1,3).

It has been suggested today that testicular germ cell cancer, semen quality, cryptorchidism and hypospadias are linked together biologically as a testicular dysgenesis syndrome (TDS) and prenatal factors appear to be important in their common aetiology although post-natal factors may also influence their progression (32).

A definite trend of decreasing semen quality has been detected in USA and Europe. The original paper by Carlsen et al (33) analysing 61 publications on sperm count indicated a

declining sperm count from 1938 to 1990, with an overall linear regression slope of -0.93 ($p < 0.01$). If studies are excluded when results are from studies that include infertile publications, there are 54 remaining papers from 1938 to 1990, which give a slope of -0.95 ($p < 0.001$). Since Carlsen's meta-analysis suggested a $>40\%$ decline in sperm counts worldwide, public and regulatory concern with regard to the possible adverse impacts of EDs has been created. Sharpe and Skakkebaek (30) expanded the concern regarding sperm counts to dysfunction of multiple organ/tissues in the male reproductive tract and the before mentioned TDS includes testicular cancer, decreased sperm counts, increased incidence of hypospadias and cryptorchidism (32). It is also postulated that these defects may reflect "the existence of a common underlying cause" and "can be a result of disruption of embryonal programming and gonadal development during fetal life" (32). It was concluded that "the rise in the incidence of the various symptoms of TDs occurred rapidly over few generations, the aetiological impact of adverse environmental factors such as hormone disrupters, probably acting upon a susceptible genetic background, must be considered" (32).

Sperm counts have been examined from various laboratories and locations (34,35,36,37,38,39,40,41,42). Several of these sperm specimens were derived from clinical material in sperm banks, fertility or vasectomy clinics, and according to the results there were both temporal increases and decreases in sperm counts. For instance, Auger et al (36) examined 1351 sperm bank donors from a clinic in Paris and showed that from 1973-1992, there was a significant decrease in sperm counts from $89 \times 10^6/\text{ml}$ to $60 \times 10^6/\text{ml}$. In contrast, using a similar method, mean sperm counts of $83.12 \times 10^6/\text{ml}$ were observed in 302 men from a clinic in Toulouse, and there were no significant alterations from 1977-1992 (43). The variability in sperm counts and quality was subsequently reported in several studies and part of this variability may be due to several factors, including measurement techniques, seasonal variability and differences between men providing samples in clinics versus random sampling. Handelsman (44) reported the results of several studies in Sydney, Australia, which recruited five different groups of volunteers for sperm donation. Sperm counts (date of collection) in studies #1 (1987-1989), #2 (1989), #3 (1990-1993), #4 (1993) and #5 (1994) were 103, 142, 84, 67 and $63 \times 10^6/\text{ml}$. It was concluded that "this highlights the invalidity of extrapolating such findings on sperm output of self-selected volunteers to the general male community from which the volunteers originated" (44).

A study by Fisch et al (45) used standardized measurement techniques in the same laboratory to investigate temporal changes in sperm counts of men from vasectomy clinics in New York, Minnesota and California. Mean sperm counts were 131.5, 100.8 and $72.7 \times 10^6/\text{ml}$,

respectively, and these were unchanged from 1970-1994 demonstrating that sperm counts were not decreasing but varied with location. This observation has now been observed in multiple studies demonstrating highly variable sperm counts within the same country and between countries (21,22,34,35,36,37,38,39,40,41,42,43,44,45). It is evident that sperm counts are highly variable within regions and countries. The differences in sperms counts within and between studies clearly show the importance of demography in this particular measure of male fertility. The reason for these differences are unknown, regional variability in organochlorine compounds such as PCBs, DDE and pesticides are minimal and therefore, are unlikely to contribute to decreased sperm counts of TDs. This was highlighted when it was shown that DDE levels in Scandinavian breast milk samples from Norway, Sweden, Denmark and Finland were comparable and reduced by $>90\%$ from the late 1960s (1,3). The DDE levels in breast milk and their temporal decline did not correlate with the greater than four-fold difference in testicular cancer rates in the Scandinavian countries and the temporal increase in the incidence of this tumor. These data further highlight differences in TDS between countries which do not correlate with exposures to a group of compounds which have been implicated in endocrine disruption in wildlife.

Skakkebaek et al. (32) suggest that TDS is a coordinate and "in-creasingly common development disorder with environmental aspects". However, correlation with so-called "environmental aspects" has not been reported. Sperm counts and testicular cancer are two male reproductive tract problems that are hypothesized to the part of TDS. The variation in sperm counts and rates of testicular cancer between different countries/regions has been reported; however, evidence for coordinate country-dependent changes in both parameters is unclear. Jorgensen et al (42) reported that the east-west gradient in semen quality in the Nordic-Baltic area (Denmark $<$ Norway $<$ Estonia \sim Finland) is "in parallel with the incidence of testicular cancer". However, East Germany, which is further west of Estonia, has a relatively high incidence of testicular cancer and similar to that observed in Denmark .

In their discussion of TDS (32), the authors stated the following: "Growing evidence from clinical observations of individual patients and from larger epidemiological studies indicates a synchronized increase in the incidence of male reproductive problems such as testicular cancer, genital abnormalities, reduced semen quality, and subfertility". Although testicular cancer incidence is increasing, the evidence that sperm counts are decreasing is lacking and confounded by large demographic effects and minimal data on normal (not self-selected) populations. One such report (41) in Sapporo, Japan, did not observe a decrease in sperm

counts in volunteers sampled between 1975-1998. International trends for hypospadias are cryptorchidism show high variability between regions/countries. It was concluded that increases in hypospadias "leveled off in many systems after 1985", whereas for cryptorchidism "since 1985, rates declined in most systems". Moreover fertility studies in Sweden and Britain (46) do not show temporal declines, and the latter study concluded that "if a decline in male fertility has occurred, it has been more than compensated for by a counter-vailing increase in couple fertility" (46).

The endocrine disruptor hypothesis regarding decreased male reproductive capacity suggest that inappropriate in utero exposure to estrogens plays a role in the TDS (32,47) tested this hypothesis by investigating the rates of testicular cancer dizygotic and monozygotic twins since studies have shown that free estrogen levels are higher in the former group. Their results showed that there was a 50% increase in the risk for testicular cancer in dizygotic compared to monozygotic twins. A recent twin study from Denmark directly tested the role of in utero exposure to estrogens on sperm counts sperm counts/quality of singletons mono- and dizygotic twin (48). Their results showed no significant differences in sperm quality in any of the three groups of men and concluded that "higher prenatal concentrations of oestrogen are not related to reduced sperm counts in adulthood" (48). Offspring of women who were prescribed high pharmacologic doses of DES or estrogens during pregnancy are among the highest in utero exposed individuals to hormones. Studies in the United States and Finland showed that fertility in these high exposure groups was not different from a control population (49).

There is indirect evidence that high oestrogen levels during the first trimester of gestation have an influence on the incidence of testicular cryptorchidism. The human fetus is bathed in oestrogens starting very early in gestation, which is mainly of fetal origin; the oestrogen is synthesized by aromatase in the human placenta from fetal adrenal androgen precursors. The exposure of male rats to the oestrogenic chemical nonylphenol during only a certain period of neonatal life resulted in reduction of the size of testes, epididymis, seminal vesicle, ventral prostate and increase in the incidence of cryptorchidism (Lee 1998). This experiment demonstrates that there is a vulnerable period during male genital tract development, during which malformations may occur after exposure to oestrogenic chemicals.

Hypospadias has also been related to prenatal oestrogen exposure in animal models. The prevalence of hypospadias is increasing, according to World Health Organization (WHO). In Norway, paternal exposure to pesticides was associated with both hypospadias and cryptorchidism. Phytoestrogens may have adverse effects on the fetus because maternal vegetarian diet during pregnancy was associated with

hypospadias (50). Phenobarbital itself is known to cause hypospadias.

The hypothesis that the increase in the incidence of testicular cancer, hypospadias and cryptorchidism and the decrease in sperm count is due to EDs needs to be tested (31). Markers of exposure and of effect should be measured and linked to health outcome. Exposure should be measured at the appropriate period of vulnerability.

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INTRA UTERUS IMPACT OF ENDOCRINE DISRUPTERS

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Η Επίδραση των Ενδοκρινικών Διαταρακτών στην Ενδομήτρια Ζωή

Η αντίληψη ότι ο πλακούντας αποτελεί φραγμό για τις τοξικές ουσίες, που κυκλοφορούν στη μητέρα του κυοφορούντος εμβρύου, προασπίζοντας την καινούργια ζωή, έχει επιστημονικά ανατραπεί.

Η επίδραση από ετερογενείς αλλά και ιατρογενείς ουσίες, όπως η άτυχη περίπτωση της Δι-αιθυλοστυλβεστρόλης (DES), αλλά και του Δυσγενετικού Ορχικού Συνδρόμου (TDS) είναι σημαντικά ιατρικά προβλήματα που αναλύονται.

INTRODUCTION

Environmental and dietary chemicals with estrogenic activity have been a matter of increasing concern, in view of their potential adverse reproductive health consequences by affecting normal developmental processes (1-5). Initial attention focused only on chemicals with estrogenic activity, but now concern has broadened to include numerous chemicals that mimic or interfere with the normal actions of all endocrine hormones. These substances, collectively referred to as endocrine disrupting chemicals (EDCs), are likely to affect the developing human organism during the critical period of organogenesis and cause a wide range of adverse outcomes following prenatal exposure. It is suggested that a large fraction of human male reproductive disorders is of antenatal origin (6). This applies not only for congenital disorders, such as hypospadias and cryptorchidism, which were reported to show a trend of increasing incidence during recent decades (7,8), but also for testicular cancer (6,9,10). In addition, there are claims that the underlying cause of male infertility is often of fetal origin (6).

This article aims to review the published evidence for the potential impact of EDCs on the developing fetus following intrauterine exposure.

THE DES SYNDROME

Diethylstilbestrol (DES) is a chemical with potent estrogenic properties and constitutes a unique example of documented developmental disruption in the human fetus. DES was prescribed for numerous indications in the 60 years that followed its synthesis. Owing to the widespread belief that it prevented spontaneous abortion, millions of pregnant women were treated with DES in Europe and the U.S.A. (11,12).

In a seminal paper of 1971 by Arthur Herbst et al. (13), DES administration during pregnancy was linked to the development of vaginal cancer in the female offspring, 15 to 30 years after the exposure in utero. This was the first example of transplacental carcinogenesis, showing that a chemical is transferred from the maternal to the fetal circulation across the placenta and affects the developing vaginal cell, resulting in a long term carcinogenic effect with the development of clear cell cervical or vaginal cancer years after birth. Clear-cell carcinoma was a rare complication (about 1 case/1200 exposures), but clear cell adenosis was a common, almost constant complication of intrauterine DES exposure before the 18th week of gestation. The lesion could be detected in fetuses from 29 to 36 weeks' gestational age and in the perinatal period, either asymptomatic or causing vaginal leukorrhea (14). The basis of DES action is believed to be related to the estrogen-binding protein (demonstrable in mice and rats), which effectively sequesters natural estrogens in the fetal and neonatal circulations. Synthetic estrogens such as DES are much less effectively bound, and their

estrogen effects are unfettered. The result, according to this widely held hypothesis, is the partial retention of müllerian epithelium in the fetal vagina. Stromal anomalies such as vaginal ridges and septa have been linked to the influence of mesenchyme on epithelial differentiation. This led to an alternative hypothesis that the primary DES-induced effect is the failure of segregation of the mesenchymal layers, which determine the ratio of mucinous to tuboendometrial epithelium in various parts of the genital tract (15). The possibility of gene imprinting by estrogenic compounds has also been sustained to explain the phenotype associated with developmental estrogenization in both females and males (16).

Genital defects were observed in men whose mothers had taken DES during pregnancy (17,18). DES-exposed men had a higher incidence of undescended testes and epididymal cysts than comparable unexposed men (17,18). Extended studies showed in addition a higher incidence of hypoplastic testes and abnormal sperm (19,20) or even a "predisposition" to testicular cancer. This possibility was raised, as there were a few case reports of seminoma development in men prenatally exposed to DES (20,21).

In mice exposed prenatally to DES or other estrogens, the male offspring exhibit structural malformations including cryptorchidism, epididymal cysts of Müllerian origin and retention of Müllerian ducts. The latter condition reflects cases of male pseudohermaphroditism with the presence of functioning testes and both male and female internal genitalia (16).

Apart from genital malformations, an increased rate of autoimmune disorders has also been associated with prenatal DES exposure (22). The literature concerning a possible neuroteratogenic or psychopathological impact on DES-children is surrounded by controversy and prevents from drawing any firm conclusions (23).

TESTICULAR DYSGENESIS SYNDROME (TDS)

Observations over many years have resulted in a build-up of evidence that there is an association amongst testicular carcinoma in situ, cryptorchidism, hypospadias and testicular dysgenesis. Further evidence is still being accrued suggesting identical or overlapping causal factors. A new concept that has recently emerged proposes that poor semen quality, testicular cancer, cryptorchidism and hypospadias are different endpoints of one underlying entity, the testicular dysgenesis syndrome (TDS), which may be increasingly common due to adverse environmental influences (6). It has also been suggested that TDS is a result of disruption of embryonal programming and gonadal development during fetal life (6). According to this hypothesis, the adults who were prenatally exposed to EDCs are prone to testicular

cancer and subfertility and their offspring sex ratio tends to have a female bias. Cryptorchidism, hypospadias and low birth weight are symptoms of the same syndrome, manifested in the perinatal period. This hypothesis has been based on suggestive evidence provided by animal studies and wildlife and derived from human experience, all reviewed in reference (6).

The possible implication of EDCs in the etiology of TDS started in the 1970s, when a role of endogenous maternal estrogens was proposed by Henderson et al (24). Subsequently, Sharpe and Skakkebaek (25) formulated the so-called estrogen hypothesis, implicating environmental estrogen-like agents. This hypothesis has been expanded to include also environmental anti-androgens as endocrine disrupters, with potential adverse effect on male reproductive health (26). Epidemiological studies have reported an increased risk of genital malformations in children of workers occupationally exposed to pesticides (27) and the clustering of cryptorchidism in areas of intense agriculture (28). Further epidemiological data (reviewed in ref. 6) suggest that all the different endpoints in the testicular dysgenesis syndrome can be caused by hormones and EDCs. Indirect evidence from experimental studies support the epidemiological conclusions, as most male reproductive problems in humans related to environmental hazards can be experimentally produced in animals by prenatal exposure to endocrine disrupters (6). However, there is still no direct evidence to confirm that the different endpoints of the TDS are caused by human exposure to specific environmental hormones. Research is needed to delineate the role of endocrine disrupters in humans and illustrate any possible causal relation between their intrauterine action and the various endpoints of the testicular dysgenesis syndrome, thus validating the formulated hypothesis.

ENVIRONMENTAL ESTROGENS AND ANTI-ANDROGENS

In addition to substances with estrogenic activity, chemicals that act as androgen receptor agonists and antagonists or inhibit fetal steroidogenesis may disrupt development in human and laboratory animals by intervening with normal sexual differentiation. Environmental chemicals with estrogenic or anti-androgenic potency, shown to produce developmental disruptions, include the following:

Oral contraceptives - 17 α -ethinyl estradiol (EE)

It is estimated that a percentage as high as 2 to 5% of women who take oral contraceptives continue their use during subclinical pregnancy, thus resulting in unintended in utero exposure of the offspring to reproductive steroidal hormones. The use of oral contraceptives during subclinical

pregnancy often continues into the first trimester, even extending into the 4th month of gestation, encompassing a critical period for developmental disruption (29). EE is a synthetic estrogen with an estrogenic potency very similar to that of diethylstilbestrol, the latter being a known human reproductive teratogen.

Prenatal exposure to oral contraceptives containing EE has generally not been associated with an increased incidence of externally observable malformations at birth. Experimental studies in male mice exposed prenatally to very low doses of EE have shown an increase in prostate weight of the exposed offspring. The same doses of EE caused a transient reduction of sperm production during adolescence (30, 31, 32). This series of studies raises the possibility that prenatal exposure of exogenous sex hormones may cause adverse effects other than externally observed gross malformations.

Dioxin and related compounds

Polychlorinated dioxins, furans and polychlorinated benzene (PCB) constitute a family of toxic persistent environmental pollutants. PCBs are a mixture of mainly phenobarbital-like and low percentage dioxin-like congeners. Some PCBs have an estrogenic effect, while dioxins have an anti-estrogenic effect (33). Intrauterine exposure to these compounds appears to result in fetal loss and developmental disruption in experimental animals. More specifically, high doses of dioxin exposure have been associated to fetal loss in multiple animal species including Rhesus monkeys (33). In rats, a single low dose of TCDD, the most toxic compound of the dioxin family, consisting in 50-100 ng TCDD/kg, alters the differentiation of androgen-dependent fetal tissues. The mechanism of action is likely to involve interaction with a nuclear transcription factor, the hormone-like receptor Ah-R, rather than the androgen receptor. Thymic atrophy and dysfunction is one of the earliest signs of immunotoxicity mediated by the Ah-receptor (34).

In humans, acute dioxin poisoning is known to produce centropalmar chloracne, hematological effects and abnormal lipid spectrum in adults. In the perinatal period, PCBs and dioxins appear to be immunotoxicants with a persistent and dose-dependent effect (35). Phenobarbital-like PCBs were suspected to cause congenital malformations like cleft-lip and palate, simulating the documented teratogenic effect of phenobarbital itself (Allen 1980). Concern on a possible intrauterine embryotoxicity of dioxins followed the accident in Seveso in 1976, with large numbers of the population exposed to high doses of dioxin. Studies on the offspring of exposed mothers have been controversial (33) and, to date, no clear evidence has been provided to justify concern. The majority of older and recent studies have shown that fetal losses and birth defects remained within the expected rates, newborn growth and development proceeded normally, while

chromosomal examinations did not reveal abnormalities in number and patterns, beyond the normally expected rate (36-39). Infant studies have shown that prenatal PCB exposure might be related to adverse effects on the neurodevelopment and behaviour of children (33), however, data are limited and any clear impact of dioxin and PCB prenatal exposure remain elusive.

Phtalate esters

They constitute a large group of chemical agents used predominantly as plasticizers and solvents. Most information relating to dose-response relationships has been obtained for di-n-butyl-phtalate (DBP) by experimental studies on male rats. Intrauterine exposure to DBP was shown to elicit marked effects on the developing male reproductive tract, including malformations of the epididymis and vas deferens and hypospadias and cryptorchidism. Retention of thoracic nipples and reductions in anogenital distance were also noted. The testes of fetal rats showed markedly reduced testosterone levels and increased Leydig cell numbers, while Leydig cell adenomas developed later in some of the male offsprings (40).

Pesticides

They constitute an important group of chemical pollutants, their relevance being due to their widespread use. Most organochlorine (OC) agropesticides behave as xenoestrogens, having long lasting effects in the biosphere. Several OC pesticides, including vinclozolin, procymidone, linuron and DDT are androgen receptor-antagonists (41). They have been shown to produce dose-dependent effects on male rat fetuses after in utero exposure, causing reproductive abnormalities such as reduction in male anogenital distance, hypospadias, agenesis of the sex accessory tissues, retained nipples, cryptorchidism and epididymal agenesis (42). Organophosphorous (OP) pesticides have largely replaced the OC pesticides, but have also been shown to be potentially mutagenic, embryotoxic and teratogenic in experimental animals (43).

Additional evidence for the hazardous effects of pesticides is provided from wildlife, with documented exposure in many aquatic and terrestrial species (44,45), raising awareness about a possible association with the increased rate of abortion, impaired reproduction, male genital deformities, thyroid abnormalities and depressed immune system function, reported in wildlife by the WWF. The effects seen in wildlife living in polluted areas may give clues as to possible effects to look for in humans. In a most recent epidemiological study though, assessment of PCBs and chlorinated pesticides in pregnant women from Western Canada failed to document increased exposure levels (46).

Bisphenols

Bisphenols are a group of chemical compounds that were initially designed as synthetic estrogenic hormones and now form a part of innumerable manufactured plastic polymeres with very different properties.

Intrauterine exposure to bisphenol-A affects the normal development of murine male and female genital tract, resulting in increased weight of prostate gland, reduced semen production, decreased testicular and epididymal weight, enlarged anogenital distance and early puberty, at a dose-dependent manner (reviewed in ref. 47). In human studies, exposure to bisphenol-A has been recently associated with recurrent abortion in a small epidemiological sample (48).

Neuroendocrine disrupters

Examples of directly and indirectly acting neuroendocrine disrupters include some PCBs, dioxins, pesticides, metals, synthetic steroids etc. Although there is evidence presented on the possible developmental neurotoxicity of EDCs (reviewed in ref. 49), the area is characterized by uncertainty, mostly due to the complexity and multicausality of neurodevelopmental disorders.

Exposure Window

Timing of exposure is critical in understanding the phenotypic variation for all effects of EDCs. There are specific critical periods of sensitivity to endocrine disruption. These might be short and specific for different organs and species (50). The differential sensitivity of the fetus to its hormonal milieu is illustrated by experimental animal studies (reviewed in ref. 49). In humans, critical periods of sensitivity have been disclosed for certain substances, such as PCBs, which appear to cause adverse effects during the prenatal and early postnatal period (51).

CONCLUSIONS and PERSPECTIVES

Assessment of exposure to EDCs is a complex issue. The evaluation of any possible impact on health following intra uterus exposure to EDCs is further complicated by the possibility of long-term effects, not readily recognizable in the perinatal period. The issue of multicausality should always be raised when dealing with developmental abnormalities. Genetic factors, environmental disruption or an interaction of both may account for any developmental alterations. Thus, widespread exposure to chemical compounds may act directly on the developing fetus or indirectly, by interfering with gene expression, even causing epigenetic transgenerational actions (52). Evidence provided by experimental animal studies leaves little doubt about the

adverse developmental effects of EDCs. Besides, several disorders observed in wildlife living in polluted areas are also suggestive of environmental endocrine disruptions. However, extrapolation to the human population is not obvious and the possible developmental impact of EDCs is hard to document, while the relevant epidemiological data remain controversial and far from being conclusive. However, awareness and concern are raised by little pieces of evidence and by the formation of plausible hypotheses connecting endocrine disrupters with adverse developmental effects, i.e. the Testicular Dysgenesis Syndrome hypothesis. Being in doubt, one should opt for the precautionary principle. The diffusion of EDCs into the environment deserves to be prevented and precautionary measures are warranted when taking decisions on human exposure. Finally, preventive actions should be mandatory when dealing with any potential threat to human health, with due regard, however, to the psychological consequences of excessive alarm.

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THYROID AND ENDOCRINE DISRUPTION a fascinating theory

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Θυρεοειδής και Ενδοκρινικοί Διαταράκτες Μιά ενδιαφέρουσα ιστορία

Η επίδραση των Ενδοκρινικών Διαταρακτών στη λειτουργία του θυρεοειδούς, ενός τόσο πολύπλοκου οργάνου που συνδέεται και με την ανάπτυξη του Κεντρικού Νευρικού Συστήματος στο έμβρυο θεωρείται, ότι είναι ένα πολύ σημαντικό θέμα της σύγχρονης βιβλιογραφίας.

ENDOCRINE DISRUPTION: THE CASE OF THE THYROID

The Endocrine Disrupters (EDs) Hypothesis is a difficult but fascinating theory; and nowhere as fascinating as when it pertains to the thyroid hormone system. A very important physiological system in its own right, the relevance of studying its disruption in humans can not be overemphasized, as it regulates brain development and maturation (Porterfield *et al*, 1993). The fact that it is well conserved in most species is a great benefit, and its molecular mechanisms are extremely well researched (Brentn, 1994). On the other hand, direct effects are not easily discernible, and therefore not easy to measure or quantify, unless if they are extreme, such as thyroid enlargement or atrophy (Khan *et al*, 1999). Such an example is congenital hypothyroidism, which in humans presents neurological effects, and extremely difficult to identify. Some signs include cognitive dysfunction, lowered IQ and even increased vulnerability to Attention Deficit/Hyperactivity Disorder (ADHD) (Porterfield, 2000). This is also seen at patients with generalized resistance to the thyroid hormone (Hauser *et al*, 1993). There are excellent reviews describing the normal function of the thyroid pathway (Zoeller *et al*, 2002). What needs to be kept in mind is that the effects of these hormones are quite specific on

different cells and tissues, and that sensitivity is also dependent on the developmental stage considered (Zoeller, 2000).

Research has focused on wildlife, for some of the observed results, such as enlarged thyroid, could be reproduced in the labs (Kudo *et al*, 2004), and even adapted to form assays for detecting contamination. Exposure of tree sparrows (*Tachycineta bicolor*) to extensive pesticide spraying (mixtures of carbamates) increased their T3 levels significantly ($p=0.02$). Exposure of freshwater catfish (*Clarias batrachus*) to another carbamate insecticide, carbaryl (12 mg/L) caused a decrease in T4 and an increase in T3 (Colborn, 2002). Even though effects were evidenced, mechanisms were not identified, although some progress is made (Yen, 2001). Another experiment involved two species of mice: white laboratory mice (*Mus musculus*) and deer mice (*Peromyscus maniculatus*) from a colony maintained at the University of Wisconsin. These adults were exposed to three chemicals in mixtures: aldicarb, atrazine and nitrate, with low and high doses (maximum: 10 parts per billion (ppb), 10 ppb and 28 parts per million, respectively). One of the most notable results was a change in free thyroid index, with a wide assortment of behavioral changes. This would indicate that even adults could be affected by relatively low doses, the highest dose being fractionally above the one observed for local groundwater (Porter *et al*, 1999). Applying different versions (Shimada N. and Yamauchi K. 2004),

Experimental models of hypothyroidism caused aspects of brain development to be delayed, such as interaction of cytoskeletal elements (Oh *et al*, 1991). Studies of the development of the rat brain, when combined with equivalent ones of human fetal brain, allowed the sketching of a timeline of the brain's development and the time and role of thyroid

hormone appearance (*Howdeshell, 2002*). Also, it has shown that maternal hormone plays an important role in fetal brain maturation, and even in children (*Rogan & Ragan, 2003*). Combined with the findings of *Zoeller et al, 2000*, where it was observed that cortical neurons differentiation and cerebellar granule cell proliferation has to be done by gene activation by maternal thyroid hormones (since the fetus only expresses receptors), a strong implication can be made for specific timescales, or 'critical windows', of possible actions of thyroid hormones, and not just a thyroid-hormone sensitivity (*Zoeller et al, 2002*). This could mean that the fetus can undergo endocrine disruption by the effects mediated through the mother's hormone levels, causing irreversible effects on brain development (*Zhou et al, 2002*).

By considering these results with epidemiological studies followed in humans, such as the Yu-Cheng cohort, a link can be drawn to contamination and effects that must have occurred in utero (*Chen et al, 1992*). A study with women consuming contaminated fish from the Great Lakes lacked any overt symptoms, but reported delays in development and decrease in intelligence of children (*Jacobson et al, 1997*). In a more recent extensive metastudy, most of the children cohorts examined for PCB contamination were reevaluated for neuropsychological function: a correlation between increased PCB content in maternal plasma and increased TSH in the infant emerged (*Schantz et al, 2003*). Also, all the cohorts evaluated indicated negative associations between PCB found prenatally and cognitive functioning of the children some years later. All of this would indicate that there is a correlation between thyroid hormone disruption and cognitive dysfunction, even though right now it is weak: many mechanisms are still unclear. The evidence available points to many potential ways for disruption to occur. A plausible correlation linking neurobehavioral effects during development and thyroid hormones is to be found in *Hauser et al, 1993*, with the observation that people with resistance to thyroid hormone seem to develop Attention Deficit and Hyperactivity Disorders (ADHD) syndrome.

CONFOUNDING FACTORS AND UNRESOLVED DIFFICULTIES

This paper demonstrates the complexity involved in performing studies lasting many years, and using results to compare different populations, even there are different behavioral and statistical tests with different criteria, reducing the impact of any evidence collected. Criticisms have been directed against the hypothesis, due to the limitations that it encounters, some of them including adults not seeming to be affected by relatively high doses of environmental EDs, and no effects can be seen from circulating contaminants. Also,

natural hormones are usually much more potent than most EDs. The metabolites of a parent compound can have different actions with different strengths and binding affinities. Also, certain weak compounds can dramatically enhance the action of other endogenous hormones. Minute doses on various stages of CNS maturation can alter neuroendocrine function drastically, but have no observable effect when maturation has already been reached (*Rice et al, 2003*). The placenta in the mother's womb does not provide protection against many of these compounds that are present in the mother's blood, which get transmitted to the child.

Some of these difficulties can be ascribed to procedural reasons, in order to explain the difficulty of assessing ED effects. Most testing in laboratories for safety evaluation usually is toxicological: short term exposure to high doses, on adult animals, when it should be the other way round. More mechanisms are being identified constantly (*Michelle and Blumberg, 2005*), especially by focussing on cellular and molecular aspects of thyroid circulation (*Shimada N. and Yamauchi K. 2004, Miyazaki et al, 2004*).

The reason for this complication is also methodological: it consists of tending to simplify the phenomenon studied by focusing on a particular question, presenting it as a unicausal problem. Usually, the case for a particular disease or condition can be caused/traced to any number of unrelated factors and parameters: this is the main reason why more and more scientists advocate multicausality.

At least these factors have started to be taken into account in the consideration of new studies (*Rice et al, 2003, Kitamura et al, 2005*), so that in the foreseeable future experimental handlings should provide better controls or procedures to minimize these effects. That has been a problem with many studies encountered, in that they didn't adopt the same methodology with each other, often generating conflicting results. The wildlife results are also taken more into account, allowing the planning of better and more performing studies (*Fort et al, 2004*).

As a conclusion, it can be affirmed that EDs can have neurological effects, as evidenced by wildlife studies on animals, and with human populations. Unfortunately, the link between human exposure and effects is not easily established, due to a lack of causal evidence. It can only be hoped that with the newer mechanisms and molecular assays developed on the body of knowledge established will pave the way to better understanding of endocrine disrupting effects and mode of actions.

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ENDOCRINE DISRUPTERS AND THE WILDLIFE

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Ενδοκρινικοί Διαταράκτες στην Άγρια Ζωή

Η επίδραση των Ενδοκρινικών Διαταρακτών στα ελεύθερα άγρια ζώα ήταν το πρώτο σημείο παρατήρησης και εντόπισης της δράσης τους, γι' αυτό και αποτελεί και ιδιαίτερο ενδιαφέρον. Περιγράφονται συνοπτικά μεν, αλλά ουσιαστικά τα παραδείγματα, τα πειραματικά μοντέλα και τα πιθανά ερευνητικά μονοπάτια του μέλλοντος.

Introduction:

The effects on endocrine disrupters (EDs) are quite complex, and not yet fully understood as to the variety of their effects. This is of particular concern when considering possible consequences for human health and reproduction. The rise of certain cancers, deformities of reproductive organs and congenital conditions would indicate a persistent trend where one wasn't identified before. Most of these effects though have first been observed and documented on wildlife, for many years. This study aims to have a brief overview of the literature reporting some of these effects on wildlife with implication of EDs, in order to give a clear idea of how EDs can act, the possible effects they can have on humans, and why animal models can be effective indications of harm

The Problem:

The usual problem of epidemiological studies consists in being able to pinpoint the deciding cause of a particular observed trend, isolate it and be able to link it to a causal chain. Living in a multifactorial world, there are multiple causes to particular events, none of them necessarily constituting by itself a deciding factor. A combination of events, either weakening or enhancing, can contribute to

create a dynamic flowing pattern of action, reaction and inaction that gives rise to a unique event, or series of events. All of these can be subtle, or even undistinguishable, from the mass of phenomena observed, and difficult to determine. Which is why causal links are difficult to establish, even more so in human studies and possible causes of disease, on a large sample or population scale.

This difficulty is especially evident for EDs, substances that can act like, mimic, antagonize, or modulate, existing hormones and their related pathways of an organism (Aleva *et al*, 1998; Fox, 2004). Exposure to EDs, such as polychlorinated biphenyls (PCBs) and dichlorodiphenyl-trichloroethane (DDT) is more and more acknowledged as being near to ubiquitous, observed in extremely varied environments, such as in snow accumulation of mountains or the deep seas (Blaise *et al*, 1998; Froescheis *et al*, 2000). The reason for that is that they usually consist of very stable organochlorine compounds, in the form of waste products from chemical usage, or just resulting from degradation of plastics and synthetic compounds. They can also assume the form of organometals, and yet affect hormone receptors, as observed with metals such as lead or Cr.

Any hormonal axis can potentially be affected, yet the most vulnerable ones remain the ones that, once affected in critical exposure times, or 'critical windows of development' such as the embryonic stage (Howdeshell, 2002), cannot be restored to normal. Those would consist of the Hypothalamic Pituitary Thyroid (HPT) axis and the Hypothalamic Pituitary Gonadal (HPG) axis; the former regulating brain development, the latter sexual organs development and reproductive ability.

Thyroid deformities are difficult to diagnose, unless if they are extreme, and manifest themselves in such overt

phenomena, such as goiter (*for a review, see Leatherland, 1999*).

For the gonadal axis, effects are a bit easier for recognition, but not necessarily obvious at first glance: examples would consist of masculinization of females, or vice versa, with various other effects (*Nikolopoulou-Stamati & Pitsos, 2001*). Behavior can also be used to determine impact caused to the gonadal axis, by any deviation or changes from established observations, of which a larger literature is available than of the molecular workings. Yet recent advances focus more and more on the molecular aspect of the actions of EDs (*Brevini et al, 2005*).

In both these systems though, severe disruption can lead to death: thyroid hormone disruption leading to motor coordination problems and brain deficiencies, being lethal (*Porterfield & Hendrich, 1993*). Disruption in the sexual organs will mean that the organism in question will not be able to reproduce efficiently, or have offspring that is viable; sterility of offspring is another possibility (*Baatrup & Junge, 2001*). Of course, this leads again to lethal consequences. In both cases, the disruption can be overlooked or attributed to other, more obvious causes, interpreting the death of the organism as a consequence of infections or predators, till a given population dwindles. By then, more studies might be conducted, once a discrepancy has been identified; but even so, it might be too late to intervene (*Rice et al, 2003*).

Considering these difficulties, this paper focuses on some of the effects of EDs, as seen through some examples in the wildlife: some of the recent studies will be cited, as many reviews have been conducted on the available literature (see *Fry, 1995*). The careful use of animal cases and systems as models for study of ED action is discussed, along with the potential benefits and drawbacks. Finally, the basis for a link of ED action in humans from wildlife studies is considered, and whether concern is justified.

Examples of EDs actions: The Wildlife Situation

The first observations occurred in populations of birds, which seemed to dwindle due to reproductive difficulties, such as thinning of eggshells. Correlations were drawn to the recent use of pesticides in the surrounding fields and plantations, and particularly DDT, exposure to which was also discovered in eggshells. Studies in different areas with similar patterns of exposure yielded similar results, and these Rachel Carson presented in her book *Silent Spring* (*Carson, 1962*). Controversial at the time, it has been vindicated many times since, as seen with studies of eggshell effects of dichlorodiphenyldichloroethylene (DDE) in peregrine falcons (*Radcliff, 1973*). This illustration represents a classical paradigm shift in environmental toxicology. Studies of

exposures on population have yielded some links to the different manners by which interactions of environmental chemicals with animals can occur, correlated with reproduction problems (*Fry, 1995*), or with thyroid ones (*Mocia et al, 1986*). These models gained prominence and can now provide explanations to the questions that have remained unanswered for decades (*Lundholm et al, 1997*). Most importantly, these new approaches can change the accepted notions of a whole discipline, and reshape that discipline (*Colborn, 2004*). A more detailed review on the effects of EDs on wildlife has been presented in Tyler et al, 1998: yet a small presentation of the most recent studies, on each individual class of animal might be necessary.

By considering birds, many deformities have been observed, particularly evident in certain areas, providing good material for studies (*Gilbertson 1975; Moccia et al, 1986*). The fact that these cases seemed to occur more frequently in the close proximity of large lakes did not go unnoticed, and various other incidents with other species led to detailed examinations of the environment of the habitat. In order to discover any similar agents. Many studies were performed in the environment of the Great Lakes in North America, due to the extensive contamination with PCBs and other chemicals (*Giesy et al, 1994*). A recent study on the disruption of gonadal development of the White Perch in that region indicated still existing severe action of EDs (*Kavanagh et al, 2004*). Which also leads to the inevitable conclusion that most of these studies are based on the aftermath of environmental disasters, and are mostly reactive in nature, highlighting one of the weaknesses of Wildlife studies: they occur most often in areas of extreme contamination.

A very famous example of such a situation consists of the cryptorchidism cases and sex change encountered in alligators from Lake Apopka, which became rapidly an area of interest after an extensive chemical spill. The population seemed in decline: males had smaller phalluses, and various abnormalities of the testes were observed, along with blood levels of estrogens similar to females. The females exhibited almost double their normal level of concentration of estrogens. These observations, done by *Guillete et al in 1994*, were soon followed by the discovery that the main chemical contaminating the lake was similar to DDT (*Kelce et al, 1995*). It was found to exert the same anti-androgenic and feminizing effects, which constitute prime functions of EDs. This is due to the fact that expression of male or female genotype requires a fine balancing of gonadal hormones, which, in tandem with receptor distribution at critical stages of development, will determine the phenotype. Perturbing this balance, especially at times when it is fragile can cause permanent changes to the phenotype itself. An example would be the sex reversal observed in *Caiman latirostris*, which were found to have tissue contaminated with high

doses of bisphenol A (BPA) (Stoker *et al*, 2003).

Effects on the HPG can also impact, directly or indirectly, the HP-Adrenal axis (HPA), modulating steroid and stress hormones, with consequences to the immune system, as observed by immune suppression in turtles of the lake Apopka. Reptiles have also been considered to be useful sentinel species, to gauge endocrine disruption and concentration of EDs in an area, by observing changes to their genital organs: testicular atrophy in freshwater turtles (*Chrysemus picta*) are an example (Rie *et al*, 2005).

The disruption of homeostasis is well studied in amphibians, upon which many studies have recently focused. Leopard frogs in specific sites in the United States of America have exhibited hermaphroditism, along with several other abnormalities documented, such as gonadal dysgenesis: these sites contained high concentrations of atrazine (Hayes *et al*, 2003). This has been proven before, with attempts to reproduce these conditions (environmental concentrations of pollutants) on laboratory animals, and have yielded similar results (Porter *et al*, 1999). Another recent communication reported a population decline in cricket frogs, linked with EDs, the consequence being the presence of intersex (Reeder, 2005).

Fishes are a prime example of animals to study, due to their exposure to various effluents in rivers, estuaries and water sources, and maturation cycle. They have been used as models for many years, with studies on the effect of EDs on early development (Weiss & Weiss, 1989). The progress of a particular substance can be traced from its origins to its effects on wildlife, as seen with androstenedione (Durhan *et al*, 2002). Two very recent studies on estuarine species implicated the action of estrogen agonists (such as 17-ethynylestradiol) and antagonists on the reproductive capacity of two species, estuarine killifish and estuarine mummichog (Boudreau *et al*, 2004, 2005).

Remaining in the same possible medium of contamination, Invertebrates also have acquired a great amount of interest, with many studies focusing on them, from population level to the individual, indicating a surge of interest (Oetken *et al*, 2004). Changes such as population variations have been observed, that can be related to fluctuations of chemical contents in the water, which is easy to monitor at different time intervals. In the case of the shore crab *Carcinus maenas*, changes between populations have been detected, that can correlate to the effects on reproductive ability by EDs (Brian, 2005). The relative advantages of invertebrates, relating to their interaction with their environment, render them ideal for modeling.

Mammals have been intensively studied due to the similarities shared with human systems, with observations

ranging from species as wide apart as sea lions to Pronghorn antelope (Dunbar, 1996) or even polar bears (Braathen *et al*, 2004), where high levels of PCBs were discovered and correlated to hermaphroditism in females. Sea lions have been detected with extremely high levels of DDT and PCB (In the case of minks and otters, shorter baculums and testes have hindered their reproduction. Many studies performed on bears had similar results, with a particular one conducted in Florida showing 11 out of 71 black bears (16%) retaining testes; clearly cryptorchidism, a congenital disorder caused by disruption of hormones (Dunbar, 1996). A different study on black bears indicated severe changes in gonadal hormones, and severe testicular recrudescence (Howell-Skalla *et al*, 2000). Pronghorn antelope populations in Oregon were seen to display signs of weakness linked to dietary deficiencies, with as yet unknown consequences, but could possibly be linked to EDs (Dunbar 1999). Mule deer also show to have been through changes, as seen by abnormal testes and antlers in 27 of 116 adult male Mule deer (Tiller *et al*, 1997). A more recent study conducted on 254 white tailed deer from Montana indicated that 67% showed genital developmental abnormalities in a period of four years (1996-2000), such as mispositioned genitals, cryptorchidism and badly formed scrotum (Hoy *et al*. 2002). This was confirmed by a similar observation in a black tail deer population in Aliliuk peninsula on Kodiak Island; 61 out of 94 were bilateral cryptorchid, 43 out of 94 had abnormal antlers, and 2 of the 10 scrotal testes examined contained precursor-cells of seminoma (Veeramanchaneni *et al*. 2005).

The Case for Wildlife Models

Laboratory animals, and even domestic animals (Magnusson, 2005), are useful for studying the effects of EDs on various systems, due to the control that can be exerted over the experimental parameters. Yet in the case of laboratory testing, thought must be given to the species used, and how representative they are of the probable multifactorial nature of the issue investigated. Also, experimental design must be quite rigorous and able to test several situations that are realistic, a tenet, which is not always applied with certain studies, by using the strict toxicological procedures of high doses over short periods of times. This paradigm is shifting, but it will take time to be applied consistently over most studies.

Wildlife studies are, in part, already done, and can be monitored over large periods of time with a minimum of exertion of control and tampering required from the human observer. The variety of species that exhibit disruption, and can be studied, represents a wealth of possible information. For instance, one of the parameters that is difficult to study in mammals is gamete viability: in wild animals it would be very

difficult to obtain such samples, and captivity would invalidate the results. In some animals, like fish, having an external reproductive cycle, it is much easier to sample and analyze, without invasive manipulations (Kime & Nash, 1999). Even then, correlating laboratory results with those seen in wildlife is difficult and challenging, if not contradictory (Mills & Chichesterb, 2005). This though just indicates that laboratory techniques and conceptual framework need to change and have some adopted guidelines.

Also, some of these species lifestyles are more representative of the possibilities of human exposure, and therefore more valuable in order to understand routes of contamination. The observations of disruptions on the estuarine mummichog led to the development of a bioassay of reproductive capacity based on levels of steroid hormone and vitellogenin (MacLatchy *et al*, 2004). Long-term exposure of zebrafishes to certain compounds, such as pharmaceutical agents like ethynylestradiol, seems to lead to reproductive failure, an example that is apt for human consumption of fishes and consequences (Nash *et al*, 2004). The obvious drawback of this is that it tries to reproduce environmental conditions in a laboratory environment, which is difficult, but at least data from wildlife studies is used in order to determine environmentally relevant quantities, which is more relevant than classical toxicological studies. To this effect, a closer monitoring of genetic factors and developmentally relevant stages has been advocated for future study designs (Naciff & Daston, 2004).

The case is being put forward for amphibians as a model of contamination through both water and air (Kloas, 2002). Invertebrates, such as crabs or mollusks, can be useful indicators of levels of certain chemicals, due to their accumulation in their tissues, and the rate at which they reproduce, but also their distribution patterns around particular estuaries and areas of possible variance for chemical concentrations (Oetken *et al*, 2004; Brian, 2005). Even though fish can be considered better and more representative models for water contamination, it has to be borne in mind that some invertebrates, even though sharing the same environment as fish, do not necessarily have the same mobility, existing in colonies that are spread by dissemination of gametes. As such, they are easier to monitor over extensive periods of time, as seen with mussels kept downstream of an area of interest (Gagne *et al*, 2004). The fact that they also can leave behind remains, such as shells, which can be analyzed for EDs concentrations, as performed with eggshells in birds, is an added benefit, providing excellent models (deFur, 2004).

Conclusions: Implications for Humans, Future

Avenues of Research

Several population studies done on humans have failed in giving clear, causal evidence of the involvement of a studied substance and a particular condition or effects. And that will probably still be the case, till the detection techniques used are changed drastically, with a proportionate improvement to our understanding of the complex paths EDs can take. The importance of this is seen when considering the state of knowledge on neurobiological development, and how EDs affect it. Studies on wildlife can, if not causally imply, at least point or direct inquiries in specific directions, allowing more elaborate and powerful modeling and hypothesis-building (Panzica, 2005). It would be reasonable to assume that if so many different species are affected all so suddenly, in such a short period of time, of drastic gonadal and endocrine abnormalities, there has to be a link with the environment, and the role we play in it. The rise of certain neurobehavioural disorders in human populations, such as ADHD, can be attributed to better diagnosis. The possibilities that they might be associated with other factors too, such as embryonic disruption or modulation of the thyroid pathway through the mother, are not extremely unlikely though, and deserve better and more insightful studies based on results from wildlife, where the worst has already happened.

The important conclusion that is to be made of these are that the causes, once again, can not be necessarily be pinpointed to simple factors: but the geographical specificity of these effects, the rapid temporal interval in which they occur and they distinctive nature they assume when they are identified, would strongly suggest the action of EDs. Furthermore, an important aspect of wildlife studies is the species considered. The lifecycle and natural environment of each different organism presenting symptoms can provide valuable clues and information as to how it got exposed to environmental agents. This, along with the disruption observed, can lead to identification of a number of compounds, allowing better study and understanding of the disrupted system in conjunction with the xenobiotics, and possibilities of using the affected organism as a model or a sentinel. This should be used for designing better studies, more suited to determine particular pollutants and their effects.

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HELLENIC SOCIETY OF ANDROLOGY
AND
EUROPEAN ACADEMY OF ANDROLOGY

POSTGRADUATE COURSE ON ERECTILE DYSFUNCTION

ATHENS, 3 – 4 FEBRUARY 2006

Organizing committee

D.A.Adamopoulos, S.C.Nicopoulou, Ch. Asvestis, N.Sophikitis, A.Lenzi, G.Forti

Local organizing committee

E.Andreou, A.Gekas, D. Goulis, D.Hatzichristou, E.Koukkou, J.Papadimas, E.Venaki,

ΑΝΑΚΟΙΝΩΣΗ

Στα πλαίσια των εκπαιδευτικών δραστηριοτήτων της, η Ελληνική Ανδρολογική Εταιρεία οργανώνει σεμινάριο με θέμα: "Διαταραχές της Στυτικής Λειτουργίας".

Το σεμινάριο θα διεξαχθεί από 3-6 Φεβρουαρίου 2006, στο Ξενοδοχείο "ΑΛΕΞΑΝΔΡΟΣ" (Πλατεία Μαβίλη).

Λόγω της φύσεως του σεμιναρίου, θα υπάρξει περιορισμένος αριθμός εγγραφών με βάση την προτεραιότητα επικοινωνίας και την ταυτόχρονη καταβολή του τέλους εγγραφής (ειδικευμένοι: 80 Ευρώ, ειδικευόμενοι: 50 Ευρώ).

Το πρόγραμμα θα αποτελείται από ομιλίες ειδικών από την Ελλάδα και το εξωτερικό, παρουσίαση εκπαιδευτικού υλικού και συνεδριάσεις αλληλοανταλλαγής (inter-active).

Το προκαταρκτικό πρόγραμμα του σεμιναρίου, συνολικής διάρκειας 12 διδακτικών ωρών, παρουσιάζεται κατωτέρω. Οι συμμετέχοντες θα λάβουν πιστοποιητικό παρακολούθησης.

Η Οργανωτική Επιτροπή

Πληροφορίες: 210 6402273 (κ. Μ. Μπόττη (10:30-13:30), κ. Σ. Νικοπούλου, κ. Ε. Βενάκη

POSTGRADUATE COURSE ON MALE ERECTILE AND SEXUAL DYSFUNCTION

DATES	FRIDAY 03 – SATURDAY 04.02.2006
DURATION	Total : 12 teaching hours - 4 sessions in physiology–aetiology–diagnosis – treatment - 4 interactive video-sessions of 40 min each. Cases: a. adult, DM – hypertension-angiopathy, b. aging, andropenia, c. drug induced, d. psychogenic - discussion after each lecture and video session

PRELIMINARY PROGRAM**FRIDAY 15:30 – 20:00**

- 1. Physiology,** Chair: J.Papadimas – A. Karanikas 5 min
- Phylogenesis - dimorphism *R. Angelopoulou* 20 + 5 min
 - Endocrine regulation of erection *C. Foresta* 20 + 5 min
 - Vascular role *S. Francavilla* 20 + 5 min
 - Composite mechanism *D. Hatzichristou* 20 + 5 min

Coffee Break - 15 min

- 2. Aetiology – Diagnosis - I,** Chair: G. Barbalias - L.Kontogeorgos
- Vascular causes *S. Francavilla* 20 + 5 min
 - Neuro-urological aetiology *G. Barbalias* 20 + 5 min
 - Endocrine aetiology *A. Lenzi* 20 + 5 min
 - Psychogenic *N. Vaidakis* 20 + 5 min

- 3. Interactive (a) adult: DM – hypertension-angiopathy,** **Video – Discussion**
Chair: S. Francavilla – Th. Alexandrides 40 min

SATURDAY 8:30 – 14:00

- 4. Aetiology–Diagnosis-II,** Chair: G. Forti - P. Nicolopoulou-Stamati 5 min
- Environmental – Drug induced *E. Koukkou* 15 + 5 min
 - Age-related-A European Perspective *G. Forti* 20 + 5 min
 - Special diagnostic tools *N. Liassis* 20 + 5 min
 - Diagnostic approach *M. Maggi* 20 + 5 min

- 5. Interactive (b) aging: andropenia,** **Video – Discussion,**
Chair: G.Forti - Ch. Asvestis 40 min

Coffee Break - 15 min

- 6. Treatment - I,** Chair: D. Panidis – D. Hatzichristou 5 min
- Sildenafil *D. Hatzichristou* 15 + 5 min
 - Vardenafil *A. Bissas* 15 + 5 min
 - Tadalafil *A. Ledda* 15 + 5 min

- 7. Interactive (c): drug induced,** **Video – Discussion,**
Chair: C. Foresta – F. Sofras 40 min

Coffee Break - 15 min

- 8. Treatment - II,** Chair: N. Sofikitis – M. Bourounis 5 min
- Local treatment *E. Constadinides* 15 + 5 min
 - Surgery *D. Katzavelos* 15 + 5 min
 - Prospectives *M. Maggi* 15 + 5 min

- 9. Interactive (d) : psychogenic,** **Video – Discussion,**
Chair: A. Lenzi - N. Vaidakis 40 min

- 10. Evaluation test for participants** 15 min

- 11. Certificates of attendance - Credits**

