Biochemical Pharmacology, Vol. 38, No. 17, pp. 2773–2779, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

PHOTO-INDUCED FORMATION OF DNA ADDUCTS OF 2,2′,4,4′,5,5′-HEXACHLOROBIPHENYL IN CULTURED HUMAN CELLS

K. BUFF,*‡ M. WEGENKE† and A. BRÜNDL*

Gesellschaft für Strahlen- und Umweltforschung München, *Abteilung für Zellchemie, †Institut für Toxikologie, D-8042 Neuherberg, Federal Republic of Germany

(*Received* 16 *December* 1988; *accepted* 24 *February* 1989)

Abstract—Association of the PCB congener 2,2′,4,4′,5,5′-hexachlorobiphenyl (6-CB) with cell nuclei has been studied in cultured monolayer human Chang liver cells. Photo-induced formation of covalent bonds determined 6-CB binding to protein of cell nuclei and to DNA. Nuclear binding of 6-CB approached equilibrium after approximately 30 min of incubation. Photo-induced binding *in vitro* to purified Chang liver cell DNA substantiated direct interaction of the PCB congener with DNA. In monolayer cells, low levels of photo-induced 6-CB DNA adducts could be detected using the very sensitive ^{32}P-postlabeling method. Adduct formation was dependent on 6-CB concentration as well as on incubation time. Highest adduct levels were in the range of 2×10^{-8}. Model reactions *in vitro* showed photo-induced binding of 6-CB to individual purine deoxyribonucleotide-3′-phosphates. The results demonstrate rapid intracellular movement of the PCB congener into the cell nucleus. The vast majority is associated with nuclear protein, minute amounts of 6-CB are found proximate to the DNA helix as evidenced by photo-induced adducts of purine nucleotides.

Transport pathways and intracellular distribution of persistent environmental chemicals have been investigated in cultured monolayer human Chang liver cells. Following rapid initial binding to the cell plasma membrane of the PCB congener 2,2′,4,4′,5,5′-hexachlorobiphenyl (6-CB)§, passive diffusion as well as temperature-dependent internalisation by membrane endocytosis were shown to constitute major uptake routes into the cell [1]. By means of photo-induced covalent binding to cell protein and lipids, intracellular migration of the compound could be followed and could be shown to slowly approach equilibrium [2]. Slow rates of PCB and 6-CB uptake have already been inferred from experiments with monolayer human lung cells [3] and rat adipose tissue slices [4], respectively. Binding to "soluble" and "particulate" cell fractions was discriminated in human skin fibroblasts [5], as was the role of exogenous lipoprotein fractions in uptake [6].

Aside from their general environmental impact, PCB have been shown to exhibit tumor-promoting activity in rat liver [7, 8]. This makes a more fundamental knowledge of intracellular distribution and nuclear association of PCB compounds desirable. In addition to a large number of chemical carcinogens and mutagens [9], nuclear association of PCB has already been observed in cultured human fibroblast cells [10]. *In vivo* binding of the metabolically stable PCB congener 6-CB to nuclear macromolecules of rat [11] and mouse liver [12] was shown, supplemented by a brief report on *in vitro* binding of 6-CB to rat liver DNA [13].

We made use of our photo-induced ligand binding technique to follow up 6-CB interaction with nuclei of monolayer cells. Reversible association with nuclear macromolecules was fixed by generating photo-induced covalent bonds of 6-CB to nuclear protein and DNA. The irreversible bonds prevent dissociation of the molecular complexes during subsequent cell fractionation. The resulting binding pattern does reflect true intracellular (and intranuclear) distribution of 6-CB and related compounds. In order to detect minute amounts of 6-CB DNA adducts, we resorted to the ^{32}P-postlabeling method developed by K. Randerath and his group [14]. The non-radioactive 2,2′,4,4′,5,5′-hexachlorobiphenyl congener is incubated with monolayer cells; after photofixation, the DNA is rigorously purified and hydrolyzed (digested). The high specific radioactivity required to detect minute amounts of DNA adducts is introduced by converting the deoxyribonucleotide-3′-phosphates of the DNA digest to the 3′,5′-diphosphates by phosphorylation with ^{32}P-ATP. Adducts are then separated from unmodified nucleotides by consecutive TLC on PEI–cellulose [14].

Using this method, we found a rapid distribution of 6-CB into the cell nuclei of monolayer cells. Moreover, differential binding to nuclear protein and DNA nucleotides is shown reflecting variable exogenous conditions of cell exposure.

MATERIALS AND METHODS

Chemicals and enzymes. 2,2′,4,4′,5,5′-Hexachlorobiphenyl (6-CB) was purchased from

‡ To whom correspondence should be addressed.

§ Abbreviations: 6-CB: 2,2′,4,4′,5,5′-hexachlorobiphenyl; ^{14}C-6-CB: ^{14}C-labeled 2,2′,4,4′,5,5′-hexachlorobiphenyl; azido-5-CB: 4-azido-2,2′,4′,5,5′-pentachlorobiphenyl; diazido-4-CB: 4,4′-diazido-2,2′,5,5′-tetrachlorobiphenyl; DMEM: Dulbecco's modified Eagle's medium supplemented with glutamine (2 mM), $NaHCO_3$ (45 mM), 8% (v/v) fetal calf serum; TLC, thin-layer chromatography; PBS: phosphate-buffered saline, pH 7.4; PEI, polyethylenimine; RAL, relative adduct level.

Promochem (Wesel); U-^{14}C-labeled 2,2',4,4',5,5'-hexachlorobiphenyl (0.35 GBq = 9.4 mCi/mmol) was from Pathfinder Laboratories (St Louis, MO). Carrier-free ^{32}P-orthophosphate was obtained from New England Nuclear (Dreieich). 4-azido-2,2',4',5,5'-pentachlorobiphenyl (azido-5-CB) and 4,4'-diazido-2,2',5,5'-tetrachlorobiphenyl (diazido-4-CB) were synthesized in our laboratory. Growth media and supplements for cell culture were obtained from Biochrom (Berlin). The enzymes used in adduct analysis were: proteinase K (EC 3.4.21.14), RNAse (EC 3.1.27.5) and spleen exonuclease (EC 3.1.16.1) from Boehringer (Mannheim); micrococcal endonuclease (EC 3.1.31.1) and apyrase (EC 3.6.1.5) from Sigma (Taufkirchen); T4 polynucleotide kinase (EC 2.7.1.78) from Amersham Buchler (Braunschweig).

Cell culture. Chang liver cells (CCL 13 of the American Type Culture Collection) had been obtained through Gibco-Europe (Karlsruhe). Monolayer cells were grown on 10 cm diameter plastic culture dishes (Greiner, Nürtingen) in Dulbecco's modified Eagle's medium supplemented with glutamine (2 mM), $NaHCO_3$ (45 mM), and 8% of fetal calf serum in an atmosphere of 95% air/5% CO_2 at 37° in a humidified incubator.

Uptake and photo-induced ^{14}C-6-CB binding to cell nuclei. Cells were plated in plastic culture dishes (10 cm diameter) at a density of 1×10^6 cells/dish and were grown in DMEM for two days. Experiments were started by replacing the growth medium with DMEM containing 10 μM of ^{14}C-2,2',4,4',5,5'-hexachlorobiphenyl. This experimental medium had been prepared the day before by adding the required amount of ^{14}C-6-CB (in a minimum volume of dimethylsulfoxide) to DMEM and by subsequent overnight equilibration at 37° [2].

Uptake was stopped by replacing the experimental medium with 10 ml of chilled phosphate-buffered saline (PBS). The cell monolayer was then irradiated for 5 sec with a Philips HPK 125-W mercury lamp positioned at a distance of 2.5 cm above the open culture dish [2]. Cells of 4–6 dishes were pooled for each experimental variable. After irradiation, the cells were scraped off in buffer (0.25 M sucrose, 10 mM Tris–Cl, 1 mM EDTA, 10 mM $MgCl_2$; pH 8.0) and homogenized by 6 strokes in a glass homogenizer with a loose-fitting Teflon-coated pestle. Crude nuclei were collected by centrifugation (800 *g*, 10 min), resuspended in the same buffer and purified by centrifugation (100,000 *g*, 60 min) through buffered 1.6 M sucrose. The isolated nuclei were washed with 4 × 1 ml of 25% ethanolic trichloroacetic acid to extract residual non-covalently bound material [2]. The final precipitate was solubilized in 0.5 ml of 0.5 N NaOH to determine protein and covalently bound radioactivity.

In vitro *photo-induced ^{14}C-binding to Chang liver cell DNA.* Cells of 20 culture dishes were pooled to isolate the DNA according to standard methods [15, 16]. The cells were lysed in 15 ml of buffer (10 mM Tris–Cl, 100 mM NaCl, 1 mM EDTA, 0.1% sodium dodecyl sulfate, 100 μg proteinase K/ml; pH 7.5). Protein was removed with 2 × 15 ml of phenol/chloroform/isoamyl alcohol = 25:24:1, followed by extraction with chloroform (2 × 15 ml). After addition of 1 ml of 0.15 M sodium acetate (pH 5.2) DNA was precipitated with 2.5 vol. ethanol. The DNA was dragged out with a glass rod and redissolved in 4 ml of buffer (10 mM Tris–Cl, 1 mM EDTA; pH 7.5). Residual protein and RNA were degraded by addition of 100 μg/ml of proteinase K and RNAse, respectively, and the entire purification procedure was repeated. The final DNA concentration was determined by comparing the absorbance at 260 nm with a standard curve.

One millilitre of the final solution containing 200 μg of purified DNA was poured into a culture dish (6 cm diameter). The required amount of ^{14}C-6-CB was added in 3 ml of ethanol, and the mixture was irradiated for 5 sec. The precipitated DNA was washed twice with 2 ml of ethanol to extract any residual non-covalently bound material. The washed precipitate was isolated and redissolved in buffer for analysis.

Photo-induced 6-CB DNA adduct formation. After incubation with 6-CB (or its azido-analogues) and irradiation, cells of 6 dishes were pooled, scraped off in PBS and pelleted. The DNA was isolated as described above; the purified nucleic acid was redissolved in 1:100 diluted 0.15 M NaCl/15 mM sodium citrate buffer for subsequent adduct analysis.

Photo-induced 6-CB DNA adducts were detected by closely executing Gupta and Randerath's modified [17, 18] basic procedure [19], as outlined in the following analysis protocol. The DNA digest [19] contained 2.5 μg of deoxyribonucleotide-3'-monophosphates in 25 μl of buffer. Five microlitres were diluted with H_2O to 500 μl and set aside for analysis of total nucleotide adducts (see below). The residual 20 μl of the digest was extracted with *n*-butanol for adduct enrichment at enhanced sensitivity of the ^{32}P-ATP labeling reaction [17]; the extract was then lyophilised. To convert the extracted deoxyribonucletoide-3'-monophosphates into the corresponding ^{32}P-labeled 3',5'-diphosphates, the following reaction mixture was prepared: 2.25 μl of buffer (200 mM Bicine–NaOH, 100 mM $MgCl_2$, 100 mM dithiothreitol, 10 mM spermidine; pH 9.5), carrier-free γ-^{32}P-ATP (225 μCi; prepared as described in Ref. 19), 0.8 of T4 polynucleotide kinase (4.5 units), and H_2O ad 7.5 μl. The labeling reaction was initiated by adding 5 μl of this reaction mixture to 10 μl of the aqueous solution of extracted deoxyribonucleotide-3'-monophosphates. Incubation and subsequent apyrase treatment were as described [19].

The entire ^{32}P-labeled sample was subjected to TLC on PEI cellulose sheets (Macherey & Nagel Düren). Adducts were separated from the bulk of normal nucleotide diphosphates and resolved from each other by consecutive TLC in different directions. The following solvent systems (slightly modified from Ref. 18) were used: I (2.3 M sodium phosphate; pH 5.8), II (3.75 M lithium formate, 8 M urea; pH 3.5), III (0.8 M LiCl, 0.5 M Tris–Cl, 8 M urea; pH 8.0), and VI (1 M sodium phosphate; pH 6.8). To improve chromatographic resolution, the different TLC runs were extended overnight, with a Whatman No. 1 wick clipped to the top of the sheet. Adduct spots were located by screen-enhanced autoradiography (Kodak XAR-5 film; 24 hr exposure/

−80°). Located spots were cut out and their radioactivity counted in a liquid scintillation counter.

Estimation of relative adduct levels. The 5 μl portion of the digest, set aside and diluted as shown above, was used for estimation of total labeling recovery. To this, the sample of 5 ng deoxyribonucleotide-3′-phosphates was incubated with 2.5 μl of the above ^{32}P–ATP reaction mixture. After dilution with water to 250 μl, 5 μl = 0.1 ng of the labeled sample was separated on PEI–cellulose in 100 mM ammonium sulfate. The ^{32}P-labeled area of total deoxyribonucleotide-3′,5′-diphosphates was located by screen-enhanced autoradiography (20 min exposure/+20°). The localised area was cut out and total incorporated ^{32}P-radioactivity counted. Relative adduct levels (RAL) were then estimated by dividing the adduct-incorporated radioactivity by total incorporated nucleotide radioactivity [19], making allowance for any dilution steps from the original digest. The resulting figure gives then the ratio of adducts to unmodified nucleotides.

In vitro *adducts of deoxyribonucleotide-3′-phosphates.* Solutions of the deoxyribonucleotide-3′-phosphates of guanine (G), adenine (A), thymine (T), and cytosine (C), each 1.5 mM in 300 μl of methanol, were made up in the absence or presence of 6-CB and azido-5-CB (each 0.06 mM), respectively. The solutions were filled into the wells of a 12-well Costar plastic dish and irradiated for 10 sec. Samples containing 5 μg of nucleotides were evaporated to dryness, taken up in water and processed for adduct analysis as described above.

RESULTS

Photo-induced ^{14}C-6-CB *binding to intact cell nuclei*

We have shown previously that transport of the PCB model compound 6-CB into monolayer Chang liver cells could be "frozen" at any time by brief high-intensity ultraviolet irradiation [2]. Photo-induced generation of covalent bonds was also of advantage in studying ^{14}C-6-CB transport into cell nuclei. As shown in Fig. 1, nuclear binding increases with incubation time; equilibrium distribution is approached after 30–45 min of incubation. In the absence of irradiation ("dark" control), binding is below 5% of the photo-induced reaction. Thus, in analogy to intact monolayer cells, photo-induced formation of covalent bonds to nuclear protein reflects intracellular movement of 6-CB to the nucleus.

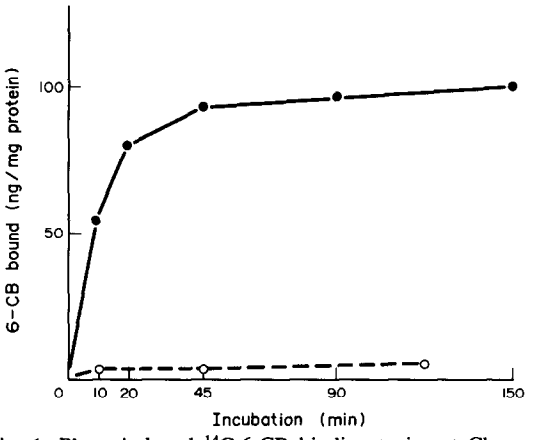

Fig. 1. Photo-induced ^{14}C-6-CB binding to intact Chang liver cell nuclei. Cells were incubated with culture medium containing 10 μM of ^{14}C-6-CB. At the indicated times, the medium was replaced by PBS, and the cell monolayer was irradiated for 5 sec. Intact cell nuclei were isolated as described in Materials and Methods. O—O, binding without irradiation ("dark" control) ●—●, photo-induced binding (after subtraction of "dark" control). Total number of experiments: 4.

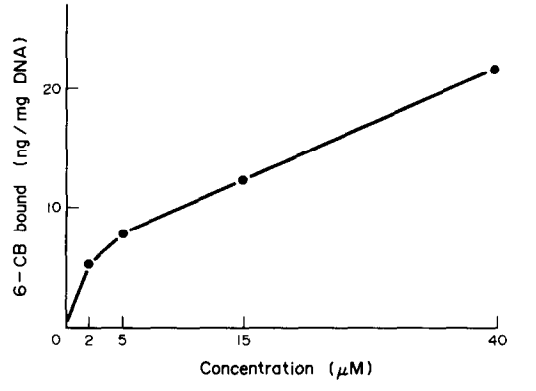

Fig. 2. Photo-induced ^{14}C-6-CB binding *in vitro* to isolated Chang liver cell DNA. The radioactive compound was added to a solution of purified Chang liver cell DNA in a culture dish. After irradiation for 5 sec, the precipitated DNA was isolated and washed with ethanol. The final DNA preparation was dissolved in buffer; aliquots were used to quantitate DNA and bound radioactivity.

In vitro 6-CB *binding to purified Chang DNA*

Covalent photobinding of 6-CB to DNA was established in a pilot experiment using purified Chang liver cell DNA. The ^{14}C-labeled PCB congener was added in ethanol to a buffered solution of the DNA. After irradiation, the amount of ^{14}C-radioactivity precipitating with DNA was measured. Simultaneously, non-covalently bound material was extracted by the added organic solvent. Concentration-dependent photobinding of 6-CB to the purified DNA is shown in Fig. 2. Binding at 20 μM is equivalent to 1 molecule of 6-CB bound per 5×10^6 DNA nucleotides.

^{14}C-6-CB *binding to DNA of monolayer cells*

Putative 6-CB DNA adduct levels were estimated by incubating Chang liver cells with the ^{14}C-labeled compound. However, the isolated and purified DNA did not contain any detectable levels of DNA-associated ^{14}C-radioactivity. Since association of 6-CB with intact nuclei and with DNA has been shown (cf. Figs 1 and 2), minor adduct levels were thought to have escaped detection as a result of too low a specific radioactivity of the ligand. Therefore, a more sensitive method was applied.

Adduct analysis after ^{32}P–*ATP-postlabeling*

The ^{32}P-postlabeling technique [14] was applied to detect low levels of nucleic acid adducts. The distribution of the hexachlorobiphenyl congener into the cell nucleus and its association with DNA is

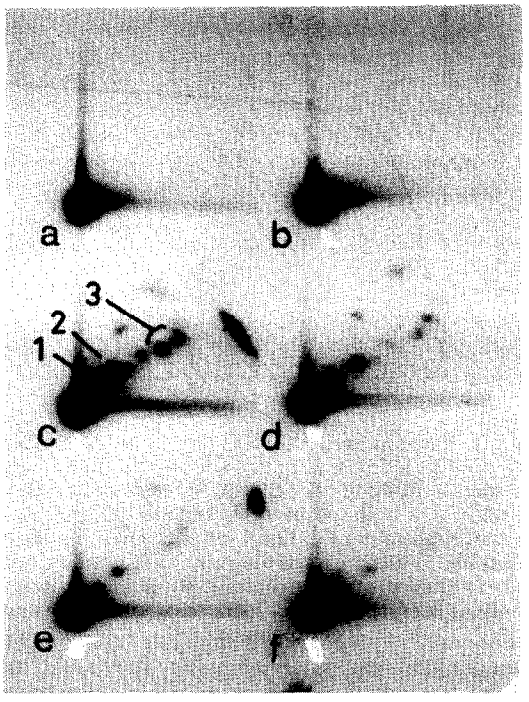

Fig. 3. Autoradiograms of ^{32}P-labeled photo-induced 6-CB DNA adducts: dependence on concentration. Photo-induced 6-CB nucleotide adducts were enriched from total Chang liver cell DNA hydrolysate by *n*-butanol extraction [17] and resolved by 2-directional TLC on PEI–cellulose. Adduct patterns are shown after autoradiography (24 hr/ −80°). Maps (a–f) show (in this sequence): "dark" control (a); cell irradiation after incubation with 0 (b), 40, 20, 10 and 5 μM of 6-CB (c–f), respectively. Irregularly-appearing spots were not considered to represent photo-induced adducts.

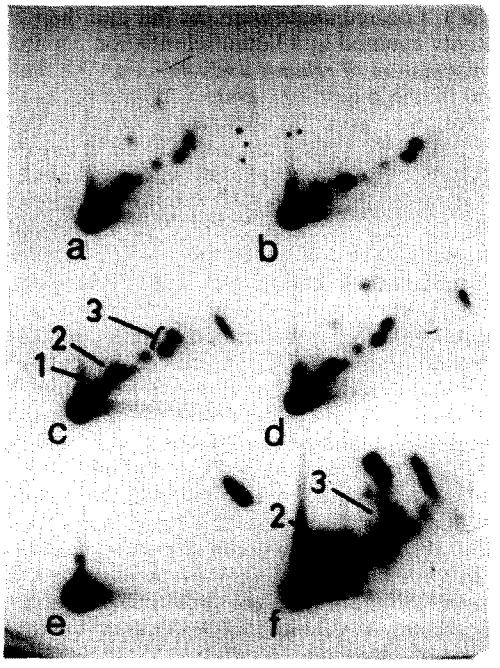

Fig. 4. Autoradiograms of ^{32}P-labeled photo-induced 6-CB DNA adducts after various periods of incubation. Autoradiographic adduct patterns are shown after various incubation times (6-CB concentration: 20 μM). Maps (a–d) show (in this sequence) adducts after 10, 20, 60 and 150 min of cell incubation. Adducts obtained after cell incubation (1 hr) with azido-5-CB (20 μM) are included in maps (e and f), showing "dark" control (e) and irradiated cells (f).

disclosed by formation of photo-induced nucleotide adducts. The chromatographic adduct pattern is shown in Fig. 3. The upper two maps (a and b) display the autoradiographic pattern of the "dark" reaction (map a, no irradiation) and the essentially blank sheet of irradiated cells in the absence of 6-CB (map b). The lower four maps (c–f) show photo-induced 6-CB DNA adduct formation after cell incubation with different 6-CB concentrations (40, 20, 10 and 5 μM of 6-CB, respectively). Whereas the number of distinct adduct spots is the same at all concentrations, spot intensity fades with decreasing concentration of the PCB compound. Thus, adduct intensity is dependent on the 6-CB concentration of the culture medium; adduct levels will be estimated as shown below.

Similarly, photo-induced 6-CB DNA adduct formation reflected the time of incubation. The upper 4 maps (a–d) of Fig. 4 show the photo-induced 6-CB DNA adduct pattern appearing after 10, 20, 60 and 150 min of cell incubation. The entire adduct pattern, as well as the intensity of each spot, come close to the final level as early as after 10 min of incubation. Rapid transport of the PCB congener through the intracellular space right into the nucleus is thus indicated. Moreover, spot distribution on each map is identical to the adduct pattern shown in Fig. 3.

As a positive control, the adduct pattern of the light-sensitive 6-CB derivative 4-azido-2,2′,4′,5,5′-pentachlorobiphenyl (azido-5-CB) is included. The two lower maps (e and f) show (from left) the autoradiographic pattern of the "dark" control (no irradiation) and of the corresponding irradiated sample. Nuclear association of the azido compound yields, indeed, a variety of photo-induced adducts with much higher intensity than the parent compound.

Adduct levels can be quantitated by calculating the ratio of adduct radioactivity to total nucleotide radioactivity [19]. This is shown in Fig. 5 for adduct spots No. 1 and No. 2. The calculated relative adduct levels (RAL) are a linear function of 6-CB concentration (Panel A), and indicate rapid distribution into cell nuclei (Panel B). At the exogenous concentration of 20 μM of 6-CB, the RALs of the two most intensive spots No. 1 and No. 2 are in the order of 2×10^{-8}, indicating 2 photo-induced adducts per 10^8 nucleotides.

The RALs accumulated from several independent experiments are listed in Table 1. The most intensive adduct spots range at $2–3 \times 10^{-8}$, with minor spots exhibiting lower RAL. The two photolabile azido-derivatives of 6-CB behaved quite differently. Whereas adduct formation of 4-azido, 2,2′,4′,5,5′-pentachlorobiphenyl is roughly 10 times more intensive than that of the parent compound, adduct levels

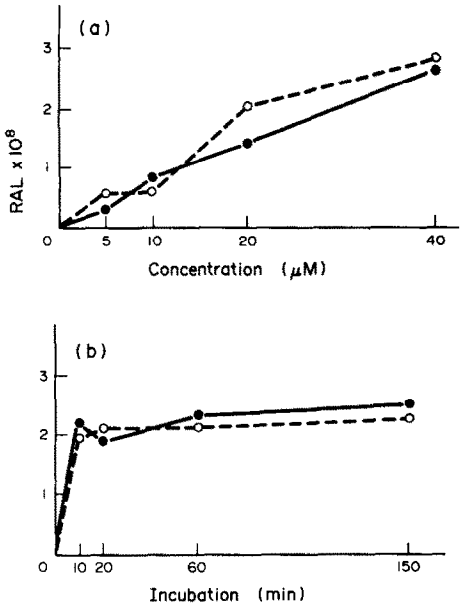

Fig. 5. Relative 6-CB DNA adduct levels (RAL). The ratio of spot radioactivity to total nucleotide radioactivity was calculated in order to estimate relative adduct levels [19]. The dependence of RAL on 6-CB concentration (panel A) and on incubation time (panel B) is shown for spots No. 1 (○—○) and No. 2 (●—●), as marked in Figs 3 and 4.

Table 1. Relative adduct levels after photo-induced binding of chlorinated biphenyls to Chang liver cell DNA

Compound	Spot No.	RAL × 10^8	N
6-CB	1	2.8 ± 1.3	7
	2	2.5 ± 1.1	7
	3	1.0 ± 0.5	7
Azido-5-CB	2	25	2
	3	6	1
Diazido-4-CB	2	1	1

The data are compiled from different experiments (concentration of individual compounds: 20 μM; incubation periods of 1–4 hr).

of the photo-activated 4,4′-diazido derivative are surprisingly low and can barely be detected (RAL $< 1 \times 10^{-8}$), despite of the potential cross-linking functions in the molecule (Table 1).

The minute amounts of autoradiographically detected adduct spots preclude any chemical structure analysis. Specific interaction with individual nucleotides can be deduced from model reactions. Consequently, photo-adducts were generated *in vitro* by irradiating a solution of 6-CB and azido-5-CB with individual deoxyribonucleotide-3′-phosphates (mimicking the composition of the DNA digest). The samples were then analyzed in analogy to an original digest. Figure 6 shows the autoradiographic adduct pattern obtained. The upper cohort (G, A, T, C) represents the control assay: no interfering substances mimicking adduct formation were detected. The following cohort (G*, A*, T*, C*) reveals that only 6-CB photoadducts with the purine nucleotides dG and dA are generated. The same is true for the azido-derivative (last cohort of G′, A′, T′, C′). Moreover, the adduct pattern of dG and dA resembles closely the adduct distribution obtained from *in vivo* DNA analysis (cf. Figs 3 and 4). The results of these experiments substantiate the formation of DNA adducts with guanine and adenine.

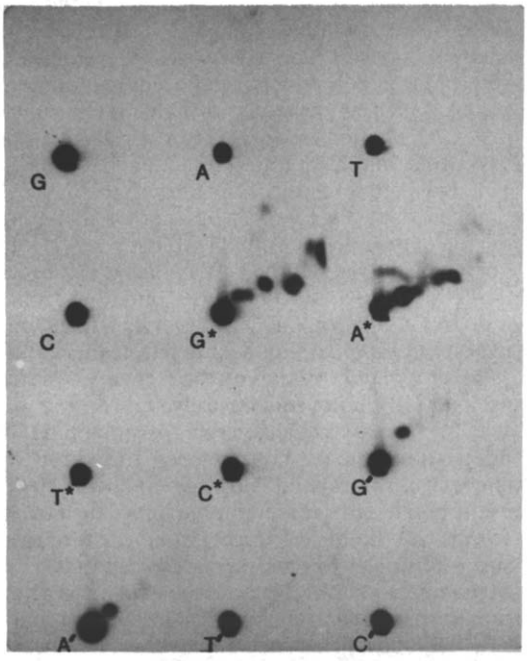

Fig. 6. Autoradiograms of *in vitro* photo-induced 6-CB and azido-5-CB adducts of deoxyribonucleotide-3′,5′-diphosphates. Individual deoxyribonucleotide-3′-phosphates were irradiated in the presence of 6-CB and azido-5-CB (see Materials and Methods). The 12 maps show autoradiograms of adducts, arranged in a page-reading frame. First cohort of G, A, T, C; blanks of nucleotides after irradiation in the absence of xenobiotics; 2nd cohort of G*, A*, T*, C*: photo-induced adducts formed in the presence of 6-CB; 3rd cohort of G′, A′, T′, C′: same, but in the presence of azido-5-CB.

DISCUSSION

We had previously shown that photo-induced covalent binding of 6-CB to components of cultured cells reflects progressive invasion of monolayer cells [2]. This technique has been used in monitoring intracellular association of 6-CB with cell nuclei. We could demonstrate time-dependent photo-induced binding to cell nuclei (Fig. 1); at equilibrium, 60–100 ng of 6-CB are bound per mg protein. This amount falls short of photobinding to total cell protein [2]; obviously, there is no significant nuclear accumulation. Since metabolism of hexachlorobiphenyls is very slow [20] photo-induced binding to DNA is direct evidence of close topologic association of the unmetabolized compound with DNA.

By means of the ^{32}P-postlabeling method low levels of photo-induced 6-CB DNA adducts in human monolayer Chang liver cells were detected. Adduct formation was controlled by experimental variables such as incidence of light, 6-CB concentration, incubation time, use of light-sensitive azido derivatives, and comparison of adduct patterns with nucleotide mixtures after *in vitro* irradiation. The combined data (Figs 3–6, Table 1) prove that a small fraction of the nuclear bound PCB congener is found proximate to the DNA double helix. Moreover, nearly identical adduct patterns obtained from *in vitro* irradiation of 6-CB with model nucleotides suggest PCB contact sites next to purine nucleotides.

Previous *in vitro* studies on the recovery and structure of 6-CB photoproducts as well as on the influence of radical scavengers on photobinding had suggested transient photo-induced formation of a number of chlorinated biphenyl radicals [2]. The results pointed to a direct interaction of such excited radicals with biological targets rather than to mechanisms of singlet oxygen-mediated reactions.

Guanine adducts have also been found in animal studies. After oral dosing of mice with 4-aminobiphenyl (300 mg/kg), binding of the carcinogen to DNA of maternal and fetal tissues was detected [21]. One of the most intensive adducts (RAL $> 2 \times 10^{-5}$) was concluded to result from guanine C-8 substitution [21].

The number of 6-CB adducts can be estimated as follows. Assuming a 5% yield of the photoreaction (taken from equilibrium binding data of Refs 1 and 2), the three major adducts identified run up in total to about 1×10^{-6}, that is 1 adduct in a million DNA nucleotides. Since binding to nuclear protein is close to the average cellular protein binding (and equivalent to a near 200-fold cellular accumulation) hexachlorobiphenyl is virtually excluded from the core of the DNA chromatin complex.

If administered *in vivo* to laboratory animals, liver DNA adducts of most carcinogenic agents are in the range of 1 adduct in 10^5–10^7 nucleotides [22]. On the other hand, incubation of HeLa cells in cell culture with 1-nitro-9-aminoacridines generated 1 adduct in 440 nucleotides [23].

It appears that adduct levels derived from experiments at optimal cell culture conditions (including uniform exposure of cells, unlimited access of the PCB compound, optimal temperature and growth conditions) are quantitatively not comparable to *in vivo* conditions. Thus, the low adduct levels of 6-CB in monolayer Chang liver cells should be taken as a maximum level which is likely never to be reached *in vivo*. For example, *in vivo* adduct levels have been reported for the structurally related 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD): rat liver DNA adduct levels of less than 1×10^{-9} can be derived from the experimental data [24]. It appears that the dense protein layer covering the DNA helix prevents the PCB congener from massive interaction with DNA nucleotides. A small fraction only is able to penetrate the protein layer and to reach DNA purine nucleotides. The association is a fairly rapid process, maximum adduct levels are approached soon after initiation of uptake. Taking "natural" conditions of exposure into consideration, the minute amounts of modified DNA nucleotides found are not thought to crucially interfere with processing of DNA.

Acknowledgements—We are greatly indebted to Mrs Heide Wanders for preparation of γ-^{32}P–ATP. Thanks go, also, to Dr Thomas Wolff, Institut für Toxikologie, for stimulating discussions. We are grateful to Prof. J. Berndt, head of the Abteilung für Zellchemie der Gesellschaft für Strahlen- und Umweltforschung München, for his courageous support.

REFERENCES

1. Mangelsdorf I, Buff K and Berndt J, Uptake of persistent environmental chemicals by cultured human cells. *Biochem Pharmacol* **36**: 2071–2078, 1987.
2. Bründl A and Buff K, Photo-induced binding of 2,2′,4,4′,5,5′-hexachlorobiphenyl to cultured human cells. *Biochem Pharmacol* **37**: 1601–1608, 1988.
3. Murakami M and Fukami J-I, Persistent pesticides and environmental chemicals are taken up to a greater extent than non-persistent compounds by cultured human cells. *Bull Environ Cont Toxicol* **78**: 423–427, 1978.
4. Francesco CD and Bickel MH, Uptake *in vitro* of lipophilic model compounds into adipose tissue preparations and lipids. *Biochem Pharmacol* **34**: 3683–3688, 1985.
5. Beranek SR, Becker MM, Kling D and Gamble W, Phospholipid and glyceride biosynthesis in 2,4,5,2′,4′,5′-hexachlorobiphenyl-treated human skin fibroblasts. *Environ Res* **34**: 103–109, 1984.
6. Shireman RB, Lipoprotein-mediated transfer of 2,4,5,2′,4′,5′-hexachlorobiphenyl into cultured human cells. *Xenobiotika* **18**: 449–457, 1988.
7. Kimbrough RD, Squire RA, Lindner RE, Strandberg JD, Montali RJ and Burse VW, Induction of liver tumors in Sherman strain female rats by polychlorinated biphenyl Aroclor 1260. *J Natl Cancer Inst* **55**: 1453–1465, 1975.
8. Oesterle D and Deml E, Dose-dependent promoting effect of polychlorinated biphenyls on enzyme-altered islands in livers of adult and weanling rats. *Carcinogenesis* **5**: 351–355, 1984.
9. Hemminki K, Nucleic acid adducts of chemical carcinogens and mutagens. *Arch Toxicol* **52**: 249–285, 1983.
10. Murakami M and Fukami J-I, Incorporation of labeled pesticides and environmental chemicals into nuclear fraction of cultured human cells. *Bull Environ Cont Toxicol* **24**: 27–30, 1980.
11. Daubeze M and Narbonne JF, Incorporation of labeled 2,4,5,2′,4′,5′-hexachlorobiphenyl into the nuclear fraction of rat hepatocytes *in vivo*. *Toxicol* **31**: 315–318, 1984.
12. Morales NM and Matthews HB, *In vivo* binding of 2,3,6,2′,3′,6′-hexachlorobiphenyl and 2,4,5,2′,4′,5′-hexachlorobiphenyl to mouse liver macromolecules. *Chem Biol Interact* **27**: 99–110, 1979.
13. Narbonne JF and Daubeze M, *In vitro* binding of hexachlorobiphenyl to DNA and proteins. *Toxicol* **16**: 173–175, 1980.
14. Randerath K, Randerath E, Agrawal HP, Gupta RC, Schurdak ME and Reddy MV, Post-labeling methods for carcinogen–DNA adduct analysis. *Environ Health Perspect* **62**: 57–65, 1985.
15. Blin N and Stafford DW, Isolation of high-molecular weight DNA. *Nucleic Acid Res* **3**: 2303–2308, 1976.
16. Maniatis T, Fritsch EF and Sambrook J. In: *Molecular Cloning (A Laboratory Manual)*. Cold Spring Harbor Laboratory, 1982.
17. Gupta RC, Enhanced sensitivity of ^{32}P-post-labeling analysis of aromatic carcinogen: DNA adducts. *Cancer*

Res **45**: 5656–5662, 1985.

18. Schurdak ME, Stong DB, Warshawsky D and Randerath K, ^{32}P-postlabeling analysis of DNA adduction in mice by synthetic metabolites of the environmental carcinogen 7H-dibenzo(c,g)carbazole: chromatographic evidence for 3-hydroxy-7H-dibenzo(c,g)carbazole being a proximate genotoxicant in liver but not skin. *Carcinogenesis* **8**: 591–597, 1987.
19. Gupta RC, Reddy MV and Randerath K, ^{32}P-post-labeling analysis of non-radioactive aromatic carcinogen–DNA adducts. *Carcinogenesis* **3**: 1081–1092, 1982.
20. Schnellmann RG, Putnam CW and Sipes IG, Metabolism of 2,2′,3,3′,6,6′-hexachlorobiphenyl and 2,2′,4,4′,5,5′-hexachlorobiphenyl by human hepatic microsomes. *Biochem Pharmacol* **32**: 3233–3239, 1983.
21. Lu L-JW, Disher RM, Reddy MV and Randerath K, ^{32}P-post-labeling assay in mice of transplacental DNA damage induced by the environmental carcinogens safrole, 4-aminobiphenyl, and benzo(*a*)pyrene. *Cancer Res* **46**: 3046–3054, 1986.
22. Watson PW, Post-radiolabeling for detecting DNA damage. *Mutagenesis* **2**: 319–331, 1987.
23. Bartoszek A and Konopa J, ^{32}P-post-labeling analysis of DNA adducts formed by antitumor 1-nitro-9-aminoacridines with DNA of HeLa S3 cells. *Biochem Pharmacol* **36**: 4169–4171, 1987.
24. Poland A and Glover E, An estimate of the maximum *in vivo* covalent binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to rat liver protein, ribosomal RNA and DNA. *Cancer Res* **39**: 3341–3344, 1979.